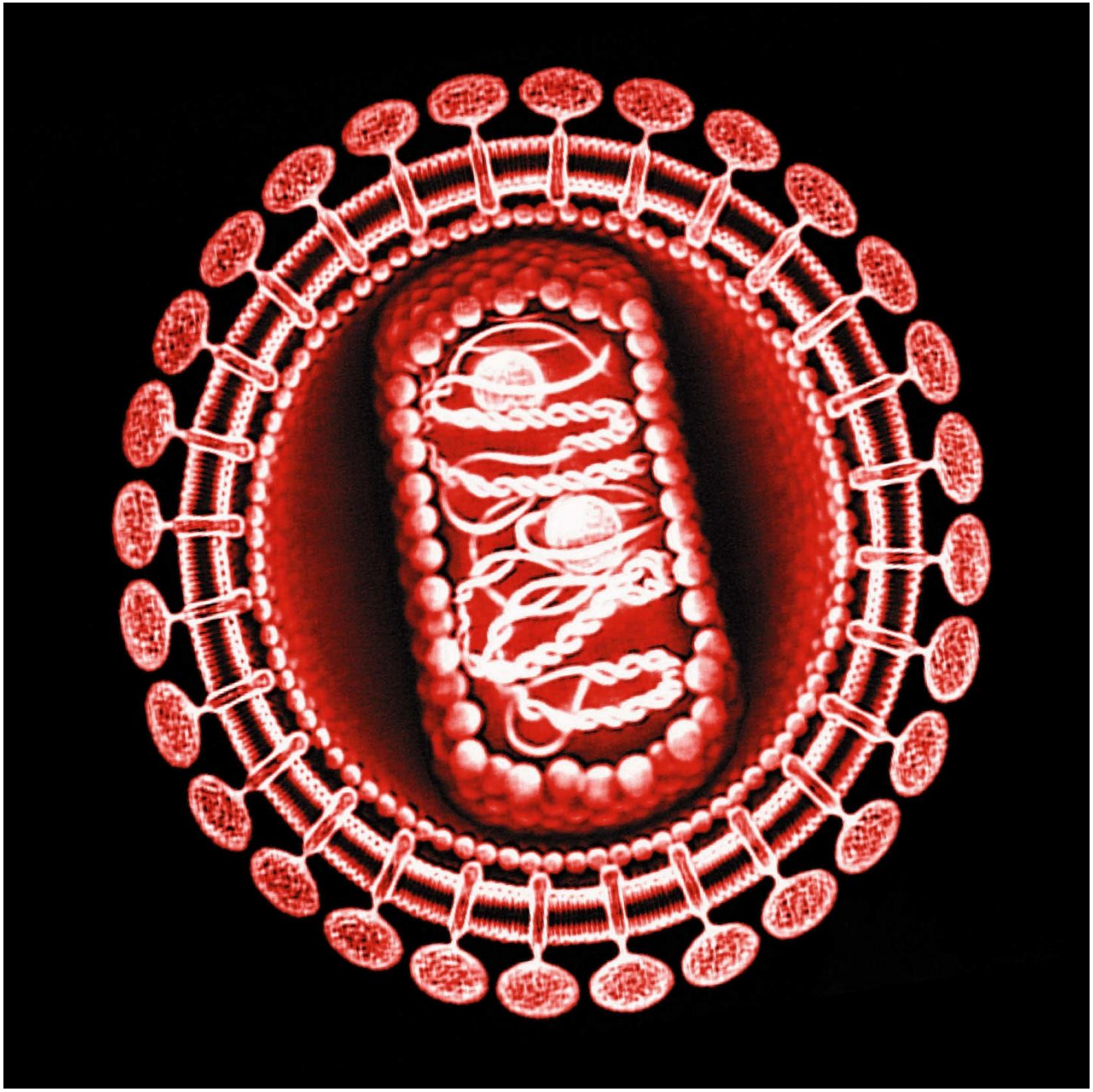
Nucleotides and Nucleic Acids



❖Nucleotides have a variety of roles in cellular metabolism.

❖They are the energy currency in metabolic transactions, the essential chemical links in the response of cells to hormones and other extracellular stimuli, and the structural components of an array of enzyme cofactors and metabolic intermediates.

❖And, last but certainly not least, they are the constituents of nucleic acids: **deoxyribonucleic acid** (DNA) and **ribonucleic acid** (RNA), the molecular repositories of genetic information.

❖The amino acid sequence of every protein in a cell, and the nucleotide sequence of every RNA, is specified by a nucleotide sequence in the cell's DNA.

❖A segment of a DNA molecule that contains the information required for the synthesis of a functional biological product, whether protein or RNA, is referred to as a **gene**.

❖A cell typically has many thousands of genes, and DNA molecules, not surprisingly, tend to be very large.

❖The storage and transmission of biological information are the only known functions of **DNA**.

❖RNAs have a broader range of functions, and several classes are found in cells.

❖**Ribosomal RNAs** (rRNAs) are components of ribosomes, the complexes that carry out the synthesis of proteins.

❖**Messenger RNAs** (mRNAs) are intermediaries, carrying genetic information from one or a few genes to a ribosome, where the corresponding proteins can be synthesized.

❖**Transfer RNAs (tRNAs)** are adapter molecules that faithfully translate the information in mRNA into a specific sequence of amino acids.

❖Nucleotides have three characteristic components: (1) a nitrogenous (nitrogen-containing) base, (2) a pentose, and (3) a phosphate. The molecule without the phosphate group is called a nucleoside.

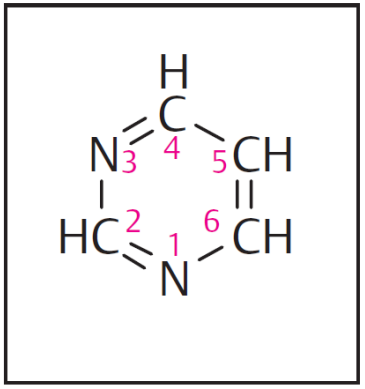
❖The nitrogenous bases are derivatives of two parent compounds, pyrimidine and purine.

❖Both DNA and RNA contain two major purine bases, adenine (A) and guanine (G), and two major pyrimidines.

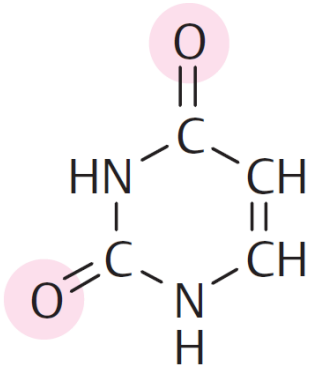
❖In both DNA and RNA one of the pyrimidines is cytosine (C), but the second major pyrimidine is not the same in both: it is thymine (T) in DNA and uracil (U) in RNA.

❖Only rarely does thymine occur in RNA or uracil in DNA.

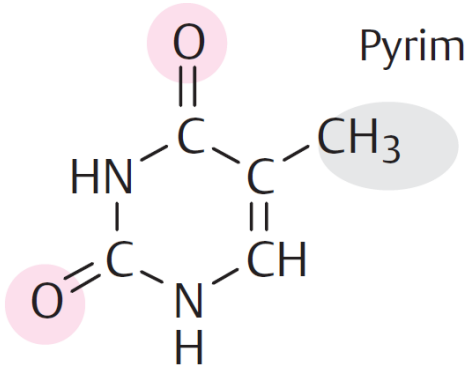
A. Nucleic acid bases



Pyrimidine

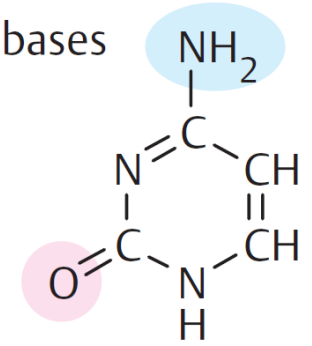


Uracil (Ura)

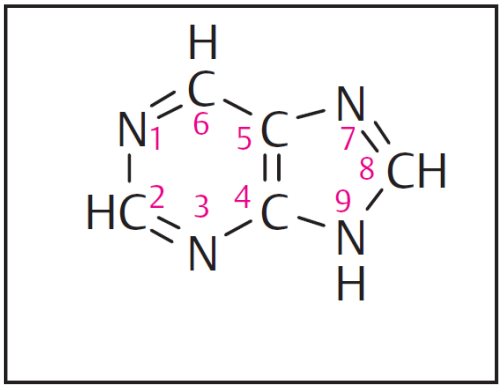


Thymine (Thy)

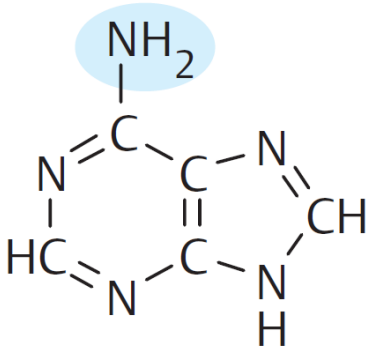
Pyrimidine bases



Cytosine (Cyt)

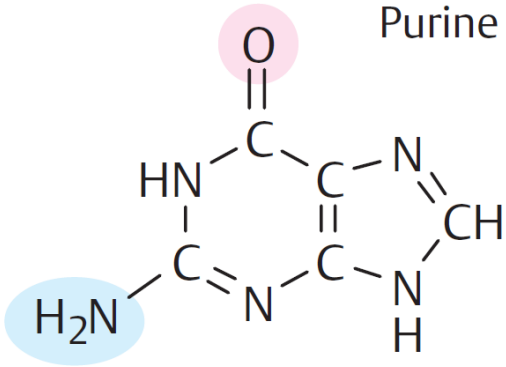


Purine

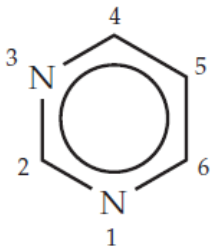


Adenine (Ade)

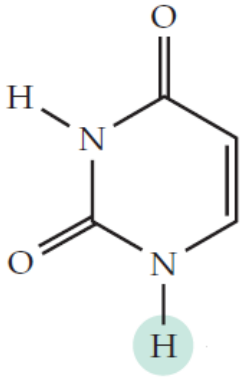
Purine bases



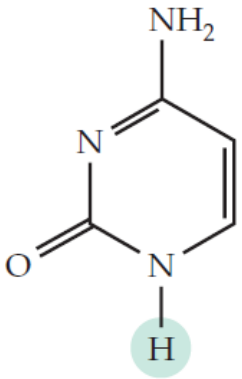
Guanine (Gua)



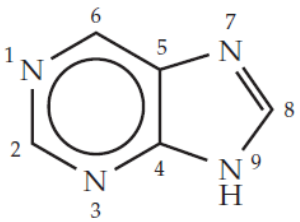
Pyrimidine



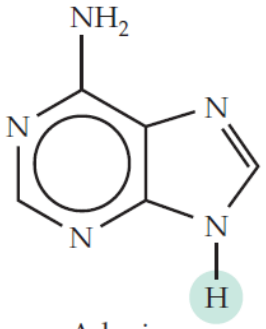
Uracil



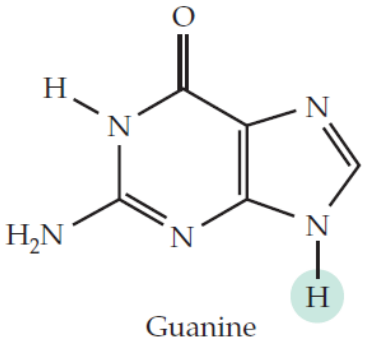
Cytosine



Purine

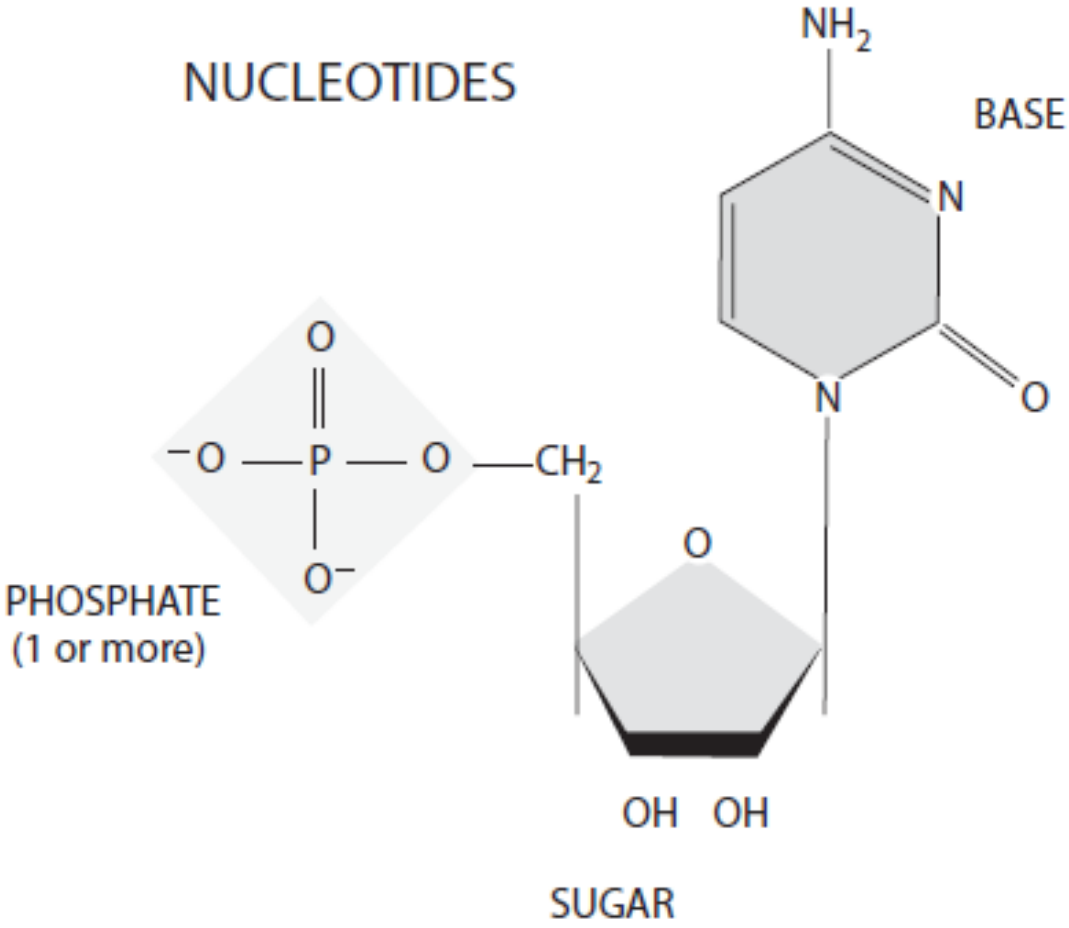


Adenine



Guanine

NUCLEOTIDES



❖Nucleic acids have two kinds of pentoses.

