Cell cycle

A. Cell cycle ◑

Proliferating cells undergo a cycle of division (the cell cycle), which lasts approximately 24 hours in mammalian cells in cell culture. The cycle is divided into four different phases (G_1, S, G_2, and M—in that sequence).

Fully differentiated animal cells only divide rarely. These cells are in the so-called **G_0 phase**, in which they can remain permanently. Some G_0 cells return to the **G_1 phase** again under the influence of mitogenic signals (growth factors, cytokines, tumor viruses, etc.), and after crossing a *control point* (G_1 to S), enter a new cycle. DNA is replicated (see p. 240) during the **S phase**, and new chromatin is formed. Particularly remarkable in morphological terms is the actual mitosis (**M phase**), in which the chromosomes separate and two daughter cells are formed. The M and S phases are separated by two segments known as the **G_1** and **G_2** phases (the G stands for "gap"). In the G_1 phase, the duration of which can vary, the cell grows by de novo synthesis of cell components. Together, the G_1, G_0, S, and G_2 phases are referred to as the **interphase**, which alternates in the cell cycle with the short M phase.

B. Control of the cell cycle ○

The progression of the cell cycle is regulated by **interconversion processes**. In each phase, special *Ser/Thr-specific protein kinases* are formed, which are known as cyclin-dependent kinases (**CDKs**). This term is used because they have to bind an activator protein (**cyclin**) in order to become active. At each control point in the cycle, specific CDKs associate with equally phase-specific cyclins. If there are no problems (e. g., DNA damage), the CDK–cyclin complex is activated by phosphorylation and/or dephosphorylation. The activated complex in turn phosphorylates **transcription factors**, which finally lead to the formation of the proteins that are required in the cell cycle phase concerned (enzymes, cytoskeleton components, other CDKs, and cyclins). The activity of the CDK–cyclin complex is then terminated again by proteolytic **cyclin degradation**.

The above outline of cell cycle progression can be examined here in more detail using the G_2–M transition as an example.

Entry of animal cells into mitosis is based on the "mitosis-promoting factor" (**MPF**). MPF consists of **CDK1** (cdc2) and **cyclin B**. The intracellular concentration of cyclin B increases constantly until mitosis starts, and then declines again rapidly (top left). MPF is initially inactive, because CDK1 is phosphorylated and cyclin B is dephosphorylated (top center). The M phase is triggered when a *protein phosphatase* [1] dephosphorylates the CDK while cyclin B is phosphorylated by a *kinase* [2]. In its active form, MPF phosphorylates various proteins that have functions in mitosis—e. g., *histone H1* (see p. 238), components of the cytoskeleton such as the *laminins* in the nuclear membrane, *transcription factors, mitotic spindle* proteins, and various *enzymes.*

When mitosis has been completed, cyclin B is marked with **ubiquitin** and broken down proteolytically by *proteasomes* (see p. 176). Protein phosphatases then regain control and dephosphorylate the proteins involved in mitosis. This returns the cell to the interphase.

Further information

The G_1–S transition (not shown) is particularly important for initiating the cell cycle. It is triggered by the CDK4–cyclin D complex, which by phosphorylating the protein **pRb** releases the transcription factor E2F previously bound to pRb. This activates the transcription of genes needed for DNA replication.

If the DNA is damaged by mutagens or ionizing radiation, the protein **p53** initially delays entry into the S phase. If the DNA repair system (see p. 256) does not succeed in removing the DNA damage, p53 forces the cell into apoptosis (see p. 396). The genes coding for pRb and p53 belong to the **tumor-suppressor genes** (see p. 398). In many tumors (see p. 400), these genes are in fact damaged by mutation.

A. Cell cycle

M phase

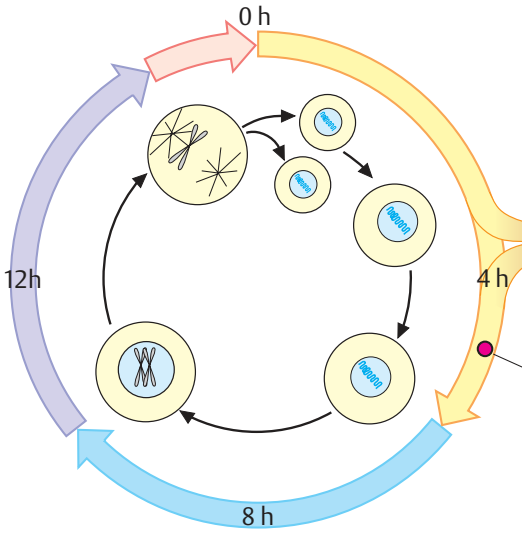
Mitosis
Chromosome separation
Cell division

G_2 phase

Preparation for mitosis

S phase

DNA replication
Histone synthesis
Centrosome formed
Chromosome duplication



G_1 phase

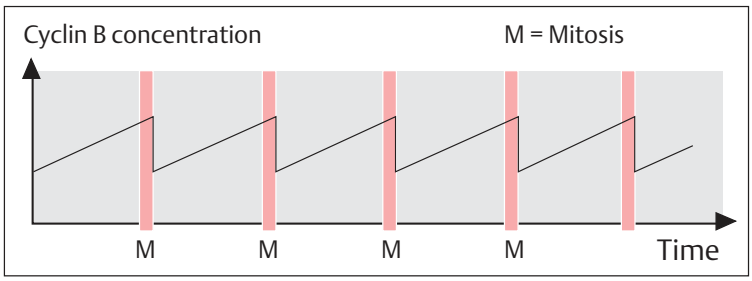
RNA and protein synthesis
Cell growth

G_0 phase

No cell division

Restriction point

B. Control of the cell cycle



Reactions early in mitosis
Reactions late in mitosis

Cyclin-dependent protein kinases
CDK 1 – 6

Cyclin
Cyclins A – E

Cyclin B
Regulatory subunit
Cyclin-dependent protein kinase (CDK1)
Catalytic subunit

Inactive protein kinase
Cyclin fragments
Proteolysis

MPF
Active protein kinase

Interphase
Protein
Histone H1
Laminin
Protein kinases
Transcription factors
Other proteins

Mitosis
Spindle formation
Chromosome condensation
Disappearance of nuclear membrane
Transcription stop
Cyclin degradation

1 Phosphoprotein phosphatase
2 Protein kinase

Apoptosis

A. Cell proliferation and apoptosis ◑

The number of cells in any tissue is mainly regulated by two processes—cell **proliferation** and *physiological cell death*, **apoptosis**. Both of these processes are regulated by stimulatory and inhibitory factors that act in solute form (growth factors and cytokines) or are presented in bound form on the surface of neighboring cells (see below).

