Molecular genetics: overview

Nucleic acids (**DNA** and various **RNAs**) are of central importance in the storage, transmission, and expression of genetic information. The decisive factor involved is their ability to enter into specific base pairings with each other (see p. 84). The individual processes involved, which are summed up in an overview here, are discussed in more detail on the following pages.

A. Expression and transmission of genetic information ●

Storage. The genetic information of all cells is stored in the base sequence of their DNA (RNA only occurs as a genetic material in viruses; see p. 404). Functional sections of DNA that code for inheritable structures or functions are referred to as **genes**. The 30 000–40 000 human genes represent only a few percent of the **genome**, which consists of approximately $5 \cdot 10^9$ base pairs (bp). Most genes code for proteins—i.e., they contain the information for the sequence of amino acid residues of a protein (its sequence). Every amino acid residue is represented in DNA by a code word (a **codon**) consisting of a sequence of three base pairs (a triplet). At the level of DNA, codons are defined as sequences of the sense strand read in the 5′→3′ direction (see p. 84). A DNA codon for the amino acid phenylalanine, for example, is thus *TTC* (**2**).

Replication. During cell division, all of the genetic information has to be passed on to the daughter cells. To achieve this, the whole of the DNA is copied during the S phase of the cell cycle (see p. 394). In this process, each strand serves as a matrix for the synthesis of a complementary second strand (**1**; see p. 240).

Transcription. For *expression* of a gene—i.e., synthesis of the coded protein—the DNA sequence information has to be converted into a protein sequence. As DNA itself is not involved in protein synthesis, the information is transferred from the nucleus to the site of synthesis in the cytoplasm. To achieve this, the *template strand* in the relevant part of the gene is transcribed into an **RNA** (hnRNA). The sequence of this RNA is thus complementary to that of the *template strand* (**3**), but—with the exception of the exchange of thymine for uracil—it is identical to that of the sense strand. In this way, the DNA triplet *TTC* gives rise in hnRNA to the RNA codon *UUC*.

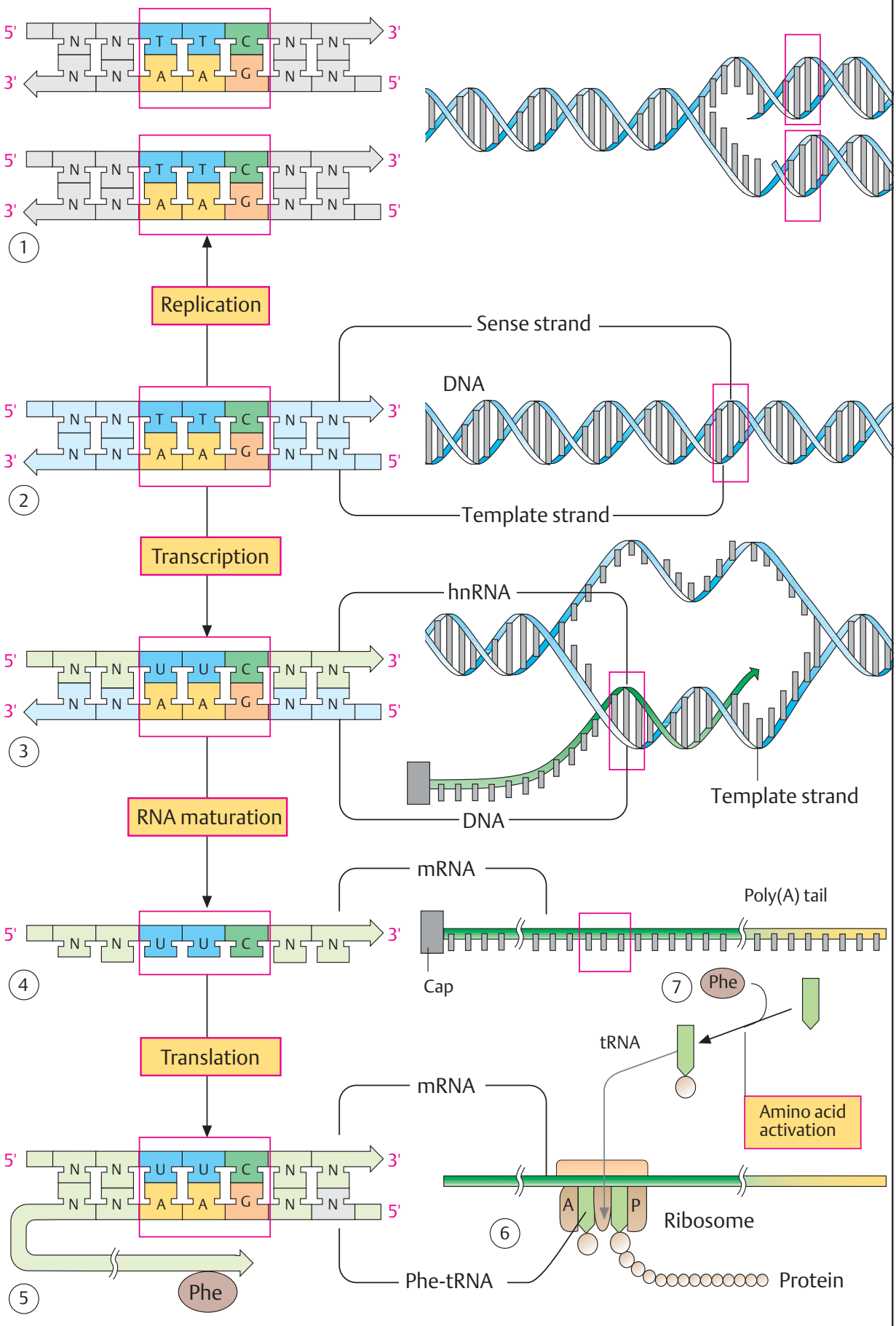
RNA maturation. In eukaryotes, the hnRNA initially formed is modified several times before it can leave the nucleus as **messenger RNA** (mRNA, **4**). During RNA maturation, superfluous ("intervening") sequences (introns) are removed from the molecule, and both ends of the transcript are protected by the addition of further nucleotides (see p. 246).

Translation. Mature mRNA enters the cytoplasm, where it binds to **ribosomes**, which convert the RNA information into a peptide sequence. The ribosomes (see p. 250) consist of more than 100 proteins and several RNA molecules (rRNA; see p. 82). rRNA plays a role as a ribosomal structural element and is also involved in the binding of mRNA to the ribosome and the formation of the peptide bond.

The actual information transfer is based on the interaction between the mRNA codons and another type of RNA, **transfer RNA** (tRNA; see p. 82). tRNAs, of which there are numerous types, always provide the correct amino acid to the ribosome according to the sequence information in the mRNA. tRNAs are loaded with an amino acid residue at the 3′ end. Approximately in the middle, they present the triplet that is complementary to each mRNA codon, known as the *anticodon* (*GAA* in the example shown). If the codon *UUC* appears on the mRNA, the anticodon binds a molecule of Phe-t-RNA^{Phe} to the mRNA (**5**) and thus brings the phenylalanine residue at the other end of the molecule into a position in which it can take over the growing polypeptide chain from the neighboring tRNA (**6**).

Amino acid activation. Before binding to the ribosomes, tRNAs are loaded with the correct amino acids by specific ligases (**7**; see p. 248). It is the amino acid tRNA ligases that carry out the transfer (translation) of the genetic information from the nucleic acid level to the protein level.

A. Expression and transmission of genetic information



Genome

A. Chromatin ◑

In the nuclei of eukaryotes (see p. 196), DNA is closely associated with proteins and RNA. These nucleoprotein complexes, with a DNA proportion of approximately one-third, are known as **chromatin**. It is only during cell division (see p. 394) that chromatin condenses into **chromosomes** that are visible under light microscopy. During interphase, most of the chromatin is loose, and in these conditions a morphological distinction can be made between tightly packed **heterochromatin** and the less dense **euchromatin**. Euchromatin is the site of active transcription.

The proteins contained in chromatin are classified as either histone or non-histone proteins. **Histones** (**B**) are small, strongly basic proteins that are directly associated with DNA. They contribute to the structural organization of chromatin, and their basic amino acids also neutralize the negatively charged phosphate groups, allowing the dense packing of DNA in the nucleus. This makes it possible for the 46 DNA molecules of the diploid human genome, with their 5 10^9 base pairs (bp) and a total length of about 2 m, to be accommodated in a nucleus with a diameter of only 10 μm. Histones also play a central role in regulating transcription (see p. 244).

Two histone molecules each of types **H2A** (blue), **H2B** (green), **H3** (yellow), and **H4** (red) form an octameric complex, around which 146 bp of DNA are wound in 1.8 turns. These particles, with a diameter of 7 nm, are referred to as **nucleosomes**. Another histone (**H1**) binds to DNA segments that are not directly in contact with the histone octamers ("linker" DNA). It covers about 20 bp and supports the formation of spirally wound superstructures with diameters of 30 nm, known as **solenoids**. When chromatin condenses into chromosomes, the solenoids form **loops** about 200 nm long, which already contain about 80 000 bp. The loops are bound to a protein framework (the **nuclear scaffolding**), which in turn organizes some 20 loops to form **minibands**. A large number of stacked minibands finally produces a chromosome. In the chromosome, the DNA is so densely packed that the smallest human chromosome already contains more than 50 million bp.

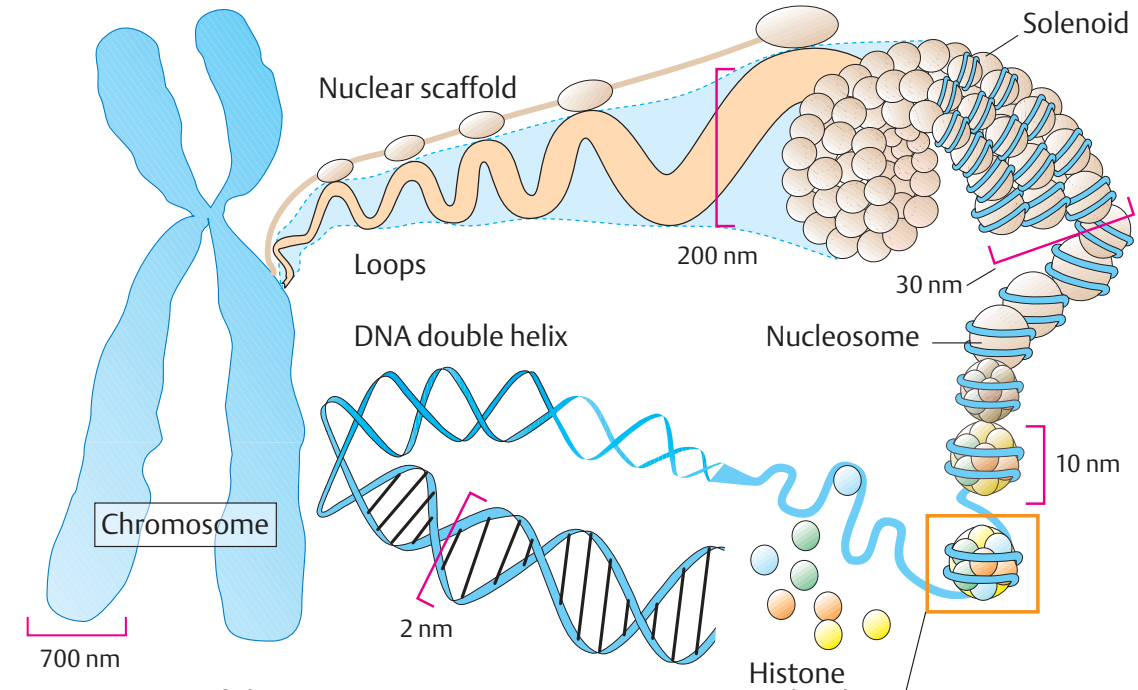
The **non-histone proteins** are very heterogeneous. This group includes *structural proteins* of the nucleus, as well as many *enzymes* and *transcription factors* (see p. 118), which selectively bind to specific segments of DNA and regulate gene expression and other processes.

B. Histones ○

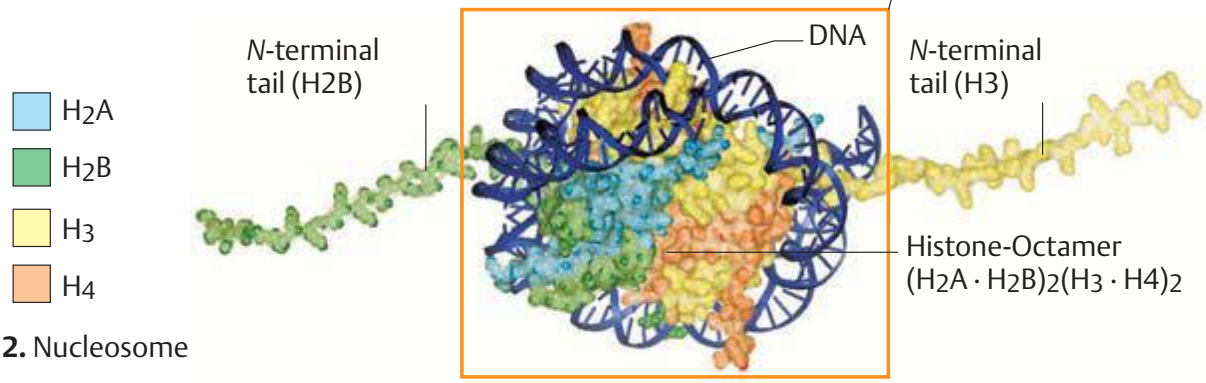
The histones are remarkable in several ways. With their high proportions of lysine and arginine (blue shading), they are strongly basic, as mentioned above. In addition, their amino acid sequence has hardly changed at all in the course of evolution. This becomes clear when one compares the histone sequences in mammals, plants, and fungi (yeasts are single-celled fungi; see p. 148). For example, the H4 histones in humans and wheat differ only in a single amino acid residue, and there are only a few changes between humans and yeast. In addition, all of these changes are "conservative"—i. e., the size and polarity barely differ. It can be concluded from this that the histones were already "optimized" when the last common predecessor of animals, plants, and fungi was alive on Earth (more than 700 million years ago). Although countless mutations in histone genes have taken place since, almost all of these evidently led to the extinction of the organisms concerned.

The histones in the octamer carry N-terminal mobile "tails" consisting of some 20 amino acid residues that project out of the nucleosomes and are important in the regulation of chromatin structure and in controlling gene expression (see **A2**; only two of the eight tails are shown in full length). For example, the condensation of chromatin into chromosomes is associated with *phosphorylation* (P) of the histones, while the transcription of genes is initiated by *acetylation* (A) of lysine residues in the N-terminal region (see p. 244).

A. Chromatin



1. Organization of chromatin



2. Nucleosome

B. Histone

A Acetylation P Phosphorylation M Methylation

	1	10	20	30	40	50	60	70	80	90	100
Animals	S G R G K G G K G L G K G G A K R H R K V L R D N I										
Plants	S G R G K G G K G L G K G G A K R H R K V L R D N I										
Yeast	S G R G K G G K G L G K G G A K R H R K I L R D N I										
Animals				Q G I T K P A I R R L A R R G G V K R I S G L I Y E							
Plants				Q G I T K P A I R R L A R R G G V K R I S G L I Y E							
Yeast				Q G I T K P A I R R L A R R G G V K R I S G L I Y E							
Animals							E T R G V L K V F L E N V I R D A V T Y T E H A K R				
Plants							E T R G V L K I F L E N V I R D A V T Y T E H A R R				
Yeast							E V R A V L K S F L E S V I R D S V T Y T E H A K R				
Animals									K T V T A M D V V Y A L K R Q G R T L Y G F G G		
Plants									K T V T A M D V V Y A L K R Q G R T L Y G F G G		
Yeast									K T V T S L D V V Y A L K R Q G R T L Y G F G G		

Amino acid sequence of histone H4

Replication

For genetic information to be passed on during cell division, a complete copy of the genome has to be produced before each mitosis. This process is known as DNA **replication**.