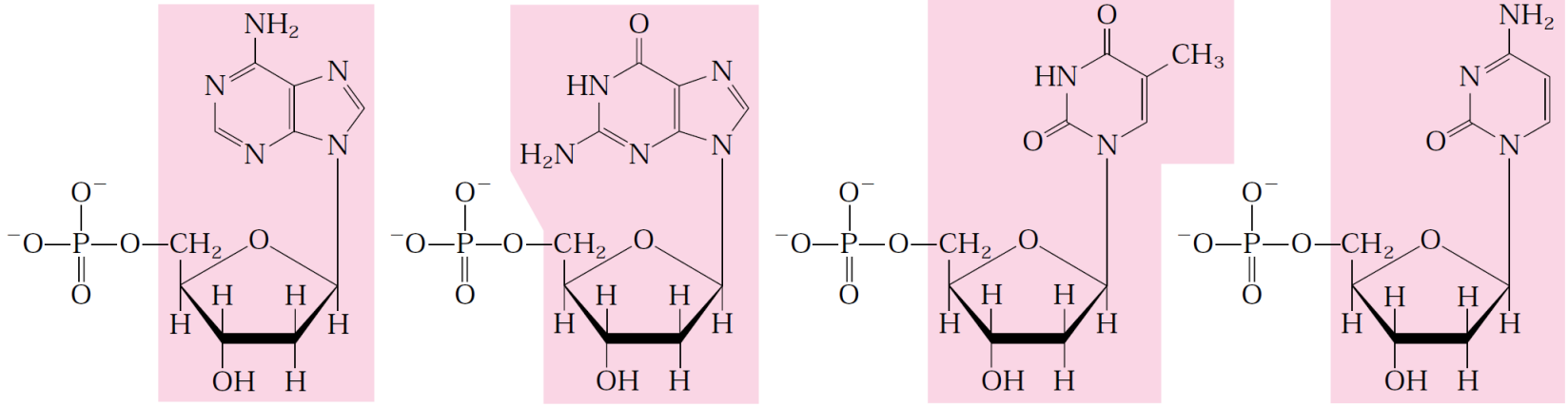
❖The recurring deoxyribonucleotide units of DNA contain 2'-deoxy-D-ribose, and the ribonucleotide units of RNA contain D-ribose.

❖In nucleotides, both types of pentoses are in their β-furanose (closed five-membered ring) form.

❖There are four major deoxyribonucleotides, the structural units of DNAs, and four major ribonucleotides, the structural units of RNAs.

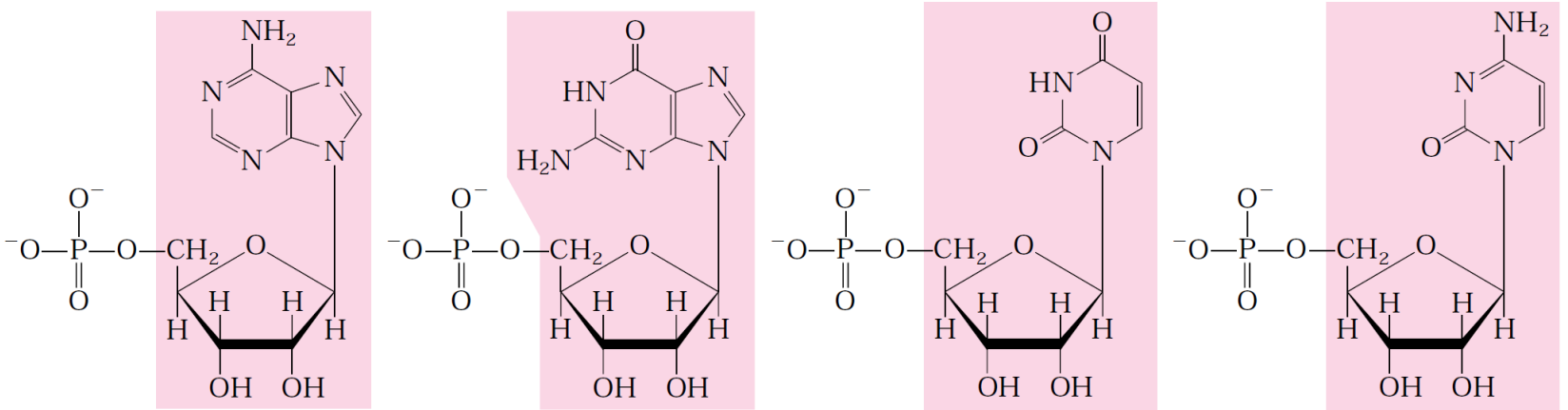
❖Specific long sequences of A, T, G, and C nucleotides in DNA are the repository of genetic information.

❖Although nucleotides bearing the major purines and pyrimidines are most common, both DNA and RNA also contain some minor bases.



	Deoxyadenylate (deoxyadenosine 5′-monophosphate)	Deoxyguanylate (deoxyguanosine 5′-monophosphate)	Deoxythymidylate (deoxythymidine 5′-monophosphate)	Deoxycytidylate (deoxycytidine 5′-monophosphate)
Symbols:	A, dA, dAMP	G, dG, dGMP	T, dT, dTMP	C, dC, dCMP
Nucleoside:	Deoxyadenosine	Deoxyguanosine	Deoxythymidine	Deoxycytidine

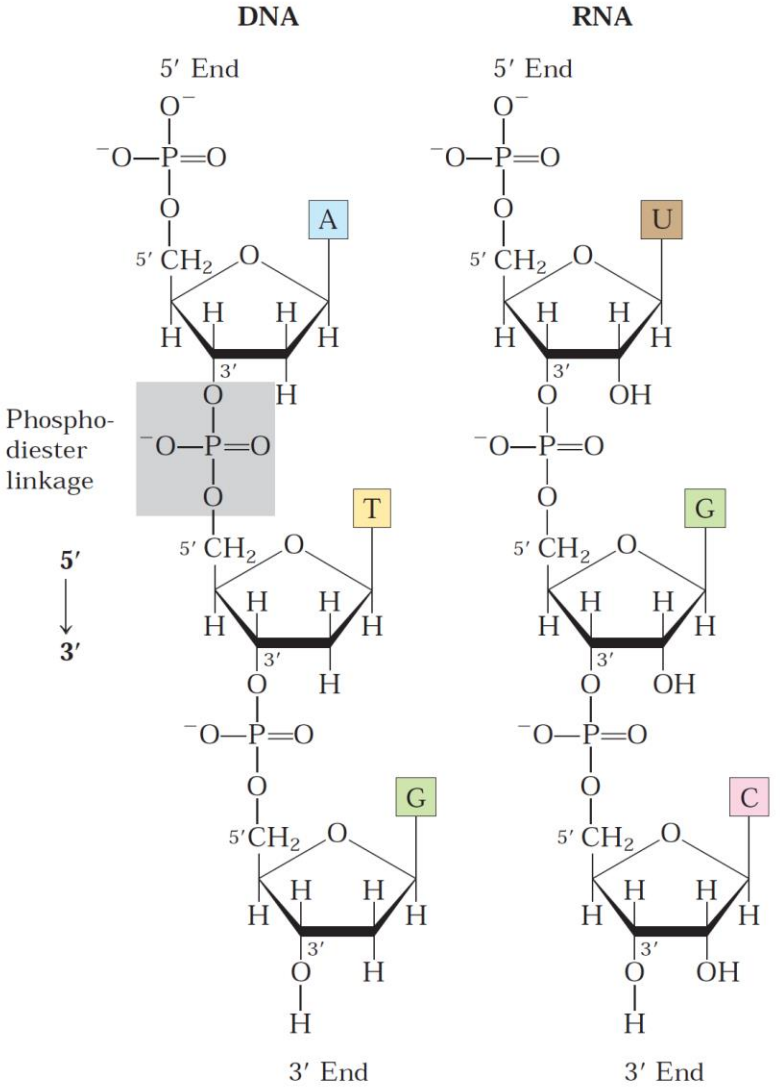
(a) Deoxyribonucleotides



	Adenylate (adenosine 5′-monophosphate)	Guanylate (guanosine 5′-monophosphate)	Uridylate (uridine 5′-monophosphate)	Cytidylate (cytidine 5′-monophosphate)
Symbols:	A, AMP	G, GMP	U, UMP	C, CMP
Nucleoside:	Adenosine	Guanosine	Uridine	Cytidine

(b) Ribonucleotides

❖The successive nucleotides of both DNA and RNA are covalently linked through phosphate-group “bridges,” in which the 5’-phosphate group of one nucleotide unit is joined to the 3’-hydroxyl group of the next nucleotide, creating a phosphodiester linkage



❖The covalent backbones of nucleic acids consist of alternating phosphate and pentose residues, and the nitrogenous bases may be regarded as side groups joined to the backbone at regular intervals.

❖The backbones of both DNA and RNA are hydrophilic.

❖The hydroxyl groups of the sugar residues form hydrogen bonds with water.

❖The phosphate groups, with a pKa near 0, are completely ionized and negatively charged at pH 7, and the negative charges are generally neutralized by ionic interactions with positive charges on proteins, metal ions, and polyamines.

❖The covalent backbone of DNA and RNA is subject to slow, nonenzymatic hydrolysis of the phosphodiester bonds.

❖In the test tube, RNA is hydrolyzed rapidly under alkaline conditions, but DNA is not; the 2’-hydroxyl groups in RNA (absent in DNA) are directly involved in the process.

❖By convention, the structure of a single strand of nucleic acid is always written in the 5'→3' direction.

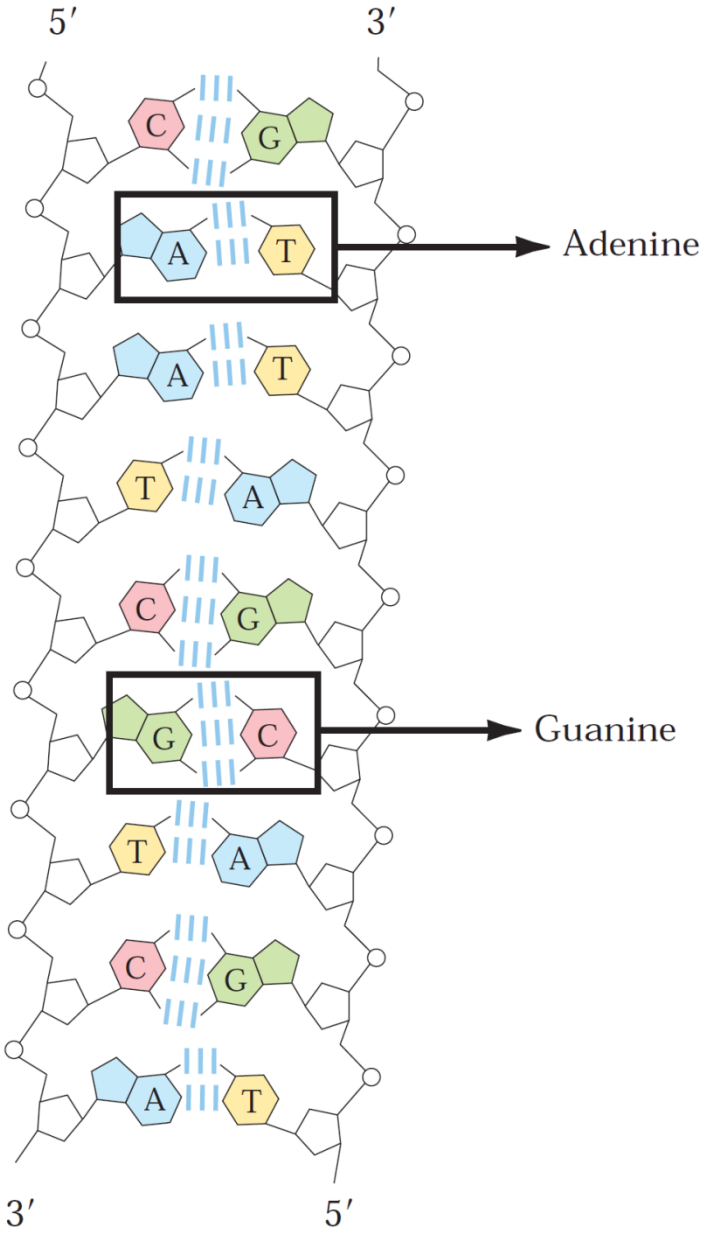
❖A short nucleic acid is referred to as an oligonucleotide.

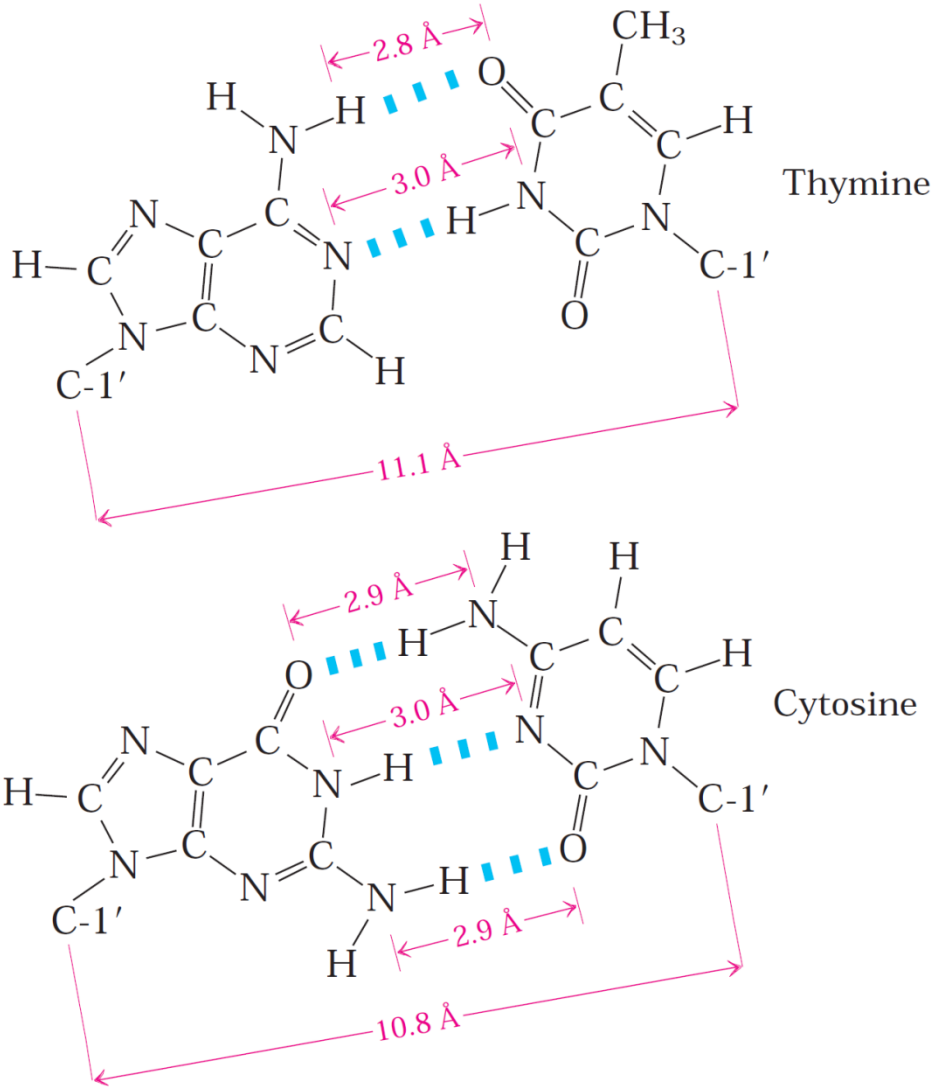
❖The definition of "short" is somewhat arbitrary, but polymers containing 50 or fewer nucleotides are generally called oligonucleotides. A longer nucleic acid is called a polynucleotide.