Apoptosis is genetically programmed cell death, which leads to "tidy" breakdown and disposal of cells. Morphologically, apoptosis is characterized by changes in the cell membrane (with the formation of small blebs known as "apoptotic bodies"), shrinking of the nucleus, chromatin condensation, and fragmentation of DNA. *Macrophages* and other phagocytic cells recognize apoptotic cells and remove them by phagocytosis without inflammatory phenomena developing.

Cell necrosis (not shown) should be distinguished from apoptosis. In cell necrosis, cell death is usually due to physical or chemical damage. Necrosis leads to swelling and bursting of the damaged cells and often triggers an inflammatory response.

The growth of tissue (or, more precisely, the number of cells) is actually regulated by apoptosis. In addition, apoptosis allows the elimination of unwanted or superfluous cells—e. g., during embryonic development or in the immune system. The contraction of the uterus after birth is also based on apoptosis. Diseased cells are also eliminated by apoptosis—e. g., tumor cells, virus-infected cells, and cells with irreparably damaged DNA. An everyday example of this is the peeling of the skin after sunburn.

B. Regulation of apoptosis ○

Apoptosis can be triggered by a number of different signals that use various transmission pathways. Other signaling pathways prevent apoptosis.

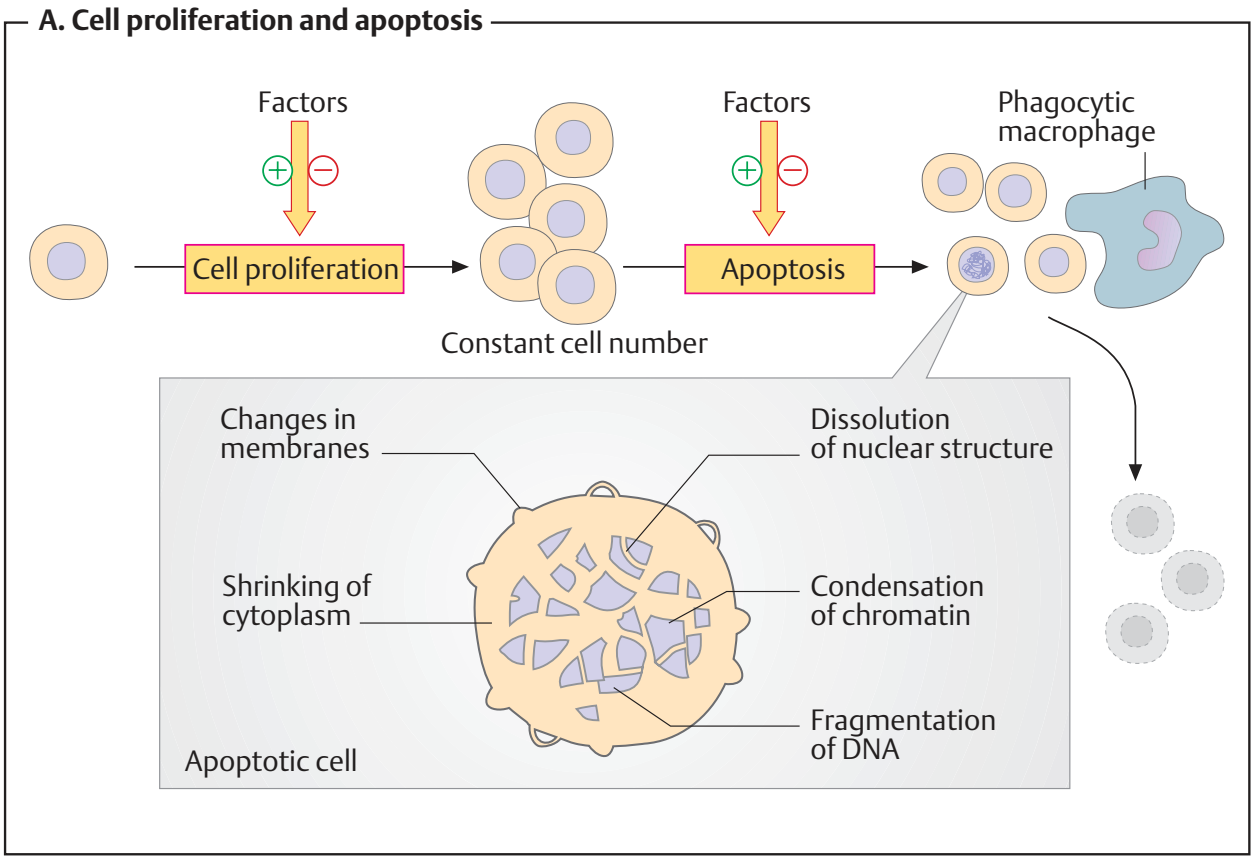
At the center of the apoptotic process lies a group of specialized *cysteine-containing aspartate proteinases* (see p. 176), known as **caspases**. These mutually activate one another, creating an *enzyme cascade* resembling the cascade involved in blood coagulation (see p. 290). Other enzymes in this group, known as **effector caspases**, cleave cell components after being activated—e. g., laminin in the nuclear membrane and snRP proteins (see p. 246)—or activate special DNases which then fragment the nuclear DNA.

