SECTION VI
Special Topics

Nutrition, Digestion, & Absorption 44

David A. Bender, PhD, & Peter A. Mayes, PhD, DSc

BIOMEDICAL IMPORTANCE

Besides water, the diet must provide metabolic fuels (mainly carbohydrates and lipids), protein (for growth and turnover of tissue proteins), fiber (for roughage), minerals (elements with specific metabolic functions), and vitamins and essential fatty acids (organic compounds needed in small amounts for essential metabolic and physiologic functions). The polysaccharides, triacylglycerols, and proteins that make up the bulk of the diet must be hydrolyzed to their constituent monosaccharides, fatty acids, and amino acids, respectively, before absorption and utilization. Minerals and vitamins must be released from the complex matrix of food before they can be absorbed and utilized.

Globally, **undernutrition** is widespread, leading to impaired growth, defective immune systems, and reduced work capacity. By contrast, in developed countries, there is often excessive food consumption (especially of fat), leading to obesity and to the development of cardiovascular disease and some forms of cancer. Deficiencies of vitamin A, iron, and iodine pose major health concerns in many countries, and deficiencies of other vitamins and minerals are a major cause of ill health. In developed countries, nutrient deficiency is rare, though there are vulnerable sections of the population at risk. Intakes of minerals and vitamins that are adequate to prevent deficiency may be inadequate to promote optimum health and longevity.

Excessive secretion of gastric acid, associated with *Helicobacter pylori* infection, can result in the development of gastric and duodenal **ulcers;** small changes in the composition of bile can result in crystallization of cholesterol as **gallstones;** failure of exocrine pancreatic secretion (as in **cystic fibrosis**) leads to undernutrition and steatorrhea. **Lactose intolerance** is due to lactase deficiency leading to diarrhea and intestinal discomfort. Absorption of intact peptides that stimulate antibody responses causes **allergic reactions,** and **celiac disease** is an allergic reaction to wheat gluten.

DIGESTION & ABSORPTION OF CARBOHYDRATES

The digestion of complex carbohydrates is by hydrolysis to liberate oligosaccharides, then free mono- and disaccharides. The increase in blood glucose after a test dose of a carbohydrate compared with that after an equivalent amount of glucose is known as the **glycemic index.** Glucose and galactose have an index of 1, as do lactose, maltose, isomaltose, and trehalose, which give rise to these monosaccharides on hydrolysis. Fructose and the sugar alcohols are absorbed less rapidly and have a lower glycemic index, as does sucrose. The glycemic index of starch varies between near 1 to near zero due to variable rates of hydrolysis, and that of nonstarch polysaccharides is zero. Foods that have a low glycemic index are considered to be more beneficial since they cause less fluctuation in insulin secretion.

Amylases Catalyze the Hydrolysis of Starch

The hydrolysis of starch by **salivary** and **pancreatic amylases** catalyze random hydrolysis of $\alpha(1\rightarrow4)$ glycoside bonds, yielding dextrins, then a mixture of glucose, maltose, and isomaltose (from the branch points in amylopectin).

Disaccharidases Are Brush Border Enzymes

The disaccharidases—**maltase, sucrase-isomaltase** (a bifunctional enzyme catalyzing hydrolysis of sucrose and isomaltose), **lactase,** and **trehalase**—are located on the brush border of the intestinal mucosal cells where the resultant monosaccharides and others arising from the diet are absorbed. In most people, apart from those of northern European genetic origin, lactase is gradually lost through adolescence, leading to **lactose intolerance.** Lactose remains in the intestinal lumen, where it is a substrate for bacterial fermentation to lactate, resulting in discomfort and diarrhea.

There Are Two Separate Mechanisms for the Absorption of Monosaccharides in the Small Intestine

Glucose and galactose are absorbed by a sodium-dependent process. They are carried by the same transport protein (SGLT 1) and compete with each other for intestinal absorption (Figure 44–1). Other monosaccharides are absorbed by carrier-mediated diffusion. Because they are not actively transported, fructose and sugar alcohols are only absorbed down their concentration gradient, and after a moderately high intake some may remain in the intestinal lumen, acting as a substrate for bacterial fermentation.

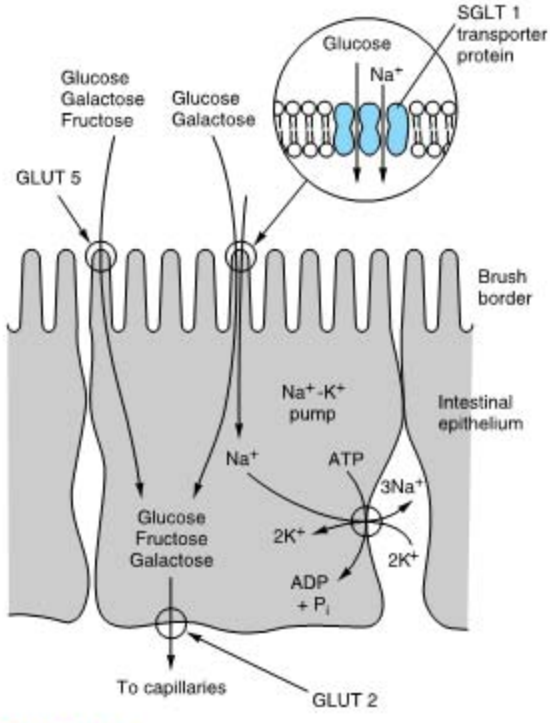

Figure 44–1. Transport of glucose, fructose, and galactose across the intestinal epithelium. The SGLT 1 transporter is coupled to the Na^+-K^+ pump, allowing glucose and galactose to be transported against their concentration gradients. The GLUT 5 Na^+-independent facilitative transporter allows fructose as well as glucose and galactose to be transported with their concentration gradients. Exit from the cell for all the sugars is via the GLUT 2 facilitative transporter.

DIGESTION & ABSORPTION OF LIPIDS

The major lipids in the diet are triacylglycerols and, to a lesser extent, phospholipids. These are hydrophobic molecules and must be hydrolyzed and emulsified to very small droplets (micelles) before they can be absorbed. The fat-soluble vitamins—A, D, E, and K—and a variety of other lipids (including cholesterol) are absorbed dissolved in the lipid micelles. Absorption of the fat-soluble vitamins is impaired on a very low fat diet.

Hydrolysis of triacylglycerols is initiated by **lingual** and **gastric lipases** that attack the *sn*-3 ester bond, forming 1,2-diacylglycerols and free fatty acids, aiding emulsification. **Pancreatic lipase** is secreted into the small intestine and requires a further pancreatic protein, **colipase,** for activity. It is specific for the primary ester links—ie, positions 1 and 3 in triacylglycerols—resulting in 2-monoacylglycerols and free fatty acids as the major end-products of luminal triacylglycerol digestion. Monoacylglycerols are hydrolyzed with difficulty to glycerol and free fatty acids, so that less than 25% of ingested triacylglycerol is completely hydrolyzed to glycerol and fatty acids (Figure 44–2). Bile salts, formed in the liver and secreted in the bile, enable emulsification of the products of lipid digestion into micelles and liposomes together with phospholipids and cholesterol from the bile. Because the micelles are soluble, they allow the products of digestion, including the fat-soluble vitamins, to be transported through the aqueous environment of the intestinal lumen and permit close contact with the brush border of the mucosal cells, allowing uptake into the epithelium, mainly of the jejunum. The bile salts pass on to the ileum, where most are absorbed into the **enterohepatic circulation** (Chapter 26). Within the intestinal epithelium, 1-monoacylglycerols are hydrolyzed to fatty acids and glycerol and 2-monoacylglycerols are re-acylated to triacylglycerols via the **monoacylglycerol pathway.** Glycerol released in the intestinal lumen is not reutilized but passes into the portal vein; glycerol released within the

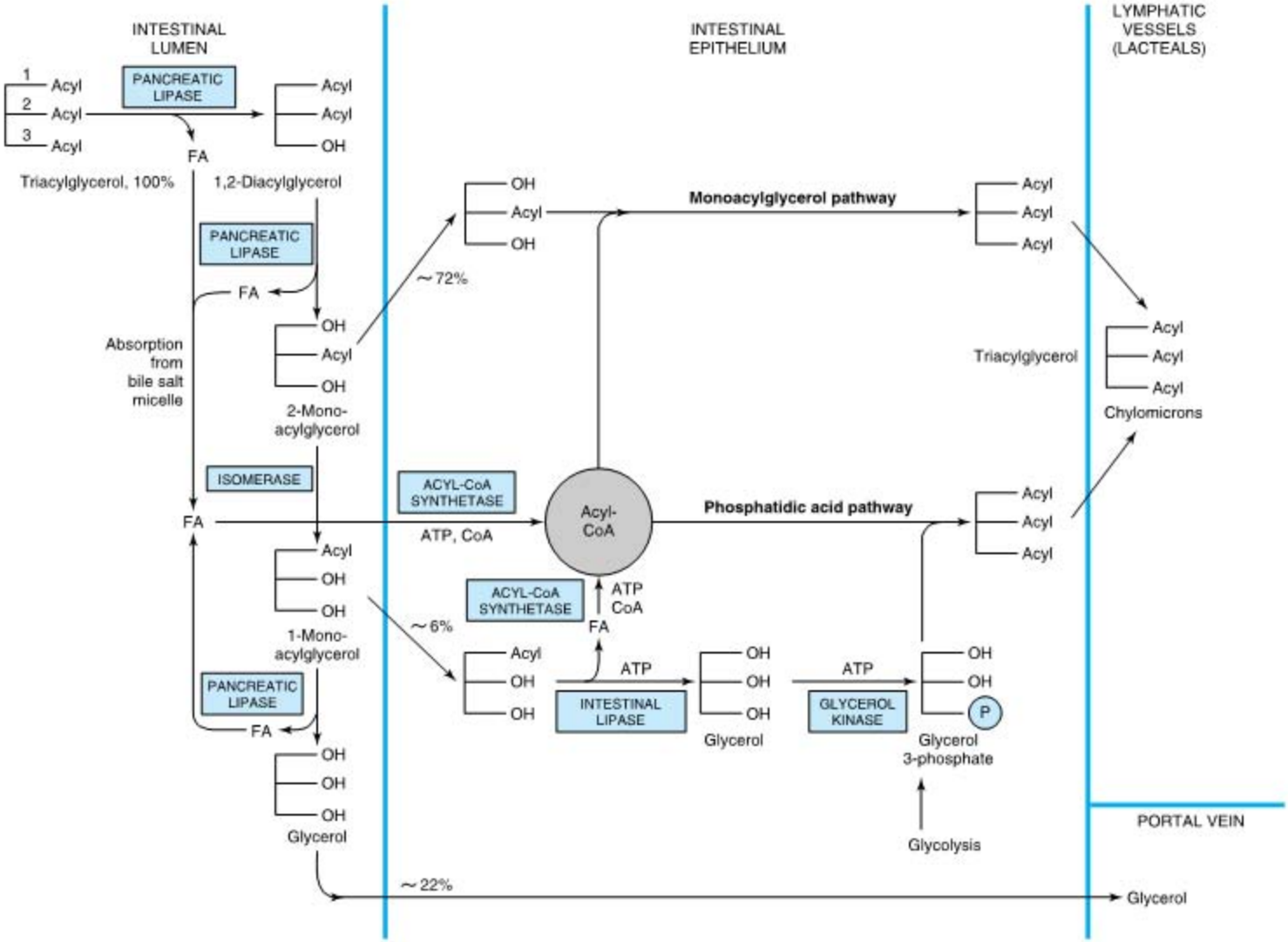

Figure 44–2. Digestion and absorption of triacylglycerols. The values given for percentage uptake may vary widely but indicate the relative importance of the three routes shown.

epithelium is reutilized for triacylglycerol synthesis via the normal phosphatidic acid pathway (Chapter 24). All long-chain fatty acids absorbed are converted to triacylglycerol in the mucosal cells and, together with the other products of lipid digestion, secreted as chylomicrons into the lymphatics, entering the blood stream via the thoracic duct (Chapter 25).

DIGESTION & ABSORPTION OF PROTEINS

Few peptide bonds that are hydrolyzed by proteolytic enzymes are accessible without prior denaturation of dietary proteins (by heat in cooking and by the action of gastric acid).

Several Groups of Enzymes Catalyze the Digestion of Proteins

There are two main classes of proteolytic digestive enzymes **(proteases),** with different specificities for the amino acids forming the peptide bond to be hydrolyzed. **Endopeptidases** hydrolyze peptide bonds between specific amino acids throughout the molecule. They are the first enzymes to act, yielding a larger number of smaller fragments, eg, **pepsin** in the gastric juice and **trypsin, chymotrypsin,** and **elastase** secreted into the small intestine by the pancreas. **Exopeptidases** catalyze the hydrolysis of peptide bonds, one at a time, from the ends of polypeptides. **Carboxypeptidases,** secreted in the pancreatic juice, release amino acids from the free carboxyl terminal, and **aminopeptidases,** secreted by the intestinal mucosal cells, release amino acids from the amino terminal. Dipeptides, which are not substrates for exopeptidases, are hydrolyzed in the brush border of intestinal mucosal cells by **dipeptidases.**

The proteases are secreted as inactive **zymogens;** the active site of the enzyme is masked by a small region of its peptide chain, which is removed by hydrolysis of a specific peptide bond. **Pepsinogen** is activated to pepsin by gastric acid and by activated pepsin (autocatalysis). In the small intestine, **trypsinogen,** the precursor of trypsin, is activated by **enteropeptidase,** which is secreted by the duodenal epithelial cells; trypsin can then activate **chymotrypsinogen** to chymotrypsin, **proelastase** to elastase, **procarboxypeptidase** to carboxypeptidase, and **proaminopeptidase** to aminopeptidase.

Free Amino Acids & Small Peptides Are Absorbed by Different Mechanisms

The end product of the action of endopeptidases and exopeptidases is a mixture of free amino acids, di- and tripeptides, and oligopeptides, all of which are absorbed. Free amino acids are absorbed across the intestinal mucosa by sodium-dependent active transport. There are several different amino acid transporters, with specificity for the nature of the amino acid side chain (large or small; neutral, acidic, or basic). The various amino acids carried by any one transporter compete with each other for absorption and tissue uptake. Dipeptides and tripeptides enter the brush border of the intestinal mucosal cells, where they are hydrolyzed to free amino acids, which are then transported into the hepatic portal vein. Relatively large peptides may be absorbed intact, either by uptake into mucosal epithelial cells (transcellular) or by passing between epithelial cells (paracellular). Many such peptides are large enough to stimulate antibody formation—this is the basis of allergic reactions to foods.

DIGESTION & ABSORPTION OF VITAMINS & MINERALS

Vitamins and minerals are released from food during digestion—though this is not complete—and the availability of vitamins and minerals depends on the type of food and, especially for minerals, the presence of chelating compounds. The fat-soluble vitamins are absorbed in the lipid micelles that result from fat digestion; water-soluble vitamins and most mineral salts are absorbed from the small intestine either by active transport or by carrier-mediated diffusion followed by binding to intracellular binding proteins to achieve concentration upon uptake. Vitamin B_{12} absorption requires a specific transport protein, **intrinsic factor;** calcium absorption is dependent on vitamin D; zinc absorption probably requires a zinc-binding ligand secreted by the exocrine pancreas; and the absorption of iron is limited.

Calcium Absorption Is Dependent on Vitamin D

In addition to its role in regulating calcium homeostasis, vitamin D is required for the intestinal absorption of calcium. Synthesis of the intracellular calcium-binding protein, **calbindin,** required for calcium absorption, is induced by vitamin D, which also affects the permeability of the mucosal cells to calcium, an effect that is rapid and independent of protein synthesis.

Phytic acid (inositol hexaphosphate) in cereals binds calcium in the intestinal lumen, preventing its absorption. Other minerals, including zinc, are also chelated by phytate. This is mainly a problem among people who consume large amounts of unleavened whole wheat products; yeast contains an enzyme, **phytase,** which dephosphorylates phytate, so rendering it inactive. High concentrations of fatty acids in the intestinal lumen—as a result of impaired fat absorption—can also reduce calcium absorption by forming insoluble calcium salts; a high intake of oxalate can sometimes cause deficiency, since calcium oxalate is insoluble.

Iron Absorption Is Limited & Strictly Controlled but Is Enhanced by Vitamin C & Ethanol

Although iron deficiency is a common problem, about 10% of the population are genetically at risk of iron overload **(hemochromatosis),** and elemental iron can lead to nonenzymic generation of free radicals. Absorption of iron is strictly regulated. Inorganic iron is accumulated in intestinal mucosal cells bound to an intracellular protein, **ferritin.** Once the ferritin in the cell is saturated with iron, no more can enter. Iron can only leave the mucosal cell if there is **transferrin** in plasma to bind to. Once transferrin is saturated with iron, any that has accumulated in the mucosal cells will be lost when the cells are shed. As a result of this mucosal barrier, only about 10% of dietary iron is normally absorbed and only 1–5% from many plant foods.

Inorganic iron is absorbed only in the Fe^{2+} (reduced) state, and for that reason the presence of reducing agents will enhance absorption. The most effective compound is **vitamin C,** and while intakes of 40–60 mg of vitamin C per day are more than adequate to meet requirements, an intake of 25–50 mg per meal will enhance iron absorption, especially when iron salts are used to treat iron deficiency anemia. Ethanol and fructose also enhance iron absorption. Heme iron from meat is absorbed separately and is considerably more available than inorganic iron. However, the absorption of both inorganic and heme iron is impaired by calcium—a glass of milk with a meal significantly reduces availability.

ENERGY BALANCE: OVER- & UNDERNUTRITION

After the provision of water, the body's first requirement is for metabolic fuels—fats, carbohydrates, and amino acids from proteins (and ethanol) (Table 27–1). Food intake in excess of energy expenditure leads to **obesity,** while intake less than expenditure leads to emaciation and wasting, as in **marasmus** and **kwashiorkor.** Both obesity and severe undernutrition are associated with increased mortality. The **body mass index,** defined as weight in kilograms divided by height in meters squared, is commonly used as a way of expressing relative obesity to height. A desirable range is between 20 and 25.

Energy Requirements Are Estimated by Measurement of Energy Expenditure

Energy expenditure can be determined directly by measuring heat output from the body but is normally estimated indirectly from the consumption of oxygen. There is an energy expenditure of 20 kJ/L of oxygen consumed regardless of whether the fuel being metabolized is carbohydrate, fat, or protein. Measurement of the ratio of the volume of carbon dioxide produced to volume of oxygen consumed (respiratory quotient; RQ) is an indication of the mixture of metabolic fuels being oxidized (Table 27–1). A more recent technique permits estimation of total energy expenditure over a period of 1–2 weeks using dual isotopically labeled water, $^{2}H_{2}{}^{18}O$. ^{2}H is lost from the body only in water, while ^{18}O is lost in both water and carbon dioxide; the difference in the rate of loss of the two labels permits estimation of total carbon dioxide production and thus oxygen consumption and energy expenditure.

Basal metabolic rate (BMR) is the energy expenditure by the body when at rest—but not asleep—under controlled conditions of thermal neutrality, measured at about 12 hours after the last meal, and depends on weight, age, and gender. Total energy expenditure depends on the basal metabolic rate, the energy required for physical activity, and the energy cost of synthesizing reserves in the fed state. It is therefore possible to calculate an individual's energy requirement from body weight, age, gender, and level of physical activity. Body weight affects BMR because there is a greater amount of active tissue in a larger body. The decrease in BMR with increasing age, even when body weight remains constant, is due to muscle tissue replacement by adipose tissue, which is metabolically much less active. Similarly, women have a significantly lower BMR than do men of the same body weight because women's bodies have proportionately more adipose tissue than men.

Energy Requirements Increase With Activity

The most useful way of expressing the energy cost of physical activities is as a multiple of BMR. Sedentary activities use only about 1.1–1.2 × BMR. By contrast, vigorous exertion, such as climbing stairs, cross-country skiing, walking uphill, etc, may use 6–8 × BMR.

Ten Percent of the Energy Yield of a Meal May Be Expended in Forming Reserves

There is a considerable increase in metabolic rate after a meal, a phenomenon known as **diet-induced thermogenesis.** A small part of this is the energy cost of secreting digestive enzymes and of active transport of the products of digestion; the major part is due to synthesizing reserves of glycogen, triacylglycerol, and protein.

There Are Two Extreme Forms of Undernutrition

Marasmus can occur in both adults and children and occurs in vulnerable groups of all populations. **Kwash-**

iorkor only affects children and has only been reported in developing countries. The distinguishing feature of kwashiorkor is that there is fluid retention, leading to edema. Marasmus is a state of extreme emaciation; it is the outcome of prolonged negative energy balance. Not only have the body's fat reserves been exhausted, but there is wastage of muscle as well, and as the condition progresses there is loss of protein from the heart, liver, and kidneys. The amino acids released by the catabolism of tissue proteins are used as a source of metabolic fuel and as substrates for gluconeogenesis to maintain a supply of glucose for the brain and red blood cells. As a result of the reduced synthesis of proteins, there is impaired immune response and more risk from infections. Impairment of cell proliferation in the intestinal mucosa occurs, resulting in reduction in surface area of the intestinal mucosa and reduction in absorption of such nutrients as are available.

Patients With Advanced Cancer & AIDS Are Malnourished

Patients with advanced cancer, HIV infection and AIDS, and a number of other chronic diseases are frequently undernourished—the condition is called **cachexia.** Physically, they show all the signs of marasmus, but there is considerably more loss of body protein than occurs in starvation. The secretion of cytokines in response to infection and cancer increases the catabolism of tissue protein. This differs from marasmus, in which protein synthesis is reduced but catabolism in unaffected. Patients are **hypermetabolic,** ie, there is a considerable increase in basal metabolic rate. Many tumors metabolize glucose anaerobically to release lactate. This is used for gluconeogenesis in the liver, which is energy-consuming with a net cost of six ATP for each mole of glucose cycled (Chapter 19). There is increased stimulation of **uncoupling proteins** by cytokines, leading to thermogenesis and increased oxidation of metabolic fuels. Futile cycling of lipids occurs because hormone-sensitive lipase is activated by a proteoglycan secreted by tumors, resulting in liberation of fatty acids from adipose tissue and ATP-expensive reesterification in the liver to triacylglycerols, which are exported in VLDL.

Kwashiorkor Affects Undernourished Children

In addition to the wasting of muscle tissue, loss of intestinal mucosa, and impaired immune responses seen in marasmus, children with **kwashiorkor** show a number of characteristic features. The defining characteristic is **edema,** associated with a decreased concentration of plasma proteins. In addition, there is enlargement of the liver due to accumulation of fat. It was formerly believed that the cause of kwashiorkor was a lack of protein, with a more or less adequate energy intake; however, analysis of the diets of affected children shows that this is not so. Children with kwashiorkor are less stunted than those with marasmus, and the edema begins to improve early in treatment, when the child is still receiving a low-protein diet. Very commonly, an infection precipitates kwashiorkor. Superimposed on general food deficiency, there is probably a deficiency of the antioxidant nutrients such as zinc, copper, carotene, and vitamins C and E. The **respiratory burst** in response to infection leads to the production of oxygen and halogen **free radicals** as part of the cytotoxic action of stimulated macrophages. This added oxidant stress may well trigger the development of kwashiorkor.

PROTEIN & AMINO ACID REQUIREMENTS

Protein Requirements Can Be Determined by Measuring Nitrogen Balance

The state of protein nutrition can be determined by measuring the dietary intake and output of nitrogenous compounds from the body. Although nucleic acids also contain nitrogen, protein is the major dietary source of nitrogen and measurement of total nitrogen intake gives a good estimate of protein intake (mg N × 6.25 = mg protein, as nitrogen is 16% of most proteins). The output of nitrogen from the body is mainly in urea and smaller quantities of other compounds in urine and undigested protein in feces, and significant amounts may also be lost in sweat and shed skin.

The difference between intake and output of nitrogenous compounds is known as **nitrogen balance.** Three states can be defined: In a healthy adult, nitrogen balance is in **equilibrium** when intake equals output, and there is no change in the total body content of protein. In a growing child, a pregnant woman, or in recovery from protein loss, the excretion of nitrogenous compounds is less than the dietary intake and there is net retention of nitrogen in the body as protein, ie, **positive nitrogen balance.** In response to trauma or infection—or if the intake of protein is inadequate to meet requirements—there is net loss of protein nitrogen from the body, ie, **negative nitrogen balance.** The continual catabolism of tissue proteins creates the requirement for dietary protein even in an adult who is not growing, though some of the amino acids released can be reutilized. Nitrogen balance studies show that the average daily requirement is 0.6 g of protein per kilogram of body weight (the factor 0.75 should be used to allow for individual variation), or approximately 50 g/d. Average intakes of protein in developed countries are about 80–100 g/d, ie, 14–15% of energy intake. Because

growing children are increasing the protein in the body, they have a proportionately greater requirement than adults and should be in positive nitrogen balance. Even so, the need is relatively small compared with the requirement for protein turnover. In some countries, protein intake may be inadequate to meet these requirements, resulting in stunting of growth.

There Is a Loss of Body Protein in Response to Trauma & Infection

One of the metabolic reactions to major trauma, such as a burn, a broken limb, or surgery, is an increase in the net catabolism of tissue proteins. As much as 6–7% of the total body protein may be lost over 10 days. Prolonged bed rest results in considerable loss of protein because of atrophy of muscles. Protein is catabolized as normal, but without the stimulus of exercise it is not completely replaced. Lost protein is replaced during convalescence, when there is positive nitrogen balance. A normal diet is adequate to permit this replacement.

The Requirement Is Not for Protein Itself but for Specific Amino Acids

Not all proteins are nutritionally equivalent. More of some than of others is needed to maintain nitrogen balance because different proteins contain different amounts of the various amino acids. The body's requirement is for specific amino acids in the correct proportions to replace the body proteins. The amino acids can be divided into two groups: **essential** and **nonessential.** There are nine essential or indispensable amino acids, which cannot be synthesized in the body: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. If one of these is lacking or inadequate, then—regardless of the total intake of protein—it will not be possible to maintain nitrogen balance since there will not be enough of that amino acid for protein synthesis.

Two amino acids—cysteine and tyrosine—can be synthesized in the body, but only from essential amino acid precursors (cysteine from methionine and tyrosine from phenylalanine). The dietary intakes of cysteine and tyrosine thus affect the requirements for methionine and phenylalanine. The remaining 11 amino acids in proteins are considered to be nonessential or dispensable, since they can be synthesized as long as there is enough total protein in the diet—ie, if one of these amino acids is omitted from the diet, nitrogen balance can still be maintained. However, only three amino acids—alanine, aspartate, and glutamate—can be considered to be truly dispensable; they are synthesized from common metabolic intermediates (pyruvate, oxaloacetate, and α-ketoglutarate, respectively). The remaining amino acids are considered as nonessential, but under some circumstances the requirement for them may outstrip the organism's capacity for synthesis.

SUMMARY

- Digestion involves hydrolyzing food molecules into smaller molecules for absorption through the gastrointestinal epithelium. Polysaccharides are absorbed as monosaccharides; triacylglycerols as 2-monoacylglycerols, fatty acids, and glycerol; and proteins as amino acids.
- Digestive disorders arise as a result of (1) enzyme deficiency, eg, lactase and sucrase; (2) malabsorption, eg, of glucose and galactose due to defects in the Na^+-glucose cotransporter (SGLT 1); (3) absorption of unhydrolyzed polypeptides, leading to immunologic responses, eg, as in celiac disease; and (4) precipitation of cholesterol from bile as gallstones.
- Besides water, the diet must provide metabolic fuels (carbohydrate and fat) for bodily growth and activity; protein for synthesis of tissue proteins; fiber for roughage; minerals for specific metabolic functions; certain polyunsaturated fatty acids of the n-3 and n-6 families for eicosanoid synthesis and other functions; and vitamins, organic compounds needed in small amounts for many varied essential functions.
- Twenty different amino acids are required for protein synthesis, of which nine are essential in the human diet. The quantity of protein required is affected by protein quality, energy intake, and physical activity.
- Undernutrition occurs in two extreme forms: marasmus in adults and children and kwashiorkor in children. Overnutrition from excess energy intake is associated with diseases such as obesity, type 2 diabetes mellitus, atherosclerosis, cancer, and hypertension.

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Vitamins & Minerals

45

David A. Bender, PhD, & Peter A. Mayes, PhD, DSc

BIOMEDICAL IMPORTANCE

Vitamins are a group of organic nutrients required in small quantities for a variety of biochemical functions and which, generally, cannot be synthesized by the body and must therefore be supplied in the diet.

The lipid-soluble vitamins are apolar hydrophobic compounds that can only be absorbed efficiently when there is normal fat absorption. They are transported in the blood, like any other apolar lipid, in lipoproteins or attached to specific binding proteins. They have diverse functions, eg, vitamin A, vision; vitamin D, calcium and phosphate metabolism; vitamin E, antioxidant; vitamin K, blood clotting. As well as dietary inadequacy, conditions affecting the digestion and absorption of the lipid-soluble vitamins—such as steatorrhea and disorders of the biliary system—can all lead to deficiency syndromes, including: night blindness and xerophthalmia (vitamin A); rickets in young children and osteomalacia in adults (vitamin D); neurologic disorders and anemia of the newborn (vitamin E); and hemorrhage of the newborn (vitamin K). Toxicity can result from excessive intake of vitamins A and D. Vitamin A and β-carotene (provitamin A), as well as vitamin E, are antioxidants and have possible roles in atherosclerosis and cancer prevention.

The water-soluble vitamins comprise the B complex and vitamin C and function as enzyme cofactors. Folic acid acts as a carrier of one-carbon units. Deficiency of a single vitamin of the B complex is rare, since poor diets are most often associated with multiple deficiency states. Nevertheless, specific syndromes are characteristic of deficiencies of individual vitamins, eg, beriberi (thiamin); cheilosis, glossitis, seborrhea (riboflavin); pellagra (niacin); peripheral neuritis (pyridoxine); megaloblastic anemia, methylmalonic aciduria, and pernicious anemia (vitamin B_{12}); and megaloblastic anemia (folic acid). Vitamin C deficiency leads to scurvy.

Inorganic mineral elements that have a function in the body must be provided in the diet. When the intake is insufficient, deficiency symptoms may arise, eg, anemia (iron), cretinism and goiter (iodine). If present in excess as with selenium, toxicity symptoms may occur.

THE DETERMINATION OF NUTRIENT REQUIREMENTS DEPENDS ON THE CRITERIA OF ADEQUACY CHOSEN

For any nutrient, particularly minerals and vitamins, there is a range of intakes between that which is clearly inadequate, leading to **clinical deficiency disease,** and that which is so much in excess of the body's metabolic capacity that there may be signs of **toxicity.** Between these two extremes is a level of intake that is adequate for normal health and the maintenance of metabolic integrity. Individuals do not all have the same requirement for nutrients even when calculated on the basis of body size or energy expenditure. There is a range of individual requirements of up to 25% around the mean. Therefore, in order to assess the adequacy of diets, it is necessary to set a reference level of intake high enough to ensure that no one will either suffer from deficiency or be at risk of toxicity. If it is assumed that individual requirements are distributed in a statistically normal fashion around the observed mean requirement, then a range of +/− 2 × the standard deviation (SD) around the mean will include the requirements of 95% of the population.

THE VITAMINS ARE A DISPARATE GROUP OF COMPOUNDS WITH A VARIETY OF METABOLIC FUNCTIONS

A vitamin is defined as an organic compound that is required in the diet in small amounts for the maintenance of normal metabolic integrity. Deficiency causes a specific disease, which is cured or prevented only by restoring the vitamin to the diet (Table 45–1). However, **vitamin D,** which can be made in the skin after exposure to sunlight, and **niacin,** which can be formed from the essential amino acid tryptophan, do not strictly conform to this definition.

Table 45–1. The vitamins.

Vitamin		Functions	Deficiency Disease
A	Retinol, β-carotene	Visual pigments in the retina; regulation of gene expression and cell differentiation; β-carotene is an antioxidant	Night blindness, xerophthalmia; keratinization of skin
D	Calciferol	Maintenance of calcium balance; enhances intestinal absorption of Ca^{2+} and mobilizes bone mineral	Rickets = poor mineralization of bone; osteomalacia = bone demineralization
E	Tocopherols, tocotrienols	Antioxidant, especially in cell membranes	Extremely rare—serious neurologic dysfunction
K	Phylloquinone, menaquinones	Coenzyme in formation of γ-carboxyglutamate in enzymes of blood clotting and bone matrix	Impaired blood clotting, hemorrhagic disease
B_1	Thiamin	Coenzyme in pyruvate and α–ketoglutarate, dehydrogenases, and transketolase; poorly defined function in nerve conduction	Peripheral nerve damage (beriberi) or central nervous system lesions (Wernicke-Korsakoff syndrome)
B_2	Riboflavin	Coenzyme in oxidation and reduction reactions; prosthetic group of flavoproteins	Lesions of corner of mouth, lips, and tongue; seborrheic dermatitis
Niacin	Nicotinic acid, nicotinamide	Coenzyme in oxidation and reduction reactions, functional part of NAD and NADP	Pellagra—photosensitive dermatitis, depressive psychosis
B_6	Pyridoxine, pyridoxal, pyridoxamine	Coenzyme in transamination and decarboxylation of amino acids and glycogen phosphorylase; role in steroid hormone action	Disorders of amino acid metabolism, convulsions
	Folic acid	Coenzyme in transfer of one-carbon fragments	Megaloblastic anemia
B_{12}	Cobalamin	Coenzyme in transfer of one-carbon fragments and metabolism of folic acid	Pernicious anemia = megaloblastic anemia with degeneration of the spinal cord
	Pantothenic acid	Functional part of CoA and acyl carrier protein: fatty acid synthesis and metabolism	
H	Biotin	Coenzyme in carboxylation reactions in gluconeogenesis and fatty acid synthesis	Impaired fat and carbohydrate metabolism, dermatitis
C	Ascorbic acid	Coenzyme in hydroxylation of proline and lysine in collagen synthesis; antioxidant; enhances absorption of iron	Scurvy—impaired wound healing, loss of dental cement, subcutaneous hemorrhage

LIPID-SOLUBLE VITAMINS

RETINOIDS & CAROTENOIDS HAVE VITAMIN A ACTIVITY (Figure 45–1)

Retinoids comprise **retinol, retinaldehyde,** and **retinoic acid** (preformed vitamin A, found only in foods of animal origin); carotenoids, found in plants, comprise carotenes and related compounds, known as provitamin A, as they can be cleaved to yield retinaldehyde and thence retinol and retinoic acid. The α-, β-, and γ-carotenes and cryptoxanthin are quantitatively the most important provitamin A carotenoids. Although it would appear that one molecule of β-carotene should yield two of retinol, this is not so in practice; 6 μg of β-carotene is equivalent to 1 μg of preformed retinol. The total amount of vitamin A in foods is therefore expressed as micrograms of retinol equivalents. Beta-carotene and other provitamin A carotenoids are cleaved in the intestinal mucosa by **carotene dioxygenase,** yielding retinaldehyde, which is reduced to retinol, esterified, and secreted in chylomi-

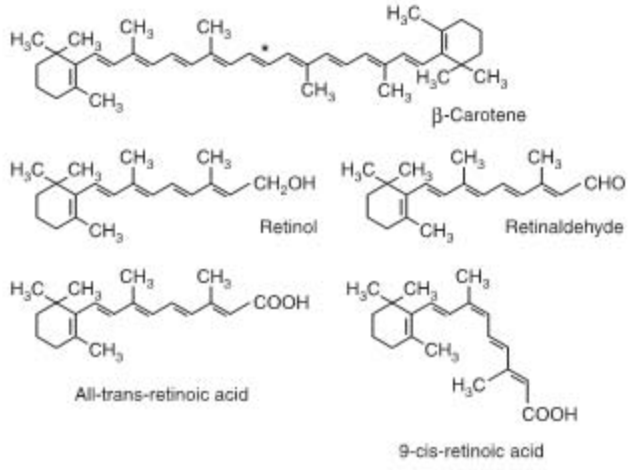

Figure 45–1. β-Carotene and the major vitamin A vitamers. * Shows the site of cleavage of β-carotene into two molecules of retinaldehyde by carotene dioxygenase.

crons together with esters formed from dietary retinol. The intestinal activity of carotene dioxygenase is low, so that a relatively large proportion of ingested β-carotene may appear in the circulation unchanged. While the principal site of carotene dioxygenase attack is the central bond of β-carotene, asymmetric cleavage may also occur, leading to the formation of 8′-, 10′-, and 12′-apo-carotenals, which are oxidized to retinoic acid but cannot be used as sources of retinol or retinaldehyde.

Vitamin A Has a Function in Vision

In the retina, retinaldehyde functions as the prosthetic group of the light-sensitive opsin proteins, forming **rhodopsin** (in rods) and **iodopsin** (in cones). Any one cone cell contains only one type of opsin and is sensitive to only one color. In the pigment epithelium of the retina, all-*trans*-retinol is isomerized to 11-*cis*-retinol and oxidized to 11-*cis*-retinaldehyde. This reacts with a lysine residue in opsin, forming the holoprotein rhodopsin. As shown in Figure 45–2, the absorption of light by rhodopsin causes isomerization of the retinaldehyde from 11-*cis* to all-*trans,* and a conformational change in opsin. This results in the release of retinaldehyde from the protein and the initiation of a nerve impulse. The formation of the initial excited form of rhodopsin, bathorhodopsin, occurs within picoseconds of illumination. There is then a series of conformational changes leading to the formation of metarhodopsin II, which initiates a guanine nucleotide amplification cascade and then a nerve impulse. The final step is hydrolysis to release all-*trans*-retinaldehyde and opsin. The key to initiation of the visual cycle is the availability of 11-*cis*-retinaldehyde, and hence vitamin A. In deficiency, both the time taken to adapt to darkness and the ability to see in poor light are impaired.

Retinoic Acid Has a Role in the Regulation of Gene Expression & Tissue Differentiation

A most important function of vitamin A is in the control of cell differentiation and turnover. All-*trans*-retinoic acid and 9-*cis*-retinoic acid (Figure 45–1) regulate growth, development, and tissue differentiation; they have different actions in different tissues. Like the steroid hormones and vitamin D, retinoic acid binds to nuclear receptors that bind to response elements of DNA and regulate the transcription of specific genes. There are two families of nuclear retinoid receptors: the retinoic acid receptors (RARs) bind all-*trans*-retinoic acid or 9-*cis*-retinoic acid, and the retinoid X receptors (RXRs) bind 9-*cis*-retinoic acid.

Vitamin A Deficiency Is a Major Public Health Problem Worldwide

Vitamin A deficiency is the most important preventable cause of blindness. The earliest sign of deficiency is a loss of sensitivity to green light, followed by impairment of adaptation to dim light, followed by night blindness. More prolonged deficiency leads to **xerophthalmia:** keratinization of the cornea and skin and blindness. Vitamin A also has an important role in differentiation of immune system cells, and mild deficiency leads to increased susceptibility to infectious diseases. Furthermore, the synthesis of retinol-binding protein in response to infection is reduced (it is a negative **acute phase protein**), decreasing the circulating vi-

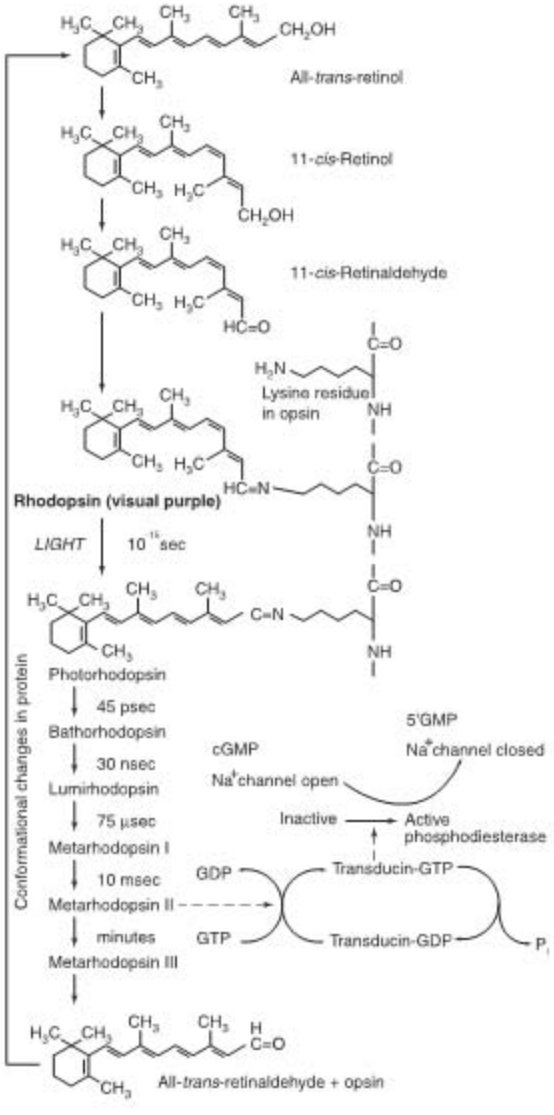

Figure 45–2. The role of retinaldehyde in vision.

tamin, and therefore there is further impairment of immune responses.

Vitamin A Is Toxic in Excess

There is only a limited capacity to metabolize vitamin A, and excessive intakes lead to accumulation beyond the capacity of binding proteins, so that unbound vitamin A causes tissue damage. Symptoms of toxicity affect the central nervous system (headache, nausea, ataxia, and anorexia, all associated with increased cerebrospinal fluid pressure), the liver (hepatomegaly with histologic changes and hyperlipidemia), calcium homeostasis (thickening of the long bones, hypercalcemia and calcification of soft tissues), and the skin (excessive dryness, desquamation, and alopecia).

VITAMIN D IS REALLY A HORMONE

Vitamin D is not strictly a vitamin since it can be synthesized in the skin, and under most conditions that is its major source. Only when sunlight is inadequate is a dietary source required. The main function of vitamin D is in the regulation of calcium absorption and homeostasis; most of its actions are mediated by way of nuclear receptors that regulate gene expression. Deficiency—leading to rickets in children and osteomalacia in adults—continues to be a problem in northern latitudes, where sunlight exposure is poor.

Vitamin D Is Synthesized in the Skin

7-Dehydrocholesterol (an intermediate in the synthesis of cholesterol that accumulates in the skin), undergoes a nonenzymic reaction on exposure to ultraviolet light, yielding previtamin D (Figure 45–3). This undergoes a further reaction over a period of hours to form the vitamin itself, cholecalciferol, which is absorbed into the bloodstream. In temperate climates, the plasma concentration of vitamin D is highest at the end of summer and lowest at the end of winter. Beyond about 40 degrees north or south in winter, there is very little ultraviolet radiation of appropriate wavelength.

Vitamin D Is Metabolized to the Active Metabolite, Calcitriol, in Liver & Kidney

In the liver, cholecalciferol, which has been synthesized in the skin or derived from food, is hydroxylated to form the 25-hydroxy derivative calcidiol (Figure 45–4). This is released into the circulation bound to a vitamin D-binding globulin which is the main storage form of the vitamin. In the kidney, calcidiol undergoes either 1-hydroxylation to yield the active metabolite 1,25-dihydroxyvitamin D (calcitriol) or 24-hydroxylation to yield an inactive metabolite, 24,25-dihydroxyvitamin D (24-hydroxycalcidiol). Ergocalciferol from fortified foods undergoes similar hydroxylations to yield ercalcitriol.

Vitamin D Metabolism Both Regulates & Is Regulated by Calcium Homeostasis

The main function of vitamin D is in the control of calcium homeostasis, and in turn vitamin D metabolism is

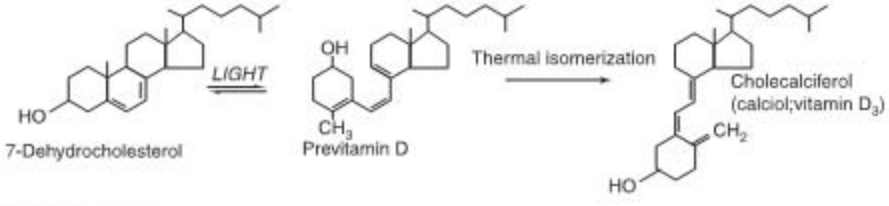

Figure 45–3. Synthesis of vitamin D in the skin.

regulated by factors that respond to plasma concentrations of calcium and phosphate. Calcitriol acts to reduce its own synthesis by inducing the 24-hydroxylase and repressing the 1-hydroxylase in the kidney. Its principal function is to maintain the plasma calcium concentration. Calcitriol achieves this in three ways: it increases intestinal absorption of calcium, reduces excretion of calcium (by stimulating resorption in the distal renal tubules), and mobilizes bone mineral. In addition, calcitriol is involved in insulin secretion, synthesis and secretion of parathyroid and thyroid hormones, inhibition of production of interleukin by activated T lymphocytes and of immunoglobulin by activated B lymphocytes, differentiation of monocyte precursor cells, and modulation of cell proliferation. In its actions, it behaves like a **steroid hormone,** binding to a nuclear receptor protein.

Vitamin D Deficiency Affects Children & Adults

In the vitamin D deficiency disease **rickets,** the bones of children are undermineralized as a result of poor absorption of calcium. Similar problems occur in adolescents who are deficient during their growth spurt. **Osteomalacia** in adults results from demineralization of bone in women who have little exposure to sunlight, often after several pregnancies. Although vitamin D is essential for prevention and treatment of osteomalacia in the elderly, there is little evidence that it is beneficial in treating **osteoporosis.**

Vitamin D Is Toxic in Excess

Some infants are sensitive to intakes of vitamin D as low as 50 µg/d, resulting in an elevated plasma concen-

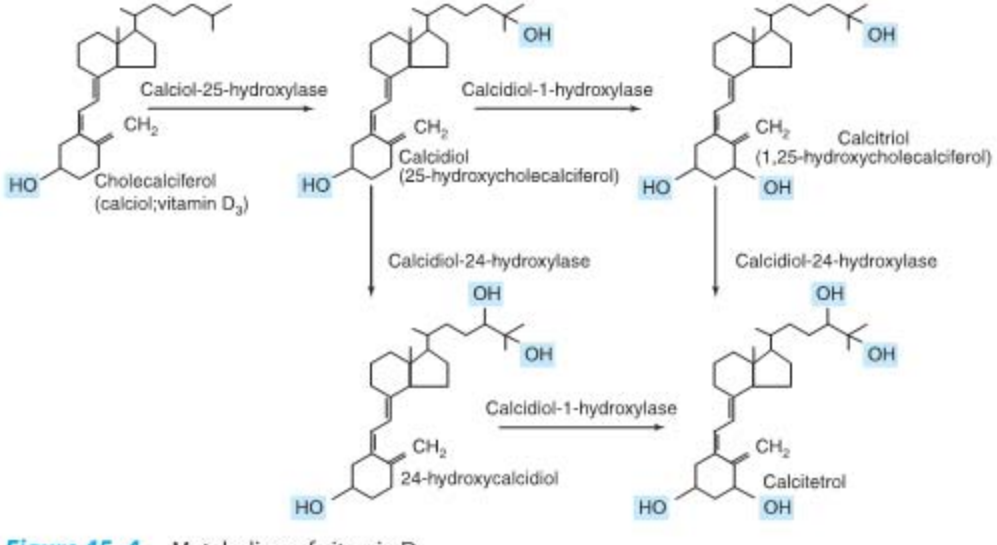

Figure 45–4. Metabolism of vitamin D.

tration of calcium. This can lead to contraction of blood vessels, high blood pressure, and **calcinosis**—the calcification of soft tissues. Although excess dietary vitamin D is toxic, excessive exposure to sunlight does not lead to vitamin D poisoning because there is a limited capacity to form the precursor 7-dehydrocholesterol and to take up cholecalciferol from the skin.

VITAMIN E DOES NOT HAVE A PRECISELY DEFINED METABOLIC FUNCTION

No unequivocal unique function for vitamin E has been defined. However, it does act as a lipid-soluble antioxidant in cell membranes, where many of its functions can be provided by synthetic antioxidants. Vitamin E is the generic descriptor for two families of compounds, the **tocopherols** and the **tocotrienols** (Figure 45–5). The different vitamers (compounds having similar vitamin activity) have different biologic potencies; the most active is D-α-tocopherol, and it is usual to express vitamin E intake in milligrams of D-α-tocopherol equivalents. Synthetic DL-α-tocopherol does not have the same biologic potency as the naturally occurring compound.

Vitamin E Is the Major Lipid-Soluble Antioxidant in Cell Membranes & Plasma Lipoproteins

The main function of vitamin E is as a chain-breaking, free radical trapping antioxidant in cell membranes and plasma lipoproteins. It reacts with the lipid peroxide radicals formed by peroxidation of polyunsaturated fatty acids before they can establish a chain reaction. The tocopheroxyl free radical product is relatively unreactive and ultimately forms nonradical compounds. Commonly, the tocopheroxyl radical is reduced back to tocopherol by reaction with vitamin C from plasma (Figure 45–6). The resultant monodehydroascorbate free radical then undergoes enzymic or nonenzymic reaction to yield ascorbate and dehydroascorbate, neither of which is a free radical. The stability of the tocopheroxyl free radical means that it can penetrate farther into cells and, potentially, propagate a chain reaction. Therefore, vitamin E may, like other antioxidants, also have pro-oxidant actions, especially at high concentrations. This may explain why, although studies have shown an association between high blood concentrations of vitamin E and a lower incidence of atherosclerosis, the effect of high doses of vitamin E have been disappointing.

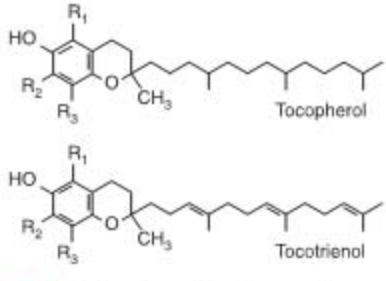

Figure 45–5. The vitamin E vitamers. In α-tocopherol and tocotrienol R_1, R_2, and R_3 are all —CH_3 groups. In the β-vitamers R_2 is H; in the γ-vitamers R_1 is H, and in the δ-vitamers R_1 and R_2 are both H.

Dietary Vitamin E Deficiency in Humans Is Unknown

In experimental animals, vitamin E deficiency results in resorption of fetuses and testicular atrophy. Dietary deficiency of vitamin E in humans is unknown, though patients with severe fat malabsorption, cystic fibrosis, and some forms of chronic liver disease suffer deficiency because they are unable to absorb the vitamin or transport it, exhibiting nerve and muscle membrane damage. Premature infants are born with inadequate reserves of the vitamin. Their erythrocyte membranes are abnormally fragile as a result of peroxidation, which leads to hemolytic anemia.

VITAMIN K IS REQUIRED FOR SYNTHESIS OF BLOOD-CLOTTING PROTEINS

Vitamin K was discovered as a result of investigations into the cause of a bleeding disorder—hemorrhagic (sweet clover) disease—of cattle, and of chickens fed on a fat-free diet. The missing factor in the diet of the chickens was vitamin K, while the cattle feed contained **dicumarol,** an antagonist of the vitamin. Antagonists of vitamin K are used to reduce blood coagulation in patients at risk of thrombosis—the most widely used agent is **warfarin.**

Three compounds have the biologic activity of vitamin K (Figure 45–7): **phylloquinone,** the normal dietary source, found in green vegetables; **menaquinones,** synthesized by intestinal bacteria, with differing lengths of side-chain; **menadione, menadiol,** and **menadiol diacetate,** synthetic compounds that can be metabolized to phylloquinone. Menaquinones are absorbed to some extent but it is not clear to what extent they are biologically active as it is possible to induce signs of vitamin K deficiency simply by feeding a phylloquinone deficient diet, without inhibiting intestinal bacterial action.

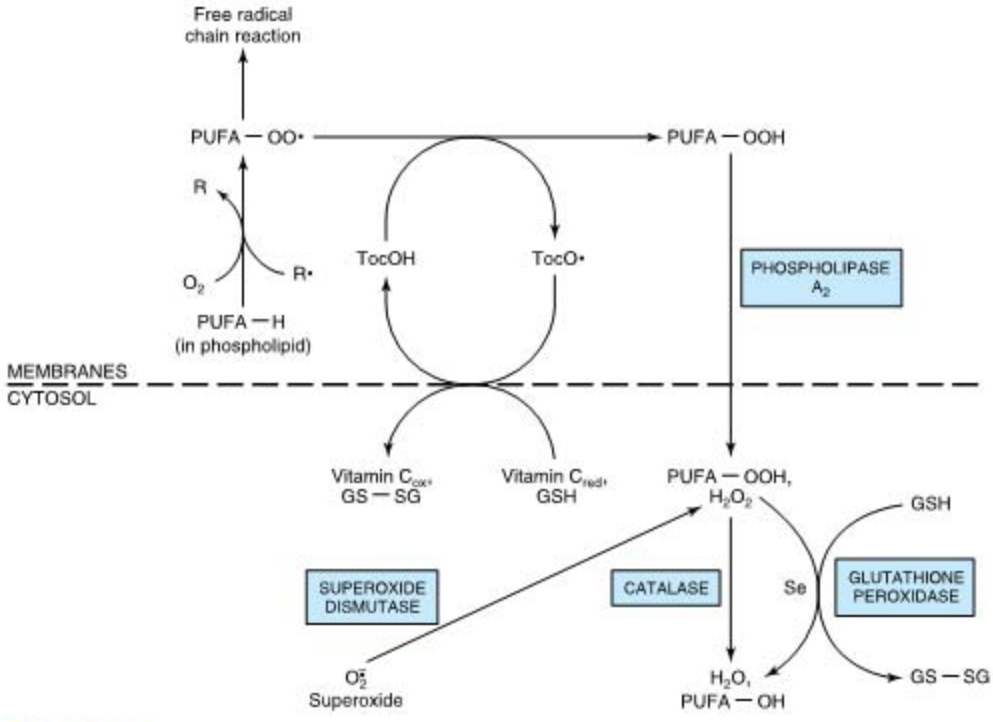

Figure 45–6. Interaction and synergism between antioxidant systems operating in the lipid phase (membranes) of the cell and the aqueous phase (cytosol). (R•, free radical; PUFA-OO•, peroxyl free radical of polyunsaturated fatty acid in membrane phospholipid; PUFA-OOH, hydroperoxy polyunsaturated fatty acid in membrane phospholipid released as hydroperoxy free fatty acid into cytosol by the action of phospholipase A_2; PUFA-OH, hydroxy polyunsaturated fatty acid; TocOH, vitamin E (α-tocopherol); TocO•, free radical of α-tocopherol; Se, selenium; GSH, reduced glutathione; GS-SG, oxidized glutathione, which is returned to the reduced state after reaction with NADPH catalyzed by glutathione reductase; PUFA-H, polyunsaturated fatty acid.)

Vitamin K Is the Coenzyme for Carboxylation of Glutamate in the Postsynthetic Modification of Calcium-Binding Proteins

Vitamin K is the cofactor for the carboxylation of glutamate residues in the post-synthetic modification of proteins to form the unusual amino acid γ-carboxyglutamate (Gla), which chelates the calcium ion. Initially, vitamin K hydroquinone is oxidized to the epoxide (Figure 45–8), which activates a glutamate residue in the protein substrate to a carbanion, that reacts nonenzymically with carbon dioxide to form γ-carboxyglutamate. Vitamin K epoxide is reduced to the quinone by a warfarin-sensitive reductase, and the quinone is reduced to the active hydroquinone by either the same warfarin-sensitive reductase or a warfarin-insensitive quinone reductase. In the presence of warfarin, vitamin K epoxide cannot be reduced but accumulates, and is excreted. If enough vitamin K (a quinone) is provided in the diet, it can be reduced to the active hydroquinone by the warfarin-insensitive enzyme, and carboxylation can continue, with stoichiometric utilization of vitamin K and excretion of the epoxide. A high dose of vitamin K is the antidote to an overdose of warfarin.

Prothrombin and several other proteins of the blood clotting system (Factors VII, IX and X, and proteins C and S) each contain between four and six γ-carboxyglutamate residues which chelate calcium ions and so permit the binding of the blood clotting proteins to membranes. In vitamin K deficiency or in the presence of warfarin, an abnormal precursor of prothrombin (preprothrombin) containing little or no γ-carboxyglutamate, and incapable of chelating calcium, is released into the circulation.

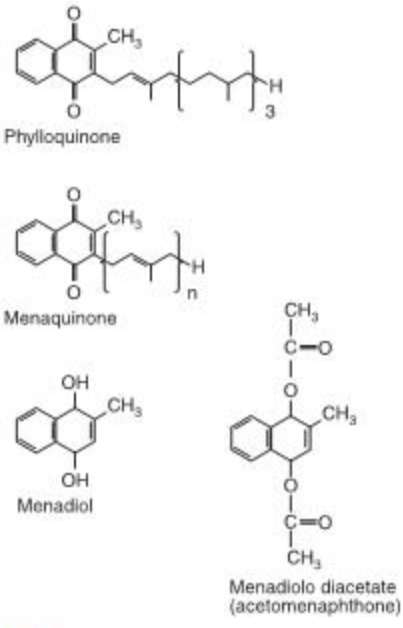

Figure 45–7. The vitamin K vitamers. Menadiol (or menadione) and menadiol diacetate are synthetic compounds that are converted to menaquinone in the liver and have vitamin K activity.

Vitamin K Is Also Important in the Synthesis of Bone Calcium-Binding Proteins

Treatment of pregnant women with warfarin can lead to fetal bone abnormalities (fetal warfarin syndrome). Two proteins are present in bone that contain γ-carboxyglutamate, osteocalcin and bone matrix Gla protein. Osteocalcin also contains hydroxyproline, so its synthesis is dependent on both vitamins K and C; in addition, its synthesis is induced by vitamin D. The release into the circulation of osteocalcin provides an index of vitamin D status.

WATER-SOLUBLE VITAMINS

VITAMIN B_1 (THIAMIN) HAS A KEY ROLE IN CARBOHYDRATE METABOLISM

Thiamin has a central role in energy-yielding metabolism, and especially the metabolism of carbohydrate (Figure 45–9). **Thiamin diphosphate** is the coenzyme for three multi-enzyme complexes that catalyze oxidative decarboxylation reactions: pyruvate dehydrogenase in carbohydrate metabolism; α-ketoglutarate dehydro-

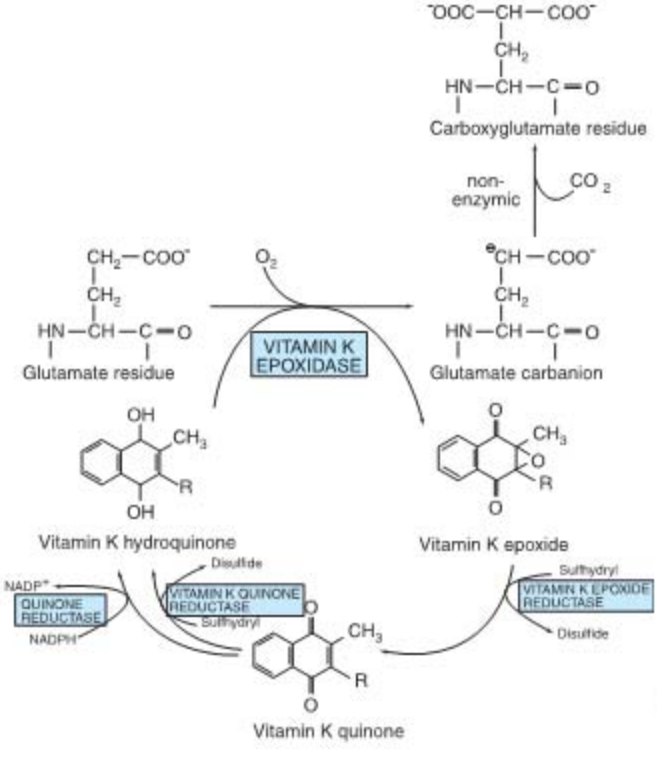

Figure 45–8. The role of vitamin K in the biosynthesis of γ-carboxyglutamate.

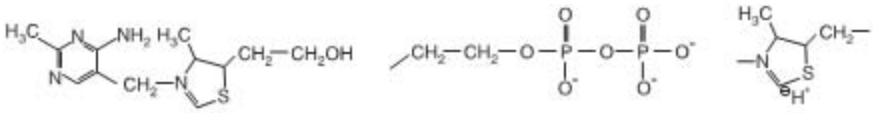

Figure 45–9. Thiamin, thiamin diphosphate, and the carbanion form.

genase in the citric acid cycle; and the branched-chain keto-acid dehydrogenase involved in the metabolism of leucine, isoleucine, and valine. It is also the coenzyme for transketolase, in the pentose phosphate pathway. In each case, the thiamin diphosphate provides a reactive carbon on the thiazole moiety that forms a carbanion, which then adds to the carbonyl group of, for instance, pyruvate. The addition compound then decarboxylates, eliminating CO_2. Electrical stimulation of nerve leads to a fall in membrane thiamin triphosphate and release of free thiamin. It is likely that thiamin triphosphate acts as a phosphate donor for phosphorylation of the nerve membrane sodium transport channel.

Thiamin Deficiency Affects the Nervous System & Heart

Thiamin deficiency can result in three distinct syndromes: a chronic peripheral neuritis, **beriberi,** which may or may not be associated with **heart failure** and **edema;** acute pernicious (fulminating) beriberi (shoshin beriberi), in which heart failure and metabolic abnormalities predominate, without peripheral neuritis; and **Wernicke's encephalopathy** with **Korsakoff's psychosis,** which is associated especially with alcohol and drug abuse. The central role of thiamin diphosphate in pyruvate dehydrogenase means that in deficiency there is impaired conversion of pyruvate to acetyl CoA. In subjects on a relatively high carbohydrate diet, this results in increased plasma concentrations of lactate and pyruvate, which may cause life-threatening **lactic acidosis.**

Thiamin Nutritional Status Can Be Assessed by Erythrocyte Transketolase Activation

The activation of apo-transketolase(the enzyme protein) in erythrocyte lysate by thiamin diphosphate added in vitro has become the accepted index of thiamin nutritional status.

VITAMIN B_2 (RIBOFLAVIN) HAS A CENTRAL ROLE IN ENERGY-YIELDING METABOLISM

Riboflavin fulfills its role in metabolism as the coenzymes **flavin mononucleotide (FMN)** and **flavin adenine dinucleotide (FAD)** (Figure 45–10). FMN is formed by ATP-dependent phosphorylation of riboflavin, whereas FAD is synthesized by further reaction of FMN with ATP in which its AMP moiety is transferred to the

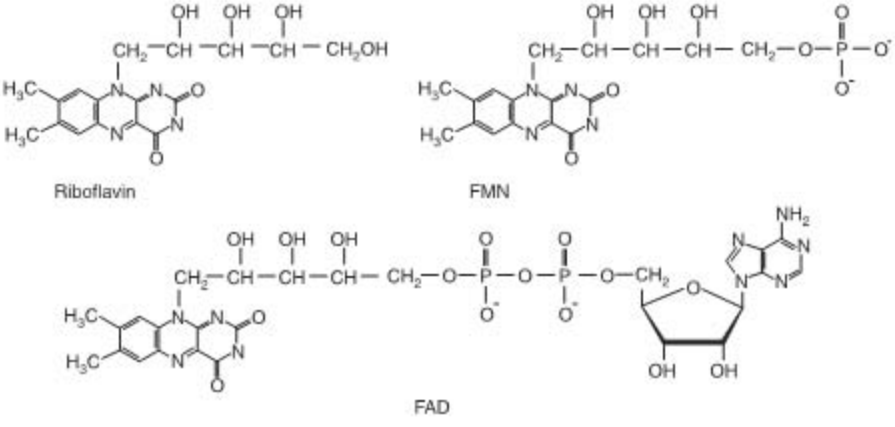

Figure 45–10. Riboflavin and the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).

FMN. The main dietary sources of riboflavin are milk and dairy products. In addition, because of its intense yellow color, riboflavin is widely used as a food additive.

Flavin Coenzymes Are Electron Carriers in Oxidoreduction Reactions

These include the mitochondrial respiratory chain, key enzymes in fatty acid and amino acid oxidation, and the citric acid cycle. Reoxidation of the reduced flavin in oxygenases and mixed-function oxidases proceeds by way of formation of the flavin radical and flavin hydroperoxide, with the intermediate generation of superoxide and perhydroxyl radicals and hydrogen peroxide. Because of this, flavin oxidases make a significant contribution to the total oxidant stress of the body.

Riboflavin Deficiency Is Widespread But Not Fatal

Although riboflavin is fundamentally involved in metabolism, and deficiencies are found in most countries, it is not fatal as there is very efficient conservation of tissue riboflavin. Riboflavin deficiency is characterized by cheilosis, lingual desquamation and a seborrheic dermatitis. Riboflavin nutritional status is assessed by measurement of the activation of erythrocyte glutathione reductase by FAD added in vitro.

NIACIN IS NOT STRICTLY A VITAMIN

Niacin was discovered as a nutrient during studies of **pellagra.** It is not strictly a vitamin since it can be synthesized in the body from the essential amino acid tryptophan. Two compounds, **nicotinic acid** and **nicotinamide,** have the biologic activity of niacin; its metabolic function is as the nicotinamide ring of the coenzymes **NAD** and **NADP** in oxidation-reduction reactions (Figure 45–11). About 60 mg of tryptophan is equivalent to 1 mg of dietary niacin. The niacin content of foods is expressed as mg niacin equivalents = mg preformed niacin + 1/60 × mg tryptophan. Because most of the niacin in cereals is biologically unavailable, this is discounted.

NAD Is the Source of ADP-Ribose

In addition to its coenzyme role, NAD is the source of ADP-ribose for the **ADP-ribosylation** of proteins and polyADP-ribosylation of nucleoproteins involved in the **DNA repair mechanism.**

Pellagra Is Caused by Deficiency of Tryptophan & Niacin

Pellagra is characterized by a photosensitive dermatitis. As the condition progresses, there is dementia, possibly

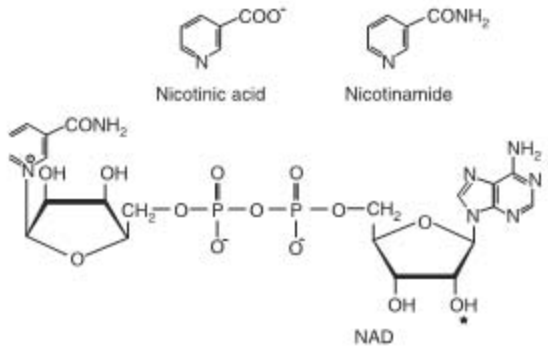

Figure 45–11. Niacin (nicotinic acid and nicotinamide) and nicotinamide adenine dinucleotide (NAD). * Shows the site of phosphorylation in NADP.

diarrhea, and, if untreated, death. Although the nutritional etiology of pellagra is well established and tryptophan or niacin will prevent or cure the disease, additional factors, including deficiency of riboflavin or vitamin B_6, both of which are required for synthesis of nicotinamide from tryptophan, may be important. In most outbreaks of pellagra twice as many women as men are affected, probably the result of inhibition of tryptophan metabolism by estrogen metabolites.

Pellagra Can Occur as a Result of Disease Despite an Adequate Intake of Tryptophan & Niacin

A number of genetic diseases that result in defects of tryptophan metabolism are associated with the development of pellagra despite an apparently adequate intake of both tryptophan and niacin. **Hartnup disease** is a rare genetic condition in which there is a defect of the membrane transport mechanism for tryptophan, resulting in large losses due to intestinal malabsorption and failure of the renal resorption mechanism. In **carcinoid syndrome** there is metastasis of a primary liver tumor of enterochromaffin cells which synthesize 5-hydroxytryptamine. Overproduction of 5-hydroxytryptamine may account for as much as 60% of the body's tryptophan metabolism, causing pellagra because of the diversion away from NAD synthesis.

Niacin Is Toxic in Excess

Nicotinic acid has been used to treat hyperlipidemia when of the order of 1–6 g/d are required, causing dilation of blood vessels and flushing, with skin irritation. Intakes of both nicotinic acid and nicotinamide in excess of 500 mg/d can cause liver damage.

VITAMIN B_6 IS IMPORTANT IN AMINO ACID & GLYCOGEN METABOLISM & IN STEROID HORMONE ACTION

Six compounds have vitamin B_6 activity (Figure 45–12): **pyridoxine, pyridoxal, pyridoxamine,** and their 5′-phosphates. The active coenzyme is pyridoxal 5′-phosphate. Approximately 80% of the body's total vitamin B_6 is present as pyridoxal phosphate in muscle, mostly associated with glycogen phosphorylase. This is not available in B_6 deficiency but is released in starvation, when glycogen reserves become depleted, and is then available, especially in liver and kidney, to meet increased requirement for gluconeogenesis from amino acids.

Vitamin B_6 Has Several Roles in Metabolism

Pyridoxal phosphate is a coenzyme for many enzymes involved in amino acid metabolism, especially in transamination and decarboxylation. It is also the cofactor of glycogen phosphorylase, where the phosphate group is catalytically important. In addition, vitamin B_6 is important in steroid hormone action where it removes the hormone-receptor complex from DNA binding, terminating the action of the hormones. In vitamin B_6 deficiency, this results in increased sensitivity to the actions of low concentrations of estrogens, androgens, cortisol, and vitamin D.

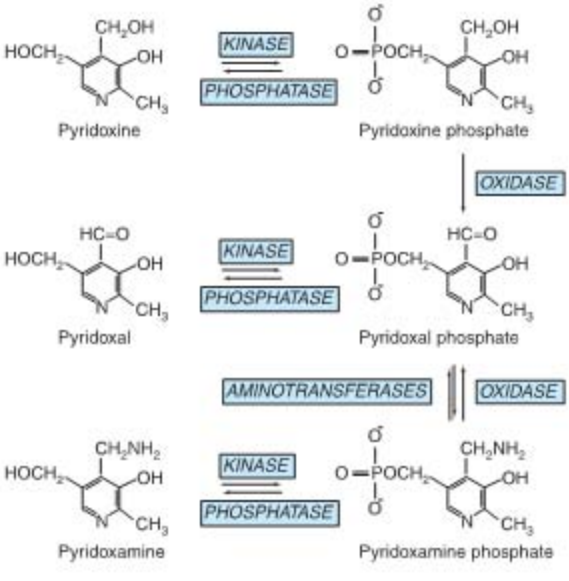

Figure 45–12. Interconversion of the vitamin B_6 vitamers.

Vitamin B_6 Deficiency Is Rare

Although clinical deficiency disease is rare, there is evidence that a significant proportion of the population have marginal vitamin B_6 status. Moderate deficiency results in abnormalities of tryptophan and methionine metabolism. Increased sensitivity to steroid hormone action may be important in the development of **hormone-dependent cancer** of the breast, uterus, and prostate, and vitamin B_6 status may affect the prognosis.

Vitamin B_6 Status Is Assessed by Assaying Erythrocyte Aminotransferases

The most widely used method of assessing vitamin B_6 status is by the activation of erythrocyte aminotransferases by pyridoxal phosphate added in vitro, expressed as the activation coefficient.

In Excess, Vitamin B_6 Causes Sensory Neuropathy

The development of sensory neuropathy has been reported in patients taking 2–7 g of pyridoxine per day for a variety of reasons (there is some slight evidence that it is effective in treating **premenstrual syndrome**). There was some residual damage after withdrawal of these high doses; other reports suggest that intakes in excess of 200 mg/d are associated with neurologic damage.

VITAMIN B_{12} IS FOUND ONLY IN FOODS OF ANIMAL ORIGIN

The term "vitamin B_{12}" is used as a generic descriptor for the **cobalamins**—those **corrinoids** (cobalt containing compounds possessing the corrin ring) having the biologic activity of the vitamin (Figure 45–13). Some corrinoids that are growth factors for microorganisms not only have no vitamin B_{12} activity but may also be antimetabolites of the vitamin. Although it is synthesized exclusively by microorganisms, for practical purposes vitamin B_{12} is found only in foods of animal origin, there being no plant sources of this vitamin. This means that strict vegetarians (vegans) are at risk of developing B_{12} deficiency. The small amounts of the vitamin formed by bacteria on the surface of fruits may be adequate to meet requirements, but preparations of vitamin B_{12} made by bacterial fermentation are available.

Vitamin B_{12} Absorption Requires Two Binding Proteins

Vitamin B_{12} is absorbed bound to **intrinsic factor,** a small glycoprotein secreted by the parietal cells of the

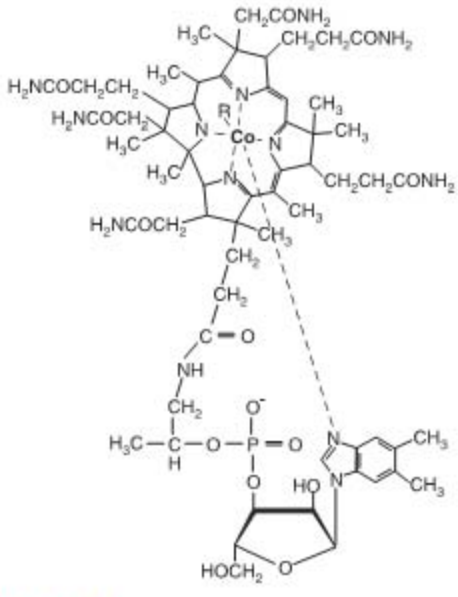

Figure 45–13. Vitamin B_{12} (cobalamin). R may be varied to give the various forms of the vitamin, eg, R = CN^- in cyanocobalamin; R = OH^- in hydroxocobalamin; R = 5′-deoxyadenosyl in 5′-deoxyadenosylcobalamin; R = H_2O in aquocobalamin; and R = CH_3 in methylcobalamin.

gastric mucosa. Gastric acid and pepsin release the vitamin from protein binding in food and make it available to bind to **cobalophilin,** a binding protein secreted in the saliva. In the duodenum, cobalophilin is hydrolyzed, releasing the vitamin for binding to intrinsic factor. **Pancreatic insufficiency** can therefore be a factor in the development of vitamin B_{12} deficiency, resulting in the excretion of cobalophilin-bound vitamin B_{12}. Intrinsic factor binds the various vitamin B_{12} vitamers, but not other corrinoids. Vitamin B_{12} is absorbed from the distal third of the ileum via receptors that bind the intrinsic factor-vitamin B_{12} complex but not free intrinsic factor or free vitamin.

There Are Three Vitamin B_{12}-Dependent Enzymes

Methylmalonyl CoA mutase, leucine aminomutase, and **methionine synthase** (Figure 45–14) are vitamin B_{12}-dependent enzymes. Methylmalonyl CoA is formed as an intermediate in the catabolism of valine and by the carboxylation of propionyl CoA arising in the catabolism of isoleucine, cholesterol, and, rarely, fatty acids with an odd number of carbon atoms—or directly from propionate, a major product of microbial fermentation in ruminants. It undergoes vitamin B_{12}-dependent rearrangement to succinyl-CoA, catalyzed by methylmalonyl-CoA isomerase (Figure 19–2). The activity of this enzyme is greatly reduced in vitamin B_{12} deficiency, leading to an accumulation of methylmalonyl-CoA and urinary excretion of methylmalonic acid, which provides a means of assessing vitamin B_{12} nutritional status.

Vitamin B_{12} Deficiency Causes Pernicious Anemia

Pernicious anemia arises when vitamin B_{12} deficiency blocks the metabolism of folic acid, leading to functional folate deficiency. This impairs erythropoiesis, causing immature precursors of erythrocytes to be released into the circulation (megaloblastic anemia). The commonest cause of pernicious anemia is failure of the absorption of vitamin B_{12} rather than dietary deficiency. This can be due to failure of intrinsic factor secretion caused by autoimmune disease of parietal cells or to generation of anti-intrinsic factor antibodies.

THERE ARE MULTIPLE FORMS OF FOLATE IN THE DIET

The active form of folic acid (pteroyl glutamate) is tetrahydrofolate (Figure 45–15). The folates in foods may have up to seven additional glutamate residues linked by γ-peptide bonds. In addition, all of the one-carbon substituted folates in Figure 45–15 may also be present in foods.

The extent to which the different forms of folate can be absorbed varies, and this must be allowed for in calculating folate intakes.

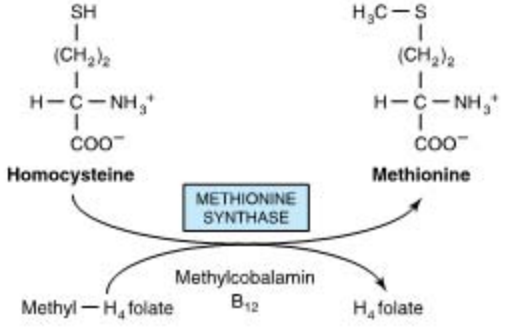

Figure 45–14. Homocysteinuria and the folate trap. Vitamin B_{12} deficiency leads to inhibition of methionine synthase activity causing homocysteinuria and the trapping of folate as methyltetrahydrofolate.

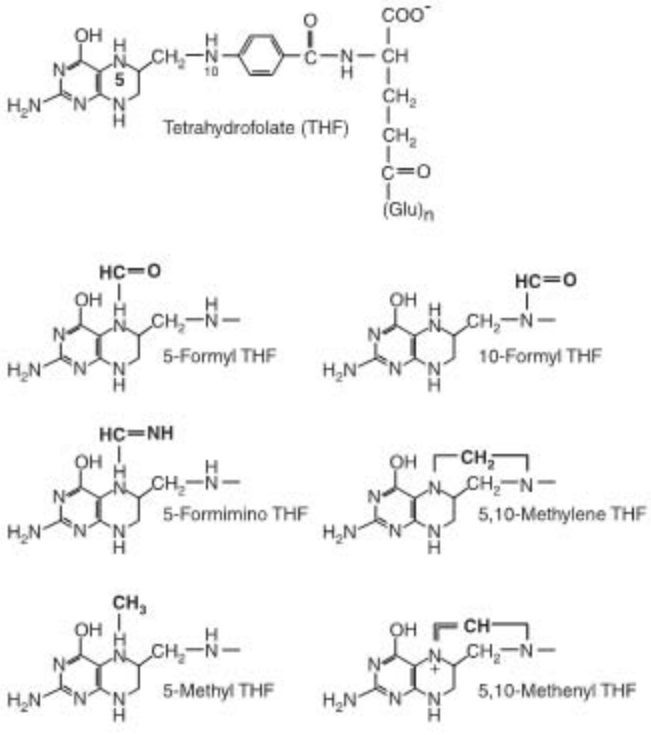

Figure 45–15. Tetrahydrofolic acid and the one-carbon substituted folates.

Tetrahydrofolate Is a Carrier of One-Carbon Units

Tetrahydrofolate can carry one-carbon fragments attached to N-5 (formyl, formimino, or methyl groups), N-10 (formyl group), or bridging N-5 to N-10 (methylene or methenyl groups). 5-Formyl-tetrahydrofolate is more stable than folate and is therefore used pharmaceutically in the agent known as **folinic acid** and in the synthetic (racemic) compound **leucovorin.**

The major point of entry for one-carbon fragments into substituted folates is methylene tetrahydrofolate (Figure 45–16), which is formed by the reaction of glycine, serine, and choline with tetrahydrofolate. Serine is the most important source of substituted folates for biosynthetic reactions, and the activity of serine hy-

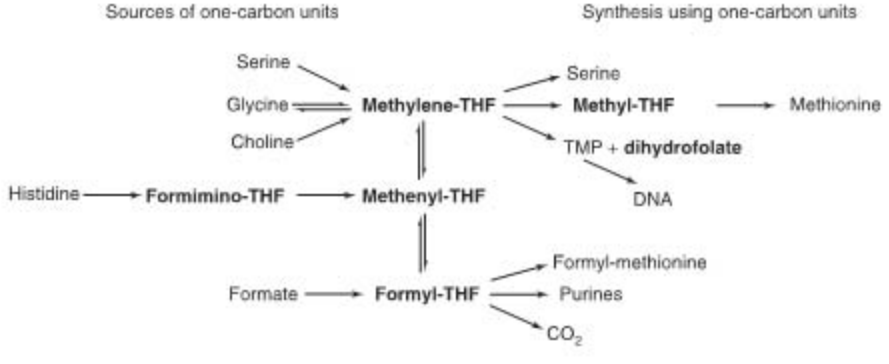

Figure 45–16. Sources and utilization of one-carbon substituted folates.

droxymethyltransferase is regulated by the state of folate substitution and the availability of folate. The reaction is reversible, and in liver it can form serine from glycine as a substrate for gluconeogenesis. Methylene, methenyl, and 10-formyl tetrahydrofolates are interconvertible. When one-carbon folates are not required, the oxidation of formyl tetrahydrofolate to yield carbon dioxide provides a means of maintaining a pool of free folate.

Inhibitors of Folate Metabolism Provide Cancer Chemotherapy & Antibacterial & Antimalarial Drugs

The methylation of deoxyuridine monophosphate (dUMP) to thymidine monophosphate (TMP), catalyzed by thymidylate synthase, is essential for the synthesis of DNA. The one-carbon fragment of methylene-tetrahydrofolate is reduced to a methyl group with release of dihydrofolate, which is then reduced back to tetrahydrofolate by **dihydrofolate reductase.** Thymidylate synthase and dihydrofolate reductase are especially active in tissues with a high rate of cell division. **Methotrexate,** an analog of 10-methyl-tetrahydrofolate, inhibits dihydrofolate reductase and has been exploited as an anticancer drug. The dihydrofolate reductases of some bacteria and parasites differ from the human enzyme; inhibitors of these enzymes can be used as antibacterial drugs, eg, **trimethoprim,** and antimalarial drugs, eg, **pyrimethamine.**

Vitamin B_{12} Deficiency Causes Functional Folate Deficiency—the Folate Trap

When acting as a methyl donor, *S*-adenosylmethionine forms homocysteine, which may be remethylated by methyltetrahydrofolate catalyzed by methionine synthase, a vitamin B_{12}-dependent enzyme (Figure 45–14). The reduction of methylene-tetrahydrofolate to methyl-tetrahydrofolate is irreversible, and since the major source of tetrahydrofolate for tissues is methyl-tetrahydrofolate, the role of methionine synthase is vital and provides a link between the functions of folate and vitamin B_{12}. Impairment of methionine synthase in B_{12} deficiency results in the accumulation of methyl-tetrahydrofolate—the "folate trap." There is therefore functional deficiency of folate secondary to the deficiency of vitamin B_{12}.