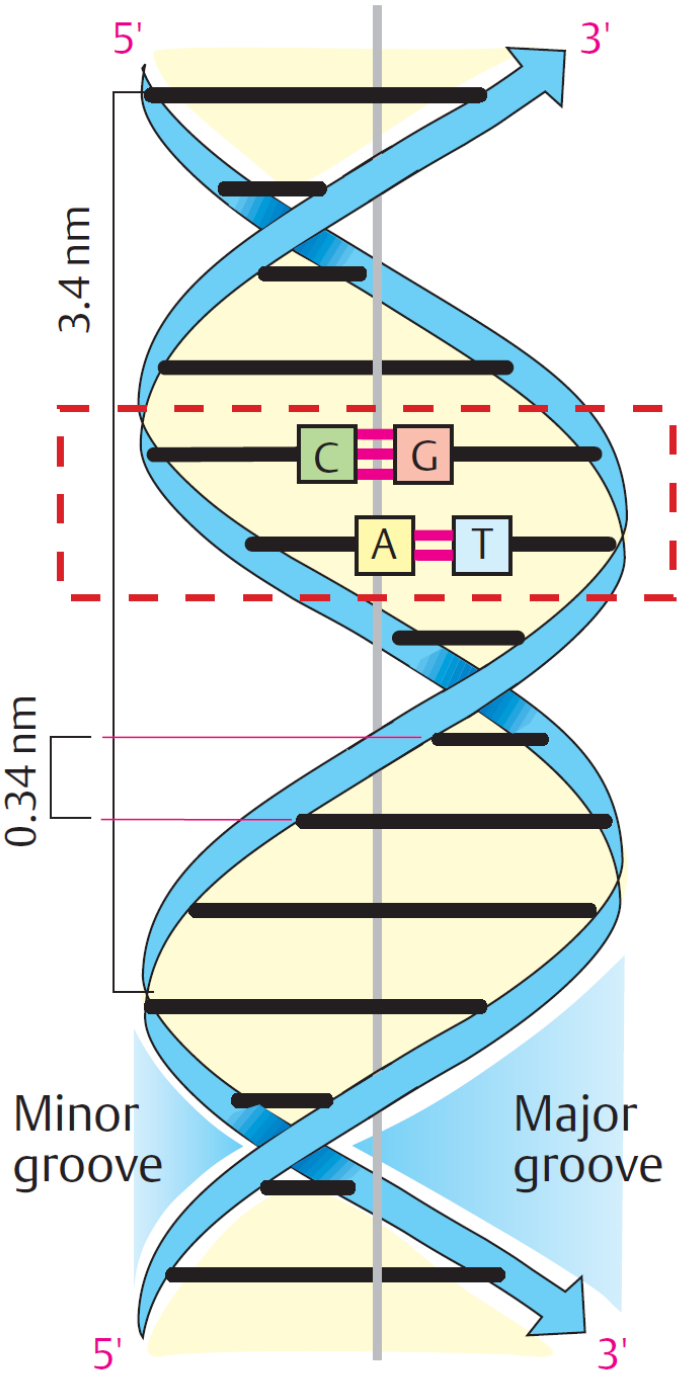
❖Free pyrimidines and purines are weakly basic compounds and are thus called bases.

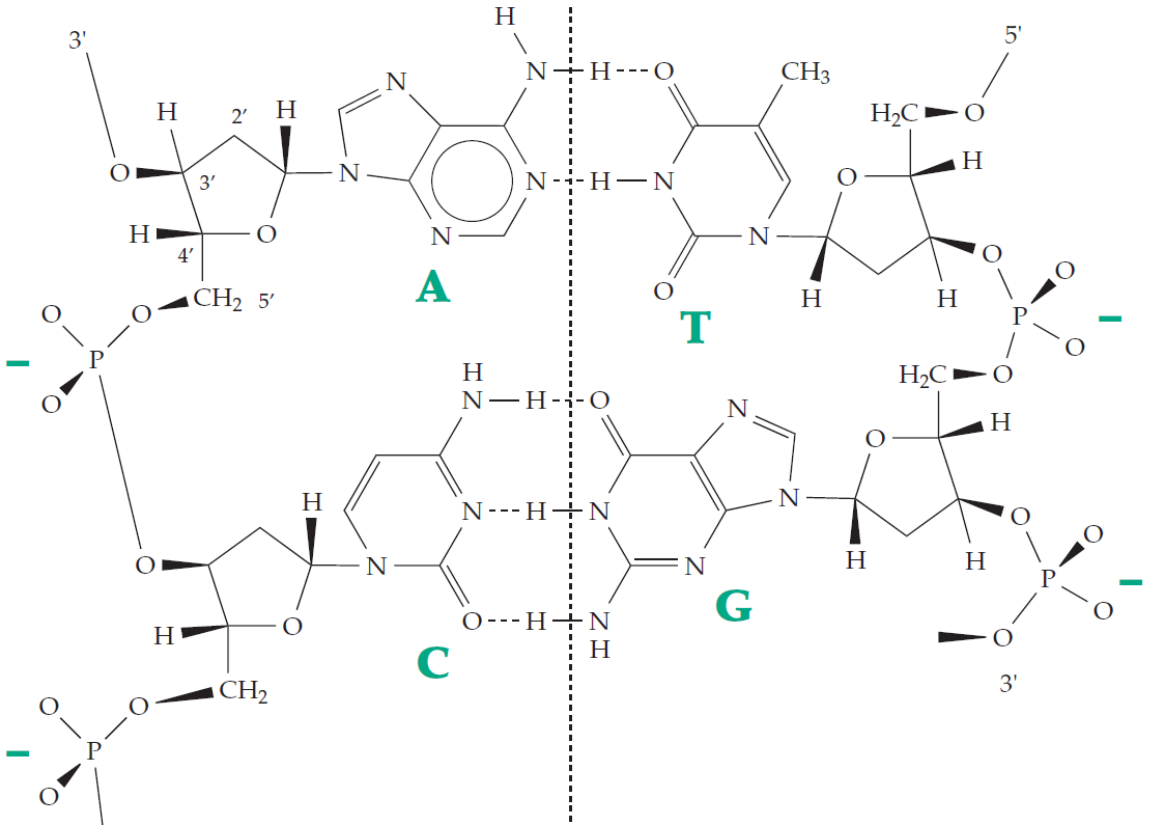
❖Hydrogen bonds between bases permit a complementary association of two (and occasionally three or four) strands of nucleic acid.

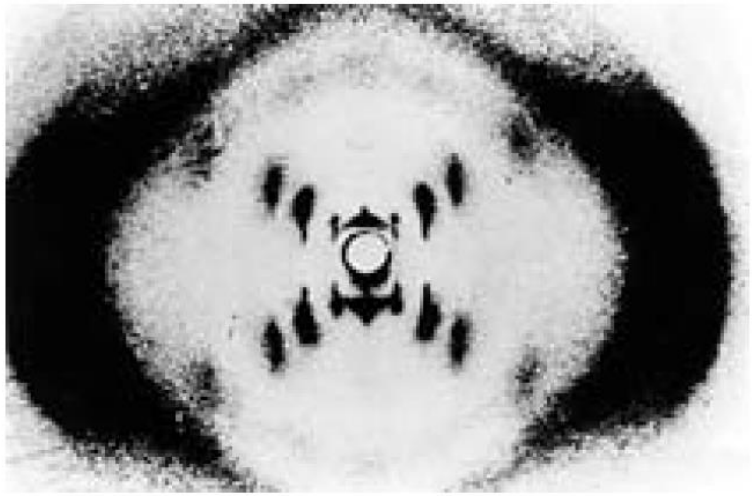
❖In double chain DNA G and C, A and T; in double chain RNA G and C and A and T pair with each other.

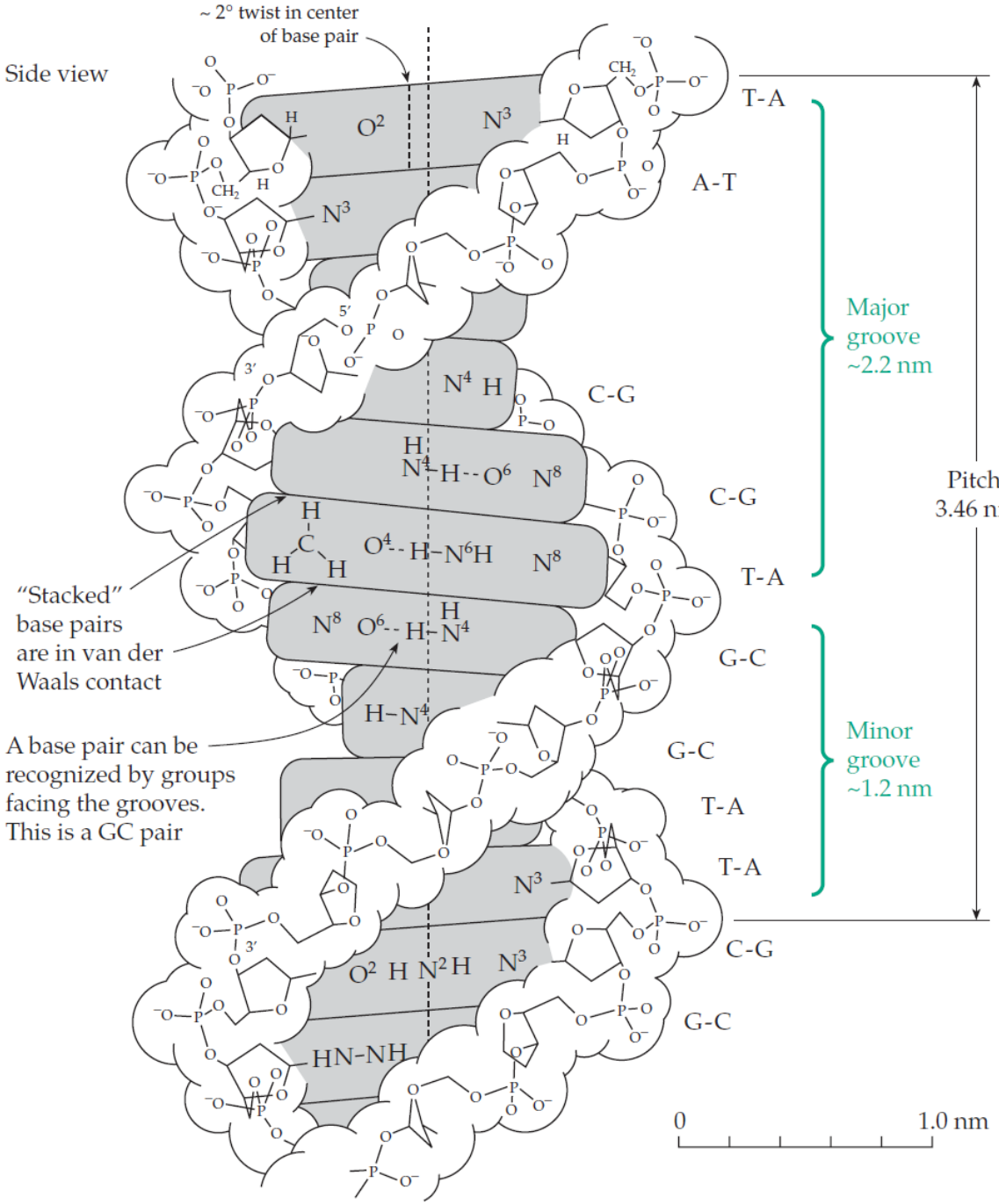

5′
3′
C
G
A
T
Adenine
A
T
T
A
C
G
G
C
Guanine
T
A
C
G
A
T
3′
5′

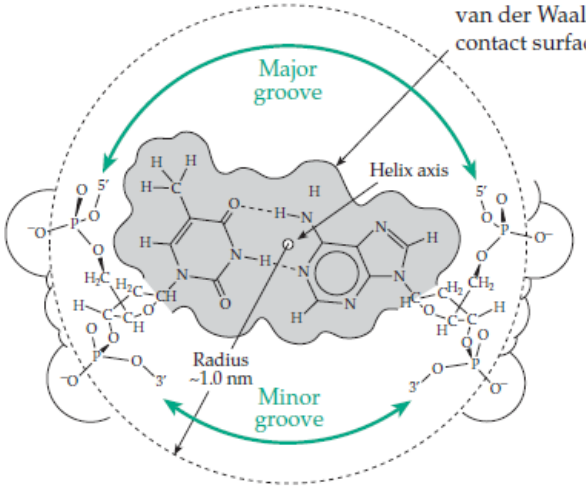

2.8 Å
3.0 Å
Thymine
11.1 Å
2.9 Å
3.0 Å
Cytosine
2.9 Å
10.8 Å


5'
3'
3.4 nm
C
G
A
T
0.34 nm
Minor groove
Major groove
5'
3'


A
T
C
G




~ 2° twist in center of base pair
Side view
T-A
A-T
C-G
C-G
T-A
G-C
G-C
T-A
T-A
C-G
G-C
Major groove ~2.2 nm
Minor groove ~1.2 nm
Pitch, 3.46 nm
"Stacked" base pairs are in van der Waals contact
A base pair can be recognized by groups facing the grooves. This is a GC pair
0
1.0 nm


van der Waals contact surface
Major groove
Helix axis
Radius ~1.0 nm
Minor groove

❖Double chain DNA is helical in structure, however DNA is a remarkably flexible molecule.