A. Mechanism of DNA polymerases ◑

Replication is catalyzed by *DNA–dependent DNA polymerases.* These enzymes require a single strand of DNA, known as the **template**. Beginning at a short starting sequence of RNA (the **primer**), they synthesize a second complementary strand on the basis of this template, and thus create a complete DNA double helix again. The substrates of the DNA polymerases are the four deoxynucleoside triphosphates **dATP**, **dGTP**, **dCTP**, and **dTTP**. In each step, base pairing first binds the nucleotide that is complementary to the current base in the template strand. The α–phosphate residue of the newly bound nucleoside triphosphate is then subjected to nucleophilic attack by the 3′–OH group of the nucleotide incorporated immediately previously. This is followed by the elimination of diphosphate and the formation of a new phosphoric acid diester bond. These steps are repeated again for each nucleotide. The mechanism described means that the matrix can only be read in the 3′→5′ direction. In other words, the newly synthesized strand always grows in the **5′→3′ direction**. The same mechanism is also used in transcription by *DNA-dependent RNA polymerases* (see p. 242). Most DNA and RNA polymerases consist of more than 10 subunits, the role of which is still unclear to some extent.

B. Replication in *E. coli* ◑

Although replication in prokaryotes is now well understood, many details in eukaryotes are still unclear. However, it is certain that the process is in principle similar. A simplified scheme of replication in the bacterium *Escherichia coli* is shown here.

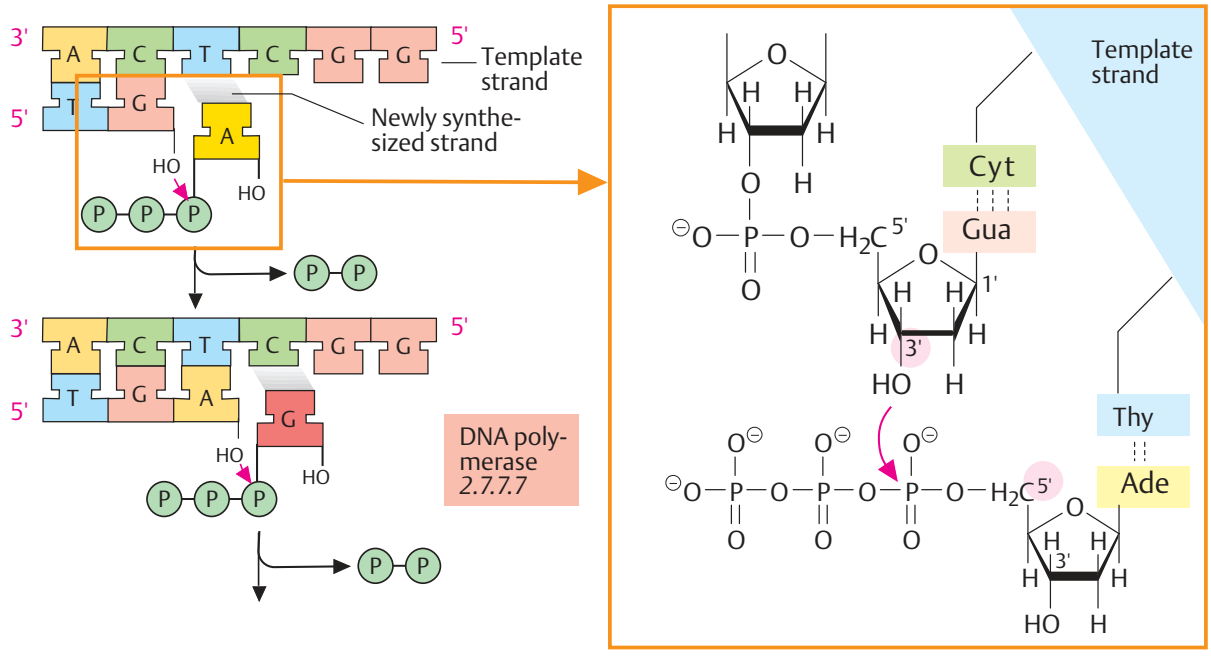
In bacteria, replication starts at a specific point in the circular DNA—the **origin of replication**—and proceeds in both directions. This results in two diverging **replication forks**, in which the two strands are replicated simultaneously. Numerous proteins are involved in the processes taking place in this type of fork, and only the most important are shown here. The two strands of the initial DNA (**1**) are shown in blue and violet, while the newly formed strands are pink and orange.

Each fork (**2**) contains two molecules of **DNA polymerase III** and a number of helper proteins. The latter include **DNA topoisomerases** and **single–strand-binding proteins**. Topoisomerases are enzymes that unwind the superhelical DNA double strand (*gyrase, topoisomerase II*) and then separate it into the two individual strands (*helicase, topoisomerase I*). Since the template strand is always read from 3′ to 5′ (see above), only one of the strands (known as the **leading** strand; violet/pink) can undergo *continuous* replication. For the **lagging strand** (light blue), the reading direction is the *opposite* of the direction of movement of the fork. In this matrix, the new strand is first synthesized in individual pieces, which are known as **Okazaki fragments** after their discoverer (green/orange).

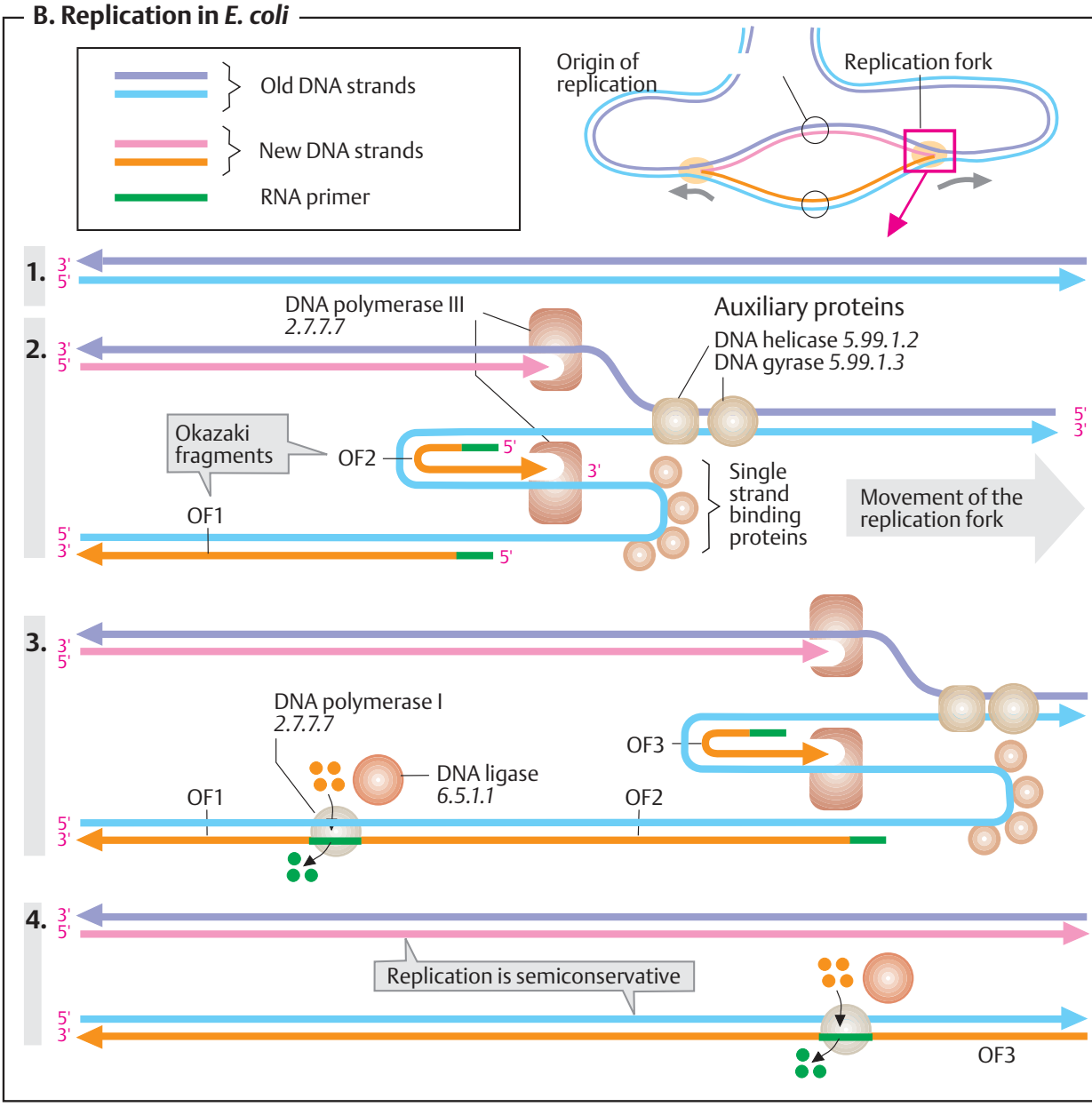
Each fragment starts with a short RNA primer (green), which is necessary for the functioning of the DNA polymerase and is synthesized by a special *RNA polymerase* (*"primase,"* not shown). The primer is then extended by *DNA polymerase III* (orange). After 1000–2000 nucleotides have been included, synthesis of the fragment is interrupted and a new one is begun, starting with another RNA primer that has been synthesized in the interim. The individual Okazaki fragments are initially not bound to one another and still have RNA at the 5′ end (**3**). At some distance from the fork, *DNA polymerase I* therefore starts to remove the RNA primer and replace it with DNA components. Finally, the gaps still remaining are closed by a *DNA ligase.* In DNA double helices formed in this way, only *one* of the strands has been newly synthesized—i. e., replication is *semiconservative*.

In bacteria, some 1000 nucleotides are replicated per second. In eukaryotes, replication takes place more slowly (about 50 nucleotides s^{-1}) and the genome is larger. Thousands of replication forks are therefore active simultaneously in eukaryotes.

A. Mechanism of DNA polymerases



B. Replication in *E. coli*



Transcription

For the genetic information stored in DNA to become effective, it has to be rewritten (transcribed) into RNA. DNA only serves as a template and is not altered in any way by the **transcription** process. Transcribable segments of DNA that code for a defined product are called **genes**. It is estimated that the mammalian genome contains 30 000–40 000 genes, which together account for less than 5% of the DNA.

A. Transcription and maturation of RNA: overview ◑

Transcription is catalyzed by *DNA–dependent RNA polymerases.* These act in a similar way to DNA polymerases (see p. 240), except that they incorporate *ribonucleotides* instead of deoxyribonucleotides into the newly synthesized strand; also, they do not require a primer. Eukaryotic cells contain at least three different types of RNA polymerase. *RNA polymerase I* synthesizes an RNA with a sedimentation coef cient (see p. 200) of 45 S, which serves as precursor for three ribosomal RNAs. The products of *RNA polymerase II* are hnRNAs, from which mRNAs later develop, as well as precursors for snRNAs. Finally, *RNA polymerase III* transcribes genes that code for tRNAs, 5S rRNA, and certain snRNAs. These precursors give rise to functional RNA molecules by a process called **RNA maturation** (see p. 246). Polymerases II and III are inhibited by *α–amanitin,* a toxin in the *Amanita phalloides* mushroom.

B. Organization of the PEP-CK gene ○

The way in which a typical eukaryotic gene is organized is illustrated here using a gene that codes for a key enzyme in gluconeogenesis (see p. 154)—the *phosphoenolpyruvate carboxykinase* (PEP-CK).

In the rat, the PEP-CK gene is nearly 7 kbp (kilobase pairs) long. Only 1863 bp, distributed over 10 coding segments (**exons**, dark blue) carry the information for the protein's 621 amino acids. The remainder is allotted to the promoter (pink) and intervening sequences (**introns**, light blue). The gene's promoter region (approximately 1 kbp) serves for regulation (see p. 188). Transcription starts at the 3′ end of the promoter ("transcription start") and continues until the polyadenylation sequence (see below) is reached. The primary transcript (**hnRNA**) still has a length of about 6.2 kbp. During RNA maturation, the noncoding sequences corresponding to the introns are removed, and the two ends of the hnRNA are modified. The translatable mRNA still has half the length of the hnRNA and is modified at both ends (see p. 246).

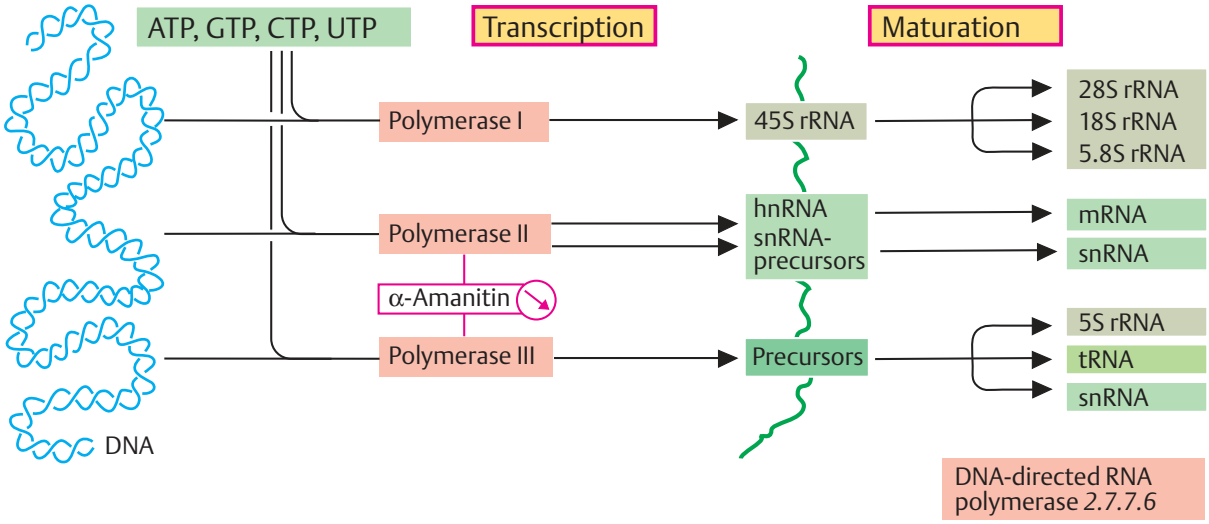
In many eukaryotic genes, the proportion of introns is even higher. For example, the gene for *dihydrofolate reductase* (see p. 402) is over 30 kbp long. The information is distributed over six exons, which together have a length of only about 6 kbp.