Folate Deficiency Causes Megaloblastic Anemia

Deficiency of folic acid itself—or deficiency of vitamin B_{12}, which leads to functional folic acid deficiency—affects cells that are dividing rapidly because they have a large requirement for thymidine for DNA synthesis. Clinically, this affects the bone marrow, leading to megaloblastic anemia.

Folic Acid Supplements Reduce the Risk of Neural Tube Defects & Hyperhomocysteinemia

Supplements of 400 µg/d of folate begun before conception result in a significant reduction in the incidence of neural tube defects as found in **spina bifida.** Elevated blood homocysteine is an associated risk factor for **atherosclerosis, thrombosis,** and **hypertension.** The condition is due to impaired ability to form methyl-tetrahydrofolate by **methylene-tetrahydrofolate reductase,** causing functional folate deficiency and resulting in failure to remethylate homocysteine to methionine. People with the causative abnormal variant of methylene-tetrahydrofolate reductase do not develop hyperhomocysteinemia if they have a relatively high intake of folate, but it is not yet known whether this affects the incidence of cardiovascular disease.

Folate Enrichment of Foods May Put Some People at Risk

Folate supplements will rectify the megaloblastic anemia of vitamin B_{12} deficiency but may hasten the development of the (irreversible) nerve damage found in B_{12} deficiency. There is also antagonism between folic acid and the anticonvulsants used in the treatment of epilepsy.

DIETARY BIOTIN DEFICIENCY IS UNKNOWN

The structures of **biotin, biocytin,** and **carboxy-biotin** (the active metabolic intermediate) are shown in Figure 45–17. Biotin is widely distributed in many foods as biocytin (ε-amino-biotinyl lysine), which is released on proteolysis. It is synthesized by intestinal flora in excess of requirements. Deficiency is unknown except among people maintained for many months on parenteral nutrition and a very small number who eat abnormally large amounts of uncooked egg white, which contains avidin, a protein that binds biotin and renders it unavailable for absorption.

Biotin Is a Coenzyme of Carboxylase Enzymes

Biotin functions to transfer carbon dioxide in a small number of carboxylation reactions. A holocarboxylase synthetase acts on a lysine residue of the apoenzymes of acetyl-CoA carboxylase, pyruvate carboxylase, propionyl-CoA carboxylase, or methylcrotonyl-CoA carboxylase to react with free biotin to form the biocytin residue of the holoenzyme. The reactive intermediate is 1-*N*-carboxybiocytin, formed from bicarbonate in an ATP-dependent reaction. The carboxyl group is then transferred to the substrate for carboxylation (Figure 21–1).

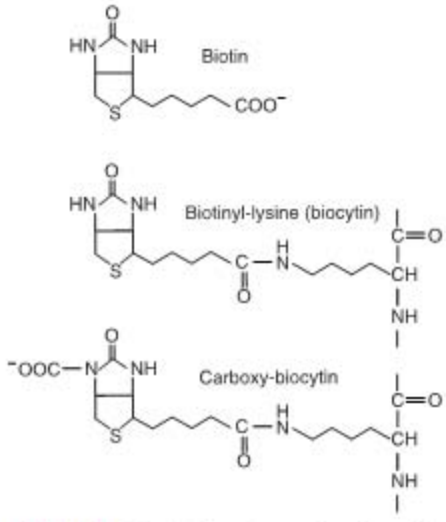

Figure 45–17. Biotin, biocytin, and carboxy-biocytin.

Biotin also has a role in regulation of the cell cycle, acting to biotinylate key nuclear proteins.

AS PART OF CoA AND ACP, PANTOTHENIC ACID ACTS AS A CARRIER OF ACYL RADICALS

Pantothenic acid has a central role in acyl group metabolism when acting as the pantetheine functional moiety of coenzyme A or acyl carrier protein (ACP) (Figure 45–18). The pantetheine moiety is formed after combination of pantothenate with cysteine, which provides the —SH prosthetic group of CoA and ACP. CoA takes part in reactions of the citric acid cycle, fatty acid synthesis and oxidation, acetylations, and cholesterol synthesis. ACP participates in fatty acid synthesis. The vitamin is widely distributed in all foodstuffs, and deficiency has not been unequivocally reported in human beings except in specific depletion studies.

ASCORBIC ACID IS A VITAMIN FOR ONLY SOME SPECIES

Vitamin C (Figure 45–19) is a vitamin for human beings and other primates, the guinea pig, bats, passerine birds, and most fishes and invertebrates; other animals synthesize it as an intermediate in the uronic acid pathway of glucose metabolism (Chapter 20). In those species for which it is a vitamin, there is a block in that pathway due to absence of gulonolactone oxidase. Both ascorbic acid and dehydroascorbic acid have vitamin activity.

Vitamin C Is the Coenzyme for Two Groups of Hydroxylases

Ascorbic acid has specific roles in the copper-containing hydroxylases and the α-ketoglutarate-linked iron-containing hydroxylases. It also increases the activity of a number of other enzymes in vitro, though this is a nonspecific reducing action. In addition, it has a number of nonenzymic effects due to its action as a reducing agent and oxygen radical quencher.

Dopamine β-hydroxylase is a copper-containing enzyme involved in the synthesis of the catecholamines norepinephrine and epinephrine from tyrosine in the adrenal medulla and central nervous system. During hydroxylation, the Cu^{+} is oxidized to Cu^{2+}; reduction back

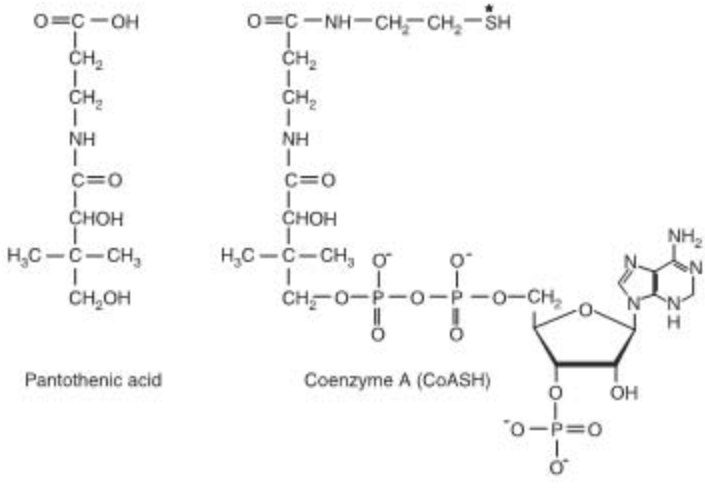

Figure 45–18. Pantothenic acid and coenzyme A. * Shows the site of acylation by fatty acids.

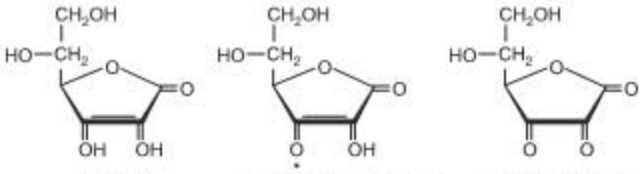

Figure 45–19. Vitamin C.

to Cu^+ specifically requires ascorbate, which is oxidized to monodehydroascorbate. Similar actions of ascorbate occur in tyrosine degradation at the *p*-hydroxyphenylpyruvate hydroxylase step and at the homogentisate dioxygenase step, which needs Fe^{2+} (Figure 30–12).

A number of peptide hormones have a carboxyl terminal amide which is derived from a glycine terminal residue. This glycine is hydroxylated on the α-carbon by a copper-containing enzyme, **peptidylglycine hydroxylase,** which, again, requires ascorbate for reduction of Cu^{2+}.

A number of iron-containing, ascorbate-requiring hydroxylases share a common reaction mechanism in which hydroxylation of the substrate is linked to decarboxylation of α-ketoglutarate (Figure 28–11). Many of these enzymes are involved in the modification of precursor proteins. **Proline and lysine hydroxylases** are required for the postsynthetic modification of **procollagen** to **collagen,** and proline hydroxylase is also required in formation of **osteocalcin** and the C1q component of **complement.** Aspartate β-hydroxylase is required for the postsynthetic modification of the precursor of protein C, the vitamin K-dependent protease which hydrolyzes activated factor V in the blood clotting cascade. Trimethyllysine and γ-butyrobetaine hydroxylases are required for the synthesis of carnitine.

Vitamin C Deficiency Causes Scurvy

Signs of vitamin C deficiency in scurvy include skin changes, fragility of blood capillaries, gum decay, tooth loss, and bone fracture, many of which can be attributed to deficient collagen synthesis.

There May Be Benefits From Higher Intakes of Vitamin C

At intakes above approximately 100 mg/d, the body's capacity to metabolize vitamin C is saturated, and any further intake is excreted in the urine. However, in addition to its other roles, vitamin C enhances the absorption of iron, and this depends on the presence of the vitamin in the gut. Therefore, increased intakes may be beneficial. Evidence is unconvincing that high doses of vitamin C prevent the common cold or reduce the duration of its symptoms.

MINERALS ARE REQUIRED FOR BOTH PHYSIOLOGIC & BIOCHEMICAL FUNCTIONS

Many of the essential minerals (Table 45–2) are widely distributed in foods, and most people eating a normal mixed diet are likely to receive adequate intakes. The

Table 45–2. Classification of essential minerals according to their function.

Function	Mineral
Structural function	Calcium, magnesium, phosphate
Involved in membrane function: principal cations of extracellular- and intracellular fluids, respectively	Sodium, potassium
Function as prosthetic groups in enzymes	Cobalt, copper, iron, molybdenum, selenium, zinc
Regulatory role or role in hormone action	Calcium, chromium, iodine, magnesium, manganese, sodium, potassium
Known to be essential, but function unknown	Silicon, vanadium, nickel, tin
Have effects in the body, but essentiality is not established	Fluoride, lithium
Without known nutritional function but toxic in excess	Aluminum, arsenic, antimony, boron, bromine, cadmium, cesium, germanium, lead, mercury, silver, strontium

amounts required vary from the order of grams per day for sodium and calcium, through milligrams per day (eg, iron) to micrograms per day for the trace elements. In general, mineral deficiencies are encountered when foods come from one region, where the soil may be deficient in some minerals, eg, iodine deficiency. Where the diet comes from a variety of different regions, mineral deficiencies are unlikely. However, iron deficiency is a general problem because if iron losses from the body are relatively high (eg, from heavy menstrual blood loss), it is difficult to achieve an adequate intake to replace the losses. Foods from soils containing high levels of selenium cause toxicity, and increased intakes of common salt (sodium chloride) cause hypertension in susceptible individuals.

SUMMARY

- Vitamins are organic nutrients with essential metabolic functions, generally required in small amounts in the diet because they cannot be synthesized by the body. The lipid-soluble vitamins (A, D, E, and K) are hydrophobic molecules requiring normal fat absorption for their efficient absorption and the avoidance of deficiency symptoms.
- Vitamin A (retinol), present in carnivorous diets, and the provitamin (β-carotene), found in plants, form retinaldehyde, utilized in vision, and retinoic acid, which acts in the control of gene expression. Vitamin D is a steroid prohormone yielding the active hormone derivative calcitriol, which regulates calcium and phosphate metabolism. Vitamin D deficiency leads to rickets and osteomalacia.
- Vitamin E (tocopherol) is the most important antioxidant in the body, acting in the lipid phase of membranes and protecting against the effects of free radicals. Vitamin K functions as cofactor to a carboxylase that acts on glutamate residues of clotting factor precursor proteins to enable them to chelate calcium.
- The water-soluble vitamins of the B complex act as enzyme cofactors. Thiamin is a cofactor in oxidative decarboxylation of α-keto acids and of transketolase in the pentose phosphate pathway. Riboflavin and niacin are important cofactors in oxidoreduction reactions, respectively present in flavoprotein enzymes and in NAD and NADP.
- Pantothenic acid is present in coenzyme A and acyl carrier protein, which act as carriers for acyl groups in metabolic reactions. Pyridoxine, as pyridoxal phosphate, is the coenzyme for several enzymes of amino acid metabolism, including the aminotransferases, and of glycogen phosphorylase. Biotin is the coenzyme for several carboxylase enzymes.
- Besides other functions, vitamin B_{12} and folic acid take part in providing one-carbon residues for DNA synthesis, deficiency resulting in megaloblastic anemia. Vitamin C is a water-soluble antioxidant that maintains vitamin E and many metal cofactors in the reduced state.
- Inorganic mineral elements that have a function in the body must be provided in the diet. When insufficient, deficiency symptoms may arise, and if present in excess they may be toxic.

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Intracellular Traffic & Sorting of Proteins

46

Robert K. Murray, MD, PhD

BIOMEDICAL IMPORTANCE

Proteins must travel from polyribosomes to many different sites in the cell to perform their particular functions. Some are destined to be components of specific organelles, others for the cytosol or for export, and yet others will be located in the various cellular membranes. Thus, there is considerable intracellular traffic of proteins. Many studies have shown that the Golgi apparatus plays a major role in the sorting of proteins for their correct destinations. A major insight was the recognition that for proteins to attain their proper locations, they generally contain information (a signal or coding sequence) that targets them appropriately. Once a number of the signals were defined, it became apparent that certain diseases result from mutations that affect these signals. In this chapter we discuss the intracellular traffic of proteins and their sorting and briefly consider some of the disorders that result when abnormalities occur.

MANY PROTEINS ARE TARGETED BY SIGNAL SEQUENCES TO THEIR CORRECT DESTINATIONS

The protein biosynthetic pathways in cells can be considered to be **one large sorting system.** Many proteins carry **signals** (usually but not always specific sequences of amino acids) that direct them to their destination, thus ensuring that they will end up in the appropriate membrane or cell compartment; these signals are a fundamental component of the sorting system. Usually the signal sequences are recognized and interact with complementary areas of proteins that serve as receptors for the proteins that contain them.

A major sorting decision is made early in protein biosynthesis, when specific proteins are synthesized either on free or on membrane-bound polyribosomes. This results in two sorting branches called the **cytosolic branch** and the **rough endoplasmic reticulum (RER) branch** (Figure 46–1). This sorting occurs because proteins synthesized on membrane-bound polyribosomes contain a **signal peptide** that mediates their attachment to the membrane of the ER. Further details on the signal peptide are given below. Proteins synthesized on **free polyribosomes** lack this particular signal peptide and are delivered into the cytosol. There they are directed to mitochondria, nuclei, and peroxisomes by specific signals—or remain in the cytosol if they lack a signal. Any protein that contains a targeting sequence that is subsequently removed is designated as a **preprotein.** In some cases a second peptide is also removed, and in that event the original protein is known as a **preproprotein** (eg, preproalbumin; Chapter 50).

Proteins synthesized and sorted in the **rough ER branch** (Figure 46–2) include many destined for various membranes (eg, of the ER, Golgi apparatus, lysosomes, and plasma membrane) and for secretion. Lysosomal enzymes are also included. Thus, such proteins may reside in the membranes or lumens of the ER or follow the major transport route of intracellular proteins to the Golgi apparatus. Further signal-mediated sorting of certain proteins occurs in the Golgi apparatus, resulting in delivery to lysosomes, membranes of the Golgi apparatus, and other sites. Proteins destined for the plasma membrane or for secretion pass through the Golgi apparatus but generally are not thought to carry specific sorting signals; they are believed to reach their destinations by default.

The entire pathway of ER → Golgi apparatus → plasma membrane is often called the **secretory** or **exocytotic pathway.** Events along this route will be given special attention. Most of the proteins reaching the Golgi apparatus or the plasma membrane are carried in **transport vesicles;** a brief description of the formation of these important particles will be given subsequently. Other proteins destined for secretion are carried in **secretory vesicles** (Figure 46–2). These are prominent in the pancreas and certain other glands. Their mobilization and discharge are regulated and often referred to as **"regulated secretion,"** whereas the secretory pathway involving transport vesicles is called **"constitutive."**

Experimental approaches that have afforded major insights to the processes described in this chapter include (1) use of yeast mutants; (2) application of recombinant DNA techniques (eg, mutating or eliminating particular sequences in proteins, or fusing new sequences onto them; and (3) development of in vitro

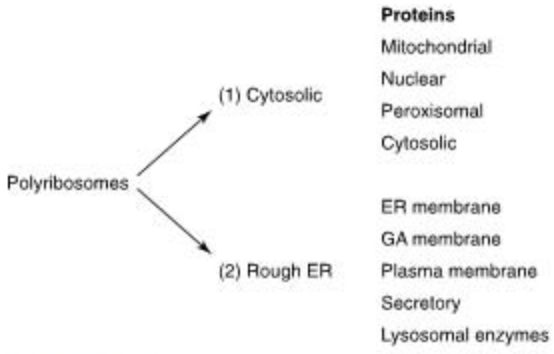

Figure 46–1. Diagrammatic representation of the two branches of protein sorting occurring by synthesis on (1) cytosolic and (2) membrane-bound polyribosomes. The mitochondrial proteins listed are encoded by nuclear genes. Some of the signals used in further sorting of these proteins are listed in Table 46–4. (ER, endoplasmic reticulum; GA, Golgi apparatus.)

systems (eg, to study translocation in the ER and mechanisms of vesicle formation).

The sorting of proteins belonging to the cytosolic branch referred to above is described next, starting with mitochondrial proteins.

THE MITOCHONDRION BOTH IMPORTS & SYNTHESIZES PROTEINS

Mitochondria contain many proteins. Thirteen proteins (mostly membrane components of the electron transport chain) are encoded by the **mitochondrial genome** and synthesized in that organelle using its own protein-synthesizing system. However, the majority (at least several hundred) are encoded by **nuclear genes,** are synthesized outside the mitochondria on cytosolic polyribosomes, and must be imported. **Yeast cells** have proved to be a particularly useful system for analyzing the mechanisms of import of mitochondrial proteins, partly because it has proved possible to generate a variety of mutants that have illuminated the fundamental processes involved. Most progress has been made in the study of proteins present in the mitochondrial matrix, such as the F_1 ATPase subunits. Only the pathway of import of matrix proteins will be discussed in any detail here.

Matrix proteins must pass from cytosolic polyribosomes through the outer and inner mitochondrial membranes to reach their destination. Passage through the two membranes is called **translocation.** They have an amino terminal leader sequence (presequence), about 20–80 amino acids in length, which is not highly conserved but contains many positively charged amino acids (eg, Lys or Arg). The presequence is equivalent to a signal peptide mediating attachment of polyribosomes to membranes of the ER (see below), but in this instance targeting proteins to the matrix; if the leader sequence is cleaved off, potential matrix proteins will not reach their destination.

Translocation is believed to occur **posttranslationally,** after the matrix proteins are released from the cytosolic polyribosomes. Interactions with a number of cytosolic proteins that act as **chaperones** (see below) and as targeting factors occur prior to translocation.

Two distinct **translocation complexes** are situated in the outer and inner mitochondrial membranes, referred to (respectively) as TOM (translocase-of-the-outer membrane) and TIM (translocase-of-the-inner membrane). Each complex has been analyzed and found to be composed of a number of proteins, some of which act as receptors for the incoming proteins and others as components of the transmembrane pores through which these proteins must pass. Proteins must be in the **unfolded state** to pass through the complexes, and this is made possible by **ATP-dependent binding to several chaperone proteins.** The roles of chaperone proteins in protein folding are discussed later in this chapter. In mitochondria, they are involved in translocation, sorting, folding, assembly, and degradation of imported proteins. A **proton-motive force** across the inner membrane is required for import; it is made up of the **electric potential** across the membrane (inside negative) and the **pH gradient** (see Chapter 12). The positively charged leader sequence may be helped through the membrane by the negative charge in the matrix. The presequence is split off in the matrix by a **matrix-processing peptidase (MPP).** Contact with **other chaperones** present in the matrix is essential to complete the overall process of import. Interaction with mt-Hsp70 (Hsp = heat shock protein) ensures proper import into the matrix and prevents misfolding or aggregation, while interaction with the mt-Hsp60-Hsp10 system ensures proper folding. The latter proteins resemble the bacterial GroEL chaperonins, a subclass of chaperones that form complex cage-like assemblies made up of heptameric ring structures. The interactions of imported proteins with the above chaperones require **hydrolysis of ATP** to drive them.

The details of how preproteins are translocated have not been fully elucidated. It is possible that the electric potential associated with the inner mitochondrial membrane causes a conformational change in the unfolded preprotein being translocated and that this helps to pull it across. Furthermore, the fact that the matrix is more negative than the intermembrane space may "attract" the positively charged amino terminal of the preprotein

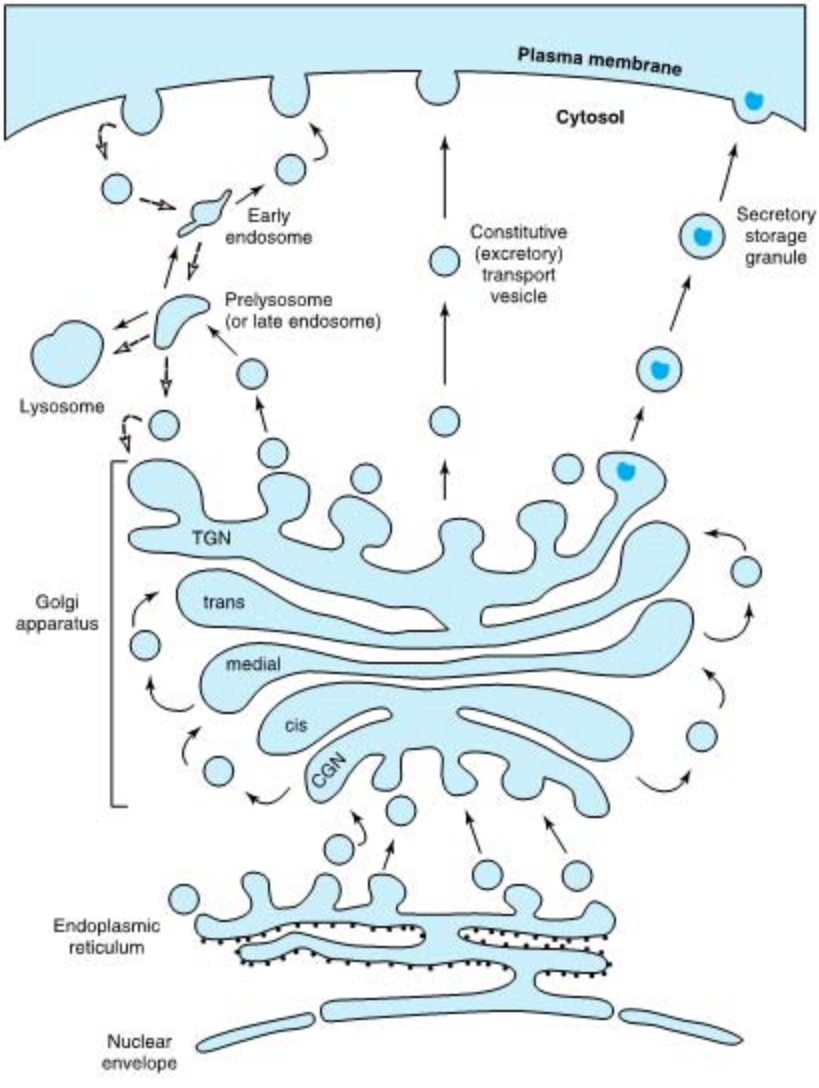

Figure 46–2. Diagrammatic representation of the rough endoplasmic reticulum branch of protein sorting. Newly synthesized proteins are inserted into the ER membrane or lumen from membrane-bound polyribosomes (small black circles studding the cytosolic face of the ER). Those proteins that are transported out of the ER (indicated by solid black arrows) do so from ribosome-free transitional elements. Such proteins may then pass through the various subcompartments of the Golgi until they reach the TGN, the exit side of the Golgi. In the TGN, proteins are segregated and sorted. Secretory proteins accumulate in secretory storage granules from which they may be expelled as shown in the upper right-hand side of the figure. Proteins destined for the plasma membrane or those that are secreted in a constitutive manner are carried out to the cell surface in transport vesicles, as indicated in the upper middle area of the figure. Some proteins may reach the cell surface via late and early endosomes. Other proteins enter prelysosomes (late endosomes) and are selectively transferred to lysosomes. The endocytic pathway illustrated in the upper left-hand area of the figure is considered elsewhere in this chapter. Retrieval from the Golgi apparatus to the ER is not considered in this scheme. (CGN, *cis*-Golgi network; TGN, *trans*-Golgi network.) (Courtesy of E Degen.)

to enter the matrix. Close contact between the membrane sites in the outer and inner membranes involved in translocation is necessary.

The above describes the major pathway of proteins destined for the mitochondrial matrix. However, certain proteins insert into the **outer mitochondrial membrane** facilitated by the TOM complex. Others stop in the **intermembrane space,** and some insert into the **inner membrane.** Yet others proceed into the matrix and then return to the inner membrane or intermembrane space. A number of proteins contain two signaling sequences—one to enter the mitochondrial matrix and the other to mediate subsequent relocation (eg, into the inner membrane). Certain mitochondrial proteins do not contain presequences (eg, cytochrome *c*, which locates in the inter membrane space), and others contain **internal presequences.** Overall, proteins employ a variety of mechanisms and routes to attain their final destinations in mitochondria.

General features that apply to the import of proteins into organelles, including mitochondria and some of the other organelles to be discussed below, are summarized in Table 46–1.

IMPORTINS & EXPORTINS ARE INVOLVED IN TRANSPORT OF MACROMOLECULES IN & OUT OF THE NUCLEUS

It has been estimated that more than a million macromolecules per minute are transported between the nucleus and the cytoplasm in an active eukaryotic cell. These macromolecules include histones, ribosomal proteins and ribosomal subunits, transcription factors, and mRNA molecules. The transport is bidirectional and occurs through the nuclear pore complexes (NPCs). These are complex structures with a mass approximately 30 times that of a ribosome and are composed of about 100 different proteins. The diameter of an NPC is approximately 9 nm but can increase up to approximately 28 nm. Molecules smaller than about 40 kDa can pass through the channel of the NPC by diffusion, but special translocation mechanisms exist for larger molecules. These mechanisms are under intensive investigation, but some important features have already emerged.

Table 46–1. Some general features of protein import to organelles.[1]

- Import of a protein into an organelle usually occurs in three stages: recognition, translocation, and maturation.
- Targeting sequences on the protein are recognized in the cytoplasm or on the surface of the organelle.
- The protein is unfolded for translocation, a state maintained in the cytoplasm by chaperones.
- Threading of the protein through a membrane requires energy and organellar chaperones on the trans side of the membrane.
- Cycles of binding and release of the protein to the chaperone result in pulling of its polypeptide chain through the membrane.
- Other proteins within the organelle catalyze folding of the protein, often attaching cofactors or oligosaccharides and assembling them into active monomers or oligomers.

[1]Data from McNew JA, Goodman JM: The targeting and assembly of peroxisomal proteins: some old rules do not apply. Trends Biochem Sci 1998;21:54.

Here we shall mainly describe **nuclear import** of certain macromolecules. The general picture that has emerged is that proteins to be imported (cargo molecules) carry a **nuclear localization signal (NLS).** One example of an NLS is the amino acid sequence $(Pro)_2$-$(Lys)_4$-Ala-Lys-Val, which is markedly rich in basic lysine residues. Depending on which NLS it contains, a cargo molecule interacts with one of a family of soluble proteins called **importins,** and the complex **docks** at the NPC. Another family of proteins called **Ran** plays a critical regulatory role in the interaction of the complex with the NPC and in its translocation through the NPC. Ran proteins are small monomeric nuclear GTPases and, like other GTPases, exist in either GTP-bound or GDP-bound states. They are themselves regulated by **guanine nucleotide exchange factors** (GEFs; eg, the protein RCC1 in eukaryotes), which are located in the nucleus, and Ran **guanine-activating proteins** (GAPs), which are predominantly cytoplasmic. The GTP-bound state of Ran is favored in the nucleus and the GDP-bound state in the cytoplasm. The conformations and activities of Ran molecules vary depending on whether GTP or GDP is bound to them (the GTP-bound state is active; see discussion of G proteins in Chapter 43). The **asymmetry** between nucleus and cytoplasm—with respect to which of these two nucleotides is bound to Ran molecules—is thought to be crucial in understanding the roles of Ran in transferring complexes unidirectionally across the NPC. When cargo molecules are released inside the nucleus, the importins recirculate to the cytoplasm to be used again. Figure 46–3 summarizes some of the principal features in the above process.

Other **small monomeric GTPases** (eg, ARF, Rab, Ras, and Rho) are important in various cellular processes such as vesicle formation and transport (ARF and Rab; see below), certain growth and differentiation processes (Ras), and formation of the actin cytoskeleton. A process involving GTP and GDP is also crucial in the transport of proteins across the membrane of the ER (see below).

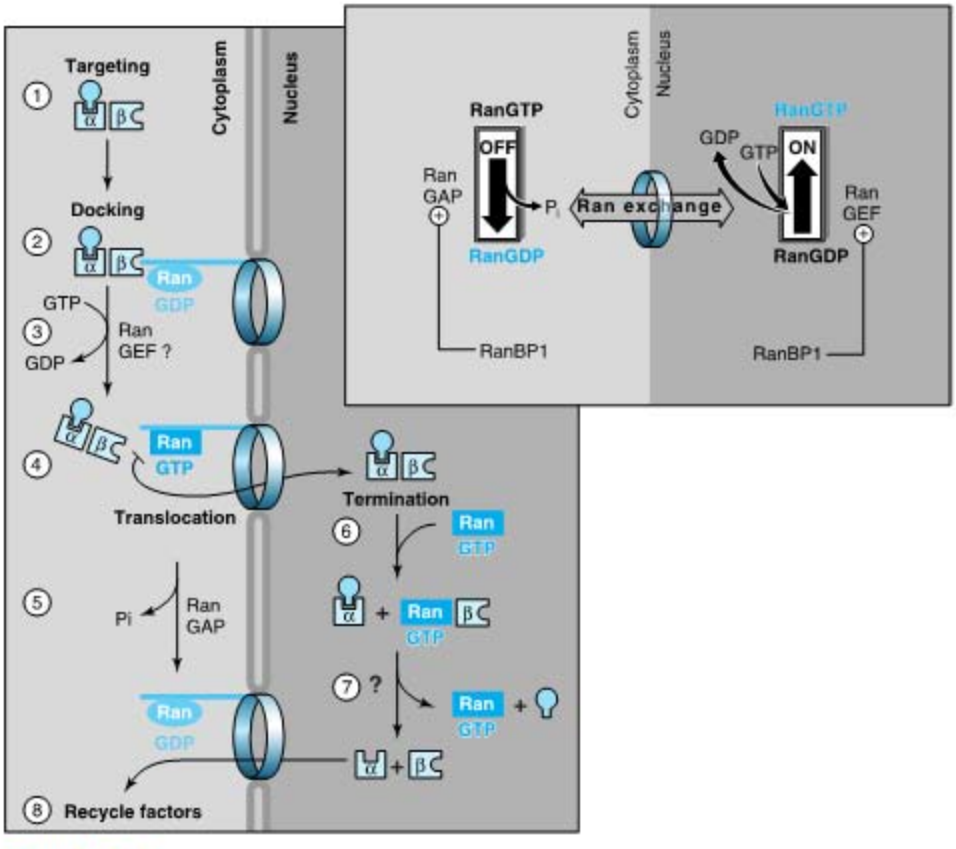

Figure 46–3. Schematic representation of the proposed role of Ran in the import of cargo carrying an NLS signal. (1) The targeting complex forms when the NLS receptor (α, an importin) binds NLS cargo and the docking factor (β). (2) Docking occurs at filamentous sites that protrude from the NPC. Ran-GDP docks independently. (3) Transfer to the translocation channel is triggered when a RanGEF converts Ran-GDP to Ran-GTP. (4) The NPC catalyzes translocation of the targeting complex. (5) Ran-GTP is recycled to Ran-GDP by docked RanGAP. (6) Ran-GTP disrupts the targeting complex by binding to a site on β that overlaps with a binding site. (7) NLS cargo dissociates from α, and Ran-GTP may dissociate from β. (8) α and β factors are recycled to the cytoplasm. *Inset:* The Ran translocation switch is off in the cytoplasm and on in the nucleus. Ran-GTP promotes NLS- and NES-directed translocation. However, cytoplasmic Ran is enriched in Ran-GDP (OFF) by an active RanGAP, and nuclear pools are enriched in Ran-GTP (ON) by an active GEF. RanBP1 promotes the contrary activities of these two factors. Direct linkage of nuclear and cytoplasmic pools of Ran occurs through the NPC by an unknown shuttling mechanism. P_i, inorganic phosphate; NLS, nuclear localization signal; NPC, nuclear pore complex; GEF, guanine nucleotide exchange factor; GAP, guanine-activating protein; NES, nuclear export signal; BP, binding protein. (Reprinted, with permission, from Goldfarb DS: Whose finger is on the switch? Science 1997;276:1814.)

Proteins similar to importins, referred to as **exportins,** are involved in export of many macromolecules from the nucleus. Cargo molecules for export carry **nuclear export signals (NESs).** Ran proteins are involved in this process also, and it is now established that the processes of import and export share a number of common features.

MOST CASES OF ZELLWEGER SYNDROME ARE DUE TO MUTATIONS IN GENES INVOLVED IN THE BIOGENESIS OF PEROXISOMES

The peroxisome is an important organelle involved in aspects of the metabolism of many molecules, including fatty acids and other lipids (eg, plasmalogens, cholesterol, bile acids), purines, amino acids, and hydrogen peroxide. The peroxisome is bounded by a single membrane and contains more than 50 enzymes; catalase and urate oxidase are marker enzymes for this organelle. Its proteins are synthesized on cytosolic polyribosomes and fold prior to import. The pathways of import of a number of its proteins and enzymes have been studied, some being **matrix components** and others **membrane components.** At least two **peroxisomal-matrix targeting sequences (PTSs)** have been discovered. One, PTS1, is a tripeptide (ie, Ser-Lys-Leu [SKL], but variations of this sequence have been detected) located at the carboxyl terminal of a number of matrix proteins, including catalase. Another, PTS2, consisting of about 26–36 amino acids, has been found in at least four matrix proteins (eg, thiolase) and, unlike PTS1, is cleaved after entry into the matrix. Proteins containing PTS1 sequences form complexes with a soluble receptor protein (PTS1R) and proteins containing PTS2 sequences complex with another, PTS2R. The resulting complexes then interact with a membrane receptor, Pex14p. Proteins involved in further transport of proteins into the matrix are also present. Most peroxisomal membrane proteins have been found to contain neither of the above two targeting sequences, but apparently contain others. The import system can handle **intact oligomers** (eg, tetrameric catalase). Import of matrix proteins requires ATP, whereas import of membrane proteins does not.

Interest in import of proteins into peroxisomes has been stimulated by studies on **Zellweger syndrome.** This condition is apparent at birth and is characterized by profound neurologic impairment, victims often dying within a year. The number of peroxisomes can vary from being almost normal to being virtually absent in some patients. Biochemical findings include an accumulation of very long chain fatty acids, abnormalities of the synthesis of bile acids, and a marked reduction of plasmalogens. The condition is believed to be due to mutations in genes encoding certain proteins—so called **peroxins**—involved in various steps of **peroxisome biogenesis** (such as the import of proteins described above), or in genes encoding certain peroxisomal enzymes themselves. Two closely related conditions are **neonatal adrenoleukodystrophy** and **infantile Refsum disease.** Zellweger syndrome and these two conditions represent a spectrum of overlapping features, with Zellweger syndrome being the most severe (many proteins affected) and infantile Refsum disease the least severe (only one or a few proteins affected). Table 46–2 lists some features of these and related conditions.

THE SIGNAL HYPOTHESIS EXPLAINS HOW POLYRIBOSOMES BIND TO THE ENDOPLASMIC RETICULUM

As indicated above, the rough ER branch is the second of the two branches involved in the synthesis and sorting of proteins. In this branch, proteins are synthesized on membrane-bound polyribosomes and translocated into the lumen of the rough ER prior to further sorting (Figure 46–2).

The **signal hypothesis** was proposed by Blobel and Sabatini partly to explain the distinction between free and membrane-bound polyribosomes. They found that proteins synthesized on membrane-bound polyribosomes contained a peptide extension **(signal peptide)**

Table 46–2. Disorders due to peroxisomal abnormalities.[1]

	MIM Number[2]
Zellweger syndrome	214100
Neonatal adrenoleukodystrophy	202370
Infantile Refsum disease	266510
Hyperpipecolic acidemia	239400
Rhizomelic chondrodysplasia punctata	215100
Adrenoleukodystrophy	300100
Pseudo-neonatal adrenoleukodystrophy	264470
Pseudo-Zellweger syndrome	261510
Hyperoxaluria type 1	259900
Acatalasemia	115500
Glutaryl-CoA oxidase deficiency	231690

[1]Reproduced, with permission, from Seashore MR, Wappner RS: *Genetics in Primary Care & Clinical Medicine.* Appleton & Lange, 1996.

[2]MIM = *Mendelian Inheritance in Man.* Each number specifies a reference in which information regarding each of the above conditions can be found.

at their amino terminals which mediated their attachment to the membranes of the ER. As noted above, proteins whose entire synthesis occurs on free polyribosomes lack this signal peptide. An important aspect of the signal hypothesis was that it suggested—as turns out to be the case—that **all ribosomes have the same structure** and that the distinction between membrane-bound and free ribosomes depends solely on the former's carrying proteins that have signal peptides. Much evidence has confirmed the original hypothesis. Because many membrane proteins are synthesized on membrane-bound polyribosomes, the signal hypothesis plays an important role in concepts of membrane assembly. Some characteristics of signal peptides are summarized in Table 46–3.

Figure 46–4 illustrates the principal features in relation to the passage of a secreted protein through the membrane of the ER. It incorporates features from the original signal hypothesis and from subsequent work. The mRNA for such a protein encodes an amino terminal **signal peptide** (also variously called a leader sequence, a transient insertion signal, a signal sequence, or a presequence). The signal hypothesis proposed that the protein is inserted into the ER membrane at the same time as its mRNA is being translated on polyribosomes, so-called **cotranslational insertion.** As the signal peptide emerges from the large subunit of the ribosome, it is recognized by a **signal recognition particle (SRP)** that blocks further translation after about 70 amino acids have been polymerized (40 buried in the large ribosomal subunit and 30 exposed). The block is referred to as **elongation arrest.** The SRP contains six proteins and has a 7S RNA associated with it that is closely related to the Alu family of highly repeated DNA sequences (Chapter 36). The SRP-imposed block is not released until the SRP-signal peptide-polyribosome complex has bound to the so-called **docking protein** (SRP-R, a receptor for the SRP) on the ER membrane; the SRP thus guides the signal peptide to the SRP-R and prevents premature folding and expulsion of the protein being synthesized into the cytosol.

The SRP-R is an integral membrane protein composed of α and β subunits. The α subunit binds GDP and the β subunit spans the membrane. When the SRP-signal peptide complex interacts with the receptor, the exchange of GDP for GTP is stimulated. This form of the receptor (with GTP bound) has a high affinity for the SRP and thus releases the signal peptide, which binds to the translocation machinery (translocon) also present in the ER membrane. The α subunit then hydrolyzes its bound GTP, restoring GDP and completing a GTP-GDP cycle. The unidirectionality of this cycle helps drive the interaction of the polyribosome and its signal peptide with the ER membrane in the forward direction.

The **translocon** consists of a number of membrane proteins that form a protein-conducting channel in the ER membrane through which the newly synthesized protein may pass. The channel appears to be open only when a signal peptide is present, preserving conductance across the ER membrane when it closes. The conductance of the channel has been measured experimentally. Specific functions of a number of components of the translocon have been identified or suggested. **TRAM** (translocating chain-associated membrane) protein may bind the signal sequence as it initially interacts with the translocon and the **Sec61p** complex (consisting of three proteins) binds the heavy subunit of the ribosome.

The insertion of the signal peptide into the conducting channel, while the other end of the parent protein is still attached to ribosomes, is termed **"cotranslational insertion."** The process of elongation of the remaining portion of the protein probably facilitates passage of the nascent protein across the lipid bilayer as the ribosomes remain attached to the membrane of the ER. Thus, the rough (or ribosome-studded) ER is formed. It is important that the protein be kept in an **unfolded state** prior to entering the conducting channel—otherwise, it may not be able to gain access to the channel.

Ribosomes remain attached to the ER during synthesis of signal peptide-containing proteins but are released and dissociated into their two types of subunits when the process is completed. The signal peptide is hydrolyzed by **signal peptidase,** located on the luminal side of the ER membrane (Figure 46–4), and then is apparently rapidly degraded by proteases.

Cytochrome P450 (Chapter 53), an integral ER membrane protein, does not completely cross the membrane. Instead, it resides in the membrane with its signal peptide intact. Its passage through the membrane is prevented by a sequence of amino acids called a halt- or stop-transfer signal.

Secretory proteins and proteins destined for membranes distal to the ER completely traverse the membrane bilayer and are discharged into the lumen of the ER. *N*-Glycan chains, if present, are added (Chapter 47) as these proteins traverse the inner part of the ER membrane—a process called "cotranslational glycosylation." Subsequently, the proteins are found in the

Table 46–3. Some properties of signal peptides.

- Usually, but not always, located at the amino terminal
- Contain approximately 12–35 amino acids
- Methionine is usually the amino terminal amino acid
- Contain a central cluster of hydrophobic amino acids
- Contain at least one positively charged amino acid near their amino terminal
- Usually cleaved off at the carboxyl terminal end of an Ala residue by signal peptidase

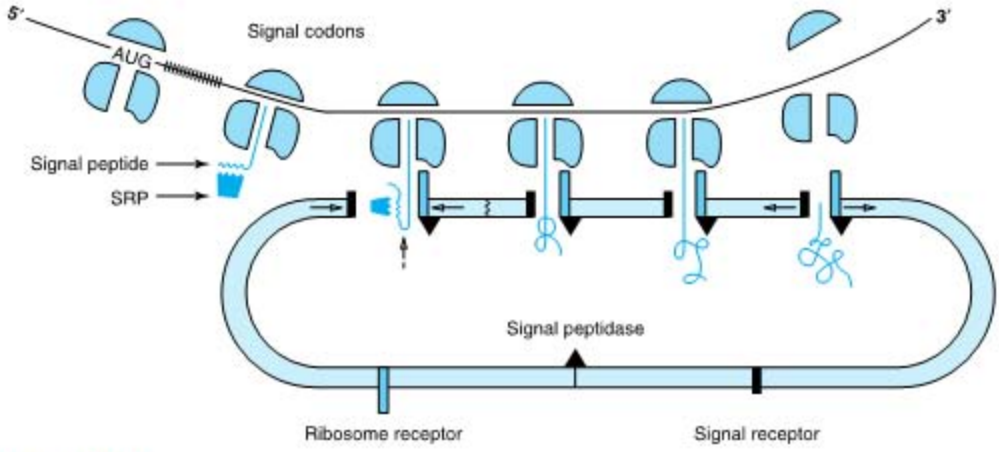

Figure 46–4. Diagram of the signal hypothesis for the transport of secreted proteins across the ER membrane. The ribosomes synthesizing a protein move along the messenger RNA specifying the amino acid sequence of the protein. (The messenger is represented by the line between 5′ and 3′.) The codon AUG marks the start of the message for the protein; the hatched lines that follow AUG represent the codons for the signal sequence. As the protein grows out from the larger ribosomal subunit, the signal sequence is exposed and bound by the signal recognition particle (SRP). Translation is blocked until the complex binds to the "docking protein," also designated SRP-R (represented by the solid bar) on the ER membrane. There is also a receptor (open bar) for the ribosome itself. The interaction of the ribosome and growing peptide chain with the ER membrane results in the opening of a channel through which the protein is transported to the interior space of the ER. During translocation, the signal sequence of most proteins is removed by an enzyme called the "signal peptidase," located at the luminal surface of the ER membrane. The completed protein is eventually released by the ribosome, which then separates into its two components, the large and small ribosomal subunits. The protein ends up inside the ER. See text for further details. (Slightly modified and reproduced, with permission, from Marx JL: Newly made proteins zip through the cell. Science 1980;207:164. Copyright © 1980 by the American Association for the Advancement of Science.)

lumen of the Golgi apparatus, where further changes in glycan chains occur (Figure 47–9) prior to intracellular distribution or secretion. There is strong evidence that the signal peptide is involved in the process of protein insertion into ER membranes. Mutant proteins, containing altered signal peptides in which a hydrophobic amino acid is replaced by a hydrophilic one, are not inserted into ER membranes. Nonmembrane proteins (eg, α-globin) to which signal peptides have been attached by genetic engineering can be inserted into the lumen of the ER or even secreted.

There is considerable evidence that a second transposon in the ER membrane is involved in **retrograde transport** of various molecules from the ER lumen to the cytosol. These molecules include unfolded or misfolded glycoproteins, glycopeptides, and oligosaccharides. Some at least of these molecules are degraded in proteasomes. Thus, there is two-way traffic across the ER membrane.

PROTEINS FOLLOW SEVERAL ROUTES TO BE INSERTED INTO OR ATTACHED TO THE MEMBRANES OF THE ENDOPLASMIC RETICULUM

The routes that proteins follow to be inserted into the membranes of the ER include the following.

A. Cotranslational Insertion

Figure 46–5 shows a variety of ways in which proteins are distributed in the plasma membrane. In particular, the amino terminals of certain proteins (eg, the LDL receptor) can be seen to be on the extracytoplasmic face, whereas for other proteins (eg, the asialoglycoprotein receptor) the carboxyl terminals are on this face. To explain these dispositions, one must consider the initial biosynthetic events at the ER membrane. The **LDL receptor** enters the ER membrane in a manner analogous to a secretory protein (Figure 46–4); it partly traverses

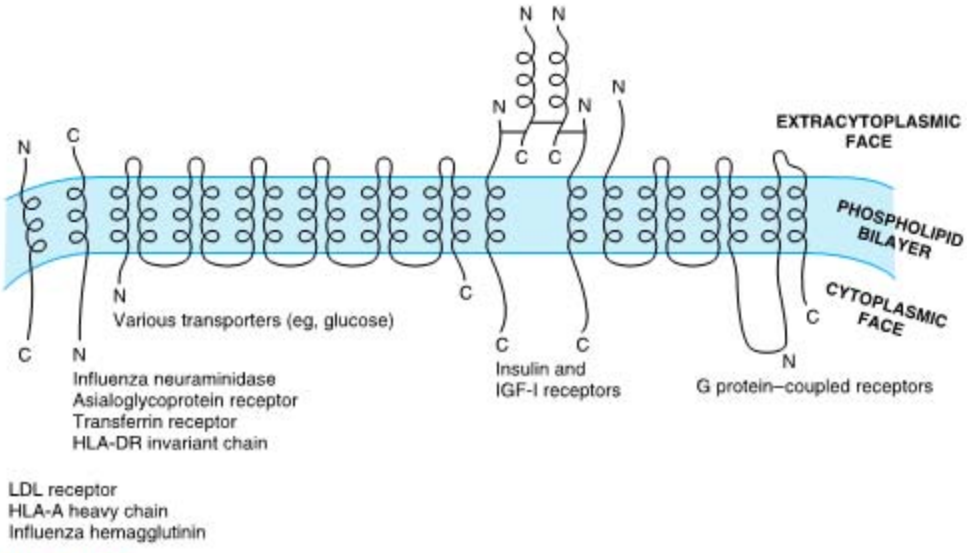

Figure 46–5. Variations in the way in which proteins are inserted into membranes. This schematic representation, which illustrates a number of possible orientations, shows the segments of the proteins within the membrane as α-helices and the other segments as lines. The LDL receptor, which crosses the membrane once and has its amino terminal on the exterior, is called a type I transmembrane protein. The asialoglycoprotein receptor, which also crosses the membrane once but has its carboxyl terminal on the exterior, is called a type II transmembrane protein. The various transporters indicated (eg, glucose) cross the membrane a number of times and are called type III transmembrane proteins; they are also referred to as polytopic membrane proteins. (N, amino terminal; C, carboxyl terminal.) (Adapted, with permission, from Wickner WT, Lodish HF: Multiple mechanisms of protein insertion into and across membranes. Science 1985;230:400. Copyright © 1985 by the American Association for the Advancement of Science.)

the ER membrane, its signal peptide is cleaved, and its amino terminal protrudes into the lumen. However, it is retained in the membrane because it contains a highly hydrophobic segment, the **halt- or stop-transfer signal.** This sequence forms the single transmembrane segment of the protein and is its membrane-anchoring domain. The small patch of ER membrane in which the newly synthesized LDL receptor is located subsequently buds off as a component of a transport vesicle, probably from the transitional elements of the ER (Figure 46–2). As described below in the discussion of asymmetry of proteins and lipids in membrane assembly, the disposition of the receptor in the ER membrane is preserved in the vesicle, which eventually fuses with the plasma membrane. In contrast, the **asialoglycoprotein receptor** possesses an internal insertion sequence, which inserts into the membrane but is not cleaved. This acts as an anchor, and its carboxyl terminal is extruded through the membrane. The more complex disposition of the **transporters** (eg, for glucose) can be explained by the fact that alternating transmembrane α-helices act as uncleaved insertion sequences and as halt-transfer signals, respectively. Each pair of helical segments is inserted as a hairpin. Sequences that determine the structure of a protein in a membrane are called **topogenic sequences.** As explained in the legend to Figure 46–5, the above three proteins are examples of **type I, type II,** and **type III** transmembrane proteins.

B. Synthesis on Free Polyribosomes & Subsequent Attachment to the Endoplasmic Reticulum Membrane

An example is cytochrome b_5, which enters the ER membrane spontaneously.

C. Retention at the Luminal Aspect of the Endoplasmic Reticulum by Specific Amino Acid Sequences

A number of proteins possess the amino acid sequence **KDEL** (Lys-Asp-Glu-Leu) at their carboxyl terminal.

This sequence specifies that such proteins will be attached to the inner aspect of the ER in a relatively loose manner. The chaperone BiP (see below) is one such protein. Actually, KDEL-containing proteins first travel to the Golgi, interact there with a specific KDEL receptor protein, and then return in transport vesicles to the ER, where they dissociate from the receptor.

D. Retrograde Transport From the Golgi Apparatus

Certain other non-KDEL-containing proteins destined for the membranes of the ER also pass to the Golgi and then return, by retrograde vesicular transport, to the ER to be inserted therein (see below).

The foregoing paragraphs demonstrate that a **variety of routes** are involved in assembly of the proteins of the ER membranes; a similar situation probably holds for other membranes (eg, the mitochondrial membranes and the plasma membrane). Precise targeting sequences have been identified in some instances (eg, KDEL sequences).

The topic of membrane biogenesis is discussed further later in this chapter.

PROTEINS MOVE THROUGH CELLULAR COMPARTMENTS TO SPECIFIC DESTINATIONS

A scheme representing the possible flow of proteins along the ER → Golgi apparatus → plasma membrane route is shown in Figure 46–6. The horizontal arrows denote transport steps that may be independent of targeting signals, whereas the vertical open arrows represent steps that depend on specific signals. Thus, flow of certain proteins (including membrane proteins) from the ER to the plasma membrane (designated **"bulk flow,"** as it is nonselective) probably occurs without any targeting sequences being involved, ie, by default. On the other hand, insertion of resident proteins into the ER and Golgi membranes is dependent upon specific signals (eg, KDEL or halt-transfer sequences for the ER). Similarly, transport of many enzymes to **lysosomes** is dependent upon the Man 6-P signal (Chapter 47), and a signal may be involved for entry of proteins into **secretory granules.** Table 46–4 summarizes information on sequences that are known to be involved in targeting various proteins to their correct intracellular sites.

CHAPERONES ARE PROTEINS THAT PREVENT FAULTY FOLDING & UNPRODUCTIVE INTERACTIONS OF OTHER PROTEINS

Exit from the ER may be the rate-limiting step in the secretory pathway. In this context, it has been found that certain proteins play a role in the assembly or proper folding of other proteins without themselves being components of the latter. Such proteins are called **molecular chaperones;** a number of important properties of these proteins are listed in Table 46–5, and the names of some of particular importance in the ER are listed in Table 46–6. Basically, they stabilize unfolded

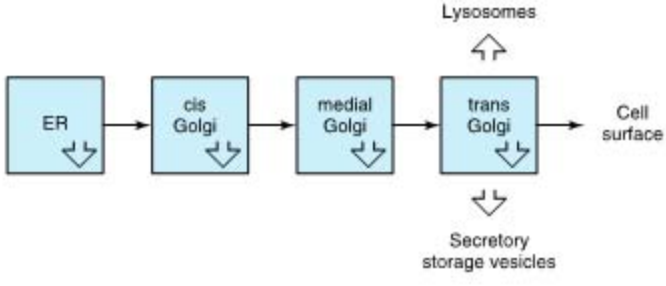

Figure 46–6. Flow of membrane proteins from the endoplasmic reticulum (ER) to the cell surface. Horizontal arrows denote steps that have been proposed to be signal independent and thus represent bulk flow. The open vertical arrows in the boxes denote retention of proteins that are resident in the membranes of the organelle indicated. The open vertical arrows outside the boxes indicate signal-mediated transport to lysosomes and secretory storage granules. (Reproduced, with permission, from Pfeffer SR, Rothman JE: Biosynthetic protein transport and sorting by the endoplasmic reticulum and Golgi. Annu Rev Biochem 1987;56:829.)

Table 46–4. Some sequences or compounds that direct proteins to specific organelles.

Targeting Sequence or Compound	Organelle Targeted
Signal peptide sequence	Membrane of ER
Amino terminal KDEL sequence (Lys-Asp-Glu-Leu)	Luminal surface of ER
Amino terminal sequence (20–80 residues)	Mitochondrial matrix
NLS[1] (eg, Pro_2-Lys_2-Ala-Lys-Val)	Nucleus
PTS[1] (eg, Ser-Lys-Leu)	Peroxisome
Mannose 6-phosphate	Lysosome

[1]NLS, nuclear localization signal; PTS, peroxisomal-matrix targeting sequence.

or partially folded intermediates, allowing them time to fold properly, and prevent inappropriate interactions, thus combating the formation of nonfunctional structures. Most chaperones exhibit **ATPase activity** and bind ADP and ATP. This activity is important for their effect on folding. The ADP-chaperone complex often has a high affinity for the unfolded protein, which, when bound, stimulates release of ADP with replacement by ATP. The ATP-chaperone complex, in turn, releases segments of the protein that have folded properly, and the cycle involving ADP and ATP binding is repeated until the folded protein is released.

Table 46–5. Some properties of chaperone proteins.

- Present in a wide range of species from bacteria to humans
- Many are so-called heat shock proteins (Hsp)
- Some are inducible by conditions that cause unfolding of newly synthesized proteins (eg, elevated temperature and various chemicals)
- They bind to predominantly hydrophobic regions of unfolded and aggregated proteins
- They act in part as a quality control or editing mechanism for detecting misfolded or otherwise defective proteins
- Most chaperones show associated ATPase activity, with ATP or ADP being involved in the protein-chaperone interaction
- Found in various cellular compartments such as cytosol, mitochondria, and the lumen of the endoplasmic reticulum

Table 46–6. Some chaperones and enzymes involved in folding that are located in the rough endoplasmic reticulum.

- BiP (immunoglobulin heavy chain binding protein)
- GRP94 (glucose-regulated protein)
- Calnexin
- Calreticulin
- PDI (protein disulfide isomerase)
- PPI (peptidyl prolyl cis-trans isomerase)

Several examples of chaperones were introduced above when the sorting of mitochondrial proteins was discussed. The **immunoglobulin heavy chain binding protein (BiP)** is located in the lumen of the ER. This protein will bind abnormally folded immunoglobulin heavy chains and certain other proteins and prevent them from leaving the ER, in which they are degraded. Another important chaperone is **calnexin,** a calcium-binding protein located in the ER membrane. This protein binds a wide variety of proteins, including mixed histocompatibility (MHC) antigens and a variety of serum proteins. As mentioned in Chapter 47, calnexin binds the monoglycosylated species of glycoproteins that occur during processing of glycoproteins, retaining them in the ER until the glycoprotein has folded properly. **Calreticulin,** which is also a calcium-binding protein, has properties similar to those of calnexin; it is not membrane-bound. Chaperones are not the only proteins in the ER lumen that are concerned with proper folding of proteins. Two enzymes are present that play an active role in folding. **Protein disulfide isomerase (PDI)** promotes rapid reshuffling of disulfide bonds until the correct set is achieved. **Peptidyl prolyl isomerase (PPI)** accelerates folding of proline-containing proteins by catalyzing the cis-trans isomerization of X-Pro bonds, where X is any amino acid residue.

TRANSPORT VESICLES ARE KEY PLAYERS IN INTRACELLULAR PROTEIN TRAFFIC

Most proteins that are synthesized on membrane-bound polyribosomes and are destined for the Golgi apparatus or plasma membrane reach these sites inside transport vesicles. The precise mechanisms by which proteins synthesized in the rough ER are inserted into these vesicles are not known. Those involved in transport from the ER to the Golgi apparatus and vice versa—and from the Golgi to the plasma membrane—are mainly clathrin-free, unlike the coated vesicles involved in endocytosis (see discussions of the LDL receptor in Chapters 25 and 26). For the sake of clarity, the non-clathrin-coated vesicles will be referred to in

this text as **transport vesicles.** There is evidence that proteins destined for the membranes of the Golgi apparatus contain specific signal sequences. On the other hand, most proteins destined for the plasma membrane or for secretion do not appear to contain specific signals, reaching these destinations by default.

The Golgi Apparatus Is Involved in Glycosylation & Sorting of Proteins

The Golgi apparatus plays two important roles in membrane synthesis. First, it is involved in the **processing of the oligosaccharide chains** of membrane and other N-linked glycoproteins and also contains enzymes involved in O-glycosylation (see Chapter 47). Second, it is involved in the **sorting** of various proteins prior to their delivery to their appropriate intracellular destinations. All parts of the Golgi apparatus participate in the first role, whereas the trans-Golgi is particularly involved in the second and is very rich in vesicles. Because of their central role in protein transport, considerable research has been conducted in recent years concerning the formation and fate of transport vesicles.

A Model of Non-Clathrin-Coated Vesicles Involves SNAREs & Other Factors

Vesicles lie at the heart of intracellular transport of many proteins. Recently, significant progress has been made in understanding the events involved in vesicle formation and transport. This has transpired because of the use of a number of approaches. These include establishment of **cell-free systems** with which to study vesicle formation. For instance, it is possible to observe, by electron microscopy, budding of vesicles from Golgi preparations incubated with cytosol and ATP. The development of genetic approaches for studying vesicles in yeast has also been crucial. The picture is complex, with its own nomenclature (Table 46–7), and involves a variety of cytosolic and membrane proteins, GTP, ATP, and accessory factors.

Based largely on a proposal by Rothman and colleagues, anterograde vesicular transport can be considered to occur in eight steps (Figure 46–7). The basic concept is that each transport vesicle bears a unique address marker consisting of one or more **v-SNARE proteins,** while each target membrane bears one or more **complementary t-SNARE proteins** with which the former interact specifically.

Step 1: **Coat assembly** is initiated when ARF is activated by binding GTP, which is exchanged for GDP. This leads to the association of GTP-bound ARF with its putative receptor (hatched in Figure 46–7) in the donor membrane.

Table 46–7. Factors involved in the formation of non-clathrin-coated vesicles and their transport.

- ARF: ADP-ribosylation factor, a GTPase
- Coatomer: A family of at least seven coat proteins (α, β, γ, δ, ε, β′, and ζ). Different transport vesicles have different complements of coat proteins.
- SNAP: Soluble NSF attachment factor
- SNARE: SNAP receptor
- v-SNARE: Vesicle SNARE
- t-SNARE: Target SNARE
- GTP-γ-S: A nonhydrolyzable analog of GTP, used to test the involvement of GTP
- NEM: *N*-Ethylmaleimide, a chemical that alkylates sulfhydryl groups
- NSF: NEM-sensitive factor, an ATPase
- Rab proteins: A family of ras-related proteins first observed in rat brain; they are GTPases and are active when GTP is found
- Sec1: A member of a family of proteins that attach to t-SNAREs and are displaced from them by Rab proteins, thereby allowing v-SNARE–t-SNARE interactions to occur.

Step 2: Membrane-associated ARF **recruits the coat proteins** that comprise the coatomer shell from the cytosol, forming a coated bud.

Step 3: The **bud pinches off** in a process involving acyl-CoA—and probably ATP—to complete the formation of the coated vesicle.

Step 4: **Coat disassembly** (involving dissociation of ARF and coatomer shell) follows hydrolysis of bound GTP; uncoating is necessary for fusion to occur.

Step 5: **Vesicle targeting** is achieved via members of a family of integral proteins, termed v-SNAREs, that tag the vesicle during its budding. v-SNAREs pair with cognate t-SNAREs in the target membrane to dock the vesicle.

It is presumed that steps 4 and 5 are closely coupled and that step 4 may follow step 5, with ARF and the coatomer shell rapidly dissociating after docking.

Step 6: The **general fusion machinery** then assembles on the paired SNARE complex; it includes an ATPase (NSF; NEM-sensitive factor) and the SNAP (soluble NSF attachment factor) proteins. SNAPs bind to the SNARE (SNAP receptor) complex, enabling NSF to bind.

Step 7: **Hydrolysis of ATP by NSF** is essential for fusion, a process that can be inhibited by NEM (*N*-ethylmaleimide). Certain other proteins and calcium are also required.

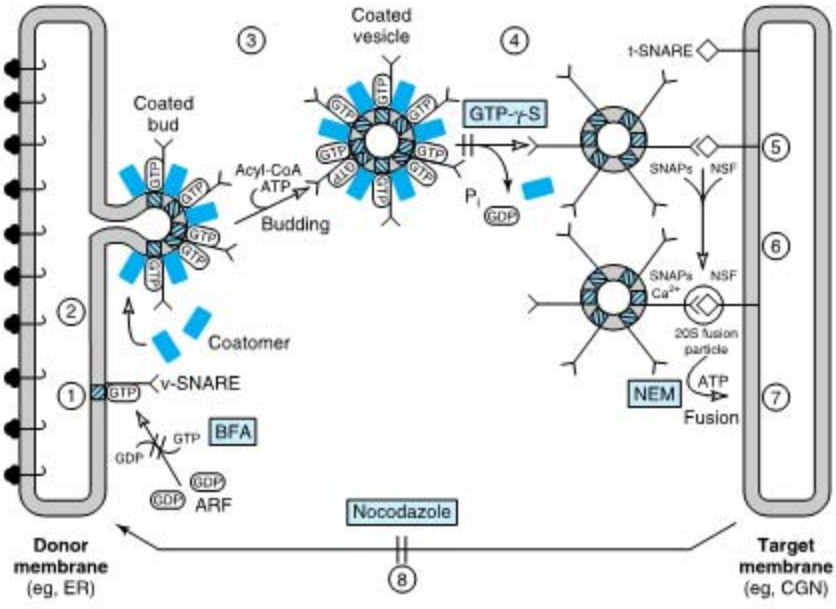

Figure 46–7. Model of the steps in a round of anterograde vesicular transport. The cycle starts in the bottom left-hand side of the figure, where two molecules of ARF are represented as small ovals containing GDP. The steps in the cycle are described in the text. Most of the abbreviations used are explained in Table 46–7. The roles of Rab and Sec1 proteins (see text) in the overall process are not dealt with in this figure. (CGN, cis-Golgi network; BFA, Brefeldin A.) (Adapted from Rothman JE: Mechanisms of intracellular protein transport. Nature 1994;372:55.) (Courtesy of E Degen.)

Step 8: **Retrograde transport** occurs to restart the cycle. This last step may retrieve certain proteins or recycle v-SNAREs. Nocodazole, a microtubule-disrupting agent, inhibits this step.

Brefeldin A Inhibits the Coating Process

The following points expand and clarify the above.

(a) To participate in step 1, ARF must first be modified by addition of **myristic acid** (C14:0), employing myristoyl-CoA as the acyl donor. Myristoylation is one of a number of enzyme-catalyzed posttranslational modifications, involving addition of certain lipids to specific residues of proteins, that facilitate the binding of proteins to the cytosolic surfaces of membranes or vesicles. Others are addition of palmitate, farnesyl, and geranylgeranyl; the two latter molecules are polyisoprenoids containing 15 and 20 carbon atoms, respectively.

(b) At least three different types of coated vesicles have been distinguished: **COPI, COPII,** and **clathrin-coated vesicles;** the first two are referred to here as transport vesicles. Many other types of vesicles no doubt remain to be discovered. COPI vesicles are involved in bidirectional transport from the ER to the Golgi and in the reverse direction, whereas COPII vesicles are involved mainly in transport in the former direction. Clathrin-containing vesicles are involved in transport from the trans-Golgi network to prelysosomes and from the plasma membrane to endosomes, respectively. Regarding **selection** of cargo molecules by vesicles, this appears to be primarily a function of the coat proteins of vesicles. **Cargo molecules** may interact with coat proteins either directly or via intermediary proteins that attach to coat proteins, and they then become enclosed in their appropriate vesicles.

(c) The fungal metabolite **brefeldin A** prevents GTP from binding to ARF in step 1 and thus inhibits the entire coating process. In its presence, the Golgi apparatus appears to disintegrate, and fragments are lost. It may do this by inhibiting the guanine nucleotide exchanger involved in step 1.

(d) **GTP-γ-S** (a nonhydrolyzable analog of GTP often used in investigations of the role of GTP in biochemical processes) blocks disassembly of the coat from coated vesicles, leading to a build-up of coated vesicles.

(e) A family of Ras-like proteins, called the **Rab protein family,** are required in several steps of intracellular protein transport, regulated secretion, and endocytosis. They are small monomeric GTPases that attach to the cytosolic faces of membranes via geranylgeranyl chains. They attach in the GTP-bound state (not shown in Figure 46–7) to the budding vesicle. Another family of proteins **(Sec1)** binds to t-SNAREs and prevents interaction with them and their complementary v-SNAREs. When a vesicle interacts with its target membrane, Rab proteins displace Sec1 proteins and the v-SNARE-t-SNARE interaction is free to occur. It appears that the Rab and Sec1 families of proteins regulate the speed of vesicle formation, opposing each other. Rab proteins have been likened to throttles and Sec1 proteins to dampers on the overall process of vesicle formation.

(f) Studies using v- and t-SNARE proteins reconstituted into separate lipid bilayer vesicles have indicated that they form **SNAREpins,** ie, SNARE complexes that link two membranes (vesicles). SNAPs and NSF are required for formation of SNAREpins, but once they have formed they can apparently lead to spontaneous fusion of membranes at physiologic temperature, suggesting that they are the minimal machinery required for membrane fusion.

(g) The fusion of synaptic vesicles with the plasma membrane of **neurons** involves a series of events similar to that described above. For example, one v-SNARE is designated **synaptobrevin** and two t-SNAREs are designated **syntaxin** and **SNAP 25** (synaptosome-associated protein of 25 kDa). **Botulinum B toxin** is one of the most lethal toxins known and the most serious cause of food poisoning. One component of this toxin is a protease that appears to cleave only synaptobrevin, thus inhibiting release of acetylcholine at the neuromuscular junction and possibly proving fatal, depending on the dose taken.

(h) Although the above model describes non-clathrin-coated vesicles, it appears likely that many of the events described above apply, at least in principle, to clathrin-coated vesicles.

THE ASSEMBLY OF MEMBRANES IS COMPLEX

There are many cellular membranes, each with its own specific features. No satisfactory scheme describing the assembly of any one of these membranes is available. How various proteins are initially inserted into the membrane of the ER has been discussed above. The transport of proteins, including membrane proteins, to various parts of the cell inside vesicles has also been described. Some general points about membrane assembly remain to be addressed.

Asymmetry of Both Proteins & Lipids Is Maintained During Membrane Assembly

Vesicles formed from membranes of the ER and Golgi apparatus, either naturally or pinched off by homogenization, exhibit **transverse asymmetries** of both lipid and protein. These asymmetries are maintained during fusion of transport vesicles with the plasma membrane. The inside of the vesicles after fusion becomes the outside of the plasma membrane, and the cytoplasmic side of the vesicles remains the cytoplasmic side of the membrane (Figure 46–8). Since the transverse asymmetry of the membranes already exists in the vesicles of the ER well before they are fused to the plasma membrane, a major problem of membrane assembly becomes understanding how the integral proteins are inserted into the lipid bilayer of the ER. This problem was addressed earlier in this chapter.

Phospholipids are the major class of lipid in membranes. The enzymes responsible for the synthesis of phospholipids reside in the cytoplasmic surface of the cisternae of the ER. As phospholipids are synthesized at that site, they probably self-assemble into thermodynamically stable bimolecular layers, thereby expanding the membrane and perhaps promoting the detachment of so-called lipid vesicles from it. It has been proposed that these vesicles travel to other sites, donating their lipids to other membranes; however, little is known about this matter. As indicated above, cytosolic proteins that take up phospholipids from one membrane and release them to another (ie, phospholipid exchange proteins) have been demonstrated; they probably play a role in contributing to the specific lipid composition of various membranes.

Lipids & Proteins Undergo Turnover at Different Rates in Different Membranes

It has been shown that the half-lives of the lipids of the ER membranes of rat liver are generally shorter than those of its proteins, so that the **turnover rates of lipids and proteins are independent.** Indeed, different lipids have been found to have different half-lives. Furthermore, the half-lives of the proteins of these membranes vary quite widely, some exhibiting short (hours) and others long (days) half-lives. Thus, individual lipids and proteins of the ER membranes appear to be inserted into it relatively independently; this is the case for many other membranes.

The biogenesis of membranes is thus a complex process about which much remains to be learned. One indication of the complexity involved is to consider the number of **posttranslational modifications** that membrane proteins may be subjected to prior to attaining their mature state. These include proteolysis, assembly

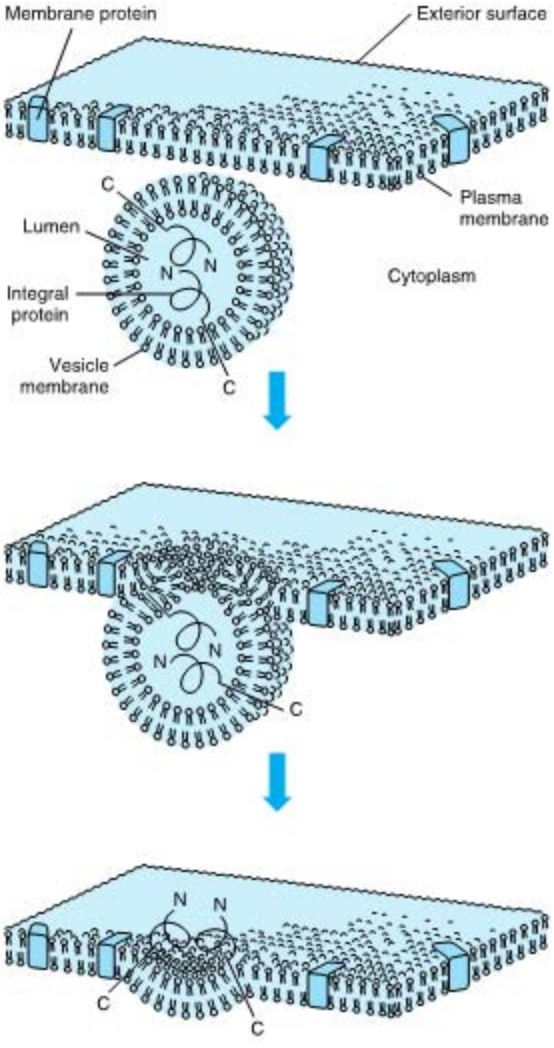

Figure 46–8. Fusion of a vesicle with the plasma membrane preserves the orientation of any integral proteins embedded in the vesicle bilayer. Initially, the amino terminal of the protein faces the lumen, or inner cavity, of such a vesicle. After fusion, the amino terminal is on the exterior surface of the plasma membrane. That the orientation of the protein has not been reversed can be perceived by noting that the other end of the molecule, the carboxyl terminal, is always immersed in the cytoplasm. The lumen of a vesicle and the outside of the cell are topologically equivalent. (Redrawn and modified, with permission, from Lodish HF, Rothman JE: The assembly of cell membranes. Sci Am [Jan] 1979;240:43.)

Table 46–8. Major features of membrane assembly.

- Lipids and proteins are inserted independently into membranes.
- Individual membrane lipids and proteins turn over independently and at different rates.
- Topogenic sequences (eg, signal [amino terminal or internal] and stop-transfer) are important in determining the insertion and disposition of proteins in membranes.
- Membrane proteins inside transport vesicles bud off the endoplasmic reticulum on their way to the Golgi; final sorting of many membrane proteins occurs in the trans-Golgi network.
- Specific sorting sequences guide proteins to particular organelles such as lysosomes, peroxisomes, and mitochondria.

into multimers, glycosylation, addition of a glycophosphatidylinositol (GPI) anchor, sulfation on tyrosine or carbohydrate moieties, phosphorylation, acylation, and prenylation—a list that is undoubtedly not complete. Nevertheless, significant progress has been made; Table 46–8 summarizes some of the major features of membrane assembly that have emerged to date.

Table 46–9. Some disorders due to mutations in genes encoding proteins involved in intracellular membrane transport.[1]

Disorder[2]	Protein Involved
Chédiak-Higashi syndrome, 214500	Lysosomal trafficking regulator
Combined deficiency of factors V and VIII, 227300	ERGIC-53, a mannose-binding lectin
Hermansky-Pudlak syndrome, 203300	AP-3 adaptor complex β3A subunit
I-cell disease, 252500	*N*-Acetylglucosamine 1-phosphotransferase
Oculocerebrorenal syndrome, 30900	OCRL-1, an inositol polyphosphate 5-phosphatase

[1]Modified from Olkonnen VM, Ikonen E: Genetic defects of intracellular-membrane transport. N Eng J Med 2000;343:1095. Certain related conditions not listed here are also described in this publication. I-cell disease is described in Chapter 47. The majority of the disorders listed above affect lysosomal function; readers should consult a textbook of medicine for information on the clinical manifestations of these conditions.

[2]The numbers after each disorder are the OMIM numbers.

Various Disorders Result From Mutations in Genes Encoding Proteins Involved in Intracellular Transport

Some of these are listed in Table 46–9; the majority affect lysosomal function. A number of other mutations affecting intracellular protein transport have been reported but are not included here.

SUMMARY

- Many proteins are targeted to their destinations by signal sequences. A major sorting decision is made when proteins are partitioned between cytosolic and membrane-bound polyribosomes by virtue of the absence or presence of a signal peptide.
- The pathways of protein import into mitochondria, nuclei, peroxisomes, and the endoplasmic reticulum are described.
- Many proteins synthesized on membrane-bound polyribosomes proceed to the Golgi apparatus and the plasma membrane in transport vesicles.
- A number of glycosylation reactions occur in compartments of the Golgi, and proteins are further sorted in the trans-Golgi network.
- Most proteins destined for the plasma membrane and for secretion appear to lack specific signals—a default mechanism.
- The role of chaperone proteins in the folding of proteins is presented, and a model describing budding and attachment of transport vesicles to a target membrane is summarized.
- Membrane assembly is discussed and shown to be complex. Asymmetry of both lipids and proteins is maintained during membrane assembly.
- A number of disorders have been shown to be due to mutations in genes encoding proteins involved in various aspects of protein traffic and sorting.

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Glycoproteins

47

Robert K. Murray, MD, PhD

BIOMEDICAL IMPORTANCE

Glycoproteins are proteins that contain oligosaccharide (glycan) chains covalently attached to their polypeptide backbones. They are one class of **glycoconjugate** or **complex carbohydrates**—equivalent terms used to denote molecules containing one or more carbohydrate chains covalently linked to protein (to form glycoproteins or proteoglycans) or lipid (to form glycolipids). (**Proteoglycans** are discussed in Chapter 48 and **glycolipids** in Chapter 14). Almost all the **plasma proteins** of humans—except albumin—are glycoproteins. Many **proteins of cellular membranes** (Chapter 41) contain substantial amounts of carbohydrate. A number of the **blood group substances** are glycoproteins, whereas others are glycosphingolipids. Certain **hormones** (eg, chorionic gonadotropin) are glycoproteins. A major problem in cancer is **metastasis,** the phenomenon whereby cancer cells leave their tissue of origin (eg, the breast), migrate through the bloodstream to some distant site in the body (eg, the brain), and grow there in an unregulated manner, with catastrophic results for the affected individual. Many cancer researchers think that alterations in the structures of glycoproteins and other glycoconjugates on the surfaces of cancer cells are important in the phenomenon of metastasis.

GLYCOPROTEINS OCCUR WIDELY & PERFORM NUMEROUS FUNCTIONS

Glycoproteins occur in most organisms, from bacteria to humans. Many viruses also contain glycoproteins, some of which have been much investigated, in part because they are very suitable for biosynthetic studies. Numerous proteins with diverse functions are glycoproteins (Table 47–1); their carbohydrate content ranges from 1% to over 85% by weight.

Many studies have been conducted in an attempt to define the precise roles oligosaccharide chains play in the functions of glycoproteins. Table 47–2 summarizes results from such studies. Some of the functions listed are firmly established; others are still under investigation.

OLIGOSACCHARIDE CHAINS ENCODE BIOLOGIC INFORMATION

An enormous number of glycosidic linkages can be generated between sugars. For example, three different hexoses may be linked to each other to form over 1000 different trisaccharides. The conformations of the sugars in oligosaccharide chains vary depending on their linkages and proximity to other molecules with which the oligosaccharides may interact. It is now established that certain oligosaccharide chains encode considerable **biologic information** and that this depends upon their constituent sugars, their sequences, and their linkages. For instance, mannose 6-phosphate residues target newly synthesized lysosomal enzymes to that organelle (see below).

TECHNIQUES ARE AVAILABLE FOR DETECTION, PURIFICATION, & STRUCTURAL ANALYSIS OF GLYCOPROTEINS

A variety of methods used in the detection, purification, and structural analysis of glycoproteins are listed in Table 47–3. The conventional methods used to purify proteins and enzymes are also applicable to the purification of glycoproteins. Once a glycoprotein has been purified, the use of **mass spectrometry** and **high-resolution NMR spectroscopy** can often identify the structures of its glycan chains. Analysis of glycoproteins can be complicated by the fact that they often exist as **glycoforms;** these are proteins with identical amino acid sequences but somewhat different oligosaccharide compositions. Although linkage details are not stressed in this chapter, it is critical to appreciate that the precise natures of the linkages between the sugars of glycoproteins are of fundamental importance in determining the structures and functions of these molecules.

Table 47–1. Some functions served by glycoproteins.

Function	Glycoproteins
Structural molecule	Collagens
Lubricant and protective agent	Mucins
Transport molecule	Transferrin, ceruloplasmin
Immunologic molecule	Immunoglobulins, histocompatibility antigens
Hormone	Chorionic gonadotropin, thyroid-stimulating hormone (TSH)
Enzyme	Various, eg, alkaline phosphatase
Cell attachment-recognition site	Various proteins involved in cell-cell (eg, sperm-oocyte), virus-cell, bacterium-cell, and hormone-cell interactions
Antifreeze	Certain plasma proteins of cold water fish
Interact with specific carbohydrates	Lectins, selectins (cell adhesion lectins), antibodies
Receptor	Various proteins involved in hormone and drug action
Affect folding of certain proteins	Calnexin, calreticulin

Table 47–2. Some functions of the oligosaccharide chains of glycoproteins.[1]

- Modulate physicochemical properties, eg, solubility, viscosity, charge, conformation, denaturation, and binding sites for bacteria and viruses
- Protect against proteolysis, from inside and outside of cell
- Affect proteolytic processing of precursor proteins to smaller products
- Are involved in biologic activity, eg, of human chorionic gonadotropin (hCG)
- Affect insertion into membranes, intracellular migration, sorting and secretion
- Affect embryonic development and differentiation
- May affect sites of metastases selected by cancer cells

[1]Adapted from Schachter H: Biosynthetic controls that determine the branching and heterogeneity of protein-bound oligosaccharides. Biochem Cell Biol 1986;64:163.

Table 47–3. Some important methods used to study glycoproteins.

Method	Use
Periodic acid-Schiff reagent	Detects glycoproteins as pink bands after electrophoretic separation.
Incubation of cultured cells with glycoproteins as radioactive bands	Leads to detection of a radioactive sugar after electrophoretic separation.
Treatment with appropriate endo- or exoglycosidase or phospholipases	Resultant shifts in electrophoretic migration help distinguish among proteins with N-glycan, O-glycan, or GPI linkages and also between high mannose and complex N-glycans.
Sepharose-lectin column chromatography	To purify glycoproteins or glycopeptides that bind the particular lectin used.
Compositional analysis following acid hydrolysis	Identifies sugars that the glycoprotein contains and their stoichiometry.
Mass spectrometry	Provides information on molecular mass, composition, sequence, and sometimes branching of a glycan chain.
NMR spectroscopy	To identify specific sugars, their sequence, linkages, and the anomeric nature of glycosidic linkages.
Methylation (linkage) analysis	To determine linkages between sugars.
Amino acid or cDNA sequencing	Determination of amino acid sequence.

EIGHT SUGARS PREDOMINATE IN HUMAN GLYCOPROTEINS

About 200 monosaccharides are found in nature; however, only eight are commonly found in the oligosaccharide chains of glycoproteins (Table 47–4). Most of these sugars were described in Chapter 13. *N*-Acetylneuraminic acid (NeuAc) is usually found at the termini of oligosaccharide chains, attached to subterminal galactose (Gal) or *N*-acetylgalactosamine (GalNAc) residues. The other sugars listed are generally found in more internal positions. **Sulfate** is often found in glycoproteins, usually attached to Gal, GalNAc, or GlcNAc.