❖Considerable rotation is possible around a number of bonds in the sugar–phosphate (phosphodeoxyribose) backbone, and thermal fluctuation can produce bending, stretching, and unpairing (melting) of the strands.

❖Many significant deviations from the Watson-Crick DNA structure are found in cellular DNA, some or all of which may play important roles in DNA metabolism.

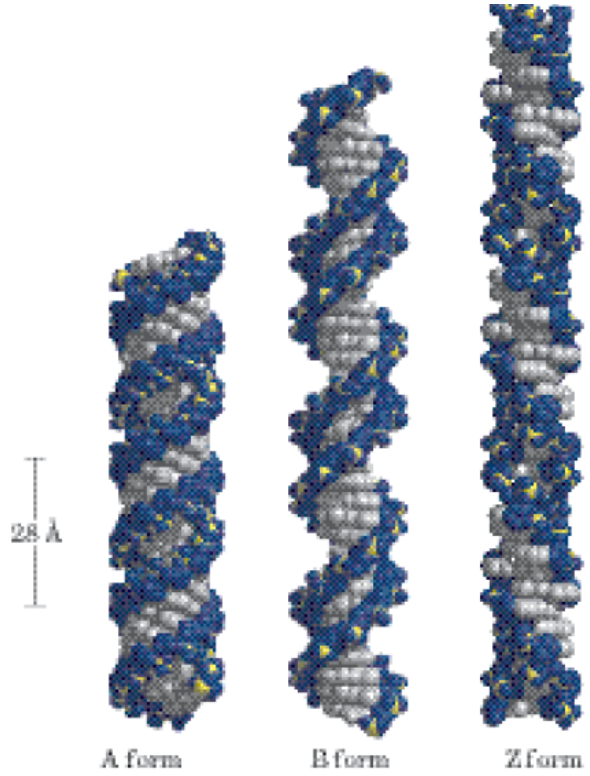
❖The Watson-Crick structure is also referred to as Bform DNA, or B-DNA.

❖The B form is the most stable structure for a random-sequence DNA molecule under physiological conditions and is therefore the standard point of reference in any study of the properties of DNA.

❖Two structural variants that have been well characterized in crystal structures are the A and Z forms.

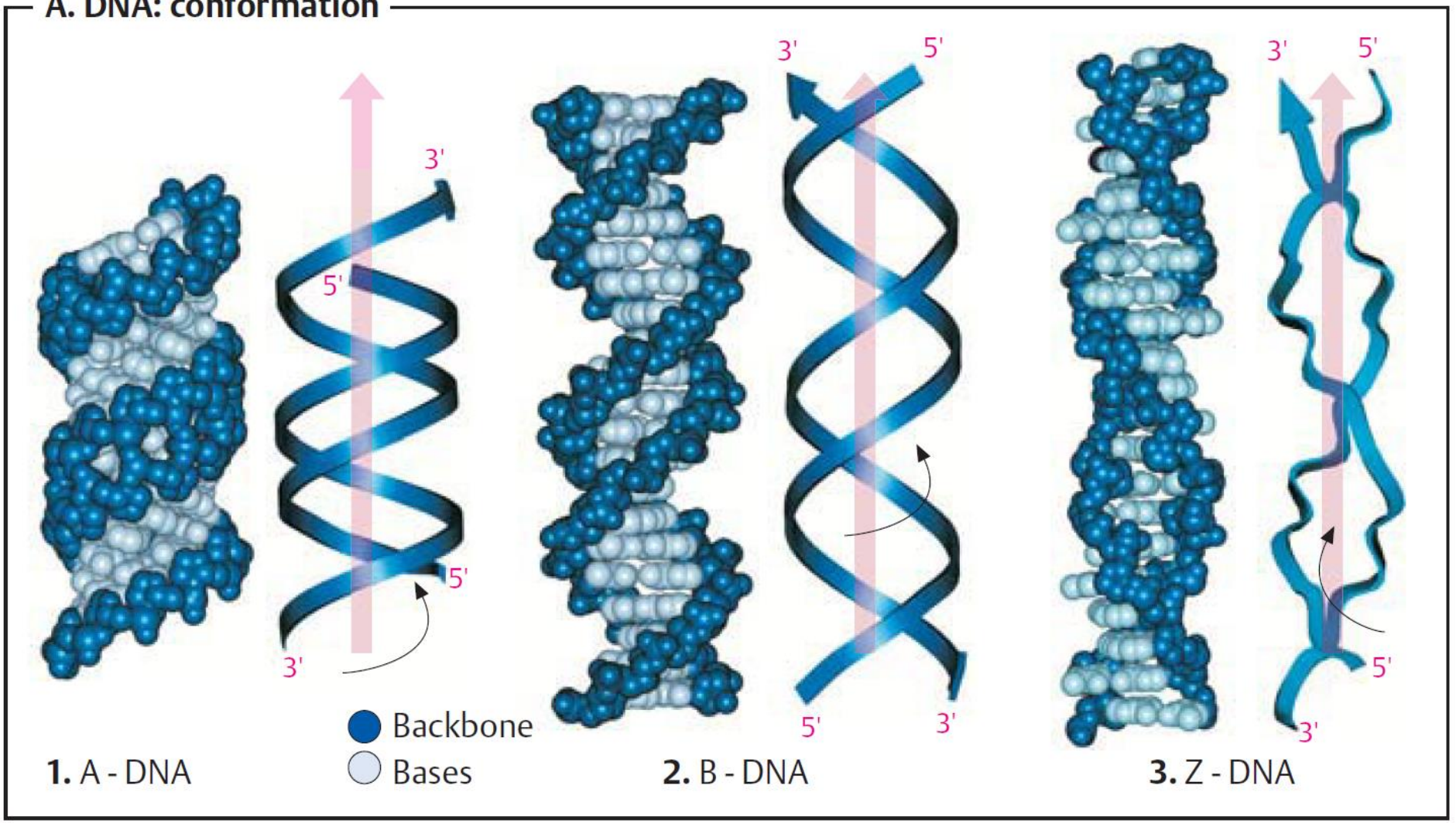
❖Z-form DNA is a more radical departure from the B structure; the most obvious distinction is the lefthanded helical rotation. The DNA backbone takes on a zigzag appearance.

❖Whether A-DNA occurs in cells is uncertain, but there is evidence for some short stretches (tracts) of Z-DNA in both prokaryotes and eukaryotes.

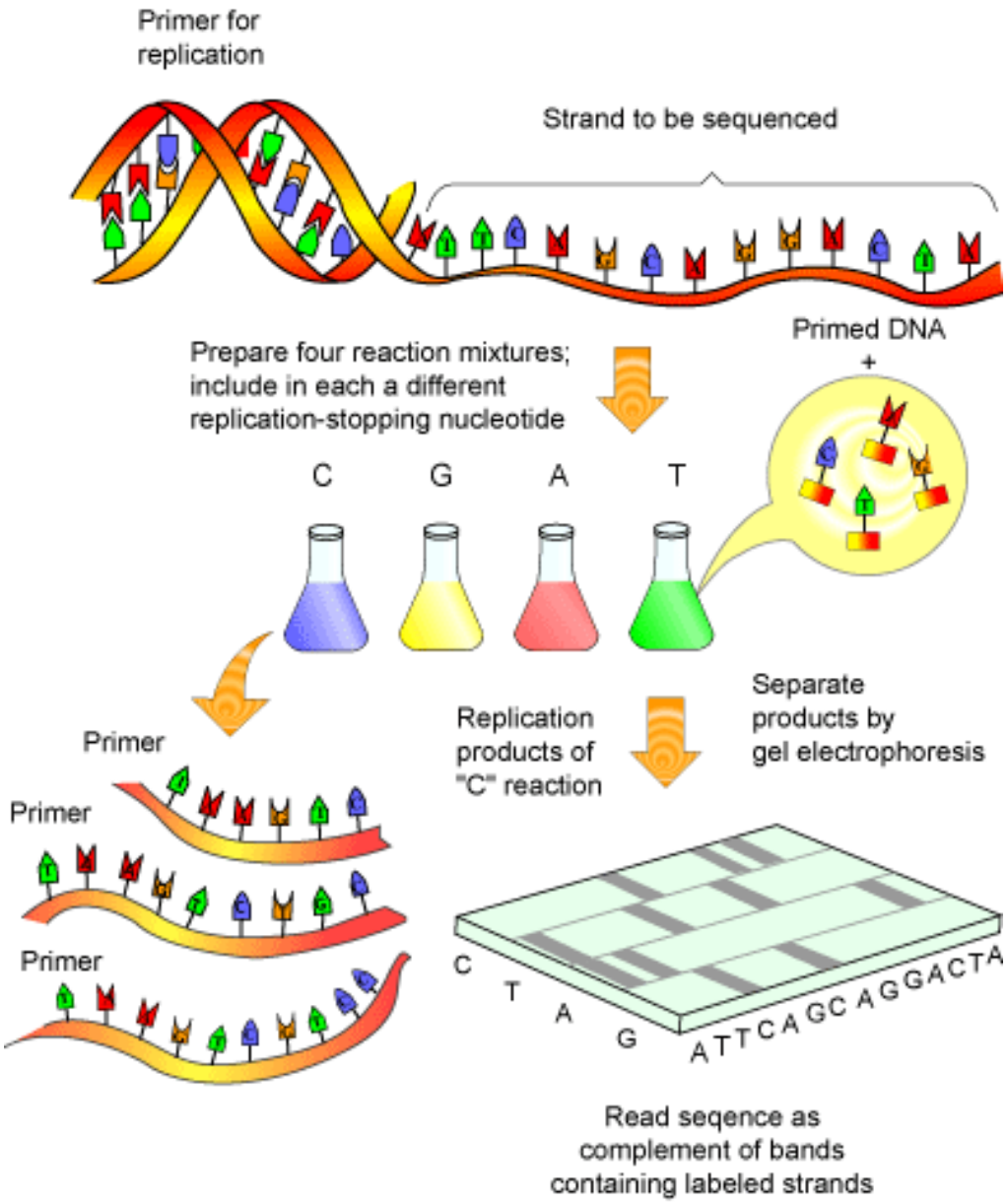


	A form	*B form*	*Z form*
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å
Base tilt normal to the helix axis	20°	6°	7°
Sugar pucker conformation	C-3′ endo	C-2′ endo	C-2′ endo for pyrimidines; C-3′ endo for purines
Glycosyl bond conformation	Anti	Anti	Anti for pyrimidines; syn for purines

FIGURE 8–19 Comparison of A, B, and Z forms of DNA. Each structure shown here has 36 base pairs. The bases are shown in gray, the phosphate atoms in yellow, and the riboses and phosphate oxygens in blue. Blue is the color used to represent DNA strands in later chapters. The table summarizes some properties of the three forms of DNA.


A. DNA: conformation
3'
5'
5'
3'
3'
5'
5'
3'
3'
5'
5'
3'
Backbone
Bases
1. A - DNA
2. B - DNA
3. Z - DNA

Nucleotides and Nucleic Acids II



- To understand how nucleic acids function, we must understand their chemical properties as well as their structures.

- The role of DNA as a repository of genetic information depends in part on its inherent stability.

- The long-term storage of information without alteration is so important to a cell, however, that even very slow reactions that alter DNA structure can be physiologically significant.

- Processes such as carcinogenesis and aging may be intimately linked to slowly accumulating, irreversible alterations of DNA.

- Other, nondestructive alterations also occur and are essential to function, such as the strand separation that must precede DNA replication or transcription.

- Just as heat and extremes of pH denature globular proteins, they also cause denaturation, or melting, of double-helical DNA.

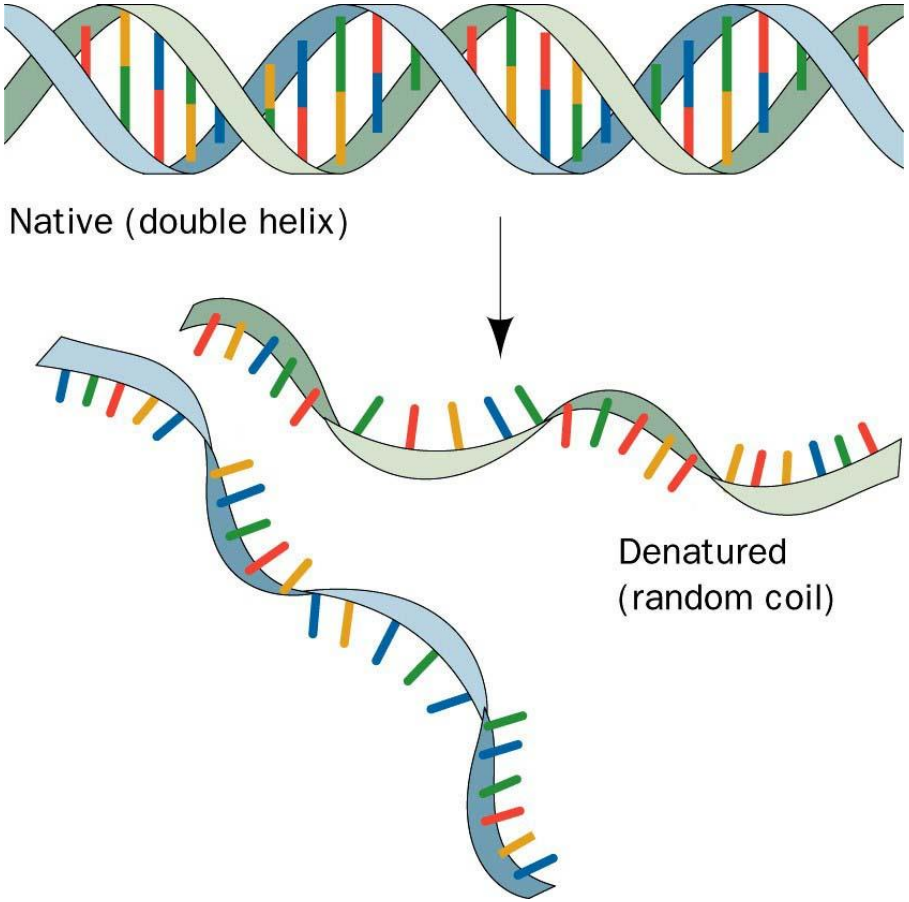
- Disruption of the hydrogen bonds between paired bases and of base stacking causes unwinding of the double helix to form two single strands, completely separate from each other along the entire length or part of the length (partial denaturation) of the molecule.