C. Transcription process ◑

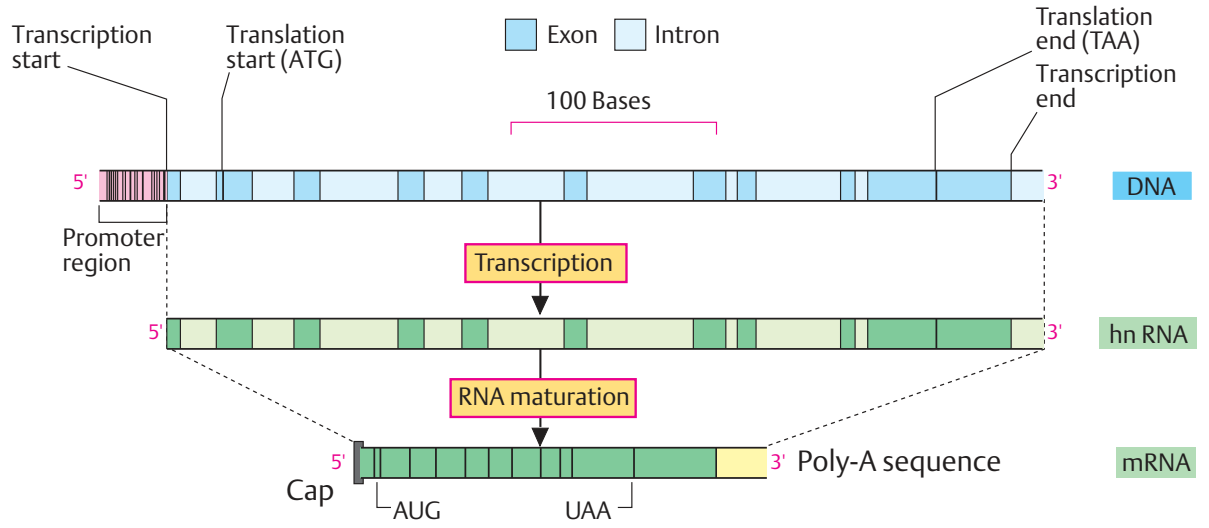
As mentioned above, *RNA polymerase II* (green) binds to the 3′ end of the promoter region. A sequence that is important for this binding is known as the **TATA box**—a short A– and T–rich sequence that varies slightly from gene to gene. A typical base sequence *("consensus sequence")* is …TATAAA… Numerous proteins known as *basal transcription factors* are necessary for the interaction of the polymerase with this region. Additional factors can promote or inhibit the process (transcriptional control; see p. 244). Together with the polymerase, they form the **basal transcription complex**.

At the end of **initiation** (**2**), the polymerase is repeatedly phosphorylated, frees itself from the basal complex, and starts moving along the DNA in the 3′ direction. The enzyme separates a short stretch of the DNA double helix into two single strands. The complementary nucleoside triphosphates are bound by base pairing in the *template strand* and are linked step by step to the hnRNA as it grows in the 5′→3′ direction (**3**). Shortly after the beginning of **elongation**, the 5′ end of the transcript is protected by a "cap" (see p. 246). Once the polyadenylation sequence has been reached (typical sequence: …AATAA…), the transcript is released (**4**). Shortly after this, the RNA polymerase stops transcribing and dissociates from the DNA.

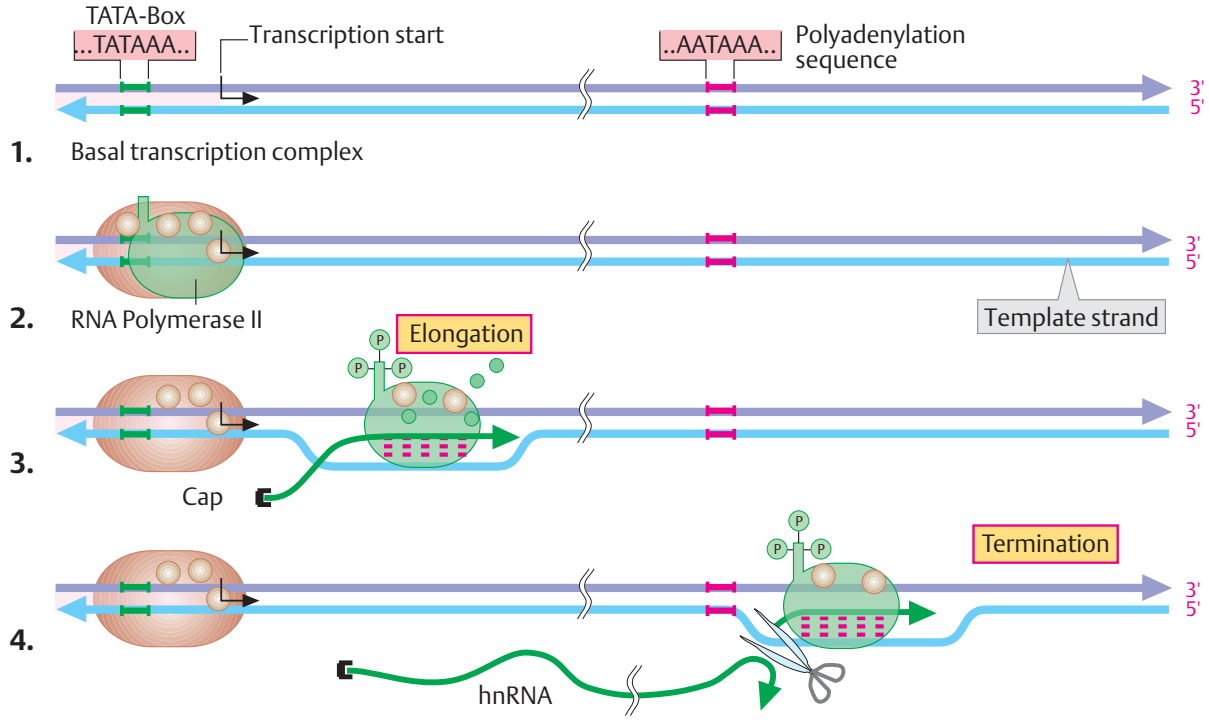
A. Transcription and maturation of RNA: overview



B. Organization of the PEP-CK gene



C. Process of transcription



Transcriptional control

Although all cells contain the complete genome, they only use a fraction of the information in it. The genes known as "housekeeping genes," which code for structural molecules and enzymes of intermediate metabolism, are the only ones that undergo constant transcription. The majority of genes are only active in certain cell types, in specific metabolic conditions, or during differentiation. Which genes are transcribed and which are not is regulated by **transcriptional control** (see also p. 118). This involves *control elements* (*cis*-active elements) in the gene's promoter region and gene-specific regulatory proteins (transcription factors, *trans* -active factors), which bind to the control elements and thereby activate or inhibit transcription.

A. Initiation of transcription ○

In the higher organisms, DNA is blocked by histones (see p. 238) and is therefore not capable of being transcribed without special positive regulation. In eukaryotes, it is therefore histones that play the role of *repressors* (see p. 118). For transcription to be set in motion at all, the chromatin first has to be restructured.

In the resting state, the lysine residues in the N-terminal "tail" of the histones (see p. 238) are not acetylated. In this state, which can be produced by *histone deacetylases* [1], the nucleosomes are stable. It is only the interaction of activator and regulator proteins with their control elements that allows the binding of coactivator complexes that have *histone acetylase* activity [2]. They acetylate the histone tails and thereby loosen the nucleosome structure suf ciently for the basal transcription complex to form.

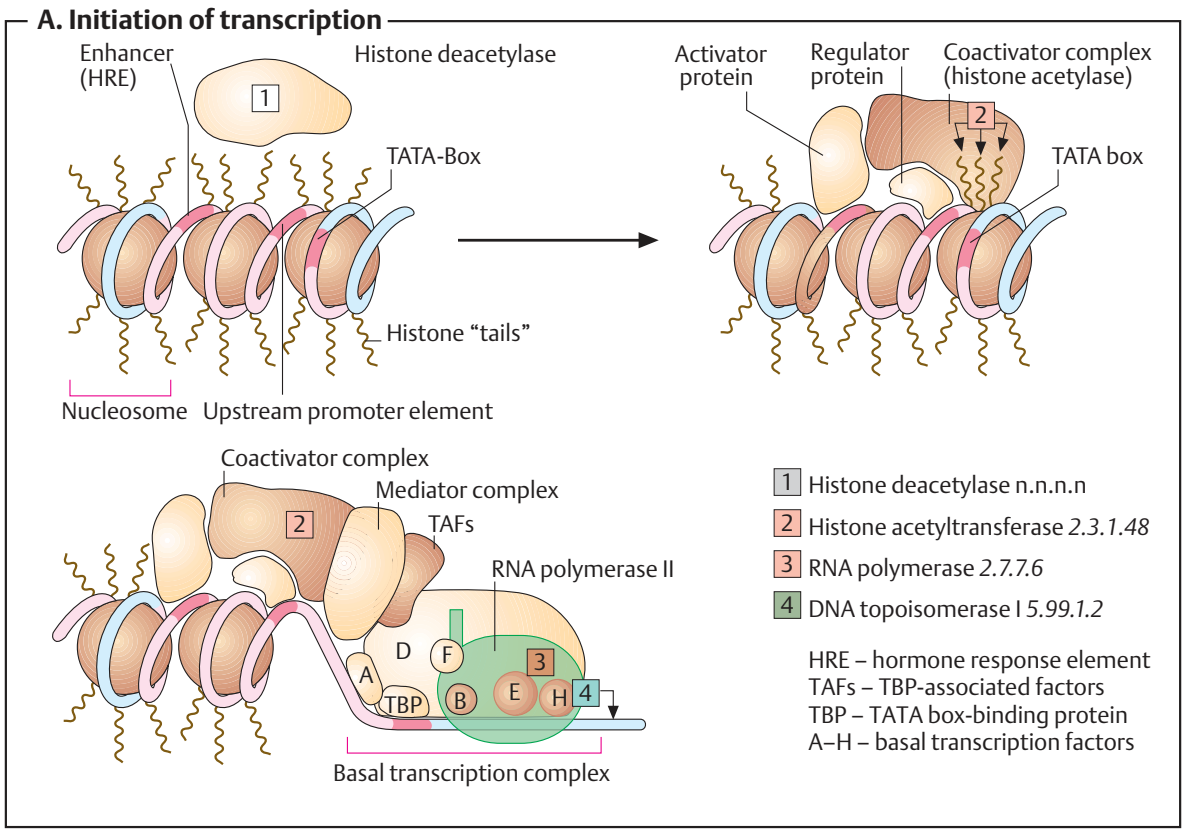
This consists of *DNA-dependent RNA polymerase II* and basal transcription factors (TFIIX, X = A – H). First, the basal factor TFIID binds to the promoter. TFIID, a large complex of numerous proteins, contains *TATA box-binding protein* (TBP) and so-called TAFs (TBP-associated factors). The polymerase is attached to this core with the help of TFIIB. Before transcription starts, additional TFs have to bind, including TFIIH, which has *helicase* activity and separates the two strands of DNA during elongation. In all, some 35 different proteins are involved in the basal complex. This alone, however, is still not suf cient for transcription to start. In addition, positive signals have to be emitted by more distant *trans*-active factors, integrated by the coactivator/mediator complex, and passed on to the basal complex (see **B**).

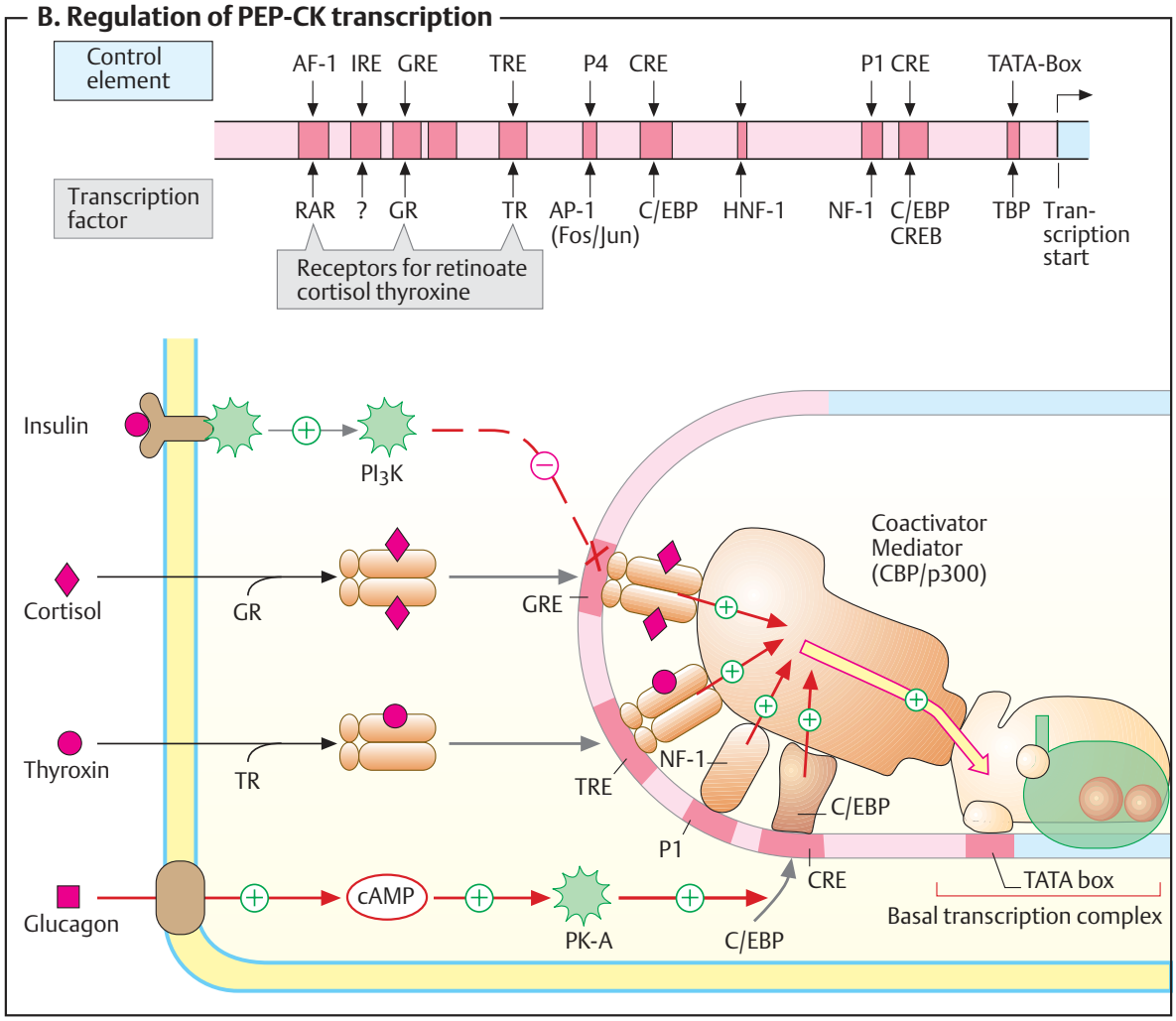
The actual signal for starting elongation consists of the multiple phosphorylation of a domain in the C-terminal region of the polymerase. In phosphorylated form, it releases itself from the basal complex along with a few TFs and starts to synthesize hnRNA.

B. Regulation of PEP-CK transcription ○

Phosphoenolpyruvate carboxykinase (PEP-CK), a key enzyme in gluconeogenesis, is regulated by several hormones, all of which affect the transcription of the PEP-CK gene. Cortisol, glucagon, and thyroxin induce PEP-CK, while insulin inhibits its induction (see p. 158).

More than ten **control elements** (dark red), distributed over approximately 1 kbp, have so far been identified in the promoter of the PEP-CK gene (top). These include response elements for the glucocorticoid receptor (GRE; see p. 378), for the thyroxin receptor (TRE), and for the steroid-like retinoic acid (AF-1). Additional control elements (CRE, cAMP-responsive element) bind the transcription factor C/EBP, which is activated by cAMP-dependent protein kinase A through phosphorylation. This is the way in which glucagon, which raises the cAMP level (see p. 158), works. Control element P1 binds the hormone-independent factor NF-1 (nuclear factor-1). All proteins that bind to the control elements mentioned above are in contact with a **coactivator/mediator complex** (CBP/p300), which integrates their input like a computer and transmits the result in the form of stronger or weaker signals to the basal transcription complex. Inhibition of PEP-CK transcription by insulin is mediated by an insulin-responsive element (IRE) in the vicinity of the GRE. Binding of an as yet unknown factor takes place here, inhibiting the binding of the glucocorticoid receptor to the GRE.