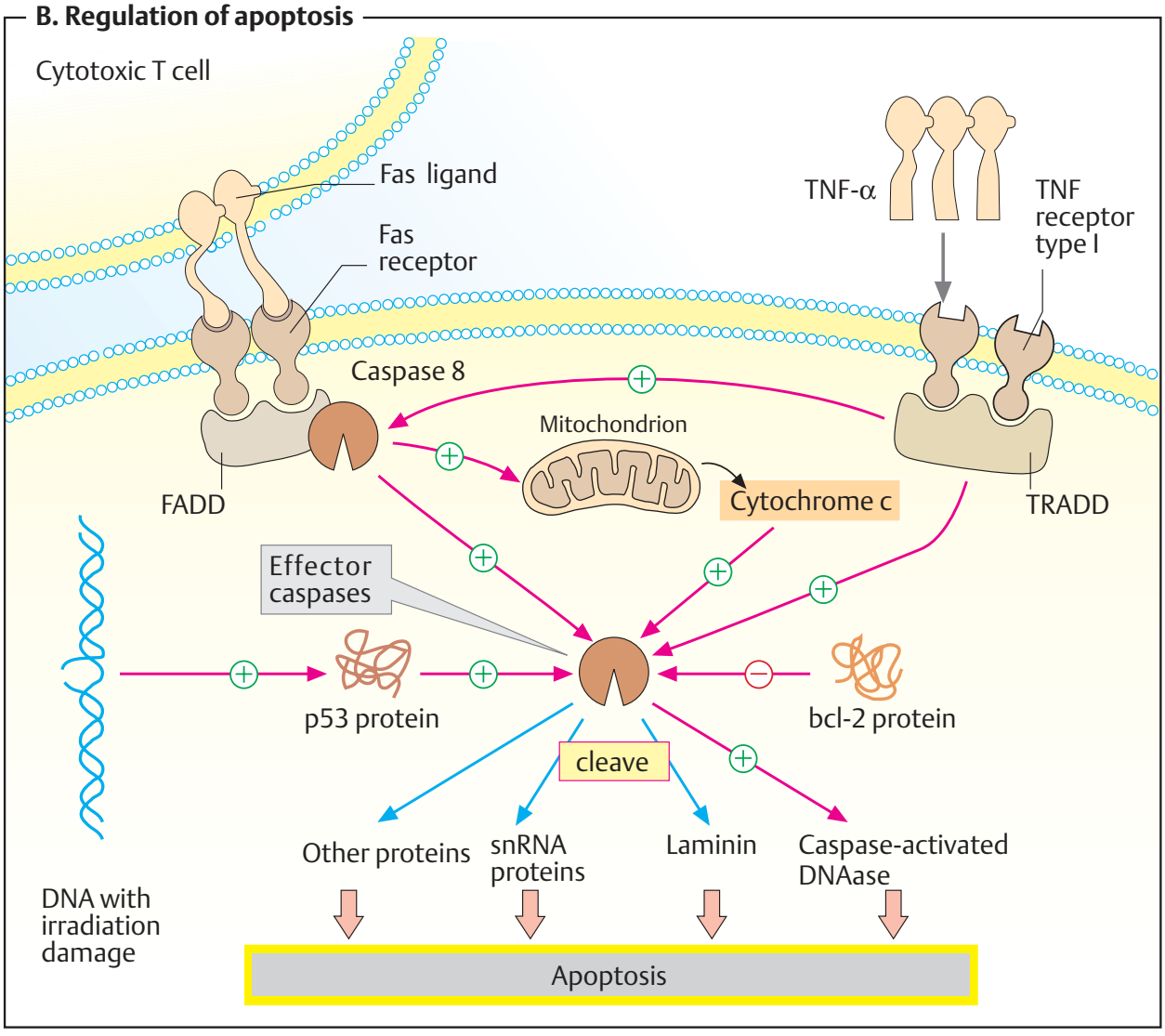
An important trigger for apoptosis is known as the **Fas system**. This is used by cytotoxic T cells, for example, which eliminate infected cells in this way (top left). Most of the body's cells have *Fas receptors* (CD 95) on their plasma membrane. If a T cell is activated by contact with an MHC presenting a viral peptide (see p. 296), binding of its *Fas ligands* occurs on the target cell's Fas receptors. Via the mediator protein FADD ("Fas-associated death domain"), this activates *caspase-8* inside the cell, setting in motion the apoptotic process.

Another trigger is provided by **tumor necrosis factor-α** (TNF-α), which acts via a similar protein (TRADD) and supports the endogenous defense system against tumors by inducing apoptosis.

Caspase-8 activates the effector caspases either directly, or indirectly by promoting the **cytochrome c** (see p. 140) from mitochondria. Once in the cytoplasm, cytochrome c binds to and activates the protein Apaf-1 (not shown) and thus triggers the caspase cascade. Apoptotic signals can also come from the cell nucleus. If irreparable DNA damage is present, the **p53 protein** (see p. 394)—the product of a *tumor suppressor gene*—promotes apoptosis and thus helps eliminate the defective cell.

There are also inhibitory factors that oppose the signals that activate apoptosis. These include **bcl-2** and related proteins. The genomes of several viruses include genes for this type of protein. The genes are expressed by the host cell and (to the benefit of the virus) prevent the host cell from being prematurely eliminated by apoptosis.





Oncogenes

Oncogenes are cellular genes that can trigger uncontrolled cell proliferation if their sequence is altered or their expression is incorrectly regulated. They were first discovered as *viral (v-) oncogenes* in retroviruses that cause tumors (tumor viruses). Viruses of this type (see p. 404) sometimes incorporate genes from the host cell into their own genome. If these genes are reincorporated into the host DNA again during later infection, tumors can then be caused in rare cases. Although virus-related tumors are rare, research into them has made a decisive contribution to our understanding of oncogenes and their functioning.

A. Proto-oncogenes: biological role ◑

The cellular form of oncogenes (known as c-oncogenes or **proto-oncogenes**) code for proteins involved in controlling growth and differentiation processes. They only become oncogenes if their sequence has been altered by *mutations* (see p. 256), *deletions,* and other processes, or when excessive amounts of the gene products have been produced as a result of *overexpression.*