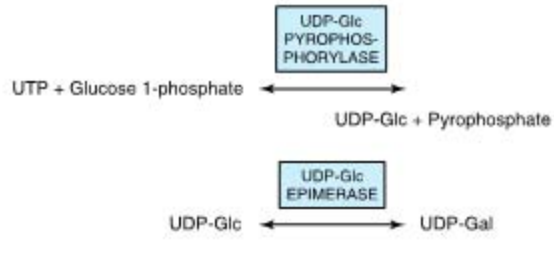
Table 47–4. The principal sugars found in human glycoproteins. Their structures are illustrated in Chapter 13.

Sugar	Type	Abbreviation	Nucleotide Sugar	Comments
Galactose	Hexose	Gal	UDP-Gal	Often found subterminal to NeuAc in N-linked glycoproteins. Also found in core trisaccharide of proteoglycans.
Glucose	Hexose	Glc	UDP-Glc	Present during the biosynthesis of N-linked glycoproteins but not usually present in mature glycoproteins. Present in some clotting factors.
Mannose	Hexose	Man	GDP-Man	Common sugar in N-linked glycoproteins.
N-Acetylneuraminic acid	Sialic acid (nine C atoms)	NeuAc	CMP-NeuAc	Often the terminal sugar in both N- and O-linked glycoproteins. Other types of sialic acid are also found, but NeuAc is the major species found in humans. Acetyl groups may also occur as O-acetyl species as well as N-acetyl.
Fucose	Deoxyhexose	Fuc	GDP-Fuc	May be external in both N- and O-linked glycoproteins or internal, linked to the GlcNAc residue attached to Asn in N-linked species. Can also occur internally attached to the OH of Ser (eg, in t-PA and certain clotting factors).
N-Acetylgalactosamine	Aminohexose	GalNAc	UDP-GalNAc	Present in both N- and O-linked glycoproteins.
N-Acetylglucosamine	Aminohexose	GlcNAc	UDP-GlcNAc	The sugar attached to the polypeptide chain via Asn in N-linked glycoproteins; also found at other sites in the oligosaccharides of these proteins. Many nuclear proteins have GlcNAc attached to the OH of Ser or Thr as a single sugar.
Xylose	Pentose	Xyl	UDP-Xyl	Xyl is attached to the OH of Ser in many proteoglycans. Xyl in turn is attached to two Gal residues, forming a link trisaccharide. Xyl is also found in t-PA and certain clotting factors.

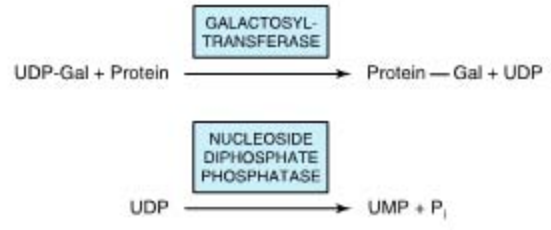
NUCLEOTIDE SUGARS ACT AS SUGAR DONORS IN MANY BIOSYNTHETIC REACTIONS

The first nucleotide sugar to be reported was uridine diphosphate glucose (UDP-Glc); its structure is shown in Figure 18–2. The common nucleotide sugars involved in the biosynthesis of glycoproteins are listed in Table 47–4; the reasons some contain UDP and others guanosine diphosphate (GDP) or cytidine monophosphate (CMP) are obscure. Many of the glycosylation reactions involved in the biosynthesis of glycoproteins utilize these compounds (see below). The anhydro nature of the linkage between the phosphate group and the sugars is of the high-energy, high-group-transfer-potential type (Chapter 10). The sugars of these compounds are thus "activated" and can be transferred to suitable acceptors provided appropriate transferases are available.

Most nucleotide sugars are formed in the cytosol, generally from reactions involving the corresponding nucleoside triphosphate. CMP-sialic acids are formed in the nucleus. Formation of uridine diphosphate galactose (UDP-Gal) requires the following two reactions in mammalian tissues:



Because many glycosylation reactions occur within the lumen of the Golgi apparatus, **carrier systems** (permeases, transporters) are necessary to transport nucleotide sugars across the Golgi membrane. Systems transporting UDP-Gal, GDP-Man, and CMP-NeuAc into the cisternae of the Golgi apparatus have been described. They are **antiport** systems; ie, the influx of one molecule of nucleotide sugar is balanced by the efflux of one molecule of the corresponding nucleotide (eg, UMP, GMP, or CMP) formed from the nucleotide sugars. This mechanism ensures an adequate concentration of each nucleotide sugar inside the Golgi apparatus. UMP is formed from UDP-Gal in the above process as follows:



EXO- & ENDOGLYCOSIDASES FACILITATE STUDY OF GLYCOPROTEINS

A number of **glycosidases** of defined specificity have proved useful in examining structural and functional aspects of glycoproteins (Table 47–5). These enzymes act at either external (exoglycosidases) or internal (endoglycosidases) positions of oligosaccharide chains. Examples of exoglycosidases are **neuraminidases** and **galactosidases;** their sequential use removes terminal NeuAc and subterminal Gal residues from most glycoproteins. **Endoglycosidases F** and **H** are examples of the latter class; these enzymes cleave the oligosaccharide chains at specific GlcNAc residues close to the polypeptide backbone (ie, at internal sites; Figure 47–5) and are thus useful in releasing large oligosaccharide chains for structural analyses. A glycoprotein can be treated with one or more of the above glycosidases to analyze the effects on its biologic behavior of removal of specific sugars.

Table 47–5. Some glycosidases used to study the structure and function of glycoproteins.[1]

Enzymes	Type
Neuraminidases	Exoglycosidase
Galactosidases	Exo- or endoglycosidase
Endoglycosidase F	Endoglycosidase
Endoglycosidase H	Endoglycosidase

[1]The enzymes are available from a variety of sources and are often specific for certain types of glycosidic linkages and also for their anomeric natures. The sites of action of endoglycosidases F and H are shown in Figure 47–5. F acts on both high-mannose and complex oligosaccharides, whereas H acts on the former.

THE MAMMALIAN ASIALOGLYCOPROTEIN RECEPTOR IS INVOLVED IN CLEARANCE OF CERTAIN GLYCOPROTEINS FROM PLASMA BY HEPATOCYTES

Experiments performed by Ashwell and his colleagues in the early 1970s played an important role in focusing attention on the functional significance of the oligosaccharide chains of glycoproteins. They treated rabbit ceruloplasmin (a plasma protein; see Chapter 50) with neuraminidase in vitro. This procedure exposed subterminal Gal residues that were normally masked by terminal NeuAc residues. Neuraminidase-treated radioactive ceruloplasmin was found to disappear rapidly from the circulation, in contrast to the slow clearance of the untreated protein. Very significantly, when the Gal residues exposed to treatment with neuraminidase were removed by treatment with a galactosidase, the clearance rate of the protein returned to normal. Further studies demonstrated that liver cells contain a **mammalian asialoglycoprotein receptor** that recognizes the Gal moiety of many desialylated plasma proteins and leads to their endocytosis. This work indicated that an individual sugar, such as Gal, could play an important role in governing at least one of the biologic properties (ie, time of residence in the circulation) of certain glycoproteins. This greatly strengthened the concept that oligosaccharide chains could contain biologic information.

LECTINS CAN BE USED TO PURIFY GLYCOPROTEINS & TO PROBE THEIR FUNCTIONS

Lectins are carbohydrate-binding proteins that agglutinate cells or precipitate glycoconjugates; a number of lectins are themselves glycoproteins. Immunoglobulins that react with sugars are not considered lectins. Lectins contain at least two sugar-binding sites; proteins with a single sugar-binding site will not agglutinate cells or precipitate glycoconjugates. The specificity of a lectin is usually defined by the sugars that are best at inhibiting its ability to cause agglutination or precipitation. Enzymes, toxins, and transport proteins can be classified as lectins if they bind carbohydrate. Lectins were first discovered in plants and microbes, but many lectins of

animal origin are now known. The mammalian asialoglycoprotein receptor described above is an important example of an animal lectin. Some important lectins are listed in Table 47–6. Much current research is centered on the roles of various animal lectins (eg, the selectins) in cell-cell interactions that occur in pathologic conditions such as inflammation and cancer metastasis (see below).

Numerous lectins have been purified and are commercially available; three plant lectins that have been widely used experimentally are listed in Table 47–7. Among many uses, lectins have been employed to purify specific glycoproteins, as tools for probing the glycoprotein profiles of cell surfaces, and as reagents for generating mutant cells deficient in certain enzymes involved in the biosynthesis of oligosaccharide chains.

THERE ARE THREE MAJOR CLASSES OF GLYCOPROTEINS

Based on the nature of the linkage between their polypeptide chains and their oligosaccharide chains, glycoproteins can be divided into three major classes (Figure 47–1): (1) those containing an O-glycosidic linkage (ie, O-linked), involving the hydroxyl side chain of serine or threonine and a sugar such as *N*-acetylgalactosamine (GalNAc-Ser[Thr]); (2) those containing an N-glycosidic linkage (ie, N-linked), involving the amide nitrogen of asparagine and *N*-acetylglucosamine (GlcNAc-Asn); and (3) those linked to the carboxyl terminal amino acid of a protein via a phosphoryl-ethanolamine moiety joined to an oligosaccharide (glycan), which in turn is linked via glucosamine to phosphatidylinositol (PI). This latter class is referred to as **glycosylphosphatidylinositol-anchored (GPI-anchored,** or **GPI-linked**) glycoproteins. Other minor classes of glycoproteins also exist.

The number of oligosaccharide chains attached to one protein can vary from one to 30 or more, with the sugar chains ranging from one or two residues in length to much larger structures. Many proteins contain more than one type of linkage; for instance, **glycophorin,** an important red cell membrane glycoprotein (Chapter 52), contains both O- and N-linked oligosaccharides.

Table 47–6. Some important lectins.

Lectins	Examples or Comments
Legume lectins	Concanavalin A, pea lectin
Wheat germ agglutinin	Widely used in studies of surfaces of normal cells and cancer cells
Ricin	Cytotoxic glycoprotein derived from seeds of the castor plant
Bacterial toxins	Heat-labile enterotoxin of *E coli* and cholera toxin
Influenza virus hemagglutinin	Responsible for host-cell attachment and membrane fusion
C-type lectins	Characterized by a Ca^{2+}-dependent carbohydrate recognition domain (CRD); includes the mammalian asialoglycoprotein receptor, the selectins, and the mannose-binding protein
S-type lectins	β-Galactoside-binding animal lectins with roles in cell-cell and cell-matrix interactions
P-type lectins	Mannose 6-P receptor
I-type lectins	Members of the immunoglobulin superfamily, eg, sialoadhesin mediating adhesion of macrophages to various cells

GLYCOPROTEINS CONTAIN SEVERAL TYPES OF O-GLYCOSIDIC LINKAGES

At least four subclasses of O-glycosidic linkages are found in human glycoproteins: (1) The **GalNAc-Ser(Thr)** linkage shown in Figure 47–1 is the predominant linkage. Two typical oligosaccharide chains found in members of this subclass are shown in Figure 47–2. Usually a Gal or a NeuAc residue is attached to the GalNAc, but many variations in the sugar compositions and lengths of such oligosaccharide chains are found. This type of linkage is found in **mucins** (see below). (2) **Proteoglycans** contain a **Gal-Gal-Xyl-Ser** trisaccharide (the so-called link trisaccharide). (3) **Collagens** contain a **Gal-hydroxylysine (Hyl)** linkage. (Subclasses [2] and [3] are discussed further in Chapter 48.) (4) Many **nuclear proteins** (eg, certain transcription factors) and **cytosolic proteins** contain side chains consisting of a single GlcNAc attached to a serine or threonine residue **(GlcNAc-Ser[Thr]).**

Table 47–7. Three plant lectins and the sugars with which they interact.[1]

Lectin	Abbreviation	Sugars
Concanavalin A	ConA	Man and Glc
Soybean lectin		Gal and GalNAc
Wheat germ agglutinin	WGA	Glc and NeuAc

[1]In most cases, lectins show specificity for the anomeric nature of the glycosidic linkage (α or β); this is not indicated in the table.

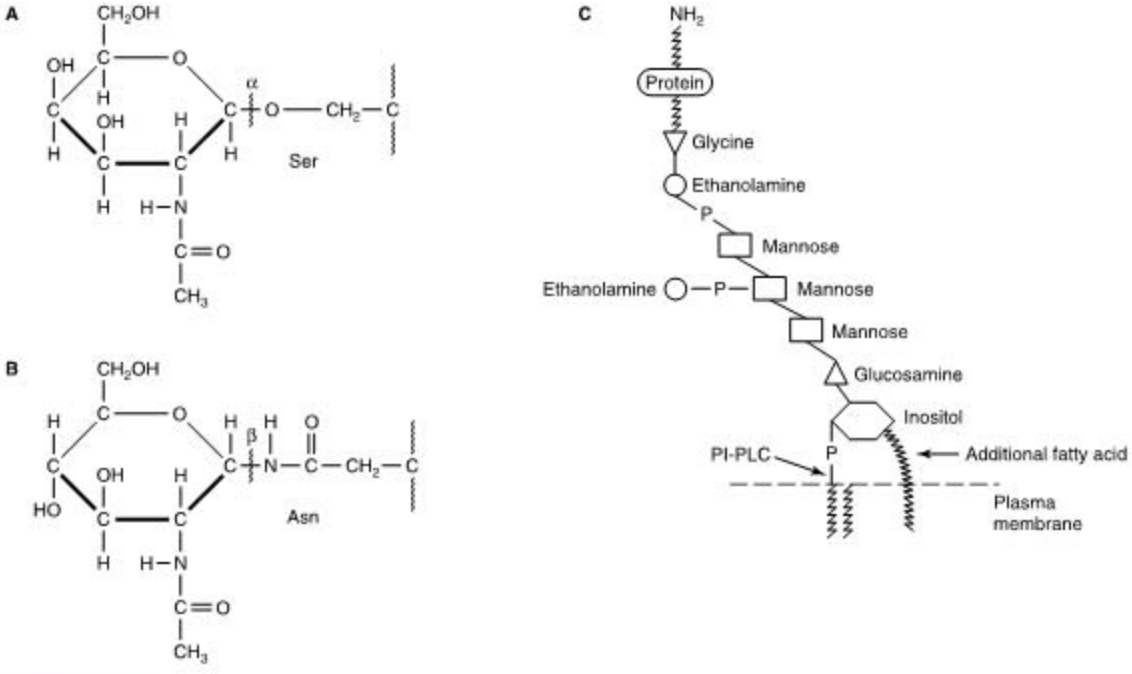


Figure 47–1. Depictions of **(A)** an O-linkage (*N*-acetylgalactosamine to serine); **(B)** an N-linkage (*N*-acetylglucosamine to asparagine) and **(C)** a glycosylphosphatidylinositol (GPI) linkage. The GPI structure shown is that linking acetylcholinesterase to the plasma membrane of the human red blood cell. The carboxyl terminal amino acid is glycine joined in amide linkage via its COOH group to the NH_2 group of phosphorylethanolamine, which in turn is joined to a mannose residue. The core glycan contains three mannose and one glucosamine residues. The glucosamine is linked to inositol, which is attached to phosphatidic acid. The site of action of PI-phospholipase C (PI-PLC) is indicated. The structure of the core glycan is shown in the text. This particular GPI contains an extra fatty acid attached to inositol and also an extra phosphorylethanolamine moiety attached to the middle of the three mannose residues. Variations found among different GPI structures include the identity of the carboxyl terminal amino acid, the molecules attached to the mannose residues, and the precise nature of the lipid moiety.

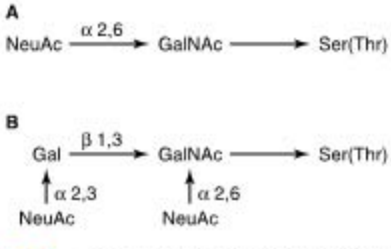


Figure 47–2. Structures of two O-linked oligosaccharides found in **(A)** submaxillary mucins and **(B)** fetuin and in the sialoglycoprotein of the membrane of human red blood cells. (Modified and reproduced, with permission, from Lennarz WJ: *The Biochemistry of Glycoproteins and Proteoglycans.* Plenum Press, 1980.)

Mucins Have a High Content of O-Linked Oligosaccharides & Exhibit Repeating Amino Acid Sequences

Mucins are glycoproteins with two major characteristics: (1) a high content of **O-linked oligosaccharides** (the carbohydrate content of mucins is generally more than 50%); and (2) the presence of **repeating amino acid sequences** (tandem repeats) in the center of their polypeptide backbones, to which the O-glycan chains are attached in clusters (Figure 47–3). These sequences are rich in serine, threonine, and proline. Although O-glycans predominate, mucins often contain a number of N-glycan chains. Both **secretory** and **membrane-bound** mucins occur. The former are found in the mucus present in the secretions of the gastrointestinal, respiratory, and reproductive tracts. **Mucus** consists of about 94% water and 5% mucins, with the remainder

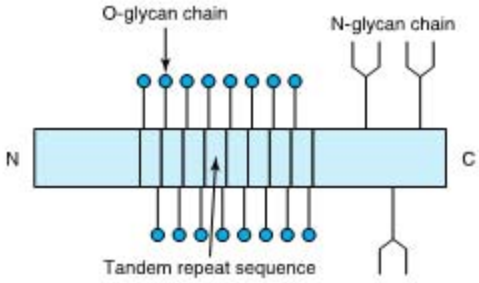


Figure 47–3. Schematic diagram of a mucin. O-glycan chains are shown attached to the central region of the extended polypeptide chain and N-glycan chains to the carboxyl terminal region. The narrow rectangles represent a series of tandem repeat amino acid sequences. Many mucins contain cysteine residues whose SH groups form interchain linkages; these are not shown in the figure. (Adapted from Strous GJ, Dekker J: Mucin-type glycoproteins. Crit Rev Biochem Mol Biol 1992;27:57.)

being a mixture of various cell molecules, electrolytes, and remnants of cells. Secretory mucins generally have an oligomeric structure and thus often have a very high molecular mass. The oligomers are composed of monomers linked by disulfide bonds. Mucus exhibits a high **viscosity** and often forms a **gel.** These qualities are functions of its content of mucins. The high content of O-glycans confers an extended structure on mucins. This is in part explained by steric interactions between their GalNAc moieties and adjacent amino acids, resulting in a chain-stiffening effect so that the conformations of mucins often become those of rigid rods. Intermolecular noncovalent interactions between various sugars on neighboring glycan chains contribute to gel formation. The high content of **NeuAc** and **sulfate** residues found in many mucins confers a negative charge on them. With regard to function, mucins help **lubricate** and form a **protective physical barrier** on epithelial surfaces. Membrane-bound mucins participate in various **cell-cell interactions** (eg, involving selectins; see below). The density of oligosaccharide chains makes it difficult for **proteases** to approach their polypeptide backbones, so that mucins are often resistant to their action. Mucins also tend to "mask" certain surface antigens. Many cancer cells form excessive amounts of mucins; perhaps the mucins may mask certain surface antigens on such cells and thus protect the cells from immune surveillance. Mucins also carry cancer-specific peptide and carbohydrate epitopes (an epitope is a site on an antigen recognized by an antibody, also called an antigenic determinant). Some of these epitopes have been used to stimulate an immune response against cancer cells.

The **genes** encoding the polypeptide backbones of a number of mucins derived from various tissues (eg, pancreas, small intestine, trachea and bronchi, stomach, and salivary glands) have been cloned and sequenced. These studies have revealed new information about the polypeptide backbones of mucins (size of tandem repeats, potential sites of N-glycosylation, etc) and ultimately should reveal aspects of their genetic control. Some important properties of mucins are summarized in Table 47–8.

The Biosynthesis of O-Linked Glycoproteins Uses Nucleotide Sugars

The polypeptide chains of O-linked and other glycoproteins are encoded by mRNA species; because most glycoproteins are membrane-bound or secreted, they are generally translated on membrane-bound polyribosomes (Chapter 38). Hundreds of different oligosaccharide chains of the O-glycosidic type exist. These glycoproteins are built up by the **stepwise donation of sugars from nucleotide sugars,** such as UDP-GalNAc, UDP-Gal, and CMP-NeuAc. The enzymes catalyzing this type of reaction are membrane-bound **glycoprotein glycosyltransferases.** Generally, synthesis of one specific type of linkage requires the activity of a correspondingly specific transferase. The factors that determine which specific serine and threonine residues are glycosylated have not been identified but are probably found in the peptide structure surrounding the glycosylation site. The enzymes assembling O-linked chains are located in the Golgi apparatus, sequentially arranged in an assembly line with terminal reactions occurring in the trans-Golgi compartments.

The major features of the biosynthesis of O-linked glycoproteins are summarized in Table 47–9.

Table 47–8. Some properties of mucins.

- Found in secretions of the gastrointestinal, respiratory, and reproductive tracts and also in membranes of various cells.
- Exhibit high content of O-glycan chains, usually containing NeuAc.
- Contain repeating amino acid sequences rich in serine, threonine, and proline.
- Extended structure contributes to their high viscoelasticity.
- Form protective physical barrier on epithelial surfaces, are involved in cell-cell interactions, and may contain or mask certain surface antigens.

Table 47–9. Summary of main features of O-glycosylation.

- Involves a battery of membrane-bound glycoprotein glycosyltransferases acting in a stepwise manner; each transferase is generally specific for a particular type of linkage.
- The enzymes involved are located in various subcompartments of the Golgi apparatus.
- Each glycosylation reaction involves the appropriate nucleotide-sugar.
- Dolichol-P-P-oligosaccharide is not involved, nor are glycosidases; and the reactions are not inhibited by tunicamycin.
- O-Glycosylation occurs posttranslationally at certain Ser and Thr residues.

N-LINKED GLYCOPROTEINS CONTAIN AN Asn-GlcNAc LINKAGE

N-Linked glycoproteins are distinguished by the presence of the Asn-GlcNAc linkage (Figure 47–1). It is the major class of glycoproteins and has been much studied, since the most readily accessible glycoproteins (eg, plasma proteins) mainly belong to this group. It includes both **membrane-bound** and **circulating** glycoproteins. The principal difference between this and the previous class, apart from the nature of the amino acid to which the oligosaccharide chain is attached (Asn versus Ser or Thr), concerns their biosynthesis.

Complex, Hybrid, & High-Mannose Are the Three Major Classes of N-Linked Oligosaccharides

There are three major classes of N-linked oligosaccharides: **complex**, **hybrid**, and **high-mannose** (Figure 47–4). Each type shares a common pentasaccharide, $Man_3GlcNAc_2$—shown within the boxed area in Figure 47–4 and depicted also in Figure 47–5—but they differ in their outer branches. The presence of the **common pentasaccharide** is explained by the fact that all three classes share an initial common mechanism of biosynthesis. Glycoproteins of the complex type generally contain terminal NeuAc residues and underlying Gal and GlcNAc residues, the latter often constituting the disaccharide *N*-acetyllactosamine. Repeating ***N*-acetyllactosamine units**—$[Gal\beta1\text{–}3/4GlcNAc\beta1\text{–}3]_n$ (poly-*N*-acetyllactosaminoglycans)—are often found on N-linked glycan chains. I/i blood group substances belong to this class. The majority of complex-type oligosaccharides contain two, three, or four outer branches (Figure 47–4), but structures containing five branches have also been described. The oligosaccharide branches are often referred to as **antennae,** so that bi-, tri-, tetra-, and penta-antennary structures may all be found. A bewildering number of chains of the complex type exist, and that indicated in Figure 47–4 is only one of many. Other complex chains may terminate in Gal or Fuc. High-mannose oligosaccharides typically have two to six additional Man residues linked to the pentasaccharide core. Hybrid molecules contain features of both of the two other classes.

The Biosynthesis of N-Linked Glycoproteins Involves Dolichol-P-P-Oligosaccharide

Leloir and his colleagues described the occurrence of a **dolichol-pyrophosphate-oligosaccharide (Dol-P-P-oligosaccharide),** which subsequent research showed to play a key role in the biosynthesis of N-linked glycoproteins. The oligosaccharide chain of this compound generally has the structure $R\text{-}GlcNAc_2Man_9Glc_3$ (R = Dol-P-P). The sugars of this compound are first assembled on the Dol-P-P backbone, and the oligosaccharide chain is then transferred en bloc to suitable Asn residues of acceptor apoglycoproteins during their synthesis on membrane-bound polyribosomes. All N-glycans have a common pentasaccharide core structure (Figure 47–5).

To form **high-mannose** chains, only the Glc residues plus certain of the peripheral Man residues are removed. To form an oligosaccharide chain of the **complex type,** the Glc residues and four of the Man residues are removed by glycosidases in the endoplasmic reticulum and Golgi. The sugars characteristic of complex chains (GlcNAc, Gal, NeuAc) are added by the action of individual glycosyltransferases located in the Golgi apparatus. The phenomenon whereby the glycan chains of N-linked glycoproteins are first partially degraded and then in some cases rebuilt is referred to as **oligosaccharide processing. Hybrid chains** are formed by partial processing, forming complex chains on one arm and Man structures on the other arm.

Thus, the initial steps involved in the biosynthesis of the N-linked glycoproteins differ markedly from those involved in the biosynthesis of the O-linked glycoproteins. The former involves Dol-P-P-oligosaccharide; the latter, as described earlier, does not.

The process of N-glycosylation can be broken down into two stages: (1) assembly of Dol-P-P-oligosaccharide and transfer of the oligosaccharide; and (2) processing of the oligosaccharide chain.

A. Assembly & Transfer of Dolichol-P-P-Oligosaccharide

Polyisoprenol compounds exist in both bacteria and eukaryotic cells. They participate in the synthesis of bacterial polysaccharides and in the biosynthesis of N-

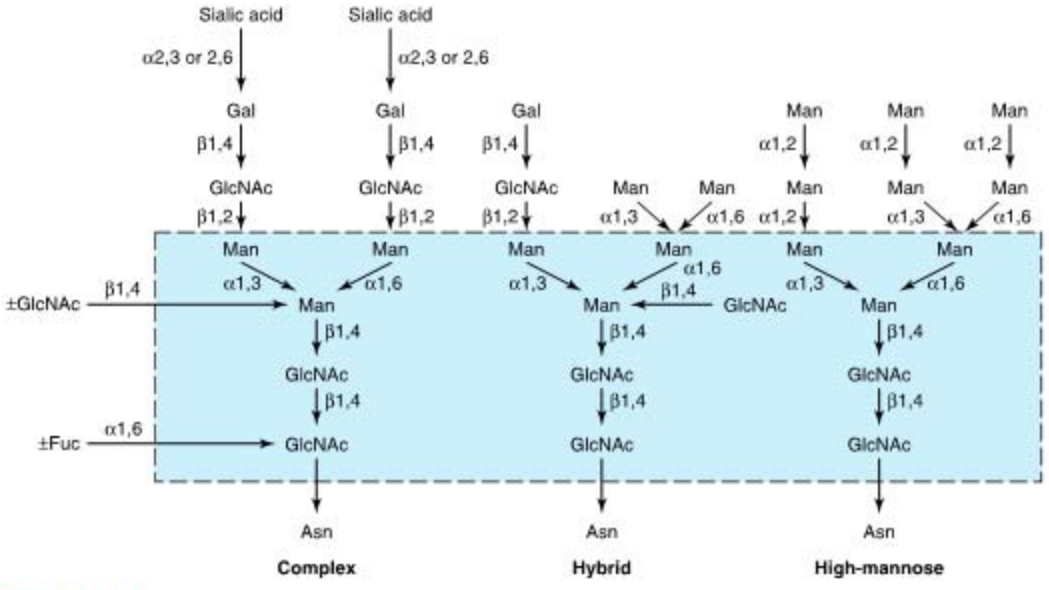


Figure 47–4. **Structures of the major types of asparagine-linked oligosaccharides. The boxed area encloses the pentasaccharide core common to all N-linked glycoproteins.** (Reproduced, with permission, from Kornfeld R, Kornfeld S: Assembly of asparagine-linked oligosaccharides. Annu Rev Biochem 1985;54:631.)

linked glycoproteins and GPI anchors. The polyisoprenol used in eukaryotic tissues is **dolichol,** which is, next to rubber, the longest naturally occurring hydrocarbon made up of a single repeating unit. Dolichol is composed of 17–20 repeating isoprenoid units (Figure 47–6).

Before it participates in the biosynthesis of Dol-P-P-oligosaccharide, dolichol must first be phosphorylated to form dolichol phosphate (Dol-P) in a reaction catalyzed by **dolichol kinase** and using ATP as the phosphate donor.

Dolichol-P-P-GlcNAc (Dol-P-P-GlcNAc) is the key lipid that acts as an acceptor for other sugars in the assembly of Dol-P-P-oligosaccharide. It is synthesized in the membranes of the endoplasmic reticulum from Dol-P and UDP-GlcNAc in the following reaction, catalyzed by GlcNAc-P transferase:

$$\text{Dol-P} + \text{UDP-GlcNAc} \rightarrow \text{Dol-P-P-GlcNAc} + \text{UMP}$$

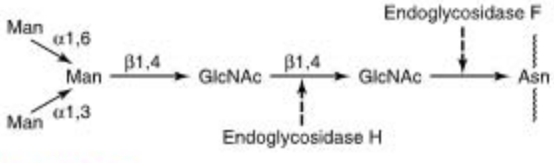


Figure 47–5. **Schematic diagram of the pentasaccharide core common to all N-linked glycoproteins and to which various outer chains of oligosaccharides may be attached. The sites of action of endoglycosidases F and H are also indicated.**

The above reaction—which is the first step in the assembly of Dol-P-P-oligosaccharide—and the other later reactions are summarized in Figure 47–7. The essential features of the subsequent steps in the assembly of Dol-P-P-oligosaccharide are as follows:

(1) A second GlcNAc residue is added to the first, again using UDP-GlcNAc as the donor.

(2) Five Man residues are added, using GDP-mannose as the donor.

(3) Four additional Man residues are next added, using Dol-P-Man as the donor. Dol-P-Man is formed by the following reaction:

$$\text{Dol-P} + \text{GDP-Man} \rightarrow \text{Dol-P-Man} + \text{GDP}$$

(4) Finally, the three peripheral glucose residues are donated by Dol-P-Glc, which is formed in a reaction analogous to that just presented except that Dol-P and UDP-Glc are the substrates.

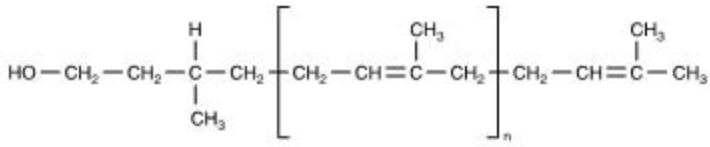


Figure 47–6. The structure of dolichol. The phosphate in dolichol phosphate is attached to the primary alcohol group at the left-hand end of the molecule. The group within the brackets is an isoprene unit (n = 17–20 isoprenoid units).

It should be noted that the first seven sugars (two GlcNAc and five Man residues) are donated by nucleotide sugars, whereas the last seven sugars (four Man and three Glc residues) added are donated by dolichol-P-sugars. The net result is assembly of the compound illustrated in Figure 47–8 and referred to in shorthand as Dol-P-P-$GlcNAc_2Man_9Glc_3$.

The oligosaccharide linked to dolichol-P-P is transferred en bloc to form an N-glycosidic bond with one or more specific Asn residues of an acceptor protein emerging from the luminal surface of the membrane of the endoplasmic reticulum. The reaction is catalyzed by **oligosaccharide:protein transferase,** a membrane-associated enzyme complex. The transferase will recognize and transfer any substrate with the general structure Dol-P-P-$(GlcNAc)_2$-R, but it has a strong preference for the Dol-P-P-$GlcNAc_2Man_9Glc_3$ structure. Glycosylation occurs at the Asn residue of an Asn-X-Ser/Thr tripeptide sequence, where X is any amino acid except proline, aspartic acid, or glutamic acid. A tripeptide site contained within a β turn is favored. Only about one-third of the Asn residues that are potential acceptor sites are actually glycosylated, suggesting that factors other than the tripeptide are also important. The acceptor proteins are of both the secretory and integral membrane class. Cytosolic proteins are rarely glycosylated. The transfer reaction and subsequent processes in the glycosylation of N-linked glycoproteins, along with their subcellular locations, are depicted in Figure 47–9. The other product of the oligosaccharide:protein transferase reaction is dolichol-P-P, which is subsequently converted to dolichol-P by a

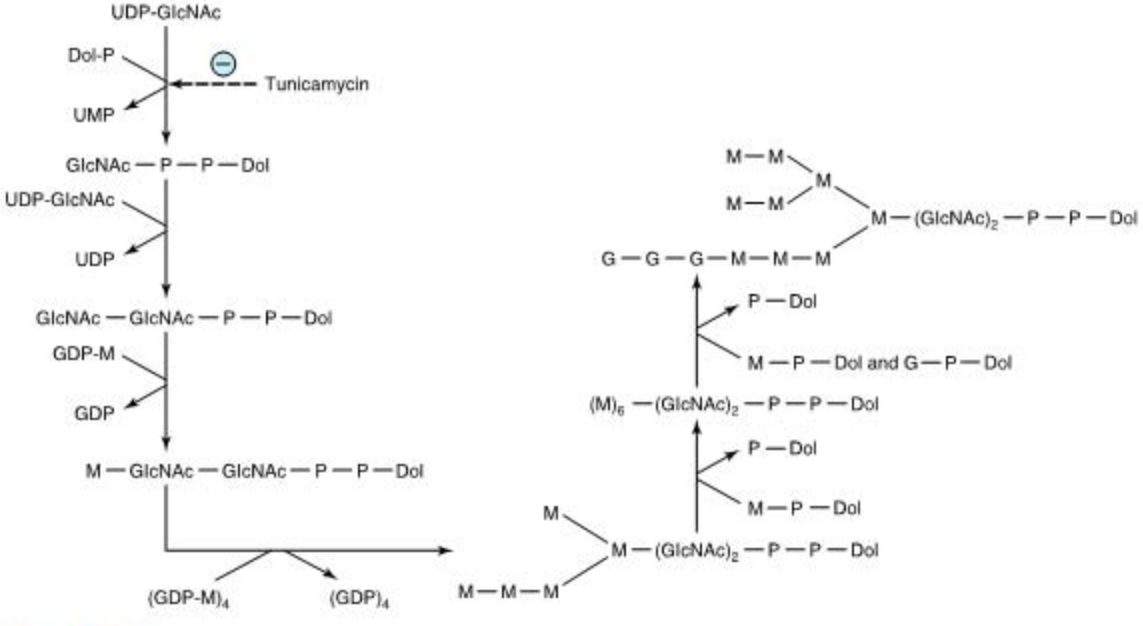


Figure 47–7. Pathway of biosynthesis of dolichol-P-P-oligosaccharide. The specific linkages formed are indicated in Figure 47–8. Note that the first five internal mannose residues are donated by GDP-mannose, whereas the more external mannose residues and the glucose residues are donated by dolichol-P-mannose and dolichol-P-glucose. (UDP, uridine diphosphate; Dol, dolichol; P, phosphate; UMP, uridine monophosphate; GDP, guanosine diphosphate; M, mannose; G, glucose.)

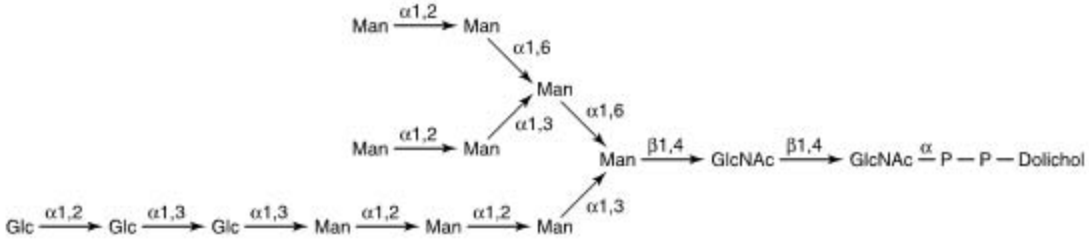


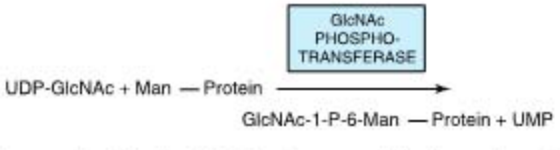
Figure 47–8. **Structure of dolichol-P-P-oligosaccharide.** (Reproduced, with permission, from Lennarz WJ: *The Biochemistry of Glycoproteins and Proteoglycans.* Plenum Press, 1980.)

phosphatase. The dolichol-P can serve again as an acceptor for the synthesis of another molecule of Dol-P-P-oligosaccharide.

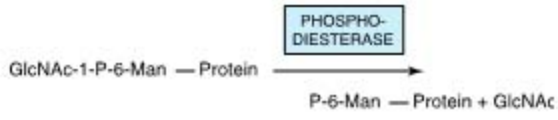
B. Processing of the Oligosaccharide Chain

1. Early phase—The various reactions involved are indicated in Figure 47–9. The oligosaccharide:protein transferase catalyzes reaction 1 (see above). Reactions 2 and 3 involve the removal of the terminal Glc residue by glucosidase I and of the next two Glc residues by glucosidase II, respectively. In the case of **high-mannose** glycoproteins, the process may stop here, or up to four Man residues may also be removed. However, to form **complex** chains, additional steps are necessary, as follows. Four external Man residues are removed in reactions 4 and 5 by at least two different mannosidases. In reaction 6, a GlcNAc residue is added to the Man residue of the Manα1–3 arm by GlcNAc transferase I. The action of this latter enzyme permits the occurrence of reaction 7, a reaction catalyzed by yet another mannosidase (Golgi α-mannosidase II) and which results in a reduction of the Man residues to the core number of three (Figure 47–5).

An important additional pathway is indicated in reactions I and II of Figure 47–9. This involves enzymes destined for **lysosomes.** Such enzymes are targeted to the lysosomes by a specific chemical marker. In reaction I, a residue of GlcNAc-1-P is added to carbon 6 of one or more specific Man residues of these enzymes. The reaction is catalyzed by a GlcNAc phosphotransferase, which uses UDP-GlcNAc as the donor and generates UMP as the other product:



In reaction II, the GlcNAc is removed by the action of a phosphodiesterase, leaving the Man residues phosphorylated in the 6 position:



Man 6-P receptors, located in the Golgi apparatus, bind the Man 6-P residue of these enzymes and direct them to the lysosomes. Fibroblasts from patients with **I-cell disease** (see below) are severely deficient in the activity of the GlcNAc phosphotransferase.

2. Late phase—To assemble a typical complex oligosaccharide chain, additional sugars must be added to the structure formed in reaction 7. Hence, in reaction 8, a second GlcNAc is added to the peripheral Man residue of the other arm of the bi-antennary structure shown in Figure 47–9; the enzyme catalyzing this step is GlcNAc transferase II. Reactions 9, 10, and 11 involve the addition of Fuc, Gal, and NeuAc residues at the sites indicated, in reactions catalyzed by fucosyl, galactosyl, and sialyl transferases, respectively. The assembly of poly-*N*-acetyllactosamine chains requires additional GlcNAc transferases.

The Endoplasmic Reticulum & Golgi Apparatus Are the Major Sites of Glycosylation

As indicated in Figure 47–9, the endoplasmic reticulum and the Golgi apparatus are the major sites involved in glycosylation processes. The assembly of Dol-P-P-oligosaccharide occurs on both the cytoplasmic and luminal surfaces of the ER membranes. Addition of the oligosaccharide to protein occurs in the rough endoplasmic reticulum during or after translation. Removal of the Glc and some of the peripheral Man residues also occurs in the endoplasmic reticulum. The Golgi apparatus is composed of cis, medial, and trans cisternae; these can be separated by appropriate centrifugation procedures. Vesicles containing glycoproteins appear to bud off in the endoplasmic reticulum and are transported to the cis Golgi. Various studies have shown

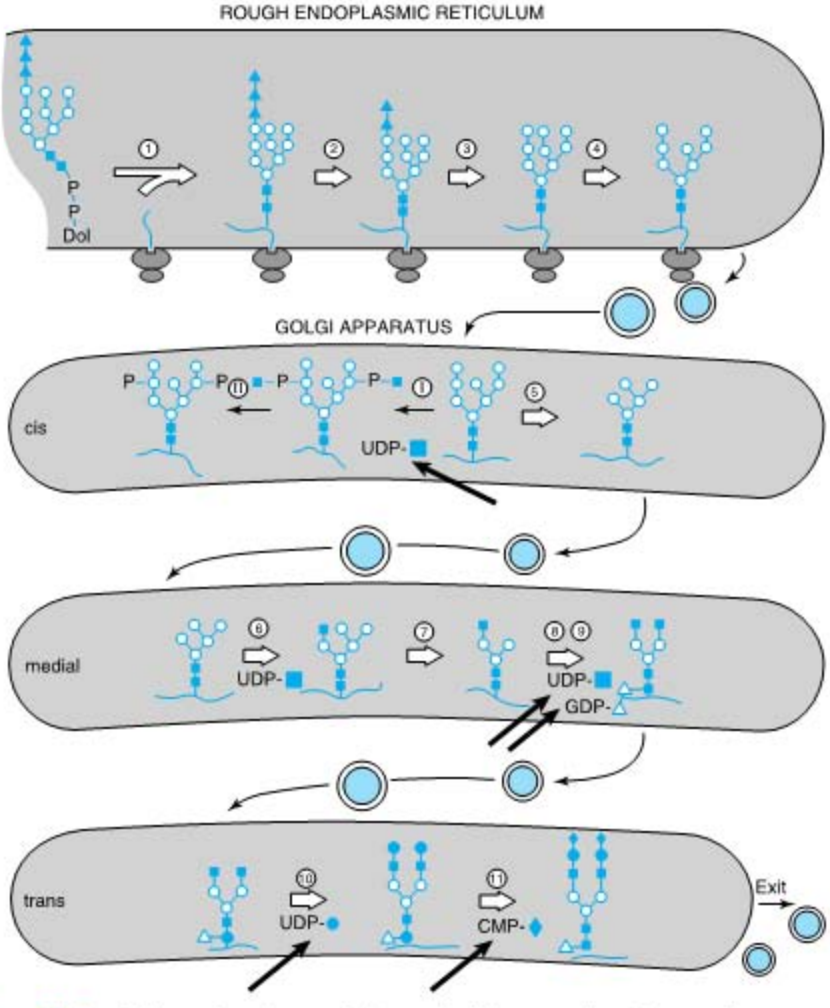


Figure 47–9. Schematic pathway of oligosaccharide processing. The reactions are catalyzed by the following enzymes: ①, oligosaccharide:protein transferase; ②, α-glucosidase I; ③, α-glucosidase II; ④, endoplasmic reticulum α1,2-mannosidase; ⑤, *N*-acetylglucosaminylphosphotransferase; ⑩, *N*-acetylglucosamine-1-phosphodiester α-*N*-acetylglucosaminidase; ⑤, Golgi apparatus α-mannosidase I; ⑥, *N*-acetylglucosaminyltransferase I; ⑦, Golgi apparatus α-mannosidase II; ⑧, *N*-acetylglucosaminyltransferase II; ⑨, fucosyltransferase; ⑩, galactosyltransferase; ⑪, sialyltransferase. The thick arrows indicate various nucleotide sugars involved in the oveall scheme. (Solid square, *N*-acetylglucosamine; open circle, mannose; solid triangle, glucose; open triangle, fucose; solid circle, galactose; solid diamond, sialic acid.) (Reproduced, with permission, from Kornfeld R, Kornfeld S: Assembly of asparagine-linked oligosaccharides. Annu Rev Biochem 1985;54:631.)

that the enzymes involved in glycoprotein processing show differential locations in the cisternae of the Golgi. As indicated in Figure 47–9, Golgi α-mannosidase I (catalyzing reaction 5) is located mainly in the cis Golgi, whereas GlcNAc transferase I (catalyzing reaction 6) appears to be located in the medial Golgi, and the fucosyl, galactosyl, and sialyl transferases (catalyzing reactions 9, 10, and 11) are located mainly in the trans Golgi. The major features of the biosynthesis of N-linked glycoproteins are summarized in Table 47–10 and should be contrasted with those previously listed (Table 47–9) for O-linked glycoproteins.

Table 47–10. Summary of main features of N-glycosylation.

- The oligosaccharide $Glc_3Man_9(GlcNAc)_2$ is transferred from dolichol-P-P-oligosaccharide in a reaction catalyzed by oligosaccharide:protein transferase, which is inhibited by tunicamycin.
- Transfer occurs to specific Asn residues in the sequence Asn-X-Ser/Thr, where X is any residue except Pro, Asp, or Glu.
- Transfer can occur cotranslationally in the endoplasmic reticulum.
- The protein-bound oligosaccharide is then partially processed by glucosidases and mannosidases; if no additional sugars are added, this results in a high-mannose chain.
- If processing occurs down to the core heptasaccharide ($Man_5[GlcNAc]_2$), complex chains are synthesized by the addition of GlcNAc, removal of two Man, and the stepwise addition of individual sugars in reactions catalyzed by specific transferases (eg, GlcNAc, Gal, NeuAc transferases) that employ appropriate nucleotide sugars.

Some Glycan Intermediates Formed During N-Glycosylation Have Specific Functions

The following are a number of specific functions of N-glycan chains that have been established or are under investigation. (1) The involvement of the **mannose 6-P signal** in targeting of certain lysosomal enzymes is clear (see above and discussion of I-cell disease, below). (2) It is likely that the large N-glycan chains present on newly synthesized glycoproteins may assist in keeping these proteins in a soluble state inside the lumen of the endoplasmic reticulum. (3) One species of N-glycan chains has been shown to play a role in the folding and retention of certain glycoproteins in the lumen of the endoplasmic reticulum. **Calnexin** is a protein present in the endoplasmic reticulum membrane that acts as a "chaperone" (Chapter 46). It has been found that calnexin will bind specifically to a number of glycoproteins (eg, the influenza virus hemagglutinin [HA]) that possess the **monoglycosylated core structure.** This species is the product of reaction 2 shown in Figure 47–9 but from which the terminal glucose residue has been removed, leaving only the innermost glucose attached. The release of fully folded HA from calnexin requires the enzymatic removal of this last glucosyl residue by α-glucosidase II. In this way, calnexin retains certain partly folded (or misfolded) glycoproteins and releases them when proper folding has occurred; it is thus an important component of the quality control systems operating in the lumen of the ER. The soluble protein **calreticulin** appears to play a similar function.

Several Factors Regulate the Glycosylation of Glycoproteins

It is evident that glycosylation of glycoproteins is a complex process involving a large number of enzymes. One index of its complexity is that more than ten distinct GlcNAc transferases involved in glycoprotein biosynthesis have been reported, and many others are theoretically possible. Multiple species of the other glycosyltransferases (eg, sialyltransferases) also exist. Controlling factors of the first stage of N-linked glycoprotein biosynthesis (ie, **oligosaccharide assembly and transfer**) include (1) the presence of suitable acceptor sites in proteins, (2) the tissue level of Dol-P, and (3) the activity of the oligosaccharide:protein transferase.

Some factors known to be involved in the regulation of **oligosaccharide processing** are listed in Table 47–11. Two of the points listed merit further comment. First, **species variations** among processing enzymes have assumed importance in relation to production of glycoproteins of therapeutic use by means of recombinant DNA technology. For instance, recombinant erythropoietin (epoetin alfa; EPO) is sometimes administered to patients with certain types of chronic anemia in order to stimulate erythropoiesis. The half-life of EPO in plasma is influenced by the nature of its glycosylation pattern, with certain patterns being associated with a short half-life, appreciably limiting its period of therapeutic effectiveness. It is thus important to harvest EPO from host cells that confer a pattern of glycosylation consistent with a normal half-life in plasma. Second, there is great interest in analysis of the activities of glycoprotein-processing enzymes in various types of cancer cells. These cells have often been found to synthesize different oligosaccharide chains (eg, they often exhibit greater branching) from those made in control cells. This could be due to cancer cells containing different patterns of glycosyltransferases from those exhibited by corresponding normal cells, due to specific gene activation or repression. The differences in oligosaccharide chains could affect adhesive interactions between cancer cells and their normal parent tissue cells, contributing to metastasis. If a correlation could be found between the activity of particular processing enzymes and the **metastatic properties** of cancer cells, this could be important as it might permit synthesis of drugs to inhibit these enzymes and, secondarily, metastasis.

The genes encoding many glycosyltransferases have already been cloned, and others are under study. Cloning has revealed new information on both protein and gene structures. The latter should also cast light on

Table 47–11. Some factors affecting the activities of glycoprotein processing enzymes.

Factor	Comment
Cell type	Different cell types contain different profiles of processing enzymes.
Previous enzyme	Certain glycosyltransferases will only act on an oligosaccharide chain if it has already been acted upon by another processing enzyme.[1]
Development	The cellular profile of processing enzymes may change during development if their genes are turned on or off.
Intracellular location	For instance, if an enzyme is destined for insertion into the membrane of the ER (eg, HMG-CoA reductase), it may never encounter Golgi-located processing enzymes.
Protein conformation	Differences in conformation of different proteins may facilitate or hinder access of processing enzymes to identical oligosaccharide chains.
Species	Same cells (eg, fibroblasts) from different species may exhibit different patterns of processing enzymes.
Cancer	Cancer cells may exhibit processing enzymes different from those of corresponding normal cells.

[1]For example, prior action of GlcNAc transferase I is necessary for the action of Golgi α-mannosidase II.

the mechanisms involved in their transcriptional control, and gene knockout studies are being used to evaluate the biologic importance of various glycosyltransferases.

Tunicamycin Inhibits N- but Not O-Glycosylation

A number of compounds are known to inhibit various reactions involved in glycoprotein processing. **Tunicamycin, deoxynojirimycin,** and **swainsonine** are three such agents. The reactions they inhibit are indicated in Table 47–12. These agents can be used experimentally to inhibit various stages of glycoprotein biosynthesis and to study the effects of specific alterations upon the process. For instance, if cells are grown in the presence of tunicamycin, no glycosylation of their normally N-linked glycoproteins will occur. In certain cases, lack of glycosylation has been shown to increase the susceptibility of these proteins to proteolysis. Inhibition of glycosylation does not appear to have a consistent effect upon the secretion of glycoproteins that are normally secreted. The inhibitors of glycoprotein processing listed in Table 47–12 do not affect the biosynthesis of O-linked glycoproteins. The extension of O-linked chains can be prevented by GalNAc-benzyl. This compound competes with natural glycoprotein substrates and thus prevents chain growth beyond GalNAc.

Table 47–12. Three inhibitors of enzymes involved in the glycosylation of glycoproteins and their sites of action.

Inhibitor	Site of Action
Tunicamycin	Inhibits GlcNAc-P transferase, the enzyme catalyzing addition of GlcNAc to dolichol-P, the first step in the biosynthesis of oligosaccharide-P-P-dolichol
Deoxynojirimycin	Inhibitor of glucosidases I and II
Swainsonine	Inhibitor of mannosidase II

SOME PROTEINS ARE ANCHORED TO THE PLASMA MEMBRANE BY GLYCOSYLPHOSPHATIDYLINOSITOL STRUCTURES

Glycosylphosphatidylinositol (GPI)-linked glycoproteins comprise the third major class of glycoprotein. The GPI structure (sometimes called a "sticky foot") involved in linkage of the enzyme acetylcholinesterase (ACh esterase) to the plasma membrane of the red blood cell is shown in Figure 47–1. GPI-linked proteins are anchored to the outer leaflet of the plasma membrane by the fatty acids of phosphatidylinositol (PI). The PI is linked via a $GlcNH_2$ moiety to a glycan chain that contains various sugars (eg, Man, $GlcNH_2$). In turn, the oligosaccharide chain is linked via phosphorylethanolamine in an amide linkage to the carboxyl terminal amino acid of the attached protein. The core of most GPI structures contains one molecule of phosphorylethanolamine, three Man residues, one molecule of $GlcNH_2$, and one molecule of phosphatidylinositol, as follows:

Ethanolamine-phospho → 6Manα1 →
2Manα1 → 6Manα1 → GlcNα1 →
6 — *myo*-inositol-1-phospholipid

Additional constituents are found in many GPI structures; for example, that shown in Figure 47–1 contains an extra phosphorylethanolamine attached to the middle of the three Man moieties of the glycan and an extra fatty acid attached to $GlcNH_2$. The functional significance of these variations among structures is not understood. This type of linkage was first detected by the use of bacterial PI-specific phospholipase C (PI-PLC), which was found to release certain proteins from the plasma membrane of cells by splitting the bond indicated in Figure 47–1. Examples of some proteins that are anchored by this type of linkage are given in Table 47–13. At least three possible functions of this type of linkage have been suggested: (1) The GPI anchor may allow greatly enhanced **mobility** of a protein in the plasma membrane compared with that observed for a protein that contains transmembrane sequences. This is perhaps not surprising, as the GPI anchor is attached only to the outer leaflet of the lipid bilayer, so that it is freer to diffuse than a protein anchored via both leaflets of the bilayer. Increased mobility may be important in facilitating rapid responses to appropriate stimuli. (2) Some GPI anchors may connect with **signal transduction** pathways. (3) It has been shown that GPI structures can target certain proteins to apical domains of the plasma membrane of certain epithelial cells. The biosynthesis of GPI anchors is complex and begins in the endoplasmic reticulum. The GPI anchor is assembled independently by a series of enzyme-catalyzed reactions and then transferred to the carboxyl terminal end of its acceptor protein, accompanied by cleavage of the preexisting carboxyl terminal hydrophobic peptide from that protein. This process is sometimes called **glypiation.** An acquired defect in an early stage of the biosynthesis of the GPI structure has been implicated in the causation of **paroxysmal nocturnal hemoglobinuria** (see below).

GLYCOPROTEINS ARE INVOLVED IN MANY BIOLOGIC PROCESSES & IN MANY DISEASES

As listed in Table 47–1, glycoproteins have many different functions; some have already been addressed in this chapter and others are described elsewhere in this text (eg, transport molecules, immunologic molecules, and hormones). Here, their involvement in two specific processes—fertilization and inflammation—will be briefly described. In addition, the bases of a number of diseases that are due to abnormalities in the synthesis and degradation of glycoproteins will be summarized.

Table 47–13. Some GPI-linked proteins.

- Acetylcholinesterase (red cell membrane)
- Alkaline phosphatase (intestinal, placental)
- Decay-accelerating factor (red cell membrane)
- 5′-Nucleotidase (T lymphocytes, other cells)
- Thy-1 antigen (brain, T lymphocytes)
- Variable surface glycoprotein (*Trypanosoma brucei*)

Glycoproteins Are Important in Fertilization

To reach the plasma membrane of an oocyte, a sperm has to traverse the **zona pellucida (ZP),** a thick, transparent, noncellular envelope that surrounds the oocyte. The zona pellucida contains three glycoproteins of interest, ZP1–3. Of particular note is ZP3, an O-linked glycoprotein that functions as a receptor for the sperm. A protein on the sperm surface, possibly galactosyl transferase, interacts specifically with oligosaccharide chains of ZP3; in at least certain species (eg, the mouse), this interaction, by transmembrane signaling, induces the **acrosomal reaction,** in which enzymes such as proteases and hyaluronidase and other contents of the acrosome of the sperm are released. Liberation of these enzymes helps the sperm to pass through the zona pellucida and reach the plasma membrane (PM) of the oocyte. In hamsters, it has been shown that another glycoprotein, PH-30, is important in both the binding of the PM of the sperm to the PM of the oocyte and also in the subsequent fusion of the two membranes. These interactions enable the sperm to enter and thus fertilize the oocyte. It may be possible to inhibit fertilization by developing drugs or antibodies that interfere with the normal functions of ZP3 and PH-30 and which would thus act as contraceptive agents.

Selectins Play Key Roles in Inflammation & in Lymphocyte Homing

Leukocytes play important roles in many inflammatory and immunologic phenomena. The first steps in many of these phenomena are interactions between circulating leukocytes and endothelial cells prior to passage of the former out of the circulation. Work done to identify specific molecules on the surfaces of the cells involved in such interactions has revealed that leukocytes and endothelial cells contain on their surfaces specific lectins, called **selectins,** that participate in their intercellular adhesion. Features of the three major classes of selectins are summarized in Table 47–14. Selectins are single-chain Ca^{2+}-binding transmembrane proteins that contain a number of domains (Figure 47–10). Their amino terminal ends contain the lectin domain, which is involved in binding to specific carbohydrate ligands.

The adhesion of neutrophils to endothelial cells of postcapillary venules can be considered to occur in four

Table 47–14. Some molecules involved in leukocyte-endothelial cell interactions.[1]

Molecule	Cell	Ligands
Selectins		
L-selectin	PMN, lymphs	CD34, Gly-CAM-1[2] Sialyl-Lewis^x and others
P-selectin	EC, platelets	P-selectin glycoprotein ligand-1 (PSGL-1) Sialyl-Lewis^x and others
E-selectin	EC	Sialyl-Lewis^x and others
Integrins		
LFA-1	PMN, lymphs	ICAM-1, ICAM-2 (CD11a/CD18)
Mac-1	PMN	ICAM-1 and others (CD11b/CD18)
Immunoglobulin superfamily		
ICAM-1	Lymphs, EC	LFA-1, Mac-1
ICAM-2	Lymphs, EC	LFA-1
PECAM-1	EC, PMN, lymphs	Various platelets

[1]Modified from Albelda SM, Smith CW, Ward PA: Adhesion molecules and inflammatory injury. FASEB J 1994;8:504.
[2]These are ligands for lymphocyte L-selectin; the ligands for neutrophil L-selectin have not been identified.
Key: PMN, polymorphonuclear leukocytes; EC, endothelial cell; lymphs, lymphocytes; CD, cluster of differentiation; ICAM, intercellular adhesion molecule; LFA-1, lymphocyte function-associated antigen-1; PECAM-1, platelet endothelial cell adhesion cell molecule-1.

stages, as shown in Figure 47–11. The initial baseline stage is succeeded by slowing or rolling of the neutrophils, mediated by selectins. Interactions between L-selectin on the neutrophil surface and CD34 and GlyCAM-1 or other glycoproteins on the endothelial surface are involved. These particular interactions are initially short-lived, and the overall binding is of relatively low affinity, permitting rolling. However, during this stage, activation of the neutrophils by various chemical mediators (discussed below) occurs, resulting in a change of shape of the neutrophils and firm adhesion of these cells to the endothelium. An additional set of adhesion molecules is involved in firm adhesion, namely, LFA-1 and Mac-1 on the neutrophils and ICAM-1 and ICAM-2 on endothelial cells. LFA-1 and Mac-1 are CD11/CD18 integrins (see Chapter 52 for a discussion of integrins), whereas ICAM-1 and ICAM-2 are members of the immunoglobulin superfamily. The fourth stage is transmigration of the neutrophils across the endothelial wall. For this to occur, the neutrophils insert pseudopods into the junctions between endothelial cells, squeeze through these junctions, cross the basement membrane, and then are free to migrate in the extravascular space. Platelet-endothelial cell adhesion molecule-1 (PECAM-1) has been found to be localized at the junctions of endothelial cells and thus may have a role in transmigration. A variety of biomolecules have been found to be involved in activation of neutrophil and endothelial cells, including tumor necrosis factor α, various interleukins, platelet activating factor (PAF), leukotriene B_4, and certain complement fragments. These compounds stimulate various signaling pathways, resulting in changes in cell shape and function, and some are also chemotactic. One important functional change is recruitment of selectins to the cell surface, as in some cases selectins are stored in granules (eg, in endothelial cells and platelets).

Figure 47–10. Schematic diagram of the structure of human L-selectin. The extracellular portion contains an amino terminal domain homologous to C-type lectins and an adjacent epidermal growth factor-like domain. These are followed by a variable number of complement regulatory-like modules (numbered circles) and a transmembrane sequence (black diamond). A short cytoplasmic sequence (open rectangle) is at the carboxyl terminal. The structures of P- and E-selectin are similar to that shown except that they contain more complement-regulatory modules. The numbers of amino acids in L-, P-, and E- selectins, as deduced from the cDNA sequences, are 385, 789, and 589, respectively. (Reproduced, with permission, from Bevilacqua MP, Nelson RM: Selectins. J Clin Invest 1993;91:370.)

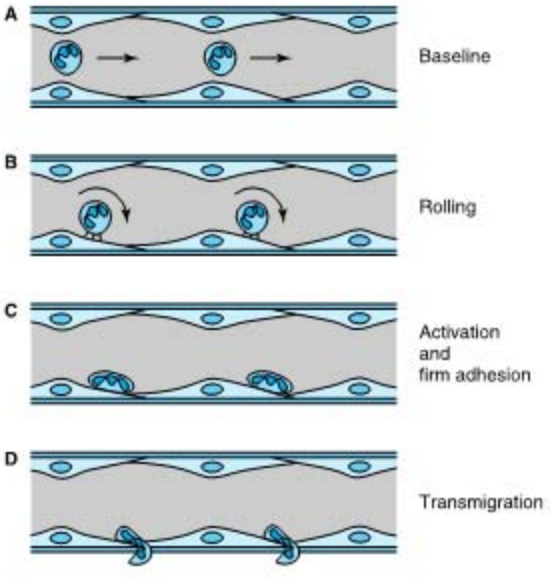


Figure 47–11. Schematic diagram of neutrophil-endothelial cell interactions. **A:** Baseline conditions: Neutrophils do not adhere to the vessel wall. **B:** The first event is the slowing or rolling of the neutrophils within the vessel (venule) mediated by selectins. **C:** Activation occurs, resulting in neutrophils firmly adhering to the surfaces of endothelial cells and also assuming a flattened shape. This requires interaction of activated CD18 integrins on neutrophils with ICAM-1 on the endothelium. **D:** The neutrophils then migrate through the junctions of endothelial cells into the interstitial tissue; this requires involvement of PECAM-1. Chemotaxis is also involved in this latter stage. (Reproduced, with permission, from Albelda SM, Smith CW, Ward PA: Adhesion molecules and inflammatory injury. FASEB J 1994;8;504.)

The precise chemical nature of some of the ligands involved in selectin-ligand interactions has been determined. All three selectins bind **sialylated and fucosylated oligosaccharides,** and in particular all three bind **sialyl-Lewis^X** (Figure 47–12), a structure present on both glycoproteins and glycolipids. Whether this compound is the actual ligand involved in vivo is not established. Sulfated molecules, such as the sulfatides (Chapter 14), may be ligands in certain instances. This basic knowledge is being used in attempts to synthesize compounds that block selectin-ligand interactions and thus may inhibit the inflammatory response. Approaches include administration of specific monoclonal antibodies or of chemically synthesized analogs of sialyl-Lewis^X, both of which bind selectins. Cancer cells often exhibit sialyl-Lewis^X and other selectin ligands on their surfaces. It is thought that these ligands play a role in the invasion and metastasis of cancer cells.

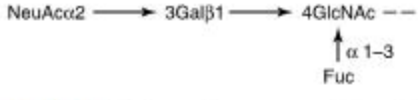


Figure 47–12. Schematic representation of the structure of sialyl-Lewis^X.

Abnormalities in the Synthesis of Glycoproteins Underlie Certain Diseases

Table 47–15 lists a number of conditions in which abnormalities in the synthesis of glycoproteins are of im-

Table 47–15. Some diseases due to or involving abnormalities in the biosynthesis of glycoproteins.

Disease	Abnormality
Cancer	Increased branching of cell surface glycans or presentation of selectin ligands may be important in metastasis.
Congenital disorders of glycosylation[1]	See Table 47–16.
HEMPAS[2] (MIM 224100)	Abnormalities in certain enzymes (eg, mannosidase II and others) involved in the biosynthesis of N-glycans, particularly affecting the red blood cell membrane.
Leukocyte adhesion deficiency, type II (MIM 266265)	Probably mutations affecting a Golgi-located GDP-fucose transporter, resulting in defective fucosylation.
Paroxysmal nocturnal hemoglobinuria (MIM 311770)	Acquired defect in biosynthesis of the GPI[3] structures of decay accelerating factor (DAF) and CD59.
I-cell disease (MIM 252500)	Deficiency of GlcNAc phosphotransferase, resulting in abnormal targeting of certain lysosomal enzymes.

[1]The MIM number for congenital disorder of glycosylation type Ia is 212065.
[2]Hereditary erythroblastic multinuclearity with a positive acidified serum lysis test (congenital dyserythropoietic anemia type II). This is a relatively mild form of anemia. It reflects at least in part the presence in the red cell membranes of various glycoproteins with abnormal N-glycan chains, which contribute to the susceptibility to lysis.
[3]Glycosylphosphatidylinositol.

portance. As mentioned above, many **cancer cells** exhibit different profiles of oligosaccharide chains on their surfaces, some of which may contribute to metastasis. The **congenital disorders of glycosylation (CDG)** are a group of disorders of considerable current interest. The major features of these conditions are summarized in Table 47–16. **Leukocyte adhesion deficiency (LAD) II** is a rare condition probably due to mutations affecting the activity of a Golgi-located GDP-fucose transporter. It can be considered a congenital disorder of glycosylation. The absence of fucosylated ligands for selectins leads to a marked decrease in neutrophil rolling. Subjects suffer life-threatening, recurrent bacterial infections and also psychomotor and mental retardation. The condition appears to respond to oral fucose. **Hereditary erythroblastic multinuclearity with a positive acidified lysis test (HEMPAS)**—congenital dyserythropoietic anemia type II—is another disorder due to abnormalities in the processing of N-glycans. Some cases have been claimed to be due to defects in alpha–mannosidase II. I-cell disease is discussed further below. **Paroxysmal nocturnal hemoglobinuria** is an acquired mild anemia characterized by the presence of hemoglobin in urine due to hemolysis of red cells, particularly during sleep. This latter phenomenon may reflect a slight drop in plasma pH during sleep, which increases susceptibility to lysis by the complement system (Chapter 50). The basic defect in paroxysmal nocturnal hemoglobinuria is the acquisition of somatic mutations in the *PIG-A* (for phosphatidylinositol glycan class A) gene of certain hematopoietic cells. The product of this gene appears to be the enzyme that links glucosamine to phosphatidylinositol in the GPI structure (Figure 47–1). Thus, proteins that are anchored by a GPI linkage are deficient in the red cell membrane. Two proteins are of particular interest: **decay accelerating factor (DAF)** and another protein designated CD59. They normally interact with certain components of the complement system (Chapter 50) to prevent the hemolytic actions of the latter. However, when they are deficient, the complement system can act on the red cell membrane to cause hemolysis. Paroxysmal nocturnal hemoglobinuria can be diagnosed relatively simply, as the red cells are much more sensitive to hemolysis in normal serum acidified to pH 6.2 (Ham's test); the complement system is activated under these conditions, but normal cells are not affected. Figure 47–13 summarizes the etiology of paroxysmal nocturnal hemoglobinuria.

Table 47–16. Major features of the congenital disorders of glycosylation.

- Autosomal recessive disorders
- Multisystem disorders that have probably not been recognized in the past
- Generally affect the central nervous system, resulting in psychomotor retardation and other features
- Type I disorders are due to mutations in genes encoding enzymes (eg, phosphomannomutase-2 [PMM-2], causing CDG Ia) involved in the synthesis of dolichol-P-P-oligosaccharide
- Type II disorders are due to mutations in genes encoding enzymes (eg, GlcNAc transferase-2, causing CDG IIa) involved in the processing of N-glycan chains
- About 11 distinct disorders have been recognized
- Isoelectric focusing of transferrin is a useful biochemical test for assisting in the diagnosis of these conditions; truncation of the oligosaccharide chains of this protein alters its isolectric focusing pattern
- Oral mannose has proved of benefit in the treatment of CDG Ia

Key: CDG, congenital disorder of glycosylation.

I-Cell Disease Results From Faulty Targeting of Lysosomal Enzymes

As indicated above, Man 6-P serves as a chemical marker to target certain lysosomal enzymes to that organelle. Analysis of cultured fibroblasts derived from patients with I-cell (inclusion cell) disease played a large part in revealing the above role of Man 6-P. I-cell disease is an uncommon condition characterized by severe progressive psychomotor retardation and a variety of physical signs, with death often occurring in the first decade. Cultured cells from patients with I-cell disease were found to lack almost all of the normal lysosomal enzymes; the lysosomes thus accumulate many different

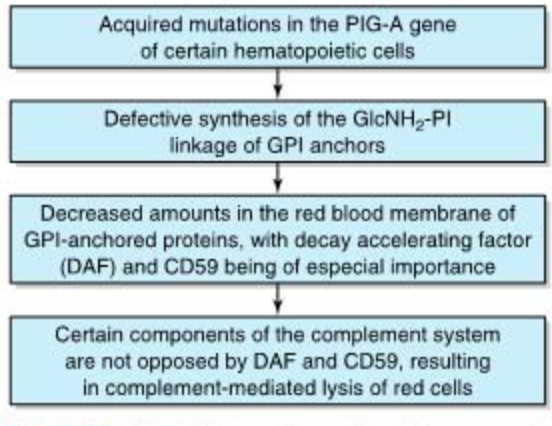


Figure 47–13. Scheme of causation of paroxysmal nocturnal hemoglobinuria (MIM 311770).

types of undegraded molecules, forming inclusion bodies. Samples of plasma from patients with the disease were observed to contain very high activities of lysosomal enzymes; this suggested that the enzymes were being synthesized but were failing to reach their proper intracellular destination and were instead being secreted. Cultured cells from patients with the disease were noted to take up exogenously added lysosomal enzymes obtained from normal subjects, indicating that the cells contained a normal receptor on their surfaces for endocytic uptake of lysosomal enzymes. In addition, this finding suggested that lysosomal enzymes from patients with I-cell disease might lack a recognition marker. Further studies revealed that lysosomal enzymes from normal individuals carried the Man 6-P recognition marker described above, which interacted with a specific intracellular protein, the Man 6-P receptor. Cultured cells from patients with I-cell disease were then found to be deficient in the activity of the cis Golgi-located GlcNAc phosphotransferase, explaining how their lysosomal enzymes failed to acquire the Man 6-P marker. It is now known that there are two Man 6-P receptor proteins, one of high (275 kDa) and one of low (46 kDa) molecular mass. These proteins are lectins, recognizing Man 6-P. The former is cation-independent and also binds IGF-II (hence it is named the Man 6-P–IGF-II receptor), whereas the latter is cation-dependent in some species and does not bind IGF-II. It appears that both receptors function in the intracellular sorting of lysosomal enzymes into clathrin-coated vesicles, which occurs in the trans Golgi subsequent to synthesis of Man 6-P in the cis Golgi. These vesicles then leave the Golgi and fuse with a prelysosomal compartment. The low pH in this compartment causes the lysosomal enzymes to dissociate from their receptors and subsequently enter into lysosomes. The receptors are recycled and reused. Only the smaller receptor functions in the endocytosis of extracellular lysosomal enzymes, which is a minor pathway for lysosomal location. Not all cells employ the Man 6-P receptor to target their lysosomal enzymes (eg, hepatocytes use a different but undefined pathway); furthermore, not all lysosomal enzymes are targeted by this mechanism. Thus, biochemical investigations of I-cell disease not only led to elucidation of its basis but also contributed significantly to knowledge of how newly synthesized proteins are targeted to specific organelles, in this case the lysosome. Figure 47–14 summarizes the causation of I-cell disease.

Pseudo-Hurler polydystrophy is another genetic disease closely related to I-cell disease. It is a milder condition, and patients may survive to adulthood. Studies have revealed that the GlcNAc phosphotransferase involved in I-cell disease has several domains, including a catalytic domain and a domain that specifically recognizes and interacts with lysosomal enzymes. It has been proposed that the defect in pseudo-Hurler polydystrophy lies in the latter domain, and the retention of some catalytic activity results in a milder condition.

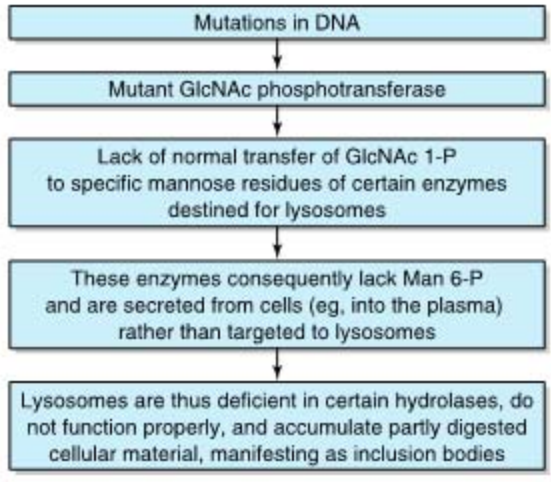


Figure 47–14. Summary of the causation of I-cell disease (MIM 252500).

Genetic Deficiencies of Glycoprotein Lysosomal Hydrolases Cause Diseases Such as α-Mannosidosis

Glycoproteins, like most other biomolecules, undergo both synthesis and degradation (ie, turnover). Degradation of the oligosaccharide chains of glycoproteins involves a battery of lysosomal hydrolases, including α-neuraminidase, β-galactosidase, β-hexosaminidase, α- and β-mannosidases, α-*N*-acetylgalactosaminidase, α-fucosidase, endo-β-*N*-acetylglucosaminidase, and aspartylglucosaminidase. The sites of action of the last two enzymes are indicated in the legend to Figure 47–5. Genetically determined defects of the activities of these enzymes can occur, resulting in abnormal degradation of glycoproteins. The accumulation in tissues of such abnormally degraded glycoproteins can lead to various diseases. Among the best-recognized of these diseases are mannosidosis, fucosidosis, sialidosis, aspartylglycosaminuria, and Schindler disease, due respectively to deficiencies of α-mannosidase, α-fucosidase, α-neuraminidase, aspartylglucosaminidase, and α-*N*-acetyl-galactosaminidase. These diseases, which are relatively uncommon, have a variety of manifestations; some of their major features are listed in Table 47–17. The fact that patients affected by these disorders all show signs referable to the central nervous sys-

Table 47–17. Major features of some diseases (eg, α-mannosidosis, β-mannosidosis, fucosidosis, sialidosis, aspartylglycosaminuria, and Schindler disease) due to deficiencies of glycoprotein hydrolases.[1]

- Usually exhibit mental retardation or other neurologic abnormalities, and in some disorders coarse features or visceromegaly (or both)
- Variations in severity from mild to rapidly progressive
- Autosomal recessive inheritance
- May show ethnic distribution (eg, aspartylglycosaminuria is common in Finland)
- Vacuolization of cells observed by microscopy in some disorders
- Presence of abnormal degradation products (eg, oligosaccharides that accumulate because of the enzyme deficiency) in urine, detectable by TLC and characterizable by GLC-MS
- Definitive diagnosis made by assay of appropriate enzyme, often using leukocytes
- Possibility of prenatal diagnosis by appropriate enzyme assays
- No definitive treatment at present

[1]MIM numbers: α-mannosidosis, 248500; β-mannosidosis, 248510; fucosidosis, 230000; sialidosis, 256550; aspartylglycosaminuria, 208400; Schindler disease, 104170.

tem reflects the importance of glycoproteins in the development and normal function of that system.

From the above, it should be apparent that glycoproteins are involved in a wide variety of biologic processes and diseases. Glycoproteins play direct or indirect roles in a number of other diseases, as shown in the following examples.

(1) The **influenza virus** possesses a neuraminidase that plays a key role in elution of newly synthesized progeny from infected cells. If this process is inhibited, spread of the virus is markedly diminished. Inhibitors of this enzyme are now available for use in treating patients with influenza.

(2) **HIV-1,** thought by many to be the causative agent of AIDS, attaches to cells via one of its surface glycoproteins, gp120.

(3) **Rheumatoid arthritis** is associated with an alteration in the glycosylation of circulating immunoglobulin-γ (IgG) molecules (Chapter 50), such that they lack galactose in their Fc regions and terminate in GlcNAc. **Mannose-binding protein** (not to be confused with the mannose-6-P receptor), a C-lectin synthesized by liver cells and secreted into the circulation, binds mannose, GlcNAc, and certain other sugars. It can thus bind the agalactosyl IgG molecules, which subsequently activate the complement system, contributing to chronic inflammation in the synovial membranes of joints. This protein can also bind the above sugars when they are present on the surfaces of certain bacteria, fungi, and viruses, preparing these pathogens for opsonization or for destruction by the complement system. This is an example of innate immunity, not involving immunoglobulins. Deficiency of this protein in young infants, due to mutation, renders them very susceptible to **recurrent infections.**

Other disorders in which glycoproteins have been implicated include hepatitis B and C, Creutzfeldt-Jakob disease, and diarrheas due to a number of bacterial enterotoxins. It is hoped that basic studies of glycoproteins and other glycoconjugates (ie, the field of glycobiology) will lead to effective treatments for diseases in which these molecules are involved. Already, at least two disorders have been found to respond to oral supplements of sugars.

The fantastic progress made in relation to the human genome has stimulated intense interest in both **genomics** and **proteomics.** It is anticipated that the pace of research in **glycomics**—characterization of the entire complement of sugar chains found in cells (the glycome)—will also accelerate markedly. For a number of reasons, this field will prove more challenging than either genomics or proteomics. These reasons include the complexity of the structures of oligosaccharide chains due to linkage variations—in contrast to the generally uniform nature of the linkages between nucleotides and between amino acids. There are also significant variations in oligosaccharide structures among cells and at different stages of development. In addition, no simple technique exists for amplifying oligosaccharides, comparable to the PCR reaction. Despite these and other problems, it seems certain that research in this area will uncover many new important biologic interactions that are sugar-dependent and will provide targets for drug and other therapies.

SUMMARY

- Glycoproteins are widely distributed proteins—with diverse functions—that contain one or more covalently linked carbohydrate chains.
- The carbohydrate components of a glycoprotein range from 1% to more than 85% of its weight and may be simple or very complex in structure.
- At least certain of the oligosaccharide chains of glycoproteins encode biologic information; they are also important to glycoproteins in modulating their solubility and viscosity, in protecting them against proteolysis, and in their biologic actions.

- The structures of many oligosaccharide chains can be elucidated by gas-liquid chromatography, mass spectrometry, and high-resolution NMR spectrometry.
- Glycosidases hydrolyze specific linkages in oligosaccharides and are used to explore both the structures and functions of glycoproteins.
- Lectins are carbohydrate-binding proteins involved in cell adhesion and other biologic processes.
- The major classes of glycoproteins are O-linked (involving an OH of serine or threonine), N-linked (involving the N of the amide group of asparagine), and glycosylphosphatidylinositol (GPI)-linked.
- Mucins are a class of O-linked glycoproteins that are distributed on the surfaces of epithelial cells of the respiratory, gastrointestinal, and reproductive tracts.
- The Golgi apparatus plays a major role in glycosylation reactions involved in the biosynthesis of glycoproteins.
- The oligosaccharide chains of O-linked glycoproteins are synthesized by the stepwise addition of sugars donated by nucleotide sugars in reactions catalyzed by individual specific glycoprotein glycosyltransferases.
- In contrast, the biosynthesis of N-linked glycoproteins involves a specific dolichol-P-P-oligosaccharide and various glycosidases. Depending on the glycosidases and precursor proteins synthesized by a tissue, it can synthesize complex, hybrid, or high-mannose types of N-linked oligosaccharides.
- Glycoproteins are implicated in many biologic processes. For instance, they have been found to play key roles in fertilization and inflammation.
- A number of diseases involving abnormalities in the synthesis and degradation of glycoproteins have been recognized. Glycoproteins are also involved in many other diseases, including influenza, AIDS, and rheumatoid arthritis.
- Developments in the new field of glycomics are likely to provide much new information on the roles of sugars in health and disease and also indicate targets for drug and other types of therapies.

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The Extracellular Matrix

48

Robert K. Murray, MD, PhD, & Frederick W. Keeley, PhD

BIOMEDICAL IMPORTANCE

Most mammalian cells are located in tissues where they are surrounded by a complex **extracellular matrix (ECM)** often referred to as **"connective tissue."** The ECM contains three major classes of biomolecules: (1) the **structural proteins,** collagen, elastin, and fibrillin; (2) certain **specialized proteins** such as fibrillin, fibronectin, and laminin; and (3) **proteoglycans,** whose chemical natures are described below. The ECM has been found to be involved in many normal and pathologic processes—eg, it plays important roles in development, in inflammatory states, and in the spread of cancer cells. Involvement of certain components of the ECM has been documented in both rheumatoid arthritis and **osteoarthritis.** Several diseases (eg, osteogenesis imperfecta and a number of types of the Ehlers-Danlos syndrome) are due to genetic disturbances of the synthesis of collagen. Specific components of proteoglycans (the glycosaminoglycans; GAGs) are affected in the group of genetic disorders known as the mucopolysaccharidoses. Changes occur in the ECM during the aging process. This chapter describes the basic biochemistry of the three major classes of biomolecules found in the ECM and illustrates their biomedical significance. Major biochemical features of two specialized forms of ECM—bone and cartilage—and of a number of diseases involving them are also briefly considered.

COLLAGEN IS THE MOST ABUNDANT PROTEIN IN THE ANIMAL WORLD

Collagen, the major component of most connective tissues, constitutes approximately 25% of the protein of mammals. It provides an extracellular framework for all metazoan animals and exists in virtually every animal tissue. At least 19 distinct types of collagen made up of 30 distinct polypeptide chains (each encoded by a separate gene) have been identified in human tissues. Although several of these are present only in small proportions, they may play important roles in determining the physical properties of specific tissues. In addition, a number of proteins (eg, the C1q component of the complement system, pulmonary surfactant proteins SP-A and SP-D) that are not classified as collagens have collagen-like domains in their structures; these proteins are sometimes referred to as "noncollagen collagens."

Table 48–1 summarizes the types of collagens found in human tissues; the nomenclature used to designate types of collagen and their genes is described in the footnote.

The 19 types of collagen mentioned above can be subdivided into a number of classes based primarily on the structures they form (Table 48–2). In this chapter, we shall be primarily concerned with the fibril-forming collagens I and II, the major collagens of skin and bone and of cartilage, respectively. However, mention will be made of some of the other collagens.