RNA maturation

Before the hnRNA produced by RNA polymerase II (see p. 242) can leave the nucleus in order to serve as a template for protein synthesis in the cytoplasm, it has to undergo several modifications first. Even during transcription, the two ends of the transcript have additional nucleotides added (**A**). The sections that correspond to the intervening gene sequences in the DNA (introns) are then cut out (splicing; see **B**). Other transcripts—e.g., the 45 S precursor of rRNA formed by polymerase I (see p. 242)—are broken down into smaller fragments by nucleases before export into the cytoplasm.

A. 5′ and 3′ modification of mRNA ◑

Shortly after transcription begins in eukaryotes, the end of the growing RNA is blocked in several reaction steps by a structure known as a "**cap**." In hnRNAs, this consists of a GTP residue that is methylated at N-7 of the guanine ring. The β-phosphate residue of the cap is linked to the free 5′-OH group of the terminal ribose via an ester bond. After the "polyadenylation signal" has been reached (typical sequence: …AAUAAA…; see p. 242), a **polyadenylate "tail"** consisting of up to 200 AMP nucleotides is also added at the free 3′ end of the transcript. This reaction is catalyzed by a special *polyadenylate polymerase*. It is only at this point that the mRNA leaves the nucleus as a complex with RNA-binding proteins.

Both the cap and the poly-A tail play a vital part in initiating eukaryotic translation (see p. 250). They help position the ribosome correctly on the mRNA near to the starting codon. The protection which the additional nucleotides provide against premature enzymatic degradation appears to be of lesser importance.

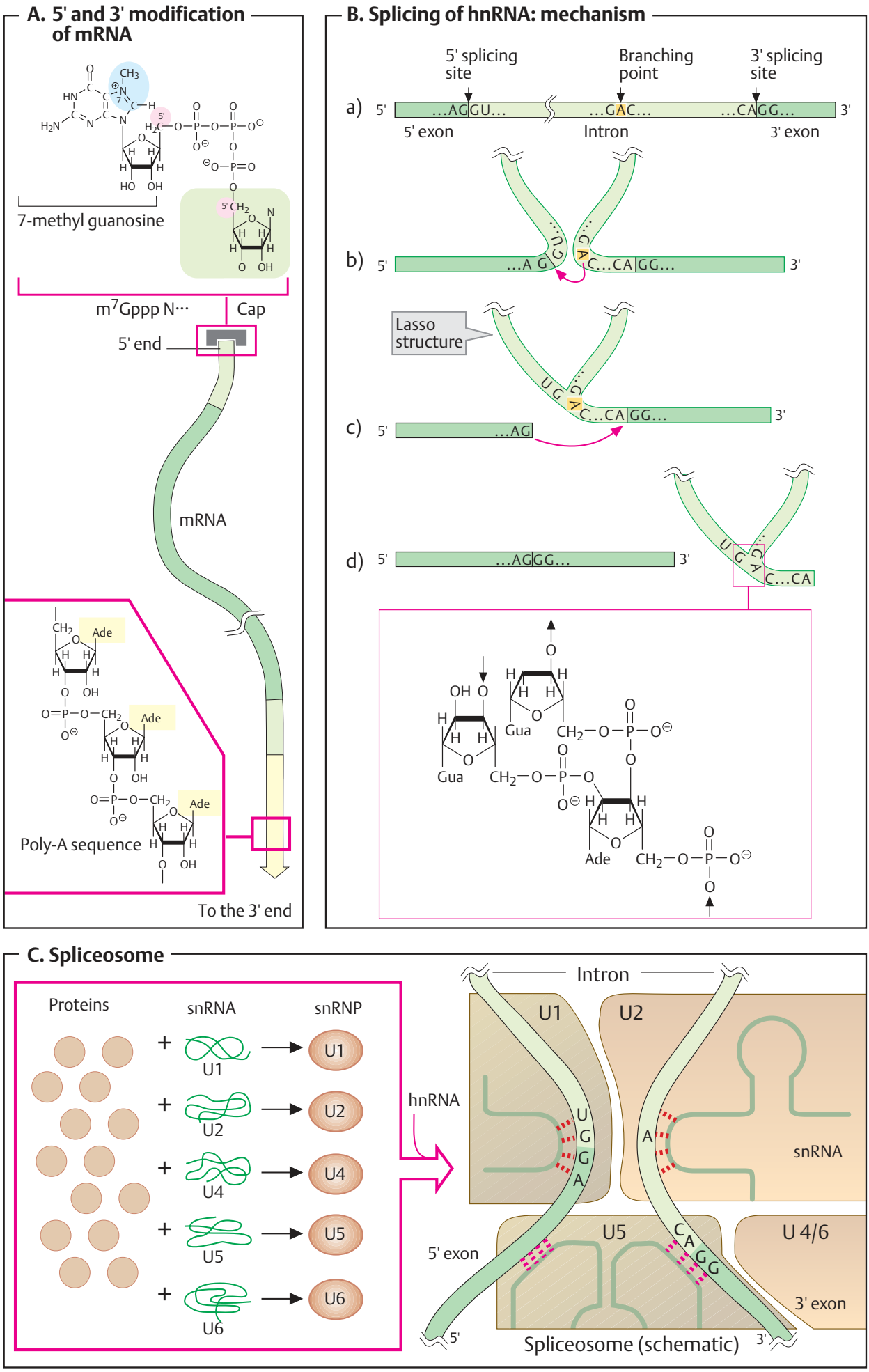
B. Splicing of hnRNA ◑

Immediately after transcription, the hnRNA introns are removed and the exons are linked to form a continuous coding sequence. This process, known as **splicing**, is supported by complicated RNA–protein complexes in the nucleus, the so-called **spliceosomes**. The components of these macromolecular machines are called **snRNPs** (*small nuclear ribonucleoprotein particles,* pronounced "snurps"). SnRNPs occur in five different forms (U1, U2, U4, U5, and U6). They consist of numerous *proteins* and one molecule of *snRNA* each (see p. 82).

To ensure that the RNA message is not destroyed, splicing has to take place in a very precise fashion. The start and end of the hnRNA introns are recognized by a characteristic sequence (…AGGT… at the 5′ end or …[C,U]AGG… at the 3′ end). Another important structure is the so-called *branching point* inside the intron. Its sequence is less conserved than the terminal splicing sites, but it always contains one adenosine residue (A). During splicing, the 2′-OH group of this residue—supported by the spliceosome (see **C**)—attacks the phosphoric acid diester bond at the 5′ end of the intron and cleaves it (**b**). Simultaneously, an unusual 2′→5′ bond is formed inside the intron, which thereby takes on a *lasso shape* (**c**; see formula). In the second step of the splicing process, the free 3′-OH group at the end of the 5′ terminal exon attacks the A–G bond at the 3′ end of the intron. As a result, the two exons are linked and the intron is released, still in a lasso shape (d).

C. Spliceosome ○

As described above, it is residues of the hnRNA that carry out bond cleavage and bond formation during the splicing process. It is therefore not a protein enzyme that acts as a catalyst here, but rather an RNA. Catalytic RNAs of this type are called *ribozymes* (see also p. 88). The task of the spliceosomes is to fix and orientate the reacting groups by establishing base pairings between snRNAs and segments of the hnRNA. The probable situation before the adenosine attack at the branching point on the 5′ splicing site (see **B**, Fig. **b**) is shown schematically on the right side of the illustration. In this phase, the U1 snRNA fixes the 5′ splicing site, U2 fixes the branching site, and U5 fixes the ends of the two exons.



Amino acid activation

A. The genetic code ◑

Most of the genetic information stored in the genome codes for the amino acid sequences of proteins. For these proteins to be expressed, a text in "nucleic acid language" therefore has to be translated into "protein language." This is the origin of the use of the term **translation** to describe protein biosynthesis. The dictionary used for the translation is the **genetic code.**

As there are 20 proteinogenic amino acids (see p. 60), the nucleic acid language has to contain at least as many words **(codons)**. However, there are only four letters in the nucleic acid alphabet (A, G, C, and U or T). To obtain 20 different words from these, each word has to be at least three letters long (with two letters, there would only be $4^2 = 16$ possibilities). And in fact the codons do consist of three sequential bases **(triplets)**.

Figure 1 shows the standard code in "DNA language" (i. e., as a sequence of triplets in the *sense strand* of DNA, read in the 5′→3′ direction; see p. 84), represented as a circular diagram. The scheme is read from the inside to the outside. For example, the triplet CAT codes for the amino acid histidine. With the exception of the exchange of U for T, the DNA codons are identical to those of mRNA.

As the genetic code provides $4^3 = 64$ codons for the 20 amino acids, there are several synonymous codons for most amino acids—the code is **degenerate**. Three triplets do not code for amino acids, but instead signal the end of translation **(stop codons)**. Another special codon, the **start codon**, marks the start of translation. The code shown here is almost universally applicable; only the mitochondria (see p. 210) and a few microorganisms deviate from it slightly.

As an example of the way in which the code is read, Fig. 2 shows small sections from the normal and a mutated form of the β-globin gene (see p. 280), as well as the corresponding mRNA and protein sequences. The **point mutation** shown, which is relatively frequent, leads to replacement of a glutamate residue in position 6 of the β-chain by valine (G**A**G → G**T**G). As a consequence, the mutated hemoglobin tends to aggregate in the deoxygenated form. This leads to sickle-shaped distortions of the erythrocytes and disturbances of O_2 transport (sickle-cell anemia).

B. Amino acid activation ◑

Some 20 different *amino acid tRNA ligases* in the cytoplasm each bind one type of tRNA (see p. 82) with the corresponding amino acid. This reaction, known as **amino acid activation**, is endergonic and is therefore coupled to ATP cleavage in two steps.

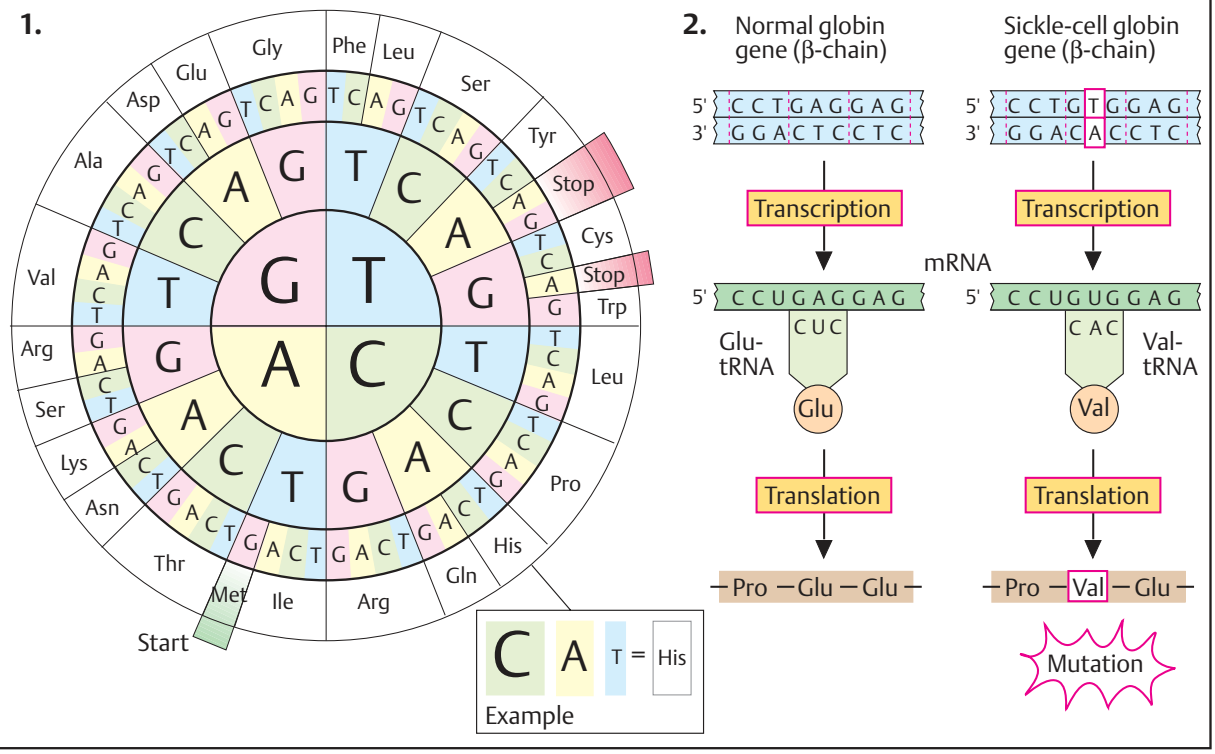
First, the amino acid is bound by the enzyme and reacts there with ATP to form diphosphate and an "energy–rich" mixed acid anhydride (**aminoacyl adenylate**). In the second step, the 3′-OH group (in other ligases it is the 2′-OH group) of the terminal ribose residue of the tRNA takes over the amino acid residue from the aminoacyl adenylate. In **aminoacyl tRNAs**, the carboxyl group of the amino acid is therefore esterified with the ribose residue of the terminal adenosine of the sequence …CCA-3′.

The accuracy of translation primarily depends on the specificity of the amino acid tRNA ligases, as incorrectly incorporated amino acid residues are not recognized by the ribosome later. A *"proofreading mechanism"* in the active center of the ligase therefore ensures that incorrectly incorporated amino acid residues are immediately removed again. On average, an error only occurs once every 1300 amino acid residues. This is a surprisingly low rate considering how similar some amino acids are—e. g., leucine and isoleucine.

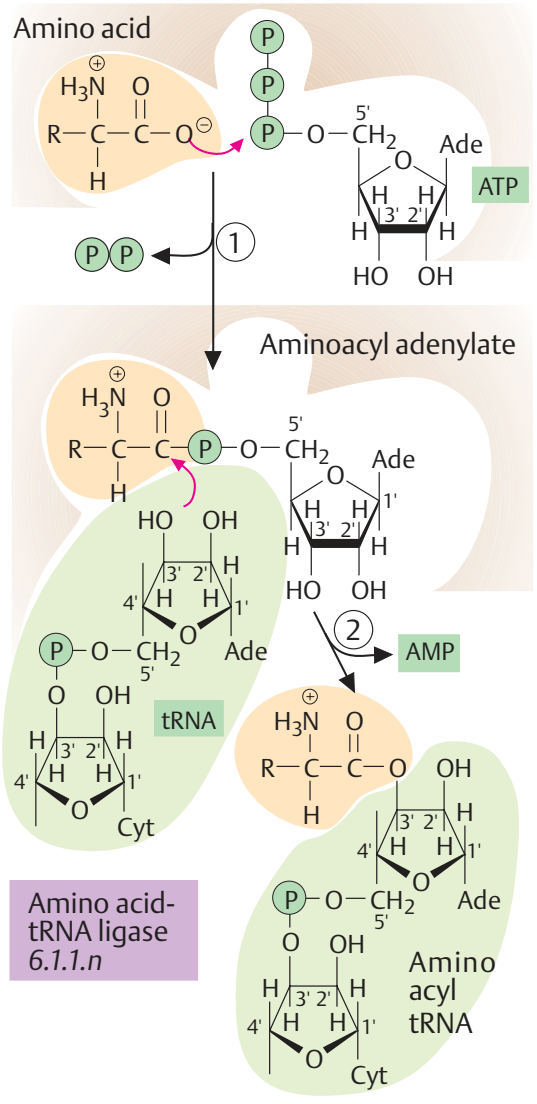
C. Asp–tRNA ligase (dimer) ○

The illustration shows the ligase responsible for the activation of aspartate. Each subunit of the dimeric enzyme (protein parts shown in orange) binds one molecule of $tRNA^{Asp}$ (blue). The active centers can be located by the bound ATP (green). They are associated with the 3′ end of the tRNA. Another domain in the protein (upper left) is responsible for "recognition" of the tRNA anticodon.