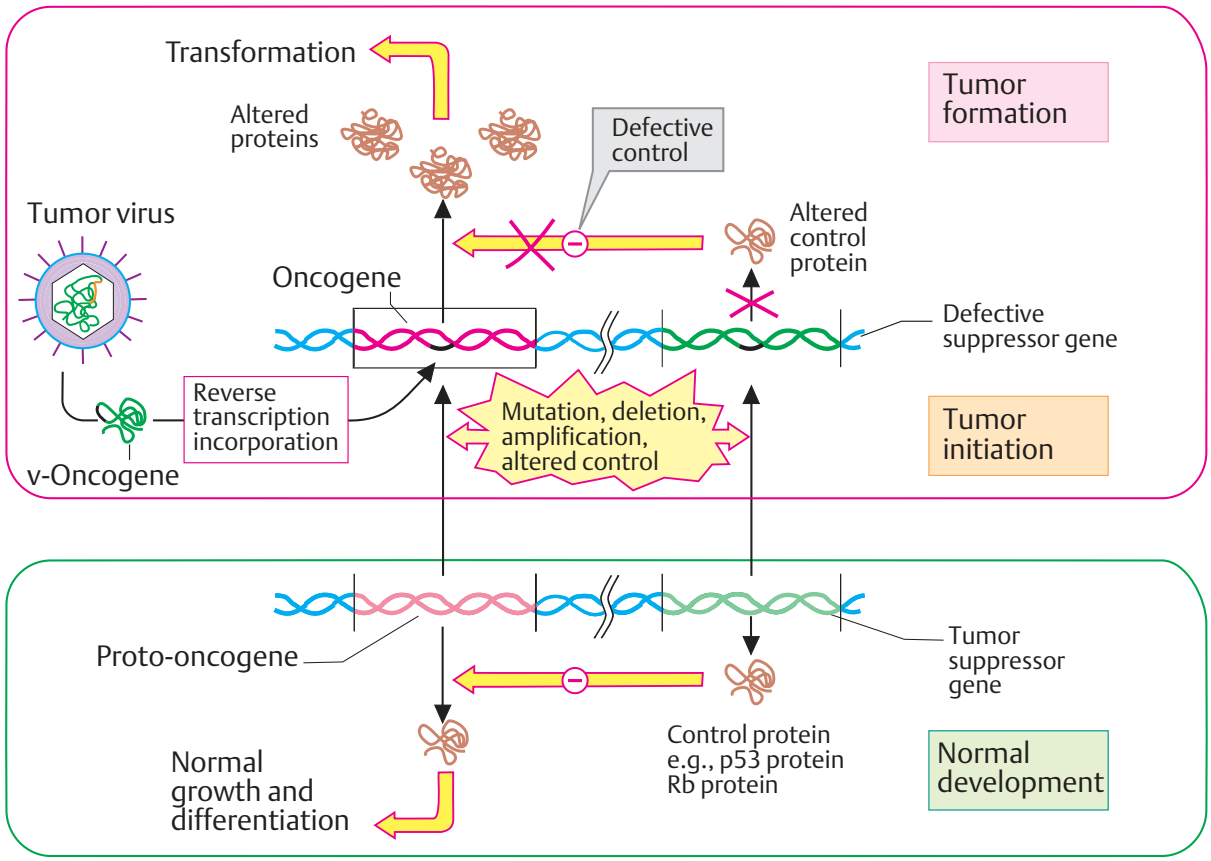
Overexpression can occur when **amplification** leads to numerous functional copies of the respective gene, or when the gene falls under the influence of a highly active promoter (see p. 244). If the control of oncogene expression by **tumor suppressor genes** (see p. 394) is also disturbed, **transformation** and unregulated proliferation of the cells can occur. A single activated oncogene does not usually lead to a loss of growth control. It only occurs when over the course of time mutations and regulation defects accumulate in one and the same cell. If the immune system does not succeed in eliminating the transformed cell, it can over the course of months or years grow into a macroscopically visible **tumor**.

B. Oncogene products: biochemical functions ◑

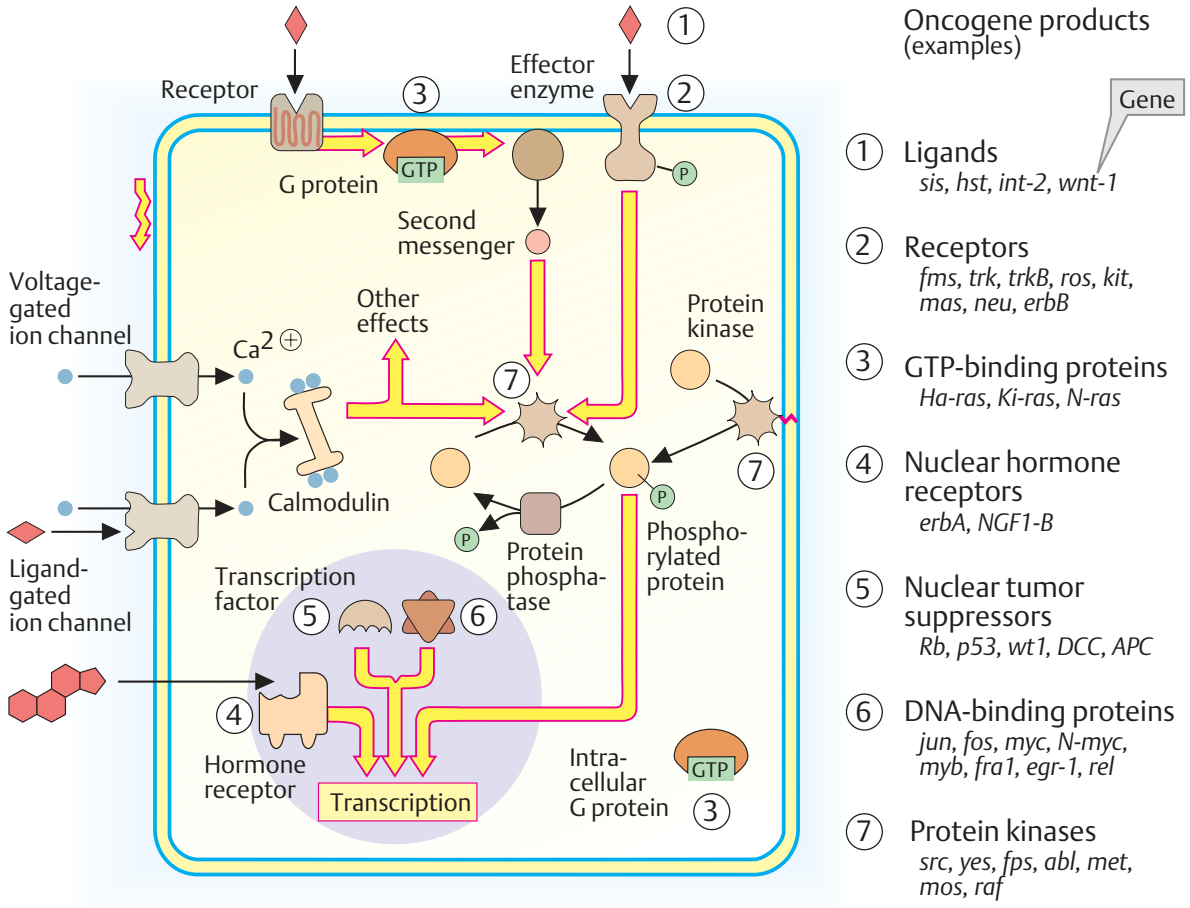
A feature common to all oncogenes is the fact that they code for proteins involved in *signal transduction processes.* The genes are designated using three-letter abbreviations that usually indicate the origin of the viral gene and are printed in italics (e.g., *myc* for myelocytomatosis, a viral disease in birds). Oncogene products can be classified into the following groups according to their functions.