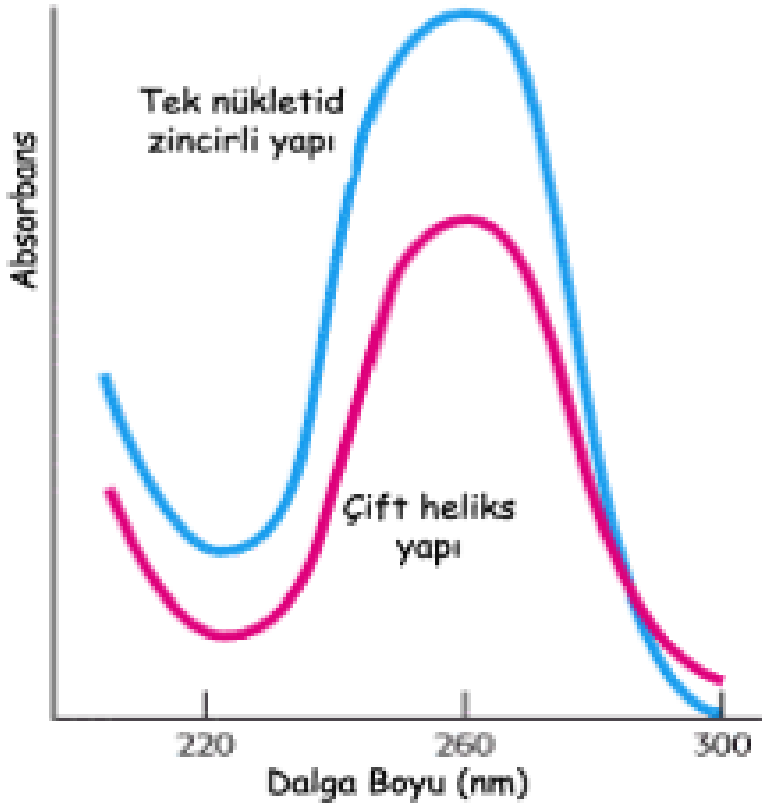
- No covalent bonds in the DNA are broken

- Renaturation of a DNA molecule is a rapid one-step process, as long as a double-helical segment of a dozen or more residues still unites the two strands.

- When the temperature or pH is returned to the range in which most organisms live, the unwound segments of the two strands spontaneously rewind, or anneal, to yield the intact duplex.

- The close interaction between stacked bases in a nucleic acid has the effect of decreasing its absorption of UV light relative to that of a solution with the same concentration of free nucleotides, and the absorption is decreased further when two complementary nucleic acids strands are paired. This is called the **hypochromic effect.**



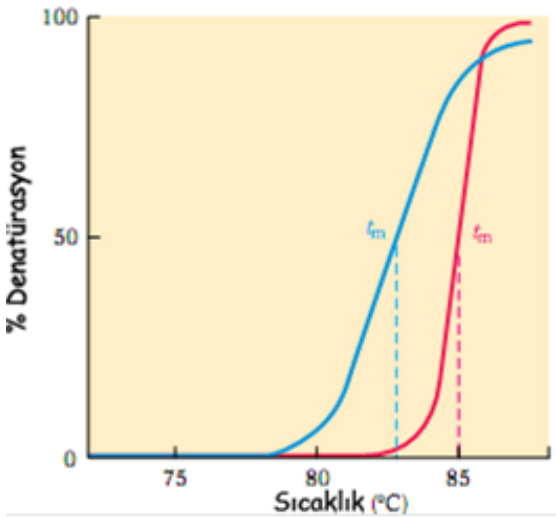


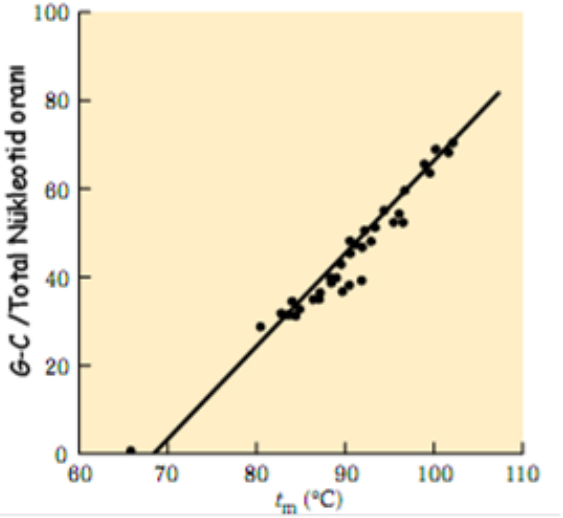
- Viral or bacterial DNA molecules in solution denature when they are heated slowly .

- Each species of DNA has a characteristic denaturation temperature, or melting point (t_m): the higher its content of G-C base pairs, the higher the melting point of the DNA.

- This is because G-C base pairs, with three hydrogen bonds, require more heat energy to dissociate than A-T base pairs.

- Careful determination of the melting point of a DNA specimen, under fixed conditions of pH and ionic strength, can yield an estimate of its base composition.





- Duplexes of two RNA strands or of one RNA strand and one DNA strand (RNA-DNA hybrids) can also be denatured.

- The ability of two complementary DNA strands to pair with one another can be used to detect similar DNA sequences in two different species or within the genome of a single species.

- If duplex DNAs isolated from human cells and from mouse cells are completely denatured by heating, then mixed and kept at 65 °C for many hours, much of the DNA will anneal.

- Some strands of the mouse DNA will associate with human DNA strands to yield **hybrid duplexes,** in which segments of a mouse DNA strand form base-paired regions with segments of a human DNA strand.

- This process called as hybridization.

- Purines and pyrimidines, along with the nucleotides of which they are a part, undergo a number of spontaneous alterations in their covalent structure.

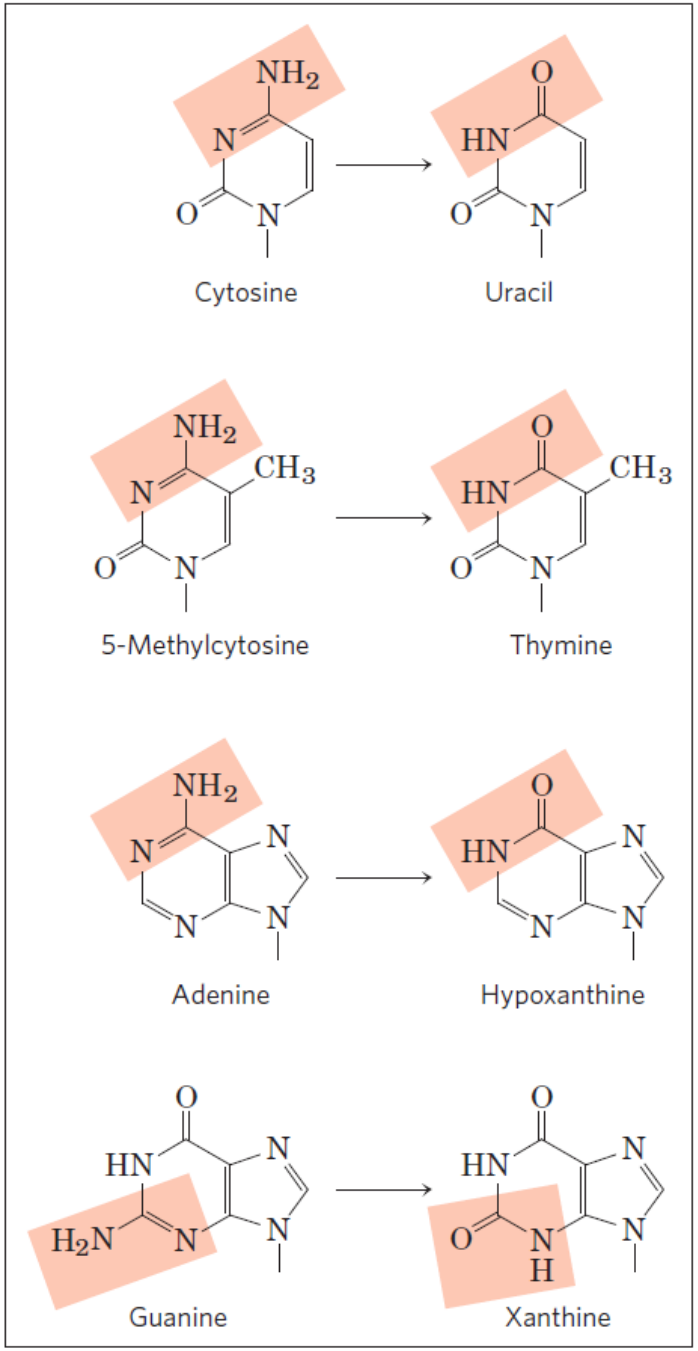
- The rate of these reactions is generally very slow, but they are physiologically significant because of the cell's very low tolerance for alterations in its genetic information.

- Alterations in DNA structure that produce permanent changes in the genetic information encoded therein are called **mutations.**

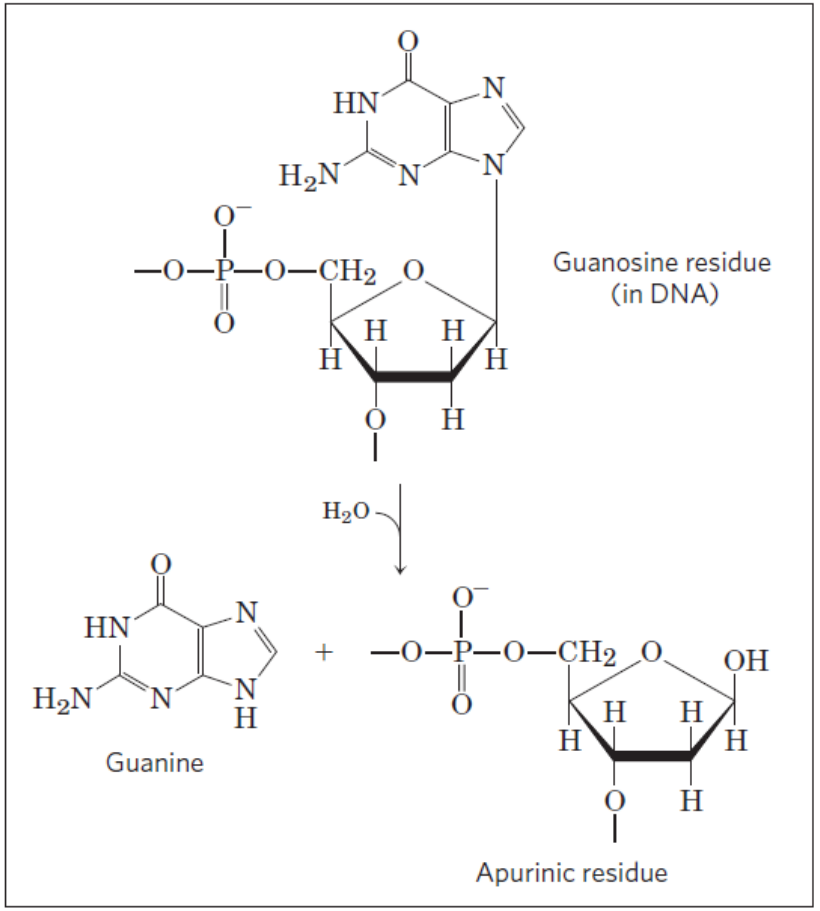
- Several nucleotide bases undergo spontaneous loss of their exocyclic amino groups (deamination).

- For example, under typical cellular conditions, deamination of cytosine (in DNA) to uracil occurs in about one of every 10^7 cytidine residues in 24 hours.

- This corresponds to about 100 spontaneous events per day, on average, in a mammalian cell.



(a) Deamination



(b) Depurination

FIGURE 8–30 Some well-characterized nonenzymatic reactions of nucleotides. **(a)** Deamination reactions. Only the base is shown. **(b)** Depurination, in which a purine is lost by hydrolysis of the *N*-β-glycosyl bond. Loss of pyrimidines via a similar reaction occurs, but much more slowly. The resulting lesion, in which the deoxyribose is present but the base is not, is called an abasic site or an AP site (apurinic site or, rarely, apyrimidinic site). The deoxyribose remaining after depurination is readily converted from the β-furanose to the aldehyde form (see Fig. 8–3), further destabilizing the DNA at this position. More nonenzymatic reactions are illustrated in Figures 8–31 and 8–32.

- Another important reaction in deoxyribonucleotides is the hydrolysis of the N-β-glycosyl bond between the base and the pentose (depurinization). This occurs at a higher rate for purines than for pyrimidines.

- Other reactions are promoted by radiation.

- UV light induces the condensation of two ethylene groups to form a cyclobutane ring.

- In the cell, the same reaction between adjacent pyrimidine bases in nucleic acids forms cyclobutane pyrimidine dimers.

- This happens most frequently between adjacent thymidine residues on the same DNA strand.

- Ionizing radiation (x rays and gamma rays) can cause ring opening and fragmentation of bases as well as breaks in the covalent backbone of nucleic acids.