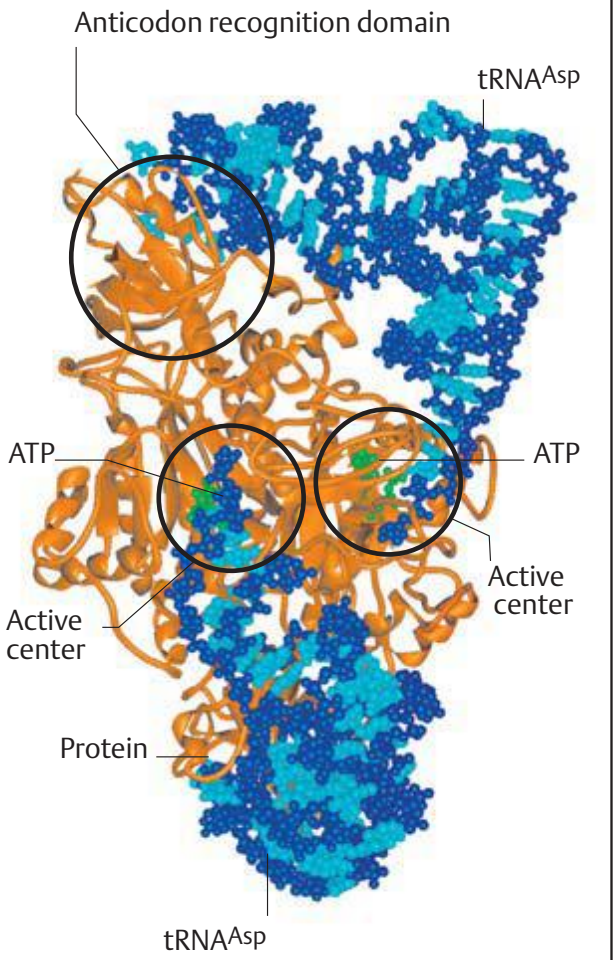
A. The genetic code



B. Amino acid activation



C. Asp-tRNA-Ligase (Dimer)



Translation I: initiation

Like amino acid activation (see p. 248), protein biosynthesis (**translation**) takes place in the cytoplasm. It is catalyzed by complex nucleoprotein particles, the **ribosomes**, and mainly requires GTP to cover its energy requirements.

A. Structure of the eukaryotic ribosome ◑

Ribosomes consist of two subunits of different size, made up of **ribosomal RNA (rRNA)** and nearly 80 **proteins** (the number of proteins applies to rat liver ribosomes). It is customary to give the sedimentation coef cients (see p. 200) of ribosomes and their components instead of their masses. For example, the eukaryotic ribosome has a sedimentation coefficient of 80 Svedberg units (80 S), while the sedimentation coef cients of its subunits are 40 S and 60 S (S values are not additive).

The smaller 40 S subunit consists of one molecule of 18 S rRNA and 33 protein molecules. The larger 60 S subunit contains three types of rRNA with sedimentation coef cients of 5 S, 5.8 S, and 28 S and 47 proteins. In the presence of mRNA, the subunits assemble to form the complete ribosome, with a mass about 650 times larger than that of a hemoglobin molecule.

The arrangement of the individual components of a ribosome has now been determined for prokaryotic ribosomes. It is known that filamentous mRNA passes through a cleft between the two subunits near the characteristic "horn" on the small subunit. tRNAs also bind near this site. The illustration shows the size of a tRNA molecule for comparison.

Prokaryotic ribosomes have a similar structure, but are somewhat smaller than those of eukaryotes (sedimentation coef cient 70 S for the complete ribosome, 30 S and 50 S for the subunits). Mitochondrial and chloroplast ribosomes are comparable to prokaryotic ones.

B. Polysomes ◑

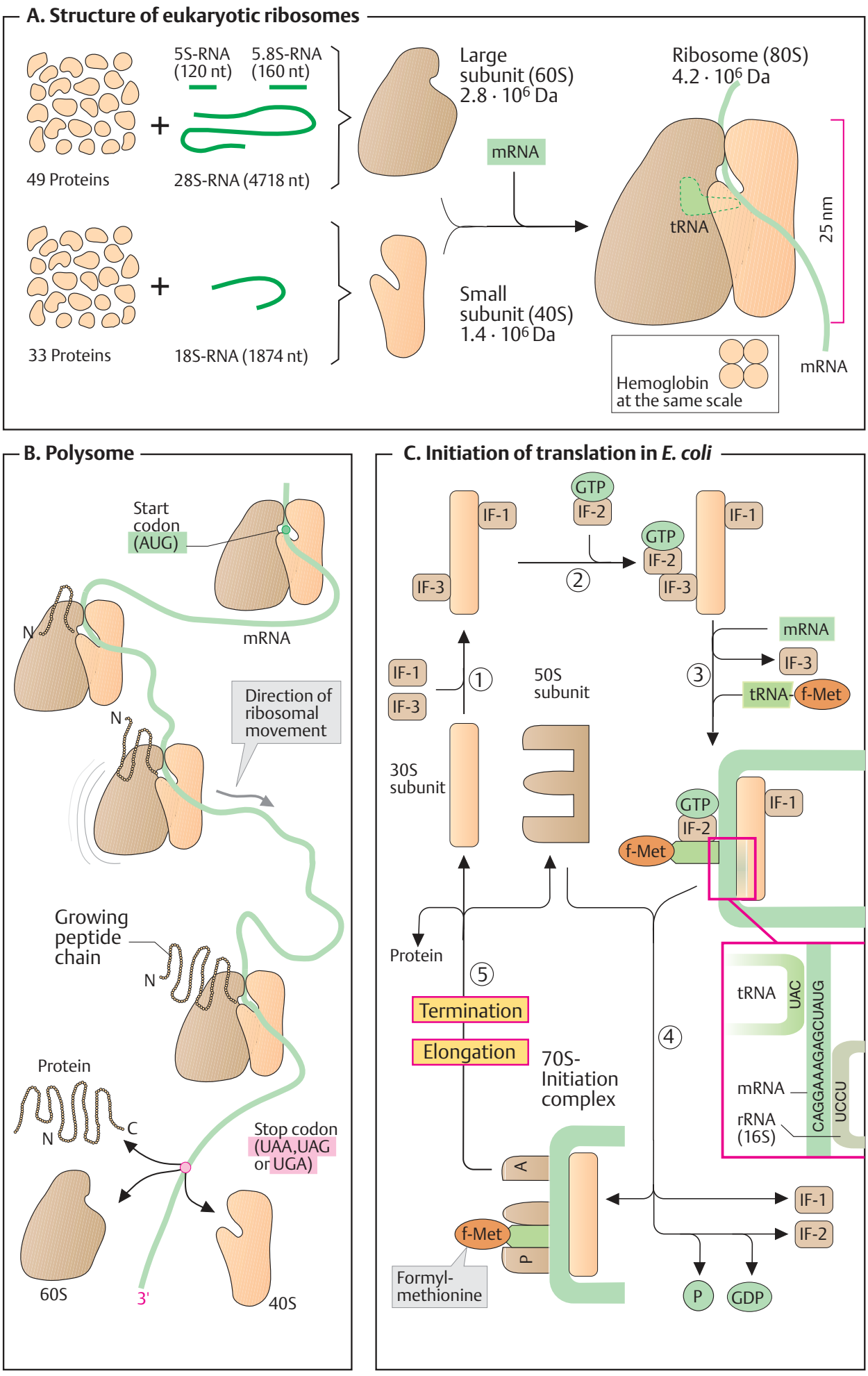
In cells that are carrying out intensive protein synthesis, ribosomes are often found in a linear arrangement like a string of pearls; these are known as **polysomes**. This arrangement arises because several ribosomes are translating a single mRNA molecule simultaneously. The ribosome first binds near the **start codon** (AUG; see p. 248) at the 5′ end of the mRNA (top). During translation, the ribosome moves in the direction of the 3′ end until it reaches a **stop codon** (UAA, UAG, or UGA). At this point, the newly synthesized chain is released, and the ribosome dissociates again into its two subunits.

C. Initiation of translation in E. coli ◑

Protein synthesis in prokaryotes is in principle the same as in eukaryotes. However, as the process is simpler and has been better studied in prokaryotes, the details involved in translation are discussed here and on p. 252 using the example of the bacterium *Escherichia coli.*

The first phase of translation, **initiation**, involves several steps. First, two proteins, **initiation factors** IF–1 and IF–3, bind to the 30 S subunit (**1**). Another factor, IF–2, binds as a complex with GTP (**2**). This allows the subunit to associate with the mRNA and makes it possible for a special tRNA to bind to the start codon (**3**). In prokaryotes, this starter tRNA carries the substituted amino acid *N-formylmethionine* (fMet). In eukaryotes, it carries an unsubstituted *methionine*. Finally, the 50 S subunit binds to the above complex (**4**). During steps 3 and 4, the initiation factors are released again, and the GTP bound to IF–2 is hydrolyzed to GDP and P_i.

In the **70 S initiation complex**, formylmethionine tRNA is initially located at a binding site known as the **peptidyl site (P)**. A second binding site, the **acceptor site (A)**, is not yet occupied during this phase of translation. Sometimes, a third tRNA binding site is defined as an *exit site (E),* from which uncharged tRNAs leave the ribosome again (see p. 252; not shown).



Translation II: elongation and termination

After translation has been initiated (see p. 250), the peptide chain is extended by the addition of further amino acid residues (**elongation**) until the ribosome reaches a stop codon on the mRNA and the process is interrupted (**termination**).

A. Elongation and termination of protein biosynthesis in E. coli ◑

Elongation can be divided into four phases:

[1] **Binding of aminoacyl tRNA.** First, the peptidyl site (P) of the ribosome is occupied by a tRNA that carries at its 3′ end the complete peptide chain formed up to this point (top left). A second tRNA, loaded with the next amino acid (Val–$tRNA^{Val}$ in the example shown), then binds via its complementary anticodon (see p. 82) to the mRNA codon exposed at the acceptor site (in this case GUG). The tRNA binds as a complex with a GTP-containing protein, the *elongation factor Tu* (EF–Tu) (**1a**). It is only after the bound GTP has been hydrolyzed to GDP and phosphate that EF–Tu dissociates again (**1b**). As the binding of the tRNA to the mRNA is still loose before this, GTP hydrolysis acts as a delaying factor, making it possible to check whether the correct tRNA has been bound. A further protein, the *elongation factor Ts* (EF-Ts), later catalyzes the exchange of GDP for GTP and in this way regenerates the EF–Tu GTP complex. EF-Tu is related to the G proteins involved in signal transduction (see p. 384).

[2] **Synthesis of the peptide bond** takes place in the next step. Ribosomal *peptidyltransferase* catalyzes (without consumption of ATP or GTP) the transfer of the peptide chain from the tRNA at the P site to the NH_2 group of the amino acid residue of the tRNA at the A site. The ribosome's peptidyltransferase activity is not located in one of the ribosomal proteins, but in the 28 S rRNA. Catalytically active RNAs of this type are known as *ribozymes* (cf. p. 246). It is thought that the few surviving ribozymes are remnants of the *"RNA world"*—an early phase of evolution in which proteins were not as important as they are today.

[3] After the transfer of the growing peptide to the A site, the free tRNA at the P site dissociates and another GTP–containing elongation factor (EF-G GTP) binds to the ribosome. Hydrolysis of the GTP in this factor provides the energy for **translocation** of the ribosome. During this process, the ribosome moves three bases along the mRNA in the direction of the 3′ end. The tRNA carrying the peptide chain is stationary relative to the mRNA and reaches the ribosome's P site during translocation, while the next mRNA codon (in this case GUG) appears at the A site.

[4] The uncharged Val-tRNA then dissociates from the E site. The ribosome is now ready for the next elongation cycle.

When one of the three stop codons (UAA, UAG, or UGA) appears at the A site, **termination** starts.

[5] There are no complementary tRNAs for the stop codons. Instead, two *releasing factors* bind to the ribosome. One of these factors (RF–1) catalyzes hydrolytic cleavage of the ester bond between the tRNA and the C–terminus of the peptide chain, thereby releasing the protein.

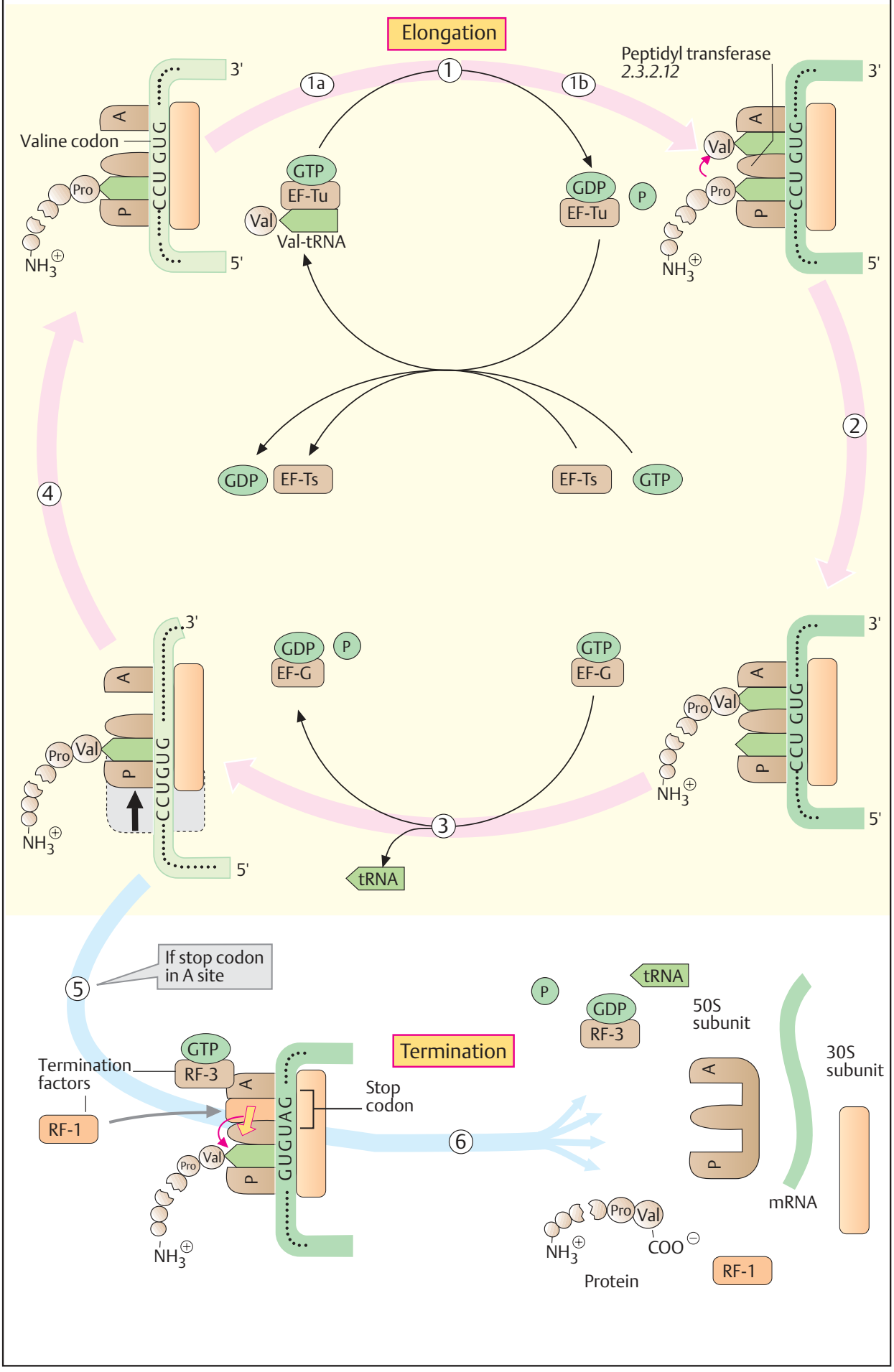
[6] Hydrolysis of GTP by factor RF–3 supplies the energy for the dissociation of the whole complex into its component parts.