1. **Ligands** such as *growth factors* and *cytokines,* which promote cell proliferation.
2. **Membrane receptors** of the 1-helix type with tyrosine kinase activity, which can bind growth factors and hormones (see p. 394).
3. **GTP-binding proteins.** This group includes the G proteins in the strict sense and related proteins such as Ras (see p. 388), the product of the oncogene c-*ras.*
4. **Receptors for lipophilic hormones** mediate the effects of steroid hormones and related signaling substances. They regulate the transcription of specific genes (see p. 378). The products of several oncogenes (e.g., *erbA*) belong to this superfamily of *ligand-controlled transcription factors.*
5. **Nuclear tumor suppressors** inhibit return to the cell cycle in fully differentiated cells. The genes that code for these proteins are referred to as *anti-oncogenes* due to this function. On the role of p53 and pRb, see p. 394.
6. **DNA-binding proteins.** A whole series of oncogenes code for *transcription factors.* Particularly important for cell proliferation are *myc,* as well as *fos* and *jun.* The protein products of the latter two genes form the transcription factor AP-1 as a heterodimer (see p. 244).
7. **Protein kinases** play a central role in intracellular signal transduction. By phosphorylating proteins, they bring about alterations in biological activity that can only be reversed again by the effects of *protein phosphatases.* The interplay between protein phosphorylation by protein kinases and dephosphorylation by protein phosphatases (*interconversion*) serves to regulate the cell cycle (see p. 394) and other important processes. The protein kinase Raf is also involved in the signal transduction of insulin (see p. 388).

A. Proto-oncogenes: biological role



B. Oncogene products: biochemical functions



Tumors

A. Division behavior of cells ◑

The body's cells are normally subject to strict "social" control. They only divide until they come into contact with neighboring cells; cell division then ceases due to *contact inhibition.* Exceptions to this rule include embryonic cells, cells of the intestinal epithelium (where the cells are constantly being replaced), cells in the bone marrow (where formation of blood cells takes place), and **tumor cells.** *Uncontrolled cell proliferation* is an important indicator of the presence of a tumor. While normal cells in cell culture only divide 20–60 times, tumor cells are potentially immortal and are not subject to contact inhibition.

In medicine, a distinction is made between *benign* and *malignant tumors.* Benign tumors consist of slowly growing, largely differentiated cells. By contrast, malignant tumors show rapid, invasive growth and tend to form *metastases* (dissemination of daughter lesions). The approximately 100 different types of tumor that exist are responsible for more than 20% of deaths in Europe and North America.

B. Transformation ◑

The transition of a normal cell into a tumor cell is referred to as **transformation.**

Normal cells have all the characteristics of fully differentiated cells specialized for a particular function. Their division is inhibited and they are usually in the G_0 phase of the cell cycle (see p. 394). Their external shape is variable and is determined by a strongly structured cytoskeleton.

In contrast, *tumor cells* divide without inhibition and are often de-differentiated—i. e., they have acquired some of the properties of embryonic cells. The surface of these cells is altered, and this is particularly evident in a disturbance of contact inhibition by neighboring cells. The cytoskeleton of tumor cells is also restructured and often reduced, giving them a rounded shape. The nuclei of tumor cells can be atypical in terms of shape, number, and size.

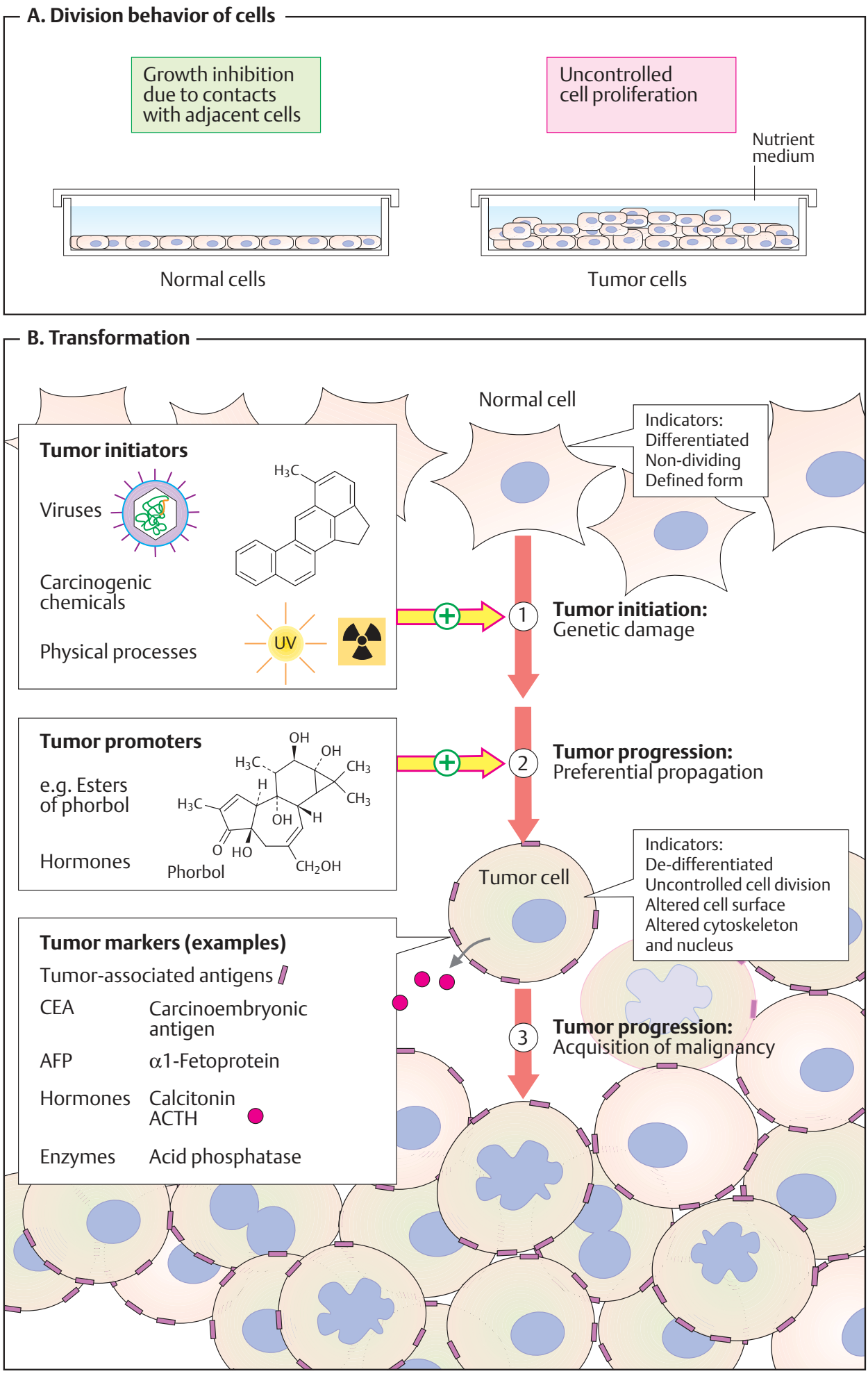
Tumor markers are clinically important for detecting certain tumors. These are proteins that are formed with increasing frequency by tumor cells (group 1) or are induced by them in other cells (group 2). Group 1 tumor markers include *tumor-associated antigens,* secreted hormones, and enzymes. The table lists a few examples.