COLLAGEN TYPE I IS COMPOSED OF A TRIPLE HELIX STRUCTURE & FORMS FIBRILS

All collagen types have a **triple helical structure.** In some collagens, the entire molecule is triple helical, whereas in others the triple helix may involve only a fraction of the structure. Mature collagen type I, containing approximately 1000 amino acids, belongs to the former type; in it, each polypeptide subunit or alpha chain is twisted into a left-handed helix of three residues per turn (Figure 48–1). Three of these alpha chains are then wound into a right-handed superhelix, forming a rod-like molecule 1.4 nm in diameter and about 300 nm long. A striking characteristic of collagen is the occurrence of **glycine** residues at every third position of the triple helical portion of the alpha chain. This is necessary because glycine is the only amino acid small enough to be accommodated in the limited space available down the central core of the triple helix. This repeating structure, represented as $(Gly\text{-}X\text{-}Y)_n$, is an absolute requirement for the formation of the triple helix. While X and Y can be any other amino acids, about 100 of the X positions are proline and about 100 of the Y positions are hydroxyproline. Proline and hydroxyproline confer rigidity on the collagen molecule. **Hydroxyproline** is formed by the posttranslational hydroxylation of peptide-bound proline residues catalyzed by the enzyme **prolyl hydroxylase,** whose cofactors are **ascorbic acid** (vitamin C) and α-ketoglutarate. Lysines

Table 48–1. Types of collagen and their genes.[1,2]

Type	Genes	Tissue
I	*COL1A1, COL1A2*	Most connective tissues, including bone
II	*COL2A1*	Cartilage, vitreous humor
III	*COL3A1*	Extensible connective tissues such as skin, lung, and the vascular system
IV	*COL4A1–COL4A6*	Basement membranes
V	*COL5A1–COL5A3*	Minor component in tissues containing collagen I
VI	*COL6A1–COL6A3*	Most connective tissues
VII	*COL7A1*	Anchoring fibrils
VIII	*COL8A1–COL8A2*	Endothelium, other tissues
IX	*COL9A1–COL9A3*	Tissues containing collagen II
X	*COL10A1*	Hypertrophic cartilage
XI	*COL11A1, COL11A2, COL2A1*	Tissues containing collagen II
XII	*COL12A1*	Tissues containing collagen I
XIII	*COL13A1*	Many tissues
XIV	*COL14A1*	Tissues containing collagen I
XV	*COL15A1*	Many tissues
XVI	*COL16A1*	Many tissues
XVII	*COL17A1*	Skin hemidesmosomes
XVIII	*COL18A1*	Many tissues (eg, liver, kidney)
XIX	*COL19A1*	Rhabdomyosarcoma cells

[1]Adapted slightly from Prockop DJ, Kivirrikko KI: Collagens: molecular biology, diseases, and potentials for therapy. Annu Rev Biochem 1995;64:403.

[2]The types of collagen are designated by Roman numerals. Constituent procollagen chains, called proα chains, are numbered using Arabic numerals, followed by the collagen type in parentheses. For instance, type I procollagen is assembled from two proα1(I) and one proα2(I) chain. It is thus a heterotrimer, whereas type 2 procollagen is assembled from three proα1(II) chains and is thus a homotrimer. The collagen genes are named according to the collagen type, written in Arabic numerals for the gene symbol, followed by an A and the number of the proα chain that they encode. Thus, the COL1A1 and COL1A2 genes encode the α1 and α2 chains of type I collagen, respectively.

Table 48–2. Classification of collagens, based primarily on the structures that they form.[1]

Class	Type
Fibril-forming	I, II, III, V, and XI
Network-like	IV, VIII, X
FACITs[2]	IX, XII, XIV, XVI, XIX
Beaded filaments	VI
Anchoring fibrils	VII
Transmembrane domain	XIII, XVII
Others	XV, XVIII

[1]Based on Prockop DJ, Kivirrikko KI: Collagens: molecular biology, diseases, and potentials for therapy. Annu Rev Biochem 1995;64:403.

[2]FACITs = fibril-associated collagens with interrupted triple helices.

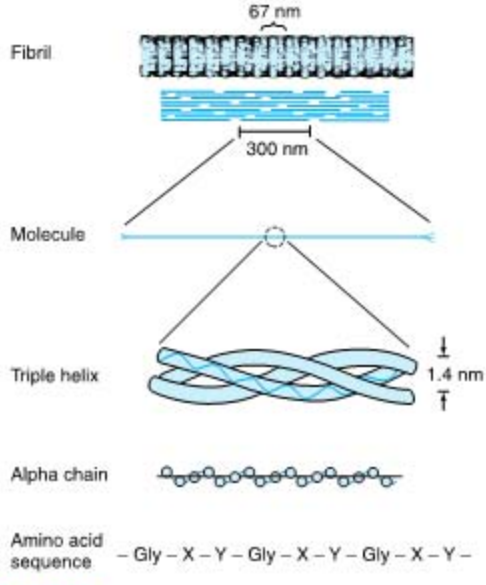

Figure 48–1. **Molecular features of collagen structure from primary sequence up to the fibril.** (Slightly modified and reproduced, with permission, from Eyre DR: Collagen: Molecular diversity in the body's protein scaffold. Science 1980;207:1315. Copyright © 1980 by the American Association for the Advancement of Science.)

in the Y position may also be posttranslationally modified to hydroxylysine through the action of **lysyl hydroxylase,** an enzyme with similar cofactors. Some of these hydroxylysines may be further modified by the addition of galactose or galactosyl-glucose through an **O-glycosidic linkage,** a glycosylation site that is unique to collagen.

Collagen types that form long rod-like fibers in tissues are assembled by lateral association of these triple helical units into a "quarter staggered" alignment such that each is displaced longitudinally from its neighbor by slightly less than one-quarter of its length (Figure 48–1, upper part). This arrangement is responsible for the banded appearance of these fibers in connective tissues. Collagen fibers are further stabilized by the formation of **covalent cross-links,** both within and between the triple helical units. These cross-links form through the action of **lysyl oxidase,** a copper-dependent enzyme that oxidatively deaminates the ε-amino groups of certain lysine and hydroxylysine residues, yielding reactive aldehydes. Such aldehydes can form aldol condensation products with other lysine- or hydroxylysine-derived aldehydes or form Schiff bases with the ε-amino groups of unoxidized lysines or hydroxylysines. These reactions, after further chemical rearrangements, result in the stable covalent cross-links that are important for the tensile strength of the fibers. Histidine may also be involved in certain cross-links.

Several collagen types do not form fibrils in tissues (Table 48–2). They are characterized by interruptions of the triple helix with stretches of protein lacking Gly-X-Y repeat sequences. These non-Gly-X-Y sequences result in areas of globular structure interspersed in the triple helical structure.

Type IV collagen, the best-characterized example of a collagen with discontinuous triple helices, is an important component of **basement membranes,** where it forms a mesh-like network.

Collagen Undergoes Extensive Posttranslational Modifications

Newly synthesized collagen undergoes extensive posttranslational modification before becoming part of a mature extracellular collagen fiber (Table 48–3). Like most secreted proteins, collagen is synthesized on ribosomes in a precursor form, **preprocollagen,** which contains a leader or signal sequence that directs the polypeptide chain into the lumen of the endoplasmic reticulum. As it enters the endoplasmic reticulum, this leader sequence is enzymatically removed. **Hydroxylation** of proline and lysine residues and **glycosylation** of hydroxylysines in the **procollagen** molecule also take place at this site. The procollagen molecule contains

Table 48–3. Order and location of processing of the fibrillar collagen precursor.

Intracellular
1. Cleavage of signal peptide
2. Hydroxylation of prolyl residues and some lysyl residues; glycosylation of some hydroxylysyl residues
3. Formation of intrachain and interchain S–S bonds in extension peptides
4. Formation of triple helix
Extracellular
1. Cleavage of amino and carboxyl terminal propeptides
2. Assembly of collagen fibers in quarter-staggered alignment
3. Oxidative deamination of ε-amino groups of lysyl and hydroxylysyl residues to aldehydes
4. Formation of intra- and interchain cross-links via Schiff bases and aldol condensation products

polypeptide extensions (extension peptides) of 20–35 kDa at both its amino and carboxyl terminal ends, neither of which is present in mature collagen. Both extension peptides contain cysteine residues. While the amino terminal propeptide forms only intrachain disulfide bonds, the carboxyl terminal propeptides form both intrachain and interchain disulfide bonds. Formation of these disulfide bonds assists in the registration of the three collagen molecules to form the triple helix, winding from the carboxyl terminal end. After formation of the triple helix, no further hydroxylation of proline or lysine or glycosylation of hydroxylysines can take place. **Self-assembly** is a cardinal principle in the biosynthesis of collagen.

Following **secretion** from the cell by way of the Golgi apparatus, extracellular enzymes called **procollagen aminoproteinase** and **procollagen carboxyproteinase** remove the extension peptides at the amino and carboxyl terminal ends, respectively. Cleavage of these propeptides may occur within crypts or folds in the cell membrane. Once the propeptides are removed, the triple helical collagen molecules, containing approximately 1000 amino acids per chain, spontaneously assemble into collagen fibers. These are further stabilized by the formation of inter- and intrachain cross-links through the action of lysyl oxidase, as described previously.

The same cells that secrete collagen also secrete **fibronectin,** a large glycoprotein present on cell surfaces, in the extracellular matrix, and in blood (see below). Fibronectin binds to aggregating precollagen fibers and alters the kinetics of fiber formation in the pericellular matrix. Associated with fibronectin and procollagen in

this matrix are the **proteoglycans** heparan sulfate and chondroitin sulfate (see below). In fact, type IX collagen, a minor collagen type from cartilage, contains attached proteoglycan chains. Such interactions may serve to regulate the formation of collagen fibers and to determine their orientation in tissues.

Once formed, collagen is relatively metabolically stable. However, its breakdown is increased during starvation and various inflammatory states. Excessive production of collagen occurs in a number of conditions, eg, hepatic cirrhosis.

A Number of Genetic Diseases Result From Abnormalities in the Synthesis of Collagen

About 30 genes encode collagen, and its pathway of biosynthesis is complex, involving at least eight enzyme-catalyzed posttranslational steps. Thus, it is not surprising that a number of diseases (Table 48–4) are due to **mutations in collagen genes** or in **genes encoding some of the enzymes** involved in these posttranslational modifications. The diseases affecting bone (eg, osteogenesis imperfecta) and cartilage (eg, the chondrodysplasias) will be discussed later in this chapter.

Ehlers-Danlos syndrome comprises a group of inherited disorders whose principal clinical features are hyperextensibility of the skin, abnormal tissue fragility, and increased joint mobility. The clinical picture is variable, reflecting underlying extensive genetic heterogeneity. At least 10 types have been recognized, most but not all of which reflect a variety of lesions in the synthesis of collagen. **Type IV** is the most serious because of its tendency for spontaneous rupture of arteries or the bowel, reflecting abnormalities in type III collagen. Patients with **type VI,** due to a deficiency of lysyl hydroxylase, exhibit marked joint hypermobility and a tendency to ocular rupture. A deficiency of procollagen N-proteinase, causing formation of abnormal thin, irregular collagen fibrils, results in **type VIIC,** manifested by marked joint hypermobility and soft skin.

Alport syndrome is the designation applied to a number of genetic disorders (both X-linked and autosomal) affecting the structure of type IV collagen fibers, the major collagen found in the basement membranes of the renal glomeruli (see discussion of laminin, below). Mutations in several genes encoding type IV collagen fibers have been demonstrated. The presenting sign is hematuria, and patients may eventually develop end-stage renal disease. Electron microscopy reveals characteristic abnormalities of the structure of the basement membrane and lamina densa.

In **epidermolysis bullosa,** the skin breaks and blisters as a result of minor trauma. The dystrophic form is due to mutations in *COL7A1,* affecting the structure of type VII collagen. This collagen forms delicate fibrils that anchor the basal lamina to collagen fibrils in the dermis. These anchoring fibrils have been shown to be markedly reduced in this form of the disease, probably resulting in the blistering. Epidermolysis bullosa simplex, another variant, is due to mutations in keratin 5 (Chapter 49).

Scurvy affects the structure of collagen. However, it is due to a deficiency of ascorbic acid (Chapter 45) and is not a genetic disease. Its major signs are bleeding

Table 48–4. Diseases caused by mutations in collagen genes or by deficiencies in the activities of posttranslational enzymes involved in the biosynthesis of collagen.[1]

Gene or Enzyme	Disease[2]
COL1A1, COL1A2	Osteogenesis imperfecta, type 1[3] (MIM 1566200) Osteoporosis[4] (MIM 166710) Ehlers-Danlos syndrome type VII autosomal dominant (130060)
COL2A1	Severe chondrodysplasias Osteoarthritis[4] (MIM 120140)
COL3A1	Ehlers-Danlos syndrome type IV (MIM 130050)
COL4A3–COL4A6	Alport syndrome (including both autosomal and X-linked forms) (MIM 104200)
COL7A1	Epidermolysis bullosa, dystrophic (MIM 131750)
COL10A1	Schmid metaphysial chondrodysplasia (MIM 156500)
Lysyl hydroxylase	Ehlers-Danlos syndrome type VI (MIM 225400)
Procollagen *N*-proteinase	Ehlers-Danlos syndrome type VII autosomal recessive (MIM 225410)
Lysyl hydroxylase	Menkes disease[5] (MIM 309400)

[1]Adapted from Prockop DJ, Kivirrikko KI: Collagens: molecular biology, diseases, and potentials for therapy. Annu Rev Biochem 1995;64:403.
[2]Genetic linkage to collagen genes has been shown for a few other conditions not listed here.
[3]At least four types of osteogenesis imperfecta are recognized; the great majority of mutations in all types are in the *COL1A1* and *COL1A2* genes.
[4]At present applies to only a relatively small number of such patients.
[5]Secondary to a deficiency of copper (Chapter 50).

gums, subcutaneous hemorrhages, and poor wound healing. These signs reflect impaired synthesis of collagen due to deficiencies of prolyl and lysyl hydroxylases, both of which require ascorbic acid as a cofactor.

ELASTIN CONFERS EXTENSIBILITY & RECOIL ON LUNG, BLOOD VESSELS, & LIGAMENTS

Elastin is a connective tissue protein that is responsible for properties of extensibility and elastic recoil in tissues. Although not as widespread as collagen, elastin is present in large amounts, particularly in tissues that require these physical properties, eg, lung, large arterial blood vessels, and some elastic ligaments. Smaller quantities of elastin are also found in skin, ear cartilage, and several other tissues. In contrast to collagen, there appears to be only one genetic type of elastin, although variants arise by alternative splicing (Chapter 37) of the hnRNA for elastin. Elastin is synthesized as a soluble monomer of 70 kDa called **tropoelastin.** Some of the prolines of tropoelastin are hydroxylated to **hydroxyproline** by prolyl hydroxylase, though hydroxylysine and glycosylated hydroxylysine are not present. Unlike collagen, tropoelastin is not synthesized in a pro- form with extension peptides. Furthermore, elastin does not contain repeat Gly-X-Y sequences, triple helical structure, or carbohydrate moieties.

After secretion from the cell, certain lysyl residues of tropoelastin are oxidatively deaminated to aldehydes by **lysyl oxidase,** the same enzyme involved in this process in collagen. However, the major cross-links formed in elastin are the **desmosines,** which result from the condensation of three of these lysine-derived aldehydes with an unmodified lysine to form a tetrafunctional cross-link unique to elastin. Once cross-linked in its mature, extracellular form, elastin is highly insoluble and extremely stable and has a very low turnover rate. Elastin exhibits a variety of random coil conformations that permit the protein to stretch and subsequently recoil during the performance of its physiologic functions.

Table 48–5 summarizes the main differences between collagen and elastin.

Deletions in the elastin gene (located at 7q11.23) have been found in approximately 90% of subjects with **Williams syndrome,** a developmental disorder affecting connective tissue and the central nervous system. The mutations, by affecting synthesis of elastin, probably play a causative role in the supravalvular aortic stenosis often found in this condition. A number of skin diseases (eg, scleroderma) are associated with accumulation of elastin. Fragmentation or, alternatively, a decrease of elastin is found in conditions such as pulmonary emphysema, cutis laxa, and aging of the skin.

Table 48–5. Major differences between collagen and elastin.

Collagen	Elastin
1. Many different genetic types	One genetic type
2. Triple helix	No triple helix; random coil conformations permitting stretching
3. $(Gly\text{-}X\text{-}Y)_n$ repeating structure	No $(Gly\text{-}X\text{-}Y)_n$ repeating structure
4. Presence of hydroxylysine	No hydroxylysine
5. Carbohydrate-containing	No carbohydrate
6. Intramolecular aldol cross-links	Intramolecular desmosine cross-links
7. Presence of extension peptides during biosynthesis	No extension peptides present during biosynthesis

MARFAN SYNDROME IS DUE TO MUTATIONS IN THE GENE FOR FIBRILLIN, A PROTEIN PRESENT IN MICROFIBRILS

Marfan syndrome is a relatively prevalent inherited disease affecting connective tissue; it is inherited as an autosomal dominant trait. It affects the eyes (eg, causing dislocation of the lens, known as ectopia lentis), the skeletal system (most patients are tall and exhibit long digits [arachnodactyly] and hyperextensibility of the joints), and the cardiovascular system (eg, causing weakness of the aortic media, leading to dilation of the ascending aorta). Abraham Lincoln may have had this condition. Most cases are caused by mutations in the gene (on chromosome 15) for fibrillin; missense mutations have been detected in several patients with Marfan syndrome.

Fibrillin is a large glycoprotein (about 350 kDa) that is a structural component of microfibrils, 10- to 12-nm fibers found in many tissues. Fibrillin is secreted (subsequent to a proteolytic cleavage) into the extracellular matrix by fibroblasts and becomes incorporated into the insoluble microfibrils, which appear to provide a scaffold for deposition of elastin. Of special relevance to Marfan syndrome, fibrillin is found in the zonular fibers of the lens, in the periosteum, and associated with elastin fibers in the aorta (and elsewhere); these locations respectively explain the ectopia lentis, arachnodactyly, and cardiovascular problems found in the syndrome. Other proteins (eg, emelin and two microfibril-associated proteins) are also present in these microfibrils, and it appears likely that abnormalities of them may cause other connective tissue disorders. An-

other gene for fibrillin exists on chromosome 5; mutations in this gene are linked to causation of congenital contractural arachnodactyly but not to Marfan syndrome. The probable sequence of events leading to Marfan syndrome is summarized in Figure 48–2.

FIBRONECTIN IS AN IMPORTANT GLYCOPROTEIN INVOLVED IN CELL ADHESION & MIGRATION

Fibronectin is a major glycoprotein of the extracellular matrix, also found in a soluble form in plasma. It consists of two identical subunits, each of about 230 kDa, joined by two disulfide bridges near their carboxyl terminals. The gene encoding fibronectin is very large, containing some 50 exons; the RNA produced by its transcription is subject to considerable alternative splicing, and as many as 20 different mRNAs have been detected in various tissues. Fibronectin contains three types of repeating motifs (I, II, and III), which are organized into functional **domains** (at least seven); functions of these domains include binding **heparin** (see below) and fibrin, collagen, DNA, and cell surfaces (Figure 48–3). The amino acid sequence of the fibronectin receptor of fibroblasts has been derived, and the protein is a member of the transmembrane integrin class of proteins (Chapter 51). The integrins are heterodimers, containing various types of α and β polypeptide chains. Fibronectin contains an Arg-Gly-Asp (RGD) sequence that binds to the receptor. The RGD sequence is shared by a number of other proteins present in the ECM that bind to integrins present in cell surfaces. Synthetic peptides containing the RGD sequence inhibit the binding of fibronectin to cell surfaces. Figure 48–4 illustrates the interaction of collagen, fibronectin, and laminin, all major proteins of the ECM, with a typical cell (eg, fibroblast) present in the matrix.

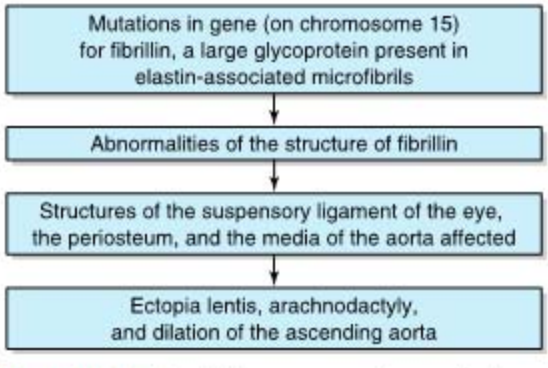

Figure 48–2. Probable sequence of events in the causation of the major signs exhibited by patients with Marfan syndrome (MIM 154700).

The fibronectin receptor interacts indirectly with **actin** microfilaments (Chapter 49) present in the cytosol (Figure 48–5). A number of proteins, collectively known as **attachment proteins,** are involved; these include talin, vinculin, an actin-filament capping protein, and α-actinin. Talin interacts with the receptor and vinculin, whereas the latter two interact with actin. The interaction of fibronectin with its receptor provides one route whereby the exterior of the cell can communicate with the interior and thus affect cell behavior. Via the interaction with its cell receptor, fibronectin plays an important role in the adhesion of cells to the ECM. It is also involved in cell migration by providing a binding site for cells and thus helping them to steer their way through the ECM. The amount of fibronectin around many transformed cells is sharply reduced, partly explaining their faulty interaction with the ECM.

LAMININ IS A MAJOR PROTEIN COMPONENT OF RENAL GLOMERULAR & OTHER BASAL LAMINAS

Basal laminas are specialized areas of the ECM that surround epithelial and some other cells (eg, muscle cells); here we discuss only the laminas found in the **renal glomerulus.** In that structure, the basal lamina is contributed by two separate sheets of cells (one endothelial and one epithelial), each disposed on opposite sides of the lamina; these three layers make up the **glomerular membrane.** The primary components of the basal lamina are three proteins—laminin, entactin, and type IV collagen—and the GAG **heparin** or **heparan sulfate.** These components are synthesized by the underlying cells.

Laminin (about 850 kDa, 70 nm long) consists of three distinct elongated polypeptide chains (A, B_1, and B_2) linked together to form an elongated cruciform shape. It has binding sites for type IV collagen, heparin, and integrins on cell surfaces. The collagen interacts with laminin (rather than directly with the cell surface), which in turn interacts with integrins or other laminin receptor proteins, thus anchoring the lamina to the cells. **Entactin,** also known as "nidogen," is a glycoprotein containing an RGD sequence; it binds to laminin and is a major cell attachment factor. The relatively thick basal lamina of the renal glomerulus has an important role in **glomerular filtration,** regulating the passage of large molecules (most plasma proteins) across the glomerulus into the renal tubule. The glomerular membrane allows small molecules, such as **inulin** (5.2 kDa), to pass through as easily as water. On the other hand, only a small amount of the protein **albumin** (69

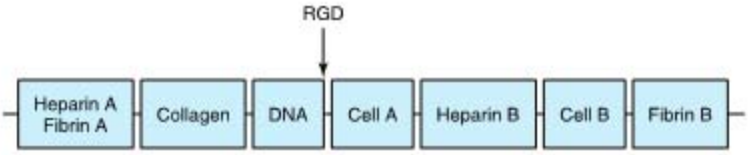

Figure 48–3. Schematic representation of fibronectin. Seven functional domains of fibronectin are represented; two different types of domain for heparin, cell-binding, and fibrin are shown. The domains are composed of various combinations of three structural motifs (I, II, and III), not depicted in the figure. Also not shown is the fact that fibronectin is a dimer joined by disulfide bridges near the carboxyl terminals of the monomers. The approximate location of the RGD sequence of fibronectin, which interacts with a variety of fibronectin integrin receptors on cell surfaces, is indicated by the arrow. (Redrawn after Yamada KM: Adhesive recognition sequences. J Biol Chem 1991;266:12809.)

kDa), the major plasma protein, passes through the normal glomerulus. This is explained by two sets of facts: (1) The pores in the glomerular membrane are large enough to allow molecules up to about 8 nm to pass through. (2) Albumin is smaller than this pore size, but it is prevented from passing through easily by the negative charges of heparan sulfate and of certain sialic acid-containing glycoproteins present in the lamina. These negative charges repel albumin and most plasma proteins, which are negatively charged at the pH of blood. The normal structure of the glomerulus may be severely damaged in certain types of **glomerulonephritis** (eg, caused by antibodies directed against various components of the glomerular membrane). This alters the pores and the amounts and dispositions of the negatively charged macromolecules referred to above, and relatively massive amounts of albumin (and of certain

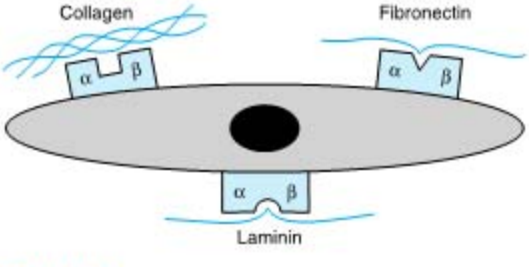

Figure 48–4. Schematic representation of a cell interacting through various integrin receptors with collagen, fibronectin, and laminin present in the ECM. (Specific subunits are not indicated.) (Redrawn after Yamada KM: Adhesive recognition sequences. J Biol Chem 1991;266:12809.)

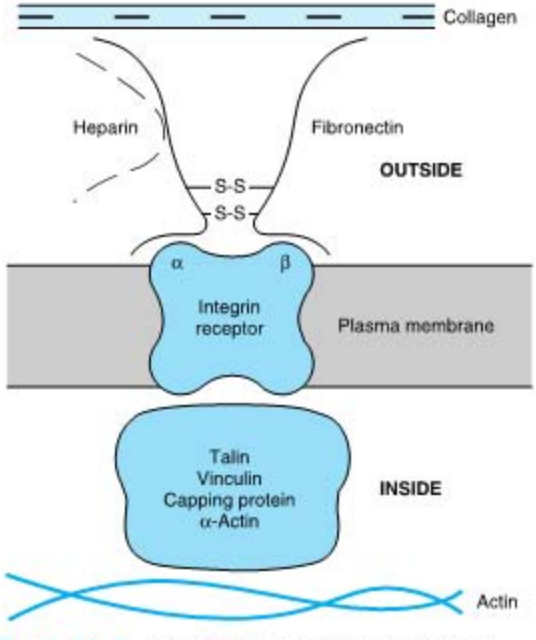

Figure 48–5. Schematic representation of fibronectin interacting with an integrin fibronectin receptor situated in the exterior of the plasma membrane of a cell of the ECM and of various attachment proteins interacting indirectly or directly with an actin microfilament in the cytosol. For simplicity, the attachment proteins are represented as a complex.

other plasma proteins) can pass through into the urine, resulting in severe **albuminuria.**

PROTEOGLYCANS & GLYCOSAMINOGLYCANS

The Glycosaminoglycans Found in Proteoglycans Are Built Up of Repeating Disaccharides

Proteoglycans are proteins that contain covalently linked glycosaminoglycans. A number of them have been characterized and given names such as syndecan, betaglycan, serglycin, perlecan, aggrecan, versican, decorin, biglycan, and fibromodulin. They vary in tissue distribution, nature of the core protein, attached glycosaminoglycans, and function. The proteins bound covalently to glycosaminoglycans are called **"core proteins";** they have proved difficult to isolate and characterize, but the use of recombinant DNA technology is beginning to yield important information about their structures. The amount of carbohydrate in a proteoglycan is usually much greater than is found in a glycoprotein and may comprise up to 95% of its weight. Figures 48–6 and 48–7 show the general structure of one particular proteoglycan, **aggrecan,** the major type found in cartilage. It is very large (about 2×10^3 kDa), with its overall structure resembling that of a bottle brush. It contains a long strand of hyaluronic acid (one type of GAG) to which link proteins are attached noncovalently. In turn, these latter interact noncovalently with core protein molecules from which chains of other GAGs (keratan sulfate and chondroitin sulfate in this case) project. More details on this macromolecule are given when cartilage is discussed below.

There are at least seven **glycosaminoglycans (GAGs):** hyaluronic acid, chondroitin sulfate, keratan sulfates I and II, heparin, heparan sulfate, and dermatan sulfate. A GAG is an unbranched polysaccharide made up of repeating disaccharides, one component of which is always an amino sugar (hence the name GAG), either D-glucosamine or D-galactosamine. The other component of the repeating disaccharide (except in the case of keratan sulfate) is a uronic acid, either L-glucuronic acid (GlcUA) or its 5′-epimer, L-iduronic acid (IdUA). With the exception of hyaluronic acid, all the GAGs contain sulfate groups, either as O-esters or as N-sulfate (in heparin and heparan sulfate). Hyaluronic acid affords another exception because there is no clear evidence that it is attached covalently to protein, as the definition of a proteoglycan given above specifies. Both GAGs and proteoglycans have proved difficult to work with, partly because of their complexity. However, they are major components of the ground substance; they have a number of important biologic roles; and they are involved in a number of disease processes—so that interest in them is increasing rapidly.

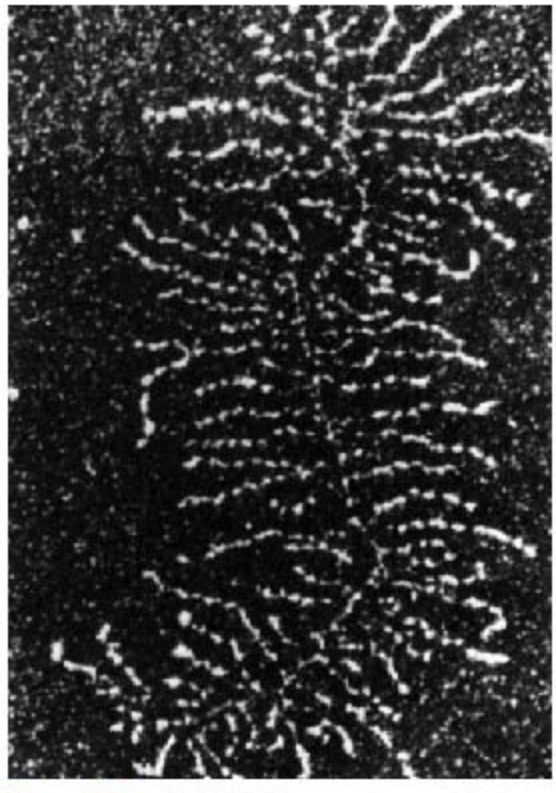

Figure 48–6. **Dark field electron micrograph of a proteoglycan aggregate in which the proteoglycan subunits and filamentous backbone are particularly well extended.** (Reproduced, with permission, from Rosenberg L, Hellman W, Kleinschmidt AK: Electron microscopic studies of proteoglycan aggregates from bovine articular cartilage. J Biol Chem 1975;250:1877.)

Biosynthesis of Glycosaminoglycans Involves Attachment to Core Proteins, Chain Elongation, & Chain Termination

A. Attachment to Core Proteins

The linkage between GAGs and their core proteins is generally one of three types.

1. An O-glycosidic bond between xylose (Xyl) and Ser, a bond that is unique to proteoglycans. This linkage is formed by transfer of a Xyl residue to Ser from UDP-xylose. Two residues of Gal are then added to the

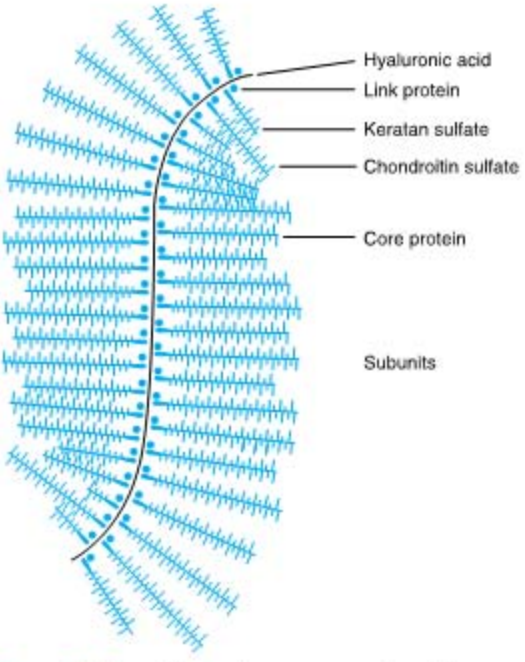

Figure 48–7. Schematic representation of the proteoglycan aggrecan. (Reproduced, with permission, from Lennarz WJ: *The Biochemistry of Glycoproteins and Proteoglycans.* Plenum Press, 1980.)

Xyl residue, forming a **link trisaccharide,** Gal-Gal-Xyl-Ser. Further chain growth of the GAG occurs on the terminal Gal.

2. An O-glycosidic bond forms between GalNAc (*N*-acetylgalactosamine) and Ser (Thr) (Figure 47–1[a]), present in keratan sulfate II. This bond is formed by donation to Ser (or Thr) of a GalNAc residue, employing UDP-GalNAc as its donor.

3. An N-glycosylamine bond between GlcNAc (*N*-acetylglucosamine) and the amide nitrogen of **Asn,** as found in N-linked glycoproteins (Figure 47–1[b]). Its synthesis is believed to involve dolichol-P-P-oligosaccharide.

The synthesis of the core proteins occurs in the **endoplasmic reticulum,** and formation of at least some of the above linkages also occurs there. Most of the later steps in the biosynthesis of GAG chains and their subsequent modifications occur in the **Golgi apparatus.**

B. Chain Elongation

Appropriate nucleotide sugars and highly specific Golgi-located glycosyltransferases are employed to synthesize the oligosaccharide chains of GAGs. The "one enzyme, one linkage" relationship appears to hold here, as in the case of certain types of linkages found in glycoproteins. The enzyme systems involved in chain elongation are capable of high-fidelity reproduction of complex GAGs.

C. Chain Termination

This appears to result from (1) sulfation, particularly at certain positions of the sugars, and (2) the progression of the growing GAG chain away from the membrane site where catalysis occurs.

D. Further Modifications

After formation of the GAG chain, numerous chemical modifications occur, such as the introduction of sulfate groups onto GalNAc and other moieties and the epimerization of GlcUA to IdUA residues. The enzymes catalyzing sulfation are designated **sulfotransferases** and use 3′-phosphoadenosine-5′-phosphosulfate (PAPS; active sulfate) as the sulfate donor. These Golgi-located enzymes are highly specific, and distinct enzymes catalyze sulfation at different positions (eg, carbons 2, 3, 4, and 6) on the acceptor sugars. An **epimerase** catalyzes conversions of glucuronyl to iduronyl residues.

The Various Glycosaminoglycans Exhibit Differences in Structure & Have Characteristic Distributions

The seven GAGs named above differ from each other in a number of the following properties: amino sugar composition, uronic acid composition, linkages between these components, chain length of the disaccharides, the presence or absence of sulfate groups and their positions of attachment to the constituent sugars, the nature of the core proteins to which they are attached, the nature of the linkage to core protein, their tissue and subcellular distribution, and their biologic functions.

The structures (Figure 48–8) and the distributions of each of the GAGs will now be briefly discussed. The major features of the seven GAGs are summarized in Table 48–6.

A. Hyaluronic Acid

Hyaluronic acid consists of an unbranched chain of repeating disaccharide units containing GlcUA and GlcNAc. Hyaluronic acid is present in bacteria and is widely distributed among various animals and tissues, including synovial fluid, the vitreous body of the eye, cartilage, and loose connective tissues.

B. Chondroitin Sulfates (Chondroitin 4-Sulfate & Chondroitin 6-Sulfate)

Proteoglycans linked to chondroitin sulfate by the Xyl-Ser O-glycosidic bond are prominent components of **cartilage** (see below). The repeating disaccharide is similar to that found in hyaluronic acid, containing

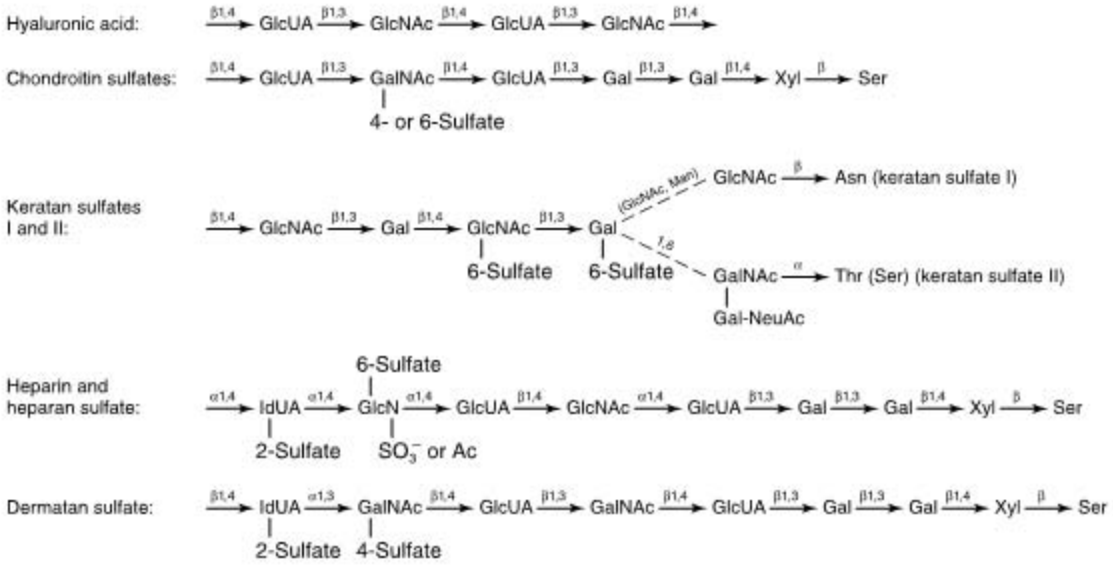

Figure 48–8. Summary of structures of glycosaminoglycans and their attachments to core proteins. (GlcUA, D-glucuronic acid; IdUA, L-iduronic acid; GlcN, D-glucosamine; GalN, D-galactosamine; Ac, acetyl; Gal, D-galactose; Xyl, D-xylose; Ser, L-serine; Thr, L-threonine; Asn, L-asparagine; Man, D-mannose; NeuAc, *N*-acetylneuraminic acid.) The summary structures are qualitative representations only and do not reflect, for example, the uronic acid composition of hybrid glycosaminoglycans such as heparin and dermatan sulfate, which contain both L-iduronic and D-glucuronic acid. Neither should it be assumed that the indicated substituents are always present, eg, whereas most iduronic acid residues in heparin carry a 2′-sulfate group, a much smaller proportion of these residues are sulfated in dermatan sulfate. The presence of link trisaccharides (Gal-Gal-Xyl) in the chondroitin sulfates, heparin, and heparan and dermatan sulfates is shown. (Slightly modified and reproduced, with permission, from Lennarz WJ: *The Biochemistry of Glycoproteins and Proteoglycans.* Plenum Press, 1980.)

Table 48–6. Major properties of the glycosaminoglycans.

GAG	Sugars	Sulfate[1]	Linkage of Protein	Location
HA	GlcNAc, GlcUA	Nil	No firm evidence	Synovial fluid, vitreous humor, loose connective tissue
CS	GalNAc, GlcUA	GalNAc	Xyl-Ser; associated with HA via link proteins	Cartilage, bone, cornea
KS I	GlcNAc, Gal	GlcNAc	GlcNAc-Asn	Cornea
KS II	GlcNAc, Gal	Same as KS I	GalNAc-Thr	Loose connective tissue
Heparin	GlcN, IdUA	GlcN GlcN IdUA	Ser	Mast cells
Heparan sulfate	GlcN, GlcUA	GlcN	Xyl-Ser	Skin fibroblasts, aortic wall
Dermatan sulfate	GalNAc, IdUA, (GlcUA)	GalNAc IdUa	Xyl-Ser	Wide distribution

[1]The sulfate is attached to various positions of the sugars indicated (see Figure 48–7).

GlcUA but with GalNAc replacing GlcNAc. The GalNAc is substituted with sulfate at either its 4′ or its 6′ position, with approximately one sulfate being present per disaccharide unit.

C. Keratan Sulfates I & II

As shown in Figure 48–8, the keratan sulfates consist of repeating Gal-GlcNAc disaccharide units containing sulfate attached to the 6′ position of GlcNAc or occasionally of Gal. Type I is abundant in **cornea,** and type II is found along with chondroitin sulfate attached to hyaluronic acid in **loose connective tissue.** Types I and II have different attachments to protein (Figure 48–8).

D. Heparin

The repeating disaccharide contains **glucosamine** (GlcN) and either of the two uronic acids (Figure 48–9). Most of the amino groups of the GlcN residues are **N-sulfated,** but a few are acetylated. The GlcN also carries a C_6 sulfate ester.

Approximately 90% of the uronic acid residues are IdUA. Initially, all of the uronic acids are GlcUA, but a 5′-epimerase converts approximately 90% of the GlcUA residues to IdUA after the polysaccharide chain is formed. The protein molecule of the heparin proteoglycan is unique, consisting exclusively of serine and glycine residues. Approximately two-thirds of the serine residues contain GAG chains, usually of 5–15 kDa but occasionally much larger. Heparin is found in the granules of **mast cells** and also in liver, lung, and skin.

E. Heparan Sulfate

This molecule is present on many **cell surfaces** as a proteoglycan and is extracellular. It contains GlcN with fewer N-sulfates than heparin, and, unlike heparin, its predominant uronic acid is GlcUA.

F. Dermatan Sulfate

This substance is widely distributed in animal tissues. Its structure is similar to that of chondroitin sulfate, except that in place of a GlcUA in β-1,3 linkage to GalNAc it contains an IdUA in an α-1,3 linkage to GalNAc. Formation of the IdUA occurs, as in heparin and heparan sulfate, by 5′-epimerization of GlcUA. Because this is regulated by the degree of sulfation and because sulfation is incomplete, dermatan sulfate contains both IdUA-GalNAc and GlcUA-GalNAc disaccharides.

Deficiencies of Enzymes That Degrade Glycosaminoglycans Result in Mucopolysaccharidoses

Both exo- and endoglycosidases degrade GAGs. Like most other biomolecules, GAGs are subject to turnover, being both synthesized and degraded. In adult tissues, GAGs generally exhibit relatively slow turnover, their half-lives being days to weeks.

Understanding of the degradative pathways for GAGs, as in the case of glycoproteins (Chapter 47) and glycosphingolipids (Chapter 24), has been greatly aided by elucidation of the specific enzyme deficiencies that occur in certain **inborn errors of metabolism.** When GAGs are involved, these inborn errors are called **mucopolysaccharidoses** (Table 48–7).

Degradation of GAGs is carried out by a battery of lysosomal hydrolases. These include certain endoglycosidases, various exoglycosidases, and sulfatases, generally acting in sequence to degrade the various GAGs. A number of them are indicated in Table 48–7.

The **mucopolysaccharidoses** share a common mechanism of causation, as illustrated in Figure 48–10. They are inherited in an autosomal recessive manner, with Hurler and Hunter syndromes being perhaps the most widely studied. None are common. In some cases, a family history of a mucopolysaccharidosis is obtained. Specific laboratory investigations of help in their diagnosis are urine testing for the presence of increased

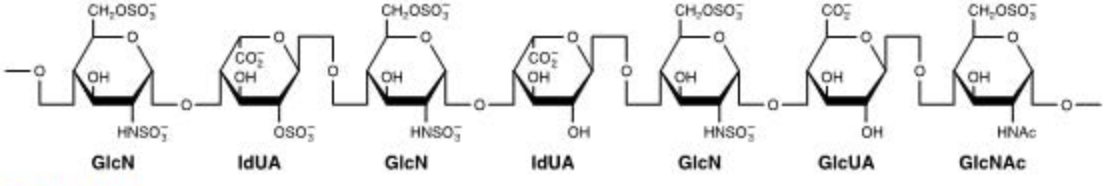

Figure 48–9. Structure of heparin. The polymer section illustrates structural features typical of heparin; however, the sequence of variously substituted repeating disaccharide units has been arbitrarily selected. In addition, non-O-sulfated or 3-O-sulfated glucosamine residues may also occur. (Modified, redrawn, and reproduced, with permission, from Lindahl U et al: Structure and biosynthesis of heparin-like polysaccharides. Fed Proc 1977;36:19.)

Table 48–7. Biochemical defects and diagnostic tests in mucopolysaccharidoses (MPS) and mucolipidoses (ML).[1]

Name	Alternative Designation[2,3]	Enzymatic Defect	Urinary Metabolites
Mucopolysaccharidoses			
Hurler, Scheie, Hurler-Scheie (MIM 252800)	MPS I	α-L-Iduronidase	Dermatan sulfate, heparan sulfate
Hunter (MIM 309900)	MPS II	Iduronate sulfatase	Dermatan sulfate, heparan sulfate
Sanfilippo A (MIM 252900)	MPS IIIA	Heparan sulfate *N*-sulfatase (sulfamidase)	Heparan sulfate
Sanfilippo B (MIM 252920)	MPS IIIB	α-*N*-Acetylglucosaminidase	Heparan sulfate
Sanfilippo C (MIM 252930)	MPS IIIC	Acetyltransferase	Heparan sulfate
Sanfilippo D (MIM 252940)	MPS IIID	*N*-Acetylglucosamine 6-sulfatase	Heparan sulfate
Morquio A (MIM 253000)	MPS IVA	Galactosamine 6-sulfatase	Keratan sulfate, chondroitin 6-sulfate
Morquio B (MIM 253010)	MPS IVB	β-Galactosidase	Keratan sulfate
Maroteaux-Lamy (MIM 253200)	MPS VI	*N*-Acetylgalactosamine 4-sulfatase (arylsulfatase B)	Dermatan sulfate
Sly (MIM 253220)	MPS VII	β-Glucuronidase	Dermatan sulfate, heparan sulfate, chondroitin 4-sulfate, chondroitin 6-sulfate
Mucolipidoses			
Sialidosis (MIM 256550)	M LI	Sialidase (neuraminidase)	Glycoprotein fragments
I-cell disease (MIM 252500)	ML II	UDP-*N*-acetylglucosamine: glycoprotein *N*-acetylglucosamininylphosphotransferase. (Acid hydrolases thus lack phosphomannosyl residues.)	Glycoprotein fragments
Pseudo-Hurler polydystrophy (MIM 252600)	ML III	As for ML II but deficiency is incomplete	Glycoprotein fragments

[1]Modified and reproduced, with permission, from DiNatale P, Neufeld EF: The biochemical diagnosis of mucopolysaccharidoses, mucolipidoses and related disorders. In: *Perspectives in Inherited Metabolic Diseases,* vol 2. Barr B et al (editors). Editiones Ermes (Milan), 1979.

[2]Fibroblasts, leukocytes, tissues, amniotic fluid cells, or serum can be used for the assay of many of the above enzymes. Patients with these disorders exhibit a variety of clinical findings that may include cloudy corneas, mental retardation, stiff joints, cardiac abnormalities, hepatosplenomegaly, and short stature, depending on the specific disease and its severity.

[3]The term MPS V is no longer used. The existence of MPS VIII (suspected glucosamine 6-sulfatase deficiency: MIM 253230) has not been confirmed. At least one case of hyaluronidase deficiency (MPS IX; MIM 601492) has been reported.

amounts of GAGs and assays of suspected enzymes in white cells, fibroblasts, or sometimes in serum. In certain cases, a tissue biopsy is performed and the GAG that has accumulated can be determined by electrophoresis. DNA tests are increasingly available. Prenatal diagnosis can be made using amniotic cells or chorionic villus biopsy.

The term **"mucolipidosis"** was introduced to denote diseases that combined features common to both mucopolysaccharidoses and sphingolipidoses (Chapter 24). Three mucolipidoses are listed in Table 48–7. In **sialidosis** (mucolipidosis I, ML-I), various oligosaccharides derived from glycoproteins and certain gangliosides can accumulate in tissues. **I-cell disease** (ML-II)

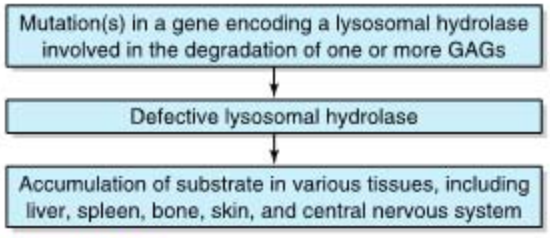

Figure 48–10. Simplified scheme of causation of a mucopolysaccharidosis, such as Hurler syndrome (MIM 252800), in which the affected enzyme is α-L-iduronidase. Marked accumulation of the GAGs in the tissues mentioned in the figure could cause hepatomegaly, splenomegaly, disturbances of growth, coarse facies, and mental retardation, respectively.

and **pseudo-Hurler polydystrophy** (ML-III) are described in Chapter 47. The term "mucolipidosis" is retained because it is still in relatively widespread clinical usage, but it is not appropriate for these two latter diseases since the mechanism of their causation involves mislocation of certain lysosomal enzymes. Genetic defects of the catabolism of the oligosaccharide chains of glycoproteins (eg, mannosidosis, fucosidosis) are also described in Chapter 47. Most of these defects are characterized by increased excretion of various fragments of glycoproteins in the urine, which accumulate because of the metabolic block, as in the case of the mucolipidoses.

Hyaluronidase is one important enzyme involved in the catabolism of both hyaluronic acid and chondroitin sulfate. It is a widely distributed endoglycosidase that cleaves hexosaminidic linkages. From hyaluronic acid, the enzyme will generate a tetrasaccharide with the structure (GlcUA-β-1,3-GlcNAc-β-1,4)$_2$, which can be degraded further by a β-glucuronidase and β-*N*-acetylhexosaminidase. Surprisingly, only one case of an apparent genetic deficiency of this enzyme appears to have been reported.

Proteoglycans Have Numerous Functions

As indicated above, proteoglycans are remarkably complex molecules and are found in every tissue of the body, mainly in the ECM or "ground substance." There they are associated with each other and also with the other major structural components of the matrix, collagen and elastin, in quite specific manners. Some proteoglycans bind to collagen and others to elastin. These interactions are important in determining the structural organization of the matrix. Some proteoglycans (eg, decorin) can also bind growth factors such as TGF-β, modulating their effects on cells. In addition, some of them interact with certain adhesive proteins such as fibronectin and laminin (see above), also found in the matrix. The GAGs present in the proteoglycans are polyanions and hence bind polycations and cations such as Na^+ and K^+. This latter ability attracts water by osmotic pressure into the extracellular matrix and contributes to its turgor. GAGs also gel at relatively low concentrations. Because of the long extended nature of the polysaccharide chains of GAGs and their ability to gel, the proteoglycans can act as sieves, restricting the passage of large macromolecules into the ECM but allowing relatively free diffusion of small molecules. Again, because of their extended structures and the huge macromolecular aggregates they often form, they occupy a large volume of the matrix relative to proteins.

A. Some Functions of Specific GAGs & Proteoglycans

Hyaluronic acid is especially high in concentration in embryonic tissues and is thought to play an important role in permitting cell migration during morphogenesis and wound repair. Its ability to attract water into the extracellular matrix and thereby "loosen it up" may be important in this regard. The high concentrations of hyaluronic acid and chondroitin sulfates present in cartilage contribute to its compressibility (see below).

Chondroitin sulfates are located at sites of calcification in endochondral bone and are also found in cartilage. They are also located inside certain neurons and may provide an endoskeletal structure, helping to maintain their shape.

Both **keratan sulfate I** and **dermatan sulfate** are present in the cornea. They lie between collagen fibrils and play a critical role in corneal transparency. Changes in proteoglycan composition found in corneal scars disappear when the cornea heals. The presence of dermatan sulfate in the sclera may also play a role in maintaining the overall shape of the eye. Keratan sulfate I is also present in cartilage.

Heparin is an important anticoagulant. It binds with factors IX and XI, but its most important interaction is with **plasma antithrombin III** (discussed in Chapter 51). Heparin can also bind specifically to lipoprotein lipase present in capillary walls, causing a release of this enzyme into the circulation.

Certain proteoglycans (eg, **heparan sulfate**) are associated with the plasma membrane of cells, with their core proteins actually spanning that membrane. In it they may act as receptors and may also participate in the mediation of cell growth and cell-cell communication. The attachment of cells to their substratum in culture is mediated at least in part by heparan sulfate. This proteoglycan is also found in the basement membrane of the kidney along with type IV collagen and laminin

(see above), where it plays a major role in determining the charge selectiveness of glomerular filtration.

Proteoglycans are also found in intracellular locations such as the nucleus; their function in this organelle has not been elucidated. They are present in some storage or secretory granules, such as the chromaffin granules of the adrenal medulla. It has been postulated that they play a role in release of the contents of such granules. The various functions of GAGs are summarized in Table 48–8.

B. Associations With Major Diseases & With Aging

Hyaluronic acid may be important in permitting **tumor cells** to migrate through the ECM. Tumor cells can induce fibroblasts to synthesize greatly increased amounts of this GAG, thereby perhaps facilitating their own spread. Some tumor cells have less heparan sulfate at their surfaces, and this may play a role in the lack of adhesiveness that these cells display.

The intima of the **arterial wall** contains hyaluronic acid and chondroitin sulfate, dermatan sulfate, and heparan sulfate proteoglycans. Of these proteoglycans, dermatan sulfate binds plasma low-density lipoproteins. In addition, dermatan sulfate appears to be the major GAG synthesized by arterial smooth muscle cells. Because it is these cells that proliferate in atherosclerotic lesions in arteries, dermatan sulfate may play an important role in development of the atherosclerotic plaque.

Table 48–8. Some functions of glycosaminoglycans and proteoglycans.[1]

- Act as structural components of the ECM
- Have specific interactions with collagen, elastin, fibronectin, laminin, and other proteins such as growth factors
- As polyanions, bind polycations and cations
- Contribute to the characteristic turgor of various tissues
- Act as sieves in the ECM
- Facilitate cell migration (HA)
- Have role in compressibility of cartilage in weight-bearing (HA, CS)
- Play role in corneal transparency (KS I and DS)
- Have structural role in sclera (DS)
- Act as anticoagulant (heparin)
- Are components of plasma membranes, where they may act as receptors and participate in cell adhesion and cell-cell interactions (eg, HS)
- Determine charge-selectiveness of renal glomerulus (HS)
- Are components of synaptic and other vesicles (eg, HS)

[1]ECM, extracellular matrix; HA, hyaluronic acid; CS, chondroitin sulfate; KS I, keratan sulfate I; DS, dermatan sulfate; HS, heparan sulfate.

In various types of **arthritis,** proteoglycans may act as autoantigens, thus contributing to the pathologic features of these conditions. The amount of chondroitin sulfate in cartilage diminishes with age, whereas the amounts of keratan sulfate and hyaluronic acid increase. These changes may contribute to the development of **osteoarthritis.** Changes in the amounts of cer-

Table 48–9. The principal proteins found in bone.[1]

Proteins	Comments
Collagens	
Collagen type I	Approximately 90% of total bone protein. Composed of two α1(I) and one α2(I) chains.
Collagen type V	Minor component.
Noncollagen proteins	
Plasma proteins	Mixture of various plasma proteins.
Proteoglycans[2]	
CS-PG I (biglycan)	Contains two GAG chains; found in other tissues.
CS-PG II (decorin)	Contains one GAG chain; found in other tissues.
CS-PG III	Bone-specific.
Bone SPARC[3] protein (osteonectin)	Not bone-specific.
Osteocalcin (bone Gla protein)	Contains γ-carboxyglutamate residues that bind to hydroxyapatite. Bone-specific.
Osteopontin	Not bone-specific. Glycosylated and phosphorylated.
Bone sialoprotein	Bone-specific. Heavily glycosylated, and sulfated on tyrosine.
Bone morphogenetic proteins (BMPs)	A family (eight or more) of secreted proteins with a variety of actions on bone; many induce ectopic bone growth.

[1]Various functions have been ascribed to the noncollagen proteins, including roles in mineralization; however, most of them are still speculative. It is considered unlikely that the noncollagen proteins that are not bone-specific play a key role in mineralization. A number of other proteins are also present in bone, including a tyrosine-rich acidic matrix protein (TRAMP), some growth factors (eg, TGFβ), and enzymes involved in collagen synthesis (eg, lysyl oxidase).
[2]CS-PG, chondroitin sulfate–proteoglycan; these are similar to the dermatan sulfate PGs (DS-PGs) of cartilage (Table 48–11).
[3]SPARC, secreted protein acidic and rich in cysteine.

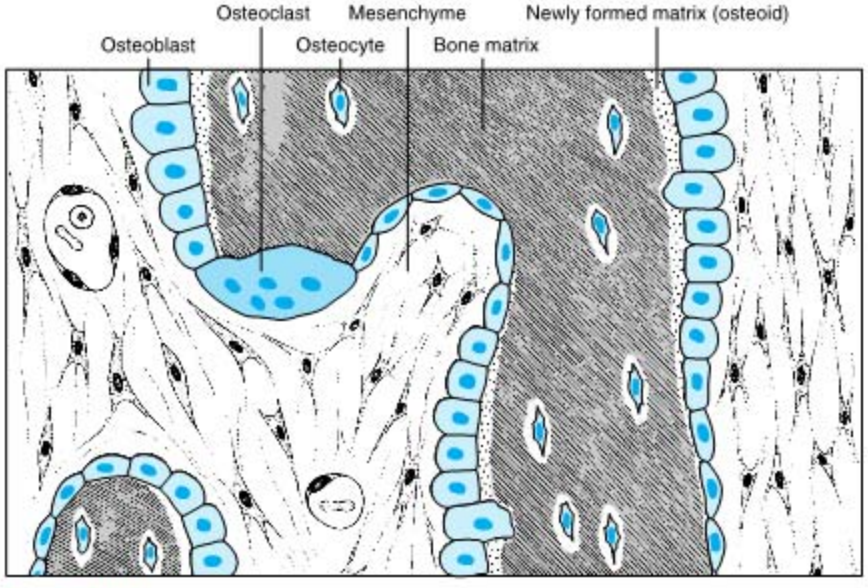

Figure 48–11. Schematic illustration of the major cells present in membranous bone. Osteoblasts (lighter color) are synthesizing type I collagen, which forms a matrix that traps cells. As this occurs, osteoblasts gradually differentiate to become osteocytes. (Reproduced, with permission, from Junqueira LC, Carneiro J: *Basic Histology: Text & Atlas,* 10th ed. McGraw-Hill, 2003.)

tain GAGs in the skin are also observed with **aging** and help to account for the characteristic changes noted in this organ in the elderly.

An exciting new phase in proteoglycan research is opening up with the findings that mutations that affect individual proteoglycans or the enzymes needed for their synthesis alter the regulation of specific signaling pathways in drosophila and *Caenorhabditis elegans,* thus affecting development; it already seems likely that similar effects exist in mice and humans.

BONE IS A MINERALIZED CONNECTIVE TISSUE

Bone contains both organic and inorganic material. The organic matter is mainly protein. The principal proteins of bone are listed in Table 48–9; **type I collagen** is the major protein, comprising 90–95% of the organic material. Type V collagen is also present in small amounts, as are a number of noncollagen proteins, some of which are relatively specific to bone. The inorganic or mineral component is mainly crystalline **hydroxyapatite**—$Ca_{10}(PO_4)_6(OH)_2$—along with sodium, magnesium, carbonate, and fluoride; approximately 99% of the body's calcium is contained in bone (Chapter 45). Hydroxyapatite confers on bone the strength and resilience required by its physiologic roles.

Bone is a dynamic structure that undergoes continuing cycles of remodeling, consisting of resorption followed by deposition of new bone tissue. This remodeling permits bone to adapt to both physical (eg, increases in weight-bearing) and hormonal signals.

The major cell types involved in bone resorption and deposition are **osteoclasts** and **osteoblasts** (Figure 48–11). The former are associated with resorption and the latter with deposition of bone. Osteocytes are descended from osteoblasts; they also appear to be involved in maintenance of bone matrix but will not be discussed further here.

Osteoclasts are multinucleated cells derived from pluripotent hematopoietic stem cells. Osteoclasts possess an apical membrane domain, exhibiting a ruffled border that plays a key role in bone resorption (Figure 48–12). A proton-translocating ATPase expels protons across the ruffled border into the resorption area, which is the microenvironment of low pH shown in the figure. This lowers the local pH to 4.0 or less, thus increasing the solubility of hydroxyapatite and allowing demineralization to occur. Lysosomal acid proteases are released that digest the now accessible matrix proteins.

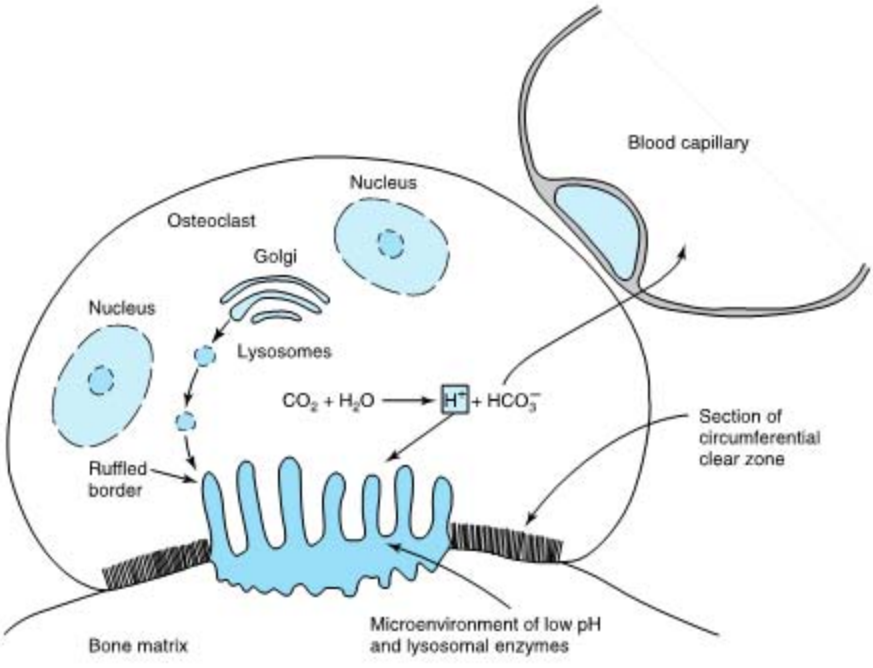

Figure 48–12. Schematic illustration of some aspects of the role of the osteoclast in bone resorption. Lysosomal enzymes and hydrogen ions are released into the confined microenvironment created by the attachment between bone matrix and the peripheral clear zone of the osteoclast. The acidification of this confined space facilitates the dissolution of calcium phosphate from bone and is the optimal pH for the activity of lysosomal hydrolases. Bone matrix is thus removed, and the products of bone resorption are taken up into the cytoplasm of the osteoclast, probably digested further, and transferred into capillaries. The chemical equation shown in the figure refers to the action of carbonic anhydrase II, described in the text. (Reproduced, with permission, from Junqueira LC, Carneiro J: *Basic Histology: Text & Atlas*, 10th ed. McGraw-Hill, 2003.)

Osteoblasts—mononuclear cells derived from pluripotent mesenchymal precursors—synthesize most of the proteins found in bone (Table 48–9) as well as various growth factors and cytokines. They are responsible for the deposition of new bone matrix (osteoid) and its subsequent mineralization. Osteoblasts control mineralization by regulating the passage of calcium and phosphate ions across their surface membranes. The latter contain alkaline phosphatase, which is used to generate phosphate ions from organic phosphates. The mechanisms involved in mineralization are not fully understood, but several factors have been implicated. Alkaline phosphatase contributes to mineralization but in itself is not sufficient. Small vesicles (matrix vesicles) containing calcium and phosphate have been described at sites of mineralization, but their role is not clear. Type I collagen appears to be necessary, with mineralization being first evident in the gaps between successive molecules. Recent interest has focused on acidic phosphoproteins, such as bone sialoprotein, acting as sites of nucleation. These proteins contain motifs (eg, poly-Asp and poly-Glu stretches) that bind calcium and may provide an initial scaffold for mineralization. Some macromolecules, such as certain proteoglycans and glycoproteins, can also act as inhibitors of nucleation.

It is estimated that approximately 4% of compact bone is renewed annually in the typical healthy adult, whereas approximately 20% of trabecular bone is replaced.

Many factors are involved in the regulation of bone metabolism, only a few of which will be mentioned here. Some stimulate osteoblasts (eg, parathyroid hormone and 1,25-dihydroxycholecalciferol) and others inhibit them (eg, corticosteroids). Parathyroid hormone and 1,25-dihydroxycholecalciferol also stimulate osteoclasts, whereas calcitonin and estrogens inhibit them.

Table 48–10. Some metabolic and genetic diseases affecting bone and cartilage.

Disease	Comments
Dwarfism	Often due to a deficiency of growth hormone, but has many other causes.
Rickets	Due to a deficiency of vitamin D during childhood.
Osteomalacia	Due to a deficiency of vitamin D during adulthood.
Hyperparathyroidism	Excess parathormone causes bone resorption.
Osteogenesis imperfecta (eg, MIM 166200)	Due to a variety of mutations in the *COL1A1* and *COL1A2* genes affecting the synthesis and structure of type I collagen.
Osteoporosis	Commonly postmenopausal or in other cases is more gradual and related to age; a small number of cases are due to mutations in the *COL1A1* and *COL1A2* genes and possibly in the vitamin D receptor gene (MIM 166710)
Osteoarthritis	A small number of cases are due to mutations in the *COL1A* genes.
Several chondrodysplasias	Due to mutations in *COL2A1* genes.
Pfeiffer syndrome[1] (MIM 100600)	Mutations in the gene encoding fibroblast growth receptor 1 (FGFR1).
Jackson-Weiss (MIM 123150) and Crouzon (MIM 123500) syndromes[1]	Mutations in the gene encoding FGFR2.
Achondroplasia (MIM 100800) and thanatophoric dysplasia[2] (MIM 187600)	Mutations in the gene encoding FGFR3.

[1]The Pfeiffer, Jackson-Weiss, and Crouzon syndromes are craniosynostosis syndromes; craniosynostosis is a term signifying premature fusion of sutures in the skull.
[2]Thanatophoric (Gk *thanatos* "death" + *phoros* "bearing") dysplasia is the most common neonatal lethal skeletal dysplasia, displaying features similar to those of homozygous achondroplasia.

MANY METABOLIC & GENETIC DISORDERS INVOLVE BONE

A number of the more important examples of metabolic and genetic disorders that affect bone are listed in Table 48–10.

Osteogenesis imperfecta (brittle bones) is characterized by abnormal fragility of bones. The scleras are often abnormally thin and translucent and may appear blue owing to a deficiency of connective tissue. Four types of this condition (mild, extensive, severe, and variable) have been recognized, of which the extensive type occurring in the newborn is the most ominous. Affected infants may be born with multiple fractures and not survive. Over 90% of patients with osteogenesis imperfecta have mutations in the *COL1A1* and *COL1A2* genes, encoding proα1(I) and proα2(I) chains, respectively. Over 100 mutations in these two genes have been documented and include partial gene deletions and duplications. Other mutations affect RNA splicing, and the most frequent type results in the replacement of glycine by another bulkier amino acid, affecting formation of the triple helix. In general, these mutations result in decreased expression of collagen or

Table 48–11. The principal proteins found in cartilage.

Proteins	Comments
Collagen proteins	
Collagen type II	90–98% of total articular cartilage collagen. Composed of three α1(II) chains.
Collagens V, VI, IX, X, XI	Type IX cross-links to type II collagen. Type XI may help control diameter of type II fibrils.
Noncollagen proteins	
Proteoglycans	
Aggrecan	The major proteoglycan of cartilage.
Large non-aggregating proteoglycan	Found in some types of cartilage.
DS-PG I (biglycan)[1]	Similar to CS-PG I of bone.
DS-PG II (decorin)	Similar to CS-PG II of bone.
Chondronectin	May play role in binding type II collagen to surface of cartilage.
Anchorin C II	May bind type II collagen to surface of chondrocyte.

[1]The core proteins of DS-PG I and DS-PG II are homologous to those of CS-PG I and CS-PG II found in bone (Table 48–9). A possible explanation is that osteoblasts lack the epimerase required to convert glucuronic acid to iduronic acid, the latter of which is found in dermatan sulfate.

in structurally abnormal proα chains that assemble into abnormal fibrils, weakening the overall structure of bone. When one abnormal chain is present, it may interact with two normal chains, but folding may be prevented, resulting in enzymatic degradation of all of the chains. This is called "procollagen suicide" and is an example of a dominant negative mutation, a result often seen when a protein consists of multiple different subunits.

Osteopetrosis (marble bone disease), characterized by increased bone density, is due to inability to resorb bone. One form occurs along with renal tubular acidosis and cerebral calcification. It is due to mutations in the gene (located on chromosome 8q22) encoding carbonic anhydrase II (CA II), one of four isozymes of carbonic anhydrase present in human tissues. The reaction catalyzed by carbonic anhydrase is shown below:

Reaction II is spontaneous. In osteoclasts involved in bone resorption, CA II apparently provides protons to neutralize the OH^- ions left inside the cell when H^+ ions are pumped across their ruffled borders (see above). Thus, if CA II is deficient in activity in osteoclasts, normal bone resorption does not occur, and osteopetrosis results. The mechanism of the cerebral calcification is not clear, whereas the renal tubular acidosis reflects deficient activity of CA II in the renal tubules.

Osteoporosis is a generalized progressive reduction in bone tissue mass per unit volume causing skeletal weakness. The ratio of mineral to organic elements is unchanged in the remaining normal bone. Fractures of various bones, such as the head of the femur, occur very easily and represent a huge burden to both the affected patients and to the health care budget of society. Among other factors, estrogens and interleukins-1 and -6 appear to be intimately involved in the causation of osteoporosis.

THE MAJOR COMPONENTS OF CARTILAGE ARE TYPE II COLLAGEN & CERTAIN PROTEOGLYCANS

The principal proteins of hyaline cartilage (the major type of cartilage) are listed in Table 48–11. Type II collagen is the principal protein (Figure 48–13), and a number of other minor types of collagen are also present. In

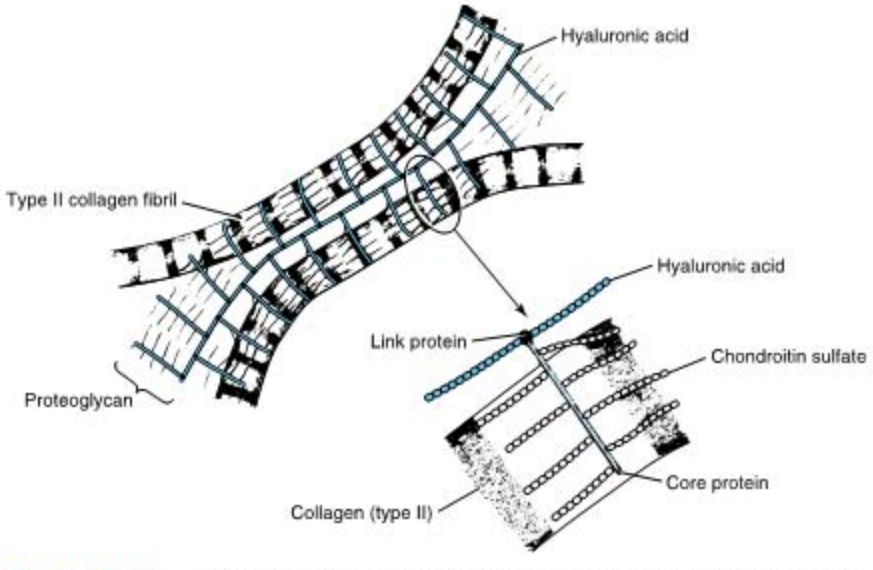

Figure 48–13. Schematic representation of the molecular organization in cartilage matrix. Link proteins noncovalently bind the core protein (lighter color) of proteoglycans to the linear hyaluronic acid molecules (darker color). The chondroitin sulfate side chains of the proteoglycan electrostatically bind to the collagen fibrils, forming a cross-linked matrix. The oval outlines the area enlarged in the lower part of the figure. (Reproduced, with permission, from Junqueira LC, Carneiro J: ***Basic Histology***: *Text & Atlas*, 10th ed. McGraw-Hill, 2003.)

addition to these components, elastic cartilage contains elastin and fibroelastic cartilage contains type I collagen. Cartilage contains a number of proteoglycans, which play an important role in its compressibility. **Aggrecan** (about 2×10^3 kDa) is the major proteoglycan. As shown in Figure 48–14, it has a very complex structure, containing several GAGs (hyaluronic acid, chondroitin sulfate, and keratan sulfate) and both link and core proteins. The core protein contains three domains: A, B, and C. The hyaluronic acid binds noncovalently to domain A of the core protein as well as to the link protein, which stabilizes the hyaluronate–core protein interactions. The keratan sulfate chains are located in domain B, whereas the chondroitin sulfate chains are located in domain C; both of these types of GAGs are bound covalently to the core protein. The core protein also contains both O- and N-linked oligosaccharide chains.

The other proteoglycans found in cartilage have simpler structures than aggrecan.

Chondronectin is involved in the attachment of type II collagen to chondrocytes.

Cartilage is an avascular tissue and obtains most of its nutrients from synovial fluid. It exhibits slow but continuous turnover. Various **proteases** (eg, collagenases and stromalysin) synthesized by chondrocytes can degrade collagen and the other proteins found in cartilage. Interleukin-1 (IL-1) and tumor necrosis factor α (TNFα) appear to stimulate the production of such proteases, whereas transforming growth factor β (TGFβ) and insulin-like growth factor 1 (IGF-I) generally exert an anabolic influence on cartilage.

THE MOLECULAR BASES OF THE CHONDRODYSPLASIAS INCLUDE MUTATIONS IN GENES ENCODING TYPE II COLLAGEN & FIBROBLAST GROWTH FACTOR RECEPTORS

Chondrodysplasias are a mixed group of hereditary disorders affecting cartilage. They are manifested by short-limbed dwarfism and numerous skeletal deformities. A number of them are due to a variety of mutations in the *COL2A1* gene, leading to abnormal forms of type II collagen. One example is **Stickler syndrome,** manifested by degeneration of joint cartilage and of the vitreous body of the eye.

The best-known of the chondrodysplasias is **achondroplasia,** the commonest cause of short-limbed dwarfism. Affected individuals have short limbs, nor-

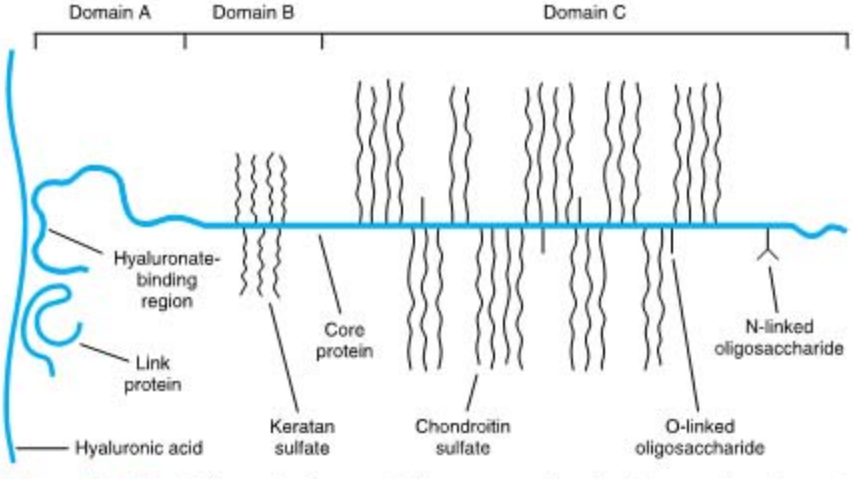

Figure 48–14. Schematic diagram of the aggrecan from bovine nasal cartilage. A strand of hyaluronic acid is shown on the left. The core protein (about 210 kDa) has three major domains. Domain A, at its amino terminal end, interacts with approximately five repeating disaccharides in hyaluronate. The link protein interacts with both hyaluronate and domain A, stabilizing their interactions. Approximately 30 keratan sulfate chains are attached, via GalNAc-Ser linkages, to domain B. Domain C contains about 100 chondroitin sulfate chains attached via Gal-Gal-Xyl-Ser linkages and about 40 O-linked oligosaccharide chains. One or more N-linked glycan chains are also found near the carboxyl terminal of the core protein. (Reproduced, with permission, from Moran LA et al: *Biochemistry,* 2nd ed. Neil Patterson Publishers, 1994.)

mal trunk size, macrocephaly, and a variety of other skeletal abnormalities. The condition is often inherited as an autosomal dominant trait, but many cases are due to new mutations. The molecular basis of achondroplasia is outlined in Figure 48–15. Achondroplasia is not a collagen disorder but is due to mutations in the gene encoding **fibroblast growth factor receptor 3 (FGFR3). Fibroblast growth factors** are a family of at least nine proteins that affect the growth and differentiation of cells of mesenchymal and neuroectodermal origin. Their receptors are transmembrane proteins and form a subgroup of the family of receptor tyrosine kinases. FGFR3 is one member of this subgroup and mediates the actions of FGF3 on cartilage. In almost all cases of achondroplasia that have been investigated, the mutations were found to involve nucleotide 1138 and resulted in substitution of arginine for glycine (residue number 380) in the transmembrane domain of the protein, rendering it inactive. No such mutation was found in unaffected individuals. As indicated in Table 48–10, other skeletal dysplasias (including certain craniosynostosis syndromes) are also due to mutations in genes encoding FGF receptors. Another type of skeletal dysplasia (diastrophic dysplasia) has been found to be due to mutation in a sulfate transporter. Thus, thanks to recombinant DNA technology, a new era in understanding of skeletal dysplasias has begun.

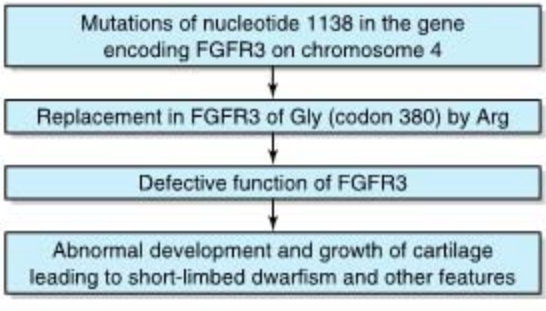

Figure 48–15. Simplified scheme of the causation of achondroplasia (MIM 100800). In most cases studied so far, the mutation has been a G to A transition at nucleotide 1138. In a few cases, the mutation was a G to C transversion at the same nucleotide. This particular nucleotide is a real "hot spot" for mutation. Both mutations result in replacement of a Gly residue by an Arg residue in the transmembrane segment of the receptor. A few cases involving replacement of Gly by Cys at codon 375 have also been reported.

SUMMARY

- The major components of the ECM are the structural proteins collagen, elastin, and fibrillin; a number of specialized proteins (eg, fibronectin and laminin); and various proteoglycans.
- Collagen is the most abundant protein in the animal kingdom; approximately 19 types have been isolated. All collagens contain greater or lesser stretches of triple helix and the repeating structure $(Gly\text{-}X\text{-}Y)_n$.
- The biosynthesis of collagen is complex, featuring many posttranslational events, including hydroxylation of proline and lysine.
- Diseases associated with impaired synthesis of collagen include scurvy, osteogenesis imperfecta, Ehlers-Danlos syndrome (many types), and Menkes disease.
- Elastin confers extensibility and elastic recoil on tissues. Elastin lacks hydroxylysine, Gly-X-Y sequences, triple helical structure, and sugars but contains desmosine and isodesmosine cross-links not found in collagen.
- Fibrillin is located in microfibrils. Mutations in the gene for fibrillin cause Marfan syndrome.
- The glycosaminoglycans (GAGs) are made up of repeating disaccharides containing a uronic acid (glucuronic or iduronic) or hexose (galactose) and a hexosamine (galactosamine or glucosamine). Sulfate is also frequently present.
- The major GAGs are hyaluronic acid, chondroitin 4- and 6-sulfates, keratan sulfates I and II, heparin, heparan sulfate, and dermatan sulfate.
- The GAGs are synthesized by the sequential actions of a battery of specific enzymes (glycosyltransferases, epimerases, sulfotransferases, etc) and are degraded by the sequential action of lysosomal hydrolases. Genetic deficiencies of the latter result in mucopolysaccharidoses (eg, Hurler syndrome).
- GAGs occur in tissues bound to various proteins (linker proteins and core proteins), constituting proteoglycans. These structures are often of very high molecular weight and serve many functions in tissues.
- Many components of the ECM bind to proteins of the cell surface named integrins; this constitutes one pathway by which the exteriors of cells can communicate with their interiors.
- Bone and cartilage are specialized forms of the ECM. Collagen I and hydroxyapatite are the major constituents of bone. Collagen II and certain proteoglycans are major constituents of cartilage.

- The molecular causes of a number of heritable diseases of bone (eg, osteogenesis imperfecta) and of cartilage (eg, the chondrodystrophies) are being revealed by the application of recombinant DNA technology.

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Muscle & the Cytoskeleton

49

Robert K. Murray, MD, PhD

BIOMEDICAL IMPORTANCE

Proteins play an important role in movement at both the organ (eg, skeletal muscle, heart, and gut) and cellular levels. In this chapter, the roles of specific proteins and certain other key molecules (eg, Ca^{2+}) in **muscular contraction** are described. A brief coverage of **cytoskeletal proteins** is also presented.

Knowledge of the molecular bases of a number of conditions that affect muscle has advanced greatly in recent years. Understanding of the molecular basis of **Duchenne-type muscular dystrophy** was greatly enhanced when it was found that it was due to mutations in the gene encoding dystrophin. Significant progress has also been made in understanding the molecular basis of **malignant hyperthermia,** a serious complication for some patients undergoing certain types of anesthesia. **Heart failure** is a very common medical condition, with a variety of causes; its rational therapy requires understanding of the biochemistry of heart muscle. One group of conditions that cause heart failure are the **cardiomyopathies,** some of which are genetically determined. **Nitric oxide** (NO) has been found to be a major regulator of smooth muscle tone. Many widely used **vasodilators**—such as nitroglycerin, used in the treatment of angina pectoris—act by increasing the formation of NO. Muscle, partly because of its mass, plays major roles in the **overall metabolism** of the body.

MUSCLE TRANSDUCES CHEMICAL ENERGY INTO MECHANICAL ENERGY

Muscle is the major biochemical transducer (machine) that converts potential (chemical) energy into kinetic (mechanical) energy. Muscle, the largest single tissue in the human body, makes up somewhat less than 25% of body mass at birth, more than 40% in the young adult, and somewhat less than 30% in the aged adult. We shall discuss aspects of the three types of muscle found in vertebrates: **skeletal, cardiac,** and **smooth.** Both skeletal and cardiac muscle appear **striated** upon microscopic observation; smooth muscle is **nonstriated.** Although skeletal muscle is under voluntary nervous control, the control of both cardiac and smooth muscle is involuntary.