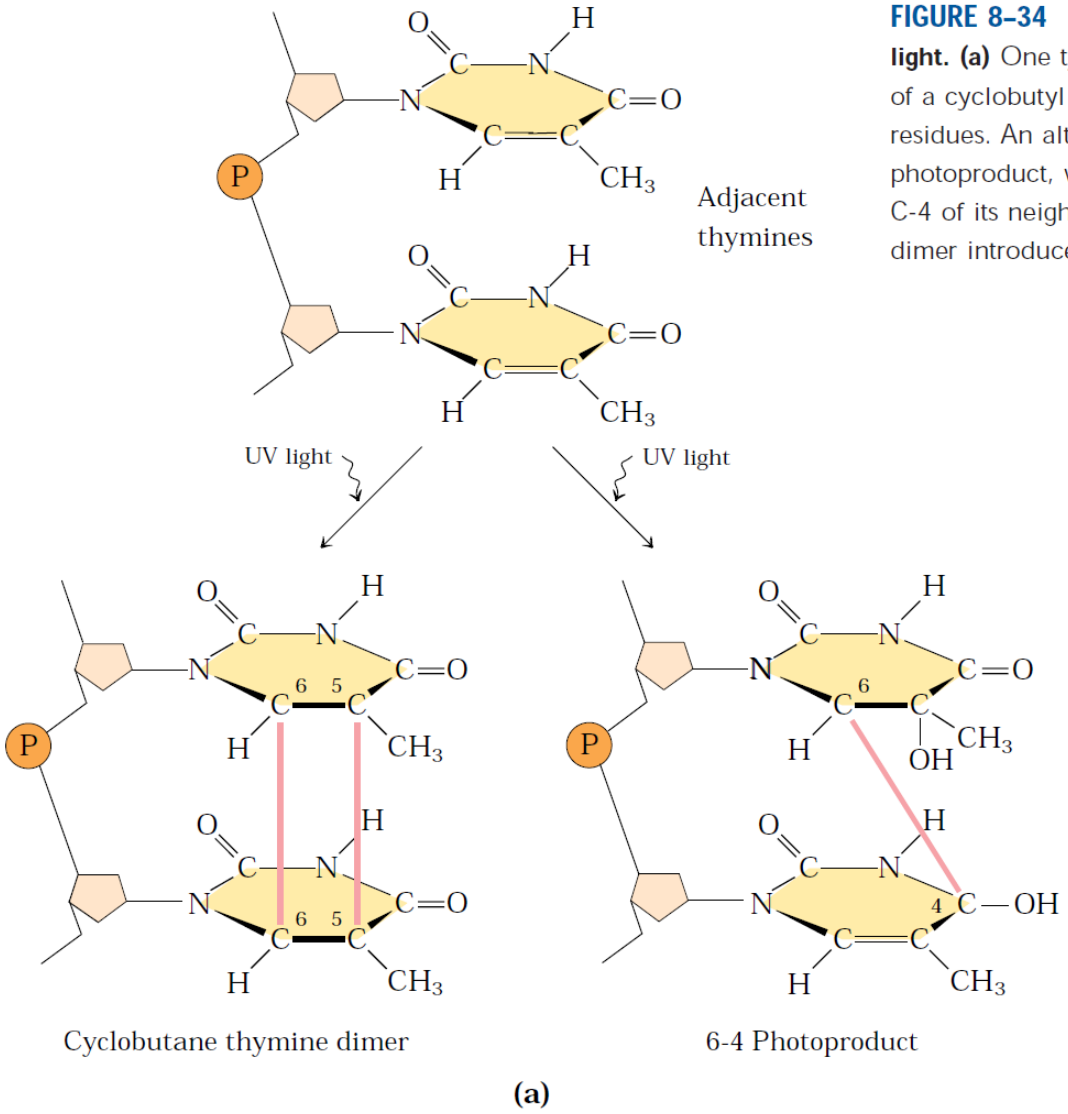
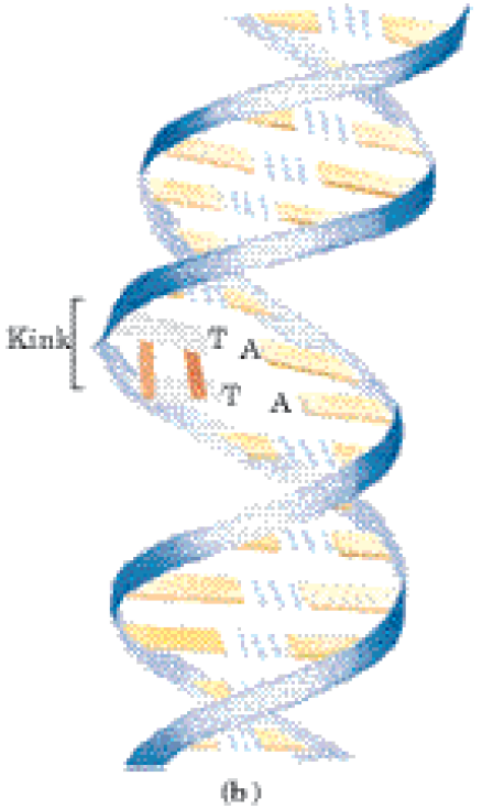


FIGURE 8–34 **Formation of pyrimidine dimers induced by UV light. (a)** One type of reaction (on the left) results in the formation of a cyclobutyl ring involving C-5 and C-6 of adjacent pyrimidine residues. An alternative reaction (on the right) results in a 6-4 photoproduct, with a linkage between C-6 of one pyrimidine and C-4 of its neighbor. **(b)** Formation of a cyclobutane pyrimidine dimer introduces a bend or kink into the DNA.



- DNA also may be damaged by reactive chemicals introduced into the environment as products of industrial activity.

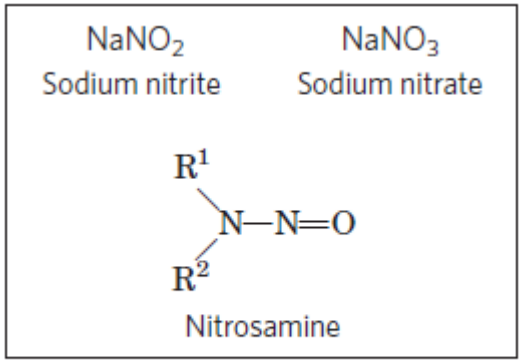
- Two prominent classes of such agents are (1) deaminating agents, particularly nitrous acid (HNO_2) or compounds that can be metabolized to nitrous acid or nitrites, and (2) alkylating agents.

- Possibly the most important source of mutagenic alterations in DNA is oxidative damage. Excited-oxygen species such as hydrogen peroxide, hydroxyl radicals, and superoxide radicals arise during irradiation or as a byproduct of aerobic metabolism.

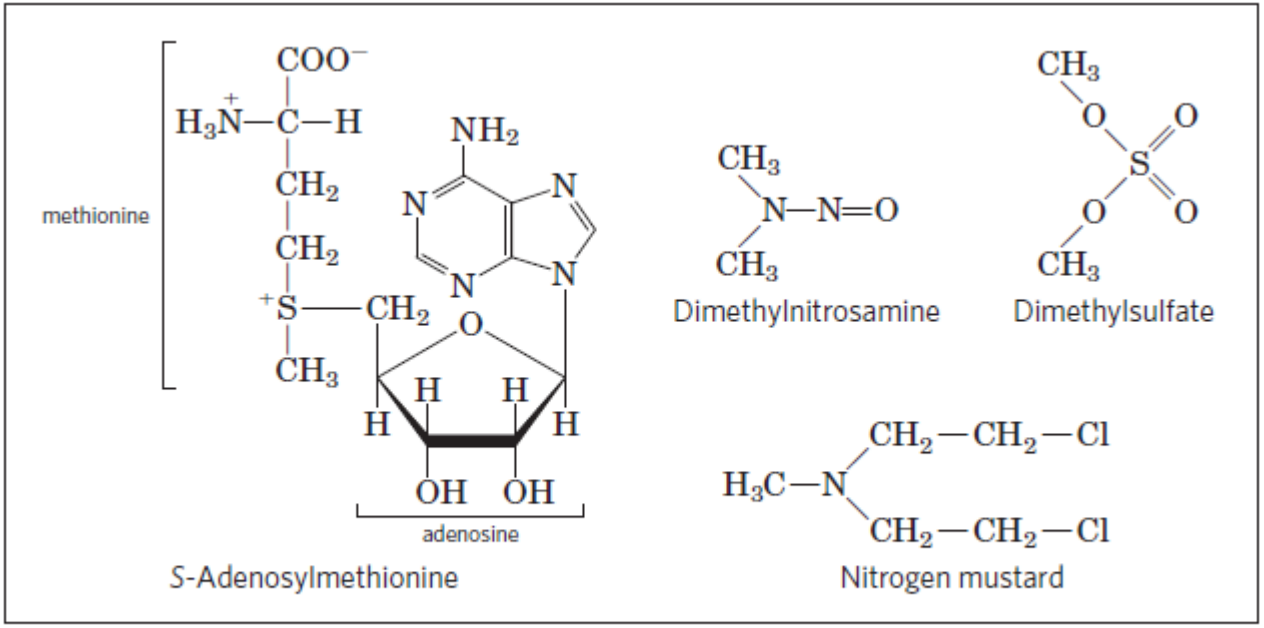
- Of these species, the hydroxyl radicals are responsible for most oxidative DNA damage.

- Cells have an elaborate defense system to destroy reactive oxygen species, including enzymes such as catalase and superoxide dismutase.

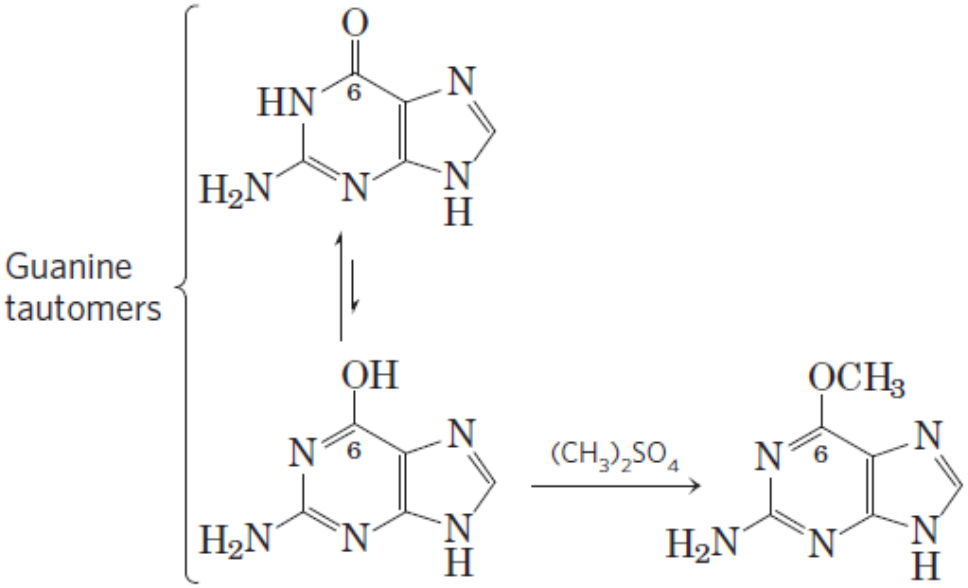
- The integrity of DNA as a polymer is better maintained than that of either RNA or protein, because DNA is the only macromolecule that has the benefit of biochemical repair systems