Energy requirements in protein synthesis are high. Four energy-rich phosphoric acid anhydride bonds are hydrolyzed for each amino acid residue. Amino acid activation uses up two energy-rich bonds per amino acid (ATP → AMP + PP; see p. 248), and two GTPs are consumed per elongation cycle. In addition, initiation and termination each require one GTP per chain.

Further information

In eukaryotic cells, the number of initiation factors is larger and initiation is therefore more complex than in prokaryotes. The cap at the 5′ end of mRNA and the polyA tail (see p. 246) play important parts in initiation. However, the elongation and termination processes are similar in all organisms. The individual steps of bacterial translation can be inhibited by antibiotics (see p. 254).

A. Elongation and termination of protein biosynthesis in *E. coli*



Antibiotics

A. Antibiotics: overview ◑

Antibiotics are substances which, even at low concentrations, inhibit the growth and reproduction of bacteria and fungi. The treatment of infectious diseases would be inconceivable today without antibiotics. Substances that only restrict the reproduction of bacteria are described as having *bacteriostatic* effects (or *fungistatic* for fungi). If the target cells are killed, then the term *bactericidal* (or *fungicidal*) is used. Almost all antibiotics are produced by microorganisms—mainly bacteria of the genus *Streptomyces* and certain fungi. However, there are also synthetic antibacterial substances, such as sulfonamides and gyrase inhibitors.

A constantly increasing problem in antibiotic treatment is the development of resistant pathogens that no longer respond to the drugs available. The illustration shows a few of the therapeutically important antibiotics and their sites of action in the bacterial metabolism.

Substances known as **intercalators**, such as *rifamycin* and *actinomycin D* (bottom) are deposited in the DNA double helix and thereby interfere with replication and transcription (**B**). As DNA is the same in all cells, intercalating antibiotics are also toxic for eukaryotes, however. They are therefore only used as cytostatic agents (see p. 402). Synthetic inhibitors of DNA topoisomerase II (see p. 240), known as **gyrase inhibitors** (center), restrict replication and thus bacterial reproduction.

A large group of antibiotics attack bacterial ribosomes. These **inhibitors of translation** (left) include the *tetracyclines*—broad-spectrum antibiotics that are effective against a large number of different pathogens. The *aminoglycosides*, of which *streptomycin* is the best-known, affect all phases of translation. *Erythromycin* impairs the normal functioning of the large ribosomal subunit. *Chloramphenicol,* one of the few natural nitro compounds, inhibits ribosomal peptidyltransferase. Finally, *puromycin* mimics an aminoacyl tRNA and therefore leads to premature interruption of elongation.

The **β–lactam antibiotics** (bottom right) are also frequently used. The members of this group, the *penicillins* and *cephalosporins,* are synthesized by fungi and have a reactive β-lactam ring. They are mainly used against Gram-positive pathogens, in which they inhibit cell wall synthesis (**C**).

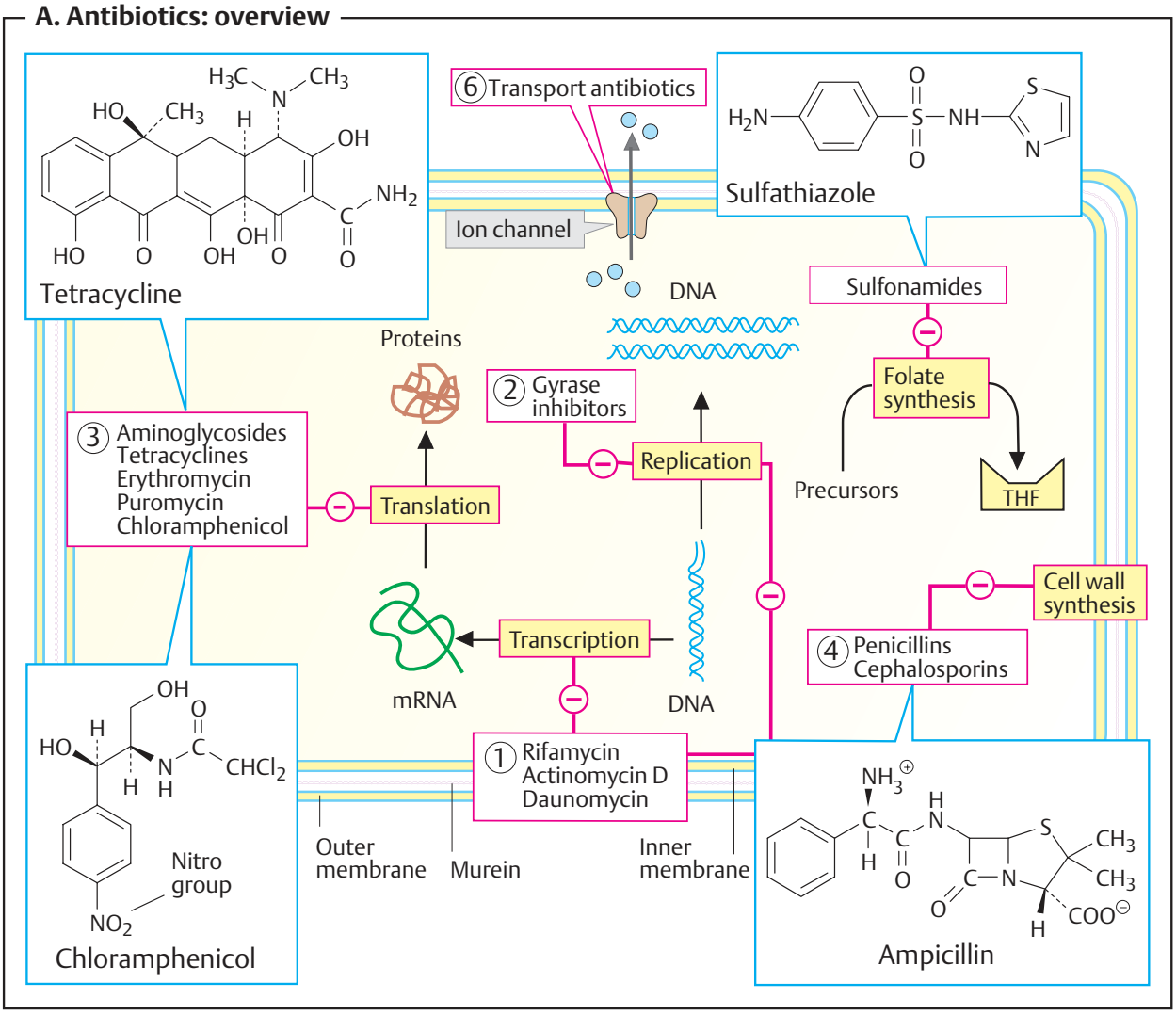
The first synthetic antibiotics were the **sulfonamides** (right). As analogues of p–aminobenzoic acid, these affect the synthesis of *folic acid,* an essential precursor of the coenzyme THF (see p. 108). **Transport antibiotics** (top center) have the properties of ion channels (see p. 222). When they are deposited in the plasma membrane, it leads to a loss of ions that damages the bacterial cells.

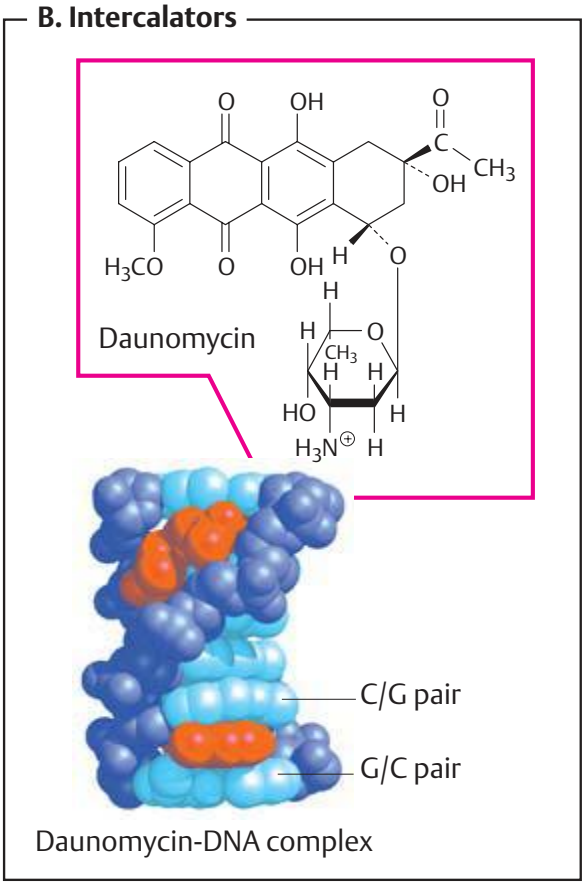
B. Intercalators ○

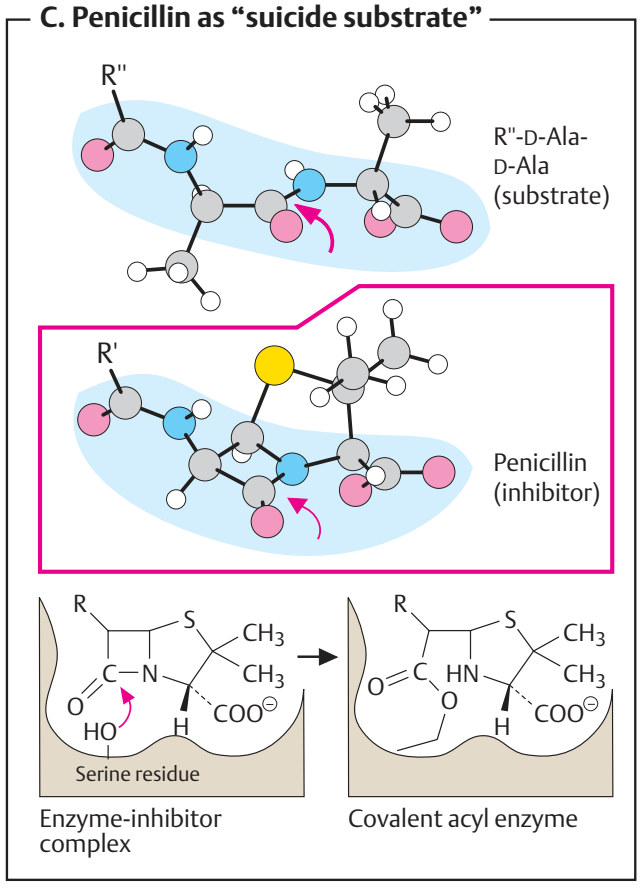
The effects of intercalators (see also p. 262) are illustrated here using the example of the **daunomycin–DNA complex**, in which two daunomycin molecules (red) are inserted in the double helix (blue). The antibiotic's ring system inserts itself between G/C base pairs (bottom), while the sugar moiety occupies the minor groove in the DNA (above). This leads to a localized change of the DNA conformation that prevents replication and transcription.

C. Penicillin as a "suicide substrate" ○

The site of action in the β–lactam antibiotics is *muramoylpentapeptide carboxypeptidase,* an enzyme that is essential for cross–linking of bacterial cell walls. The antibiotic resembles the substrate of this enzyme (a peptide with the C-terminal sequence D-Ala–D–Ala) and is therefore reversibly bound in the active center. This brings the β–lactam ring into proximity with an essential serine residue of the enzyme. Nucleophilic substitution then results in the formation of a stable covalent bond between the enzyme and the inhibitor, blocking the active center (see p. 96). In dividing bacteria, the loss of activity of the enzyme leads to the formation of unstable cell walls and eventually death.







Mutation and repair

Genetic information is set down in the base sequence of DNA. Changes in the DNA bases or their sequence therefore have *mutagenic* effects. Mutagens often also damage growth regulation in cells, and they are then also *carcinogenic* (see p. 400). Gene alterations (**mutations**) are one of the decisive positive factors in biological evolution. On the other hand, an excessive mutation frequency would threaten the survival of individual organisms or entire species. For this reason, every cell has **repair mechanisms** that eliminate most of the DNA changes arising from mutations (**C**).

A. Mutagenic agents ◑

Mutations can arise as a result of physical or chemical effects, or they can be due to accidental errors in DNA replication and recombination.

The principal physical mutagen is **ionizing radiation** (α, β, and γ radiation, X-rays). In cells, it produces **free radicals** (molecules with unpaired electrons), which are extremely reactive and can damage DNA. Short-wavelength **ultraviolet light** (UV light) also has mutagenic effects, mainly in skin cells (sunburn). The most common chemical change due to UV exposure is the formation of **thymine dimers**, in which two neighboring thymine bases become covalently linked to one another (**2**). This results in errors when the DNA is read during replication and transcription.

Only a few examples of the group of **chemical mutagens** are shown here. *Nitrous acid* (HNO_2; salt: nitrite) and *hydroxylamine* (NH_2OH) both deaminate bases; they convert cytosine to uracil and adenine to inosine.

Alkylating compounds carry reactive groups that can form covalent bonds with DNA bases (see also p. 402). *Methylnitrosamines* (**3**) release the reactive methyl cation (CH_3^+), which methylates OH and NH_2 groups in DNA. The dangerous carcinogen *benzo*[a]*pyrene* is an aromatic hydrocarbon that is only converted into the active form in the organism (**4**; see p. 316). Multiple hydroxylation of one of the rings produces a reactive epoxide that can react with NH_2 groups in guanine residues, for example. Free radicals of benzo[*a*]pyrene also contribute to its toxicity.