The Sarcoplasm of Muscle Cells Contains ATP, Phosphocreatine, & Glycolytic Enzymes

Striated muscle is composed of multinucleated muscle fiber cells surrounded by an electrically excitable plasma membrane, the **sarcolemma.** An individual muscle fiber cell, which may extend the entire length of the muscle, contains a bundle of many **myofibrils** arranged in parallel, embedded in intracellular fluid termed **sarcoplasm.** Within this fluid is contained glycogen, the high-energy compounds ATP and phosphocreatine, and the enzymes of glycolysis.

The Sarcomere Is the Functional Unit of Muscle

An overall view of voluntary muscle at several levels of organization is presented in Figure 49–1.

When the myofibril is examined by electron microscopy, alternating dark and light bands (anisotropic bands, meaning birefringent in polarized light; and isotropic bands, meaning not altered by polarized light) can be observed. These bands are thus referred to as **A and I bands,** respectively. The central region of the A band (the H band) appears less dense than the rest of the band. The I band is bisected by a very dense and narrow **Z line** (Figure 49–2).

The **sarcomere** is defined as the region between two Z lines (Figures 49–1 and 49–2) and is repeated along the axis of a fibril at distances of 1500–2300 nm depending upon the state of contraction.

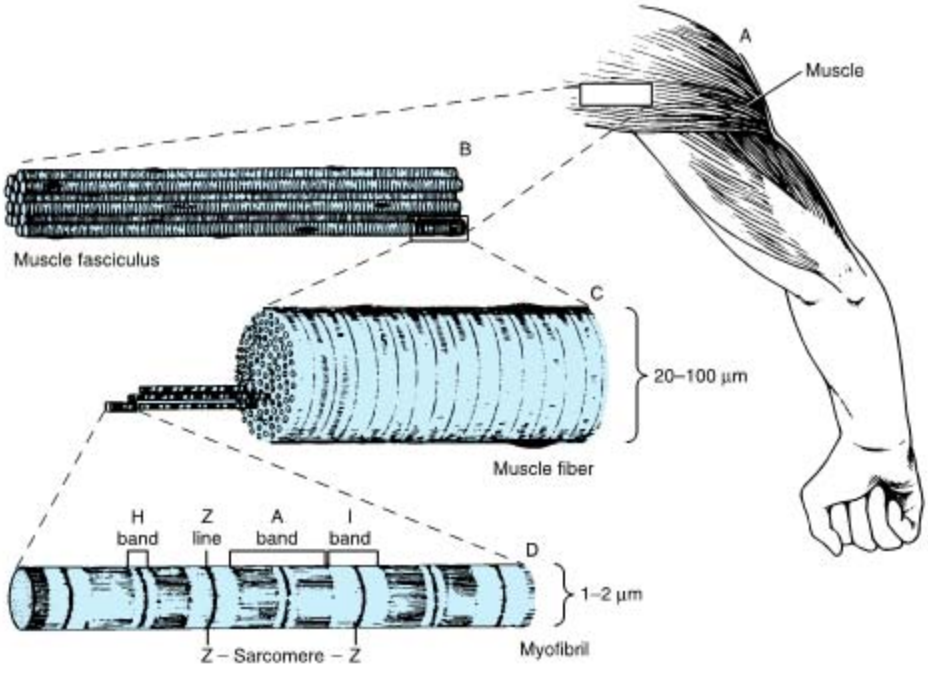

Figure 49–1. **The structure of voluntary muscle. The sarcomere is the region between the Z lines.** (Drawing by Sylvia Colard Keene. Reproduced, with permission, from Bloom W, Fawcett DW: *A Textbook of Histology*, 10th ed. Saunders, 1975.)

The striated appearance of voluntary and cardiac muscle in light microscopic studies results from their high degree of organization, in which most muscle fiber cells are aligned so that their sarcomeres are in parallel register (Figure 49–1).

Thick Filaments Contain Myosin; Thin Filaments Contain Actin, Tropomyosin, & Troponin

When myofibrils are examined by electron microscopy, it appears that each one is constructed of two types of longitudinal filaments. One type, the **thick filament,** confined to the A band, contains chiefly the protein myosin. These filaments are about 16 nm in diameter and arranged in cross-section as a hexagonal array (Figure 49–2, center; right-hand cross-section).

The **thin filament** (about 7 nm in diameter) lies in the I band and extends into the A band but not into its H zone (Figure 49–2). Thin filaments contain the proteins actin, tropomyosin, and troponin (Figure 49–3). In the A band, the thin filaments are arranged around the thick (myosin) filament as a secondary hexagonal array. Each thin filament lies symmetrically between three thick filaments (Figure 49–2, center; mid cross-section), and each thick filament is surrounded symmetrically by six thin filaments.

The thick and thin filaments interact via cross-bridges that emerge at intervals of 14 nm along the thick filaments. As depicted in Figure 49–2, the cross-bridges (drawn as arrowheads at each end of the myosin filaments, but not shown extending fully across to the thin filaments) have opposite polarities at the two ends of the thick filaments. The two poles of the thick filaments are separated by a 150-nm segment (the M band, not labeled in the figure) that is free of projections.

The Sliding Filament Cross-Bridge Model Is the Foundation on Which Current Thinking About Muscle Contraction Is Built

This model was proposed independently in the 1950s by Henry Huxley and Andrew Huxley and their colleagues. It was largely based on careful morphologic observations on resting, extended, and contracting muscle. Basically, when muscle contracts, there is no change in the lengths of the thick and thin filaments, but the H zones and the I bands shorten (see legend to Fig-

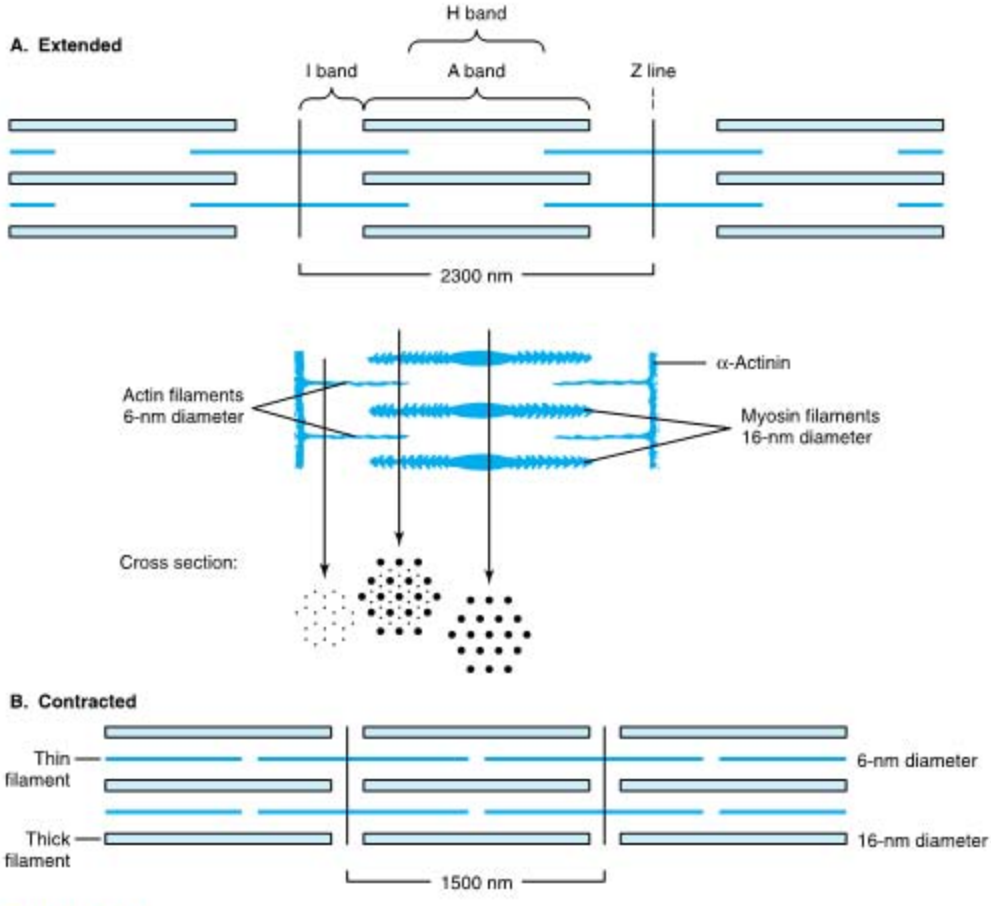

Figure 49–2. Arrangement of filaments in striated muscle. **A:** Extended. The positions of the I, A, and H bands in the extended state are shown. The thin filaments partly overlap the ends of the thick filaments, and the thin filaments are shown anchored in the Z lines (often called Z disks). In the lower part of Figure 49–2A, "arrowheads," pointing in opposite directions, are shown emanating from the myosin (thick) filaments. Four actin (thin) filaments are shown attached to two Z lines via α-actinin. The central region of the three myosin filaments, free of arrowheads, is called the M band (not labeled). Cross-sections through the M bands, through an area where myosin and actin filaments overlap and through an area in which solely actin filaments are present, are shown. **B:** Contracted. The actin filaments are seen to have slipped along the sides of the myosin fibers toward each other. The lengths of the thick filaments (indicated by the A bands) and the thin filaments (distance between Z lines and the adjacent edges of the H bands) have not changed. However, the lengths of the sarcomeres have been reduced (from 2300 nm to 1500 nm), and the lengths of the H and I bands are also reduced because of the overlap between the thick and thin filaments. These morphologic observations provided part of the basis for the sliding filament model of muscle contraction.

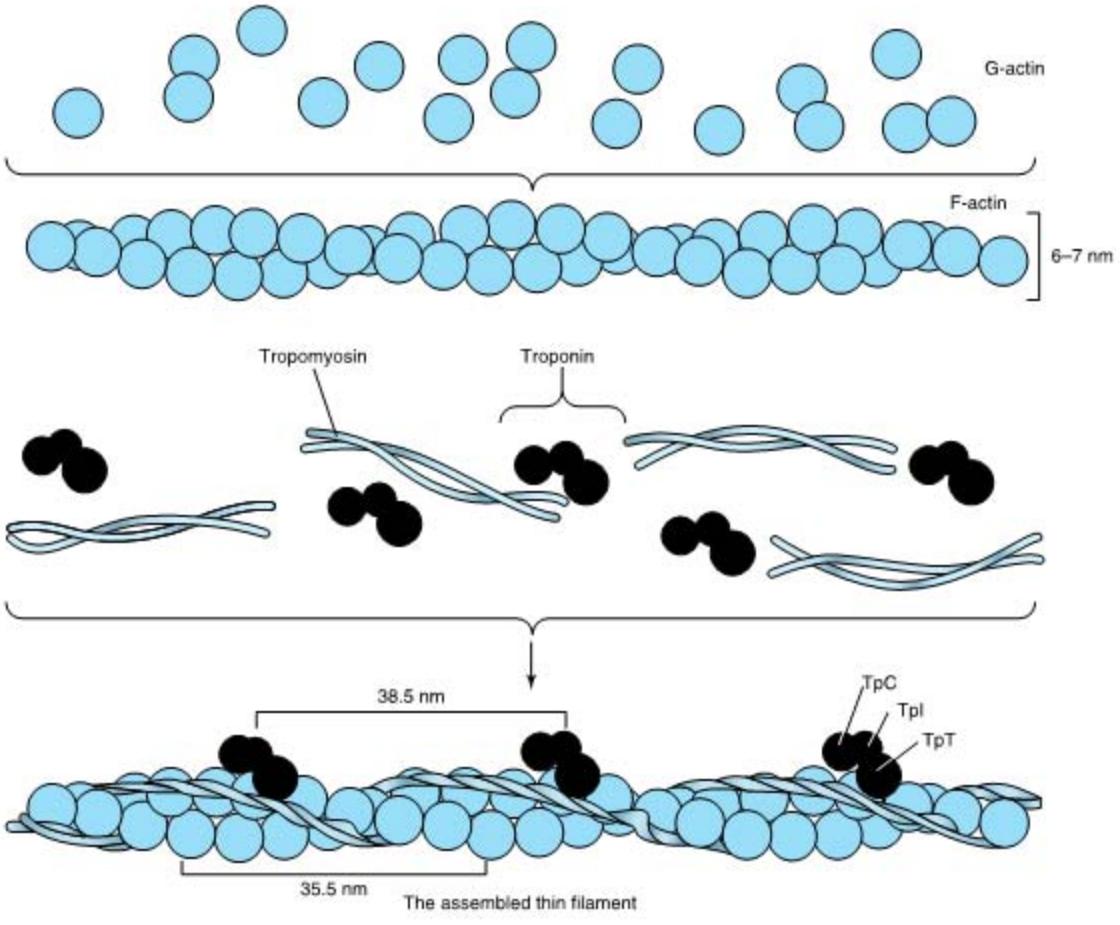

Figure 49–3. Schematic representation of the thin filament, showing the spatial configuration of its three major protein components: actin, myosin, and tropomyosin. The upper panel shows individual molecules of G-actin. The middle panel shows actin monomers assembled into F-actin. Individual molecules of tropomyosin (two strands wound around one another) and of troponin (made up of its three subunits) are also shown. The lower panel shows the assembled thin filament, consisting of F-actin, tropomyosin, and the three subunits of troponin (TpC, TpI, and TpT).

ure 49–2). Thus, the arrays of interdigitating filaments must slide past one another during contraction. Cross-bridges that link thick and thin filaments at certain stages in the contraction cycle generate and sustain the tension. The tension developed during muscle contraction is proportionate to the filament overlap and to the number of cross-bridges. Each cross-bridge head is connected to the thick filament via a flexible fibrous segment that can bend outward from the thick filament. This flexible segment facilitates contact of the head with the thin filament when necessary but is also sufficiently pliant to be accommodated in the interfilament spacing.

ACTIN & MYOSIN ARE THE MAJOR PROTEINS OF MUSCLE

The mass of a muscle is made up of 75% water and more than 20% protein. The two major proteins are actin and myosin.

Monomeric **G-actin** (43 kDa; G, globular) makes up 25% of muscle protein by weight. At physiologic ionic strength and in the presence of Mg^{2+}, G-actin polymerizes noncovalently to form an insoluble double helical filament called F-actin (Figure 49–3). The **F-actin** fiber is 6–7 nm thick and has a pitch or repeating structure every 35.5 nm.

Myosins constitute a family of proteins, with at least 15 members having been identified. The myosin discussed in this chapter is myosin-II, and when myosin is referred to in this text, it is this species that is meant unless otherwise indicated. Myosin-I is a monomeric species that binds to cell membranes. It may serve as a linkage between microfilaments and the cell membrane in certain locations.

Myosin contributes 55% of muscle protein by weight and forms the thick filaments. It is an asymmetric hexamer with a molecular mass of approximately 460 kDa. Myosin has a fibrous tail consisting of two intertwined helices. Each helix has a globular head portion attached at one end (Figure 49–4). The hexamer consists of one pair of **heavy (H) chains** each of approximately 200 kDA molecular mass, and two pairs of **light (L) chains** each with a molecular mass of approximately 20 kDa. The L chains differ, one being called the essential light chain and the other the regulatory light chain. Skeletal muscle myosin binds actin to form actomyosin (actin-myosin), and its intrinsic ATPase activity is markedly enhanced in this complex. Isoforms of myosin exist whose amounts can vary in different anatomic, physiologic, and pathologic situations.

The structures of actin and of the head of myosin have been determined by x-ray crystallography; these studies have confirmed a number of earlier findings concerning their structures and have also given rise to much new information.

Limited Digestion of Myosin With Proteases Has Helped to Elucidate Its Structure & Function

When myosin is digested with trypsin, two myosin fragments (meromyosins) are generated. **Light meromyosin** (LMM) consists of aggregated, insoluble α-helical fibers from the tail of myosin (Figure 49–4). LMM

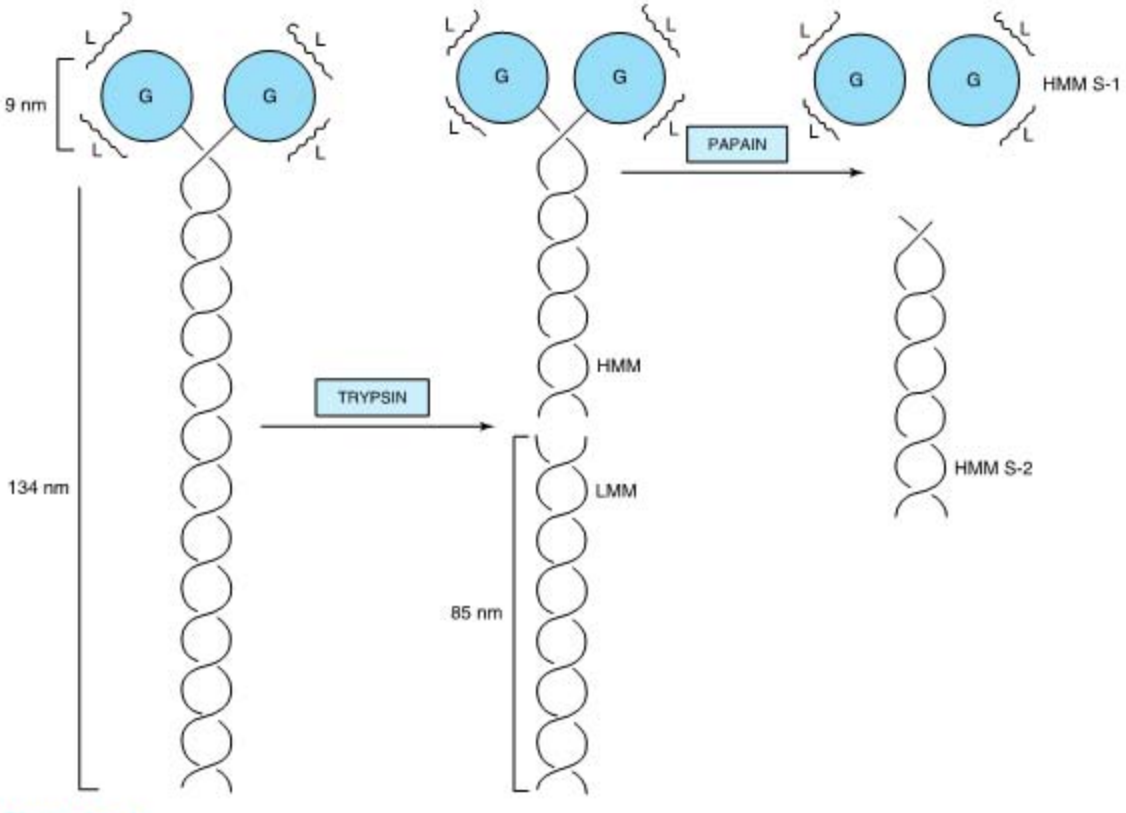

Figure 49–4. Diagram of a myosin molecule showing the two intertwined α-helices (fibrous portion), the globular region or head (G), the light chains (L), and the effects of proteolytic cleavage by trypsin and papain. The globular region (myosin head) contains an actin-binding site and an L chain-binding site and also attaches to the remainder of the myosin molecule.

exhibits no ATPase activity and does not bind to F-actin.

Heavy meromyosin (HMM; molecular mass about 340 kDa) is a soluble protein that has both a fibrous portion and a globular portion (Figure 49–4). It exhibits ATPase activity and binds to F-actin. Digestion of HMM with papain generates two subfragments, S-1 and S-2. The S-2 fragment is fibrous in character, has no ATPase activity, and does not bind to F-actin.

S-1 (molecular mass approximately 115 kDa) does exhibit ATPase activity, binds L chains, and in the absence of ATP will bind to and decorate actin with "arrowheads" (Figure 49–5). Both S-1 and HMM exhibit ATPase activity, which is accelerated 100- to 200-fold by complexing with F-actin. As discussed below, F-actin greatly enhances the rate at which myosin ATPase releases its products, ADP and P_i. Thus, although F-actin does not affect the hydrolysis step per se, its ability to promote release of the products produced by the ATPase activity greatly accelerates the overall rate of catalysis.

CHANGES IN THE CONFORMATION OF THE HEAD OF MYOSIN DRIVE MUSCLE CONTRACTION

How can hydrolysis of ATP produce macroscopic movement? Muscle contraction essentially consists of the cyclic attachment and detachment of the S-1 head of myosin to the F-actin filaments. This process can also be referred to as the making and breaking of cross-bridges. The attachment of actin to myosin is followed by conformational changes which are of particular importance in the S-1 head and are dependent upon which nucleotide is present (ADP or ATP). These changes result in the **power stroke,** which drives movement of actin filaments past myosin filaments. The energy for the power stroke is ultimately supplied by **ATP,** which is hydrolyzed to ADP and P_i. However, the power stroke itself occurs as a result of **conformational changes** in the myosin head when **ADP** leaves it.

The major biochemical events occurring during one cycle of muscle contraction and relaxation can be represented in the five steps shown in Figure 49–6:

(1) In the **relaxation phase** of muscle contraction, the S-1 head of myosin hydrolyzes ATP to ADP and P_i, but these products remain bound. The resultant ADP-P_i-myosin complex has been energized and is in a so-called high-energy conformation.

(2) When **contraction** of muscle is stimulated (via events involving Ca^{2+}, troponin, tropomyosin, and actin, which are described below), actin becomes accessible and the S-1 head of myosin finds it, binds it, and forms the actin-myosin-ADP-P_i complex indicated.

(3) Formation of this complex **promotes the release of P_i,** which initiates the power stroke. This is followed by release of ADP and is accompanied by a large conformational change in the head of myosin in relation to its tail (Figure 49–7), pulling actin about 10 nm toward the center of the sarcomere. This is the power stroke. The myosin is now in a so-called low-energy state, indicated as actin-myosin.

(4) Another molecule of ATP binds to the S-1 head, forming an actin-myosin-ATP complex.

(5) Myosin-ATP has a low affinity for actin, and **actin is thus released.** This last step is a key component of relaxation and is dependent upon the binding of ATP to the actin-myosin complex.

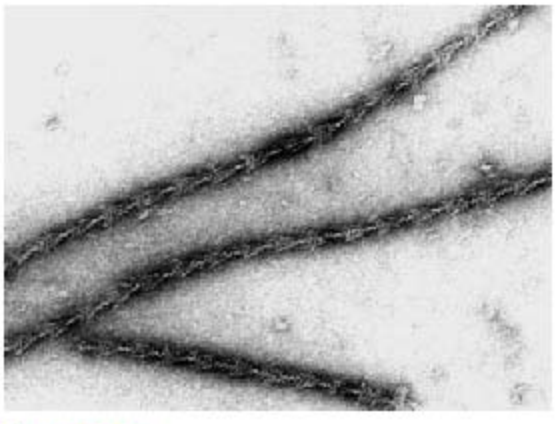

Figure 49–5. The decoration of actin filaments with the S-1 fragments of myosin to form "arrowheads." (Courtesy of JA Spudich.)

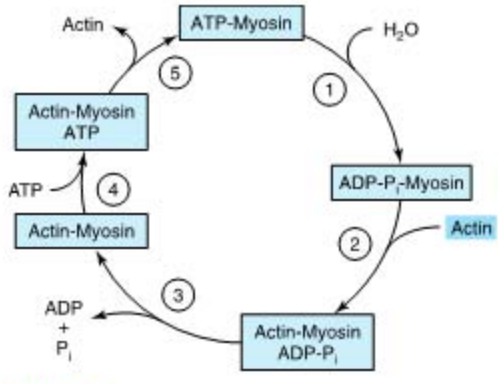

Figure 49–6. The hydrolysis of ATP drives the cyclic association and dissociation of actin and myosin in five reactions described in the text. (Modified from Stryer L: *Biochemistry,* 2nd ed. Freeman, 1981.)

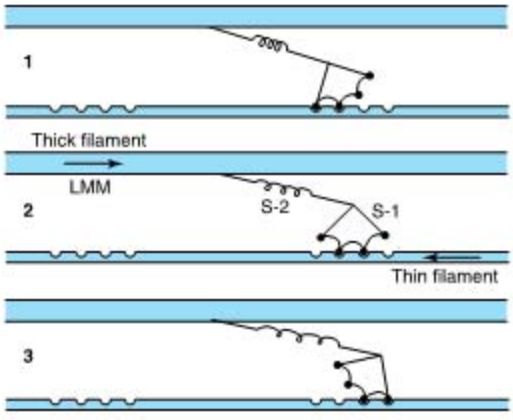

Figure 49–7. Representation of the active cross-bridges between thick and thin filaments. This diagram was adapted by AF Huxley from HE Huxley: The mechanism of muscular contraction. Science 1969;164:1356. The latter proposed that the force involved in muscular contraction originates in a tendency for the myosin head (S-1) to rotate relative to the thin filament and is transmitted to the thick filament by the S-2 portion of the myosin molecule acting as an inextensible link. Flexible points at each end of S-2 permit S-1 to rotate and allow for variations in the separation between filaments. The present figure is based on HE Huxley's proposal but also incorporates elastic (the coils in the S-2 portion) and stepwise-shortening elements (depicted here as four sites of interaction between the S-1 portion and the thin filament). (See Huxley AF, Simmons RM: Proposed mechanism of force generation in striated muscle. Nature [Lond] 1971;233:533.) The strengths of binding of the attached sites are higher in position 2 than in position 1 and higher in position 3 than position 2. The myosin head can be detached from position 3 with the utilization of a molecule of ATP; this is the predominant process during shortening. The myosin head is seen to vary in its position from about 90° to about 45°, as indicated in the text. (S-1, myosin head; S-2, portion of the myosin molecule; LMM, light meromyosin) (see legend to Figure 49–4). (Reproduced from Huxley AF: Muscular contraction. J Physiol 1974; 243:1. By kind permission of the author and the *Journal of Physiology*.)

Another cycle then commences with the hydrolysis of ATP (step 1 of Figure 49–6), re-forming the high-energy conformation.

Thus, hydrolysis of ATP is used to drive the cycle, with the actual power stroke being the conformational change in the S-1 head that occurs upon the release of ADP. The hinge regions of myosin (referred to as flexible points at each end of S-2 in the legend to Figure 49–7) permit the large range of movement of S-1 and also allow S-1 to find actin filaments.

If intracellular levels of ATP drop (eg, after death), ATP is not available to bind the S-1 head (step 4 above), actin does not dissociate, and relaxation (step 5) does not occur. This is the explanation for **rigor mortis,** the stiffening of the body that occurs after death.

Calculations have indicated that the efficiency of contraction is about 50%; that of the internal combustion engine is less than 20%.

Tropomyosin & the Troponin Complex Present in Thin Filaments Perform Key Functions in Striated Muscle

In striated muscle, there are two other proteins that are minor in terms of their mass but important in terms of their function. **Tropomyosin** is a fibrous molecule that consists of two chains, alpha and beta, that attach to F-actin in the groove between its filaments (Figure 49–3). Tropomyosin is present in all muscular and muscle-like structures. The **troponin complex** is unique to striated muscle and consists of three polypeptides. **Troponin T** (TpT) binds to tropomyosin as well as to the other two troponin components. **Troponin I** (TpI) inhibits the F-actin-myosin interaction and also binds to the other components of troponin. **Troponin C** (TpC) is a calcium-binding polypeptide that is structurally and functionally analogous to **calmodulin,** an important calcium-binding protein widely distributed in nature. Four molecules of calcium ion are bound per molecule of troponin C or calmodulin, and both molecules have a molecular mass of 17 kDa.

Ca^{2+} Plays a Central Role in Regulation of Muscle Contraction

The contraction of muscles from all sources occurs by the general mechanism described above. Muscles from different organisms and from different cells and tissues within the same organism may have different molecular mechanisms responsible for the regulation of their contraction and relaxation. In all systems, Ca^{2+} plays a key regulatory role. There are two general mechanisms of regulation of muscle contraction: **actin-based** and **myosin-based.** The former operates in skeletal and cardiac muscle, the latter in smooth muscle.

Actin-Based Regulation Occurs in Striated Muscle

Actin-based regulation of muscle occurs in vertebrate skeletal and cardiac muscles, both striated. In the gen-

eral mechanism described above (Figure 49–6), the only potentially limiting factor in the cycle of muscle contraction might be ATP. The skeletal muscle system is **inhibited** at rest; this inhibition is relieved to activate contraction. The inhibitor of striated muscle is the **troponin system,** which is bound to tropomyosin and F-actin in the thin filament (Figure 49–3). In striated muscle, there is no control of contraction unless the tropomyosin-troponin systems are present along with the actin and myosin filaments. As described above, **tropomyosin** lies along the groove of F-actin, and the three components of **troponin**—TpT, TpI, and TpC—are bound to the F-actin–tropomyosin complex. TpI prevents binding of the myosin head to its F-actin attachment site either by altering the conformation of F-actin via the tropomyosin molecules or by simply rolling tropomyosin into a position that directly blocks the sites on F-actin to which the myosin heads attach. Either way prevents activation of the myosin ATPase that is mediated by binding of the myosin head to F-actin. Hence, the TpI system blocks the contraction cycle at step 2 of Figure 49–6. This accounts for the inhibited state of relaxed striated muscle.

The Sarcoplasmic Reticulum Regulates Intracellular Levels of Ca^{2+} in Skeletal Muscle

In the sarcoplasm of resting muscle, the concentration of Ca^{2+} is 10^{-8} to 10^{-7} mol/L. The resting state is achieved because Ca^{2+} is pumped into the sarcoplasmic reticulum through the action of an active transport system, called the Ca^{2+} ATPase (Figure 49–8), initiating relaxation. The sarcoplasmic reticulum is a network of fine membranous sacs. Inside the sarcoplasmic reticulum, Ca^{2+} is bound to a specific Ca^{2+}-binding protein designated **calsequestrin.** The sarcomere is surrounded by an excitable membrane (the T tubule system) composed of transverse (T) channels closely associated with the sarcoplasmic reticulum.

When the sarcolemma is excited by a nerve impulse, the signal is transmitted into the T tubule system and a **Ca^{2+} release channel** in the nearby sarcoplasmic reticulum opens, releasing Ca^{2+} from the sarcoplasmic reticulum into the sarcoplasm. The concentration of Ca^{2+} in the sarcoplasm rises rapidly to 10^{-5} mol/L. The Ca^{2+}-binding sites on TpC in the thin filament are quickly occupied by Ca^{2+}. The TpC-4Ca^{2+} interacts with TpI and TpT to alter their interaction with tropomyosin. Accordingly, tropomyosin moves out of the way or alters the conformation of F-actin so that the myosin head-ADP-P_i (Figure 49–6) can interact with F-actin to start the contraction cycle.

The Ca^{2+} release channel is also known as the **ryanodine receptor** (RYR). There are two isoforms of this

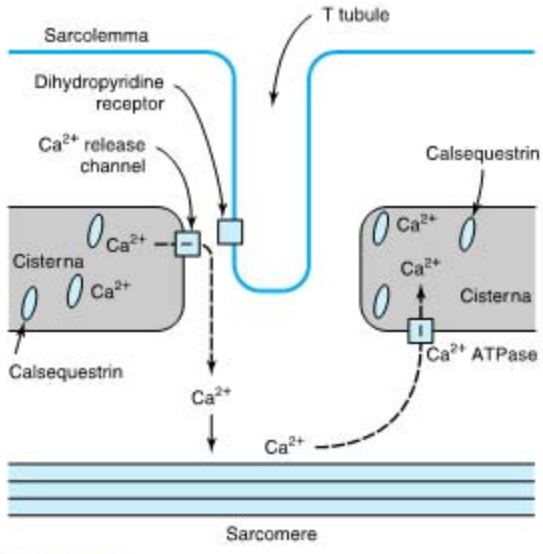

Figure 49–8. Diagram of the relationships among the sarcolemma (plasma membrane), a T tubule, and two cisternae of the sarcoplasmic reticulum of skeletal muscle (not to scale). The T tubule extends inward from the sarcolemma. A wave of depolarization, initiated by a nerve impulse, is transmitted from the sarcolemma down the T tubule. It is then conveyed to the Ca^{2+} release channel (ryanodine receptor), perhaps by interaction between it and the dihydropyridine receptor (slow Ca^{2+} voltage channel), which are shown in close proximity. Release of Ca^{2+} from the Ca^{2+} release channel into the cytosol initiates contraction. Subsequently, Ca^{2+} is pumped back into the cisternae of the sarcoplasmic reticulum by the Ca^{2+} ATPase (Ca^{2+} pump) and stored there, in part bound to calsequestrin.

receptor, RYR1 and RYR2, the former being present in skeletal muscle and the latter in heart muscle and brain. **Ryanodine** is a plant alkaloid that binds to RYR1 and RYR2 specifically and modulates their activities. The Ca^{2+} release channel is a homotetramer made up of four subunits of kDa 565. It has transmembrane sequences at its carboxyl terminal, and these probably form the Ca^{2+} channel. The remainder of the protein protrudes into the cytosol, bridging the gap between the sarcoplasmic reticulum and the transverse tubular membrane. The channel is ligand-gated, Ca^{2+} and ATP working synergistically in vitro, although how it operates in vivo is not clear. A possible sequence of events leading to opening of the channel is shown in Figure 49–9. The channel lies very close to the **dihydropyridine receptor** (DHPR; a voltage-gated slow K type

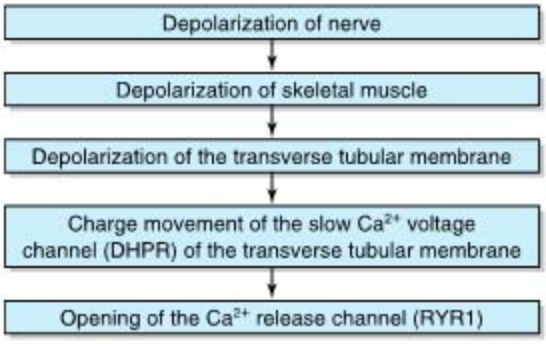

Figure 49–9. Possible chain of events leading to opening of the Ca^{2+} release channel. As indicated in the text, the Ca^{2+} voltage channel and the Ca^{2+} release channel have been shown to interact with each other in vitro via specific regions in their polypeptide chains. (DHPR, dihydropyridine receptor; RYR1, ryanodine receptor 1.)

Ca^{2+} channel) of the transverse tubule system (Figure 49–8). Experiments in vitro employing an affinity column chromatography approach have indicated that a 37-amino-acid stretch in RYR1 interacts with one specific loop of DHPR.

Relaxation occurs when sarcoplasmic Ca^{2+} falls below 10^{-7} mol/L owing to its resequestration into the sarcoplasmic reticulum by Ca^{2+} ATPase. TpC.4Ca^{2+} thus loses its Ca^{2+}. Consequently, troponin, via interaction with tropomyosin, inhibits further myosin head and F-actin interaction, and in the presence of ATP the myosin head detaches from the F-actin.

Thus, Ca^{2+} controls skeletal muscle contraction and relaxation by an allosteric mechanism mediated by TpC, TpI, TpT, tropomyosin, and F-actin.

A decrease in the concentration of **ATP** in the sarcoplasm (eg, by excessive usage during the cycle of contraction-relaxation or by diminished formation, such as might occur in ischemia) has two major effects: (1) The Ca^{2+} ATPase (Ca^{2+} pump) in the sarcoplasmic reticulum ceases to maintain the low concentration of Ca^{2+} in the sarcoplasm. Thus, the interaction of the myosin heads with F-actin is promoted. (2) The ATP-dependent detachment of myosin heads from F-actin cannot occur, and rigidity (contracture) sets in. The condition of **rigor mortis,** following death, is an extension of these events.

Muscle contraction is a delicate dynamic balance of the attachment and detachment of myosin heads to F-actin, subject to fine regulation via the nervous system.

Table 49–1 summarizes the overall events in contraction and relaxation of skeletal muscle.

Mutations in the Gene Encoding the Ca^{2+} Release Channel Are One Cause of Human Malignant Hyperthermia

Some genetically predisposed patients experience a severe reaction, designated malignant hyperthermia, on exposure to certain anesthetics (eg, halothane) and depolarizing skeletal muscle relaxants (eg, succinylcholine). The reaction consists primarily of rigidity of skeletal muscles, hypermetabolism, and high fever. A **high cytosolic concentration of Ca^{2+}** in skeletal muscle is a major factor in its causation. Unless malignant hyperthermia is recognized and treated immediately, patients may die acutely of ventricular fibrillation or survive to succumb subsequently from other serious complications. Appropriate treatment is to stop the anesthetic and administer the drug **dantrolene** intravenously. Dantrolene is a skeletal muscle relaxant that acts to inhibit release of Ca^{2+} from the sarcoplasmic reticulum into the cytosol, thus preventing the increase of cytosolic Ca^{2+} found in malignant hyperthermia.

Table 49–1. Sequence of events in contraction and relaxation of skeletal muscle.[1]

Steps in contraction
(1) Discharge of motor neuron
(2) Release of transmitter (acetylcholine) at motor endplate
(3) Binding of acetylcholine to nicotinic acetylcholine receptors
(4) Increased Na^+ and K^+ conductance in endplate membrane
(5) Generation of endplate potential
(6) Generation of action potential in muscle fibers
(7) Inward spread of depolarization along T tubules
(8) Release of Ca^{2+} from terminal cisterns of sarcoplasmic reticulum and diffusion to thick and thin filaments
(9) Binding of Ca^{2+} to troponin C, uncovering myosin binding sites of actin
(10) Formation of cross-linkages between actin and myosin and sliding of thin on thick filaments, producing shortening
Steps in relaxation
(1) Ca^{2+} pumped back into sarcoplasmic reticulum
(2) Release of Ca^{2+} from troponin
(3) Cessation of interaction between actin and myosin

[1]Reproduced, with permission, from Ganong WF: *Review of Medical Physiology,* 21st ed. McGraw-Hill, 2003.

Malignant hyperthermia also occurs in swine. Susceptible animals homozygous for malignant hyperthermia respond to stress with a fatal reaction **(porcine stress syndrome)** similar to that exhibited by humans. If the reaction occurs prior to slaughter, it affects the quality of the pork adversely, resulting in an inferior product. Both events can result in considerable economic losses for the swine industry.

The finding of a high level of cytosolic Ca^{2+} in muscle in malignant hyperthermia suggested that the condition might be caused by abnormalities of the Ca^{2+} ATPase or of the Ca^{2+} release channel. No abnormalities were detected in the former, but sequencing of cDNAs for the latter protein proved insightful, particularly in swine. All cDNAs from swine with malignant hyperthermia so far examined have shown a substitution of T for C1843, resulting in the substitution of Cys for Arg^{615} in the Ca^{2+} release channel. The mutation affects the function of the channel in that it opens more easily and remains open longer; the net result is massive release of Ca^{2+} into the cytosol, ultimately causing sustained muscle contraction.

The picture is more complex in humans, since malignant hyperthermia exhibits **genetic heterogeneity.** Members of a number of families who suffer from malignant hyperthermia have not shown genetic linkage to the *RYR1* gene. Some humans susceptible to malignant hyperthermia have been found to exhibit the same mutation found in swine, and others have a variety of point mutations at different loci in the *RYR1* gene. Certain families with malignant hypertension have been found to have mutations affecting the DHPR. Figure 49–10 summarizes the probable chain of events in malignant hyperthermia. The major promise of these findings is that, once additional mutations are detected, it will be possible to **screen,** using suitable DNA probes, for individuals at risk of developing malignant hyperthermia during anesthesia. Current screening tests (eg, the in vitro caffeine-halothane test) are relatively unreliable. Affected individuals could then be given alternative anesthetics, which would not endanger their lives. It should also be possible, if desired, to eliminate malignant hyperthermia from swine populations using suitable breeding practices.

Another condition due to mutations in the *RYR1* gene is **central core disease.** This is a rare myopathy presenting in infancy with hypotonia and proximal muscle weakness. Electron microscopy reveals an absence of mitochondria in the center of many type I (see below) muscle fibers. Damage to mitochondria induced by high intracellular levels of Ca^{2+} secondary to abnormal functioning of *RYR1* appears to be responsible for the morphologic findings.

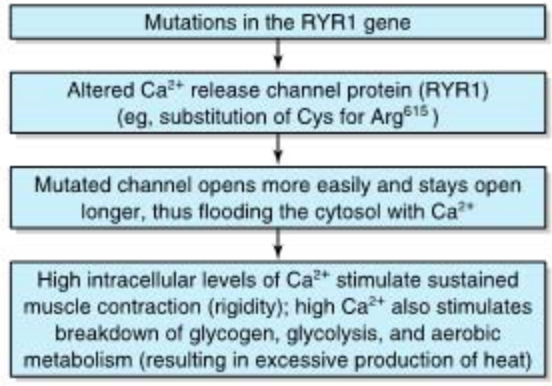

Figure 49–10. Simplified scheme of the causation of malignant hyperthermia (MIM 145600). At least 17 different point mutations have been detected in the *RYR1* gene, some of which are associated with central core disease (MIM 117000). It is estimated that at least 50% of families with members who have malignant hyperthermia are linked to the *RYR1* gene. Some individuals with mutations in the gene encoding DHPR have also been detected; it is possible that mutations in other genes for proteins involved in certain aspects of muscle metabolism will also be found.

MUTATIONS IN THE GENE ENCODING DYSTROPHIN CAUSE DUCHENNE MUSCULAR DYSTROPHY

A number of additional proteins play various roles in the structure and function of muscle. They include titin (the largest protein known), nebulin, α-actinin, desmin, dystrophin, and calcineurin. Some properties of these proteins are summarized in Table 49–2.

Dystrophin is of special interest. Mutations in the gene encoding this protein have been shown to be the cause of Duchenne muscular dystrophy and the milder Becker muscular dystrophy (see Figure 49–11). They are also implicated in some cases of dilated cardiomyopathy (see below). The gene encoding dystrophin is the largest gene known (= 2300 kb) and is situated on the X chromosome, accounting for the maternal inheritance pattern of Duchenne and Becker muscular dystrophies. As shown in Figure 49–12, dystrophin forms part of a large complex of proteins that attach to or interact with the plasmalemma. Dystrophin links the actin cytoskeleton to the ECM and appears to be needed for assembly of the synaptic junction. Impairment of these processes by formation of defective dystrophin is presumably critical in the causation of

Table 49–2. Some other important proteins of muscle.

Protein	Location	Comment or Function
Titin	Reaches from the Z line to the M line	Largest protein in body. Role in relaxation of muscle.
Nebulin	From Z line along length of actin filaments	May regulate assembly and length of actin filaments.
α-Actinin	Anchors actin to Z lines	Stabilizes actin filaments.
Desmin	Lies alongside actin filaments	Attaches to plasma membrane (plasmalemma).
Dystrophin	Attached to plasmalemma	Deficient in Duchenne muscular dystrophy. Mutations of its gene can also cause dilated cardiomyopathy.
Calcineurin	Cytosol	A calmodulin-regulated protein phosphatase. May play important roles in cardiac hypertrophy and in regulating amounts of slow and fast twitch muscles.
Myosin-binding protein C	Arranged transversely in sarcomere A-bands	Binds myosin and titin. Plays a role in maintaining the structural integrity of the sarcomere.

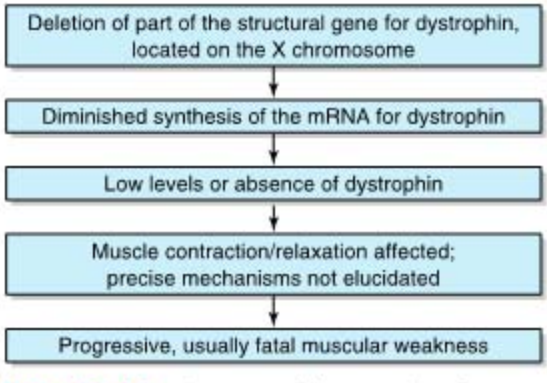

Figure 49–11. Summary of the causation of Duchenne muscular dystrophy (MIM 310200).

Duchenne muscular dystrophy. Mutations in the genes encoding some of the components of the sarcoglycan complex shown in Figure 49–12 are responsible for limb-girdle and certain other congenital forms of muscular dystrophy.

CARDIAC MUSCLE RESEMBLES SKELETAL MUSCLE IN MANY RESPECTS

The general picture of muscle contraction in the heart resembles that of skeletal muscle. Cardiac muscle, like skeletal muscle, is **striated** and uses the actin-myosin-tropomyosin-troponin system described above. Unlike skeletal muscle, cardiac muscle exhibits intrinsic rhythmicity, and individual myocytes communicate with each other because of its syncytial nature. The **T tubular system** is more developed in cardiac muscle, whereas the **sarcoplasmic reticulum** is less extensive and consequently the intracellular supply of Ca^{2+} for contraction is less. Cardiac muscle thus relies on **extracellular Ca^{2+}** for contraction; if isolated cardiac muscle is deprived of Ca^{2+}, it ceases to beat within approximately 1 minute, whereas skeletal muscle can continue to contract without an extracellular source of Ca^{2+}. **Cyclic AMP** plays a more prominent role in cardiac than in skeletal muscle. It modulates intracellular levels of Ca^{2+} through the activation of protein kinases; these enzymes phosphorylate various transport proteins in the sarcolemma and sarcoplasmic reticulum and also in the troponin-tropomyosin regulatory complex, affecting intracellular levels of Ca^{2+} or responses to it. There is a rough correlation between the phosphorylation of TpI and the increased contraction of cardiac muscle induced by catecholamines. This may account for the **inotropic effects** (increased contractility) of β-adrenergic compounds on the heart. Some differences among skeletal, cardiac, and smooth muscle are summarized in Table 49–3.

Ca^{2+} Enters Myocytes via Ca^{2+} Channels & Leaves via the Na^{+}-Ca^{2+} Exchanger & the Ca^{2+} ATPase

As stated above, extracellular Ca^{2+} plays an important role in contraction of cardiac muscle but not in skeletal muscle. This means that Ca^{2+} both enters and leaves myocytes in a regulated manner. We shall briefly consider three transmembrane proteins that play roles in this process.

A. Ca^{2+} Channels

Ca^{2+} enters myocytes via these channels, which allow entry only of Ca^{2+} ions. The major portal of entry is the

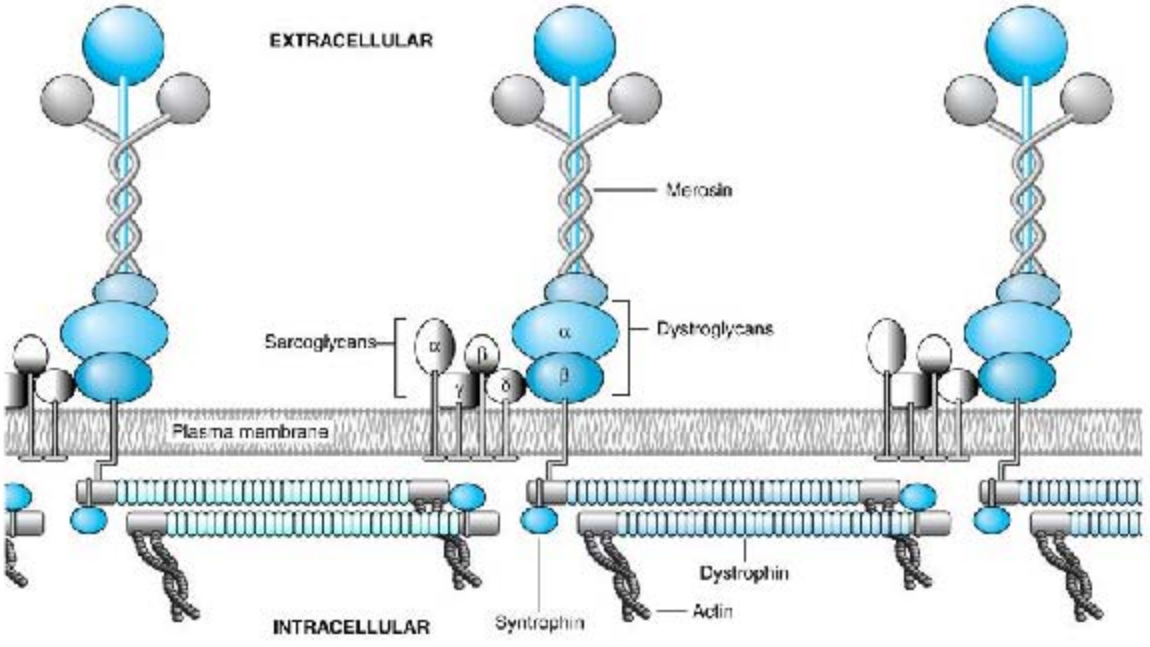

Figure 49–12. Organization of dystrophin and other proteins in relation to the plasma membrane of muscle cells. Dystrophin is part of a large oligomeric complex associated with several other protein complexes. The dystroglycan complex consists of α-dystroglycan, which associates with the basal lamina protein merosin, and β-dystroglycan, which binds α-dystroglycan and dystrophin. Syntrophin binds to the carboxyl terminal of dystrophin. The sarcoglycan complex consists of four transmembrane proteins: α-, β-, γ-, and δ-sarcoglycan. The function of the sarcoglycan complex and the nature of the interactions within the complex and between it and the other complexes are not clear. The sarcoglycan complex is formed only in striated muscle, and its subunits preferentially associate with each other, suggesting that the complex may function as a single unit. Mutations in the gene encoding dystrophin cause Duchenne and Becker muscular dystrophy; mutations in the genes encoding the various sarcoglycans have been shown to be responsible for limb-girdle dystrophies (eg, MIM 601173). (Reproduced, with permission, from Duggan DJ et al: Mutations in the sarcoglycan genes in patients with myopathy. N Engl J Med 1997;336:618.)

L-type (long-duration current, large conductance) or slow Ca^{2+} channel, which is voltage-gated, opening during depolarization induced by spread of the cardiac action potential and closing when the action potential declines. These channels are equivalent to the dihydropyridine receptors of skeletal muscle (Figure 49–8). Slow Ca^{2+} channels are regulated by cAMP-dependent protein kinases (stimulatory) and cGMP-protein kinases (inhibitory) and are blocked by so-called calcium channel blockers (eg, verapamil). Fast (or T, transient) Ca^{2+} channels are also present in the plasmalemma, though in much lower numbers; they probably contribute to the early phase of increase of myoplasmic Ca^{2+}.

The resultant increase of Ca^{2+} in the myoplasm acts on the Ca^{2+} release channel of the sarcoplasmic reticulum to open it. This is called Ca^{2+}-induced Ca^{2+} release (CICR). It is estimated that approximately 10% of the Ca^{2+} involved in contraction enters the cytosol from the extracellular fluid and 90% from the sarcoplasmic reticulum. However, the former 10% is important, as the rate of increase of Ca^{2+} in the myoplasm is important, and entry via the Ca^{2+} channels contributes appreciably to this.

B. Ca^{2+}-Na^+ Exchanger

This is the principal route of exit of Ca^{2+} from myocytes. In resting myocytes, it helps to maintain a low level of free intracellular Ca^{2+} by exchanging one Ca^{2+} for three Na^+. The energy for the uphill movement of Ca^{2+} out of the cell comes from the downhill movement of Na^+ into the cell from the plasma. This exchange contributes to relaxation but may run in the re-

Table 49–3. Some differences between skeletal, cardiac, and smooth muscle.

Skeletal Muscle	Cardiac Muscle	Smooth Muscle
1. Striated.	1. Striated.	1. Nonstriated.
2. No syncytium.	2. Syncytial.	2. Syncytial.
3. Small T tubules.	3. Large T tubules.	3. Generally rudimentary T tubules.
4. Sarcoplasmic reticulum well-developed and Ca^{2+} pump acts rapidly.	4. Sarcoplasmic reticulum present and Ca^{2+} pump acts relatively rapidly.	4. Sarcoplasmic reticulum often rudimentary and Ca^{2+} pump acts slowly.
5. Plasmalemma lacks many hormone receptors.	5. Plasmalemma contains a variety of receptors (eg, α- and β-adrenergic).	5. Plasmalemma contains a variety of receptors (eg, α- and β-adrenergic).
6. Nerve impulse initiates contraction.	6. Has intrinsic rhythmicity.	6. Contraction initiated by nerve impulses, hormones, etc.
7. Extracellular fluid Ca^{2+} not important for contraction.	7. Extracellular fluid Ca^{2+} important for contraction.	7. Extracellular fluid Ca^{2+} important for contraction.
8. Troponin system present.	8. Troponin system present.	8. Lacks troponin system; uses regulatory head of myosin.
9. Caldesmon not involved.	9. Caldesmon not involved.	9. Caldesmon is important regulatory protein.
10. Very rapid cycling of the cross-bridges.	10. Relatively rapid cycling of the cross-bridges.	10. Slow cycling of the cross-bridges permits slow prolonged contraction and less utilization of ATP.

verse direction during excitation. Because of the Ca^{2+}-Na^+ exchanger, anything that causes intracellular Na^+ (Na^+_i) to rise will secondarily cause Ca^{2+}_i to rise, causing more forceful contraction. This is referred to as a positive inotropic effect. One example is when the drug **digitalis** is used to treat heart failure. Digitalis inhibits the sarcolemmal Na^+-K^+ ATPase, diminishing exit of Na^+ and thus increasing Na^+_i. This in turn causes Ca^{2+} to increase, via the Ca^{2+}-Na^+ exchanger. The increased Ca^{2+}_i results in increased force of cardiac contraction, of benefit in heart failure.

C. Ca^{2+} ATPase

This Ca^{2+} pump, situated in the sarcolemma, also contributes to Ca^{2+} exit but is believed to play a relatively minor role as compared with the Ca^{2+}-Na^+ exchanger.

It should be noted that there are a variety of **ion channels** (Chapter 41) in most cells, for Na^+, K^+, Ca^{2+}, etc. Many of them have been cloned in recent years and their dispositions in their respective membranes worked out (number of times each one crosses its membrane, location of the actual ion transport site in the protein, etc). They can be classified as indicated in Table 49–4. Cardiac muscle is rich in ion channels, and they are also important in skeletal muscle. Mutations in genes encoding ion channels have been shown to be responsible for a number of relatively rare conditions affecting muscle. These and other diseases due to mutations of ion channels have been termed **channelopathies;** some are listed in Table 49–5.

Table 49–4. Major types of ion channels found in cells.

Type	Comment
External ligand-gated	Open in response to a specific extracellular molecule, eg, acetylcholine.
Internal ligand-gated	Open or close in response to a specific intracellular molecule, eg, a cyclic nucleotide.
Voltage-gated	Open in response to a change in membrane potential, eg, Na^+, K^+, and Ca^{2+} channels in heart.
Mechanically gated	Open in response to change in mechanical pressure.

Table 49–5. Some disorders (channelopathies) due to mutations in genes encoding polypeptide constituents of ion channels.[1]

Disorder[2]	Ion Channel and Major Organs Involved
Central core disease (MIM 117000)	Ca^{2+} release channel (RYR1) Skeletal muscle
Cystic fibrosis (MIM 219700)	CFTR (Cl^- channel) Lungs, pancreas
Hyperkalemic periodic paralysis (MIM 170500)	Sodium channel Skeletal muscle
Hypokalemic periodic paralysis (MIM 114208)	Slow Ca^{2+} voltage channel (DHPR) Skeletal muscle
Malignant hyperthermia (MIM 180901)	Ca^{2+} release channel (RYR1) Skeletal muscle
Myotonia congenita (MIM 160800)	Chloride channel Skeletal muscle

[1]Data in part from Ackerman NJ, Clapham DE: Ion channels—basic science and clinical disease. N Engl J Med 1997;336:1575.
[2]Other channelopathies include the long QT syndrome (MIM 192500); pseudoaldosteronism (Liddle syndrome, MIM 177200); persistent hyperinsulinemic hypoglycemia of infancy (MIM 601820); hereditary X-linked recessive type II nephrolithiasis of infancy (Dent syndrome, MIM 300009); and generalized myotonia, recessive (Becker disease, MIM 255700). The term "myotonia" signifies any condition in which muscles do not relax after contraction.

Inherited Cardiomyopathies Are Due to Disorders of Cardiac Energy Metabolism or to Abnormal Myocardial Proteins

An inherited cardiomyopathy is any structural or functional abnormality of the ventricular myocardium due to an inherited cause. There are nonheritable types of cardiomyopathy, but these will not be described here. As shown in Table 49–6, the causes of inherited cardiomyopathies fall into two broad classes: (1) disorders of cardiac energy metabolism, mainly reflecting mutations in genes encoding enzymes or proteins involved in fatty acid oxidation (a major source of energy for the myocardium) and oxidative phosphorylation; and (2) mutations in genes encoding proteins involved in or affecting myocardial contraction, such as myosin, tropomyosin, the troponins, and cardiac myosin-binding protein C. Mutations in the genes encoding these latter proteins cause familial hypertrophic cardiomyopathy, which will now be discussed.

Table 49–6. Biochemical causes of inherited cardiomyopathies.[1,2]

Cause	Proteins or Process Affected
Inborn errors of fatty acid oxidation	Carnitine entry into cells and mitochondria Certain enzymes of fatty acid oxidation
Disorders of mitochondrial oxidative phosphorylation	Proteins encoded by mitochondrial genes Proteins encoded by nuclear genes
Abnormalities of myocardial contractile and structural proteins	β-Myosin heavy chains, troponin, tropomyosin, dystrophin

[1]Based on Kelly DP, Strauss AW: Inherited cardiomyopathies. N Engl J Med 1994;330:913.
[2]Mutations (eg, point mutations, or in some cases deletions) in the genes (nuclear or mitochondrial) encoding various proteins, enzymes, or tRNA molecules are the fundamental causes of the inherited cardiomyopathies. Some conditions are mild, whereas others are severe and may be part of a syndrome affecting other tissues.

Mutations in the Cardiac β-Myosin Heavy Chain Gene Are One Cause of Familial Hypertrophic Cardiomyopathy

Familial hypertrophic cardiomyopathy is one of the most frequent hereditary cardiac diseases. Patients exhibit hypertrophy—often massive—of one or both ventricles, starting early in life, and not related to any extrinsic cause such as hypertension. Most cases are transmitted in an autosomal dominant manner; the rest are sporadic. Until recently, its cause was obscure. However, this situation changed when studies of one affected family showed that a **missense mutation** (ie, substitution of one amino acid by another) in the β-myosin heavy chain gene was responsible for the condition. Subsequent studies have shown a number of missense mutations in this gene, all coding for highly conserved residues. Some individuals have shown other mutations, such as formation of an α/β-myosin heavy chain hybrid gene. Patients with familial hypertrophic cardiomyopathy can show great variation in clinical picture. This in part reflects genetic heterogeneity; ie, mutation in a number of **other genes** (eg, those encoding cardiac actin, tropomyosin, cardiac troponins I and T, essential and regulatory myosin light chains, and cardiac myosin-binding protein C) may also cause familial hypertrophic

cardiomyopathy. In addition, mutations at different sites in the gene for β-myosin heavy chain may affect the function of the protein to a greater or lesser extent. The missense mutations are clustered in the head and head-rod regions of myosin heavy chain. One hypothesis is that the mutant polypeptides ("poison polypeptides") cause formation of abnormal myofibrils, eventually resulting in compensatory hypertrophy. Some mutations alter the charge of the amino acid (eg, substitution of arginine for glutamine), presumably affecting the conformation of the protein more markedly and thus affecting its function. Patients with these mutations have a significantly shorter life expectancy than patients in whom the mutation produced no alteration in charge. Thus, definition of the precise mutations involved in the genesis of FHC may prove to be of important prognostic value; it can be accomplished by appropriate use of the polymerase chain reaction on genomic DNA obtained from one sample of blood lymphocytes. Figure 49–13 is a simplified scheme of the events causing familial hypertrophic cardiomyopathy.

Another type of cardiomyopathy is termed **dilated cardiomyopathy.** Mutations in the genes encoding dystrophin, muscle LIM protein (so called because it was found to contain a cysteine-rich domain originally detected in three proteins: Lin-II, Isl-1, and Mec-3), and the cyclic response-element binding protein (CREB) have been implicated in the causation of this condition. The first two proteins help organize the contractile apparatus of cardiac muscle cells, and CREB is involved in the regulation of a number of genes in these cells. Current research is not only elucidating the molecular causes of the cardiomyopathies but is also disclosing mutations that cause **cardiac developmental disorders** (eg, septal defects) and arrhythmias (eg, due to mutations affecting ion channels).

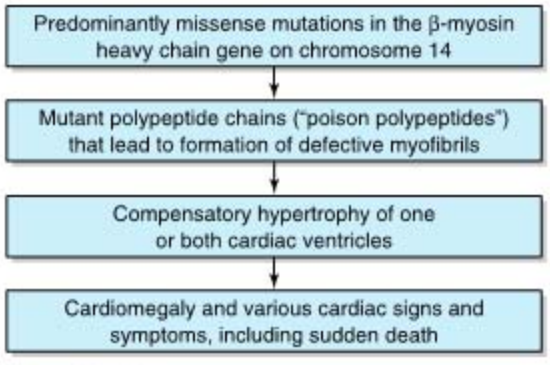

Figure 49–13. Simplified scheme of the causation of familial hypertrophic cardiomyopathy (MIM 192600) due to mutations in the gene encoding β-myosin heavy chain. Mutations in genes encoding other proteins, such as the troponins, tropomyosin, and cardiac myosin-binding protein C can also cause this condition. Mutations in genes encoding yet other proteins (eg, dystrophin) are involved in the causation of dilated cardiomyopathy.

Ca^{2+} Also Regulates Contraction of Smooth Muscle

While all muscles contain actin, myosin, and tropomyosin, only vertebrate **striated** muscles contain the troponin system. Thus, the mechanisms that regulate contraction must differ in various contractile systems.

Smooth muscles have molecular structures similar to those in striated muscle, but the sarcomeres are not aligned so as to generate the striated appearance. Smooth muscles contain α-actinin and tropomyosin molecules, as do skeletal muscles. They do not have the troponin system, and the light chains of smooth muscle myosin molecules differ from those of striated muscle myosin. Regulation of smooth muscle contraction is **myosin-based,** unlike striated muscle, which is actin-based. However, like striated muscle, smooth muscle contraction is regulated by Ca^{2+}.

Phosphorylation of Myosin Light Chains Initiates Contraction of Smooth Muscle

When smooth muscle myosin is bound to F-actin in the absence of other muscle proteins such as tropomyosin, there is no detectable ATPase activity. This absence of activity is quite unlike the situation described for striated muscle myosin and F-actin, which has abundant ATPase activity. Smooth muscle myosin contains light chains that prevent the binding of the myosin head to F-actin; they must be phosphorylated before they allow F-actin to activate myosin ATPase. The ATPase activity then attained hydrolyzes ATP about tenfold more slowly than the corresponding activity in skeletal muscle. The phosphate on the myosin light chains may form a chelate with the Ca^{2+} bound to the tropomyosin-TpC-actin complex, leading to an increased rate of formation of cross-bridges between the myosin heads and actin. The phosphorylation of light chains initiates the attachment-detachment contraction cycle of smooth muscle.

Myosin Light Chain Kinase Is Activated by Calmodulin-4Ca^{2+} & Then Phosphorylates the Light Chains

Smooth muscle sarcoplasm contains a myosin light chain kinase that is calcium-dependent. The Ca^{2+} activation of myosin light chain kinase requires binding of **calmodulin-4Ca^{2+}** to its kinase subunit (Figure 49–14).

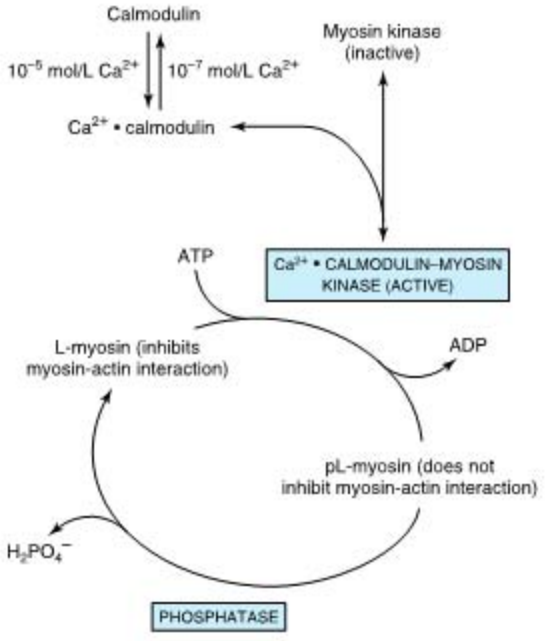

Figure 49–14. **Regulation of smooth muscle contraction by Ca^{2+}. pL-myosin is the phosphorylated light chain of myosin; L-myosin is the dephosphorylated light chain.** (Adapted from Adelstein RS, Eisenberg R: Regulation and kinetics of actin-myosin ATP interaction. Annu Rev Biochem 1980;49:921.)

The calmodulin-4Ca^{2+}-activated light chain kinase phosphorylates the light chains, which then ceases to inhibit the myosin–F-actin interaction. The contraction cycle then begins.

Smooth Muscle Relaxes When the Concentration of Ca^{2+} Falls Below 10^{-7} Molar

Relaxation of smooth muscle occurs when sarcoplasmic Ca^{2+} falls below 10^{-7} mol/L. The Ca^{2+} dissociates from calmodulin, which in turn dissociates from the myosin light chain kinase, inactivating the kinase. No new phosphates are attached to the p-light chain, and light chain protein phosphatase, which is continually active and calcium-independent, removes the existing phosphates from the light chains. Dephosphorylated myosin p-light chain then inhibits the binding of myosin heads to F-actin and the ATPase activity. The myosin head detaches from the F-actin in the presence of ATP, but it cannot reattach because of the presence of dephosphorylated p-light chain; hence, relaxation occurs.

Table 49–7 summarizes and compares the regulation of actin-myosin interactions (activation of myosin ATPase) in striated and smooth muscles.

The myosin light chain kinase is not directly affected or activated by cAMP. However, cAMP-activated protein kinase can phosphorylate the myosin light chain kinase (not the light chains themselves). The phosphorylated myosin light chain kinase exhibits a significantly lower affinity for calmodulin-Ca^{2+} and thus is less sensitive to activation. Accordingly, an increase in cAMP dampens the contraction response of smooth muscle to a given elevation of sarcoplasmic Ca^{2+}. This molecular mechanism can explain the relaxing effect of β-adrenergic stimulation on smooth muscle.

Another protein that appears to play a Ca^{2+}-dependent role in the regulation of smooth muscle contraction is **caldesmon** (87 kDa). This protein is ubiquitous in smooth muscle and is also found in nonmuscle tissue. At low concentrations of Ca^{2+}, it binds to tropomyosin and actin. This prevents interaction of actin with myosin, keeping muscle in a relaxed state. At higher concentrations of Ca^{2+}, Ca^{2+}-calmodulin binds caldesmon, releasing it from actin. The latter is then free to bind to myosin, and contraction can occur. Caldesmon is also subject to phosphorylation-dephosphorylation; when phosphorylated, it cannot bind actin, again freeing the latter to interact with myosin. Caldesmon may also participate in organizing the structure of the contractile apparatus in smooth muscle. Many of its effects have been demonstrated in vitro, and its physiologic significance is still under investigation.

As noted in Table 49–3, slow cycling of the crossbridges permits slow prolonged contraction of smooth muscle (eg, in viscera and blood vessels) with less utilization of ATP compared with striated muscle. The ability of smooth muscle to maintain force at reduced velocities of contraction is referred to as the **latch state;** this is an important feature of smooth muscle, and its precise molecular bases are under study.

Nitric Oxide Relaxes the Smooth Muscle of Blood Vessels & Also Has Many Other Important Biologic Functions

Acetylcholine is a vasodilator that acts by causing relaxation of the smooth muscle of blood vessels. However, it does not act directly on smooth muscle. A key observation was that if endothelial cells were stripped away from underlying smooth muscle cells, acetylcholine no longer exerted its vasodilator effect. This finding indicated that vasodilators such as acetylcholine initially interact with the endothelial cells of small blood vessels via receptors. The receptors are coupled to the phosphoinositide cycle, leading to the intracellular release of

Table 49–7. Actin-myosin interactions in striated and smooth muscle.

	Striated Muscle	Smooth Muscle (and Nonmuscle Cells)
Proteins of muscle filaments	Actin Myosin Tropomyosin Troponin (Tpl, TpT, TpC)	Actin Myosin[1] Tropomyosin
Spontaneous interaction of F-actin and myosin alone (spontaneous activation of myosin ATPase by F-actin	Yes	No
Inhibitor of F-actin–myosin interaction (inhibitor of F-actin–dependent activation of ATPase)	Troponin system (Tpl)	Unphosphorylated myosin light chain
Contraction activated by	Ca^{2+}	Ca^{2+}
Direct effect of Ca^{2+}	$4Ca^{2+}$ bind to TpC	$4Ca^{2+}$ bind to calmodulin
Effect of protein-bound Ca^{2+}	TpC · $4Ca^{2+}$ antagonizes Tpl inhibition of F-actin–myosin interaction (allows F-actin activation of ATPase)	Calmodulin · $4Ca^{2+}$ activates myosin light chain kinase that phosphorylates myosin p-light chain. The phosphorylated p-light chain no longer inhibits F-actin–myosin interaction (allows F-actin activation of ATPase).

[1]Light chains of myosin are different in striated and smooth muscles.

Ca^{2+} through the action of inositol trisphosphate. In turn, the elevation of Ca^{2+} leads to the liberation of **endothelium-derived relaxing factor (EDRF),** which diffuses into the adjacent smooth muscle. There, it reacts with the heme moiety of a soluble guanylyl cyclase, resulting in activation of the latter, with a consequent elevation of intracellular levels of cGMP (Figure 49–15). This in turn stimulates the activities of certain cGMP-dependent protein kinases, which probably phosphorylate specific muscle proteins, causing relaxation; however, the details are still being clarified. The important coronary artery vasodilator **nitroglycerin,** widely used to relieve angina pectoris, acts to increase intracellular release of EDRF and thus of cGMP.

Quite unexpectedly, EDRF was found to be the gas **nitric oxide (NO).** NO is formed by the action of the enzyme NO synthase, which is cytosolic. The endothelial and neuronal forms of NO synthase are activated by Ca^{2+} (Table 49–8). The substrate is arginine, and the products are citrulline and NO:

$$\text{Arginine} \xrightarrow{\text{NO SYNTHASE}} \text{Citrulline} + \text{NO}$$

NO synthase catalyzes a five-electron oxidation of an amidine nitrogen of arginine. L-Hydroxyarginine is an intermediate that remains tightly bound to the enzyme. NO synthase is a very complex enzyme, employing five redox cofactors: NADPH, FAD, FMN, heme, and tetrahydrobiopterin. NO can also be formed from **nitrite,** derived from vasodilators such as glyceryl trinitrate during their metabolism. NO has a very short half-life (approximately 3–4 seconds) in tissues because it reacts with oxygen and superoxide. The product of the reaction with superoxide is peroxynitrite ($ONOO^-$), which decomposes to form the highly reactive $OH^•$ radical. NO is inhibited by hemoglobin and other heme proteins, which bind it tightly. Chemical inhibitors of NO synthase are now available that can markedly decrease formation of NO. Administration of such inhibitors to animals and humans leads to vasoconstriction and a marked elevation of blood pressure, indicating that NO is of major importance in the maintenance of blood pressure in vivo. Another important cardiovascular effect is that by increasing synthesis of cGMP, it acts as an inhibitor of platelet aggregation (Chapter 51).

Since the discovery of the role of NO as a vasodilator, there has been intense experimental interest in this substance. It has turned out to have a variety of physiologic roles, involving virtually every tissue of the body (Table 49–9). Three major isoforms of NO synthase have been identified, each of which has been cloned, and the chromosomal locations of their genes in hu-

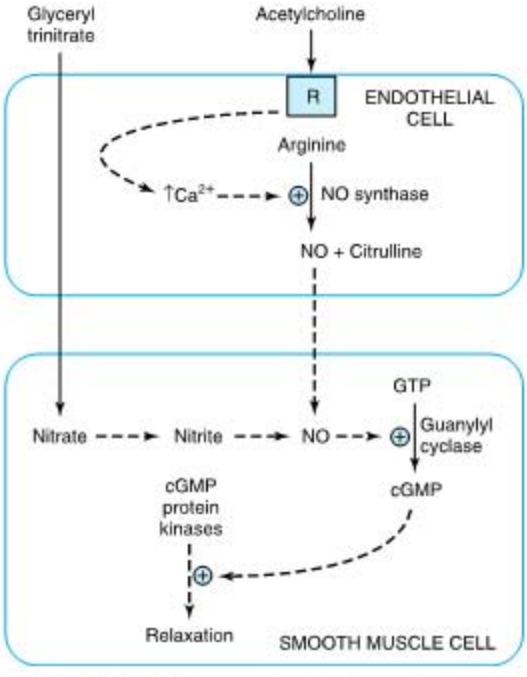

Figure 49–15. Diagram showing formation in an endothelial cell of nitric oxide (NO) from arginine in a reaction catalyzed by NO synthase. Interaction of an agonist (eg, acetylcholine) with a receptor (R) probably leads to intracellular release of Ca^{2+} via inositol trisphosphate generated by the phosphoinositide pathway, resulting in activation of NO synthase. The NO subsequently diffuses into adjacent smooth muscle, where it leads to activation of guanylyl cyclase, formation of cGMP, stimulation of cGMP-protein kinases, and subsequent relaxation. The vasodilator nitroglycerin is shown entering the smooth muscle cell, where its metabolism also leads to formation of NO.

mans have been determined. Gene knockout experiments have been performed on each of the three isoforms and have helped establish some of the postulated functions of NO.

To summarize, research in the past decade has shown that NO plays an important role in many physiologic and pathologic processes.

SEVERAL MECHANISMS REPLENISH STORES OF ATP IN MUSCLE

The ATP required as the constant energy source for the contraction-relaxation cycle of muscle can be generated (1) by glycolysis, using blood glucose or muscle glycogen, (2) by oxidative phosphorylation, (3) from creatine phosphate, and (4) from two molecules of ADP in a reaction catalyzed by adenylyl kinase (Figure 49–16). The amount of ATP in skeletal muscle is only sufficient to provide energy for contraction for a few seconds, so that ATP must be constantly renewed from one or more of the above sources, depending upon metabolic conditions. As discussed below, there are at least two distinct types of fibers in skeletal muscle, one predominantly active in aerobic conditions and the other in anaerobic conditions; not unexpectedly, they use each of the above sources of energy to different extents.

Skeletal Muscle Contains Large Supplies of Glycogen

The sarcoplasm of skeletal muscle contains large stores of glycogen, located in granules close to the I bands. The release of glucose from glycogen is dependent on a specific muscle glycogen phosphorylase (Chapter 18), which can be activated by Ca^{2+}, epinephrine, and AMP. To generate glucose 6-phosphate for glycolysis in skeletal muscle, glycogen phosphorylase b must be activated to phosphorylase a via phosphorylation by phosphorylase b kinase (Chapter 18). Ca^{2+} promotes the activation of phosphorylase b kinase, also by phosphorylation. Thus, Ca^{2+} both initiates muscle contraction and activates a pathway to provide necessary energy. The hormone epinephrine also activates glycogenolysis in muscle. AMP, produced by breakdown of ADP during muscular exercise, can also activate phosphorylase b without causing phosphorylation. Muscle glycogen phosphorylase b is inactive in **McArdle disease,** one of the glycogen storage diseases (Chapter 18).

Under Aerobic Conditions, Muscle Generates ATP Mainly by Oxidative Phosphorylation

Synthesis of ATP via oxidative phosphorylation requires a supply of oxygen. Muscles that have a high demand for oxygen as a result of sustained contraction (eg, to maintain posture) store it attached to the heme moiety of **myoglobin.** Because of the heme moiety, muscles containing myoglobin are red, whereas muscles with little or no myoglobin are white. Glucose, derived from the blood glucose or from endogenous glycogen, and fatty acids derived from the triacylglycerols of adipose tissue are the principal substrates used for aerobic metabolism in muscle.

Creatine Phosphate Constitutes a Major Energy Reserve in Muscle

Creatine phosphate prevents the rapid depletion of ATP by providing a readily available high-energy phosphate that can be used to regenerate ATP from ADP.

Table 49–8. Summary of the nomenclature of the NO synthases and of the effects of knockout of their genes in mice.[1]

Subtype	Name[2]	Comments	Result of Gene Knockout in Mice[3]
1	nNOS	Activity depends on elevated Ca^{2+}. First identified in neurons. Calmodulin-activated.	Pyloric stenosis, resistant to vascular stroke, aggressive sexual behavior (males).
2	iNOS[4]	Independent of elevated Ca^{2+}. Prominent in macrophages.	More susceptible to certain types of infection.
3	eNOS	Activity depends on elevated Ca^{2+}. First identified in endothelial cells.	Elevated mean blood pressure.

[1]Adapted from Snyder SH: No endothelial NO. Nature 1995;377:196.
[2]n, neuronal; i, inducible; e, endothelial.
[3]Gene knockouts were performed by homologous recombination in mice. The enzymes are characterized as neuronal, inducible (macrophage), and endothelial because these were the sites in which they were first identified. However, all three enzymes have been found in other sites, and the neuronal enzyme is also inducible. Each gene has been cloned, and its chromosomal location in humans has been determined.
[4]iNOS is Ca^{2+} -independent but binds calmodulin very tightly.

Creatine phosphate is formed from ATP and creatine (Figure 49–16) at times when the muscle is relaxed and demands for ATP are not so great. The enzyme catalyzing the phosphorylation of creatine is creatine kinase (CK), a muscle-specific enzyme with clinical utility in the detection of acute or chronic diseases of muscle.

SKELETAL MUSCLE CONTAINS SLOW (RED) & FAST (WHITE) TWITCH FIBERS

Different types of fibers have been detected in skeletal muscle. One classification subdivides them into type I (slow twitch), type IIA (fast twitch-oxidative), and type IIB (fast twitch-glycolytic). For the sake of simplicity, we shall consider only two types: type I (slow twitch, oxidative) and type II (fast twitch, glycolytic) (Table 49–10). The type I fibers are red because they contain myoglobin and mitochondria; their metabolism is aerobic, and they maintain relatively sustained contractions. The type II fibers, lacking myoglobin and containing few mitochondria, are white: they derive their energy from anaerobic glycolysis and exhibit relatively short durations of contraction. The proportion of these two types of fibers varies among the muscles of the body, depending on function (eg, whether or not a muscle is involved in sustained contraction, such as maintaining posture). The proportion also varies with training; for example, the number of type I fibers in certain leg muscles increases in athletes training for marathons, whereas the number of type II fibers increases in sprinters.

Table 49–9. Some physiologic functions and pathologic involvements of nitric oxide (NO).

- Vasodilator, important in regulation of blood pressure
- Involved in penile erection; sildenafil citrate (Viagra) affects this process by inhibiting a cGMP phosphodiesterase
- Neurotransmitter in the brain and peripheral autonomic nervous system
- Role in long-term potentiation
- Role in neurotoxicity
- Low level of NO involved in causation of pylorospasm in infantile hypertrophic pyloric stenosis
- May have role in relaxation of skeletal muscle
- May constitute part of a primitive immune system
- Inhibits adhesion, activation, and aggregation of platelets

A Sprinter Uses Creatine Phosphate & Anaerobic Glycolysis to Make ATP, Whereas a Marathon Runner Uses Oxidative Phosphorylation

In view of the two types of fibers in skeletal muscle and of the various energy sources described above, it is of interest to compare their involvement in a sprint (eg, 100 meters) and in the marathon (42.2 km; just over 26 miles) (Table 49–11).

The major sources of energy in the **100-m sprint** are **creatine phosphate** (first 4–5 seconds) and then **anaerobic glycolysis,** using muscle glycogen as the source of glucose. The two main sites of metabolic control are at glycogen phosphorylase and at PFK-1. The former is activated by Ca^{2+} (released from the sarcoplasmic reticulum during contraction), epinephrine, and

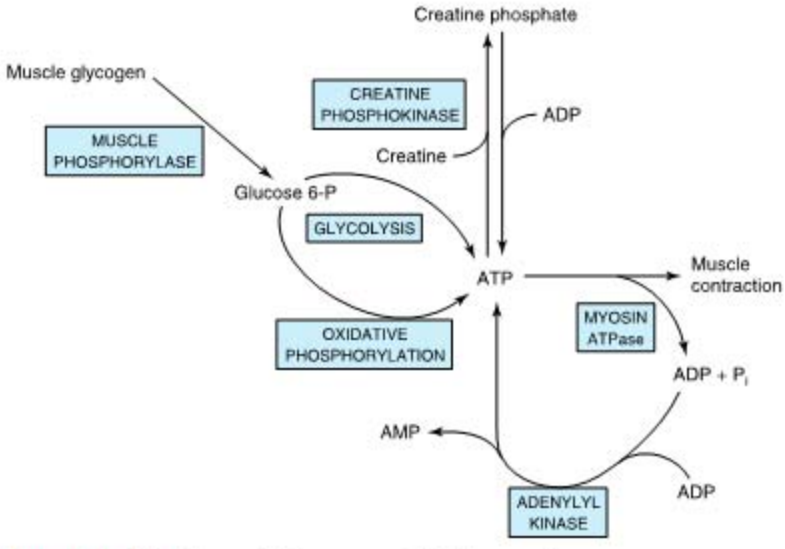

Figure 49–16. The multiple sources of ATP in muscle.

AMP. PFK-1 is activated by AMP, P_i, and NH_3. Attesting to the efficiency of these processes, the flux through glycolysis can increase as much as 1000-fold during a sprint.

In contrast, in the **marathon,** aerobic metabolism is the principal source of ATP. The major fuel sources are blood glucose and free fatty acids, largely derived from the breakdown of triacylglycerols in adipose tissue, stimulated by epinephrine. Hepatic glycogen is degraded to maintain the level of blood glucose. Muscle glycogen is also a fuel source, but it is degraded much more gradually than in a sprint. It has been calculated that the amounts of glucose in the blood, of glycogen in the liver, of glycogen in muscle, and of triacylglycerol in adipose tissue are sufficient to supply muscle with energy during a marathon for 4 minutes, 18 minutes, 70 minutes, and approximately 4000 minutes, respectively. However, the rate of oxidation of fatty acids by muscle is slower than that of glucose, so that oxidation of glucose and of fatty acids are both major sources of energy in the marathon.