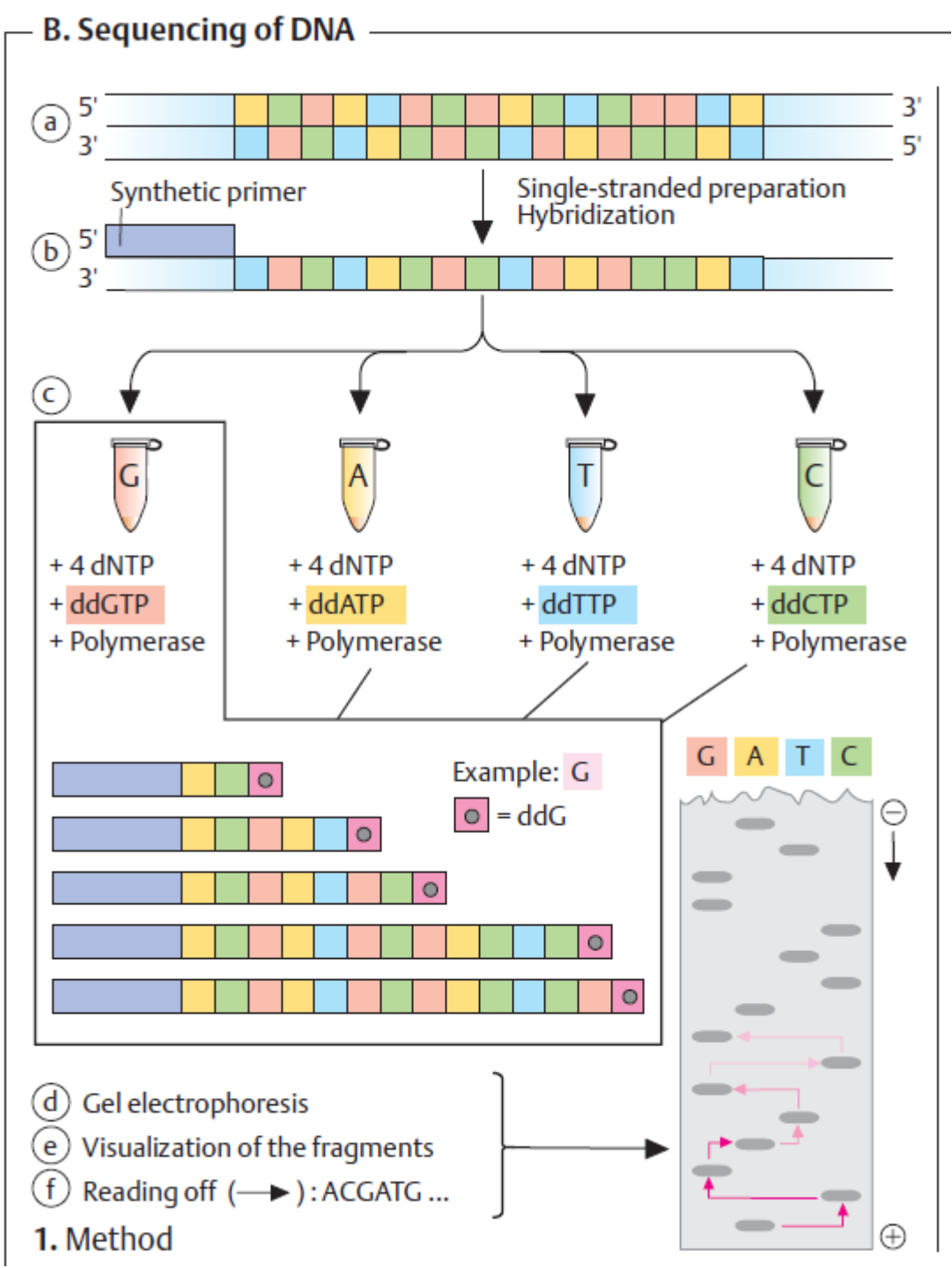
(a) Nitrous acid precursors



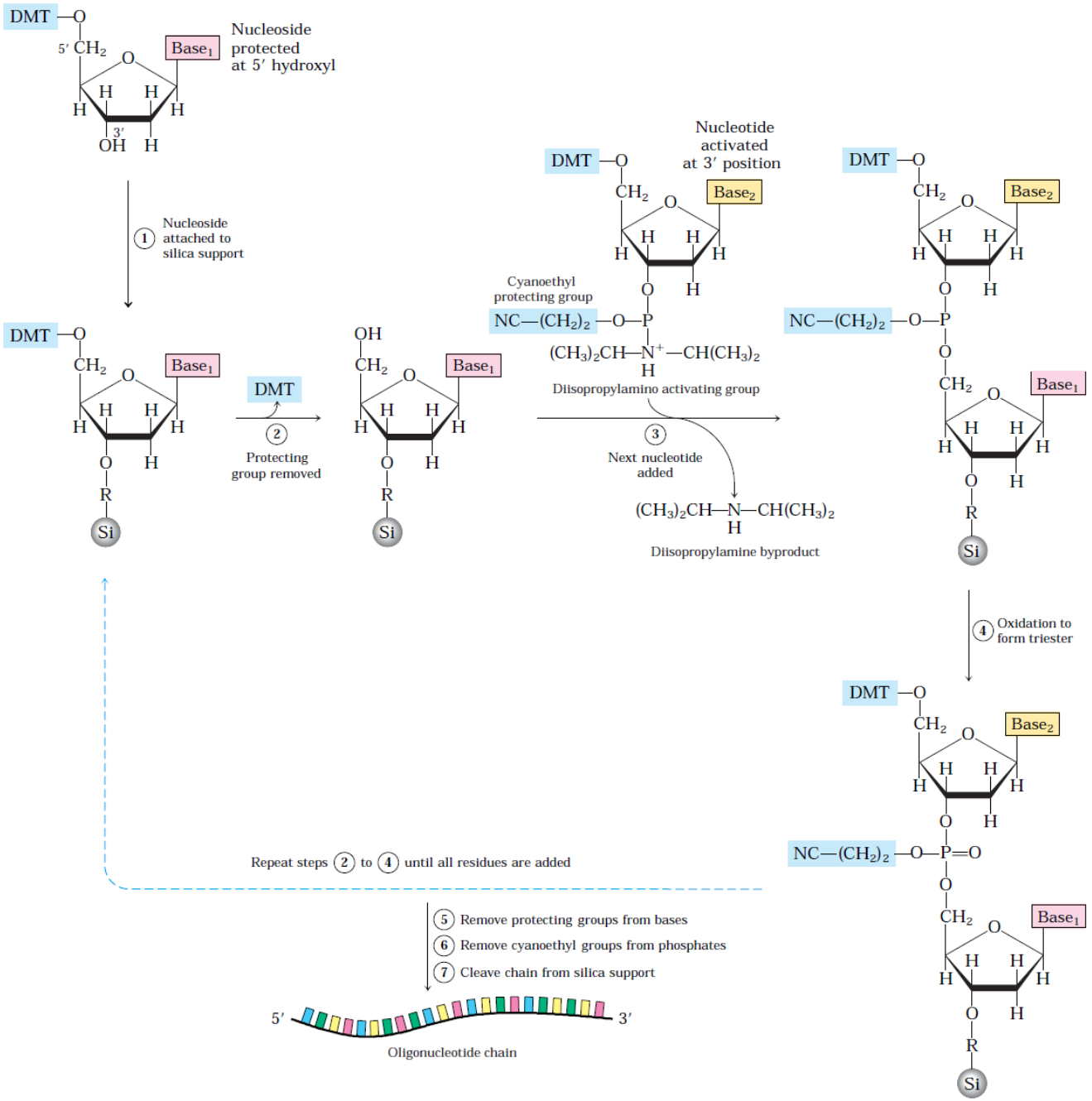
(b) Alkylating agents



- DNA molecule's most important property is its nucleotide sequence.
- In both Sanger and Maxam-Gilbert sequencing, the general principle is to reduce the DNA to four sets of labeled fragments.
- The reaction producing each set is base-specific, so the lengths of the fragments correspond to positions in the DNA sequence where a certain base occurs.

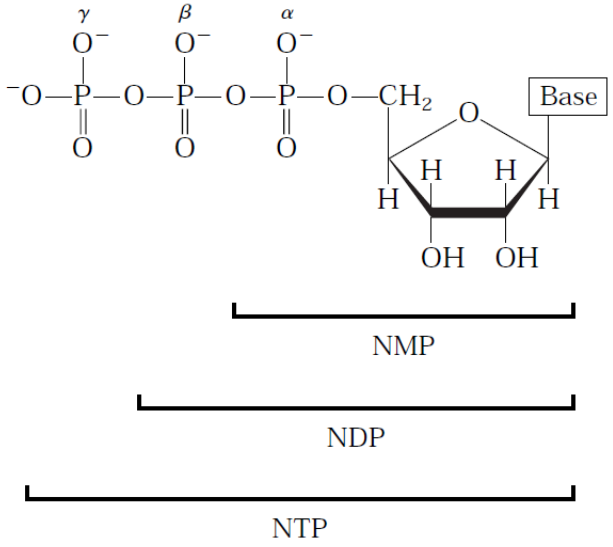


❖ The chemical methods for synthesizing nucleic acids were also developed.

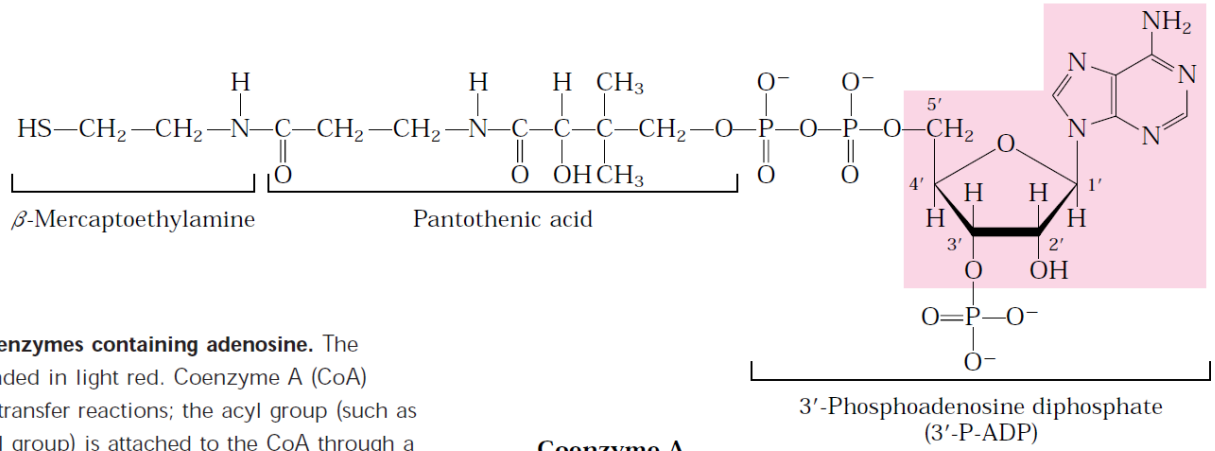


- In addition to their roles as the subunits of nucleic acids, nucleotides have a variety of other functions in every cell: as energy carriers, components of enzyme cofactors, and chemical messengers.

- Nucleoside mono-, di-, and triphosphates provides the chemical energy to drive a wide variety of cellular reactions.

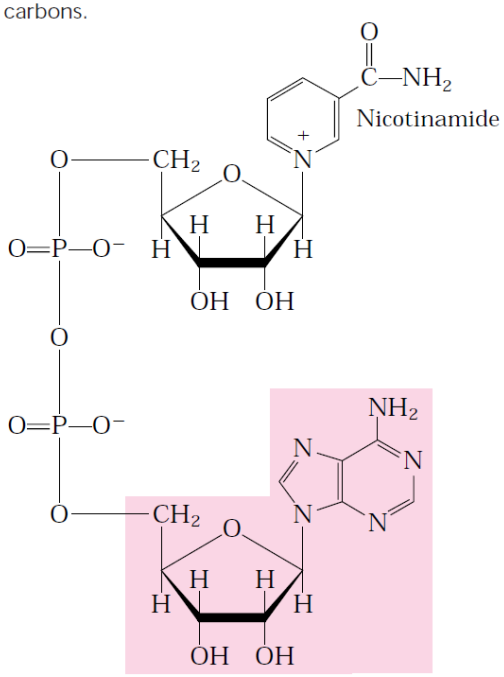


- A variety of enzyme cofactors serving a wide range of chemical functions include adenosine as part of their structure.

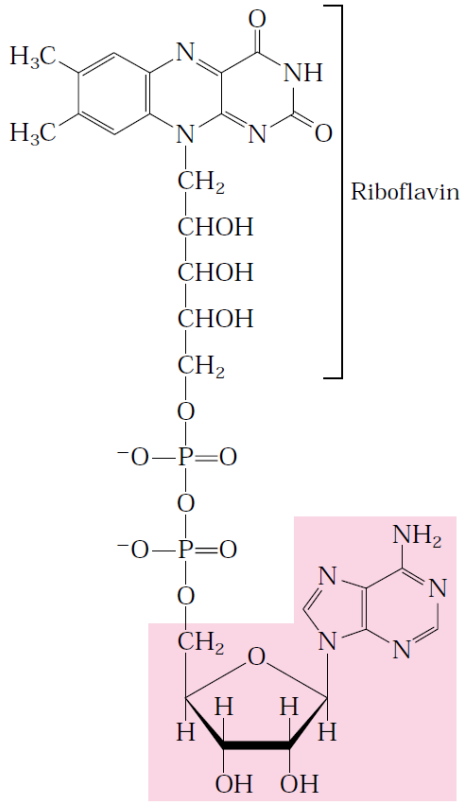


Coenzyme A

FIGURE 8–41 **Some coenzymes containing adenosine.** The adenosine portion is shaded in light red. Coenzyme A (CoA) functions in acyl group transfer reactions; the acyl group (such as the acetyl or acetoacetyl group) is attached to the CoA through a thioester linkage to the β-mercaptoethylamine moiety. NAD^+ functions in hydride transfers, and FAD, the active form of vitamin B_2 (riboflavin), in electron transfers. Another coenzyme incorporating adenosine is 5′-deoxyadenosylcobalamin, the active form of vitamin B_{12} (see Box 17-2), which participates in intramolecular group transfers between adjacent carbons.



Nicotinamide adenine dinucleotide (NAD^+)

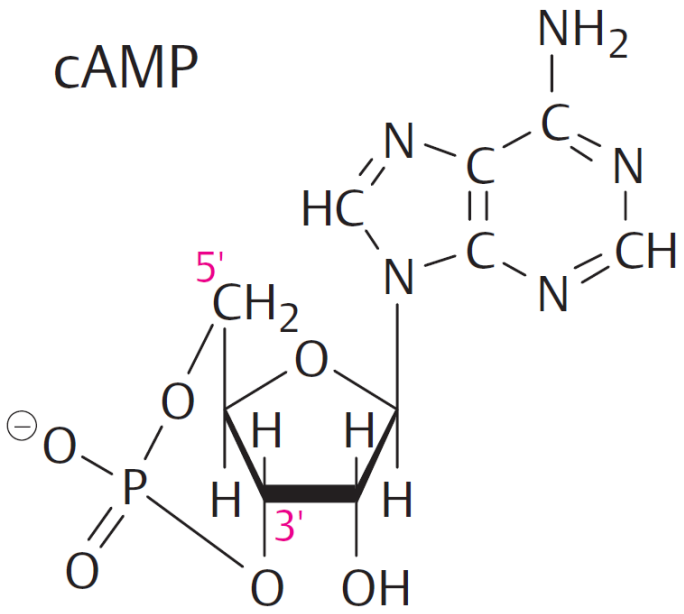


Flavin adenine dinucleotide (FAD)

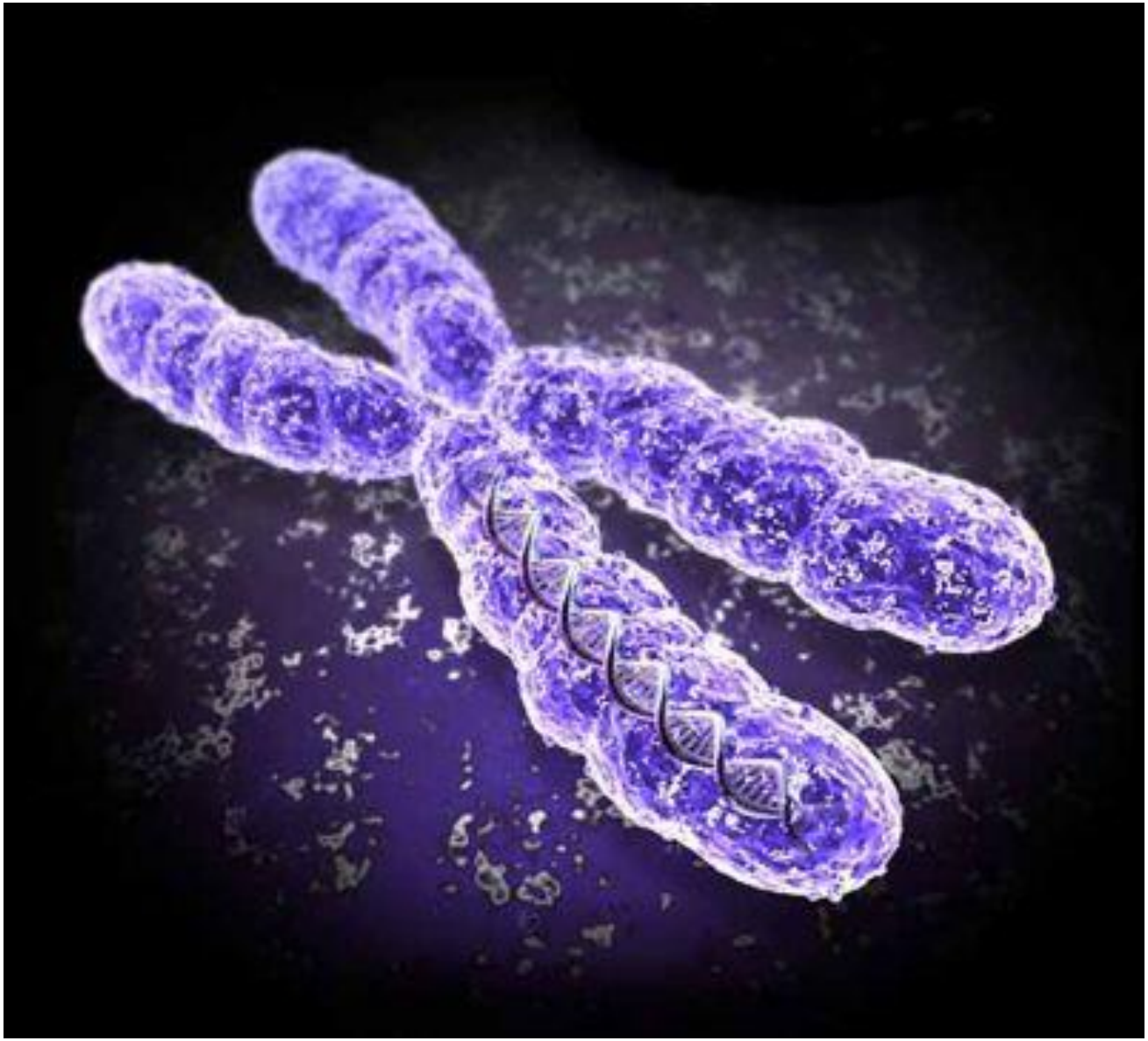
- Cells respond to their environment by taking cues from hormones or other external chemical signals.