B. Effects ◑

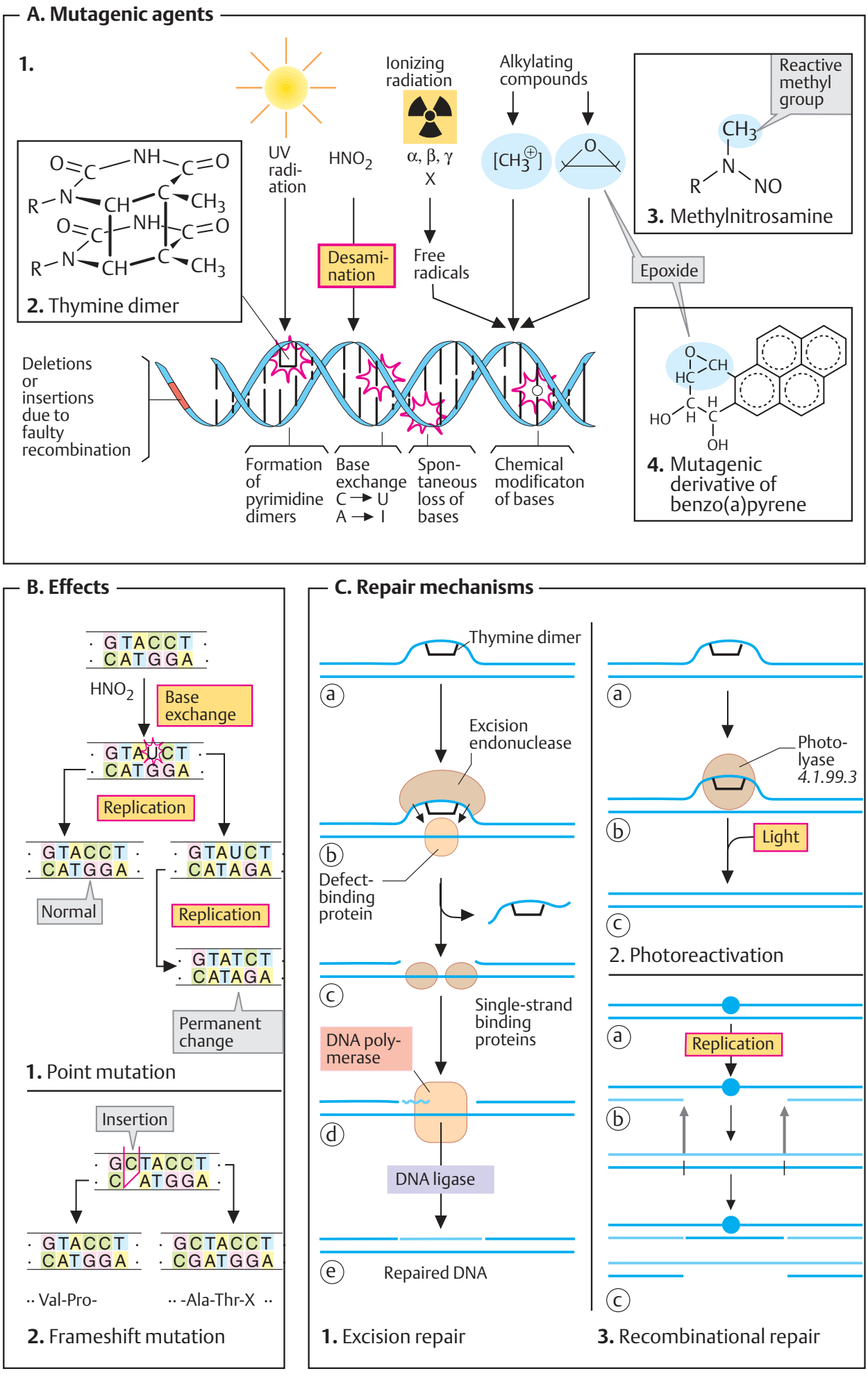
Nitrous acid causes **point mutations** (**1**). For example, C is converted to U, which in the next replication pairs with A instead of G. The alteration thus becomes permanent. Mutations in which a number of nucleotides not divisible by three are inserted or removed lead to reading errors in whole segments of DNA, as they move the reading frame (**frameshift mutations**). This is shown in Fig. **2** using a simple example. From the inserted C onwards, the resulting mRNA is interpreted differently during translation, producing a completely new protein sequence.

C. Repair mechanisms ○

An important mechanism for the removal of DNA damage is **excision repair** (**1**). In this process, a specific *excision endonuclease* removes a complete segment of DNA on both sides of the error site. Using the sequence of the opposite strand, the missing segment is then replaced by a *DNA polymerase*. Finally, a *DNA ligase* closes the gaps again.

Thymine dimers can be removed by **photoreactivation** (**2**). A specific *photolyase* binds at the defect and, when illuminated, cleaves the dimer to yield two single bases again.

A third mechanism is **recombination repair** (**3**, shown in simplified form). In this process, the defect is omitted during replication. The gap is closed by shifting the corresponding sequence from the correctly replicated second strand. The new gap that results is then filled by polymerases and ligases. Finally, the original defect is corrected by excision repair as in Fig. **1** (not shown).



DNA cloning

The growth of molecular genetics since 1970 has mainly been based on the development and refinement of methods of analyzing and manipulating DNA. **Genetic engineering** has practical applications in many fields. For example, it has provided new methods of diagnosing and treating diseases, and it is now also possible to create targeted changes in specific characteristics of organisms. Since biological risks cannot be completely ruled out with these procedures, it is particularly important to act responsibly when dealing with genetic engineering. A short overview of important methods involved in genetic engineering is provided here and on the following pages.

A. Restriction endonucleases ◑

In many genetic engineering procedures, defined DNA fragments have to be isolated and then newly combined with other DNA segments. For this purpose, enzymes are used that can cut DNA and join it together again inside the cell. Of particular importance are *restriction endonucleases*—a group of bacterial enzymes that cleave the DNA double strand in a sequence–specific way. The numerous restriction enzymes known are named using abbreviations based on the organism from which they originate. The example used here is *Eco*RI, a nuclease isolated from the bacterium *Escherichia coli.*

Like many other restriction endonucleases, EcoRI cleaves DNA at the site of a *palindrome*—i. e., a short segment of DNA in which both the strand and counter-strand have the same sequence (each read in the 5′→3′ direction). In this case, the sequence is 5′-GAATTC-3′. *Eco*RI, a homodimer, cleaves the phosphoric acid diester bonds in both strands between G and A. This results in the formation of complementary *overhanging* or *"sticky" ends* (AATT), which are held together by base pairing. However, they are easily separated—e. g. by heating. When the fragments are cooled, the overhanging ends hybridize again in the correct arrangement. The cleavage sites can then be sealed again by a *DNA ligase*.

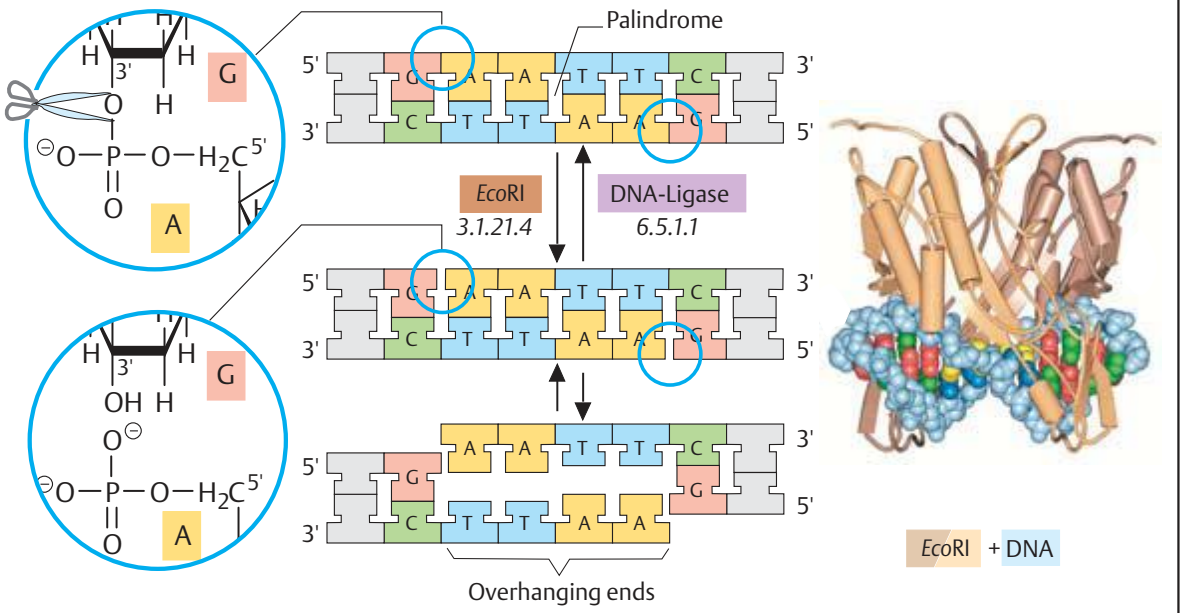
B. DNA cloning ○

Most DNA segments—e. g., genes—occur in very small quantities in the cell. To be able to work with them experimentally, a large number of identical copies (**"clones"**) first have to be produced. The classic procedure for cloning DNA takes advantage of the ability of bacteria to take up and replicate short, circular DNA fragments known as **plasmids**.

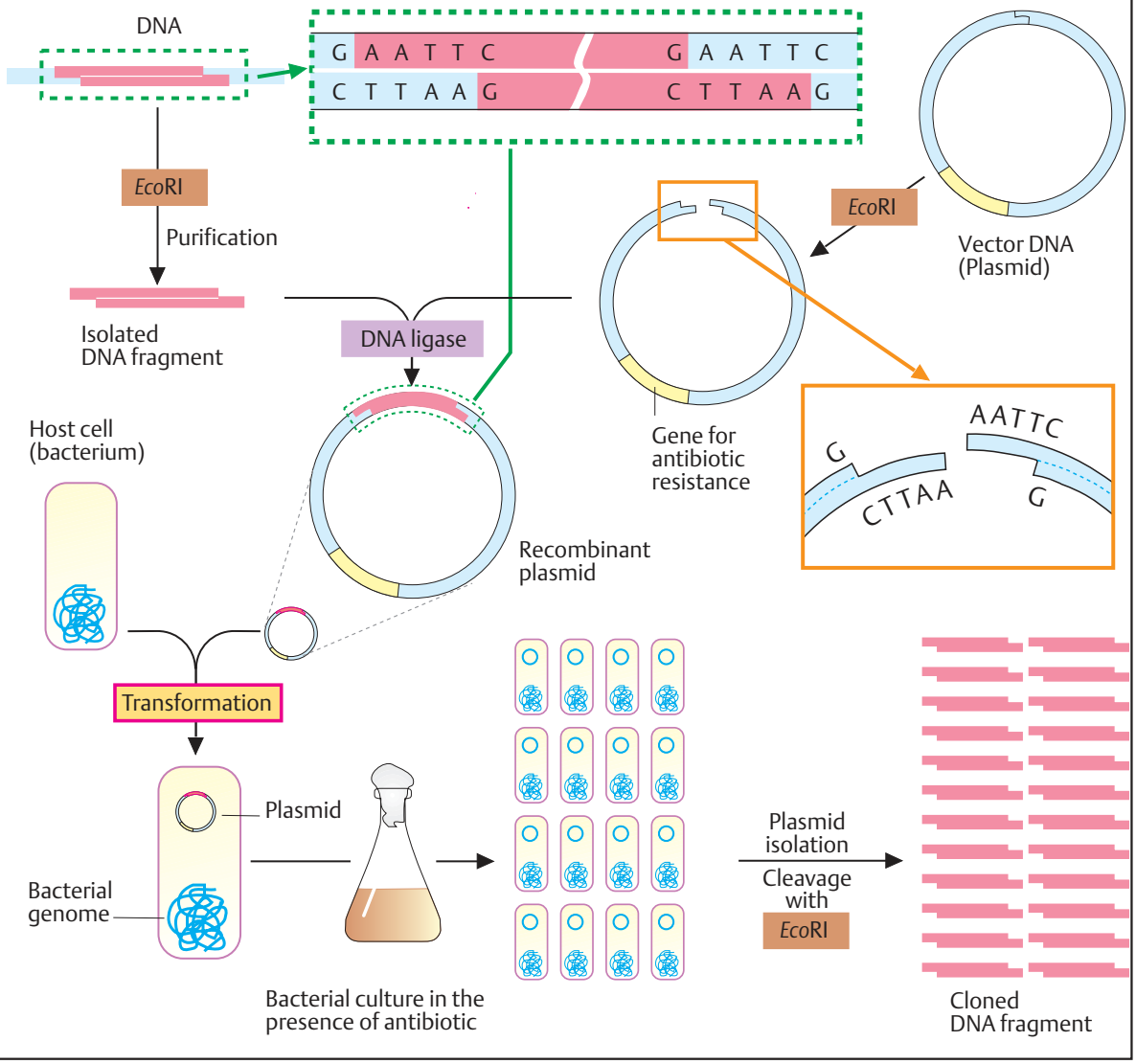
The segment to be cloned is first cut out of the original DNA using restriction endonucleases (see above; for the sake of simplicity, cleavage using *Eco*RI alone is shown here, but in practice two different enzymes are usually used). As a vehicle (*"vector"*), a plasmid is needed that has only *one Eco*RI cleavage site. The plasmid rings are first opened by cleavage with *Eco*RI and then mixed with the isolated DNA fragments. Since the fragment and the vector have the same overhanging ends, some of the molecules will hybridize in such a way that the fragment is incorporated into the vector DNA. When the cleavage sites are now closed again using *DNA ligase,* a newly combined *("recombinant") plasmid* arises.

By pretreating a large number of host cells, one can cause some of them to take up the plasmid (a process known as **transformation**) and replicate it along with their own genome when reproducing. To ensure that only host bacteria that contain the plasmid replicate, plasmids are used that give the host *resistance* to a particular antibiotic. When the bacteria are incubated in the presence of this antibiotic, only the cells containing the plasmid will replicate. The plasmid is then isolated from these cells, cleaved with *Eco*RI again, and the fragments are separated using agarose gel electrophoresis (see p. 262). The desired fragment can be identified using its size and then extracted from the gel and used for further experiments.

A. Restriction endonucleases



B. DNA cloning



DNA sequencing

A. Gene libraries ○

It is often necessary in genetic engineering to isolate a DNA segment when its details are not fully known—e.g., in order to determine its nucleotide sequence. In this case, one can use what are known as **DNA libraries**. A DNA library consists of a large number of *vector DNA molecules* containing different fragments of *foreign DNA.* For example, it is possible to take all of the mRNA molecules present in a cell and transcribe them into DNA. These DNA fragments (known as copy DNA or **cDNA**) are then randomly introduced into vector molecules.

A library of genomic DNA can be established by cleaving the total DNA from a cell into small fragments using restriction endonucleases (see p. 258), and then incorporating these into vector DNA. Suitable vectors for gene libraries include **bacteriophages**, for example ("phages" for short). Phages are viruses that only infect bacteria and are replicated by them (see p. 404). Gene libraries have the advantage that they can be searched for specific DNA segments, using hybridization with oligonucleotides.

The first step is to strongly dilute a small part of the library (10^5–10^6 phages in a small volume), mix it with host bacteria, and plate out the mixture onto nutrient medium. The bacteria grow and form a continuous cloudy layer of cells. Bacteria infected by phages grow more slowly. In their surroundings, the bacterial "lawn" is less dense, and a clearer circular zone known as a **plaque** forms. The bacteria in this type of plaque exclusively contain the offspring of a single phage from the library.

The next step is to make an impression of the plate on a plastic foil, which is then heated. This causes the phage DNA to adhere to the foil. When the foil is incubated with a DNA fragment that hybridizes to the DNA segment of interest (a **gene probe**), the probe binds to the sites on the imprint at which the desired DNA is attached. Binding of the gene probe can be detected by prior radioactive or other labeling of the probe. Phages from the positive plaques in the original plate are then isolated and replicated. Restriction cleavage finally provides large amounts of the desired DNA.