The transition from a normal to a transformed state is a process involving several steps.

1. Tumor initiation. Almost every tumor begins with damage to the DNA of an individual cell. The genetic defect is almost always caused by environmental factors. These can include tumor-inducing chemicals (*carcinogens*—e. g., components of tar from tobacco), physical processes (e. g., UV light, X-ray radiation; see p. 256), or in rare cases tumor viruses (see p. 398). Most of the approximately 10^{14} cells in the human body probably suffer this type of DNA damage during the average lifespan, but it is usually repaired again (see p. 256). It is mainly defects in **proto-oncogenes** (see p. 398) that are relevant to tumor initiation; these are the decisive cause of *transformation.* Loss of an **anti-oncogene** (a tumor-suppressor gene) can also contribute to tumor initiation.

2. Tumor promotion is preferential proliferation of a cell damaged by transformation. It is a very slow process that can take many years. Certain substances are able to strongly accelerate it—e. g., *phorbol esters.* These occur in plants (e. g., *Euphorbia* species) and act as activators of protein kinase C (see p. 386).

3. Tumor progression finally leads to a macroscopically visible tumor as a result of growth. When solid tumors of this type exceed a certain size, they form their own vascular network that supplies them with blood (angiogenesis). *Collagenases* (matrix metalloproteinases, MMPs) play a special role in the metastatic process, by loosening surrounding connective tissue and thereby allowing tumor cells to disseminate and enter the bloodstream. New approaches to combating tumors have been aimed at influencing tumor angiogenesis and metastatic processes.



Cytostatic drugs

Tumors (see p. 400) arise from degenerated (transformed) cells that grow in an uncontrolled way as a result of genetic defects. Most transformed cells are recognized by the immune system and eliminated (see p. 294). If endogenous defense is not sufficiently effective, rapid tumor growth can occur. Attempts are then made to inhibit growth by physical or chemical treatment.

A frequently used procedure is targeted irradiation with γ-rays, which block cell reproduction due their mutagenic effect (see p. 256). Another approach is to inhibit cell growth by chemotherapy. The growth-inhibiting substances used are known as **cytostatic drugs**. Unfortunately, neither radiotherapy nor chemotherapy act selectively—i.e., they damage normal cells as well, and are therefore often associated with severe side effects.

Most cytostatic agents directly or indirectly inhibit DNA replication in the S phase of the cell cycle (see p. 394). The first group (**A**) lead to chemical changes in cellular DNA that impede transcription and replication. A second group of cytostatic agents (**B**) inhibit the synthesis of DNA precursors.

A. Alkylating agents, anthracyclines ○

Alkylating agents are compounds capable of reacting covalently with DNA bases. If a compound of this type contains *two* reactive groups, intramolecular or intermolecular *crosslinking* of the DNA double helix and "bending" of the double strand occurs. Examples of this type shown here are **cyclophosphamide** and the inorganic complex **cisplatin**. Anthracyclines such as **doxorubicin** (adriamycin) insert themselves non-covalently between the bases and thus lead to local alterations in the DNA structure (see p. 254 B).

B. Antimetabolites ○

Antimetabolites are enzyme inhibitors (see p. 96) that selectively block metabolic pathways. The majority of clinically important cytostatic drugs act on *nucleotide biosynthesis*. Many of these are modified nucleobases or nucleotides that *competitively* inhibit their target enzymes (see p. 96). Many are also incorporated into the DNA, thereby preventing replication.

The cytostatic drugs administered (indicated by a syringe in the illustration) are often not active themselves but are only converted into the actual active agent in the metabolism. This also applies to the adenine analogue **6-mercaptopurine**, which is initially converted to the mononucleotide tIMP (thioinosine monophosphate). Via several intermediate steps, tIMP gives rise to tdGTP, which is incorporated into the DNA and leads to crosslinks and other anomalies in it. The second effective metabolite of 6-mercaptopurine is *S*-methylated tIMP, an inhibitor of *amidophosphoribosyl transferase* (see p. 188).

Hydroxyurea selectively inhibits *ribonucleotide reductase* (see p. 190). As a radical scavenger, it removes the tyrosine radicals that are indispensable for the functioning of the reductase.