A number of procedures have been used by athletes to counteract muscle fatigue and inadequate strength. These include carbohydrate loading, soda (sodium bi-

Table 49–10. Characteristics of type I and type II fibers of skeletal muscle.

	Type I Slow Twitch	Type II Fast Twitch
Myosin ATPase	Low	High
Energy utilization	Low	High
Mitochondria	Many	Few
Color	Red	White
Myoglobin	Yes	No
Contraction rate	Slow	Fast
Duration	Prolonged	Short

Table 49–11. Types of muscle fibers and major fuel sources used by a sprinter and by a marathon runner.

Sprinter (100 m)	Marathon Runner
Type II (glycolytic) fibers are used predominantly.	Type I (oxidative) fibers are used predominantly.
Creatine phosphate is the major energy source during the first 4–5 seconds.	ATP is the major energy source throughout.
Glucose derived from muscle glycogen and metabolized by anaerobic glycolysis is the major fuel source.	Blood glucose and free fatty acids are the major fuel sources.
Muscle glycogen is rapidly depleted.	Muscle glycogen is slowly depleted.

carbonate) loading, blood doping (administration of red blood cells), and ingestion of creatine and androstenedione. Their rationales and efficacies will not be discussed here.

SKELETAL MUSCLE CONSTITUTES THE MAJOR RESERVE OF PROTEIN IN THE BODY

In humans, skeletal muscle protein is the major nonfat source of stored energy. This explains the very large losses of muscle mass, particularly in adults, resulting from prolonged caloric undernutrition.

The study of tissue protein breakdown in vivo is difficult, because amino acids released during intracellular breakdown of proteins can be extensively reutilized for protein synthesis within the cell, or the amino acids may be transported to other organs where they enter anabolic pathways. However, actin and myosin are methylated by a posttranslational reaction, forming **3-methylhistidine.** During intracellular breakdown of actin and myosin, 3-methylhistidine is released and excreted into the urine. The urinary output of the methylated amino acid provides a reliable index of the rate of myofibrillar protein breakdown in the musculature of human subjects.

Various features of muscle metabolism, most of which are dealt with in other chapters of this text, are summarized in Table 49–12.

Table 49–12. Summary of major features of the biochemistry of skeletal muscle related to its metabolism.[1]

- Skeletal muscle functions under both aerobic (resting) and anaerobic (eg, sprinting) conditions, so both aerobic and anaerobic glycolysis operate, depending on conditions.
- Skeletal muscle contains myoglobin as a reservoir of oxygen.
- Skeletal muscle contains different types of fibers primarily suited to anaerobic (fast twitch fibers) or aerobic (slow twitch fibers) conditions.
- Actin, myosin, tropomyosin, troponin complex (TpT, TpI, and TpC), ATP, and Ca^{2+} are key constituents in relation to contraction.
- The Ca^{2+} ATPase, the Ca^{2+} release channel, and calsequestrin are proteins involved in various aspects of Ca^{2+} metabolism in muscle.
- Insulin acts on skeletal muscle to increase uptake of glucose.
- In the fed state, most glucose is used to synthesize glycogen, which acts as a store of glucose for use in exercise; "preloading" with glucose is used by some long-distance athletes to build up stores of glycogen.
- Epinephrine stimulates glycogenolysis in skeletal muscle, whereas glucagon does not because of absence of its receptors.
- Skeletal muscle cannot contribute directly to blood glucose because it does not contain glucose-6-phosphatase.
- Lactate produced by anaerobic metabolism in skeletal muscle passes to liver, which uses it to synthesize glucose, which can then return to muscle (the Cori cycle).
- Skeletal muscle contains phosphocreatine, which acts as an energy store for short-term (seconds) demands.
- Free fatty acids in plasma are a major source of energy, particularly under marathon conditions and in prolonged starvation.
- Skeletal muscle can utilize ketone bodies during starvation.
- Skeletal muscle is the principal site of metabolism of branched-chain amino acids, which are used as an energy source.
- Proteolysis of muscle during starvation supplies amino acids for gluconeogenesis.
- Major amino acids emanating from muscle are alanine (destined mainly for gluconeogenesis in liver and forming part of the glucose-alanine cycle) and glutamine (destined mainly for the gut and kidneys).

[1]This table brings together material from various chapters in this book.

THE CYTOSKELETON PERFORMS MULTIPLE CELLULAR FUNCTIONS

Nonmuscle cells perform mechanical work, including self-propulsion, morphogenesis, cleavage, endocytosis, exocytosis, intracellular transport, and changing cell shape. These cellular functions are carried out by an extensive intracellular network of filamentous structures constituting the **cytoskeleton.** The cell cytoplasm is not a sac of fluid, as once thought. Essentially all eukaryotic cells contain three types of filamentous structures: actin filaments (7–9.5 nm in diameter; also known as microfilaments), microtubules (25 nm), and intermediate filaments (10–12 nm). Each type of filament can be distinguished biochemically and by the electron microscope.

Nonmuscle Cells Contain Actin That Forms Microfilaments

G-actin is present in most if not all cells of the body. With appropriate concentrations of magnesium and potassium chloride, it spontaneously polymerizes to form double helical F-actin filaments like those seen in muscle. There are at least two types of actin in nonmus-

cle cells: β-actin and γ-actin. Both types can coexist in the same cell and probably even copolymerize in the same filament. In the cytoplasm, F-actin forms microfilaments of 7–9.5 nm that frequently exist as bundles of a tangled-appearing meshwork. These bundles are prominent just underlying the plasma membrane of many cells and are there referred to as stress fibers. The stress fibers disappear as cell motility increases or upon malignant transformation of cells by chemicals or oncogenic viruses.

Although not organized as in muscle, actin filaments in nonmuscle cells interact with myosin to cause cellular movements.

Microtubules Contain α- & β-Tubulins

Microtubules, an integral component of the cellular cytoskeleton, consist of cytoplasmic tubes 25 nm in diameter and often of extreme length. Microtubules are necessary for the formation and function of the **mitotic spindle** and thus are present in all eukaryotic cells. They are also involved in the intracellular movement of endocytic and exocytic vesicles and form the major structural components of cilia and flagella. Microtubules are a major component of axons and dendrites, in which they maintain structure and participate in the axoplasmic flow of material along these neuronal processes.

Microtubules are cylinders of 13 longitudinally arranged protofilaments, each consisting of dimers of α-tubulin and β-tubulin, closely related proteins of approximately 50 kDa molecular mass. The tubulin dimers assemble into protofilaments and subsequently into sheets and then cylinders. A microtubule-organizing center, located around a pair of centrioles, nucleates the growth of new microtubules. A third species of tubulin, **γ-tubulin,** appears to play an important role in this assembly. GTP is required for assembly. A variety of proteins are associated with microtubules (microtubule-associated proteins [MAPs], one of which is tau) and play important roles in microtubule assembly and stabilization. Microtubules are in a state of dynamic instability, constantly assembling and disassembling. They exhibit polarity (plus and minus ends); this is important in their growth from centrioles and in their ability to direct intracellular movement. For instance, in axonal transport, the protein **kinesin,** with a myosin-like ATPase activity, uses hydrolysis of ATP to move vesicles down the axon toward the positive end of the microtubular formation. Flow of materials in the opposite direction, toward the negative end, is powered by **cytosolic dynein,** another protein with ATPase activity. Similarly, **axonemal dyneins** power ciliary and flagellar movement. Another protein, **dynamin,** uses GTP and is involved in endocytosis. Kinesins, dyneins, dynamin, and myosins are referred to as **molecular motors.**

An absence of dynein in cilia and flagella results in immotile cilia and flagella, leading to male sterility and chronic respiratory infection, a condition known as **Kartagener syndrome.**

Certain **drugs** bind to microtubules and thus interfere with their assembly or disassembly. These include colchicine (used for treatment of acute gouty arthritis), vinblastine (a vinca alkaloid used for treating certain types of cancer), paclitaxel (Taxol) (effective against ovarian cancer), and griseofulvin (an antifungal agent).

Intermediate Filaments Differ From Microfilaments & Microtubules

An intracellular fibrous system exists of filaments with an axial periodicity of 21 nm and a diameter of 8–10 nm that is intermediate between that of microfilaments (6 nm) and microtubules (23 nm). Four classes of intermediate filaments are found, as indicated in Table 49–13. They are all elongated, fibrous molecules, with a central rod domain, an amino terminal head, and a carboxyl terminal tail. They form a structure like a rope, and the mature filaments are composed of tetramers packed together in a helical manner. They are important structural components of cells, and most are relatively stable components of the cytoskeleton, not undergoing rapid assembly and disassembly and not

Table 49–13. Classes of intermediate filaments of eukaryotic cells and their distributions.

Proteins	Molecular Mass	Distributions
Keratins		
Type I (acidic)	40–60 kDa	Epithelial cells, hair, nails
Type II (basic)	50–70 kDa	
Vimentin-like		
Vimentin	54 kDa	Various mesenchymal cells
Desmin	53 kDa	Muscle
Glial fibrillary acid protein	50 kDa	Glial cells
Peripherin	66 kDa	Neurons
Neurofilaments		
Low (L), medium (M), and high (H)[1]	60–130 kDa	Neurons
Lamins		
A, B, and C	65–75 kDa	Nuclear lamina

[1]Refers to their molecular masses.

disappearing during mitosis, as do actin and many microtubular filaments. An important exception to this is provided by the lamins, which, subsequent to phosphorylation, disassemble at mitosis and reappear when it terminates.

Keratins form a large family, with about 30 members being distinguished. As indicated in Table 49–13, two major types of keratins are found; all individual keratins are heterodimers made up of one member of each class.

Vimentins are widely distributed in mesodermal cells, and desmin, glial fibrillary acidic protein, and peripherin are related to them. All members of the vimentin-like family can copolymerize with each other. Intermediate filaments are very prominent in nerve cells; neurofilaments are classified as low, medium, and high on the basis of their molecular masses. **Lamins** form a meshwork in apposition to the inner nuclear membrane. The distribution of intermediate filaments in normal and abnormal (eg, cancer) cells can be studied by the use of immunofluorescent techniques, using antibodies of appropriate specificities. These antibodies to specific intermediate filaments can also be of use to pathologists in helping to decide the origin of certain dedifferentiated malignant tumors. These tumors may still retain the type of intermediate filaments found in their cell of origin.

A number of **skin diseases,** mainly characterized by blistering, have been found to be due to mutations in genes encoding various keratins. Three of these disorders are epidermolysis bullosa simplex, epidermolytic hyperkeratosis, and epidermolytic palmoplantar keratoderma. The blistering probably reflects a diminished capacity of various layers of the skin to resist mechanical stresses due to abnormalities in microfilament structure.

SUMMARY

- The myofibrils of skeletal muscle contain thick and thin filaments. The thick filaments contain myosin. The thin filaments contain actin, tropomyosin, and the troponin complex (troponins T, I, and C).
- The sliding filament cross-bridge model is the foundation of current thinking about muscle contraction. The basis of this model is that the interdigitating filaments slide past one another during contraction and cross-bridges between myosin and actin generate and sustain the tension.
- The hydrolysis of ATP is used to drive movement of the filaments. ATP binds to myosin heads and is hydrolyzed to ADP and P_i by the ATPase activity of the actomyosin complex.
- Ca^{2+} plays a key role in the initiation of muscle contraction by binding to troponin C. In skeletal muscle, the sarcoplasmic reticulum regulates distribution of Ca^{2+} to the sarcomeres, whereas inflow of Ca^{2+} via Ca^{2+} channels in the sarcolemma is of major importance in cardiac and smooth muscle.
- Many cases of malignant hyperthermia in humans are due to mutations in the gene encoding the Ca^{2+} release channel.
- A number of differences exist between skeletal and cardiac muscle; in particular, the latter contains a variety of receptors on its surface.
- Some cases of familial hypertrophic cardiomyopathy are due to missense mutations in the gene coding for β-myosin heavy chain.
- Smooth muscle, unlike skeletal and cardiac muscle, does not contain the troponin system; instead, phosphorylation of myosin light chains initiates contraction.
- Nitric oxide is a regulator of vascular smooth muscle; blockage of its formation from arginine causes an acute elevation of blood pressure, indicating that regulation of blood pressure is one of its many functions.
- Duchenne-type muscular dystrophy is due to mutations in the gene, located on the X chromosome, encoding the protein dystrophin.
- Two major types of muscle fibers are found in humans: white (anaerobic) and red (aerobic). The former are particularly used in sprints and the latter in prolonged aerobic exercise. During a sprint, muscle uses creatine phosphate and glycolysis as energy sources; in the marathon, oxidation of fatty acids is of major importance during the later phases.
- Nonmuscle cells perform various types of mechanical work carried out by the structures constituting the cytoskeleton. These structures include actin filaments (microfilaments), microtubules (composed primarily of α- tubulin and β-tubulin), and intermediate filaments. The latter include keratins, vimentin-like proteins, neurofilaments, and lamins.

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Plasma Proteins & Immunoglobulins 50

Robert K. Murray, MD, PhD

BIOMEDICAL IMPORTANCE

The fundamental role of blood in the maintenance of **homeostasis** and the ease with which blood can be obtained have meant that the study of its constituents has been of central importance in the development of biochemistry and clinical biochemistry. The basic properties of a number of plasma proteins, including the immunoglobulins (antibodies), are described in this chapter. Changes in the amounts of various plasma proteins and immunoglobulins occur in many diseases and can be monitored by electrophoresis or other suitable procedures. As indicated in an earlier chapter, alterations of the activities of certain **enzymes** found in plasma are of diagnostic use in a number of pathologic conditions.

THE BLOOD HAS MANY FUNCTIONS

The functions of blood—except for specific cellular ones such as oxygen transport and cell-mediated immunologic defense—are carried out by plasma and its constituents (Table 50–1).

Plasma consists of water, electrolytes, metabolites, nutrients, proteins, and hormones. The water and electrolyte composition of plasma is practically the same as that of all extracellular fluids. Laboratory determinations of levels of Na^+, K^+, Ca^{2+}, Cl^-, HCO_3^-, $PaCO_2$, and of blood pH are important in the management of many patients.

PLASMA CONTAINS A COMPLEX MIXTURE OF PROTEINS

The concentration of total protein in human plasma is approximately 7.0–7.5 g/dL and comprises the major part of the solids of the plasma. The proteins of the plasma are actually a complex mixture that includes not only simple proteins but also conjugated proteins such as **glycoproteins** and various types of **lipoproteins.** Thousands of **antibodies** are present in human plasma, though the amount of any one antibody is usually quite low under normal circumstances. The relative dimensions and molecular masses of some of the most important plasma proteins are shown in Figure 50–1.

The **separation** of individual proteins from a complex mixture is frequently accomplished by the use of solvents or electrolytes (or both) to remove different protein fractions in accordance with their solubility characteristics. This is the basis of the so-called salting-out methods, which find some usage in the determination of protein fractions in the clinical laboratory. Thus, one can separate the proteins of the plasma into three major groups—**fibrinogen, albumin,** and **globulins**—by the use of varying concentrations of sodium or ammonium sulfate.

The most common method of analyzing plasma proteins is by **electrophoresis.** There are many types of electrophoresis, each using a different supporting medium. In clinical laboratories, **cellulose acetate** is widely used as a supporting medium. Its use permits resolution, after staining, of plasma proteins into five bands, designated albumin, α_1, α_2, β, and γ fractions, respectively (Figure 50–2). The stained strip of cellulose acetate (or other supporting medium) is called an electrophoretogram. The amounts of these five bands can be conveniently quantified by use of densitometric scanning machines. Characteristic changes in the amounts of one or more of these five bands are found in many diseases.

The Concentration of Protein in Plasma Is Important in Determining the Distribution of Fluid Between Blood & Tissues

In arterioles, the **hydrostatic pressure** is about 37 mm Hg, with an interstitial (tissue) pressure of 1 mm Hg opposing it. The **osmotic pressure** (oncotic pressure) exerted by the plasma proteins is approximately 25 mm Hg. Thus, a net outward force of about 11 mm Hg drives fluid out into the interstitial spaces. In venules, the hydrostatic pressure is about 17 mm Hg, with the oncotic and interstitial pressures as described above; thus, a net force of about 9 mm Hg attracts water back into the circulation. The above pressures are often referred to as the **Starling forces.** If the concentration of plasma proteins is markedly diminished (eg, due to severe protein malnutrition), fluid is not attracted back into the intravascular compartment and accumulates in the extravascular tissue spaces, a condition known as **edema.** Edema has many causes; protein deficiency is one of them.

Table 50–1. Major functions of blood.

(1)	**Respiration**—transport of oxygen from the lungs to the tissues and of CO_2 from the tissues to the lungs
(2)	**Nutrition**—transport of absorbed food materials
(3)	**Excretion**—transport of metabolic waste to the kidneys, lungs, skin, and intestines for removal
(4)	Maintenance of the normal **acid-base balance** in the body
(5)	Regulation of **water balance** through the effects of blood on the exchange of water between the circulating fluid and the tissue fluid
(6)	Regulation of **body temperature** by the distribution of body heat
(7)	**Defense** against infection by the white blood cells and circulating antibodies
(8)	Transport of **hormones** and regulation of metabolism
(9)	Transport of **metabolites**
(10)	**Coagulation**

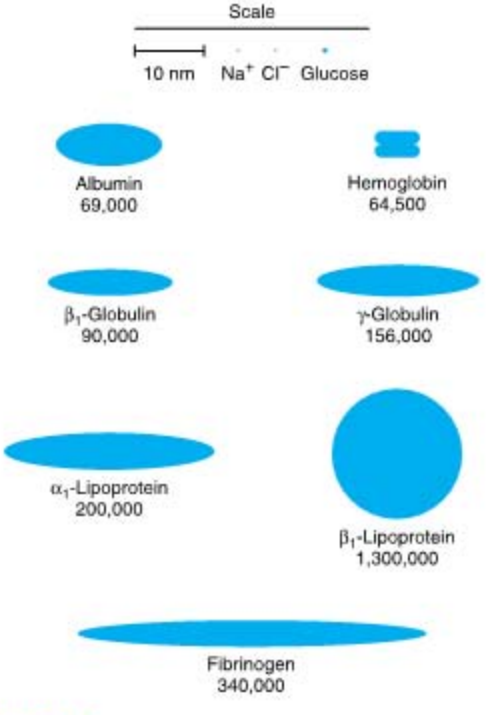

Figure 50–1. Relative dimensions and approximate molecular masses of protein molecules in the blood (Oncley).

Plasma Proteins Have Been Studied Extensively

Because of the relative ease with which they can be obtained, plasma proteins have been studied extensively in both humans and animals. Considerable information is available about the biosynthesis, turnover, structure, and functions of the major plasma proteins. Alterations of their amounts and of their metabolism in many disease states have also been investigated. In recent years, many of the genes for plasma proteins have been cloned and their structures determined.

The preparation of **antibodies** specific for the individual plasma proteins has greatly facilitated their study, allowing the precipitation and isolation of pure proteins from the complex mixture present in tissues or plasma. In addition, the use of **isotopes** has made possible the determination of their pathways of biosynthesis and of their turnover rates in plasma.

The following generalizations have emerged from studies of plasma proteins.

A. Most Plasma Proteins Are Synthesized in the Liver

This has been established by experiments at the whole-animal level (eg, hepatectomy) and by use of the isolated perfused liver preparation, of liver slices, of liver homogenates, and of in vitro translation systems using preparations of mRNA extracted from liver. However, the γ-globulins are synthesized in plasma cells and certain plasma proteins are synthesized in other sites, such as endothelial cells.

B. Plasma Proteins Are Generally Synthesized on Membrane-Bound Polyribosomes

They then traverse the major secretory route in the cell (rough endoplasmic membrane → smooth endoplasmic membrane → Golgi apparatus → secretory vesicles) prior to entering the plasma. Thus, most plasma proteins are synthesized as **preproteins** and initially contain amino terminal signal peptides (Chapter 46). They are usually subjected to various posttranslational modifications (proteolysis, glycosylation, phosphorylation, etc) as they travel through the cell. Transit times through the hepatocyte from the site of synthesis to the plasma vary from 30 minutes to several hours or more for individual proteins.

C. Most Plasma Proteins Are Glycoproteins

Accordingly, they generally contain either N- or O-linked oligosaccharide chains, or both (Chapter 47). Albumin is the major exception; it does not contain sugar residues. The oligosaccharide chains have various functions (Table 47–2). Removal of terminal sialic acid

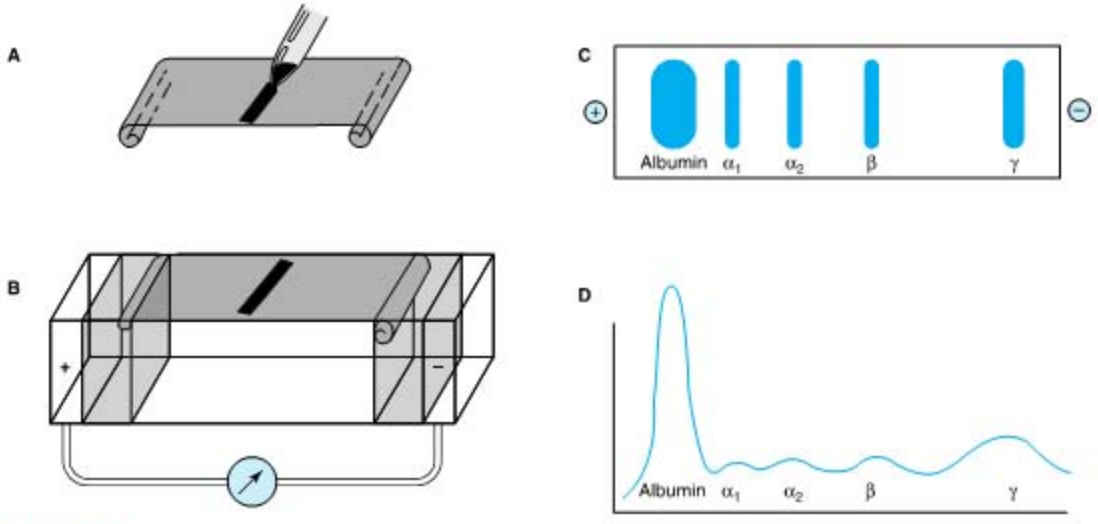

Figure 50–2. Technique of cellulose acetate zone electrophoresis. **A:** A small amount of serum or other fluid is applied to a cellulose acetate strip. **B:** Electrophoresis of sample in electrolyte buffer is performed. **C:** Separated protein bands are visualized in characteristic positions after being stained. **D:** Densitometer scanning from cellulose acetate strip converts bands to characteristic peaks of albumin, α_1-globulin, α_2-globulin, β-globulin, and γ-globulin. (Reproduced, with permission, from Parslow TG et al [editors]: *Medical Immunology*, 10th ed. McGraw-Hill, 2001.)

residues from certain plasma proteins (eg, ceruloplasmin) by exposure to neuraminidase can markedly shorten their half-lives in plasma (Chapter 47).

D. Many Plasma Proteins Exhibit Polymorphism

A polymorphism is a mendelian or monogenic trait that exists in the population in at least two phenotypes, neither of which is rare (ie, neither of which occurs with frequency of less than 1–2%). The ABO blood group substances (Chapter 52) are the best-known examples of human polymorphisms. Human plasma proteins that exhibit polymorphism include α_1-antitrypsin, haptoglobin, transferrin, ceruloplasmin, and immunoglobulins. The polymorphic forms of these proteins can be distinguished by different procedures (eg, various types of electrophoresis or isoelectric focusing), in which each form may show a characteristic migration. Analyses of these human polymorphisms have proved to be of genetic, anthropologic, and clinical interest.

E. Each Plasma Protein Has a Characteristic Half-Life in the Circulation

The half-life of a plasma protein can be determined by labeling the isolated pure protein with ^{131}I under mild, nondenaturing conditions. This isotope unites covalently with tyrosine residues in the protein. The labeled protein is freed of unbound ^{131}I and its specific activity (disintegrations per minute per milligram of protein) determined. A known amount of the radioactive protein is then injected into a normal adult subject, and samples of blood are taken at various time intervals for determinations of radioactivity. The values for radioactivity are plotted against time, and the half-life of the protein (the time for the radioactivity to decline from its peak value to one-half of its peak value) can be calculated from the resulting graph, discounting the times for the injected protein to equilibrate (mix) in the blood and in the extravascular spaces. The half-lives obtained for albumin and haptoglobin in normal healthy adults are approximately 20 and 5 days, respectively. In certain diseases, the half-life of a protein may be markedly altered. For instance, in some gastrointestinal diseases such as regional ileitis (Crohn disease), considerable amounts of plasma proteins, including albumin, may be lost into the bowel through the inflamed intestinal mucosa. Patients with this condition have a **protein-losing gastroenteropathy,** and the half-life of injected iodinated albumin in these subjects may be reduced to as little as 1 day.

F. The Levels of Certain Proteins in Plasma Increase During Acute Inflammatory States or Secondary to Certain Types of Tissue Damage

These proteins are called **"acute phase proteins"** (or reactants) and include C-reactive protein (CRP, so-named because it reacts with the C polysaccharide of pneumococci), α_1-antitrypsin, haptoglobin, α_1-acid glycoprotein, and fibrinogen. The elevations of the levels of these proteins vary from as little as 50% to as much as 1000-fold in the case of CRP. Their levels are also usually elevated during chronic inflammatory states and in patients with cancer. These proteins are believed to play a role in the body's response to inflammation. For example, C-reactive protein can stimulate the classic complement pathway, and α_1-antitrypsin can neutralize certain proteases released during the acute inflammatory state. CRP is used as a marker of tissue injury, infection, and inflammation, and there is considerable interest in its use as a predictor of certain types of cardiovascular conditions secondary to atherosclerosis. Interleukin-1 (IL-1), a polypeptide released from mononuclear phagocytic cells, is the principal—but not the sole—stimulator of the synthesis of the majority of acute phase reactants by hepatocytes. Additional molecules such as IL-6 are involved, and they as well as IL-1 appear to work at the level of gene transcription.

Table 50–2 summarizes the functions of many of the plasma proteins. The remainder of the material in this chapter presents basic information regarding selected plasma proteins: albumin, haptoglobin, transferrin, ceruloplasmin, α_1-antitrypsin, α_2-macroglobulin, the immunoglobulins, and the complement system. The lipoproteins are discussed in Chapter 25.

Table 50–2. Some functions of plasma proteins.

Function	Plasma Proteins
Antiproteases	Antichymotrypsin α_1-Antitrypsin (α_1-antiproteinase) α_2-Macroglobulin Antithrombin
Blood clotting	Various coagulation factors, fibrinogen
Enzymes	Function in blood, eg, coagulation factors, cholinesterase Leakage from cells or tissues, eg, aminotransferases
Hormones	Erythropoietin[1]
Immune defense	Immunoglobulins, complement proteins, β_2-microglobulin
Involvement in inflammatory responses	Acute phase response proteins (eg, C-reactive protein, α_1-acid glycoprotein [orosomucoid])
Oncofetal	α_1-Fetoprotein (AFP)
Transport or binding proteins	Albumin (various ligands, including bilirubin, free fatty acids, ions [Ca^{2+}], metals [eg, Cu^{2+}, Zn^{2+}], metheme, steroids, other hormones, and a variety of drugs Ceruloplasmin (contains Cu^{2+}; albumin probably more important in physiologic transport of Cu^{2+}) Corticosteroid-binding globulin (transcortin) (binds cortisol) Haptoglobin (binds extracorpuscular hemoglobin) Lipoproteins (chylomicrons, VLDL, LDL, HDL) Hemopexin (binds heme) Retinol-binding protein (binds retinol) Sex hormone-binding globulin (binds testosterone, estradiol) Thyroid-binding globulin (binds T_4, T_3) Transferrin (transport iron) Transthyretin (formerly prealbumin; binds T_4 and forms a complex with retinol-binding protein)

[1]Various other protein hormones circulate in the blood but are not usually designated as plasma proteins. Similarly, ferritin is also found in plasma in small amounts, but it too is not usually characterized as a plasma protein.

Albumin Is the Major Protein in Human Plasma

Albumin (69 kDa) is the major protein of human plasma (3.4–4.7 g/dL) and makes up approximately 60% of the total plasma protein. About 40% of albumin is present in the plasma, and the other 60% is present in the extracellular space. The liver produces about 12 g of albumin per day, representing about 25% of total hepatic protein synthesis and half its secreted protein. Albumin is initially synthesized as a **preproprotein.** Its **signal peptide** is removed as it passes into the cisternae of the rough endoplasmic reticulum, and a **hexapeptide** at the resulting amino terminal is subsequently cleaved off farther along the secretory pathway. The synthesis of albumin is depressed in a variety of diseases, particularly those of the liver. The plasma of patients with liver disease often shows a decrease in the ratio of albumin to globulins (decreased albumin-globulin ratio). The synthesis of albumin decreases rela-

tively early in conditions of protein malnutrition, such as kwashiorkor.

Mature human albumin consists of one polypeptide chain of 585 amino acids and contains 17 disulfide bonds. By the use of proteases, albumin can be subdivided into three **domains,** which have different functions. Albumin has an ellipsoidal shape, which means that it does not increase the viscosity of the plasma as much as an elongated molecule such as fibrinogen does. Because of its relatively low molecular mass (about 69 kDa) and high concentration, albumin is thought to be responsible for 75–80% of the **osmotic pressure** of human plasma. Electrophoretic studies have shown that the plasma of certain humans lacks albumin. These subjects are said to exhibit **analbuminemia.** One cause of this condition is a mutation that affects splicing. Subjects with analbuminemia show only moderate edema, despite the fact that albumin is the major determinant of plasma osmotic pressure. It is thought that the amounts of the other plasma proteins increase and compensate for the lack of albumin.

Another important function of albumin is its ability to **bind various ligands**. These include free fatty acids (FFA), calcium, certain steroid hormones, bilirubin, and some of the plasma tryptophan. In addition, albumin appears to play an important role in transport of copper in the human body (see below). A variety of drugs, including sulfonamides, penicillin G, dicumarol, and aspirin, are bound to albumin; this finding has important pharmacologic implications.

Preparations of human albumin have been widely used in the treatment of hemorrhagic shock and of burns. However, this treatment is under review because some recent studies have suggested that administration of albumin in these conditions may increase mortality rates.

Haptoglobin Binds Extracorpuscular Hemoglobin, Preventing Free Hemoglobin From Entering the Kidney

Haptoglobin (Hp) is a plasma glycoprotein that binds extracorpuscular hemoglobin (Hb) in a tight noncovalent complex (Hb-Hp). The amount of haptoglobin in human plasma ranges from 40 mg to 180 mg of hemoglobin-binding capacity per deciliter. Approximately 10% of the hemoglobin that is degraded each day is released into the circulation and is thus extracorpuscular. The other 90% is present in old, damaged red blood cells, which are degraded by cells of the histiocytic system. The molecular mass of hemoglobin is approximately 65 kDa, whereas the molecular mass of the simplest polymorphic form of haptoglobin (Hp 1-1) found in humans is approximately 90 kDa. Thus, the Hb-Hp complex has a molecular mass of approximately 155 kDa. Free hemoglobin passes through the glomerulus of the kidney, enters the tubules, and tends to precipitate therein (as can happen after a massive incompatible blood transfusion, when the capacity of haptoglobin to bind hemoglobin is grossly exceeded) (Figure 50–3). However, the Hb-Hp complex is too large to pass through the glomerulus. The function of Hp thus appears to be to prevent loss of free hemoglobin into the kidney. This conserves the valuable iron present in hemoglobin, which would otherwise be lost to the body.

Human haptoglobin exists in three polymorphic forms, known as Hp 1-1, Hp 2-1, and Hp 2-2. Hp 1-1 migrates in starch gel electrophoresis as a single band, whereas Hp 2-1 and Hp 2-2 exhibit much more complex band patterns. Two genes, designated Hp^1 and Hp^2, direct these three phenotypes, with Hp 2-1 being the heterozygous phenotype. It has been suggested that the haptoglobin polymorphism may be associated with the prevalence of many inflammatory diseases.

The levels of haptoglobin in human plasma vary and are of some diagnostic use. Low levels of haptoglobin are found in patients with **hemolytic anemias.** This is explained by the fact that whereas the half-life of haptoglobin is approximately 5 days, the half-life of the Hb-Hp complex is about 90 minutes, the complex being rapidly removed from plasma by hepatocytes. Thus, when haptoglobin is bound to hemoglobin, it is cleared from the plasma about 80 times faster than normally. Accordingly, the level of haptoglobin falls rapidly in situations where hemoglobin is constantly being released from red blood cells, such as occurs in hemolytic anemias. Haptoglobin is an acute phase protein, and its plasma level is elevated in a variety of inflammatory states.

Certain other plasma proteins bind heme but not hemoglobin. Hemopexin is a β_1-globulin that binds free heme. Albumin will bind some metheme (ferric heme) to form methemalbumin, which then transfers the metheme to hemopexin.

Absorption of Iron From the Small Intestine Is Tightly Regulated

Transferrin (Tf) is a plasma protein that plays a central role in transporting iron around the body to sites where

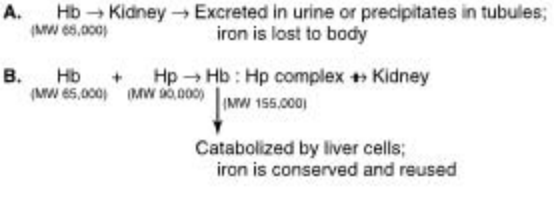

Figure 50–3. Different fates of free hemoglobin and of the hemoglobin-haptoglobin complex.

it is needed. Before we discuss it further, certain aspects of iron metabolism will be reviewed.

Iron is important in the human body because of its occurrence in many hemoproteins such as hemoglobin, myoglobin, and the cytochromes. It is ingested in the diet either as heme or nonheme iron (Figure 50–4); as shown, these different forms involve separate pathways. Absorption of iron in the proximal duodenum is tightly regulated, as there is no physiologic pathway for its excretion from the body. Under normal circumstances, the body guards its content of iron zealously, so that a healthy adult male loses only about 1 mg/d, which is replaced by absorption. Adult females are more prone to states of iron deficiency because some may lose excessive blood during menstruation. The amounts of iron in various body compartments are shown in Table 50–3.

Table 50–3. Distribution of iron in a 70-kg adult male.[1]

Transferrin	3–4 mg
Hemoglobin in red blood cells	2500 mg
In myoglobin and various enzymes	300 mg
In stores (ferritin and hemosiderin)	1000 mg
Absorption	1 mg/d
Losses	1 mg/d

[1]In an adult female of similar weight, the amount in stores would generally be less (100–400 mg) and the losses would be greater (1.5–2 mg/d).

Enterocytes in the proximal duodenum are responsible for absorption of iron. Incoming iron in the Fe^{3+} state is reduced to Fe^{2+} by a **ferrireductase** present on the surface of enterocytes. Vitamin C in food also favors reduction of ferric iron to ferrous iron. The transfer of iron from the apical surfaces of enterocytes into their interiors is performed by a proton-coupled divalent metal transporter (DMT1). This protein is not specific for iron, as it can transport a wide variety of divalent cations.

Once inside an enterocyte, iron can either be stored as ferritin or transferred across the basolateral membrane into the plasma, where it is carried by transferrin (see below). Passage across the basolateral membrane appears to be carried out by another protein, possibly iron regulatory protein 1 (IREG1). This protein may interact with the copper-containing protein hephaestin, a protein similar to ceruloplasmin (see below). Hephaestin is thought to have a ferroxidase activity, which is important in the release of iron from cells. Thus, Fe^{2+} is converted back to Fe^{3+}, the form in which it is transported in the plasma by transferrin.

Overall regulation of iron absorption is complex and not well understood mechanistically. It occurs at

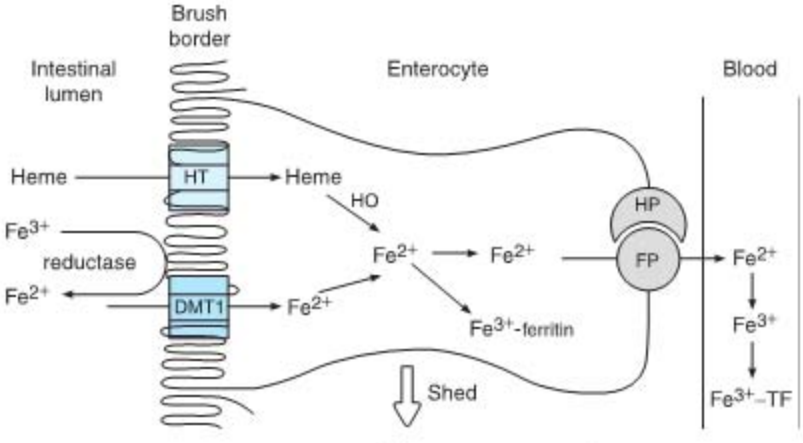

Figure 50–4. Absorption of iron. Fe^{3+} is converted to Fe^{2+} by ferric reductase, and Fe^{2+} is transported into the enterocyte by the apical membrane iron transporter DMT1. Heme is transported into the enterocyte by a separate heme transporter (HT), and heme oxidase (HO) releases Fe^{2+} from the heme. Some of the intracellular Fe^{2+} is converted to Fe^{3+} and bound by ferritin. The remainder binds to the basolateral Fe^{2+} transporter (FP) and is transported into the bloodstream, aided by hephaestin (HP). In plasma, Fe^{3+} is bound to the iron transport protein transferrin (TF). (Reproduced, with permission, from Ganong WF: *Review of Medical Physiology*, 21st ed. McGraw-Hill, 2003.)

the level of the enterocyte, where further absorption of iron is blocked if a sufficient amount has been taken up (so-called dietary regulation exerted by "mucosal block"). It also appears to be responsive to the overall requirement of erythropoiesis for iron (erythropoietic regulation). Absorption is excessive in hereditary hemochromatosis (see below).

Transferrin Shuttles Iron to Sites Where It Is Needed

Transferrin (Tf) is a β_1-globulin with a molecular mass of approximately 76 kDa. It is a glycoprotein and is synthesized in the liver. About 20 polymorphic forms of transferrin have been found. It plays a central role in the body's metabolism of iron because it transports iron (2 mol of Fe^{3+} per mole of Tf) in the circulation to sites where iron is required, eg, from the gut to the bone marrow and other organs. Approximately 200 billion red blood cells (about 20 mL) are catabolized per day, releasing about 25 mg of iron into the body—most of which will be transported by transferrin.

There are receptors (TfRs) on the surfaces of many cells for transferrin. It binds to these receptors and is internalized by receptor-mediated endocytosis (compare the fate of LDL; Chapter 25). The acid pH inside the lysosome causes the iron to dissociate from the protein. The dissociated iron leaves the endosome via DMT1 to enter the cytoplasm. Unlike the protein component of LDL, apoTf is not degraded within the lysosome. Instead, it remains associated with its receptor, returns to the plasma membrane, dissociates from its receptor, reenters the plasma, picks up more iron, and again delivers the iron to needy cells.

Abnormalities of the glycosylation of transferrin occur in the congenital disorders of glycosylation (Chapter 47) and in chronic alcohol abuse. Their detection by, for example, isoelectric focusing is used to help diagnose these conditions.

Iron Deficiency Anemia Is Extremely Prevalent

Attention to iron metabolism is **particularly important in women** for the reason mentioned above. Additionally, in **pregnancy,** allowances must be made for the growing fetus. Older people with poor dietary habits ("tea and toasters") may develop iron deficiency. Iron deficiency anemia due to inadequate intake, inadequate utilization, or excessive loss of iron is one of the most prevalent conditions seen in medical practice.

The concentration of transferrin in plasma is approximately 300 mg/dL. This amount of transferrin can bind 300 µg of iron per deciliter, so that this represents the **total iron-binding capacity** of plasma. However, the protein is normally only one-third saturated with iron. In **iron deficiency anemia,** the protein is even less saturated with iron, whereas in conditions of storage of excess iron in the body (eg, hemochromatosis) the saturation with iron is much greater than one-third.

Ferritin Stores Iron in Cells

Ferritin is another protein that is important in the metabolism of iron. Under normal conditions, it stores iron that can be called upon for use as conditions require. In conditions of excess iron (eg, hemochromatosis), body stores of iron are greatly increased and much more ferritin is present in the tissues, such as the liver and spleen. Ferritin contains approximately 23% iron, and **apoferritin** (the protein moiety free of iron) has a molecular mass of approximately 440 kDa. Ferritin is composed of 24 subunits of 18.5 kDa, which surround in a micellar form some 3000–4500 ferric atoms. Normally, there is a little ferritin in human plasma. However, in patients with excess iron, the amount of ferritin in plasma is markedly elevated. The amount of ferritin in plasma can be conveniently measured by a sensitive and specific radioimmunoassay and serves as an index of body iron stores.

Synthesis of the **transferrin receptor (TfR)** and that of **ferritin** are reciprocally linked to cellular iron content. Specific untranslated sequences of the mRNAs for both proteins (named **iron response elements)** interact with a cytosolic protein sensitive to variations in levels of cellular iron (iron-responsive element-binding protein). When iron levels are high, cells use stored ferritin mRNA to synthesize ferritin, and the TfR mRNA is degraded. In contrast, when iron levels are low, the TfR mRNA is stabilized and increased synthesis of receptors occurs, while ferritin mRNA is apparently stored in an inactive form. This is an important example of control of expression of proteins at the **translational** level.

Hemosiderin is a somewhat ill-defined molecule; it appears to be a partly degraded form of ferritin but still containing iron. It can be detected by histologic stains (eg, Prussian blue) for iron, and its presence is determined histologically when excessive storage of iron occurs.

Hereditary Hemochromatosis Is Due to Mutations in the *HFE* Gene

Hereditary (primary) hemochromatosis is a very prevalent autosomal recessive disorder in certain parts of the world (eg, Scotland, Ireland, and North America). It is characterized by excessive storage of iron in tissues, leading to tissue damage. Total body iron ranges between 2.5 g and 3.5 g in normal adults; in primary hemochromatosis it usually exceeds 15 g. The accumulated iron

damages organs and tissues such as the liver, pancreatic islets, and heart, perhaps in part due to effects on free radical production (Chapter 52). Melanin and various amounts of iron accumulate in the skin, accounting for the slate-gray color often seen. The precise cause of melanin accumulation is not clear. The frequent coexistence of diabetes mellitus (due to islet damage) and the skin pigmentation led to use of the term **bronze diabetes** for this condition. In 1995, Feder and colleagues isolated a gene, now known as *HFE,* located on chromosome 6 close to the major histocompatibility complex genes. The encoded protein (HFE) was found to be related to MHC class 1 antigens. Initially, two different missense mutations were found in *HFE* in individuals with hereditary hemochromatosis. The more frequent mutation was one that changed cysteinyl residue 282 to a tyrosyl residue (CY282Y), disrupting the structure of the protein. The other mutation changed histidyl residue 63 to an aspartyl residue (H63D). Some patients with hereditary hemochromatosis have neither mutation, perhaps due to other mutations in *HFE* or because one or more other genes may be involved in its causation. Genetic screening for this condition has been evaluated but is not presently recommended. However, testing for *HFE* mutations in individuals with elevated serum iron concentrations may be useful.

HFE has been shown to be located in cells in the crypts of the small intestine, the site of iron absorption. There is evidence that it associates with β_2-microglobulin, an association that may be necessary for its stability, intracellular processing, and cell surface expression. The complex interacts with the transferrin receptor (TfR); how this leads to excessive storage of iron when *HFE* is altered by mutation is under close study. The mouse homolog of *HFE* has been knocked out, resulting in a potentially useful animal model of hemochromatosis.

A scheme of the likely main events in the causation of hereditary hemochromatosis is set forth in Figure 50–5.

Secondary hemochromatosis can occur after repeated transfusions (eg, for treatment of sickle cell anemia), excessive oral intake of iron (eg, by African Bantu peoples who consume alcoholic beverages fermented in containers made of iron), or a number of other conditions.

Table 50–4 summarizes laboratory tests useful in the assessment of patients with abnormalities of iron metabolism.

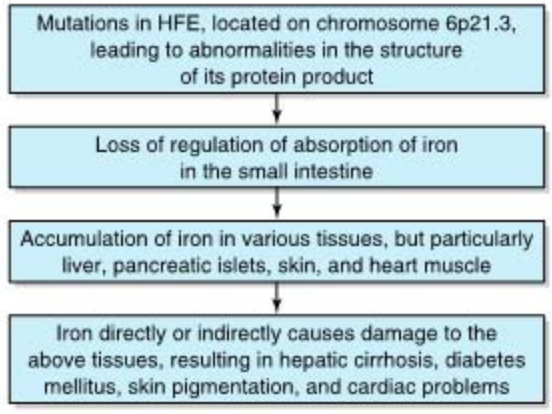

Figure 50–5. Tentative scheme of the main events in causation of primary hemochromatosis (MIM 235200). The two principal mutations are CY282Y and H63D (see text). Mutations in genes other than *HFE* are also involved in some cases.

Ceruloplasmin Binds Copper, & Low Levels of This Plasma Protein Are Associated With Wilson Disease

Ceruloplasmin (about 160 kDa) is an α_2-globulin. It has a blue color because of its high copper content and carries 90% of the copper present in plasma. Each molecule of ceruloplasmin binds six atoms of copper very tightly, so that the copper is not readily exchangeable. Albumin carries the other 10% of the plasma copper but binds the metal less tightly than does ceruloplasmin. Albumin thus donates its copper to tissues more readily than ceruloplasmin and appears to be more important than ceruloplasmin in copper transport in the human body. Ceruloplasmin exhibits a copper-dependent **oxidase** activity, but its physiologic significance has not been clarified. The amount of ceruloplasmin in plasma is decreased in liver disease. In particular, low levels of ceruloplasmin are found in **Wilson disease** (hepatolenticular degeneration), a disease due to abnormal metabolism of copper. In order to clarify the description of Wilson disease, we shall first consider the metabolism of copper in the human body and then **Menkes disease**, another condition involving abnormal copper metabolism.

Table 50–4. Laboratory tests for assessing patients with disorders of iron metabolism.

• Red blood cell count and estimation of hemoglobin
• Determinations of plasma iron, total iron-binding capacity (TIBC), and % transferrin saturation
• Determination of ferritin in plasma by radioimmunoassay
• Prussian blue stain of tissue sections
• Determination of amount of iron (μg/g) in a tissue biopsy

Copper Is a Cofactor for Certain Enzymes

Copper is an essential trace element. It is required in the diet because it is the metal cofactor for a variety of enzymes (see Table 50–5). Copper accepts and donates electrons and is involved in reactions involving dismutation, hydroxylation, and oxygenation. However, excess copper can cause problems because it can oxidize proteins and lipids, bind to nucleic acids, and enhance the production of free radicals. It is thus important to have mechanisms that will maintain the amount of copper in the body within normal limits. The body of the normal adult contains about 100 mg of copper, located mostly in bone, liver, kidney, and muscle. The daily intake of copper is about 2–4 mg, with about 50% being absorbed in the stomach and upper small intestine and the remainder excreted in the feces. Copper is carried to the liver bound to albumin, taken up by liver cells, and part of it is excreted in the bile. Copper also leaves the liver attached to **ceruloplasmin,** which is synthesized in that organ.

The Tissue Levels of Copper & of Certain Other Metals Are Regulated in Part by Metallothioneins

Metallothioneins are a group of small proteins (about 6.5 kDa), found in the cytosol of cells, particularly of liver, kidney, and intestine. They have a high content of cysteine and can bind copper, zinc, cadmium, and mercury. The SH groups of cysteine are involved in binding the metals. Acute intake (eg, by injection) of copper and of certain other metals increases the amount (induction) of these proteins in tissues, as does administration of certain hormones or cytokines. These proteins may function to store the above metals in a nontoxic form and are involved in their overall metabolism in the body. Sequestration of copper also diminishes the amount of this metal available to generate free radicals.

Menkes Disease Is Due to Mutations in the Gene Encoding a Copper-Binding P-Type ATPase

Menkes disease ("kinky" or "steely" hair disease) is a disorder of copper metabolism. It is X-linked, affects only male infants, involves the nervous system, connective tissue, and vasculature, and is usually fatal in infancy. In 1993, it was reported that the basis of Menkes disease was mutations in the gene for a copper-binding P-type ATPase. Interestingly, the enzyme showed structural similarity to certain metal-binding proteins in microorganisms. This ATPase is thought to be responsible for directing the efflux of copper from cells. When altered by mutation, copper is not mobilized normally from the intestine, in which it accumulates, as it does in a variety of other cells and tissues, from which it cannot exit. Despite the accumulation of copper, the activities of many copper-dependent enzymes are decreased, perhaps because of a defect of its incorporation into the apoenzymes. Normal liver expresses very little of the ATPase, which explains the absence of hepatic involvement in Menkes disease. This work led to the suggestion that liver might contain a different copper-binding ATPase, which could be involved in the causation of Wilson disease. As described below, this turned out to be the case.

Table 50–5. Some important enzymes that contain copper.

- Amine oxidase
- Copper-dependent superoxide dismutase
- Cytochrome oxidase
- Tyrosinase

Wilson Disease Is Also Due to Mutations in a Gene Encoding a Copper-Binding P-Type ATPase

Wilson disease is a genetic disease in which copper fails to be excreted in the bile and accumulates in liver, brain, kidney, and red blood cells. It can be regarded as an inability to maintain a near-zero copper balance, resulting in **copper toxicosis.** The increase of copper in liver cells appears to inhibit the coupling of copper to apoceruloplasmin and leads to low levels of ceruloplasmin in plasma. As the amount of copper accumulates, patients may develop a hemolytic anemia, chronic liver disease (cirrhosis, hepatitis), and a neurologic syndrome owing to accumulation of copper in the basal ganglia and other centers. A frequent clinical finding is the **Kayser-Fleischer ring.** This is a green or golden pigment ring around the cornea due to deposition of copper in Descemet's membrane. The major laboratory tests of copper metabolism are listed in Table 50–6. If Wilson disease is suspected, a liver biopsy should be performed; a value for liver copper of over 250 µg per gram dry weight along with a plasma level of ceruloplasmin of under 20 mg/dL is diagnostic.

The cause of Wilson disease was also revealed in 1993, when it was reported that a variety of mutations in a gene encoding a copper-binding P-type ATPase were responsible. The gene is estimated to encode a protein of 1411 amino acids, which is highly homologous to the product of the gene affected in Menkes disease. In a manner not yet fully explained, a nonfunctional ATPase causes defective excretion of copper into the bile, a reduction of incorporation of copper into

Table 50–6. Major laboratory tests used in the investigation of diseases of copper metabolism.[1]

Test	Normal Adult Range
Serum copper	10–22 µmol/L
Ceruloplasmin	200–600 mg/L
Urinary copper	< 1 µmol/24 h
Liver copper	20–50 µg/g dry weight

[1]Based on Gaw A et al: *Clinical Biochemistry.* Churchill Livingstone, 1995.

apoceruloplasmin, and the accumulation of copper in liver and subsequently in other organs such as brain.

Treatment for Wilson disease consists of a diet low in copper along with lifelong administration of **penicillamine,** which chelates copper, is excreted in the urine, and thus depletes the body of the excess of this mineral.

Another condition involving ceruloplasmin is **aceruloplasminemia.** In this genetic disorder, levels of ceruloplasmin are very low and consequently its ferroxidase activity is markedly deficient. This leads to failure of release of iron from cells, and iron accumulates in certain brain cells, hepatocytes, and pancreatic islet cells. Affected individuals show severe neurologic signs and have diabetes mellitus. Use of a chelating agent or administration of plasma or ceruloplasmin concentrate may be beneficial.

Deficiency of α_1-Antiproteinase (α_1-Antitrypsin) Is Associated With Emphysema & One Type of Liver Disease

α_1-Antiproteinase (about 52 kDa) was formerly called **α_1-antitrypsin,** and this name is retained here. It is a single-chain protein of 394 amino acids, contains three oligosaccharide chains, and is the major component (> 90%) of the α_1 fraction of human plasma. It is synthesized by hepatocytes and macrophages and is the principal **serine protease inhibitor** (serpin, or Pi) of human plasma. It inhibits trypsin, elastase, and certain other proteases by forming complexes with them. At least 75 polymorphic forms occur, many of which can be separated by electrophoresis. The major genotype is MM, and its phenotypic product is PiM. There are two areas of clinical interest concerning α_1-antitrypsin. A deficiency of this protein has a role in certain cases (approximately 5%) of **emphysema.** This occurs mainly in subjects with the **ZZ genotype,** who synthesize PiZ, and also in PiSZ heterozygotes, both of whom secrete considerably less protein than PiMM individuals. Considerably less of this protein is secreted as compared with PiM. When the amount of α_1-antitrypsin is deficient and polymorphonuclear white blood cells increase in the lung (eg, during pneumonia), the affected individual lacks a countercheck to proteolytic damage of the lung by proteases such as elastase (Figure 50–6). It is of considerable interest that a particular methionine (residue 358) of α_1-antitrypsin is involved in its binding to proteases. Smoking oxidizes this methionine to methionine sulfoxide and thus inactivates it. As a result, affected molecules of α_1-antitrypsin no longer neutralize proteases. This is particularly devastating in patients (eg, PiZZ phenotype) who already have low levels of α_1-antitrypsin. The further diminution in α_1-antitrypsin brought about by smoking results in increased proteolytic destruction of lung tissue, accelerating the development of emphysema. Intravenous administration of α_1-antitrypsin (augmentation therapy) has been used as an adjunct in the treatment of patients with emphysema due to α_1-antitrypsin deficiency. Attempts are being made, using the techniques of protein engineering, to replace methionine 358 by another residue that would not be subject to oxidation. The resulting "mutant" α_1-antitrypsin would thus afford protection against proteases for a much longer period of time than would native α_1-antitrypsin. Attempts are also being made to develop **gene therapy** for this condition. One approach is to use a modified adenovirus (a pathogen of the respiratory tract) into which the gene for α_1-antitrypsin has been inserted. The virus would then be introduced into the respiratory tract (eg, by an aerosol). The hope is that pulmonary epithelial cells would express the gene and secrete α_1-antitrypsin locally. Experiments in animals have indicated the feasibility of this approach.

Deficiency of α_1-antitrypsin is also implicated in one type of **liver disease** (α_1-antitrypsin deficiency liver

A. Active elastase + α_1–AT → Inactive elastase: α_1–AT complex → No proteolysis of lung → No tissue damage

B. Active elastase + ↓ or no α_1–AT → Active elastase → Proteolysis of lung → Tissue damage

Figure 50–6. Scheme illustrating **(A)** normal inactivation of elastase by α_1-antitrypsin and **(B)** situation in which the amount of α_1-antitrypsin is substantially reduced, resulting in proteolysis by elastase and leading to tissue damage.

disease). In this condition, molecules of the ZZ phenotype accumulate and aggregate in the cisternae of the endoplasmic reticulum of hepatocytes. Aggregation is due to formation of polymers of mutant α_1-antitrypsin, the polymers forming via a strong interaction between a specific loop in one molecule and a prominent β-pleated sheet in another (loop-sheet polymerization). By mechanisms that are not understood, **hepatitis** results with consequent **cirrhosis** (accumulation of massive amounts of collagen, resulting in fibrosis). It is possible that administration of a synthetic peptide resembling the loop sequence could inhibit loop-sheet polymerization. Diseases such as α_1-antitrypsin deficiency, in which cellular pathology is primarily caused by the presence of aggregates of aberrant forms of individual proteins, have been named **conformational diseases.** Most appear to be due to the formation by conformationally unstable proteins of β sheets, which in turn leads to formation of aggregates. Other members of this group of conditions include Alzheimer disease, Parkinson disease, and Huntington disease.

At present, severe α_1-antitrypsin deficiency liver disease can be successfully treated by liver transplantation. In the future, introduction of the gene for normal α_1-antitrypsin into hepatocytes may become possible, but this would not stop production of the PiZ protein. Figure 50–7 is a scheme of the causation of this disease.

α_2-Macroglobulin Neutralizes Many Proteases & Targets Certain Cytokines to Tissues

α_2-Macroglobulin is a large plasma glycoprotein (720 kDa) made up of four identical subunits of 180 kDa. It comprises 8–10% of the total plasma protein in humans. Approximately 10% of the zinc in plasma is transported by α_2-macroglobulin, the remainder being transported by albumin. The protein is synthesized by a variety of cell types, including monocytes, hepatocytes, and astrocytes. It is the major member of a group of plasma proteins that include complement proteins C3 and C4. These proteins contain a unique internal cyclic thiol ester bond (formed between a cysteine and a glutamine residue) and for this reason have been designated as the **thiol ester plasma protein family**.

α_2-Macroglobulin binds many proteinases and is thus an important **panproteinase inhibitor.** The α_2-macroglobulin-proteinase complexes are rapidly cleared from the plasma by a receptor located on many cell types. In addition, α_2-macroglobulin binds many **cytokines** (platelet-derived growth factor, transforming growth factor-β, etc) and appears to be involved in targeting them toward particular tissues or cells. Once taken up by cells, the cytokines can dissociate from α_2-macroglobulin and subsequently exert a variety of effects on cell growth and function. The binding of proteinases and cytokines by α_2-macroglobulin involves different mechanisms that will not be considered here.

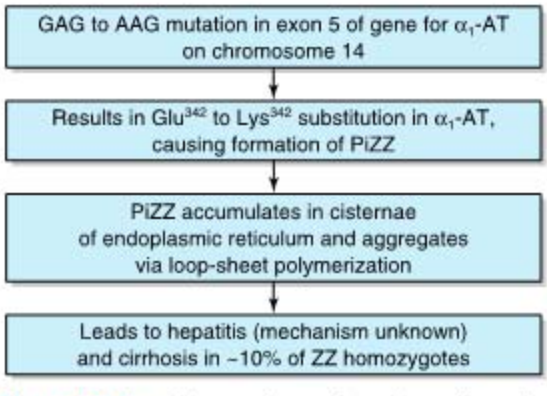

Figure 50–7. Scheme of causation of α_1-antitrypsin-deficiency liver disease. The mutation shown causes formation of PiZZ (MIM 107400). (α_1-AT, α_1-antitrypsin.)

Amyloidosis Occurs by the Deposition of Fragments of Various Plasma Proteins in Tissues

Amyloidosis is the accumulation of various insoluble fibrillar proteins between the cells of tissues to an extent that affects function. The fibrils generally represent proteolytic fragments of various plasma proteins and possess a β-pleated sheet structure. The term "amyloidosis" is a misnomer, as it was originally thought that the fibrils were starch-like in nature. Among the most common **precursor proteins** are immunoglobulin light chains (see below), amyloid-associated protein derived from serum amyloid-associated protein (a plasma glycoprotein), and transthyretin (Table 50–2). The precursor proteins in plasma are generally either increased in amount (eg, immunoglobulin light chains in multiple myeloma or β_2-microglobulin in patients being maintained on chronic dialysis) or mutant forms (eg, of transthyretin in familial amyloidotic neuropathies). The precise factors that determine the deposition of proteolytic fragments in tissues await elucidation. Other proteins have been found in amyloid fibrils, such as calcitonin and amyloid β protein (not derived from a plasma protein) in Alzheimer disease; a total of about 15 different proteins have been found. All fibrils have a **P component** associated with them, which is derived from serum amyloid P component, a plasma protein closely related to C-reactive protein. Tissue sections containing amyloid fibrils interact with Congo red stain

and display striking green birefringence when viewed by polarizing microscopy. Deposition of amyloid occurs in patients with a variety of disorders; treatment of the underlying disorder should be provided if possible.

PLASMA IMMUNOGLOBULINS PLAY A MAJOR ROLE IN THE BODY'S DEFENSE MECHANISMS

The immune system of the body consists of two major components: **B lymphocytes** and **T lymphocytes.** The B lymphocytes are mainly derived from bone marrow cells in higher animals and from the bursa of Fabricius in birds. The T lymphocytes are of thymic origin. The **B cells** are responsible for the synthesis of circulating, humoral antibodies, also known as **immunoglobulins.** The **T cells** are involved in a variety of important **cell-mediated immunologic processes** such as graft rejection, hypersensitivity reactions, and defense against malignant cells and many viruses. This section considers only the plasma immunoglobulins, which are synthesized mainly in **plasma cells.** These are specialized cells of B cell lineage that synthesize and secrete immunoglobulins into the plasma in response to exposure to a variety of **antigens.**

All Immunoglobulins Contain a Minimum of Two Light & Two Heavy Chains

Immunoglobulins contain a minimum of two identical light (L) chains (23 kDa) and two identical heavy (H) chains (53–75 kDa), held together as a tetramer (L_2H_2) by disulfide bonds The structure of IgG is shown in Figure 50–8; it is Y-shaped, with binding of antigen occurring at both tips of the Y. Each chain can be divided conceptually into specific domains, or regions, that have structural and functional significance. The half of the light (L) chain toward the carboxyl terminal is referred to as the constant region (C_L), while the amino terminal half is the variable region of the light chain (V_L). Approximately one-quarter of the heavy (H) chain at the amino terminals is referred to as its variable region (V_H), and the other three-quarters of the heavy chain are referred to as the constant regions (C_H1, C_H2, C_H3) of that H chain. The portion of the immunoglobulin molecule that binds the specific antigen is formed by the amino terminal portions (variable regions) of both the H and L chains—ie, the V_H and V_L domains. The domains of the protein chains consist of two sheets of antiparallel distinct stretches of amino acids that bind antigen.

As depicted in Figure 50–8, digestion of an immunoglobulin by the enzyme **papain** produces two antigen-binding fragments (Fab) and one crystallizable fragment (Fc), which is responsible for functions of immunoglobulins other than direct binding of antigens. Because there are two Fab regions, IgG molecules bind two molecules of antigen and are termed divalent. The site on the antigen to which an antibody binds is termed an **antigenic determinant,** or **epitope.** The area in which papain cleaves the immunoglobulin molecule—ie, the region between the C_H1 and C_H2 domains—is referred to as the **"hinge region."** The hinge region confers flexibility and allows both Fab arms to move independently, thus helping them to bind to antigenic sites that may be variable distances apart (eg, on bacterial surfaces). Fc and hinge regions differ in the different classes of antibodies, but the overall model of antibody structure for each class is similar to that shown in Figure 50–8 for IgG.

All Light Chains Are Either Kappa or Lambda in Type

There are two general types of light chains, kappa (κ) and lambda (λ), which can be distinguished on the basis of structural differences in their C_L regions. A given immunoglobulin molecule always contains two κ or two λ light chains—never a mixture of κ and λ. In humans, the κ chains are more frequent than λ chains in immunoglobulin molecules.

The Five Types of Heavy Chain Determine Immunoglobulin Class

Five classes of H chain have been found in humans (Table 50–7), distinguished by differences in their C_H regions. They are designated γ, α, μ δ, and ε. The μ and ε chains each have four C_H domains rather than the usual three. The type of H chain determines the class of immunoglobulin and thus its effector function. There are thus five immunoglobulin classes: **IgG, IgA, IgM, IgD,** and **IgE.** The biologic functions of these five classes are summarized in Table 50–8.

No Two Variable Regions Are Identical

The variable regions of immunoglobulin molecules consist of the V_L and V_H domains and are quite heterogeneous. In fact, no two variable regions from different humans have been found to have identical amino acid sequences. However, amino acid analyses have shown that the variable regions are comprised of relatively invariable regions and other hypervariable regions (Figure 50–9). L chains have three hypervariable regions (in V_L) and H chains have four (in V_H). These hypervariable regions comprise the antigen-binding site (located at the tips of the Y shown in Figure 50–8) and dictate the amazing specificity of antibodies. For this reason, hypervariable regions are also termed **complementar-**

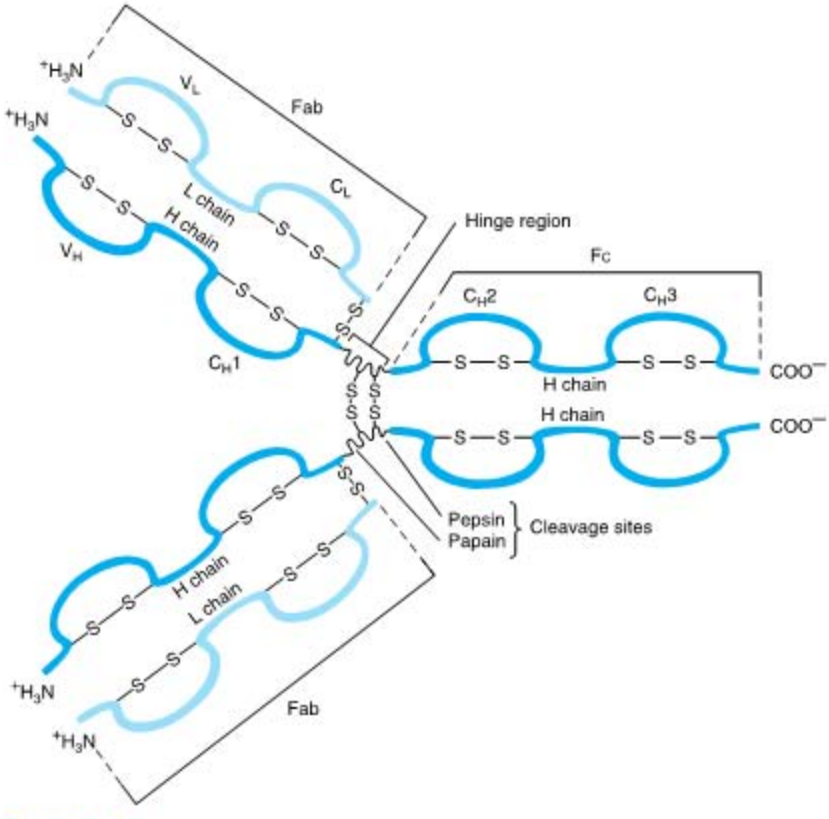

Figure 50–8. Structure of IgG. The molecule consists of two light (L) chains and two heavy (H) chains. Each light chain consists of a variable (V_L) and a constant (C_L) region. Each heavy chain consists of a variable region (V_H) and a constant region that is divided into three domains (C_H1, C_H2, and C_H3). The C_H2 domain contains the complement-binding site and the C_H3 domain contains a site that attaches to receptors on neutrophils and macrophages. The antigen-binding site is formed by the hypervariable regions of both the light and heavy chains, which are located in the variable regions of these chains (see Figure 50–9). The light and heavy chains are linked by disulfide bonds, and the heavy chains are also linked to each other by disulfide bonds. (Reproduced, with permission, from Parslow TG et al [editors]: *Medical Immunology*, 10th ed. McGraw-Hill, 2001.)

ity-determining regions (CDRs). About five to ten amino acids in each hypervariable region (CDR) contribute to the antigen-binding site. CDRs are located on small loops of the variable domains, the surrounding polypeptide regions between the hypervariable regions being termed **framework regions.** CDRs from both V_H and V_L domains, brought together by folding of the polypeptide chains in which they are contained, form a single hypervariable surface comprising the antigen-binding site. Various combinations of H and L chain CDRs can give rise to many antibodies of different specificities, a feature that contributes to the tremendous diversity of antibody molecules and is termed **combinatorial diversity.** Large antigens interact with all of the CDRs of an antibody, whereas small ligands may interact with only one or a few CDRs that form a pocket or groove in the antibody molecule. The essence of antigen-antibody interactions is mutual complementarity between the surfaces of CDRs and epitopes. The interactions between antibodies and antigens involve noncovalent forces and bonds (electrostatic and van der Waals forces and hydrogen and hydrophobic bonds).

Table 50–7. Properties of human immunoglobulins.[1]

Property	IgG	IgA	IgM	IgD	IgE
Percentage of total immunoglobulin in serum (approximate)	75	15	9	0.2	0.004
Serum concentration (mg/dL) (approximate)	1000	200	120	3	0.05
Sedimentation coefficient	7S	7S or 11S[2]	19S	7S	8S
Molecular weight (× 1000)	150	170 or 400[2]	900	180	190
Structure	Monomer	Monomer or dimer	Monomer or dimer	Monomer	Monomer
H-chain symbol	γ	α	μ	δ	ε
Complement fixation	+	–	+	–	–
Transplacental passage	+	–	–	?	–
Mediation of allergic responses	–	–	–	–	+
Found in secretions	–	+	–	–	–
Opsonization	+	–	–[3]	–	–
Antigen receptor on B cell	–	–	+	?	–
Polymeric form contains J chain	–	+	+	–	–

[1]Reproduced, with permission, from Levinson W, Jawetz E: *Medical Microbiology and Immunology,* 7th ed. McGraw-Hill, 2002.
[2]The 11S form is found in secretions (eg, saliva, milk, tears) and fluids of the respiratory, intestinal, and genital tracts.
[3]IgM opsonizes indirectly by activating complement. This produces C3b, which is an opsonin.

The Constant Regions Determine Class-Specific Effector Functions

The constant regions of the immunoglobulin molecules, particularly the C_H2 and C_H3 (and C_H4 of IgM and IgE), which constitute the Fc fragment, are responsible for the class-specific effector functions of the different immunoglobulin molecules (Table 50–7, bottom part), eg, complement fixation or transplacental passage.

Some immunoglobulins such as immune IgG exist only in the basic tetrameric structure, while others such as IgA and IgM can exist as higher order polymers of two, three (IgA), or five (IgM) tetrameric units (Figure 50–10).

The L chains and H chains are synthesized as separate molecules and are subsequently assembled within the B cell or plasma cell into mature immunoglobulin molecules, all of which are **glycoproteins.**

Both Light & Heavy Chains Are Products of Multiple Genes

Each immunoglobulin light chain is the product of at least three separate structural genes: a variable region (V_L) gene, a joining region *(J)* gene (bearing no relationship to the J chain of IgA or IgM), and a constant region (C_L) gene. Each heavy chain is the product of at least four different genes: a variable region (V_H) gene, a diversity region *(D)* gene, a joining region *(J)* gene, and a constant region (C_H) gene. Thus, the "one gene, one protein" concept is not valid. The molecular mechanisms responsible for the generation of the single immunoglobulin chains from multiple structural genes are discussed in Chapters 36 and 39.

Antibody Diversity Depends on Gene Rearrangements

Each person is capable of generating antibodies directed against perhaps 1 million different antigens. The generation of such immense antibody diversity depends upon a number of factors including the existence of multiple gene segments (V, C, J, and D segments), their recombinations (see Chapters 36 and 39), the combinations of different L and H chains, a high frequency of somatic mutations in immunoglobulin genes, and **junctional diversity.** The latter reflects the addi-

Table 50–8. Major functions of immunoglobulins.[1]

Immunoglobulin	Major Functions
IgG	Main antibody in the secondary response. Opsonizes bacteria, making them easier to phagocytose. Fixes complement, which enhances bacterial killing. Neutralizes bacterial toxins and viruses. Crosses the placenta.
IgA	Secretory IgA prevents attachment of bacteria and viruses to mucous membranes. Does not fix complement.
IgM	Produced in the primary response to an antigen. Fixes complement. Does not cross the placenta. Antigen receptor on the surface of B cells.
IgD	Uncertain. Found on the surface of many B cells as well as in serum.
IgE	Mediates immediate hypersensitivity by causing release of mediators from mast cells and basophils upon exposure to antigen (allergen). Defends against worm infections by causing release of enzymes from eosinophils. Does not fix complement. Main host defense against helminthic infections.

[1]Reproduced, with permission, from Levinson W, Jawetz E: *Medical Microbiology and Immunology,* 7th ed. McGraw-Hill, 2002.

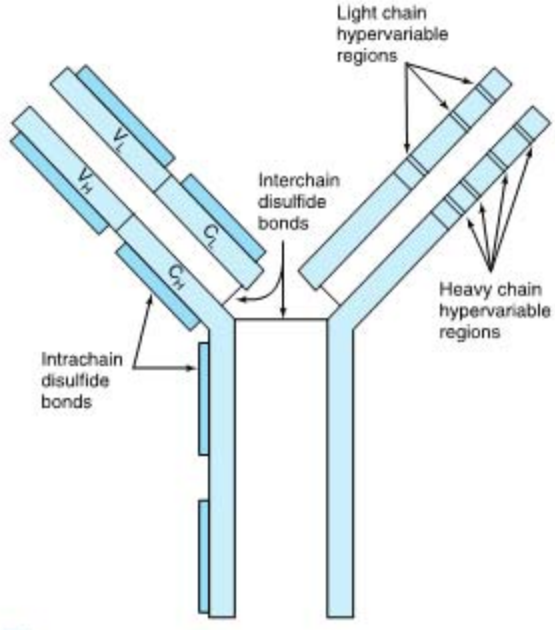

Figure 50–9. Schematic model of an IgG molecule showing approximate positions of the hypervariable regions in heavy and light chains. The antigen-binding site is formed by these hypervariable regions. The hypervariable regions are also called complementarity-determining regions (CDRs). (Modified and reproduced, with permission, from Parslow TG et al [editors]: *Medical Immunology,* 10th ed. McGraw-Hill, 2001.)

tion or deletion of a random number of nucleotides when certain gene segments are joined together, and introduces an additional degree of diversity. Thus, the above factors ensure that a vast number of antibodies can be synthesized from several hundred gene segments.

Class (Isotype) Switching Occurs During Immune Responses

In most humoral immune responses, antibodies with identical specificity but of different classes are generated in a specific chronologic order in response to the immunogen (immunizing antigen). For instance, antibodies of the IgM class normally precede molecules of the IgG class. The switch from one class to another is designated **"class or isotype switching,"** and its molecular basis has been investigated extensively. A single type of immunoglobulin light chain can combine with an antigen-specific μ chain to generate a specific IgM molecule. Subsequently, the same antigen-specific light chain combines with a γ chain with an identical V_H region to generate an IgG molecule with antigen specificity identical to that of the original IgM molecule. The same light chain can also combine with an α heavy chain, again containing the identical V_H region, to form an IgA molecule with identical antigen specificity. These three classes (IgM, IgG, and IgA) of immunoglobulin molecules against the same antigen have identical variable domains of both their light (V_L) chains and heavy (V_H) chains and are said to share an **idiotype.** (Idiotypes are the antigenic determinants formed by the specific amino acids in the hypervariable regions.) The different classes of these three immunoglobulins (called **isotypes**) are thus determined by their different C_H regions, which are combined with the same antigen-specific V_H regions.

Both Over- & Underproduction of Immunoglobulins May Result in Disease States

Disorders of immunoglobulins include increased production of specific classes of immunoglobulins or even

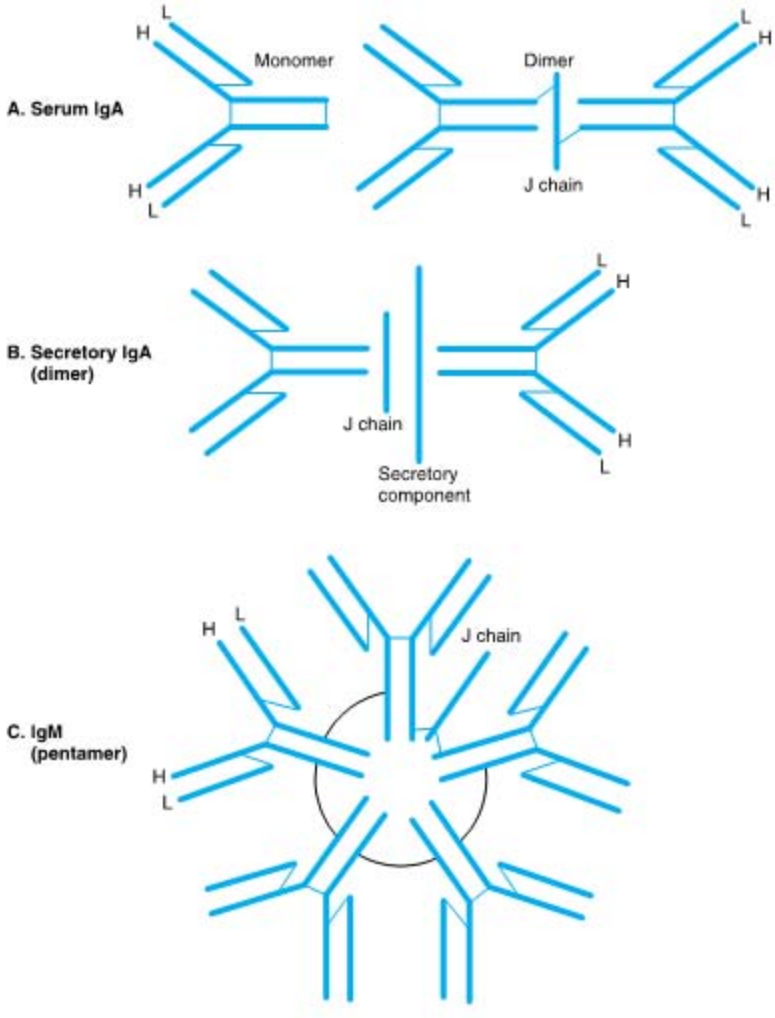

Figure 50–10. Schematic representation of serum IgA, secretory IgA, and IgM. Both IgA and IgM have a J chain, but only secretory IgA has a secretory component. Polypeptide chains are represented by thick lines; disulfide bonds linking different polypeptide chains are represented by thin lines. (Reproduced, with permission, from Parslow TG et al [editors]: *Medical Immunology*, 10th ed. McGraw-Hill, 2001.)

specific immunoglobulin molecules, the latter by clonal tumors of plasma cells called myelomas. **Multiple myeloma** is a neoplastic condition; electrophoresis of serum or urine will usually reveal a large increase of one particular immunoglobulin or one particular light chain (the latter termed a Bence Jones protein). Decreased production may be restricted to a single class of immunoglobulin molecules (eg, IgA or IgG) or may involve underproduction of all classes of immunoglobulins (IgA, IgD, IgE, IgG, and IgM). A severe reduction in synthesis of an immunoglobulin class due to a genetic abnormality can result in a serious immunodeficiency disease—eg, **agammaglobulinemia,** in which production of IgG is markedly affected—because of impairment of the body's defense against microorganisms.

Hybridomas Provide Long-Term Sources of Highly Useful Monoclonal Antibodies

When an antigen is injected into an animal, the resulting antibodies are polyclonal, being synthesized by a mixture of B cells. Polyclonal antibodies are directed against a number of different sites (epitopes or determinants) on the antigen and thus are not monospecific. However, by means of a method developed by Kohler and Milstein, large amounts of a single monoclonal antibody specific for one epitope can be obtained.

The method involves **cell fusion,** and the resulting permanent cell line is called a **hybridoma.** Typically, B cells are obtained from the spleen of a mouse (or other suitable animal) previously injected with an antigen or mixture of antigens (eg, foreign cells). The B cells are

mixed with mouse **myeloma cells** and exposed to polyethylene glycol, which causes cell fusion. A summary of the principles involved in generating hybridoma cells is given in Figure 50–11. Under the conditions used, only the hybridoma cells multiply in cell culture. This involves plating the hybrid cells into hypoxanthine-aminopterin-thymidine (HAT)-containing medium at a concentration such that each dish contains approximately one cell. Thus, a **clone** of hybridoma cells multiplies in each dish. The culture medium is harvested and screened for antibodies that react with the original antigen or antigens. If the immunogen is a mixture of many antigens (eg, a cell membrane preparation), an individual culture dish will contain a clone of hybridoma cells synthesizing a monoclonal antibody to one specific antigenic determinant of the mixture. By harvesting the media from many culture dishes, a battery of monoclonal antibodies can be obtained, many of which are specific for individual components of the immunogenic mixture. The hybridoma cells can be frozen and stored and subsequently thawed when more of the antibody is required; this ensures its long-term supply. The hybridoma cells can also be grown in the abdomen of mice, providing relatively large supplies of antibodies.

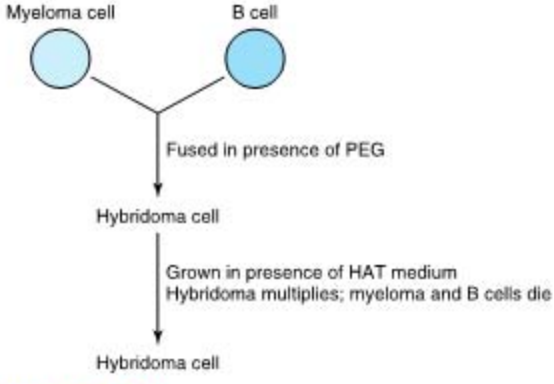

Figure 50–11. Scheme of production of a hybridoma cell. The myeloma cells are immortalized, do not produce antibody, and are $HGPRT^-$ (rendering the salvage pathway of purine synthesis [Chapter 34] inactive). The B cells are not immortalized, each produces a specific antibody, and they are $HGPRT^+$. Polyethylene glycol (PEG) stimulates cell fusion. The resulting hybridoma cells are immortalized (via the parental myeloma cells), produce antibody, and are $HGPRT^+$ (both latter properties gained from the parental B cells). The B cells will die in the medium because they are not immortalized. In the presence of HAT, the myeloma cells will also die, since the aminopterin in HAT suppresses purine synthesis by the de novo pathway by inhibiting reutilization of tetrahydrofolate (Chapter 34). However, the hybridoma cells will survive, grow (because they are $HGPRT^+$), and—if cloned—produce monoclonal antibody. (HAT, hypoxanthine, aminopterin, and thymidine; HGPRT, hypoxanthine-guanine phosphoribosyl transferase.)

Because of their specificity, monoclonal antibodies have become useful reagents in many areas of biology and medicine. For example, they can be used to measure the amounts of many individual proteins (eg, plasma proteins), to determine the nature of infectious agents (eg, types of bacteria), and to subclassify both normal (eg, lymphocytes) and tumor cells (eg, leukemic cells). In addition, they are being used to direct therapeutic agents to tumor cells and also to accelerate removal of drugs from the circulation when they reach toxic levels (eg, digoxin).