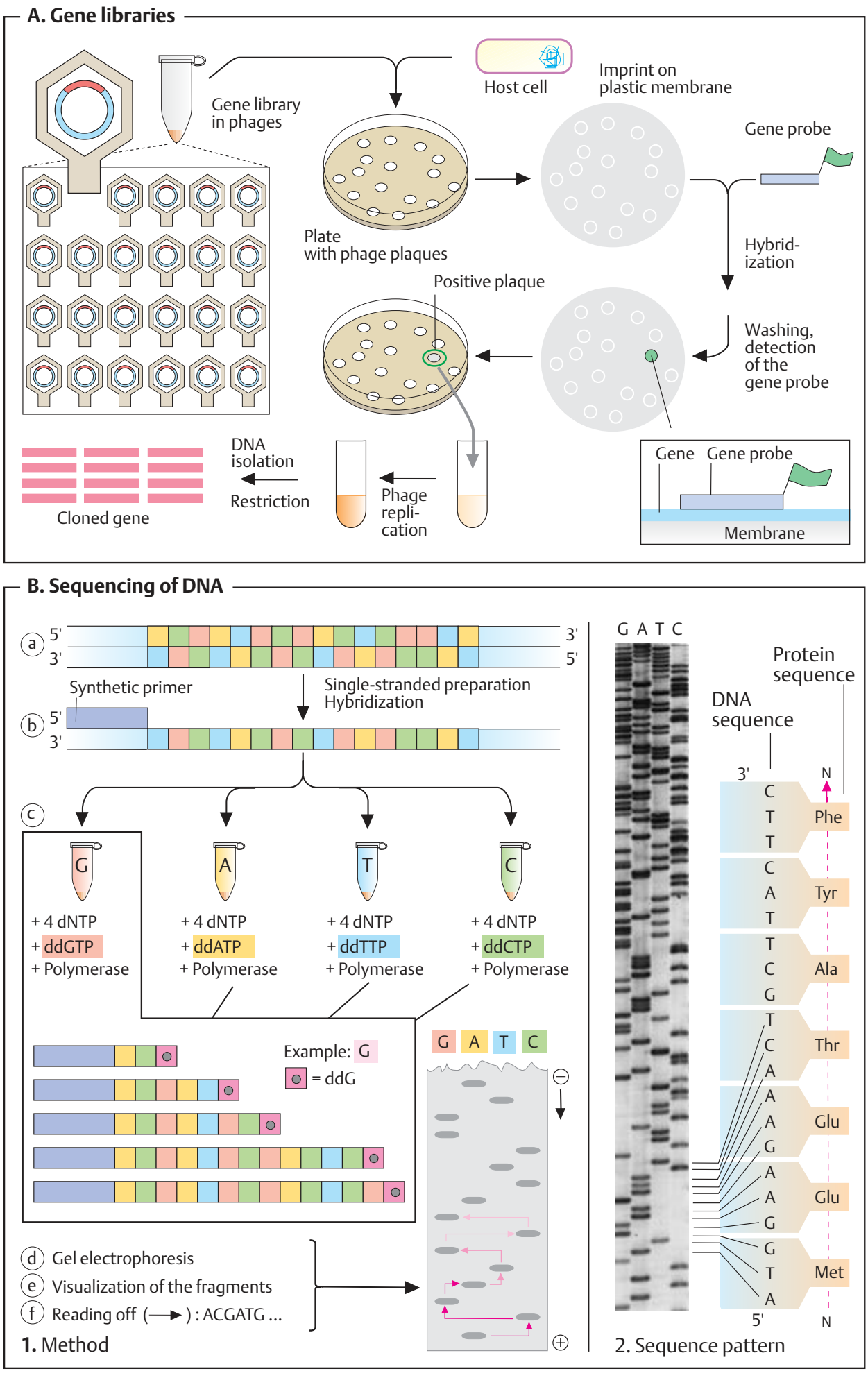
B. Sequencing of DNA ○

The nucleotide sequence of DNA is nowadays usually determined using the so-called **chain termination method**. In single-strand sequencing, the DNA fragment (**a**) is cloned into the DNA of **phage M13** (see p. 404), from which the coded single strand can be easily isolated. This is *hybridized* with a **primer**—a short, synthetically produced DNA fragment that binds to 3′ end of the introduced DNA segment (**b**).

Based on this hybrid, the missing second strand can now be generated in the test tube by adding the four *deoxyribonucleoside triphosphates* (*dNTP*) and a suitable *DNA polymerase* (**c**). The trick lies in also adding small amounts of *dideoxynucleoside triphosphates (ddNTP).* Incorporating a ddNTP leads to the *termination of second-strand synthesis.* This can occur whenever the corresponding dNTP ought to be incorporated. The illustration shows this in detail using the example of ddGTP. In this case, fragments are obtained that each include the primer plus three, six, eight, 13, or 14 additional nucleotides. *Four separate reactions,* each with a different ddNTP, are carried out (**c**), and the products are placed side by side on a supporting material. The fragments are then separated by *gel electrophoresis* (see p. 76), in which they move in relation to their length.

Following *visualization* (**d**), the sequence of the fragments in the individual lanes is simply read from bottom to top (**e**) to directly obtain the nucleotide sequence. A detail from such a sequencing gel and the corresponding protein sequence are shown in Fig. **2**.

In a more modern procedure, the four ddNTPs are covalently marked with fluorescent dyes, which produce a different color for each ddNTP on laser illumination. This allows the sequence in which the individual fragments appear at the lower end of the gel to be continuously recorded and directly stored in digital form.



PCR and protein expression

A. Polymerase chain reaction (PCR) ◑

The polymerase chain reaction (PCR) is an important procedure in genetic engineering that allows any DNA segment to be replicated (**amplified**) without the need for restriction enzymes, vectors, or host cells (see p. 258). However, the nucleotide sequence of the segment has to be known. Two oligonucleotides (**primers**) are needed, which each hybridize with one of the strands at each end of the DNA segment to be amplified; also needed are sufficient quantities of the four **deoxyribonucleoside triphosphates** and a special heat-tolerant DNA polymerase. The primers are produced by chemical synthesis, and the polymerase is obtained from thermostable bacteria.

First, the starter is heated to around 90 °C to separate the DNA double helix into single strands (**a**; cf. p. 84). The mixture is then cooled to allow hybridization of the primers (**b**). Starting from the primers, complementary DNA strands are now synthesized in both directions by the polymerase (**c**). This *cycle* (cycle 1) is *repeated 20–30 times* with the same reaction mixture (cycle 2 and subsequent cycles). The cyclic heating and cooling are carried out by *computer-controlled thermostats.*

After only the third cycle, double strands start to form with a length equal to the distance between the two primers. The proportion of these approximately doubles during each cycle, until almost all of the newly synthesized segments have the correct length.

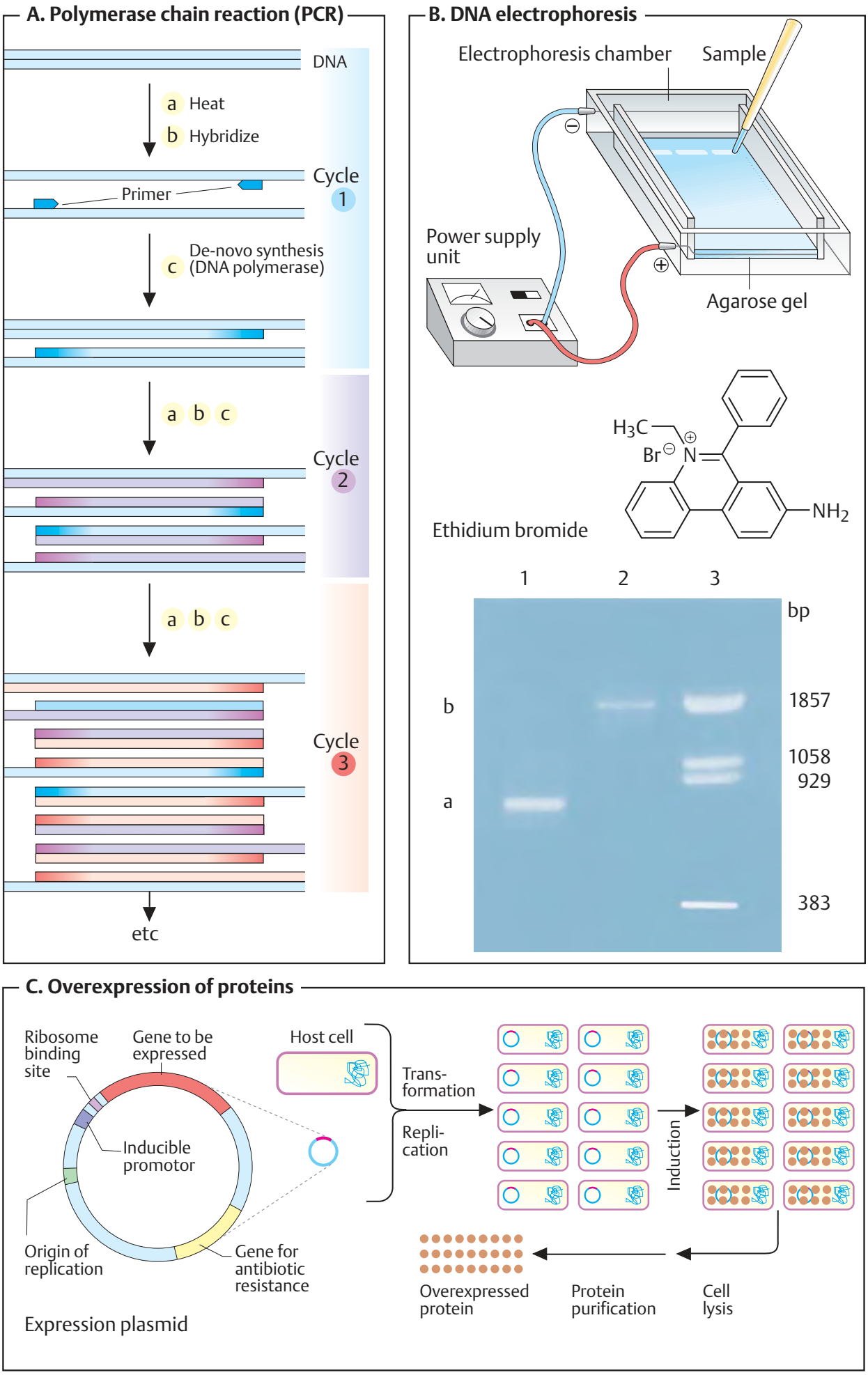
B. DNA electrophoresis ○

The separation of DNA fragments by electrophoresis is technically simpler than protein electrophoresis (see p. 78). The mobility of molecules in an electrical field of a given strength depends on the size and shape of the molecules, as well as their charge. In contrast to proteins, in which all three factors vary, the ratio of mass to charge in nucleic acids is constant, as all of the nucleotide components have similar masses and carry one negative charge. When electrophoresis is carried out in a wide-meshed support material that does not separate according to size and shape, the mobility of the molecules depends on their mass alone. The supporting material generally used in genetic engineering is a gel of the polysaccharide **agarose** (see p. 40). Agarose gels are not very stable and are therefore poured horizontally into a plastic chamber in which they are used for separation (top).

To make the separated fragments visible, after running the procedure the gels are placed in solutions of **ethidium bromide**. This is an intercalator (see p. 254) that shows strong fluorescence in UV light after binding to DNA, although it barely fluoresces in an aqueous solution. The result of separating two PCR amplificates (lanes 1 and 2) is shown in the lower part of the illustration. Comparing their distances with those of polynucleotides of known lengths (lane 3; bp = base pairs) yields lengths of approximately 800 bp for fragment 1 and 1800 bp for fragment 2. After staining, the bands can be cut out of the gel and the DNA can be extracted from them and used for further experiments.

C. Overexpression of proteins ◑

To treat some diseases, proteins are needed that occur in such small quantities in the organism that isolating them on a large scale would not be economically feasible. Proteins of this type can be obtained by *overexpression* in bacteria or eukaryotic cells. To do this, the corresponding *gene* is isolated from human DNA and cloned into an **expression plasmid** as described on p. 258. In addition to the gene itself, the plasmid also has to contain DNA segments that allow replication by the host cell and transcription of the gene. After *transformation* and *replication* of suitable host cells, **induction** is used in a targeted fashion to trigger ef cient *transcription* of the gene. *Translation* of the mRNA formed in the host cell then gives rise to large amounts of the desired protein. Human insulin (see p. 76), plasminogen activators for dissolving blood clots (see p. 292), and the growth hormone somatotropin are among the proteins produced in this way.



Genetic engineering in medicine

Genetic engineering procedures are becoming more and more important in medicine for diagnostic purposes (**A**–**C**). New genetic approaches to the treatment of severe diseases are still in the developmental stage ("gene therapy," **D**).

A. DNA fingerprinting ○

DNA fingerprinting is used to link small amounts of biological material—e. g., traces from the site of a crime—to a specific person. The procedure now used is based on the fact that the human genome contains non-coding repetitive DNA sequences, the length of which varies from individual to individual. **Short tandem repeats** (**STRs**) thus exist in which dinucleotides (e. g., -T-X-) are frequently repeated. Each STR can occur in five to 15 different lengths (alleles), of which one individual possesses only one or two. When the various allele combinations for several STRs are determined after PCR amplification of the DNA being investigated, a "genetic fingerprint" of the individual from whom the DNA originates is obtained. Using comparative material—e. g., saliva samples—definite identification is then possible.

B. Diagnosis of sickle-cell anemia using RFLP ○

This example illustrates a procedure for diagnosing a point mutation in the β-globin gene that leads to sickle-cell anemia (see p. 248). The mutation in the first exon of the gene destroys a cleavage site for the restriction endonuclease *Mst*II (see p. 258). When the DNA of healthy and diseased individuals is cleaved with *Mst*II, different fragments are produced in the region of the β-globin gene, which can be separated by electrophoresis and then demonstrated using specific probes (see p. 260). In addition, heterozygotic carriers of the sickle-cell gene can be distinguished from homozygotic ones.

C. Identification of viral DNA using RT-PCR ○

In viral infections, it is often dif cult to determine the species of the pathogen precisely. **RT-PCR** can be used to identify RNA viruses. In this procedure, reverse transcriptase (see p. 404) is used to transcribe the viral RNA into dsDNA, and then PCR is employed to amplify a segment of this DNA with virus-specific primers. In this way, an amplificate with a characteristic length can be obtained for each pathogen and identified using gel electrophoresis as described above.

D. Gene therapy ◑

Many diseases, such as hereditary metabolic defects and tumors, can still not be adequately treated. About 10 years ago, projects were therefore initiated that aimed to treat diseases of this type by transferring genes into the affected cells (gene therapy). The illustration combines conceivable and already implemented approaches to gene therapy for metabolic defects (left) and tumors (right). None of these procedures has yet become established in clinical practice.

If a mutation leads to failure of an enzyme E1 (left), its substrate B will no longer be converted into C and will accumulate. This can lead to cell damage by B itself or by a toxic product formed from it. Treatment with intact E1 is not possible, as the proteins are not capable of passing through the cell membrane. By contrast, it is in principle possible to introduce **foreign genes** into the cell using viruses as vectors (adenoviruses or retroviruses are mainly used). Their gene products could replace the defective E1 or convert B into a harmless product. Another approach uses the so-called **antisense DNA** (bottom right). This consists of polynucleotides that hybridize with the mRNA for specific cellular proteins and thereby prevent their translation. In the case shown, the synthesis of E2 could be blocked, for example.

The main problem in chemotherapy for **tumors** is the lack of tumor-specificity in the highly toxic cytostatic agents used (see p. 402). Attempts are therefore being made to introduce into tumor cells genes with products that are only released from a precursor to form active cytostatics once they have reached their target (left). Other gene products are meant to force the cells into apoptosis (see p. 396) or make them more susceptible to attack by the immune system. To steer the viral vectors to the tumor (targeting), attempts are being made to express proteins on the virus surface that are bound by tumor-specific receptors. Fusion with a tumor-specific promoter could also help limit the effect of the foreign gene to the tumor cells.