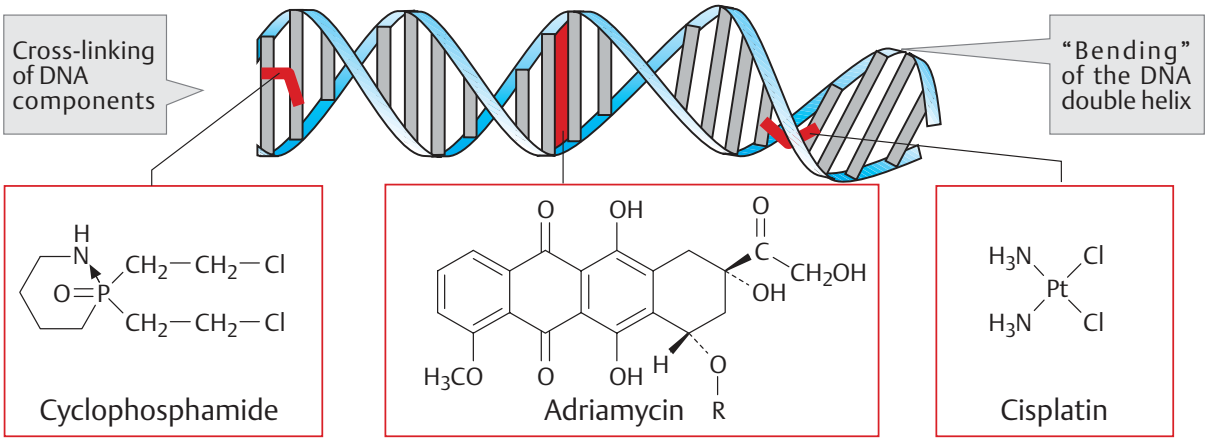
Two other important cytostatic agents target the synthesis of DNA-typical thymine, which takes place at the level of the deoxymononucleotide (see p. 190). The deoxymononucleotide formed by **5-fluorouracil** or the corresponding nucleoside inhibits *thymidylate synthase*. This inhibition is based on the fact that the fluorine atom in the pyrimidine ring cannot be substituted by a methyl group. In addition, the fluorine analogue is also incorporated into the DNA.

Dihydrofolate reductase acts as an auxiliary enzyme for thymidylate synthase. It is involved in the regeneration of the coenzyme N^5,N^{10}-methylene-THF, initially reducing DHF to THF with NADPH as the reductant (see p. 418). The folic acid analogue **methotrexate**, a frequently used cytostatic agent, is an extremely effective competitive inhibitor of dihydrofolate reductase. It leads to the depletion of N^5,N^{10}-methylene-THF in the cells and thus to cessation of DNA synthesis.

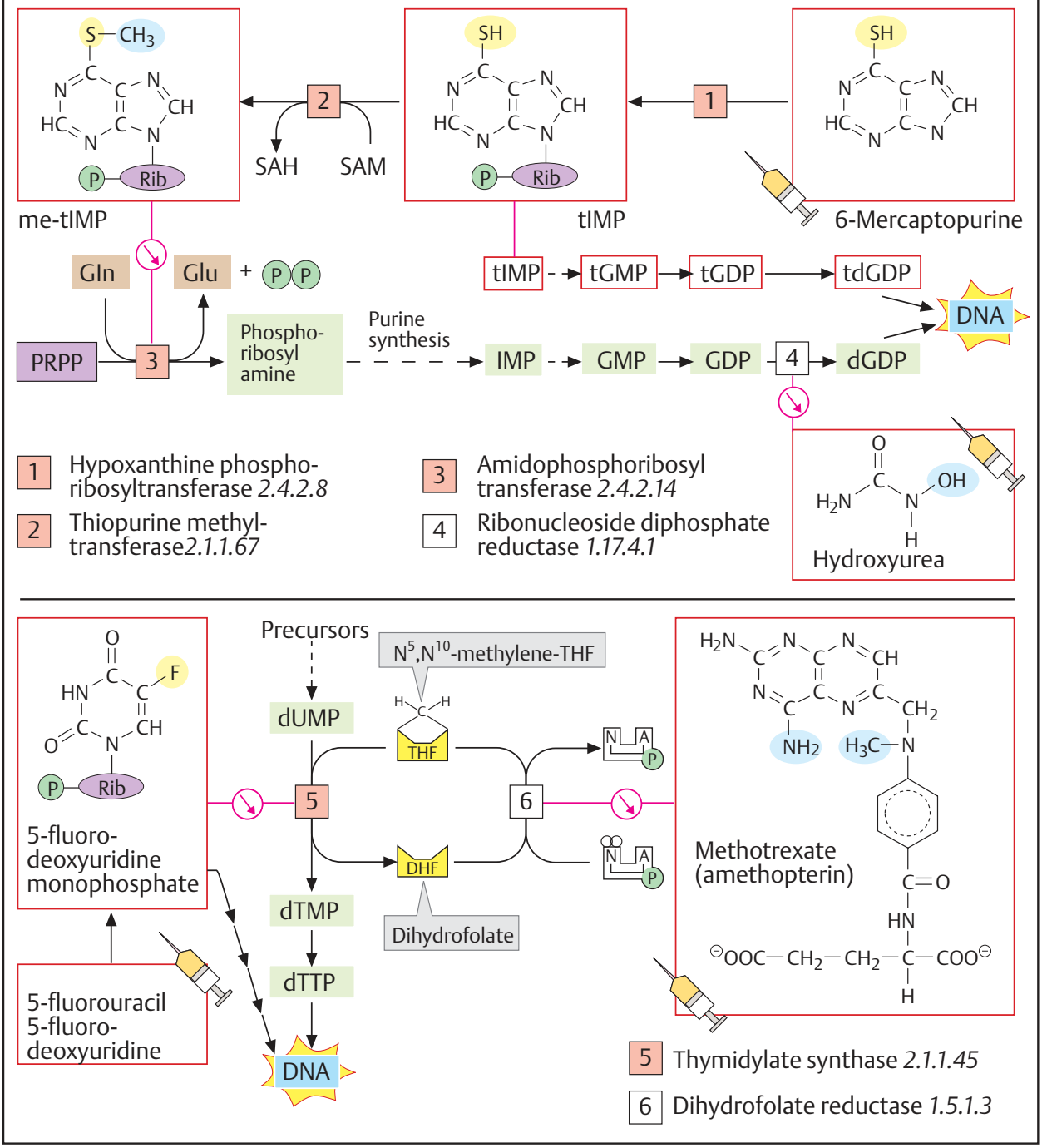
Further information

To reduce the side effects of cytostatic agents, new approaches are currently being developed on the basis of **gene therapy** (see p. 264). Attempts are being made, for example, to administer drugs in the form of precursors (known as prodrugs), which only become active in the tumor itself ("tumor targeting").