The Complement System Comprises About 20 Plasma Proteins & Is Involved in Cell Lysis, Inflammation, & Other Processes

Plasma contains approximately 20 proteins that are members of the complement system. This system was discovered when it was observed that addition of fresh serum containing antibodies directed to a bacterium caused its lysis. Unlike antibodies, the factor was labile when heated at 56 °C. Subsequent work has resolved the proteins of the system and how they function; most have been cloned and sequenced. The major protein components are designated C1–9, with C9 associated with the C5–8 complex (together constituting the **membrane attack complex**) being involved in generating a lipid-soluble pore in the cell membrane that causes **osmotic lysis.**

The details of this system are relatively complex, and a textbook of immunology should be consulted. The basic concept is that the normally inactive proteins of the system, when triggered by a stimulus, become activated by proteolysis and interact in a specific sequence with one or more of the other proteins of the system. This results in cell lysis and generation of **peptide or polypeptide fragments** that are involved in various aspects of inflammation (chemotaxis, phagocytosis, etc). The system has other functions, such as clearance of antigen-antibody complexes from the circulation. Activation of the complement system is triggered by one of two routes, called the **classic** and the **alternative pathways.** The first involves interaction of C1 with antigen-antibody complexes, and the second (not involving antibody) involves direct interaction of bacterial cell surfaces or polysaccharides with a component designated C3b.

The complement system resembles blood coagulation (Chapter 51) in that it involves both conversion of inactive precursors to active products by proteases and a cascade with amplification.

SUMMARY

- Plasma contains many proteins with a variety of functions. Most are synthesized in the liver and are glycosylated.
- Albumin, which is not glycosylated, is the major protein and is the principal determinant of intravascular osmotic pressure; it also binds many ligands, such as drugs and bilirubin.
- Haptoglobin binds extracorpuscular hemoglobin, prevents its loss into the kidney and urine, and hence preserves its iron for reutilization.
- Transferrin binds iron, transporting it to sites where it is required. Ferritin provides an intracellular store of iron. Iron deficiency anemia is a very prevalent disorder. Hereditary hemochromatosis has been shown to be due to mutations in *HFE*, a gene encoding the protein HFE, which appears to play an important role in absorption of iron.
- Ceruloplasmin contains substantial amounts of copper, but albumin appears to be more important with regard to its transport. Both Wilson disease and Menkes disease, which reflect abnormalities of copper metabolism, have been found to be due to mutations in genes encoding copper-binding P-type ATPases.
- α_1-Antitrypsin is the major serine protease inhibitor of plasma, in particular inhibiting the elastase of neutrophils. Genetic deficiency of this protein is a cause of emphysema and can also lead to liver disease.
- α_2-Macroglobulin is a major plasma protein that neutralizes many proteases and targets certain cytokines to specific organs.
- Immunoglobulins play a key role in the defense mechanisms of the body, as do proteins of the complement system. Some of the principal features of these proteins are described.

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Hemostasis & Thrombosis

51

Margaret L. Rand, PhD, & Robert K. Murray, MD, PhD

BIOMEDICAL IMPORTANCE

Basic aspects of the proteins of the blood coagulation system and of fibrinolysis are described in this chapter. Some fundamental aspects of platelet biology are also presented. Hemorrhagic and thrombotic states can cause serious medical emergencies, and thromboses in the coronary and cerebral arteries are major causes of death in many parts of the world. Rational management of these conditions requires a clear understanding of the bases of blood clotting and fibrinolysis.

HEMOSTASIS & THROMBOSIS HAVE THREE COMMON PHASES

Hemostasis is the cessation of bleeding from a cut or severed vessel, whereas thrombosis occurs when the endothelium lining blood vessels is damaged or removed (eg, upon rupture of an atherosclerotic plaque). These processes encompass blood clotting (coagulation) and involve blood vessels, platelet aggregation, and plasma proteins that cause formation or dissolution of platelet aggregates.

In hemostasis, there is initial vasoconstriction of the injured vessel, causing diminished blood flow distal to the injury. Then hemostasis and thrombosis share three phases:

(1) Formation of a loose and temporary platelet aggregate at the site of injury. Platelets bind to collagen at the site of vessel wall injury and are activated by thrombin (the mechanism of activation of platelets is described below), formed in the coagulation cascade at the same site, or by ADP released from other activated platelets. Upon activation, platelets change shape and, in the presence of fibrinogen, aggregate to form the hemostatic plug (in hemostasis) or thrombus (in thrombosis).

(2) Formation of a fibrin mesh that binds to the platelet aggregate, forming a more stable hemostatic plug or thrombus.

(3) Partial or complete dissolution of the hemostatic plug or thrombus by plasmin.

There Are Three Types of Thrombi

Three types of thrombi or clots are distinguished. All three contain **fibrin** in various proportions.

(1) The **white** thrombus is composed of platelets and fibrin and is relatively poor in erythrocytes. It forms at the site of an injury or abnormal vessel wall, particularly in areas where blood flow is rapid (arteries).

(2) The **red** thrombus consists primarily of red cells and fibrin. It morphologically resembles the clot formed in a test tube and may form in vivo in areas of retarded blood flow or stasis (eg, veins) with or without vascular injury, or it may form at a site of injury or in an abnormal vessel in conjunction with an initiating platelet plug.

(3) A third type is a disseminated **fibrin deposit** in very small blood vessels or capillaries.

We shall first describe the coagulation pathway leading to the formation of fibrin. Then we shall briefly describe some aspects of the involvement of platelets and blood vessel walls in the overall process. This separation of clotting factors and platelets is artificial, since both play intimate and often mutually interdependent roles in hemostasis and thrombosis, but it facilitates description of the overall processes involved.

Both Intrinsic & Extrinsic Pathways Result in the Formation of Fibrin

Two pathways lead to fibrin clot formation: the intrinsic and the extrinsic pathways. These pathways are not independent, as previously thought. However, this artificial distinction is retained in the following text to facilitate their description.

Initiation of the fibrin clot in response to tissue injury is carried out by the extrinsic pathway. How the intrinsic pathway is activated in vivo is unclear, but it involves a negatively charged surface. The intrinsic and extrinsic pathways converge in a **final common pathway** involving the activation of prothrombin to thrombin and the thrombin-catalyzed cleavage of fibrinogen to form the fibrin clot. The intrinsic, extrinsic, and final common pathways are complex and involve many different proteins (Figure 51–1 and Table 51–1). In

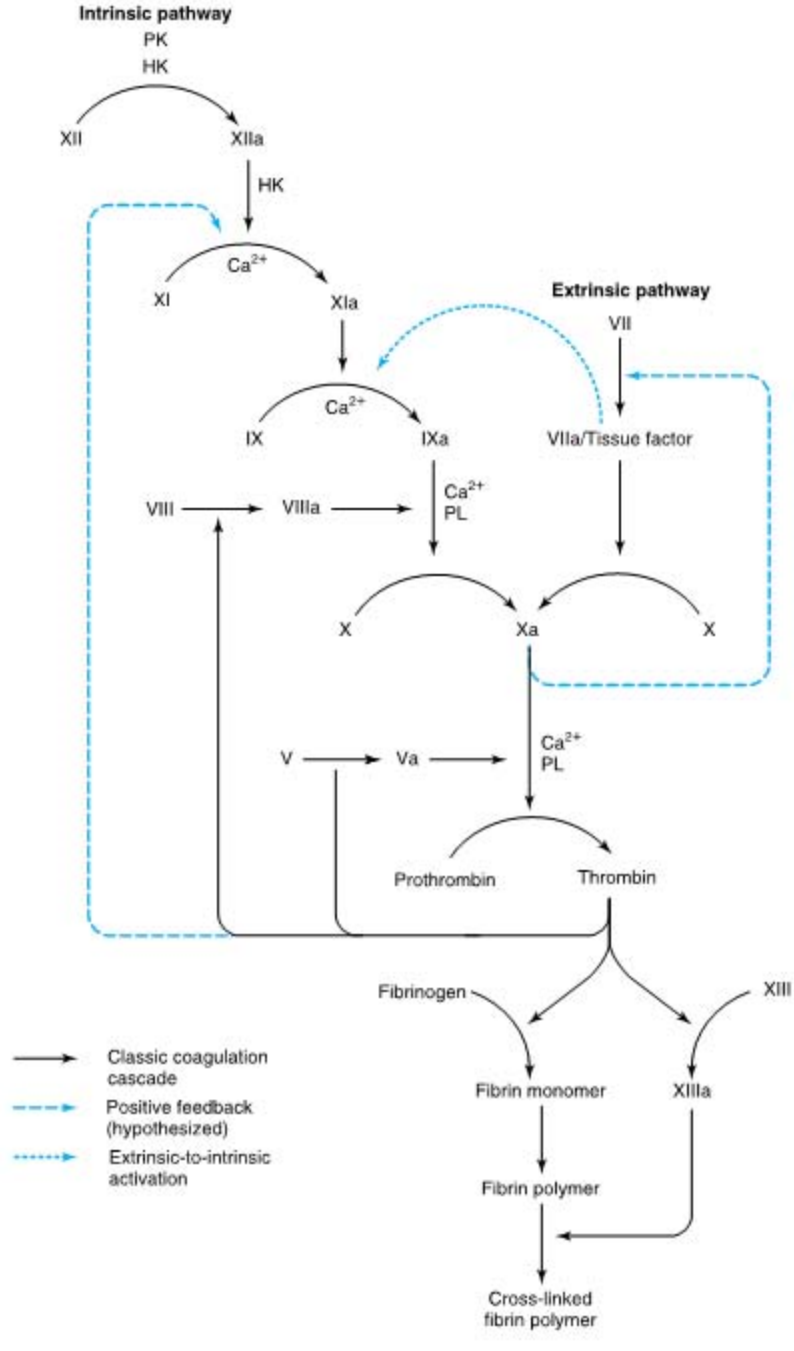


Figure 51–1. The pathways of blood coagulation. The intrinsic and extrinsic pathways are indicated. The events depicted below factor Xa are designated the final common pathway, culminating in the formation of cross-linked fibrin. New observations (dotted arrow) include the finding that complexes of tissue factor and factor VIIa activate not only factor X (in the classic extrinsic pathway) but also factor IX in the intrinsic pathway. In addition, thrombin and factor Xa feedback-activate at the two sites indicated (dashed arrows). (PK, prekallikrein; HK, HMW kininogen; PL, phospholipids.) (Reproduced, with permission, from Roberts HR, Lozier JN: New perspectives on the coagulation cascade. Hosp Pract [Off Ed] 1992 Jan;27:97.)

Table 51–1. Numerical system for nomenclature of blood clotting factors. The numbers indicate the order in which the factors have been discovered and bear no relationship to the order in which they act.

Factor	Common Name
I	Fibrinogen } These factors are usually referred
II	Prothrombin } to by their common names.
III	Tissue factor } These factors are usually not re-
IV	Ca^{2+} } ferred to as coagulation factors.
V	Proaccelerin, labile factor, accelerator (Ac-) globulin
VII[1]	Proconvertin, serum prothrombin conversion accelerator (SPCA), cothromboplastin
VIII	Antihemophilic factor A, antihemophilic globulin (AHG)
IX	Antihemophilic factor B, Christmas factor, plasma thromboplastin component (PTC)
X	Stuart-Prower factor
XI	Plasma thromboplastin antecedent (PTA)
XII	Hageman factor
XIII	Fibrin stabilizing factor (FSF), fibrinoligase

[1]There is no factor VI.

general, as shown in Table 51–2, these proteins can be classified into five types: (1) zymogens of serine-dependent proteases, which become activated during the process of coagulation; (2) cofactors; (3) fibrinogen; (4) a transglutaminase, which stabilizes the fibrin clot; and (5) regulatory and other proteins.

The Intrinsic Pathway Leads to Activation of Factor X

The intrinsic pathway (Figure 51–1) involves factors XII, XI, IX, VIII, and X as well as prekallikrein, high-molecular-weight (HMW) kininogen, Ca^{2+}, and platelet phospholipids. It results in the production of factor Xa (by convention, activated clotting factors are referred to by use of the suffix a).

This pathway commences with the "contact phase" in which prekallikrein, HMW kininogen, factor XII, and factor XI are exposed to a negatively charged activating surface. In vivo, the proteins probably assemble on endothelial cell membranes, whereas glass or kaolin can be used for in vitro tests of the intrinsic pathway. When the components of the contact phase assemble on the activating surface, factor XII is activated to factor XIIa upon proteolysis by kallikrein. This factor XIIa, generated by kallikrein, attacks prekallikrein to generate more kallikrein, setting up a reciprocal activation. Factor XIIa, once formed, activates factor XI to XIa and also releases bradykinin (a nonapeptide with potent vasodilator action) from HMW kininogen.

Table 51–2. The functions of the proteins involved in blood coagulation.

Zymogens of serine proteases	
Factor XII	Binds to negatively charged surface at site of vessel wall injury; activated by high-MW kininogen and kallikrein.
Factor XI	Activated by factor XIIa.
Factor IX	Activated by factor XIa in presence of Ca^{2+}.
Factor VII	Activated thrombin in presence of Ca^{2+}.
Factor X	Activated on surface of activated platelets by tenase complex (Ca^{2+}, factors VIIIa and IXa) and by factor VIIa in presence of tissue factor and Ca^{2+}.
Factor II	Activated on surface of activated platelets by prothrombinase complex (Ca^{2+}, factors Va and Xa). [Factors II, VII, IX, and X are Gla-containing zymogens.] (Gla = γ-carboxyglutamate.)
Cofactors	
Factor VIII	Activated by thrombin; factor VIIIa is a cofactor in the activation of factor X by factor IXa.
Factor V	Activated by thrombin; factor Va is a cofactor in the activation of prothrombin by factor Xa.
Tissue factor (factor III)	A glycoprotein expressed on the surface of injured or stimulated endothelial cells to act as a cofactor for factor VIIa.
Fibrinogen	
Factor I	Cleaved by thrombin to form fibrin clot.
Thiol-dependent transglutaminase	
Factor XIII	Activated by thrombin in presence of Ca^{2+}; stabilizes fibrin clot by covalent cross-linking.
Regulatory and other proteins	
Protein C	Activated to protein Ca by thrombin bound to thrombomodulin; then degrades factors VIIIa and Va.
Protein S	Acts as a cofactor of protein C; both proteins contain Gla (γ-carboxyglutamate) residues.
Thrombomodulin	Protein on the surface of endothelial cells; binds thrombin, which then activates protein C.

Factor XIa in the presence of Ca^{2+} activates factor IX (55 kDa, a zymogen containing vitamin K-dependent γ-carboxyglutamate [Gla] residues; see Chapter 45), to the serine protease, factor IXa. This in turn cleaves an Arg-Ile bond in factor X (56 kDa) to produce the two-chain serine protease, factor Xa. This latter reaction requires the assembly of components, called **the tenase**

complex, on the surface of activated platelets: Ca^{2+} and factor VIIIa, as well as factors IXa and X. It should be noted that in all reactions involving the Gla-containing zymogens (factors II, VII, IX, and X), the Gla residues in the amino terminal regions of the molecules serve as high-affinity binding sites for Ca^{2+}. For assembly of the tenase complex, the platelets must first be activated to expose the acidic (anionic) phospholipids, **phosphatidylserine** and **phosphatidylinositol,** that are normally on the internal side of the plasma membrane of resting, nonactivated platelets. Factor VIII (330 kDa), a glycoprotein, is not a protease precursor but a cofactor that serves as a receptor for factors IXa and X on the platelet surface. Factor VIII is activated by minute quantities of thrombin to form factor VIIIa, which is in turn inactivated upon further cleavage by thrombin.

The Extrinsic Pathway Also Leads to Activation of Factor X But by a Different Mechanism

Factor Xa occurs at the site where the intrinsic and extrinsic pathways converge (Figure 51–1) and lead into the final common pathway of blood coagulation. The extrinsic pathway involves tissue factor, factors VII and X, and Ca^{2+} and results in the production of factor Xa. It is initiated at the site of tissue injury with the exposure of **tissue factor** (Figure 51–1) on subendothelial cells. Tissue factor interacts with and activates factor VII (53 kDa), a circulating Gla-containing glycoprotein synthesized in the liver. Tissue factor acts as a cofactor for factor VIIa, enhancing its enzymatic activity to activate factor X. The association of tissue factor and factor VIIa is called **tissue factor complex.** Factor VIIa cleaves the same Arg-Ile bond in factor X that is cleaved by the tenase complex of the intrinsic pathway. Activation of factor X provides an important link between the intrinsic and extrinsic pathways.

Another important interaction between the extrinsic and intrinsic pathways is that complexes of tissue factor and factor VIIa also activate factor IX in the intrinsic pathway. Indeed, **the formation of complexes between tissue factor and factor VIIa is now considered to be the key process involved in initiation of blood coagulation in vivo.** The physiologic significance of the initial steps of the intrinsic pathway, in which factor XII, prekallikrein, and HMW kininogen are involved, has been called into question because patients with a hereditary deficiency of these components do not exhibit bleeding problems. Similarly, patients with a deficiency of factor XI may not have bleeding problems. The intrinsic pathway may actually be more important in fibrinolysis (see below) than in coagulation, since kallikrein, factor XIIa, and factor XIa can cleave plasminogen and kallikrein can activate single-chain urokinase.

Tissue factor pathway inhibitor (TFPI) is a major physiologic inhibitor of coagulation. It is a protein that circulates in the blood associated with lipoproteins. TFPI directly inhibits factor Xa by binding to the enzyme near its active site. This factor Xa-TFPI complex then inhibits the factor VIIa-tissue factor complex.

The Final Common Pathway of Blood Clotting Involves Activation of Prothrombin to Thrombin

In the final common pathway, factor Xa, produced by either the intrinsic or the extrinsic pathway, activates **prothrombin** (factor II) to **thrombin** (factor IIa), which then converts fibrinogen to fibrin (Figure 51–1).

The activation of prothrombin, like that of factor X, occurs on the surface of activated platelets and requires the assembly of **a prothrombinase complex,** consisting of platelet anionic phospholipids, Ca^{2+}, factor Va, factor Xa, and prothrombin.

Factor V (330 kDa), a glycoprotein with homology to factor VIII and ceruloplasmin, is synthesized in the liver, spleen, and kidney and is found in platelets as well as in plasma. It functions as a cofactor in a manner similar to that of factor VIII in the tenase complex. When activated to factor Va by traces of thrombin, it binds to specific receptors on the platelet membrane (Figure 51–2) and forms a complex with factor Xa and prothrombin. It is subsequently inactivated by further action of thrombin, thereby providing a means of limiting the activation of prothrombin to thrombin. **Prothrombin** (72 kDa; Figure 51–3) is a single-chain glycoprotein synthesized in the liver. The amino terminal region of prothrombin (1 in Figure 51–3) contains ten Gla residues, and the serine-dependent active protease site (indicated by the arrowhead) is in the carboxyl terminal region of the molecule. Upon binding to the complex of factors Va and Xa on the platelet membrane, prothrombin is cleaved by factor Xa at two sites (Figure 51–2) to generate the active, two-chain thrombin molecule, which is then released from the platelet surface. The A and B chains of thrombin are held together by a disulfide bond.

Conversion of Fibrinogen to Fibrin Is Catalyzed by Thrombin

Fibrinogen (factor I, 340 kDa; see Figures 51–1 and 51–4 and Tables 51–1 and 51–2) is a soluble plasma glycoprotein that consists of three nonidentical pairs of polypeptide chains $(A\alpha,B\beta\gamma)_2$ covalently linked by disulfide bonds. The $B\beta$ and γ chains contain asparagine-linked complex oligosaccharides. All three

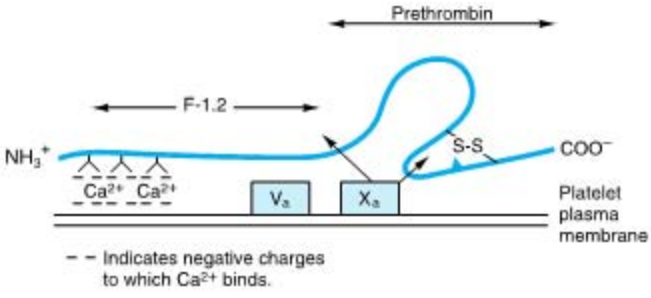


Figure 51-2. Diagrammatic representation (not to scale) of the binding of factors Va, Xa, Ca^{2+}, and prothrombin to the plasma membrane of the activated platelet. The sites of cleavage of prothrombin by factor Xa are indicated by two arrows. The part of prothrombin destined to form thrombin is labeled prethrombin. The Ca^{2+} is bound to anionic phospholipids of the plasma membrane of the activated platelet.

chains are synthesized in the liver; the three structural genes involved are on the same chromosome, and their expression is coordinately regulated in humans. The amino terminal regions of the six chains are held in close proximity by a number of disulfide bonds, while the carboxyl terminal regions are spread apart, giving rise to a highly asymmetric, elongated molecule (Figure 51–4). The A and B portions of the Aα and Bβ chains, designated **fibrinopeptides A (FPA) and B (FPB),** respectively, at the amino terminal ends of the chains, bear excess negative charges as a result of the presence of aspartate and glutamate residues, as well as an unusual tyrosine O-sulfate in FPB. These negative charges contribute to the solubility of fibrinogen in plasma and also serve to prevent aggregation by causing electrostatic repulsion between fibrinogen molecules.

Thrombin (34 kDa), a serine protease formed by the prothrombinase complex, hydrolyzes the four Arg-Gly bonds between the fibrinopeptides and the α and β portions of the Aα and Bβ chains of fibrinogen (Figure 51–5A). The release of the fibrinopeptides by thrombin generates fibrin monomer, which has the subunit structure $(\alpha, \beta, \gamma)_2$. Since FPA and FPB contain only 16 and 14 residues, respectively, the fibrin molecule retains 98% of the residues present in fibrinogen. The removal of the fibrinopeptides exposes binding sites that allow the molecules of fibrin monomers to aggregate spontaneously in a regularly staggered array, forming an insoluble fibrin clot. It is the formation of this insoluble fibrin polymer that traps platelets, red cells, and other components to form the white or red thrombi. This initial fibrin clot is rather weak, held together only by the noncovalent association of fibrin monomers.

In addition to converting fibrinogen to fibrin, thrombin also converts factor XIII to factor XIIIa. This factor is a highly specific **transglutaminase** that covalently cross-links fibrin molecules by forming peptide bonds between the amide groups of glutamine and the ε-amino groups of lysine residues (Figure 51–5B), yielding a more stable fibrin clot with increased resistance to proteolysis.

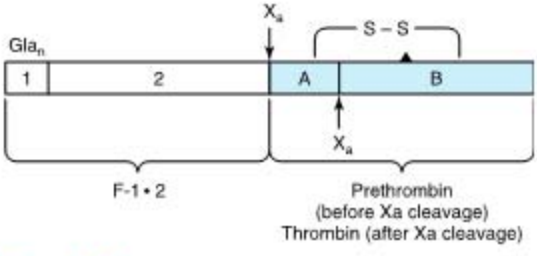


Figure 51–3. Diagrammatic representation (not to scale) of prothrombin. The amino terminal is to the left; region 1 contains all ten Gla residues. The sites of cleavage by factor Xa are shown and the products named. The site of the catalytically active serine residue is indicated by the solid triangle. The A and B chains of active thrombin (shaded) are held together by the disulfide bridge.

Levels of Circulating Thrombin Must Be Carefully Controlled or Clots May Form

Once active thrombin is formed in the course of hemostasis or thrombosis, its concentration must be carefully controlled to prevent further fibrin formation or platelet activation. This is achieved in two ways. Thrombin circulates as its inactive precursor, prothrombin, which is activated as the result of a cascade of enzymatic reactions, each converting an inactive zymogen to an active enzyme and leading finally to the conversion of prothrombin to thrombin (Figure 51–1). At each point in the cascade, **feedback mechanisms** produce a delicate balance of activation and inhibition. The concentration of factor XII in plasma is approximately 30 μg/mL, while that of fibrinogen is 3 mg/mL, with intermediate clotting factors increasing in concentration as one proceeds down the cascade, showing that the clotting cascade provides amplification. The second means of controlling thrombin activity is the inactivation of any thrombin formed by **circulating inhibi-**

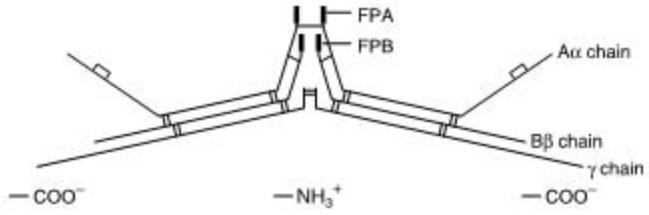


Figure 51–4. Diagrammatic representation (not to scale) of fibrinogen showing pairs of Aα, Bβ, and γ chains linked by disulfide bonds. (FPA, fibrinopeptide A; FPB, fibrinopeptide B.)

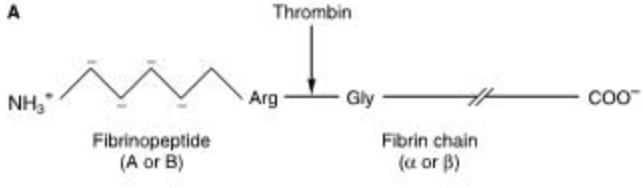
tors, the most important of which is antithrombin III (see below).

The Activity of Antithrombin III, an Inhibitor of Thrombin, Is Increased by Heparin

Four naturally occurring thrombin inhibitors exist in normal plasma. The most important is **antithrombin III** (often called simply antithrombin), which contributes approximately 75% of the antithrombin activity. Antithrombin III can also inhibit the activities of factors IXa, Xa, XIa, XIIa, and VIIa complexed with tissue factor. **α_2-Macroglobulin** contributes most of the remainder of the antithrombin activity, with **heparin cofactor II** and **α_1-antitrypsin** acting as minor inhibitors under physiologic conditions.

The endogenous activity of antithrombin III is greatly potentiated by the presence of acidic proteoglycans such as **heparin** (Chapter 48). These bind to a specific cationic site of antithrombin III, inducing a conformational change and promoting its binding to thrombin as well as to its other substrates. This is the basis for the use of heparin in clinical medicine to inhibit coagulation. The anticoagulant effects of heparin can be antagonized by strongly cationic polypeptides such as **protamine,** which bind strongly to heparin, thus inhibiting its binding to antithrombin III. Individuals with inherited deficiencies of antithrombin III are prone to develop venous thrombosis, providing evidence that antithrombin III has a physiologic function and that the coagulation system in humans is normally in a dynamic state.

Thrombin is involved in an additional regulatory mechanism that operates in coagulation. It combines with **thrombomodulin,** a glycoprotein present on the surfaces of endothelial cells. The complex activates **protein C.** In combination with **protein S,** activated protein C (APC) degrades factors Va and VIIIa, limiting their actions in coagulation. A genetic deficiency of either protein C or protein S can cause venous thrombosis. Furthermore, patients with **factor V Leiden** (which has a glutamine residue in place of an arginine at position 506) have an increased risk of venous thrombotic



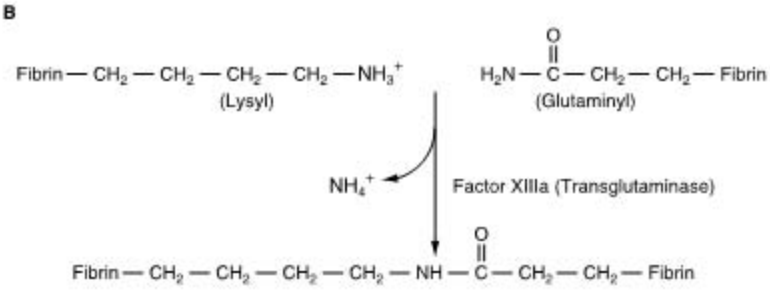


Figure 51-5. Formation of a fibrin clot. **A:** Thrombin-induced cleavage of Arg-Gly bonds of the Aα and Bβ chains of fibrinogen to produce fibrinopeptides (left-hand side) and the α and β chains of fibrin monomer (right-hand side). **B:** Cross-linking of fibrin molecules by activated factor XIII (factor XIIIa).

disease because factor V Leiden is resistant to inactivation by APC. This condition is termed APC resistance.

Coumarin Anticoagulants Inhibit the Vitamin K-Dependent Carboxylation of Factors II, VII, IX, & X

The coumarin drugs (eg, warfarin), which are used as anticoagulants, inhibit the vitamin K-dependent carboxylation of Glu to Gla residues (see Chapter 45) in the amino terminal regions of factors II, VII, IX, and X and also proteins C and S. These proteins, all of which are synthesized in the liver, are dependent on the Ca^{2+}-binding properties of the Gla residues for their normal function in the coagulation pathways. The coumarins act by inhibiting the reduction of the quinone derivatives of vitamin K to the active hydroquinone forms (Chapter 45). Thus, the administration of vitamin K will bypass the coumarin-induced inhibition and allow maturation of the Gla-containing factors. Reversal of coumarin inhibition by vitamin K requires 12–24 hours, whereas reversal of the anticoagulant effects of heparin by protamine is almost instantaneous.

Heparin and warfarin are widely used in the treatment of thrombotic and thromboembolic conditions, such as deep vein thrombosis and pulmonary embolus. Heparin is administered first, because of its prompt onset of action, whereas warfarin takes several days to reach full effect. Their effects are closely monitored by use of appropriate tests of coagulation (see below) because of the risk of producing hemorrhage.

Hemophilia A Is Due to a Genetically Determined Deficiency of Factor VIII

Inherited deficiencies of the clotting system that result in bleeding are found in humans. The most common is deficiency of factor VIII, causing **hemophilia A,** an X chromosome-linked disease that has played a major role in the history of the royal families of Europe. **Hemophilia B** is due to a deficiency of factor IX; its clinical features are almost identical to those of hemophilia A, but the conditions can be separated on the basis of specific assays that distinguish between the two factors.

The gene for human factor VIII has been cloned and is one of the largest so far studied, measuring 186 kb in length and containing 26 exons. A variety of mutations have been detected leading to diminished activity of factor VIII; these include partial gene deletions and point mutations resulting in premature chain termination. Prenatal diagnosis by DNA analysis after chorionic villus sampling is now possible.

In past years, treatment for patients with hemophilia A has consisted of administration of cryoprecipitates (enriched in factor VIII) prepared from individual donors or lyophilized factor VIII concentrates prepared from plasma pools of up to 5000 donors. It is now possible to prepare factor VIII by **recombinant DNA technology.** Such preparations are free of contaminating viruses (eg, hepatitis A, B, C, or HIV-1) found in human plasma but are at present expensive; their use may increase if cost of production decreases.

Fibrin Clots Are Dissolved by Plasmin

As stated above, the coagulation system is normally in a state of dynamic equilibrium in which fibrin clots are constantly being laid down and dissolved. This latter process is termed **fibrinolysis. Plasmin,** the serine protease mainly responsible for degrading fibrin and fibrinogen, circulates in the form of its inactive zymogen, **plasminogen** (90 kDa), and any small amounts of plasmin that are formed in the fluid phase under physiologic conditions are rapidly inactivated by the fast-acting plasmin inhibitor, α_2-antiplasmin. Plasminogen binds to fibrin and thus becomes incorporated in clots as they are produced; since plasmin that is formed when bound to fibrin is protected from α_2-antiplasmin, it remains active. **Activators of plasminogen** of various types are found in most body tissues, and all cleave the same Arg-Val bond in plasminogen to produce the two-chain serine protease, plasmin (Figure 51–6).

Tissue plasminogen activator (alteplase; t-PA) is a serine protease that is released into the circulation from vascular endothelium under conditions of injury or

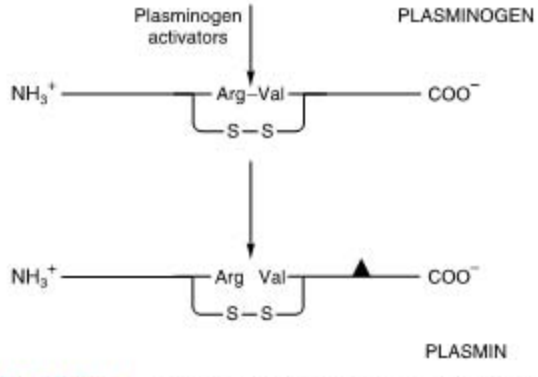


Figure 51–6. Activation of plasminogen. The same Arg-Val bond is cleaved by all plasminogen activators to give the two-chain plasmin molecule. The solid triangle indicates the serine residue of the active site. The two chains of plasmin are held together by a disulfide bridge.

stress and is catalytically inactive unless bound to fibrin. Upon binding to fibrin, t-PA cleaves plasminogen within the clot to generate plasmin, which in turn digests the fibrin to form soluble degradation products and thus dissolves the clot. Neither plasmin nor the plasminogen activator can remain bound to these degradation products, and so they are released into the fluid phase, where they are inactivated by their natural inhibitors. Prourokinase is the precursor of a second activator of plasminogen, **urokinase.** Originally isolated from urine, it is now known to be synthesized by cell types such as monocytes and macrophages, fibroblasts, and epithelial cells. Its main action is probably in the degradation of extracellular matrix. Figure 51–7 indicates the sites of action of five proteins that influence the formation and action of plasmin.

Recombinant t-PA & Streptokinase Are Used as Clot Busters

Alteplase (t-PA), produced by recombinant DNA technology, is used therapeutically as a fibrinolytic agent, as is **streptokinase.** However, the latter is less selective than t-PA, activating plasminogen in the fluid phase (where it can degrade circulating fibrinogen) as well as plasminogen that is bound to a fibrin clot. The amount of plasmin produced by therapeutic doses of streptokinase may exceed the capacity of the circulating α_2-antiplasmin, causing fibrinogen as well as fibrin to be degraded and resulting in the bleeding often encountered during fibrinolytic therapy. Because of its **selectivity** for degrading fibrin, there is considerable therapeutic interest in the use of recombinant t-PA to restore the patency of coronary arteries following thrombosis. If administered early enough, before irreversible damage of heart muscle occurs (about 6 hours after onset of thrombosis), t-PA can significantly reduce the mortality rate from myocardial damage following coronary thrombosis. t-PA is more effective than streptokinase at restoring full patency and also appears to result in a slightly better survival rate. Table 51–3 compares some thrombolytic features of streptokinase and t-PA.

There are a number of disorders, including cancer and shock, in which **the concentrations of plasminogen activators increase.** In addition, the antiplasmin activities contributed by α_1-antitrypsin and α_2-antiplasmin may be impaired in diseases such as cirrhosis. Since certain bacterial products, such as streptokinase, are capable of activating plasminogen, they may be responsible for the diffuse hemorrhage sometimes observed in patients with disseminated bacterial infections.

Activation of Platelets Involves Stimulation of the Polyphosphoinositide Pathway

Platelets normally circulate in an unstimulated disk-shaped form. During hemostasis or thrombosis, they become activated and help form hemostatic plugs or thrombi. Three major steps are involved: (1) adhesion to exposed collagen in blood vessels, (2) release of the contents of their granules, and (3) aggregation.

Platelets adhere to collagen via specific receptors on the platelet surface, including the glycoprotein complex GPIa–IIa ($\alpha_2\beta_1$ integrin; Chapter 52), in a reaction that involves **von Willebrand factor.** This is a glycoprotein, secreted by endothelial cells into the plasma, which stabilizes factor VIII and binds to collagen and the subendothelium. Platelets bind to von Willebrand factor via a glycoprotein complex (GPIb–V–IX) on the platelet surface; this interaction is especially important in platelet adherence to the subendothelium under conditions of high shear stress that occur in small vessels and stenosed arteries.

Platelets adherent to collagen change shape and spread out on the subendothelium. They release the contents of their storage granules (the dense granules and the alpha granules); secretion is also stimulated by thrombin.

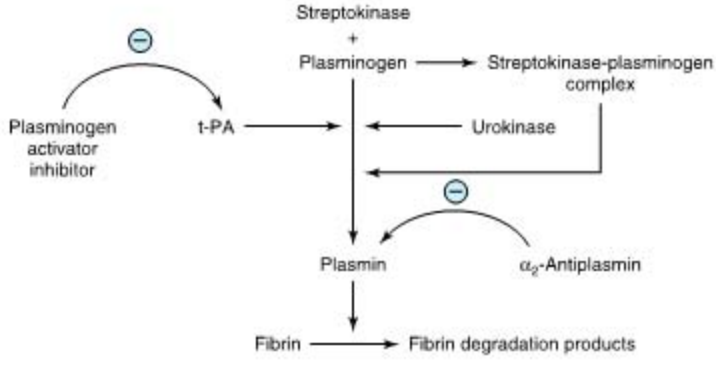


Figure 51–7. Scheme of sites of action of streptokinase, tissue plasminogen activator (t-PA), urokinase, plasminogen activator inhibitor, and α_2-antiplasmin (the last two proteins exert inhibitory actions). Streptokinase forms a complex with plasminogen, which exhibits proteolytic activity; this cleaves some plasminogen to plasmin, initiating fibrinolysis.

Table 51–3. Comparison of some properties of streptokinase (SK) and tissue plasminogen activator (t-PA) with regard to their use as thrombolytic agents.[1]

	SK	t-PA
Selective for fibrin clot	–	+
Produces plasminemia	+	–
Reduces mortality	+	+
Causes allergic reaction	+	–
Causes hypotension	+	–
Cost per treatment (approximate)	Relatively low	Relatively high

[1]Data from Webb J, Thompson C: Thrombolysis for acute myocardial infarction. Can Fam Physician 1992;38:1415.

Thrombin, formed from the coagulation cascade, is the most potent activator of platelets and initiates platelet activation by interacting with its receptor on the plasma membrane (Figure 51–8). The further events leading to platelet activation are examples of **transmembrane signaling,** in which a chemical messenger outside the cell generates effector molecules inside the cell. In this instance, thrombin acts as the external chemical messenger (stimulus or agonist). The interaction of thrombin with its receptor stimulates the activity of an intracellular **phospholipase Cβ.** This enzyme hydrolyzes the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2, a polyphosphoinositide) to form the two internal effector molecules, 1,2-diacylglycerol and 1,4,5-inositol trisphosphate.

Hydrolysis of PIP_2 is also involved in the action of many hormones and drugs. Diacylglycerol stimulates

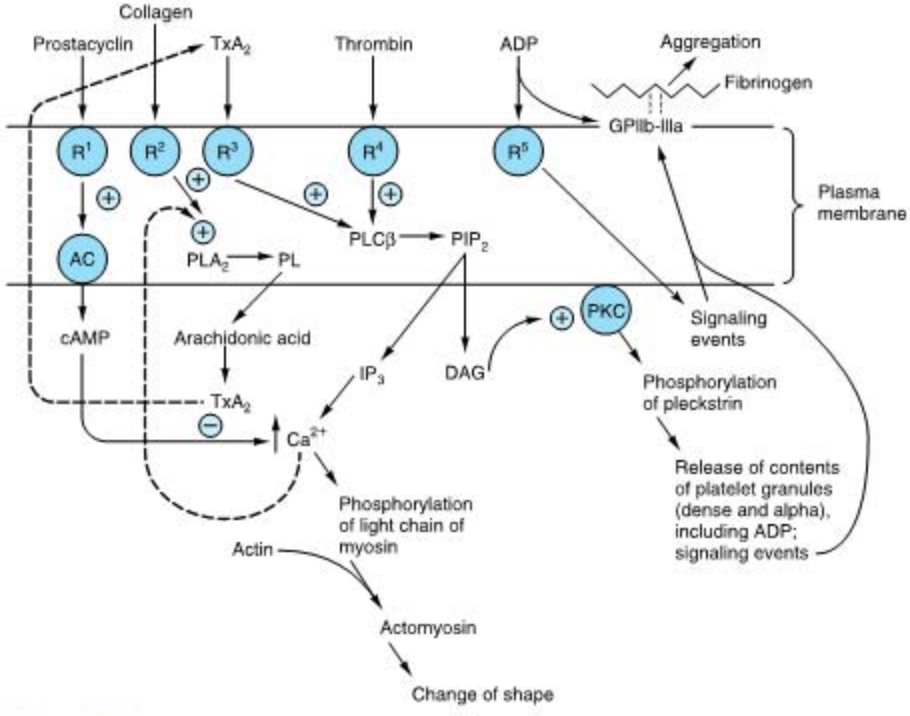


Figure 51–8. Diagrammatic representation of platelet activation. The external environment, the plasma membrane, and the inside of a platelet are depicted from top to bottom. Thrombin and collagen are the two most important platelet activators. ADP is considered a weak agonist; it causes aggregation but not granule release. (GP, glycoprotein; R^1–R^5, various receptors; AC, adenylyl cyclase; PLA_2, phospholipase A_2; PL, phospholipids; PLCβ, phospholipase Cβ; PIP_2, phosphatidylinositol 4,5-bisphosphate; cAMP, cyclic AMP; PKC, protein kinase C; TxA_2, thromboxane A_2; IP_3, inositol 1,4,5-trisphosphate; DAG, 1,2-diacylglycerol. The G proteins that are involved are not shown.)

protein kinase C, which phosphorylates the protein **pleckstrin** (47 kDa). This results in aggregation and release of the contents of the storage granules. ADP released from dense granules can also activate platelets, resulting in aggregation of additional platelets. IP_3 causes release of Ca^{2+} into the cytosol mainly from the dense tubular system (or residual smooth endoplasmic reticulum from the megakaryocyte), which then interacts with calmodulin and myosin light chain kinase, leading to phosphorylation of the light chains of myosin. These chains then interact with actin, causing changes of platelet shape.

Collagen-induced activation of a platelet phospholipase A_2 by increased levels of cytosolic Ca^{2+} results in liberation of arachidonic acid from platelet phospholipids, leading to the formation of **thromboxane A_2** (Chapter 23), which in turn, in a receptor-mediated fashion, can further activate phospholipase C, promoting platelet aggregation.

Activated platelets, besides forming a platelet aggregate, are required, via newly expressed anionic phospholipids on the membrane surface, for acceleration of the activation of factors X and II in the coagulation cascade (Figure 51–1).

All of the aggregating agents, including thrombin, collagen, ADP, and others such as platelet-activating factor, modify the platelet surface so that fibrinogen can bind to a glycoprotein complex, **GPIIb–IIIa** ($\alpha_{IIb}\beta_3$ integrin; Chapter 52), on the activated platelet surface. Molecules of divalent fibrinogen then link adjacent activated platelets to each other, forming a platelet aggregate. Some agents, including epinephrine, serotonin, and vasopressin, exert synergistic effects with other aggregating agents.

Endothelial Cells Synthesize Prostacyclin & Other Compounds That Affect Clotting & Thrombosis

The endothelial cells in the walls of blood vessels make important contributions to the overall regulation of hemostasis and thrombosis. As described in Chapter 23, these cells synthesize **prostacyclin** (PGI_2), a potent inhibitor of platelet aggregation, opposing the action of thromboxane A_2. Prostacyclin acts by stimulating the activity of adenylyl cyclase in the surface membranes of platelets. The resulting increase of intraplatelet cAMP opposes the increase in the level of intracellular Ca^{2+} produced by IP_3 and thus inhibits platelet activation (Figure 51–8). Endothelial cells play other roles in the regulation of thrombosis. For instance, these cells possess an ADPase, which hydrolyzes ADP, and thus opposes its aggregating effect on platelets. In addition, these cells appear to synthesize heparan sulfate, an anticoagulant, and they also synthesize plasminogen activators, which may help dissolve thrombi. Table 51–4 lists some molecules produced by endothelial cells that affect thrombosis and fibrinolysis. Endothelium-derived relaxing factor (nitric oxide) is discussed in Chapter 49.

Analysis of the mechanisms of uptake of atherogenic lipoproteins, such as LDL, by endothelial, smooth muscle, and monocytic cells of arteries, along with detailed studies of how these lipoproteins damage such cells is a key area of study in elucidating the mechanisms of **atherosclerosis** (Chapter 26).

Aspirin Is an Effective Antiplatelet Drug

Certain drugs (antiplatelet drugs) modify the behavior of platelets. The most important is aspirin (acetylsalicylic acid), which irreversibly acetylates and thus inhibits the platelet cyclooxygenase system involved in formation of thromboxane A_2 (Chapter 14), a potent aggregator of platelets and also a vasoconstrictor. Platelets are very sensitive to aspirin; as little as 30 mg/d (one aspirin tablet usually contains 325 mg) effectively eliminates the synthesis of thromboxane A_2. Aspirin also inhibits production of prostacyclin (PGI_2, which opposes platelet aggregation and is a vasodilator) by en-

Table 51–4. Molecules synthesized by endothelial cells that play a role in the regulation of thrombosis and fibrinolysis.[1]

Molecule	Action
ADPase (an ectoenzyme)	Degrades ADP (an aggregating agent of platelets) to AMP + P_i
Endothelium-derived relaxing factor (nitric oxide)	Inhibits platelet adhesion and aggregation by elevating levels of cGMP
Heparan sulfate (a glycosaminoglycan)	Anticoagulant; combines with antithrombin III to inhibit thrombin
Prostacyclin (PGI_2, a prostaglandin)	Inhibits platelet aggregation by increasing levels of cAMP
Thrombomodulin (a glycoprotein)	Binds protein C, which is then cleaved by thrombin to yield activated protein C; this in combination with protein S degrades factors Va and VIIIa, limiting their actions
Tissue plasminogen activator (t-PA, a protease)	Activates plasminogen to plasmin, which digests fibrin; the action of t-PA is opposed by plasminogen activator inhibitor-1 (PAI-1)

[1]Adapted from Wu KK: Endothelial cells in hemostasis, thrombosis and inflammation. Hosp Pract (Off Ed) 1992 Apr; 27:145.

dothelial cells, but unlike platelets, these cells regenerate cyclooxygenase within a few hours. Thus, the overall balance between thromboxane A_2 and prostacyclin can be shifted in favor of the latter, opposing platelet aggregation. Indications for treatment with aspirin thus include management of angina and evolving myocardial infarction and also prevention of stroke and death in patients with transient cerebral ischemic attacks.

Laboratory Tests Measure Coagulation & Thrombolysis

A number of laboratory tests are available to measure the phases of hemostasis described above. The tests include platelet count, bleeding time, activated partial thromboplastin time (aPTT or PTT), prothrombin time (PT), thrombin time (TT), concentration of fibrinogen, fibrin clot stability, and measurement of fibrin degradation products. The platelet count quantitates the number of platelets, and the bleeding time is an overall test of platelet function. aPTT is a measure of the intrinsic pathway and PT of the extrinsic pathway. PT is used to measure the effectiveness of oral anticoagulants such as warfarin, and aPTT is used to monitor heparin therapy. The reader is referred to a textbook of hematology for a discussion of these tests.

SUMMARY

- Hemostasis and thrombosis are complex processes involving coagulation factors, platelets, and blood vessels.
- Many coagulation factors are zymogens of serine proteases, becoming activated during the overall process.
- Both intrinsic and extrinsic pathways of coagulation exist, the latter initiated by tissue factor. The pathways converge at factor Xa, embarking on the common final pathway resulting in thrombin-catalyzed conversion of fibrinogen to fibrin, which is strengthened by cross-linking, catalyzed by factor XIII.
- Genetic disorders of coagulation factors occur, and the two most common involve factors VIII (hemophilia A) and IX (hemophilia B).
- An important natural inhibitor of coagulation is antithrombin III; genetic deficiency of this protein can result in thrombosis.
- For activity, factors II, VII, IX, and X and proteins C and S require vitamin K-dependent γ-carboxylation of certain glutamate residues, a process that is inhibited by the anticoagulant warfarin.
- Fibrin is dissolved by plasmin. Plasmin exists as an inactive precursor, plasminogen, which can be activated by tissue plasminogen activator (t-PA). Both t-PA and streptokinase are widely used to treat early thrombosis in the coronary arteries.
- Thrombin and other agents cause platelet aggregation, which involves a variety of biochemical and morphologic events. Stimulation of phospholipase C and the polyphosphoinositide pathway is a key event in platelet activation, but other processes are also involved.
- Aspirin is an important antiplatelet drug that acts by inhibiting production of thromboxane A_2.

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Red & White Blood Cells

52

Robert K. Murray, MD, PhD

BIOMEDICAL IMPORTANCE

Blood cells have been studied intensively because they are obtained easily, because of their functional importance, and because of their involvement in many disease processes. The structure and function of hemoglobin, the porphyrias, jaundice, and aspects of iron metabolism are discussed in previous chapters. Reduction of the number of red blood cells and of their content of hemoglobin is the cause of the anemias, a diverse and important group of conditions, some of which are seen very commonly in clinical practice. Certain of the blood group systems, present on the membranes of erythrocytes and other blood cells, are of extreme importance in relation to blood transfusion and tissue transplantation. Table 52–1 summarizes the causes of a number of important diseases affecting red blood cells; some are discussed in this chapter, and the remainder are discussed elsewhere in this text. Every organ in the body can be affected by inflammation; neutrophils play a central role in acute inflammation, and other white blood cells, such as lymphocytes, play important roles in chronic inflammation. Leukemias, defined as malignant neoplasms of blood-forming tissues, can affect precursor cells of any of the major classes of white blood cells; common types are acute and chronic myelocytic leukemia, affecting precursors of the neutrophils; and acute and chronic lymphocytic leukemias. Combination chemotherapy, using combinations of various chemotherapeutic agents, all of which act at one or more biochemical loci, has been remarkably effective in the treatment of certain of these types of leukemias. Understanding the role of red and white cells in health and disease requires a knowledge of certain fundamental aspects of their biochemistry.

THE RED BLOOD CELL IS SIMPLE IN TERMS OF ITS STRUCTURE & FUNCTION

The major functions of the red blood cell are relatively simple, consisting of delivering oxygen to the tissues and of helping in the disposal of carbon dioxide and protons formed by tissue metabolism. Thus, it has a much simpler structure than most human cells, being essentially composed of a membrane surrounding a solution of hemoglobin (this protein forms about 95% of the intracellular protein of the red cell). There are no intracellular organelles, such as mitochondria, lysosomes, or Golgi apparatus. Human red blood cells, like most red cells of animals, are nonnucleated. However, the red cell is not metabolically inert. ATP is synthesized from glycolysis and is important in processes that help the red blood cell maintain its biconcave shape and also in the regulation of the transport of ions (eg, by the Na^+-K^+ ATPase and the anion exchange protein [see below]) and of water in and out of the cell. The biconcave shape increases the surface-to-volume ratio of the red blood cell, thus facilitating gas exchange. The red cell contains cytoskeletal components (see below) that play an important role in determining its shape.

About Two Million Red Blood Cells Enter the Circulation per Second

The life span of the normal red blood cell is 120 days; this means that slightly less than 1% of the population of red cells (200 billion cells, or 2 million per second) is replaced daily. The new red cells that appear in the circulation still contain ribosomes and elements of the endoplasmic reticulum. The RNA of the ribosomes can be detected by suitable stains (such as cresyl blue), and cells containing it are termed reticulocytes; they normally number about 1% of the total red blood cell count. The life span of the red blood cell can be dramatically shortened in a variety of **hemolytic anemias.** The number of reticulocytes is markedly increased in these conditions, as the bone marrow attempts to compensate for rapid breakdown of red blood cells by increasing the amount of new, young red cells in the circulation.

Erythropoietin Regulates Production of Red Blood Cells

Human erythropoietin is a glycoprotein of 166 amino acids (molecular mass about 34 kDa). Its amount in plasma can be measured by radioimmunoassay. It is the major regulator of human erythropoiesis. Erythropoietin is synthesized mainly by the kidney and is released in response to hypoxia into the bloodstream, in which it travels to the bone marrow. There it interacts with progenitors of red blood cells via a specific receptor. The receptor is a transmembrane protein consisting of two different subunits and a number of domains. It is not a tyrosine kinase, but it stimulates the activities of specific

Table 52–1. Summary of the causes of some important disorders affecting red blood cells.

Disorder	Sole or Major Cause
Iron deficiency anemia	Inadequate intake or excessive loss of iron
Methemoglobinemia	Intake of excess oxidants (various chemicals and drugs) Genetic deficiency in the NADH-dependent methemoglobin reductase system (MIM 250800) Inheritance of HbM (MIM 141800)
Sickle cell anemia (MIM 141900)	Sequence of codon 6 of the β chain changed from GAG in the normal gene to GTG in the sickle cell gene, resulting in substitution of valine for glutamic acid
α-Thalassemias (MIM 141800)	Mutations in the α-globin genes, mainly unequal crossing-over and large deletions and less commonly nonsense and frameshift mutations
β-Thalassemia (MIM 141900)	A very wide variety of mutations in the β-globin gene, including deletions, nonsense and frameshift mutations, and others affecting every aspect of its structure (eg, splice sites, promoter mutants)
Megaloblastic anemias Deficiency of vitamin B_{12}	Decreased absorption of B_{12}, often due to a deficiency of intrinsic factor, normally secreted by gastric parietal cells
Deficiency of folic acid	Decreased intake, defective absorption, or increased demand (eg, in pregnancy) for folate
Hereditary spherocytosis[1]	Deficiencies in the amount or in the structure of α or β spectrin, ankyrin, band 3 or band 4.1
Glucose-6-phosphate dehydrogenase (G6PD) deficiency[1] (MIM 305900)	A variety of mutations in the gene (X-linked) for G6PD, mostly single point mutations
Pyruvate kinase (PK) deficiency[1] (MIM 255200)	Presumably a variety of mutations in the gene for the R (red cell) isozyme of PK
Paroxysmal nocturnal hemoglobinemia[1] (MIM 311770)	Mutations in the PIG-A gene, affecting synthesis of GPI-anchored proteins

[1]The last four disorders cause hemolytic anemias, as do a number of the other disorders listed. Most of the above conditions are discussed in other chapters of this text. MIM numbers apply only to disorders with a genetic basis.

members of this class of enzymes involved in downstream signal transduction. Erythropoietin interacts with a red cell progenitor, known as the burst-forming unit-erythroid (BFU-E), causing it to proliferate and differentiate. In addition, it interacts with a later progenitor of the red blood cell, called the colony-forming unit-erythroid (CFU-E), also causing it to proliferate and further differentiate. For these effects, erythropoietin requires the cooperation of other factors (eg, interleukin-3 and insulin-like growth factor; Figure 52–1).

The availability of a cDNA for erythropoietin has made it possible to produce substantial amounts of this hormone for analysis and for therapeutic purposes; previously the isolation of erythropoietin from human urine yielded very small amounts of the protein. The major use of **recombinant erythropoietin** has been in the treatment of a small number of **anemic states,** such as that due to renal failure.

MANY GROWTH FACTORS REGULATE PRODUCTION OF WHITE BLOOD CELLS

A large number of **hematopoietic growth factors** have been identified in recent years in addition to erythropoietin. This area of study adds to knowledge about the differentiation of blood cells, provides factors that may be useful in treatment, and also has implications for understanding of the abnormal growth of blood cells (eg, the leukemias). Like erythropoietin, most of the growth factors isolated have been glycoproteins, are very active in vivo and in vitro, interact with their target cells via specific cell surface receptors, and ultimately (via intracellular signals) affect gene expression, thereby promoting differentiation. Many have been cloned, permitting their production in relatively large amounts. Two of particular interest are **granulocyte-** and **granulocyte-macrophage colony-stimulating factors** (G-CSF and GM-CSF, respectively). G-CSF is relatively specific, inducing mainly granulocytes. GM-CSF affects a variety of progenitor cells and induces granulocytes, macrophages, and eosinophils. When the production of neutrophils is severely depressed, this condition is referred to as **neutropenia.** It is particularly likely to occur in patients treated with certain chemotherapeutic regimens and after bone marrow transplantation. These patients are liable to develop overwhelming infections. G-CSF has been administered to such patients to boost production of neutrophils.

THE RED BLOOD CELL HAS A UNIQUE & RELATIVELY SIMPLE METABOLISM

Various aspects of the metabolism of the red cell, many of which are discussed in other chapters of this text, are summarized in Table 52–2.

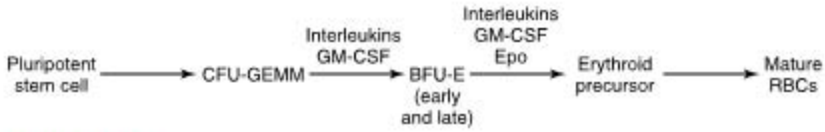

Figure 52–1. Greatly simplified scheme of differentiation of stem cells to red blood cells. Various interleukins (ILs), such as IL-3, IL-4, IL-9, and IL-11, are involved at different steps of the overall process. Erythroid precursors include the pronormoblast, basophilic, polychromatophilic, and orthochromatophilic normoblasts, and the reticulocyte. Epo acts on basophilic normoblasts but not on later erythroid cells. (CFU-GEMM, colony-forming unit whose cells give rise to granulocytes, erythrocytes, macrophages, and megakaryocytes; BFU-E, burst-forming unit-erythroid; GM-CSF, granulocyte-macrophage colony-stimulating factor; Epo, erythropoietin; RBC, red blood cell.)

The Red Blood Cell Has a Glucose Transporter in Its Membrane

The entry rate of glucose into red blood cells is far greater than would be calculated for simple diffusion. Rather, it is an example of **facilitated diffusion** (Chapter 41). The specific protein involved in this process is called the **glucose transporter** or glucose permease. Some of its properties are summarized in Table 52–3. The process of entry of glucose into red blood cells is of major importance because it is the major fuel supply for these cells. About seven different but related glucose transporters have been isolated from various tissues; unlike the red cell transporter, some of these are insulin-dependent (eg, in muscle and adipose tissue). There is considerable interest in the latter types of transporter because defects in their recruitment from intracellular sites to the surface of skeletal muscle cells may help explain the **insulin resistance** displayed by patients with type 2 diabetes mellitus.

Reticulocytes Are Active in Protein Synthesis

The mature red blood cell cannot synthesize protein. Reticulocytes are active in protein synthesis. Once reticulocytes enter the circulation, they lose their intracellular organelles (ribosomes, mitochondria, etc) within about 24 hours, becoming young red blood cells and concomitantly losing their ability to synthesize protein. Extracts of rabbit reticulocytes (obtained by injecting rabbits with a chemical—phenylhydrazine—that causes a severe hemolytic anemia, so that the red cells are almost completely replaced by reticulocytes) are widely used as an in vitro system for synthesizing proteins. Endogenous mRNAs present in these reticulocytes are destroyed by use of a nuclease, whose activity can be inhibited by addition of Ca^{2+}. The system is then programmed by adding purified mRNAs or whole-cell extracts of mRNAs, and radioactive proteins are synthesized in the presence of ^{35}S-labeled L-methionine or other radiolabeled amino acids. The radioactive proteins synthesized are separated by SDS-PAGE and detected by radioautography.

Superoxide Dismutase, Catalase, & Glutathione Protect Blood Cells From Oxidative Stress & Damage

Several powerful oxidants are produced during the course of metabolism, in both blood cells and most other cells of the body. These include superoxide (O_2^-), hydrogen peroxide (H_2O_2), peroxyl radicals ($ROO^\bullet$), and hydroxyl radicals ($OH^\bullet$). The last is a particularly reactive molecule and can react with proteins, nucleic acids, lipids, and other molecules to alter their structure and produce tissue damage. The reactions listed in Table 52–4 play an important role in forming these oxidants and in disposing of them; each of these reactions will now be considered in turn.

Superoxide is formed (reaction 1) in the red blood cell by the auto-oxidation of hemoglobin to methemoglobin (approximately 3% of hemoglobin in human red blood cells has been calculated to auto-oxidize per day); in other tissues, it is formed by the action of enzymes such as cytochrome P450 reductase and xanthine oxidase. When stimulated by contact with bacteria, neutrophils exhibit a respiratory burst (see below) and produce superoxide in a reaction catalyzed by NADPH oxidase (reaction 2). Superoxide spontaneously dismutates to form H_2O_2 and O_2; however, the rate of this same reaction is speeded up tremendously by the action of the enzyme superoxide dismutase (reaction 3). Hydrogen peroxide is subject to a number of fates. The enzyme **catalase,** present in many types of cells, converts

Table 52–2. Summary of important aspects of the metabolism of the red blood cell.

- The RBC is highly dependent upon glucose as its energy source; its membrane contains high affinity glucose transporters.
- Glycolysis, producing lactate, is the site of production of ATP.
- Because there are no mitochondria in RBCs, there is no production of ATP by oxidative phosphorylation.
- The RBC has a variety of transporters that maintain ionic and water balance.
- Production of 2,3-bisphosphoglycerate, by reactions closely associated with glycolysis, is important in regulating the ability of Hb to transport oxygen.
- The pentose phosphate pathway is operative in the RBC (it metabolizes about 5–10% of the total flux of glucose) and produces NADPH; hemolytic anemia due to a deficiency of the activity of glucose-6-phosphate dehydrogenase is common.
- Reduced glutathione (GSH) is important in the metabolism of the RBC, in part to counteract the action of potentially toxic peroxides; the RBC can synthesize GSH and requires NADPH to return oxidized glutathione (G-S-S-G) to the reduced state.
- The iron of Hb must be maintained in the ferrous state; ferric iron is reduced to the ferrous state by the action of an NADH-dependent methemoglobin reductase system involving cytochrome b_5 reductase and cytochrome b_5.
- Synthesis of glycogen, fatty acids, protein, and nucleic acids does not occur in the RBC; however, some lipids (eg, cholesterol) in the red cell membrane can exchange with corresponding plasma lipids.
- The RBC contains certain enzymes of nucleotide metabolism (eg, adenosine deaminase, pyrimidine nucleotidase, and adenylyl kinase); deficiencies of these enzymes are involved in some cases of hemolytic anemia.
- When RBCs reach the end of their life span, the globin is degraded to amino acids (which are reutilized in the body), the iron is released from heme and also reutilized, and the tetrapyrrole component of heme is converted to bilirubin, which is mainly excreted into the bowel via the bile.

Table 52–3. Some properties of the glucose transporter of the membrane of the red blood cell.

- It accounts for about 2% of the protein of the membrane of the RBC.
- It exhibits specificity for glucose and related D-hexoses (L-hexoses are not transported).
- The transporter functions at approximately 75% of its V_{max} at the physiologic concentration of blood glucose, is saturable and can be inhibited by certain analogs of glucose.
- At least seven similar but distinct glucose transporters have been detected to date in mammalian tissues, of which the red cell transporter is one.
- It is not dependent upon insulin, unlike the corresponding carrier in muscle and adipose tissue.
- Its complete amino acid sequence (492 amino acids) has been determined.
- It transports glucose when inserted into artificial liposomes.
- It is estimated to contain 12 transmembrane helical segments.
- It functions by generating a gated pore in the membrane to permit passage of glucose; the pore is conformationally dependent on the presence of glucose and can oscillate rapidly (about 900 times/s).

it to H_2O and O_2 (reaction 4). Neutrophils possess a unique enzyme, myeloperoxidase, that uses H_2O_2 and halides to produce hypohalous acids (reaction 5); this subject is discussed further below. The selenium-containing enzyme glutathione peroxidase (Chapter 20) will also act on reduced glutathione (GSH) and H_2O_2 to produce oxidized glutathione (GSSG) and H_2O (reaction 6); this enzyme can also use other peroxides as substrates. $OH^•$ and OH^- can be formed from H_2O_2 in a nonenzymatic reaction catalyzed by Fe^{2+} (the **Fenton reaction,** reaction 7). O_2^- and H_2O_2 are the substrates in the iron-catalyzed **Haber-Weiss reaction** (reaction 8), which also produces $OH^•$ and OH^-. Superoxide can release iron ions from ferritin. Thus, production of $OH^•$ may be one of the mechanisms involved in tissue injury due to iron overload (eg, hemochromatosis; Chapter 50).

Chemical compounds and reactions capable of generating potential toxic oxygen species can be referred to as **pro-oxidants.** On the other hand, compounds and reactions disposing of these species, scavenging them, suppressing their formation, or opposing their actions are **antioxidants** and include compounds such as NADPH, GSH, ascorbic acid, and vitamin E. In a normal cell, there is an appropriate pro-oxidant:antioxidant balance. However, this balance can be shifted toward the pro-oxidants when production of oxygen species is increased greatly (eg, following ingestion of certain chemicals or drugs) or when levels of antioxidants are diminished (eg, by inactivation of enzymes involved in disposal of oxygen species and by conditions that cause low levels of the antioxidants mentioned above). This state is called **"oxidative stress"** and can result in serious cell damage if the stress is massive or prolonged.

Oxygen species are now thought to play an important role in many types of **cellular injury** (eg, resulting from administration of various toxic chemicals or from ischemia), some of which can result in cell death. Indirect evidence supporting a role for these species in gen-

Table 52–4. Reactions of importance in relation to oxidative stress in blood cells and various tissues.

(1) Production of superoxide (by-product of various reactions)	$O_2 + e^- \rightarrow O_2^{\cdot-}$
(2) NADPH-oxidase	$2\ O_2 + NADPH \rightarrow 2\ O_2^{\cdot-} + NADP + H^+$
(3) Superoxide dismutase	$O_2^{\cdot-} + O_2^{\cdot-} + 2\ H^+ \rightarrow H_2O_2 + O_2$
(4) Catalase	$H_2O_2 \rightarrow 2\ H_2O + O_2$
(5) Myeloperoxidase	$H_2O_2 + X^- + H^+ \rightarrow HOX + H_2O$ ($X^- = Cl^-, Br^-, SCN^-$)
(6) Glutathione peroxidase (Se-dependent)	$2\ GSH + R\text{-}O\text{-}OH \rightarrow GSSG + H_2O + ROH$
(7) Fenton reaction	$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^-$
(8) Iron-catalyzed Haber-Weiss reaction	$O_2^{\cdot-} + H_2O_2 \rightarrow O_2 + OH^{\cdot} + OH^-$
(9) Glucose-6-phosphate dehydrogenase (G6PD)	$G6P + NADP \rightarrow 6\ Phosphogluconate + NADPH + H^+$
(10) Glutathione reductase	$G\text{-}S\text{-}S\text{-}G + NADPH + H^+ \rightarrow 2\ GSH + NADP$

erating cell injury is provided if administration of an enzyme such as superoxide dismutase or catalase is found to protect against cell injury in the situation under study.

Deficiency of Glucose-6-Phosphate Dehydrogenase Is Frequent in Certain Areas & Is an Important Cause of Hemolytic Anemia

NADPH, produced in the reaction catalyzed by the X-linked glucose-6-phosphate dehydrogenase (Table 52–4, reaction 9) in the pentose phosphate pathway (Chapter 20), plays a key role in supplying reducing equivalents in the red cell and in other cells such as the hepatocyte. Because the pentose phosphate pathway is virtually its sole means of producing NADPH, the red blood cell is very sensitive to oxidative damage if the function of this pathway is impaired (eg, by enzyme deficiency). One function of NADPH is to reduce GSSG to GSH, a reaction catalyzed by glutathione reductase (reaction 10).

Deficiency of the activity of glucose-6-phosphate dehydrogenase, owing to mutation, is extremely frequent in some regions of the world (eg, tropical Africa, the Mediterranean, certain parts of Asia, and in North America among blacks). It is the most common of all enzymopathies (diseases caused by abnormalities of enzymes), and over 300 genetic variants of the enzyme have been distinguished; at least 100 million people are deficient in this enzyme owing to these variants. The disorder resulting from deficiency of glucose-6-phosphate dehydrogenase is **hemolytic anemia.** Consumption of broad beans *(Vicia faba)* by individuals deficient in activity of the enzyme can precipitate an attack of hemolytic anemia (most likely because the beans contain potential oxidants). In addition, a number of drugs (eg, the antimalarial drug **primaquine** [the condition caused by intake of primaquine is called **primaquine-sensitive hemolytic anemia**] and **sulfonamides**) and chemicals (eg, naphthalene) precipitate an attack, because their intake leads to generation of H_2O_2 or $O_2^{\cdot-}$. Normally, H_2O_2 is disposed of by catalase and glutathione peroxidase (Table 52–4, reactions 4 and 6), the latter causing increased production of GSSG. GSH is regenerated from GSSG by the action of the enzyme glutathione reductase, which depends on the availability of NADPH (reaction 10). The red blood cells of individuals who are deficient in the activity of glucose-6-phosphate dehydrogenase cannot generate sufficient NADPH to regenerate GSH from GSSG, which in turn impairs their ability to dispose of H_2O_2 and of oxygen radicals. These compounds can cause oxidation of critical SH groups in proteins and possibly peroxidation of lipids in the membrane of the red cell, causing lysis of the red cell membrane. Some of the SH groups of hemoglobin become oxidized, and the protein precipitates inside the red blood cell, forming **Heinz bodies,** which stain purple with cresyl violet. The presence of Heinz bodies indicates that red blood cells have been subjected to oxidative stress. Figure 52–2 summarizes the possible chain of events in hemolytic anemia due to deficiency of glucose-6-phosphate dehydrogenase.

Methemoglobin Is Useless in Transporting Oxygen

The ferrous iron of hemoglobin is susceptible to oxidation by superoxide and other oxidizing agents, forming methemoglobin, which cannot transport oxygen. Only a very small amount of methemoglobin is present in normal blood, as the red blood cell possesses an effective system (the NADH-cytochrome b_5 methemoglobin reductase system) for reducing heme Fe^{3+} back to the Fe^{2+} state. This system consists of NADH (generated by glycolysis), a flavoprotein named cytochrome b_5 reductase (also known as methemoglobin reductase), and cytochrome b_5. The Fe^{3+} of methemoglobin is reduced

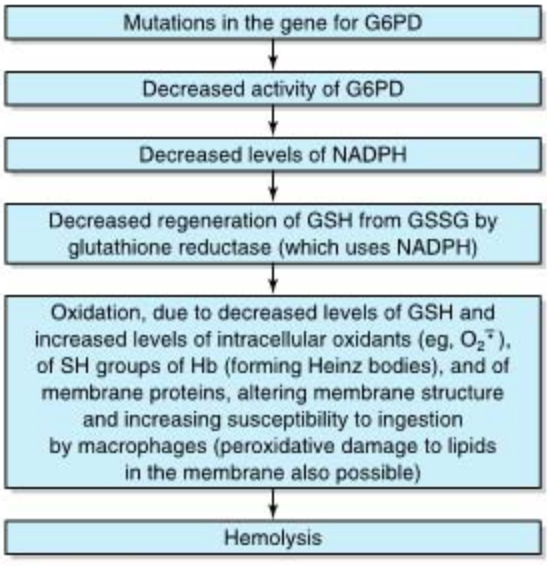

Figure 52–2. Summary of probable events causing hemolytic anemia due to deficiency of the activity of glucose-6-phosphate dehydrogenase (G6PD) (MIM 305900).

back to the Fe^{2+} state by the action of reduced cytochrome b_5:

$$\text{Hb-Fe}^{3+} + \text{Cyt } b_{5\ \text{red}} \rightarrow \text{Hb-Fe}^{2+} + \text{Cyt } b_{5\ \text{ox}}$$

Reduced cytochrome b_5 is then regenerated by the action of cytochrome b_5 reductase:

$$\text{Cyt } b_{5\ \text{ox}} + \text{NADH} \rightarrow \text{Cyt } b_{5\ \text{red}} + \text{NAD}$$

Methemoglobinemia Is Inherited or Acquired

Methemoglobinemia can be classified as either inherited or acquired by ingestion of certain drugs and chemicals. Neither type occurs frequently, but physicians must be aware of them. The inherited form is usually due to deficient activity of methemoglobin reductase, transmitted in an autosomal recessive manner. Certain abnormal hemoglobins (Hb M) are also rare causes of methemoglobinemia. In Hb M, mutation changes the amino acid residue to which heme is attached, thus altering its affinity for oxygen and favoring its oxidation. Ingestion of certain drugs (eg, sulfonamides) or chemicals (eg, aniline) can cause acquired methemoglobinemia. Cyanosis (bluish discoloration of the skin and mucous membranes due to increased amounts of deoxygenated hemoglobin in arterial blood, or in this case due to increased amounts of methemoglobin) is usually the presenting sign in both types and is evident when over 10% of hemoglobin is in the "met" form. Diagnosis is made by spectroscopic analysis of blood, which reveals the characteristic absorption spectrum of methemoglobin. Additionally, a sample of blood containing methemoglobin cannot be fully reoxygenated by flushing oxygen through it, whereas normal deoxygenated blood can. Electrophoresis can be used to confirm the presence of an abnormal hemoglobin. Ingestion of methylene blue or ascorbic acid (reducing agents) is used to treat mild methemoglobinemia due to enzyme deficiency. Acute massive methemoglobinemia (due to ingestion of chemicals) should be treated by intravenous injection of methylene blue.

MORE IS KNOWN ABOUT THE MEMBRANE OF THE HUMAN RED BLOOD CELL THAN ABOUT THE SURFACE MEMBRANE OF ANY OTHER HUMAN CELL

A variety of biochemical approaches have been used to study the membrane of the red blood cell. These include analysis of membrane proteins by SDS-PAGE, the use of specific enzymes (proteinases, glycosidases, and others) to determine the location of proteins and glycoproteins in the membrane, and various techniques to study both the lipid composition and disposition of individual lipids. Morphologic (eg, electron microscopy, freeze-fracture electron microscopy) and other techniques (eg, use of antibodies to specific components) have also been widely used. When red blood cells are lysed under specific conditions, their membranes will reseal in their original orientation to form **ghosts** (right-side-out ghosts). By altering the conditions, ghosts can also be made to reseal with their cytosolic aspect exposed on the exterior (inside-out ghosts). Both types of ghosts have been useful in analyzing the disposition of specific proteins and lipids in the membrane. In recent years, cDNAs for many proteins of this membrane have become available, permitting the deduction of their amino sequences and domains. All in all, more is known about the membrane of the red blood cell than about any other membrane of human cells (Table 52–5).

Analysis by SDS-PAGE Resolves the Proteins of the Membrane of the Red Blood Cell

When the membranes of red blood cells are analyzed by SDS-PAGE, about ten major proteins are resolved

Table 52–5. Summary of biochemical information about the membrane of the human red blood cell.

- The membrane is a bilayer composed of about 50% lipid and 50% protein.
- The major lipid classes are phospholipids and cholesterol; the major phospholipids are phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (PS) along with sphingomyelin (Sph).
- The choline-containing phospholipids, PC and Sph, predominate in the outer leaflet and the amino-containing phospholipids (PE and PS) in the inner leaflet.
- Glycosphingolipids (GSLs) (neutral GSLs, gangliosides, and complex species, including the ABO blood group substances) constitute about 5–10% of the total lipid.
- Analysis by SDS-PAGE shows that the membrane contains about 10 major proteins and more than 100 minor species.
- The major proteins (which include spectrin, ankyrin, the anion exchange protein, actin, and band 4.1) have been studied intensively, and the principal features of their disposition (eg, integral or peripheral), structure, and function have been established.
- Many of the proteins are glycoproteins (eg, the glycophorins) containing O- or N-linked (or both) oligosaccharide chains located on the external surface of the membrane.

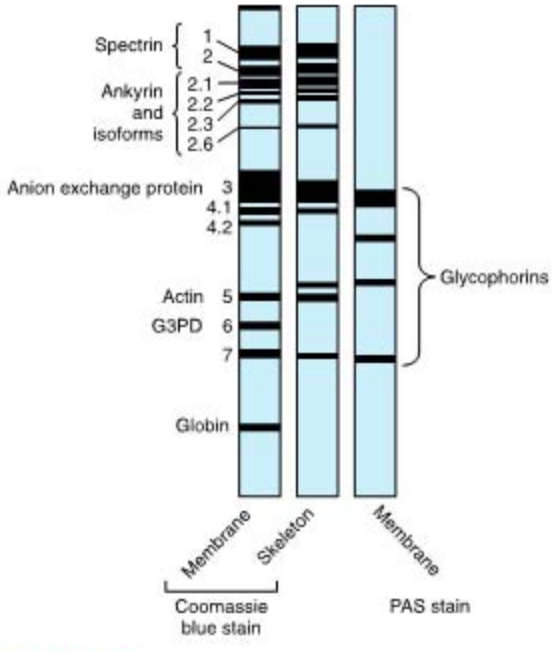

Figure 52–3. Diagrammatic representation of the major proteins of the membrane of the human red blood cell separated by SDS-PAGE. The bands detected by staining with Coomassie blue are shown in the two left-hand channels, and the glycoproteins detected by staining with periodic acid-Schiff (PAS) reagent are shown in the right-hand channel. (Reproduced, with permission, from Beck WS, Tepper RI: Hemolytic anemias III: membrane disorders. In: *Hematology*, 5th ed. Beck WS [editor]. The MIT Press, 1991.)

(Figure 52–3), several of which have been shown to be **glycoproteins.** Their migration on SDS-PAGE was used to name these proteins, with the slowest migrating (and hence highest molecular mass) being designated band 1 or **spectrin.** All these major proteins have been isolated, most of them have been identified, and considerable insight has been obtained about their functions (Table 52–6). Many of their amino acid sequences also have been established. In addition, it has been determined which are integral or peripheral membrane proteins, which are situated on the external surface, which are on the cytosolic surface, and which span the membrane (Figure 52–4). Many minor components can also be detected in the red cell membrane by use of sensitive staining methods or two-dimensional gel electrophoresis. One of these is the glucose transporter described above.

The Major Integral Proteins of the Red Blood Cell Membrane Are the Anion Exchange Protein & the Glycophorins

The anion exchange protein (band 3) is a transmembrane glycoprotein, with its carboxyl terminal end on the external surface of the membrane and its amino terminal end on the cytoplasmic surface. It is an example of a **multipass** membrane protein, extending across the bilayer at least ten times. It probably exists as a dimer in the membrane, in which it forms a tunnel, permitting the exchange of chloride for bicarbonate. Carbon dioxide, formed in the tissues, enters the red cell as bicarbonate, which is exchanged for chloride in the lungs, where carbon dioxide is exhaled. The amino terminal end binds many proteins, including hemoglobin, proteins 4.1 and 4.2, ankyrin, and several glycolytic enzymes. Purified band 3 has been added to lipid vesicles in vitro and has been shown to perform its transport functions in this reconstituted system.

Glycophorins A, B, and C are also transmembrane glycoproteins but of the **single-pass** type, extending across the membrane only once. A is the major glycophorin, is made up of 131 amino acids, and is heavily glycosylated (about 60% of its mass). Its amino terminal end, which contains 16 oligosaccharide chains (15 of which are O-glycans), extrudes out from the surface of

Table 52–6. Principal proteins of the red cell membrane.[1]

Band Number[2]	Protein	Integral (I) or Peripheral (P)	Approximate Molecular Mass (kDa)
1	Spectrin (α)	P	240
2	Spectrin (β)	P	220
2.1	Ankyrin	P	210
2.2	"	P	195
2.3	"	P	175
2.6	"	P	145
3	Anion exchange protein	I	100
4.1	Unnamed	P	80
5	Actin	P	43
6	Glyceraldehyde-3-phosphate dehydrogenase	P	35
7	Tropomyosin	P	29
8	Unnamed	P	23
	Glycophorins A, B, and C	I	31, 23, and 28

[1]Adapted from Lux DE, Becker PS: Disorders of the red cell membrane skeleton: hereditary spherocytosis and hereditary elliptocytosis. Chapter 95 in: *The Metabolic Basis of Inherited Disease*, 6th ed. Scriver CR et al (editors). McGraw-Hill, 1989.

[2]The band number refers to the position of migration on SDS-PAGE (see Figure 52–3). The glycophorins are detected by staining with the periodic acid-Schiff reagent. A number of other components (eg, 4.2 and 4.9) are not listed. Native spectrin is $\alpha_2\beta_2$.

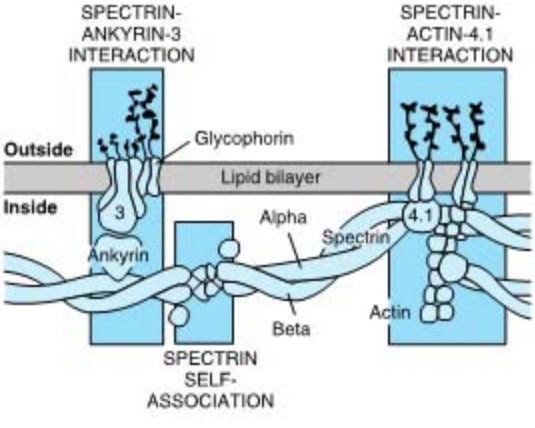

Figure 52–4. Diagrammatic representation of the interaction of cytoskeletal proteins with each other and with certain integral proteins of the membrane of the red blood cell. (Reproduced, with permission, from Beck WS, Tepper RI: Hemolytic anemias III: membrane disorders. In: *Hematology*, 5th ed. Beck WS [editor]. The MIT Press, 1991.)

the red blood cell. Approximately 90% of the sialic acid of the red cell membrane is located in this protein. Its transmembrane segment (23 amino acids) is α-helical. The carboxyl terminal end extends into the cytosol and binds to protein 4.1, which in turn binds to spectrin. Polymorphism of this protein is the basis of the MN blood group system (see below). Glycophorin A contains binding sites for influenza virus and for *Plasmodium falciparum*, the cause of one form of malaria. Intriguingly, the function of red blood cells of individuals who lack glycophorin A does not appear to be affected.

Spectrin, Ankyrin, & Other Peripheral Membrane Proteins Help Determine the Shape & Flexibility of the Red Blood Cell

The red blood cell must be able to squeeze through some tight spots in the microcirculation during its numerous passages around the body; the sinusoids of the spleen are of special importance in this regard. For the red cell to be easily and reversibly deformable, its membrane must be both fluid and flexible; it should also preserve its biconcave shape, since this facilitates gas exchange. Membrane lipids help determine membrane fluidity. Attached to the inner aspect of the membrane of the red blood cell are a number of peripheral cytoskeletal proteins (Table 52–6) that play important roles in respect to preserving shape and flexibility; these will now be described.

Spectrin is the major protein of the cytoskeleton. It is composed of two polypeptides: spectrin 1 (α chain) and spectrin 2 (β chain). These chains, measuring approximately 100 nm in length, are aligned in an antiparallel manner and are loosely intertwined, forming a dimer. Both chains are made up of segments of 106 amino acids that appear to fold into triple-stranded α-helical coils joined by nonhelical segments. One dimer interacts with another, forming a head-to-head tetramer. The overall shape confers flexibility on the protein and in turn on the membrane of the red blood cell. At least four binding sites can be defined in spectrin: (1) for self-association, (2) for ankyrin (bands 2.1, etc), (3) for actin (band 5), and (4) for protein 4.1.

Ankyrin is a pyramid-shaped protein that binds spectrin. In turn, ankyrin binds tightly to band 3, securing attachment of spectrin to the membrane. Ankyrin is sensitive to proteolysis, accounting for the appearance of bands 2.2, 2.3, and 2.6, all of which are derived from band 2.1.

Actin (band 5) exists in red blood cells as short, double-helical filaments of F-actin. The tail end of spectrin dimers binds to actin. Actin also binds to protein 4.1.

Protein 4.1, a globular protein, binds tightly to the tail end of spectrin, near the actin-binding site of the latter, and thus is part of a protein 4.1-spectrin-actin ternary complex. Protein 4.1 also binds to the integral proteins, glycophorins A and C, thereby attaching the ternary complex to the membrane. In addition, protein 4.1 may interact with certain membrane phospholipids, thus connecting the lipid bilayer to the cytoskeleton.

Certain other proteins (4.9, adducin, and tropomyosin) also participate in cytoskeletal assembly.

Abnormalities in the Amount or Structure of Spectrin Cause Hereditary Spherocytosis & Elliptocytosis

Hereditary spherocytosis is a genetic disease, transmitted as an autosomal dominant, that affects about 1:5000 North Americans. It is characterized by the presence of spherocytes (spherical red blood cells, with a low surface-to-volume ratio) in the peripheral blood, by a hemolytic anemia, and by splenomegaly. The spherocytes are not as deformable as are normal red blood cells, and they are subject to destruction in the spleen, thus greatly shortening their life in the circulation. Hereditary spherocytosis is curable by splenectomy because the spherocytes can persist in the circulation if the spleen is absent.

The spherocytes are much more susceptible to osmotic lysis than are normal red blood cells. This is assessed in the **osmotic fragility test,** in which red blood cells are exposed in vitro to decreasing concentrations of NaCl. The physiologic concentration of NaCl is 0.85 g/dL. When exposed to a concentration of NaCl of 0.5 g/dL, very few normal red blood cells are hemolyzed, whereas approximately 50% of spherocytes would lyse under these conditions. The explanation is that the spherocyte, being almost circular, has little potential extra volume to accommodate additional water and thus lyses readily when exposed to a slightly lower osmotic pressure than is normal.

One cause of hereditary spherocytosis (Figure 52–5) is a deficiency in the amount of spectrin or abnormalities of its structure, so that it no longer tightly binds the other proteins with which it normally interacts. This weakens the membrane and leads to the spherocytic shape. Abnormalities of ankyrin and of bands 3 and 4.1 are involved in other cases.

Hereditary elliptocytosis is a genetic disorder that is similar to hereditary spherocytosis except that affected red blood cells assume an elliptic, disk-like shape, recognizable by microscopy. It is also due to abnormalities in spectrin; some cases reflect abnormalities of band 4.1 or of glycophorin C.

THE BIOCHEMICAL BASES OF THE ABO BLOOD GROUP SYSTEM HAVE BEEN ESTABLISHED

At least 21 human blood group systems are recognized, the best known of which are the ABO, Rh (Rhesus), and MN systems. The term "blood group" applies to a defined system of red blood cell antigens (blood group substances) controlled by a genetic locus having a variable number of alleles (eg, A, B, and O in the ABO system). The term "blood type" refers to the antigenic phenotype, usually recognized by the use of appropriate

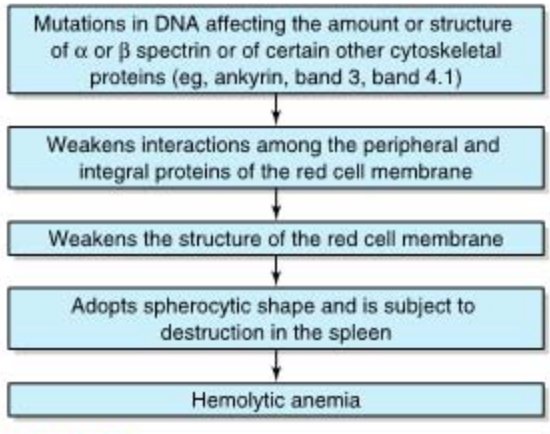

Figure 52–5. Summary of the causation of hereditary spherocytosis (MIM 182900).

antibodies. For purposes of blood transfusion, it is particularly important to know the basics of the ABO and Rh systems. However, knowledge of blood group systems is also of biochemical, genetic, immunologic, anthropologic, obstetric, pathologic, and forensic interest. Here, we shall discuss only some key features of the ABO system. From a biochemical viewpoint, the major interests in the ABO substances have been in isolating and determining their structures, elucidating their pathways of biosynthesis, and determining the natures of the products of the A, B, and O genes.

The ABO System Is of Crucial Importance in Blood Transfusion

This system was first discovered by Landsteiner in 1900 when investigating the basis of compatible and incompatible transfusions in humans. The membranes of the red blood cells of most individuals contain one blood group substance of type A, type B, type AB, or type O. Individuals of type A have anti-B antibodies in their plasma and will thus agglutinate type B or type AB blood. Individuals of type B have anti-A antibodies and will agglutinate type A or type AB blood. Type AB blood has neither anti-A nor anti-B antibodies and has been designated the **universal recipient.** Type O blood has neither A nor B substances and has been designated the **universal donor.** The explanation of these findings is related to the fact that the body does not usually produce antibodies to its own constituents. Thus, individuals of type A do not produce antibodies to their own blood group substance, A, but do possess antibodies to the foreign blood group substance, B, possibly because similar structures are present in microorganisms to which the body is exposed early in life. Since individuals of type O have neither A nor B substances, they possess antibodies to both these foreign substances. The above description has been simplified considerably; eg, there are two subgroups of type A: A_1 and A_2.

The genes responsible for production of the ABO substances are present on the long arm of chromosome 9. There are three alleles, two of which are codominant (A and B) and the third (O) recessive; these ultimately determine the four phenotypic products: the A, B, AB, and O substances.

The ABO Substances Are Glycosphingolipids & Glycoproteins Sharing Common Oligosaccharide Chains

The ABO substances are complex oligosaccharides present in most cells of the body and in certain secretions. On membranes of red blood cells, the oligosaccharides that determine the specific natures of the ABO substances appear to be mostly present in **glycosphingolipids,** whereas in secretions the same oligosaccharides are present in **glycoproteins.** Their presence in secretions is determined by a gene designated ***Se*** (for **secretor**), which codes for a specific **fucosyl (Fuc) transferase** in secretory organs, such as the exocrine glands, but which is not active in red blood cells. Individuals of *SeSe* or *Sese* genotypes secrete A or B antigens (or both), whereas individuals of the *sese* genotype do not secrete A or B substances, but their red blood cells can express the A and B antigens.

H Substance Is the Biosynthetic Precursor of Both the A & B Substances

The ABO substances have been isolated and their structures determined; simplified versions, showing only their nonreducing ends, are presented in Figure 52–6. It is important to first appreciate the structure of the H substance, since it is the precursor of both the A and B substances and is the blood group substance found in persons of type O. H substance itself is formed by the action of a **fucosyltransferase,** which catalyzes the addition of the terminal fucose in $\alpha 1 \rightarrow 2$ linkage onto the terminal Gal residue of its precursor:

$$\underset{\text{Precursor}}{\text{GDP-Fuc} + \text{Gal-}\beta\text{-R}} \rightarrow \underset{\text{H substance}}{\text{Fuc-}\alpha 1,2\text{-Gal-}\beta\text{-R}} + \text{GDP}$$

The H locus codes for this fucosyltransferase. The *h* allele of the H locus codes for an inactive fucosyltransferase; therefore, individuals of the *hh* genotype cannot generate H substance, the precursor of the A and B antigens. Thus, individuals of the *hh* genotype will have red blood cells of type O, even though they may possess the enzymes necessary to make the A or B substances (see below).

The *A* Gene Encodes a GalNAc Transferase, the *B* Gene a Gal Transferase, & the *O* Gene an Inactive Product

In comparison with H substance (Figure 52–6), **A substance** contains an additional GalNAc and **B substance** an additional Gal, linked as indicated. Anti-A antibodies are directed to the additional GalNAc residue found in the A substance, and anti-B antibodies are directed toward the additional Gal residue found in the B substance. Thus, GalNAc is the **immunodominant sugar** (ie, the one determining the specificity of the antibody formed) of blood group A substance, whereas Gal is the immunodominant sugar of the B substance. In view of the structural findings, it is not surprising that A substance can be synthesized in vitro from O substance in a reaction catalyzed by a GalNAc transferase, employing UDP-GalNAc as the sugar donor. Similarly, blood

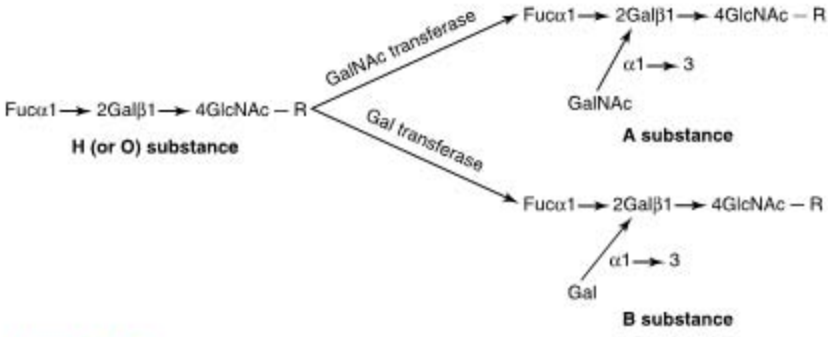

Figure 52–6. Diagrammatic representation of the structures of the H, A, and B blood group substances. R represents a long complex oligosaccharide chain, joined either to ceramide where the substances are glycosphingolipids, or to the polypeptide backbone of a protein via a serine or threonine residue where the substances are glycoproteins. Note that the blood group substances are biantennary; ie, they have two arms, formed at a branch point (not indicated) between the GlcNAc—R, and only one arm of the branch is shown. Thus, the H, A, and B substances each contain two of their respective short oligosaccharide chains shown above. The AB substance contains one type A chain and one type B chain.

group B can be synthesized from O substance by the action of a Gal transferase, employing UDP-Gal. It is crucial to appreciate that the product of the *A* gene is the GalNAc transferase that adds the terminal GalNAc to the O substance. Similarly, the product of the *B* gene is the Gal transferase adding the Gal residue to the O substance. Individuals of type AB possess both enzymes and thus have two oligosaccharide chains (Figure 52–6), one terminated by a GalNAc and the other by a Gal. Individuals of type O apparently synthesize an inactive protein, detectable by immunologic means; thus, H substance is their ABO blood group substance.

In 1990, a study using cloning and sequencing technology described the nature of the differences between the glycosyltransferase products of the *A, B,* and *O* genes. A difference of four nucleotides is apparently responsible for the distinct specificities of the A and B glycosyltransferases. On the other hand, the *O* allele has a single base-pair mutation, causing a **frameshift mutation** resulting in a protein lacking transferase activity.

HEMOLYTIC ANEMIAS ARE CAUSED BY ABNORMALITIES OUTSIDE, WITHIN, OR INSIDE THE RED BLOOD CELL MEMBRANE

Causes outside the membrane include hypersplenism, a condition in which the spleen is enlarged from a variety of causes and red blood cells become sequestered in it. Immunologic abnormalities (eg, transfusion reactions, the presence in plasma of warm and cold antibodies that lyse red blood cells, and unusual sensitivity to complement) also fall in this class, as do toxins released by various infectious agents, such as certain bacteria (eg, clostridium). Some snakes release venoms that act to lyse the red cell membrane (eg, via the action of phospholipases or proteinases).

Causes within the membrane include abnormalities of proteins. The most important conditions are hereditary spherocytosis and hereditary elliptocytosis, principally caused by abnormalities in the amount or structure of spectrin (see above).

Causes inside the red blood cell include **hemoglobinopathies** and **enzymopathies.** Sickle cell anemia is the most important hemoglobinopathy. Abnormalities of enzymes in the pentose phosphate pathway and in glycolysis are the most frequent enzymopathies involved, particularly the former. Deficiency of glucose-6-phosphate dehydrogenase is prevalent in certain parts of the world and is a frequent cause of hemolytic anemia (see above). Deficiency of pyruvate kinase is not frequent, but it is the second commonest enzyme deficiency resulting in hemolytic anemia; the mechanism appears to be due to impairment of glycolysis, resulting in decreased formation of ATP, affecting various aspects of membrane integrity.

Laboratory investigations that aid in the diagnosis of hemolytic anemia are listed in Table 52–7.

Table 52–7. Laboratory investigations that assist in the diagnosis of hemolytic anemia.

General tests and findings
Increased nonconjugated (indirect) bilirubin
Shortened red cell survival time as measured by injection of autologous ^{51}Cr-labeled red cells
Reticulocytosis
Hemoglobinemia
Low level of plasma haptoglobin
Specific tests and findings
Hb electrophoresis (eg, HbS)
Red cell enzymes (eg, G6PD or PK deficiency)
Osmotic fragility (eg, hereditary spherocytosis)
Coombs test[†]
Cold agglutinins

[†]The direct Coombs test detects the presence of antibodies on red cells, whereas the indirect test detects the presence of circulating antibodies to antigens present on red cells.

NEUTROPHILS HAVE AN ACTIVE METABOLISM & CONTAIN SEVERAL UNIQUE ENZYMES & PROTEINS

The major biochemical features of neutrophils are summarized in Table 52–8. Prominent features are active aerobic glycolysis, active pentose phosphate pathway, moderately active oxidative phosphorylation (because mitochondria are relatively sparse), and a high content of lysosomal enzymes. Many of the enzymes listed in Table 52–4 are also of importance in the oxidative metabolism of neutrophils (see below). Table 52–9 summarizes the functions of some proteins that are relatively unique to neutrophils.

Neutrophils Are Key Players in the Body's Defense Against Bacterial Infection

Neutrophils are motile phagocytic cells that play a key role in acute inflammation. When bacteria enter tissues, a number of phenomena result that are collectively known as the "acute inflammatory response." They include (1) increase of vascular permeability, (2) entry of activated neutrophils into the tissues, (3) activation of platelets, and (4) spontaneous subsidence (resolution) if the invading microorganisms have been dealt with successfully.

Table 52–8. Summary of major biochemical features of neutrophils.

• Active glycolysis
• Active pentose phosphate pathway
• Moderate oxidative phosphorylation
• Rich in lysosomes and their degradative enzymes
• Contain certain unique enzymes (eg, myeloperoxidase and NADPH-oxidase) and proteins
• Contain CD 11/CD18 integrins in plasma membrane

A variety of molecules are released from cells and plasma proteins during acute inflammation whose net overall effect is to increase vascular permeability, resulting in tissue edema (Table 52–10).

In acute inflammation, neutrophils are recruited from the bloodstream into the tissues to help eliminate the foreign invaders. The neutrophils are attracted into the tissues by **chemotactic factors,** including complement fragment C5a, small peptides derived from bacteria (eg, *N*-formyl-methionyl-leucyl-phenylalanine), and a number of leukotrienes. To reach the tissues, circulating neutrophils must pass through the capillaries. To achieve this, they marginate along the vessel walls and then adhere to endothelial (lining) cells of the capillaries.

Integrins Mediate Adhesion of Neutrophils to Endothelial Cells

Adhesion of neutrophils to endothelial cells employs specific adhesive proteins (integrins) located on their surface and also specific receptor proteins in the endothelial cells. (See also the discussion of selectins in Chapter 47.)

The integrins are a superfamily of surface proteins present on a wide variety of cells. They are involved in the adhesion of cells to other cells or to specific components of the extracellular matrix. They are heterodimers, containing an α and a β subunit linked noncovalently. The subunits contain extracellular, transmembrane, and intracellular segments. The extracellular segments bind to a variety of ligands such as specific proteins of the extracellular matrix and of the surfaces of other cells. These ligands often contain Arg-Gly-Asp (R-G-D) sequences. The intracellular domains bind to various proteins of the cytoskeleton, such as actin and vinculin. The integrins are proteins that link the outsides of cells to their insides, thereby helping to integrate responses of cells (eg, movement, phagocytosis) to changes in the environment.

Three subfamilies of integrins were recognized initially. Members of each subfamily were distinguished by containing a common β subunit, but they differed in their α subunits. However, more than three β subunits have now been identified, and the classification of integrins has become rather complex. Some integrins of specific interest with regard to neutrophils are listed in Table 52–11.

A deficiency of the β_2 subunit (also designated CD18) of LFA-1 and of two related integrins found in

Table 52–9. Some important enzymes and proteins of neutrophils.[1]

Enzyme or Protein	Reaction Catalyzed or Function	Comment
Myeloperoxidase (MPO)	$H_2O_2 + X^- \text{(halide)} + H^+ \rightarrow HOX + H_2O$ (where $X^- = Cl^-$, HOX = hypochlorous acid)	Responsible for the green color of pus Genetic deficiency can cause recurrent infections
NADPH-oxidase	$2O_2 + NADPH \rightarrow 2O_2^- + NADP + H^+$	Key component of the respiratory burst Deficient in chronic granulomatous disease
Lysozyme	Hydrolyzes link between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine found in certain bacterial cell walls	Abundant in macrophages
Defensins	Basic antibiotic peptides of 20–33 amino acids	Apparently kill bacteria by causing membrane damage
Lactoferrin	Iron-binding protein	May inhibit growth of certain bacteria by binding iron and may be involved in regulation of proliferation of myeloid cells
CD11a/CD18, CD11b/CD18, CD11c/CD18[2]	Adhesion molecules (members of the integrin family)	Deficient in leukocyte adhesion deficiency type I (MIM 116920)
Receptors for Fc fragments of IgGs	Bind Fc fragments of IgG molecules	Target antigen-antibody complexes to myeloid and lymphoid cells, eliciting phagocytosis and other responses

[1]The expression of many of these molecules has been studied during various stages of differentiation of normal neutrophils and also of corresponding leukemic cells employing molecular biology techniques (eg, measurements of their specific mRNAs). For the majority, cDNAs have been isolated and sequenced, amino acid sequences deduced, genes have been localized to specific chromosomal locations, and exons and intron sequences have been defined. Some important proteinases of neutrophils are listed in Table 52–12.
[2]CD = cluster of differentiation. This refers to a uniform system of nomenclature that has been adopted to name surface markers of leukocytes. A specific surface protein (marker) that identifies a particular lineage or differentiation stage of leukocytes and that is recognized by a group of monoclonal antibodies is called a member of a cluster of differentiation. The system is particularly helpful in categorizing subclasses of lymphocytes. Many CD antigens are involved in cell-cell interactions, adhesion, and transmembrane signaling.

neutrophils and macrophages, Mac-1 (CD11b/CD18) and p150,95 (CD11c/CD18), causes **type 1 leukocyte adhesion deficiency,** a disease characterized by recurrent bacterial and fungal infections. Among various results of this deficiency, the adhesion of affected white blood cells to endothelial cells is diminished, and lower numbers of neutrophils thus enter the tissues to combat infection.

Once having passed through the walls of small blood vessels, the neutrophils migrate toward the highest concentrations of the chemotactic factors, encounter the invading bacteria, and attempt to attack and destroy them. The neutrophils must be activated in order to turn on many of the metabolic processes involved in phagocytosis and killing of bacteria.

Activation of Neutrophils Is Similar to Activation of Platelets & Involves Hydrolysis of Phosphatidylinositol Bisphosphate

The mechanisms involved in platelet activation are discussed in Chapter 51 (see Figure 51–8). The process involves interaction of the stimulus (eg, thrombin) with a receptor, activation of G proteins, stimulation of phospholipase C, and liberation from phosphatidylinositol

Table 52–10. Sources of biomolecules with vasoactive properties involved in acute inflammation.

Mast Cells and Basophils	Platelets	Neutrophils	Plasma Proteins
Histamine	Serotonin	Platelet-activating factor (PAF) Eicosanoids (various prostaglandins and leukotrienes)	C3a, C4a, and C5a from the complement system Bradykinin and fibrin split products from the coagulation system

Table 52–11. Examples of integrins that are important in the function of neutrophils, of other white blood cells, and of platelets.[1]

Integrin	Cell	Subunit	Ligand	Function
VLA-1 (CD49a)	WBCs, others	α1β1	Collagen, laminin	Cell-ECM adhesion
VLA-5 (CD49e)	WBCs, others	α5β1	Fibronectin	Cell-ECM adhesion
VLA-6 (CD49f)	WBCs, others	α6β1	Laminin	Cell-ECM adhesion
LFA-1 (CD11a)	WBCs	αLβ2	ICAM-1	Adhesion of WBCs
Glycoprotein IIb/IIIa	Platelets	αIIbβ3	ICAM-2 Fibrinogen, fibronectin, von Willebrand factor	Platelet adhesion and aggregation

[1]LFA-1, lymphocyte function-associated antigen 1; VLA, very late antigen; CD, cluster of differentiation; ICAM, intercellular adhesion molecule; ECM, extracellular matrix. A deficiency of LFA-1 and related integrins is found in type I leukocyte adhesion deficiency (MIM 116290). A deficiency of platelet glycoprotein IIb/IIIa complex is found in Glanzmann thrombasthenia (MIM 273800), a condition characterized by a history of bleeding, a normal platelet count, and abnormal clot retraction. These findings illustrate how fundamental knowledge of cell surface adhesion proteins is shedding light on the causation of a number of diseases.

bisphosphate of inositol triphosphate and diacylglycerol. These two second messengers result in an elevation of intracellular Ca^{2+} and activation of protein kinase C. In addition, activation of phospholipase A_2 produces arachidonic acid that can be converted to a variety of biologically active eicosanoids.

The process of activation of neutrophils is essentially similar. They are activated, via specific receptors, by interaction with bacteria, binding of chemotactic factors, or antibody-antigen complexes. The resultant rise in intracellular Ca^{2+} affects many processes in neutrophils, such as assembly of microtubules and the actin-myosin system. These processes are respectively involved in secretion of contents of granules and in motility, which enables neutrophils to seek out the invaders. The activated neutrophils are now ready to destroy the invaders by mechanisms that include production of active derivatives of oxygen.

The Respiratory Burst of Phagocytic Cells Involves NADPH Oxidase & Helps Kill Bacteria

When neutrophils and other phagocytic cells engulf bacteria, they exhibit a rapid increase in oxygen consumption known as the respiratory burst. This phenomenon reflects the rapid utilization of oxygen (following a lag of 15–60 seconds) and production from it of large amounts of reactive derivatives, such as O_2^-, H_2O_2, $OH^•$, and OCl^- (hypochlorite ion). Some of these products are potent microbicidal agents.

The **electron transport chain system** responsible for the respiratory burst (named NADPH oxidase) is composed of several components. One is **cytochrome b_{558},** located in the plasma membrane; it is a heterodimer, containing two polypeptides of 91 kDa and 22 kDa. When the system is activated (see below), two cytoplasmic polypeptides of 47 kDa and 67 kDa are recruited to the plasma membrane and, together with cytochrome b_{558}, form the NADPH oxidase responsible for the respiratory burst. The reaction catalyzed by NADPH oxidase, involving formation of superoxide anion, is shown in Table 52–4 (reaction 2). This system catalyzes the one-electron reduction of oxygen to superoxide anion. The NADPH is generated mainly by the pentose phosphate cycle, whose activity increases markedly during phagocytosis.

The above reaction is followed by the spontaneous production (by spontaneous dismutation) of **hydrogen peroxide** from two molecules of superoxide:

$$O_2^{-} + O_2^{-} + 2\,H^+ \rightarrow H_2O_2 + O_2$$

The superoxide ion is discharged to the outside of the cell or into phagolysosomes, where it encounters ingested bacteria. Killing of bacteria within phagolysosomes appears to depend on the combined action of elevated pH, superoxide ion, or further oxygen derivatives (H_2O_2, $OH^•$, and HOCl [hypochlorous acid; see below]) and on the action of certain bactericidal peptides (defensins) and other proteins (eg, cathepsin G and certain cationic proteins) present in phagocytic cells. Any superoxide that enters the cytosol of the phagocytic cell is converted to H_2O_2 by the action of **superoxide dismutase,** which catalyzes the same reaction as the spontaneous dismutation shown above. In turn, H_2O_2 is used by myeloperoxidase (see below) or disposed of by the action of glutathione peroxidase or catalase.

NADPH oxidase is inactive in resting phagocytic cells and is activated upon contact with various ligands (complement fragment C5a, chemotactic peptides, etc)

with receptors in the plasma membrane. The events resulting in activation of the oxidase system have been much studied and are similar to those described above for the process of activation of neutrophils. They involve **G proteins,** activation of **phospholipase C,** and generation of **inositol 1,4,5-triphosphate** (IP_3). The last mediates a transient increase in the level of cytosolic Ca^{2+}, which is essential for induction of the respiratory burst. **Diacylglycerol** is also generated and induces the translocation of protein kinase C into the plasma membrane from the cytosol, where it catalyzes the **phosphorylation** of various proteins, some of which are components of the oxidase system. A second pathway of activation not involving Ca^{2+} also operates.

Mutations in the Genes for Components of the NADPH Oxidase System Cause Chronic Granulomatous Disease

The importance of the NADPH oxidase system was clearly shown when it was observed that the respiratory burst was defective in chronic granulomatous disease, a relatively uncommon condition characterized by recurrent infections and widespread granulomas (chronic inflammatory lesions) in the skin, lungs, and lymph nodes. The granulomas form as attempts to wall off bacteria that have not been killed, owing to genetic deficiencies in the NADPH oxidase system. The disorder is due to mutations in the genes encoding the four polypeptides that constitute the NADPH oxidase system. Some patients have responded to treatment with gamma interferon, which may increase transcription of the 91-kDa component if it is affected. The probable sequence of events involved in the causation of chronic granulomatous disease is shown in Figure 52–7.

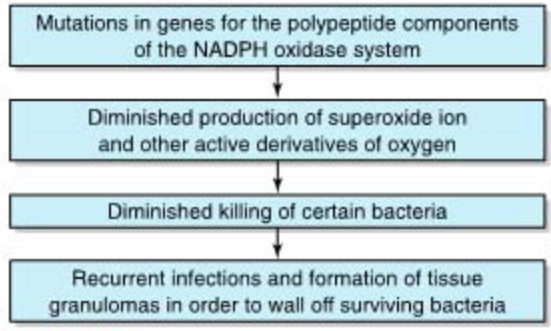

Figure 52–7. Simplified scheme of the sequence of events involved in the causation of chronic granulomatous disease (MIM 306400). Mutations in any of the genes for the four polypeptides involved (two are components of cytochrome b_{558} and two are derived from the cytoplasm) can cause the disease. The polypeptide of 91 kDa is encoded by a gene in the X chromosome; approximately 60% of cases of chronic granulomatous disease are X-linked, with the remainder being inherited in an autosomal recessive fashion.

Neutrophils Contain Myeloperoxidase, Which Catalyzes the Production of Chlorinated Oxidants

The enzyme myeloperoxidase, present in large amounts in neutrophil granules and responsible for the green color of pus, can act on H_2O_2 to produce hypohalous acids:

$$H_2O_2 + X^- + H^+ \xrightarrow{\text{MYELOPEROXIDASE}} HOX + H_2O$$

(X^- = Cl^-, Br^-, I^- or SCN^-; HOCl = hypochlorous acid)

The H_2O_2 used as substrate is generated by the NADPH oxidase system. Cl^- is the halide usually employed, since it is present in relatively high concentration in plasma and body fluids. HOCl, the active ingredient of household liquid bleach, is a powerful oxidant and is highly microbicidal. When applied to normal tissues, its potential for causing damage is diminished because it reacts with primary or secondary amines present in neutrophils and tissues to produce various nitrogen-chlorine derivatives; these chloramines are also oxidants, though less powerful than HOCl, and act as microbicidal agents (eg, in sterilizing wounds) without causing tissue damage.

The Proteinases of Neutrophils Can Cause Serious Tissue Damage If Their Actions Are Not Checked

Neutrophils contain a number of proteinases (Table 52–12) that can hydrolyze elastin, various types of collagens, and other proteins present in the extracellular matrix. Such enzymatic action, if allowed to proceed unopposed, can result in serious damage to tissues. Most of these proteinases are **lysosomal enzymes** and exist mainly as inactive precursors in normal neutrophils. Small amounts of these enzymes are released into normal tissues, with the amounts increasing markedly during inflammation. The activities of elastase and other proteinases are normally kept in check by a number of **antiproteinases** (also listed in Table 52–12) present in plasma and the extracellular fluid. Each of them can combine—usually forming a noncovalent complex—with one or more specific proteinases and thus cause inhibition. In Chapter 50 it was shown that a genetic deficiency of **α_1-antiproteinase inhibitor** (α_1-antitrypsin) permits elastase to act unopposed and digest pulmonary tissue, thereby participating in

Table 52–12. Proteinases of neutrophils and antiproteinases of plasma and tissues.[1]

Proteinases	Antiproteinases
Elastase	α_1-Antiproteinase (α_1-antitrypsin)
Collagenase	α_2-Macroglobulin
Gelatinase	Secretory leukoproteinase inhibitor
Cathepsin G	α_1-Antichymotrypsin
Plasminogen activator	Plasminogen activator inhibitor–1
	Tissue inhibitor of metalloproteinase

[1]The table lists some of the important proteinases of neutrophils and some of the proteins that can inhibit their actions. Most of the proteinases listed exist inside neutrophils as precursors. Plasminogen activator is not a proteinase, but it is included because it influences the activity of plasmin, which is a proteinase. The proteinases listed can digest many proteins of the extracellular matrix, causing tissue damage. The overall balance of proteinase:antiproteinase action can be altered by activating the precursors of the proteinases, or by inactivating the antiproteinases. The latter can be caused by proteolytic degradation or chemical modification, eg, Met-358 of α_1-antiproteinase inhibitor is oxidized by cigarette smoke.

the causation of emphysema. **α_2-Macroglobulin** is a plasma protein that plays an important role in the body's defense against excessive action of proteases; it combines with and thus neutralizes the activities of a number of important proteases (Chapter 50).

When increased amounts of chlorinated oxidants are formed during inflammation, they affect the proteinase:antiproteinase equilibrium, tilting it in favor of the former. For instance, certain of the proteinases listed in Table 52–12 are activated by HOCl, whereas certain of the antiproteinases are inactivated by this compound. In addition, the tissue inhibitor of metalloproteinases and α_1-antichymotrypsin can be hydrolyzed by activated elastase, and α_1-antiproteinase inhibitor can be hydrolyzed by activated collagenase and gelatinase. In most circumstances, an appropriate balance of proteinases and antiproteinases is achieved. However, in certain instances, such as in the lung when α_1-antiproteinase inhibitor is deficient or when large amounts of neutrophils accumulate in tissues because of inadequate drainage, considerable tissue damage can result from the unopposed action of proteinases.

RECOMBINANT DNA TECHNOLOGY HAS HAD A PROFOUND IMPACT ON HEMATOLOGY

Recombinant DNA technology has had a major impact on many aspects of hematology. The bases of the thalassemias and of many disorders of coagulation (Chapter 51) have been greatly clarified by investigations using cloning and sequencing. The study of oncogenes and chromosomal translocations has advanced understanding of the leukemias. As discussed above, cloning techniques have made available therapeutic amounts of erythropoietin and other growth factors. Deficiency of adenosine deaminase, which affects lymphocytes particularly, is the first disease to be treated by gene therapy. Like many other areas of biology and medicine, hematology has been and will continue to be revolutionized by this technology.

SUMMARY

- The red blood cell is simple in terms of its structure and function, consisting principally of a concentrated solution of hemoglobin surrounded by a membrane.
- The production of red cells is regulated by erythropoietin, whereas other growth factors (eg, granulocyte- and granulocyte-macrophage colony-stimulating factors) regulate the production of white blood cells.
- The red cell contains a battery of cytosolic enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, to dispose of powerful oxidants generated during its metabolism.
- Genetically determined deficiency of the activity of glucose-6-phosphate dehydrogenase, which produces NADPH, is an important cause of hemolytic anemia.
- Methemoglobin is unable to transport oxygen; both genetic and acquired causes of methemoglobinemia are recognized. Considerable information has accumulated concerning the proteins and lipids of the red cell membrane. A number of cytoskeletal proteins, such as spectrin, ankyrin, and actin, interact with specific integral membrane proteins to help regulate the shape and flexibility of the membrane.
- Deficiency of spectrin results in hereditary spherocytosis, another important cause of hemolytic anemia.
- The ABO blood group substances in the red cell membrane are complex glycosphingolipids; the immunodominant sugar of A substance is *N*-acetylgalactosamine, whereas that of the B substance is galactose.
- Neutrophils play a major role in the body's defense mechanisms. Integrins on their surface membranes determine specific interactions with various cell and tissue components.
- Leukocytes are activated on exposure to bacteria and other stimuli; NADPH oxidase plays a key role in the process of activation (the respiratory burst). Mutations in this enzyme and associated proteins cause chronic granulomatous disease.

- The proteinases of neutrophils can digest many tissue proteins; normally, this is kept in check by a battery of antiproteinases. However, this defense mechanism can be overcome in certain circumstances, resulting in extensive tissue damage.
- The application of recombinant DNA technology is revolutionizing the field of hematology.

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Metabolism of Xenobiotics

53

Robert K. Murray, MD, PhD

BIOMEDICAL IMPORTANCE

Increasingly, humans are subjected to exposure to various foreign chemicals (xenobiotics)—drugs, food additives, pollutants, etc. The situation is well summarized in the following quotation from Rachel Carson: "As crude a weapon as the cave man's club, the chemical barrage has been hurled against the fabric of life." Understanding how xenobiotics are handled at the cellular level is important in learning how to cope with the chemical onslaught.

Knowledge of the metabolism of xenobiotics is basic to a rational understanding of pharmacology and therapeutics, pharmacy, toxicology, management of cancer, and drug addiction. All these areas involve administration of, or exposure to, xenobiotics.

HUMANS ENCOUNTER THOUSANDS OF XENOBIOTICS THAT MUST BE METABOLIZED BEFORE BEING EXCRETED

A xenobiotic (Gk *xenos* "stranger") is a compound that is foreign to the body. The principal classes of xenobiotics of medical relevance are drugs, chemical carcinogens, and various compounds that have found their way into our environment by one route or another, such as polychlorinated biphenyls (PCBs) and certain insecticides. More than 200,000 manufactured environmental chemicals exist. Most of these compounds are subject to metabolism (chemical alteration) in the human body, with the liver being the main organ involved; occasionally, a xenobiotic may be excreted unchanged. At least 30 different enzymes catalyze reactions involved in xenobiotic metabolism; however, this chapter will only cover a selected group of them.

It is convenient to consider the metabolism of xenobiotics in two phases. In phase 1, the major reaction involved is **hydroxylation,** catalyzed by members of a class of enzymes referred to as **monooxygenases** or **cytochrome P450s.** Hydroxylation may terminate the action of a drug, though this is not always the case. In addition to hydroxylation, these enzymes catalyze a wide range of reactions, including those involving deamination, dehalogenation, desulfuration, epoxidation, peroxygenation, and reduction. Reactions involving hydrolysis (eg, catalyzed by esterases) and certain other non-P450-catalyzed reactions also occur in phase 1.

In phase 2, the hydroxylated or other compounds produced in phase 1 are converted by specific enzymes to various polar metabolites by **conjugation** with glucuronic acid, sulfate, acetate, glutathione, or certain amino acids, or by **methylation.**

The overall purpose of the two phases of metabolism of xenobiotics is to increase their **water solubility (polarity)** and thus **excretion** from the body. Very hydrophobic xenobiotics would persist in adipose tissue almost indefinitely if they were not converted to more polar forms. In certain cases, phase 1 metabolic reactions convert xenobiotics from **inactive** to **biologically active** compounds. In these instances, the original xenobiotics are referred to as **"prodrugs"** or **"procarcinogens."** In other cases, additional phase 1 reactions (eg, further hydroxylation reactions) convert the active compounds to less active or inactive forms prior to conjugation. In yet other cases, it is the conjugation reactions themselves that convert the active products of phase 1 reactions to less active or inactive species, which are subsequently excreted in the urine or bile. In a very few cases, conjugation may actually increase the biologic activity of a xenobiotic.

The term **"detoxification"** is sometimes used for many of the reactions involved in the metabolism of xenobiotics. However, the term is not always appropriate because, as mentioned above, in some cases the reactions to which xenobiotics are subject actually increase their biologic activity and toxicity.

ISOFORMS OF CYTOCHROME P450 HYDROXYLATE A MYRIAD OF XENOBIOTICS IN PHASE 1 OF THEIR METABOLISM

Hydroxylation is the chief reaction involved in phase 1. The responsible enzymes are called **monooxygenases** or **cytochrome P450s;** the human genome encodes at least 14 families of these enzymes. Estimates of the number of distinct cytochrome P450s in human tissues range from approximately 35 to 60. The reaction catalyzed by a monooxygenase (cytochrome P450) is as follows:

$$RH + O_2 + NADPH + H^+ \rightarrow R-OH + H_2O + NADP$$

RH above can represent a very wide variety of xenobiotics, including drugs, carcinogens, pesticides, petroleum products, and pollutants (such as a mixture of PCBs). In addition, **endogenous compounds,** such as certain steroids, eicosanoids, fatty acids, and retinoids, are also substrates. The substrates are generally **lipophilic** and are rendered more **hydrophilic** by hydroxylation.

Cytochrome P450 is considered the **most versatile biocatalyst** known. The actual reaction mechanism is complex and has been briefly described previously (Figure 11–6). It has been shown by the use of $^{18}O_2$ that one atom of oxygen enters R—OH and one atom enters water. This dual fate of the oxygen accounts for the former naming of monooxygenases as **"mixed-function oxidases."** The reaction catalyzed by cytochrome P450 can also be represented as follows:

$$\text{Reduced cytochrome P450} \quad \text{Oxidized cytochrome P450}$$

$$RH + O_2 \rightarrow R\text{—}OH + H_2O$$

The major monooxygenases in the endoplasmic reticulum are **cytochrome P450s**—so named because the enzyme was discovered when it was noted that preparations of microsomes that had been chemically reduced and then exposed to carbon monoxide exhibited a distinct peak at 450 nm. Among reasons that this enzyme is important is the fact that approximately 50% of the drugs humans ingest are metabolized by isoforms of cytochrome P450; these enzymes also act on various carcinogens and pollutants.

Isoforms of Cytochrome P450 Make Up a Superfamily of Heme-Containing Enzymes

The following are important points concerning cytochrome P450s.

(1) Because of the large number of isoforms (about 150) that have been discovered, it became important to have a **systematic nomenclature** for isoforms of P450 and for their genes. This is now available and in wide use and is based on structural homology. The abbreviated root symbol CYP denotes a cytochrome P450. This is followed by an Arabic number designating the **family;** cytochrome P450s are included in the same family if they exhibit 40% or more sequence identity. The Arabic number is followed by a capital letter indicating the **subfamily,** if two or more members exist; P450s are in the same subfamily if they exhibit greater than 55% sequence identity. The **individual** P450s are then arbitrarily assigned Arabic numerals. Thus, CYP1A1 denotes a cytochrome P450 that is a member of family 1 and subfamily A and is the first individual member of that subfamily. The nomenclature for the **genes** encoding cytochrome P450s is identical to that described above except that italics are used; thus, the gene encoding CYP1A1 is *CYP1A1*.

(2) Like **hemoglobin,** they are hemoproteins.

(3) They are widely distributed across species. Bacteria possess cytochrome P450s, and $P450_{cam}$ (involved in the metabolism of camphor) of *Pseudomonas putida* is the only P450 isoform whose crystal structure has been established.

(4) They are present in highest amount in **liver** and **small intestine** but are probably present in all tissues. In liver and most other tissues, they are present mainly in the **membranes of the smooth endoplasmic reticulum,** which constitute part of the **microsomal fraction** when tissue is subjected to subcellular fractionation. In hepatic microsomes, cytochrome P450s can comprise as much as 20% of the total protein. P450s are found in most tissues, though often in low amounts compared with liver. In the **adrenal,** they are found in **mitochondria** as well as in the endoplasmic reticulum; the various hydroxylases present in that organ play an important role in cholesterol and steroid biosynthesis. The mitochondrial cytochrome P450 system differs from the microsomal system in that it uses an NADPH-linked flavoprotein, **adrenodoxin reductase,** and a nonheme iron-sulfur protein, **adrenodoxin.** In addition, the specific P450 isoforms involved in steroid biosynthesis are generally much more restricted in their substrate specificity.

(5) At least six isoforms of cytochrome P450 are present in the endoplasmic reticulum of human liver, each with wide and somewhat overlapping **substrate specificities** and acting on both xenobiotics and endogenous compounds. The genes for many isoforms of P450 (from both humans and animals such as the rat) have been isolated and studied in detail in recent years.

(6) **NADPH,** not NADH, is involved in the reaction mechanism of cytochrome P450. The enzyme that uses NADPH to yield the reduced cytochrome P450, shown at the left-hand side of the above equation, is called **NADPH-cytochrome P450 reductase.** Electrons are transferred from NADPH to NADPH-cytochrome P450 reductase and then to cytochrome P450. This leads to the **reductive activation of molecular oxygen,** and one atom of oxygen is subsequently inserted into the substrate. **Cytochrome b_5,** another hemoprotein found in the membranes of the smooth endoplasmic reticulum (Chapter 11), may be involved as an electron donor in some cases.

(7) **Lipids** are also components of the cytochrome P450 system. The preferred lipid is **phosphatidylcholine,** which is the major lipid found in membranes of the endoplasmic reticulum.

(8) Most isoforms of cytochrome P450 are **inducible.** For instance, the administration of phenobarbital or of many other drugs causes hypertrophy of the

smooth endoplasmic reticulum and a three- to fourfold increase in the amount of cytochrome P450 within 4–5 days. The mechanism of induction has been studied extensively and in most cases involves increased transcription of mRNA for cytochrome P450. However, certain cases of induction involve stabilization of mRNA, enzyme stabilization, or other mechanisms (eg, an effect on translation).

Induction of cytochrome P450 has important clinical implications, since it is a biochemical mechanism of **drug interaction.** A drug interaction has occurred when the effects of one drug are altered by prior, concurrent, or later administration of another. As an illustration, consider the situation when a patient is taking the anticoagulant **warfarin** to prevent blood clotting. This drug is metabolized by CYP2C9. Concomitantly, the patient is started on **phenobarbital** (an inducer of this P450) to treat a certain type of epilepsy, but the dose of warfarin is not changed. After 5 days or so, the level of CYP2C9 in the patient's liver will be elevated three- to fourfold. This in turn means that warfarin will be metabolized much more quickly than before, and its dosage will have become inadequate. Therefore, the dose must be increased if warfarin is to be therapeutically effective. To pursue this example further, a problem could arise later on if the phenobarbital is discontinued but the increased dosage of warfarin stays the same. The patient will be at risk of bleeding, since the high dose of warfarin will be even more active than before, because the level of CYP2C9 will decline once phenobarbital has been stopped.

Another example of enzyme induction involves **CYP2E1,** which is induced by consumption of **ethanol.** This is a matter for concern, because this P450 metabolizes certain widely used solvents and also components found in tobacco smoke, many of which are established **carcinogens.** Thus, if the activity of CYP2E1 is elevated by induction, this may increase the risk of carcinogenicity developing from exposure to such compounds.

(9) Certain isoforms of cytochrome P450 (eg, CYP1A1) are particularly involved in the metabolism of polycyclic aromatic hydrocarbons (PAHs) and related molecules; for this reason they were formerly called aromatic hydrocarbon hydroxylases (AHHs). This enzyme is important in the metabolism of PAHs and in carcinogenesis produced by these agents. For example, in the lung it may be involved in the conversion of inactive PAHs (procarcinogens), inhaled by smoking, to active carcinogens by hydroxylation reactions. Smokers have higher levels of this enzyme in some of their cells and tissues than do nonsmokers. Some reports have indicated that the activity of this enzyme may be elevated (induced) in the placenta of a woman who smokes, thus potentially altering the quantities of metabolites of PAHs (some of which could be harmful) to which the fetus is exposed.

(10) Certain cytochrome P450s exist in polymorphic forms (genetic isoforms), some of which exhibit low catalytic activity. These observations are one important explanation for the variations in drug responses noted among many patients. One P450 exhibiting polymorphism is CYP2D6, which is involved in the metabolism of debrisoquin (an antihypertensive drug; see Table 53–2) and sparteine (an antiarrhythmic and oxytocic drug). Certain polymorphisms of CYP2D6 cause poor metabolism of these and a variety of other drugs so that they can accumulate in the body, resulting in untoward consequences. Another interesting polymorphism is that of CYP2A6, which is involved in the metabolism of nicotine to conitine. Three *CYP2A6* alleles have been identified: a wild type and two null or inactive alleles. It has been reported that individuals with the null alleles, who have impaired metabolism of nicotine, are apparently protected against becoming tobacco-dependent smokers (Table 53–2). These individuals smoke less, presumably because their blood and brain concentrations of nicotine remain elevated longer than those of individuals with the wild-type allele. It has been speculated that inhibiting CYP2A6 may be a novel way to help prevent and to treat smoking.

Table 53–1 summarizes some principal features of cytochrome P450s.

CONJUGATION REACTIONS PREPARE XENOBIOTICS FOR EXCRETION IN PHASE 2 OF THEIR METABOLISM

In phase 1 reactions, xenobiotics are generally converted to more polar, hydroxylated derivatives. In phase 2 reactions, these derivatives are conjugated with molecules such as glucuronic acid, sulfate, or glutathione. This renders them even more water-soluble, and they are eventually excreted in the urine or bile.

Five Types of Phase 2 Reactions Are Described Here

A. Glucuronidation

The glucuronidation of bilirubin is discussed in Chapter 32; the reactions whereby xenobiotics are glucuronidated are essentially similar. UDP-glucuronic acid is the glucuronyl donor, and a variety of glucuronosyltransferases, present in both the endoplasmic reticulum and cytosol, are the catalysts. Molecules such as 2-acetylaminofluorene (a carcinogen), aniline, benzoic acid, meprobamate (a tranquilizer), phenol, and

Table 53–1. Some properties of human cytochrome P450s.

- Involved in phase I of the metabolism of innumerable xenobiotics, including perhaps 50% of the drugs administered to humans
- Involved in the metabolism of many endogenous compounds (eg, steroids)
- All are hemoproteins
- Often exhibit broad substrate specificity, thus acting on many compounds; consequently, different P450s may catalyze formation of the same product
- Extremely versatile catalysts, perhaps catalyzing about 60 types of reactions
- However, basically they catalyze reactions involving introduction of one atom of oxygen into the substrate and one into water
- Their hydroxylated products are more water-soluble than their generally lipophilic substrates, facilitating excretion
- Liver contains highest amounts, but found in most if not all tissues, including small intestine, brain, and lung
- Located in the smooth endoplasmic reticulum or in mitochondria (steroidogenic hormones)
- In some cases, their products are mutagenic or carcinogenic
- Many have a molecular mass of about 55 kDa
- Many are inducible, resulting in one cause of drug interactions
- Many are inhibited by various drugs or their metabolic products, providing another cause of drug interactions
- Some exhibit genetic polymorphisms, which can result in atypical drug metabolism
- Their activities may be altered in diseased tissues (eg, cirrhosis), affecting drug metabolism
- Genotyping the P450 profile of patients (eg, to detect polymorphisms) may in the future permit individualization of drug therapy

many steroids are excreted as glucuronides. The glucuronide may be attached to oxygen, nitrogen, or sulfur groups of the substrates. Glucuronidation is probably the most frequent conjugation reaction.

B. Sulfation

Some alcohols, arylamines, and phenols are sulfated. The **sulfate donor** in these and other biologic sulfation reactions (eg, sulfation of steroids, glycosaminoglycans, glycolipids, and glycoproteins) is **adenosine 3′-phosphate-5′-phosphosulfate (PAPS)** (Chapter 24); this compound is called "active sulfate."

C. Conjugation With Glutathione

Glutathione (γ-glutamyl-cysteinylglycine) is a **tripeptide** consisting of glutamic acid, cysteine, and glycine (Figure 3–3). Glutathione is commonly abbreviated GSH (because of the sulfhydryl group of its cysteine, which is the business part of the molecule). A number of potentially toxic electrophilic xenobiotics (such as certain carcinogens) are conjugated to the nucleophilic GSH in reactions that can be represented as follows:

$$R + GSH \rightarrow R—S—G$$

where R = an electrophilic xenobiotic. The enzymes catalyzing these reactions are called **glutathione S-transferases** and are present in high amounts in liver cytosol and in lower amounts in other tissues. A variety of glutathione S-transferases are present in human tissue. They exhibit different substrate specificities and can be separated by electrophoretic and other techniques. If the potentially toxic xenobiotics were not conjugated to GSH, they would be free to combine covalently with DNA, RNA, or cell protein and could thus lead to serious cell damage. GSH is therefore an important **defense mechanism** against certain toxic compounds, such as some drugs and carcinogens. If the levels of GSH in a tissue such as liver are lowered (as can be achieved by the administration to rats of certain compounds that react with GSH), then that tissue can be shown to be more susceptible to injury by various chemicals that would normally be conjugated to GSH. Glutathione conjugates are subjected to further metabolism before excretion. The glutamyl and glycinyl groups belonging to glutathione are removed by specific enzymes, and an acetyl group (donated by acetyl-CoA) is added to the amino group of the remaining cysteinyl moiety. The resulting compound is a **mercapturic acid,** a conjugate of L-acetylcysteine, which is then excreted in the urine.

Glutathione has other important functions in human cells apart from its role in xenobiotic metabolism.

1. It participates in the decomposition of potentially toxic **hydrogen peroxide** in the reaction catalyzed by glutathione peroxidase (Chapter 20).
2. It is an important **intracellular reductant,** helping to maintain essential SH groups of enzymes in their reduced state. This role is discussed in Chapter 20, and its involvement in the hemolytic anemia caused by deficiency of glucose-6-phosphate dehydrogenase is discussed in Chapters 20 and 52.
3. A metabolic cycle involving GSH as a carrier has been implicated in the **transport of certain amino acids** across membranes in the kidney. The first reaction of the cycle is shown below.

$$\text{Amino acid} + GSH \rightarrow \gamma\text{-Glutamyl amino acid} + \text{Cysteinylglycine}$$

This reaction helps transfer certain amino acids across the plasma membrane, the amino acid being subsequently hydrolyzed from its complex with GSH and the GSH being resynthesized from cysteinylglycine. The enzyme catalyzing the above reaction is **γ-glutamyltransferase (GGT).** It is present in the plasma membrane of renal tubular cells and bile ductule cells, and in the endoplasmic reticulum of hepatocytes. The enzyme has diagnostic value because it is released into the blood from hepatic cells in various hepatobiliary diseases.

D. Other Reactions

The two most important other reactions are acetylation and methylation.

1. Acetylation—Acetylation is represented by

$$\text{X} + \text{Acetyl-CoA} \rightarrow \text{Acetyl-X} + \text{CoA}$$

where X represents a xenobiotic. As for other acetylation reactions, **acetyl-CoA** (active acetate) is the acetyl donor. These reactions are catalyzed by **acetyltransferases** present in the cytosol of various tissues, particularly liver. The drug **isoniazid,** used in the treatment of tuberculosis, is subject to acetylation. **Polymorphic types** of acetyltransferases exist, resulting in individuals who are classified as **slow or fast acetylators,** and influence the rate of clearance of drugs such as isoniazid from blood. Slow acetylators are more subject to certain toxic effects of isoniazid because the drug persists longer in these individuals.

2. Methylation—A few xenobiotics are subject to methylation by methyltransferases, employing *S*-adenosylmethionine (Figure 30–17) as the methyl donor.

THE ACTIVITIES OF XENOBIOTIC-METABOLIZING ENZYMES ARE AFFECTED BY AGE, SEX, & OTHER FACTORS

Various factors affect the activities of the enzymes metabolizing xenobiotics. The activities of these enzymes may differ substantially among species. Thus, for example, the possible toxicity or carcinogenicity of xenobiotics cannot be extrapolated freely from one species to another. There are significant differences in enzyme activities among individuals, many of which appear to be due to **genetic factors.** The activities of some of these enzymes vary according to **age** and **sex.**

Intake of various xenobiotics such as phenobarbital, PCBs, or certain hydrocarbons can cause **enzyme induction.** It is thus important to know whether or not an individual has been exposed to these inducing agents in evaluating biochemical responses to xenobiotics. Metabolites of certain xenobiotics can inhibit or stimulate the activities of xenobiotic-metabolizing enzymes. Again, this can affect the doses of certain drugs that are administered to patients. Various diseases (eg, cirrhosis of the liver) can affect the activities of drug-metabolizing enzymes, sometimes necessitating adjustment of dosages of various drugs for patients with these disorders.

RESPONSES TO XENOBIOTICS INCLUDE PHARMACOLOGIC, TOXIC, IMMUNOLOGIC, & CARCINOGENIC EFFECTS

Xenobiotics are metabolized in the body by the reactions described above. When the xenobiotic is a drug, phase 1 reactions may produce its active form or may diminish or terminate its action if it is pharmacologically active in the body without prior metabolism. The diverse effects produced by drugs comprise the area of study of pharmacology; here it is important to appreciate that drugs act primarily through biochemical mechanisms. Table 53–2 summarizes four important reactions to drugs that reflect **genetically determined differences** in enzyme and protein structure among individuals—part of the field of study known as **pharmacogenetics** (see below).

Table 53–2. Some important drug reactions due to mutant or polymorphic forms of enzymes or proteins.[1]

Enzyme or Protein Affected	Reaction or Consequence
Glucose-6-phosphate dehydrogenase (G6PD) [mutations] (MIM 305900)	Hemolytic anemia following ingestion of drugs such as primaquine
Ca^{2+} release channel (ryanodine receptor) in the sarcoplasmic reticulum [mutations] (MIM 180901)	Malignant hyperthermia (MIM 145600) following administration of certain anesthetics (eg, halothane)
CYP2D6 [polymorphisms] (MIM 124030)	Slow metabolism of certain drugs (eg, debrisoquin), resulting in their accumulation
CYP2A6 [polymorphisms] (MIM 122720)	Impaired metabolism of nicotine, resulting in protection against becoming a tobacco-dependent smoker

[1]G6PD deficiency is discussed in Chapters 20 and 52 and malignant hyperthermia in Chapter 49. At least one gene other than that encoding the ryanodine receptor is involved in certain cases of malignant hypertension. Many other examples of drug reactions based on polymorphism or mutation are available.

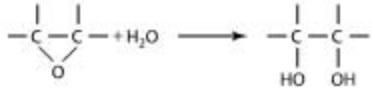
Certain xenobiotics are very toxic even at low levels (eg, cyanide). On the other hand, there are few xenobiotics, including drugs, that do not exert some toxic effects if sufficient amounts are administered. The **toxic effects of xenobiotics** cover a wide spectrum, but the major effects can be considered under three general headings (Figure 53–1).

The first is **cell injury** (cytotoxicity), which can be severe enough to result in cell death. There are many mechanisms by which xenobiotics injure cells. The one considered here is **covalent binding to cell macromolecules** of reactive species of xenobiotics produced by metabolism. These macromolecular targets include **DNA, RNA,** and **protein.** If the macromolecule to which the reactive xenobiotic binds is essential for short-term cell survival, eg, a protein or enzyme involved in some critical cellular function such as oxidative phosphorylation or regulation of the permeability of the plasma membrane, then severe effects on cellular function could become evident quite rapidly.

Second, the reactive species of a xenobiotic may bind to a protein, altering its **antigenicity.** The xenobiotic is said to act as a **hapten,** ie, a small molecule that by itself does not stimulate antibody synthesis but will combine with antibody once formed. The resulting **antibodies** can then damage the cell by several immunologic mechanisms that grossly perturb normal cellular biochemical processes.

Third, reactions of activated species of chemical carcinogens with **DNA** are thought to be of great importance in **chemical carcinogenesis.** Some chemicals (eg, benzo[α]pyrene) require activation by monooxygenases in the endoplasmic reticulum to become carcinogenic (they are thus called **indirect carcinogens**). The activities of the monooxygenases and of other xenobiotic-metabolizing enzymes present in the endoplasmic reticulum thus help to determine whether such compounds become carcinogenic or are "detoxified." Other chemicals (eg, various alkylating agents) can react directly (direct carcinogens) with DNA without undergoing intracellular chemical activation.

The enzyme **epoxide hydrolase** is of interest because it can exert a protective effect against certain carcinogens. The products of the action of certain monooxygenases on some procarcinogen substrates are **epoxides.** Epoxides are highly reactive and mutagenic or carcinogenic or both. Epoxide hydrolase—like cytochrome P450, also present in the membranes of the endoplasmic reticulum—acts on these compounds, converting them into much less reactive dihydrodiols. The reaction catalyzed by epoxide hydrolase can be represented as follows:



Epoxide **Dihydrodiol**

PHARMACOGENOMICS WILL DRIVE THE DEVELOPMENT OF NEW & SAFER DRUGS

As indicated above, **pharmacogenetics** is the study of the roles of genetic variations in the responses to drugs. As a result of the progress made in sequencing the

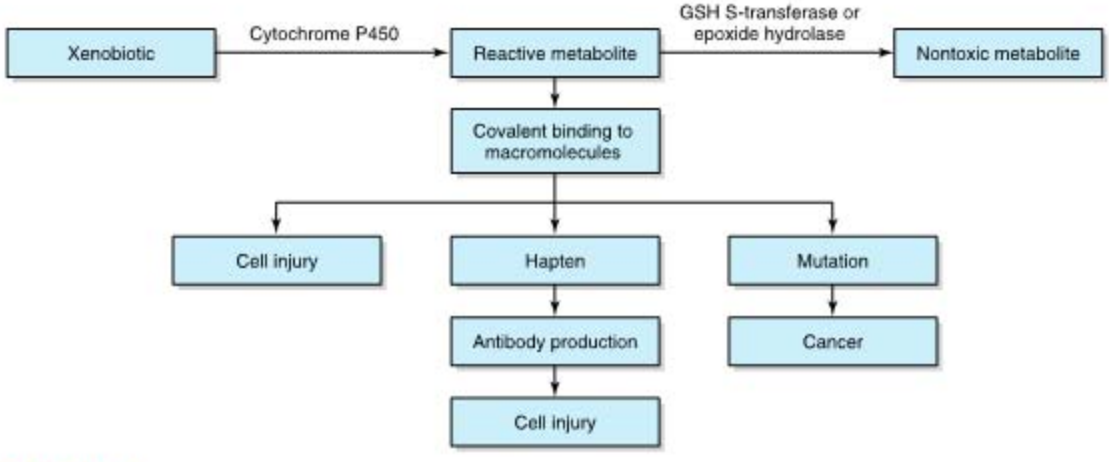


Figure 53–1. Simplified scheme showing how metabolism of a xenobiotic can result in cell injury, immunologic damage, or cancer. In this instance, the conversion of the xenobiotic to a reactive metabolite is catalyzed by a cytochrome P450, and the conversion of the reactive metabolite (eg, an epoxide) to a nontoxic metabolite is catalyzed either by a GSH S-transferase or by epoxide hydrolase.

human genome, a new field of study—**pharmacogenomics**—has developed recently. It includes pharmacogenetics but covers a much wider sphere of activity. Information from genomics, proteomics, bioinformatics, and other disciplines such as biochemistry and toxicology will be integrated to make possible the synthesis of newer and safer drugs. As the sequences of all our genes and their encoded proteins are determined, this will reveal many new targets for drug actions. It will also reveal polymorphisms (this term is briefly discussed in Chapter 50) of enzymes and proteins related to drug metabolism, action, and toxicity. DNA probes capable of detecting them will be synthesized, permitting screening of individuals for potentially harmful polymorphisms prior to the start of drug therapy. As the structures of relevant proteins and their polymorphisms are revealed, model building and other techniques will permit the design of drugs that take into account both normal protein targets and their polymorphisms. At least to some extent, drugs will be tailor-made for individuals based on their genetic profiles. A new era of rational drug design built on information derived from genomics and proteomics has already commenced.

SUMMARY

- Xenobiotics are chemical compounds foreign to the body, such as drugs, food additives, and environmental pollutants; more than 200,000 have been identified.
- Xenobiotics are metabolized in two phases. The major reaction of phase 1 is hydroxylation catalyzed by a variety of monooxygenases, also known as the cytochrome P450s. In phase 2, the hydroxylated species are conjugated with a variety of hydrophilic compounds such as glucuronic acid, sulfate, or glutathione. The combined operation of these two phases renders lipophilic compounds into water-soluble compounds that can be eliminated from the body.
- Cytochrome P450s catalyze reactions that introduce one atom of oxygen derived from molecular oxygen into the substrate, yielding a hydroxylated product. NADPH and NADPH-cytochrome P450 reductase are involved in the complex reaction mechanism.
- All cytochrome P450s are hemoproteins and generally have a wide substrate specificity, acting on many exogenous and endogenous substrates. They represent the most versatile biocatalyst known.
- Members of at least 11 families of cytochrome P450 are found in human tissue.
- Cytochrome P450s are generally located in the endoplasmic reticulum of cells and are particularly enriched in liver.
- Many cytochrome P450s are inducible. This has important implications in phenomena such as drug interaction.
- Mitochondrial cytochrome P450s also exist and are involved in cholesterol and steroid biosynthesis. They use a nonheme iron-containing sulfur protein, adrenodoxin, not required by microsomal isoforms.
- Cytochrome P450s, because of their catalytic activities, play major roles in the reactions of cells to chemical compounds and in chemical carcinogenesis.
- Phase 2 reactions are catalyzed by enzymes such as glucuronosyltransferases, sulfotransferases, and glutathione *S*-transferases, using UDP-glucuronic acid, PAPS (active sulfate), and glutathione, respectively, as donors.
- Glutathione not only plays an important role in phase 2 reactions but is also an intracellular reducing agent and is involved in the transport of certain amino acids into cells.
- Xenobiotics can produce a variety of biologic effects, including pharmacologic responses, toxicity, immunologic reactions, and cancer.
- Catalyzed by the progress made in sequencing the human genome, the new field of pharmacogenomics offers the promise of being able to make available a host of new rationally designed, safer drugs.

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The Human Genome Project

54

Robert K. Murray, MD, PhD

BIOMEDICAL SIGNIFICANCE

The information deriving from determination of the sequences of the human genome and those of other organisms will change biology and medicine for all time. For example, with reference to the human genome, new information on our origins, on disease genes, on diagnosis, and possible approaches to therapy are already flooding in. Progress in fields such as genomics, proteomics, bioinformatics, biotechnology, and pharmacogenomics is accelerating rapidly.

The aims of this chapter are to briefly summarize the major findings of the Human Genome Project (HGP) and indicate their implications for biology and medicine.

THE HUMAN GENOME PROJECT HAS A VARIETY OF GOALS

The HGP, which started in 1990, is an international effort whose principal goals were to sequence the entire human genome and the genomes of several other model organisms that have been basic to the study of genetics (eg, *Escherichia coli, Saccharomyces cerevisiae* [a yeast], *Drosophila melanogaster* [the fruit fly], *Caenorhabditis elegans* [the roundworm], and *Mus musculus* [the common house mouse]). Most of these goals have been accomplished. In the United States, the National Center for Human Genome Research (NCHGR) was established in 1989, initially directed by James D. Watson and subsequently by Francis Collins. The NCHGR played a leading role in directing the United States effort in the HGP. In 1997, it became the National Human Genome Research Institute (NHGRI). The international collaboration—involving groups from the USA, UK, Japan, France, Germany, and China—came to be known as the International Human Genome Sequencing Consortium (IHGSC). Initially, a number of short-term goals were established for the United States effort—eg, producing a human genetic map with markers 2–5 centimorgans (cM) apart and constructing a physical map of all 24 human chromosomes (22 autosomal plus X and Y) with markers spaced at approximately 100,000 base pairs (bp). Figure 54–1 summarizes the differences between a genetic map, a cytogenetic map, and a physical map of a chromosome. These and other initial goals were achieved and surpassed by the mid-nineties. In 1998, new goals for the United States wing of the HGP were announced. These included the aim of completing the entire sequence by the end of 2003 or sooner. Other specific objectives concerned sequencing technology, comparative genomics, bioinformatics, ethical considerations, and other issues. By the fall of 1998, about 6% of the human genome sequence had been completed and the foundations for future work laid. Further progress was catalyzed by the announcement that a second group, the private company Celera Genomics, led by Craig Venter, had undertaken the objective of sequencing the human genome. Venter and colleagues had published in 1995 the entire genome sequences of *Haemophilus influenzae* and *Mycoplasma genitalium*, the first of many species to have their genomic sequences determined. An important factor in the success of these workers was the use of a shotgun approach, ie, sonicating the DNA, sequencing the fragments, and reassembling the sequence, based on overlaps. For comparison, a variety of approaches that have been used at different times to study normal and disease genes are listed in Table 54–1.

A Draft Sequence of the Human Genome Was Announced in June 2000

In June 2000, leaders of the IHGSC and the personnel at Celera Genomics announced completion of working drafts of the sequence of the human genome, covering more than 90% of it. The principal findings of the two groups were published separately in February 2001 in special issues of Nature (the IHGSC) and Science (Celera). The draft published by the Consortium was the product of at least 10 years of work involving 20 sequencing centers located in six countries. That published by Celera and associates was the product of some 3 years or less of work; it relied in part on data obtained by the IHGSC. The combined achievement has been hailed, among other descriptions, as providing a Library of Life, supplying a Periodic Table of Life, and finding the Holy Grail of Human Genetics.

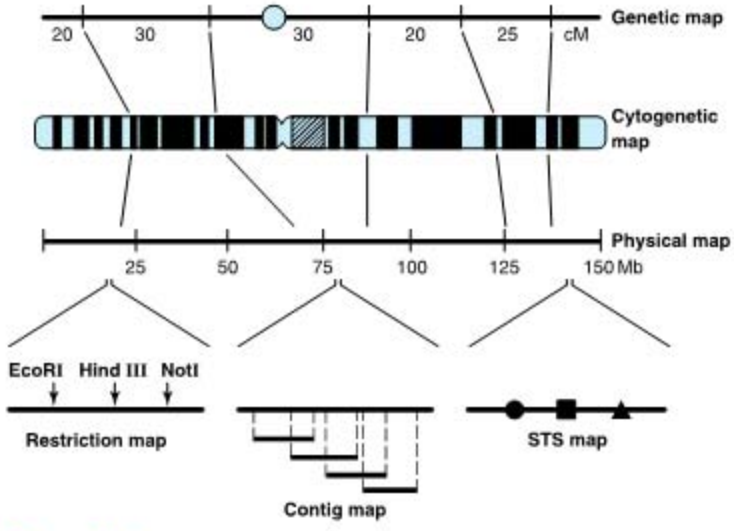

Figure 54–1. Principal methods used to identify and isolate normal and disease genes. For the genetic map, the positions of several hypothetical genetic markers are shown, along with the genetic distances in centimorgans between them. The circle shows the position of the centromere. For the cytogenetic map, the classic banding pattern of a hypothetical chromosome is shown. For the physical map, the approximate physical positions of the above genetic markers are shown, along with the relative physical distances in megabase pairs. Examples of a restriction map, a contig mark, and an STS map are also shown. (Reproduced, with permission, from Green ED, Waterston RH: The Human Genome Project: Prospects and implications for clinical medicine. JAMA 1991;266: 1966. Copyright © 1991 by the American Medical Association.)

Different Approaches Were Used by the Two Groups

We shall summarize the major findings reported in the two drafts and comment on their implications. While there are differences between the drafts, they will not be dwelt on here, as the areas of general agreement are much more extensive. It is worthwhile, however, to summarize the different approaches used by the two groups. Basically, the IHGSC employed a **map first, sequence later approach.** In part, this was because sequencing was a slow process when the public project started, and the strategy of the Consortium evolved over time as advances were made in sequencing and other techniques. The overall approach, referred to as **hierarchical shotgun sequencing,** consisted of fragmenting the entire genome into pieces of approximately 100–200 kb and inserting them into bacterial artificial chromosomes (BACs). The BACs were then positioned on individual chromosomes by looking for marker sequences known as sequence-tagged sites (STSs), whose locations had been already determined. STSs are short (usually < 500 bp), unique genomic loci for which a PCR assay is available. Clones of the BACs were then broken into small fragments (shotgunning). Each fragment was then sequenced, and computer algorithms were used that recognized matching sequence information from overlapping fragments to piece together the complete sequence.

Celera used the **whole genome shotgun approach,** in effect bypassing the mapping step. Shotgun fragments were assembled by algorithms onto large scaffolds, and the correct position of these scaffolds in the genome was determined using STSs. A scaffold is a series of "contigs" that are in the right order but not necessarily connected in one continuous sequence. Contigs are contiguous sequences of DNA made by assembling overlapping sequenced fragments of a natural chromosome or a BAC. The availability of **high-throughput sequenators, powerful computer programs,** the element of competition, and other factors accounted for the rapid progress made by both groups from 1998 onward.

Table 54–1. Principal methods used to identify and isolate normal and disease genes.

Procedure	Comments
Detection of specific cytogenetic abnormalities	For instance, a small deletion of band Xp21.2 was important in cloning the gene involved in Duchenne muscular dystrophy.
Extensive linkage studies	Large families with defined pedigrees are desirable. Dominant genes are easier to recognize than recessives.
Use of probes to define marker loci	Probes identify STSs, RFLPs, SNPs,[1] etc; thousands, covering all the chromosomes, are now available. It is desirable to flank the gene on both sides, clearly delineating it.
Radiation hybrid mapping[2]	Now the most rapid method of localizing a gene or DNA fragment to a subregion of a human chromosome and constructing a physical map.
Use of rodent or human somatic cell hybrids	Permits assignment of a gene to one specific chromosome but not to a subregion.
Fluorescence in situ hybridization	Permits localization of a gene to one chromosomal band.
Use of pulsed-field gel electrophoresis (PFGE) to separate large DNA fragments	Permits isolation of large DNA fragments obtained by use of restriction endonucleases (rare cutters) that result in very limited cutting of DNA.
Chromosome walking	Involves repeated cloning of overlapping DNA segments; the procedure is laborious and can usually cover only 100–200 kb.
Chromosome jumping	By cutting DNA into relatively large fragments and circularizing it, one can move more quickly and cover greater lengths of DNA than with chromosomal walking.
Cloning via YACs, BACs, cosmids, phages, and plasmids	Permits isolation of fragments of varying lengths.
Detection of expression of mRNAs in tissues by Northern blotting using one or more fragments of the gene as a probe	The mRNA should be expressed in affected tissues.
PCR	Can be used to amplify fragments of the gene; also many other applications.
DNA sequencing	Establishes the highest resolution physical map. Identifies open reading frame. Facilities with many high throughput instruments could sequence millions of base pairs per day.
Databases	Comparison of DNA and protein sequences obtained from unknown gene with known sequences in databases can facilitate gene identification.

Abbreviations: STS, sequence tagged site; RFLP, restriction fragment linked polymorphism; SNP, single nucleotide polymorphism; YAC, yeast artificial chromosome; BAC, bacterial artificial chromosome; PCR, polymerase chain reaction.

[1]Many single nucleotide polymorphisms (SNPs) are being detected and catalogued. These are stable and frequent, and their detection can be automated. It is anticipated that they will be particularly useful for mapping complex traits such as diabetes mellitus.

[2]Radiation hybrid mapping (consult http://compgen.rutgers.edu/rhmap/ for a detailed bibliography of this technique) makes use of a panel of somatic cell hybrids, with each cell line containing a random set of irradiated human genomic DNA in a hamster background. Briefly, the radiation fragments the DNA into small pieces of variable length; if a gene is located close to another known gene, it is likely that the two will remain linked (compare genetic linkage) on the same fragment. An STS marker is typed against a radiation hybrid panel by using its two oligonucleotide primers to perform a PCR assay against the DNA from each hybrid cell line of the panel. If enough markers are typed on one panel, continuous linkage can be established along each arm of a chromosome, and the markers can be assembled into the map as a single linkage group.

DETERMINATION OF THE SEQUENCE OF THE HUMAN GENOME HAS PRODUCED A WEALTH OF NEW FINDINGS

Only a small fraction of the findings can be covered here. The interested reader is referred to the original articles. Table 54–2 summarizes a number of the highlights, which can now be described.

Most of the Human Genome Has Been Sequenced

Over 90% of the human genome had been sequenced by July 2000. This is by far the largest genome sequenced, with an estimated size of approximately 3.2 gigabases (Gb). Prior to the human genome, that of the fruit fly had been the largest (~180 Mb) sequenced. Gaps still exist, small and large, and the quality of some of the sequencing data will be refined since some of the findings are probably not exactly right.

The Human Genome Is Estimated to Encode About 30,000–40,000 Proteins

The greatest surprise provided by the results to date has been the apparently low number of genes encoding proteins, estimated to lie between 30,000 and 40,000. The higher number could increase as new data are obtained. This number is approximately twice that found in the roundworm (19,099) and three times that of the fruit fly (13,061). The figures suggest that the complexity of humans compared with that of the two simpler organisms must have explanations other than strictly gene number.

Table 54–2. Major findings reported in the rough drafts of the human genome.

- More than 90% of the genome has been sequenced; gaps, large and small, remain to be filled in.
- Estimated number of protein-coding genes ranges from 30,000 to 40,000.
- Only 1.1–1.5% of the genome codes for proteins.
- There are wide variations in features of individual chromosomes (eg, in gene number per Mb, SNP density, GC content, numbers of transposable elements and CpG islands, recombination rate).
- Human genes do more work than those of the roundworm or fruit fly (eg, alternative splicing is used more frequently).
- The human proteome is more complex than that found in invertebrates.
- Repeat sequences probably constitute more than 50% of the genome.
- Approximately 100 coding regions have been copied and moved by RNA-based transposons.
- Approximately 200 genes may be derived from bacteria by lateral transfer.
- More than 3 million SNPs have been identified.

Only 1.1–1.5% of the Human Genome Encodes Proteins

Analyses of the available data reveal that 1.1–1.5% of the genome consists of exons. About 24% consists of introns, and 75% of sequences lying between genes (intergenic). Comparisons with the data on the roundworm and fruit fly have shown that exon size across the three species is relatively constant (mean size of 145 bp in humans). However, intron size in humans is much more variable (mean size of over 3300 bp), resulting in great variation in gene size.

The Landscape of Human Chromosomes Varies Widely

There are marked differences among individual chromosomes in many features, such as gene number per megabase, density of single nucleotide polymorphisms (SNPs), GC content, number of transposable elements and CpG islands, and recombination rate. To take one example, chromosome 19 has the richest gene content (23 genes per megabase), whereas chromosome 13 and the Y chromosome have the sparsest content (5 genes per megabase). Explanations for these variations are not apparent at this time.

Human Genes Do More Work Than Those of Simpler Organisms

Alternative splicing appears to be more prevalent in humans, involving at least 35% of their genes. Data indicate that the average number of distinct transcripts per gene for chromosomes 22 and 19 were 2.6 and 3.2, respectively. These figures are higher than for the roundworm, where only 12.2% of genes appear to be alternatively spliced and only 1.34 splice variants per gene were noted.

The Human Proteome Is More Complex Than That of Invertebrates

Relatively few new protein domains appear to have emerged among vertebrates. However, the number of distinct domain architectures (~1800) in human proteins is 1.8 times that of the roundworm or fruit fly. About 90 vertebrate-specific families of proteins have been identified, and these have been found to be enriched in proteins of the immune and nervous systems.

The results of the two drafts are rich in information about protein families and classes. One example is shown in Table 54–3, in which the major classes of proteins encoded by human genes are listed. As can be seen, the largest class is "unknown." Identification of these unknown proteins will be a major focus of activity for many laboratories.

Repeat Sequences Probably Constitute More Than 50% of the Human Genome

Repeat sequences probably account for at least half of the genome. They fall into five classes: (1) transposon-derived repeats (interspersed repeats); (2) processed pseudogenes; (3) simple sequence repeats; (4) segmental duplications, made up of 10–300 kb that have been copied from one region of the genome into another; and (5) blocks of tandemly repeated sequences, found at centromeres, telomeres, and other areas. Considerable information on most of the above classes of repeat sequences—of great value in understanding the architecture and development of the human genome—is reported in the drafts. Only two points of interest will be mentioned here. It is speculated that Alu elements, the most prominent members (about 10% of the total genome) of the short interspersed elements (SINEs), may be present in GC-rich areas because of positive selection, implying that they are of benefit to the host.

Table 54–3. Major classes of proteins encoded by human genes.[1]

Class of Protein	Number (%)[2]
Unknown	12,809 (41%)
Nucleic acid enzymes	2,308 (7.5%)
Transcription factors	1,850 (6%)
Receptors	1,543 (5%)
Hydrolases	1,227 (4.0%)
Select regulatory molecules (eg, G proteins, cell cycle regulators)	988 (3.2%)
Protooncogenes	902 (2.9%)
Cytoskeletal structural proteins	876 (2.8%)
Kinases	868 (2.8%)

[1]Data from Venter JC et al: The sequence of the human genome. Science 2001;291:1304.

[2]The percentages are derived from a total of 26,383 genes reported in the rough draft by Celera Genomics. Classes containing more than 2.5% of the total proteins encoded by the genes identified when this rough draft was written are arbitrarily listed as major.

Segmental duplications have been found to be much more common than in the roundworm or fruit fly. It is possible that these structures may be involved in exon shuffling and the increased diversity of proteins found in humans.

Other Findings of Interest

The last three major points of interest listed in Table 54–2 will be briefly described together.

Approximately 100 coding regions are estimated to have been copied and moved by RNA-based transposons (retrotransposons). It is possible that some of these genes may adopt new roles in the course of time. A surprising finding is that over 200 genes may be derived from bacteria by lateral transfer. None of these genes are present in nonvertebrate eukaryotes. More than 3 million SNPs have been identified. It is likely that they will prove invaluable for certain aspects of gene mapping.

It is stressed that the findings listed here are only a few of those reported in the drafts, and the reader is urged to consult the original reports (see References, below).

FURTHER WORK IS PLANNED ON THE HUMAN & OTHER GENOMES

The IHGSC has indicated that it will determine the complete sequence, it is hoped, by 2003. The task involves filling in the gaps and identifying new genes, their locations, and functions. Regulatory regions will be identified, and the sequences of other large genomes (eg, of the house mouse; of *Rattus norvegicus,* the Norway rat; of *Danio rerio,* the zebra fish; of *Fugu rubripes,* the tiger puffer fish; and of one or more primates) will be obtained; indeed, a draft version of the genome of the tiger puffer fish was published in 2002. Additional SNPs will be identified; a complete catalog of these variants is expected to be of great value in mapping genes associated with complex traits and for other uses as well. Along with the above, existing databases will be added to as new information flows in, and new databases will probably be established to serve specific purposes. A variety of studies in functional genomics (ie, the study of genomes to determine the functions of all their genes and their products) will also be undertaken.

IMPLICATIONS FOR PROTEOMICS, BIOTECHNOLOGY, & BIOINFORMATICS

Many fields will be influenced by knowledge of the human genome. Only a few are briefly discussed here.

Proteomics (see Chapter 4) in its broadest sense is the study of all the proteins encoded in an organism (ie,

the proteome), including their structures, modifications, functions, and interactions. In a narrower sense, it involves the identification and study of multiple proteins linked through cellular actions—but not necessarily the entire proteome. With regard to humans, many individual proteins will be identified and characterized; their interactions and levels will be determined in physiologic and pathologic states, and the resulting information will be entered into appropriate databases. Techniques such as two-dimensional electrophoresis, a variety of modes of mass spectrometry, and antibody arrays will be central to expansion of this rapidly growing field. Overall, proteomics will greatly advance our knowledge of proteins at the basic level and will also nourish **biotechnology** as new proteins that are likely to have diagnostic, therapeutic, and other uses are discovered and methods for their economic production are developed. Specialists in **bioinformatics** will be in demand, as this field rapidly gears up to manage, analyze, and utilize the flood of data from genomic and proteomic studies.

IMPLICATIONS FOR MEDICINE

Practically every area of medicine will be affected by the new information accruing from knowledge of the human genome. The **tracking of disease genes** will be enormously facilitated. As mentioned above, SNP maps should greatly assist determination of genes involved in complex diseases. Probes for any gene will be available if needed, leading to **improved diagnostic testing** for disease susceptibility genes and for genes directly involved in the causation of specific diseases. The field of **pharmacogenomics** (see Chapter 53) is already expanding greatly, and it is possible that in the future drugs will be tailored to accommodate the variations in enzymes and other proteins involved in drug action and metabolism found among individuals. Studies of genes involved in **behavior** may lead to new insights into the causation and possible treatment of psychiatric disorders. Many **ethical issues**—eg, privacy concerns and the use of genomic information for commercial purposes—will have to be addressed. It will also be important that medical and economic benefits accrue to individuals in **Third World countries** from the anticipated effects on health services and the diagnosis and treatment of disease.

SUMMARY

- Determination of the complete sequence of the human genome, now almost completed, is one of the most significant scientific achievements of all time.
- Many important findings have already emerged. The one to date that has generated the most discussion is that the number of human genes may be only two to three times that estimated for the roundworm and the fruit fly.
- Information flowing from the Human Genome Project is having major influences in fields such as proteomics, bioinformatics, biotechnology, and pharmacogenomics as well as all areas of biology and medicine.
- It is hoped that the knowledge derived will be used wisely and fairly and that the benefits that will ensue regarding health, disease, and other matters will be made available to all people everywhere.

REFERENCES

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Nature 2001;409(6822) (February 15), and Science 2001;291(5507) (February 16). (These two issues present the rough drafts prepared by the IHGSC and Celera, respectively, along with many other articles analyzing the meaning and significance of the findings.)

Science 2001;294(5540) (October 5). (This issue contains a number of articles under the title Genome: Unlocking Biology's Storehouse. They describe new ideas, approaches, and research related to genome information.)