A. Alkylating agents, anthracyclines



B. Antimetabolites



Viruses

Viruses are *parasitic nucleoprotein complexes.* They often consist of only a single nucleic acid molecule (DNA or RNA, never both) and a protein coat. Viruses have *no metabolism of their own,* and can therefore only replicate themselves with the help of host cells. They are therefore not regarded as independent organisms. Viruses that damage the host cell when they replicate are *pathogens.* Diseases caused by viruses include AIDS, rabies, poliomyelitis, measles, German measles, smallpox, influenza, and the common cold.

A. Viruses: examples ◑

Only a few examples from the large number of known viruses are illustrated here. They are all shown on the same scale.

Viruses that only replicate in bacteria are known as **bacteriophages** (or "phages" for short). An example of a phage with a simple structure is **M13.** It consists of a single-stranded DNA molecule (ssDNA) of about 7000 bp with a coat made up of 2700 helically arranged protein subunits. The coat of a virus is referred to as a *capsid,* and the complete structure as a *nucleocapsid.* In genetic engineering, M13 is important as a *vector* for foreign DNA (see p. 258).

The phage **T4** (bottom left), one of the largest viruses known, has a much more complex structure with around 170 000 base pairs (bp) of double-stranded DNA (dsDNA) contained within its "head."

The **tobacco mosaic virus** (center right), a plant pathogen, has a structure similar to that of M13, but contains ssRNA instead of DNA. The **poliovirus**, which causes poliomyelitis, is also an RNA virus. In the **influenza virus**, the pathogen that causes viral flu, the nucleocapsid is additionally surrounded by a *coat* derived from the plasma membrane of the host cell (**C**). The coat carries viral proteins that are involved in the infection process.

B. Capsid of the rhinovirus ○

Rhinoviruses cause the common cold. In these viruses, the capsid is shaped like an *icosahedron*—i. e., an object made up of 20 equilateral triangles. Its surface is formed from three different proteins, which associate with one another to form pentamers and hexamers. In all, 60 protein molecules are involved in the structure of the capsid.

C. Life cycle of HIV ◑

The *human immunodeficiency virus (HIV)* causes the immunodeficiency disease known as **AIDS** (*acquired immune deficiency syndrome*). The structure of this virus is similar to that of the influenza virus (**A**).

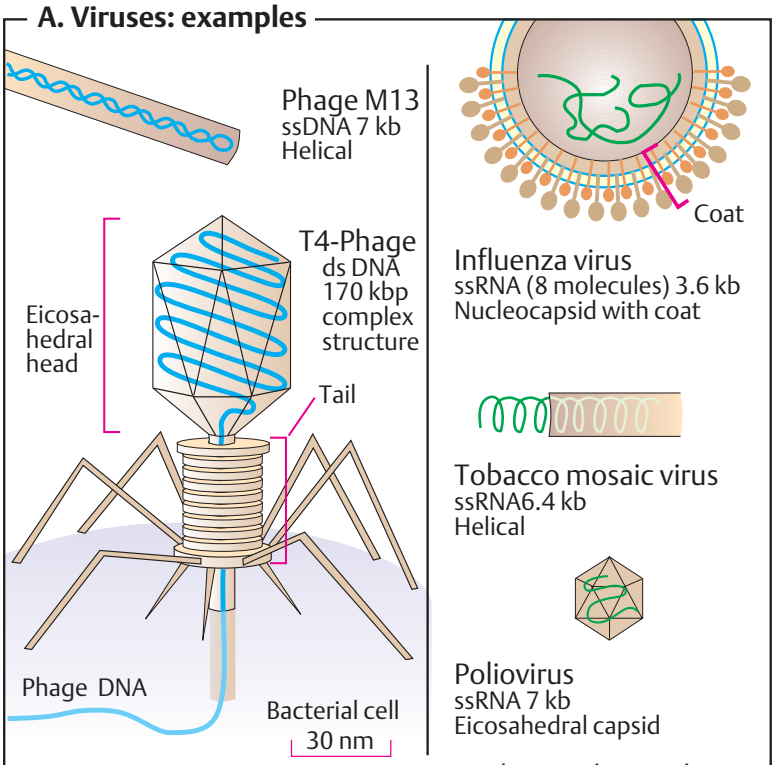
The HIV genome consists of two molecules of ssRNA (each 9.2 kb). It is enclosed by a double-layered capsid and a protein-containing coating membrane. HIV mainly infects T helper cells (see p. 294) and can thereby lead to failure of the immune system in the longer term.

During infection (**1**), the virus's coating membrane fuses with the target cell's plasma membrane, and the core of the nucleocapsid enters the cytoplasm (**2**). In the cytoplasm, the viral RNA is initially transcribed into an RNA/DNA hybrid (**3**) and then into dsDNA (**4**). Both of these reactions are catalyzed by *reverse transcriptase,* an enzyme deriving from the virus. The dsDNA formed is integrated into the host cell genome (**5**), where it can remain in an inactive state for a long time.

When viral replication occurs, the DNA segment corresponding to the viral genome is first transcribed by host cell enzymes (**6**). This gives rise not only to viral ssRNA, but also to transcription of mRNAs for precursors of the viral proteins (**7**). These precursors are integrated into the plasma membrane (**8**, **9**) before undergoing proteolytic modification (**10**). The cycle is completed by the release of new virus particles (**11**).

The group of RNA viruses to which HIV belongs are called **retroviruses**, because DNA is produced from RNA in their replication cycle—the reverse of the usual direction of transcription (DNA → RNA).

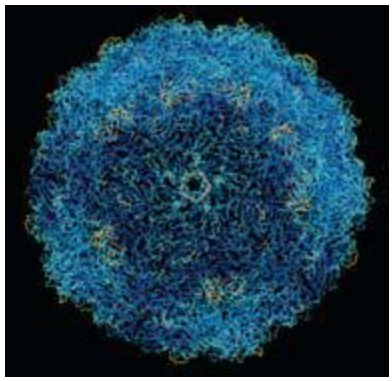
A. Viruses: examples



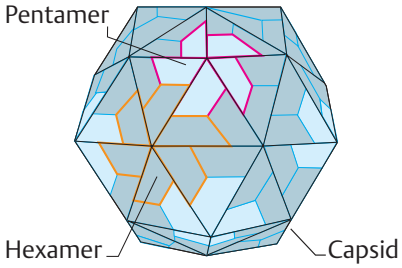
1. Bacteriophages

2. Plant and animal pathogenic viruses

B. Capsid of the rhinovirus



1. Structure



2. Diagram

C. Life cycle of the human immunodeficiency virus (HIV)