- The interaction of these extracellular chemical signals ("first messengers") with receptors on the cell surface often leads to the production of second messengers inside the cell, which in turn leads to adaptive changes in the cell interior.

- Often, the second messenger is a nucleotide. One of the most common is adenosine 3',5'-cyclic monophosphate (cyclic AMP, or cAMP), formed from ATP.



Genes and Chromosomes



- Almost every cell of a multicellular organism contains the same complement of genetic material—its **genome**.

- Chromosomes, the nucleic acid molecules that are the repository of an organism's genetic information, are the largest molecules in a cell and may contain thousands of genes.

- Human chromosomes range up to 279 million bp.

- Cellular DNA contains genes and intergenic regions, both of which may serve functions vital to the cell.

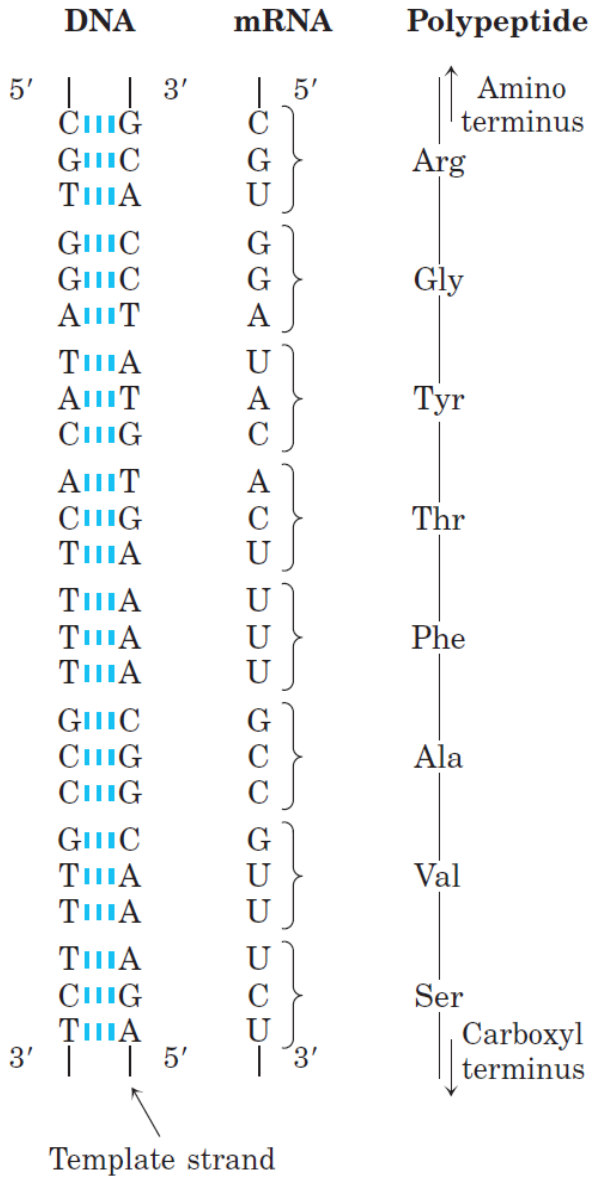
- A **gene** is all the DNA that encodes the primary sequence of some final gene product, which can be either a polypeptide or an RNA with a structural or catalytic function.

- DNA also contains other segments or sequences that have a purely regulatory function

- Regulatory sequences provide signals that may denote the beginning or the end of genes, or influence the transcription of genes, or function as initiation points for replication or recombination

- Each amino acid of a polypeptide chain is coded for by a sequence of three consecutive nucleotides in a single strand of DNA, which called as **codon.**

- Many genes in eukaryotes and a few in prokaryotes are interrupted by noncoding DNA segments.



- Viruses are not free-living organisms; rather, they are infectious parasites that use the resources of a host cell to carry out many of the processes they require to propagate. Many viral particles consist of no more than a genome (usually a single RNA or DNA molecule) surrounded by a protein coat.

- The genomes of DNA viruses vary greatly in size (5.000-150.000 bp).

- Bacteria contains very large, circular DNA chromosome in their nucleoid (for *E. Coli* 4.500.000 bp).

- Many bacteria contain one or more small circular DNA molecules that are free in the cytosol. These extrachromosomal elements are called plasmids (1.000-10.000 bp).

- Some plasmids carry genes that are useful to the host bacterium such as antibiotic resistance.

- Eukaryotes; A yeast cell, one of the simplest eukaryotes, has 2.6 times more DNA in its genome than an *E. coli* cell.

- Cells of Drosophila, the fruit fly used in classical genetic studies, contain more than 35 times as much DNA as *E. coli* cells, and human cells have almost 700 times as much.

- The cells of many plants and amphibians contain even more.

- The genetic material of eukaryotic cells is apportioned into chromosomes, the diploid (2n) number depending on the species.

- A human somatic cell, for example, has 46 chromosomes.

- Each chromosome of a eukaryotic cell, contains a single, very large, duplex DNA molecule.

- The DNA molecules in the 24 different types of human chromosomes (22 matching pairs plus the X and Y sex chromosomes) vary in length over a 25-fold range.

(a)

(b)

- Eukaryotic cells also have organelles, mitochondria and chloroplasts, that contain DNA.

- Mitochondrial DNA (mtDNA) molecules are much smaller than the nuclear chromosomes.

- In animal cells, mtDNA contains fewer than 20,000 bp (16,569 bp in human mtDNA) and is a circular duplex.

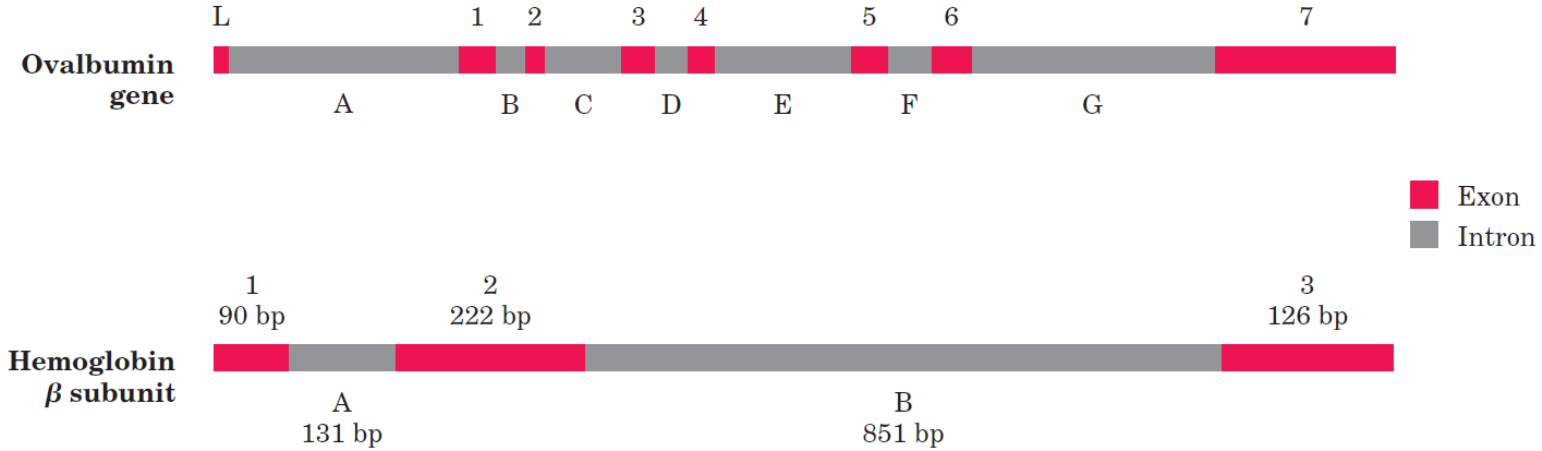
- Plant cell mtDNA ranges in size from 200,000 to 2,500,000 bp.

- Chloroplast DNA (cpDNA) also exists as circular duplexes and ranges in size from 120,000 to 160,000 bp.

- Many bacterial species have only one chromosome per cell and, in nearly all cases, each chromosome contains only one copy of each gene.

- Many, if not most, eukaryotic genes contain one or more intervening segments of DNA that do not code for the amino acid sequence of the polypeptide product.

- Such nontranslated DNA segments in genes are called intervening sequences or introns, and the coding segments are called exons.

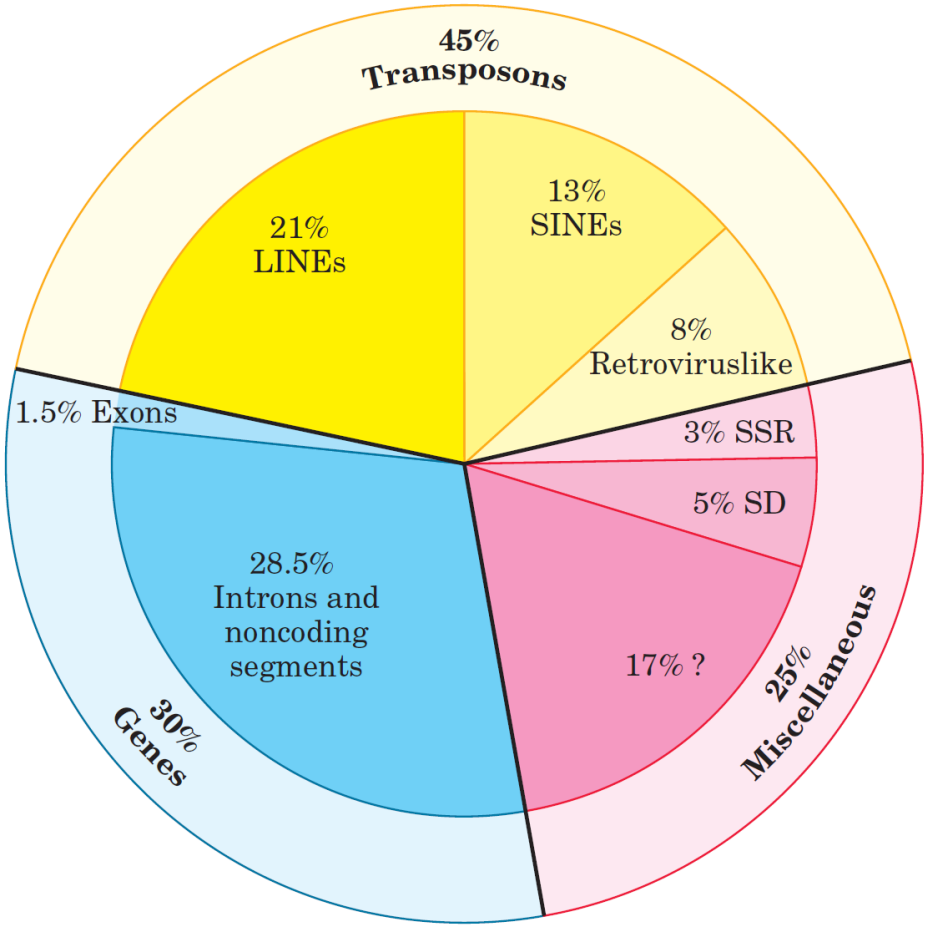


- In higher eukaryotes, the typical gene has much more intron sequence than sequences devoted to exons.

- In total, only about 1.5% of human DNA is “coding” or exon DNA, carrying information for protein or RNA products.

- Much of the nongene DNA is in the form of repeated sequences of several kinds.

- Half the human genome is made up of moderately repeated sequences that are derived from transposable elements—segments of DNA, that can move from one location to another in the genome.



- Another 3% or so of the human genome consists of highly repetitive sequences, also referred to as simple-sequence DNA or simple sequence repeats (SSR).

- Much of SSR is associated with two defining features of eukaryotic chromosomes: centromeres and telomeres.

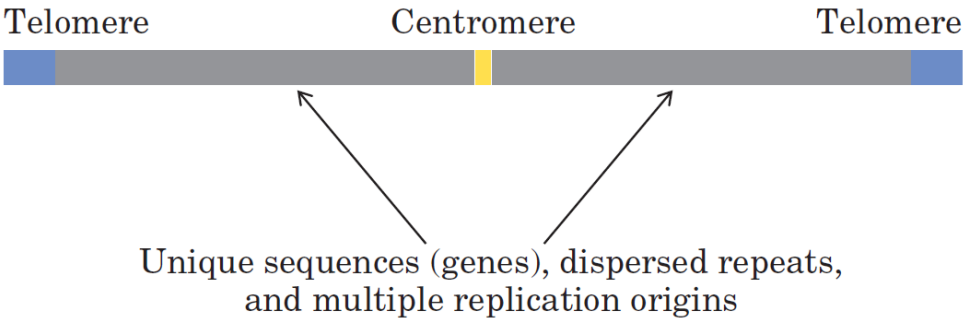


FIGURE 24–9 **Important structural elements of a yeast chromosome.**

- The **centromere** is a sequence of DNA that functions during cell division as an attachment point for proteins that link the chromosome to the mitotic spindle.

- Telomeres are sequences at the ends of eukaryotic chromosomes that help stabilize the chromosome.

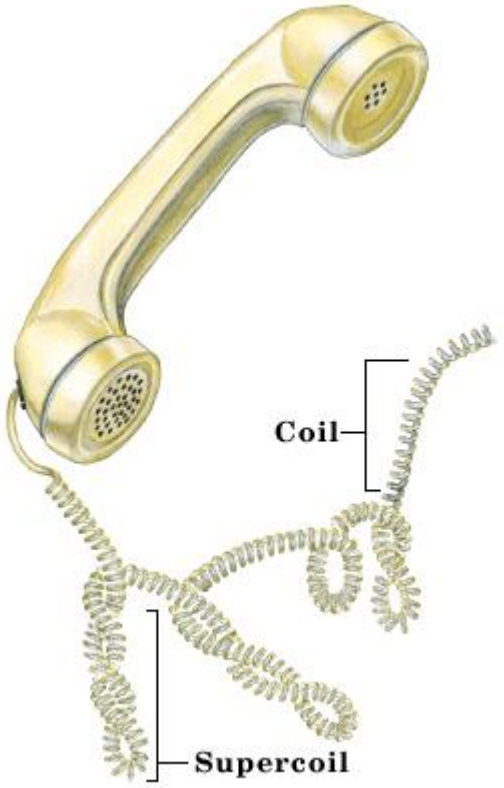
- Cellular DNA, as we have seen, is extremely compacted, implying a high degree of structural organization.

- The folding mechanism must not only pack the DNA but also permit access to the information in the DNA.

- Supercoiling means the coiling of a coil.

- DNA is coiled in the form of a double helix, with both strands of the DNA coiling around an axis.

- The further coiling of that axis upon itself produces DNA supercoiling.



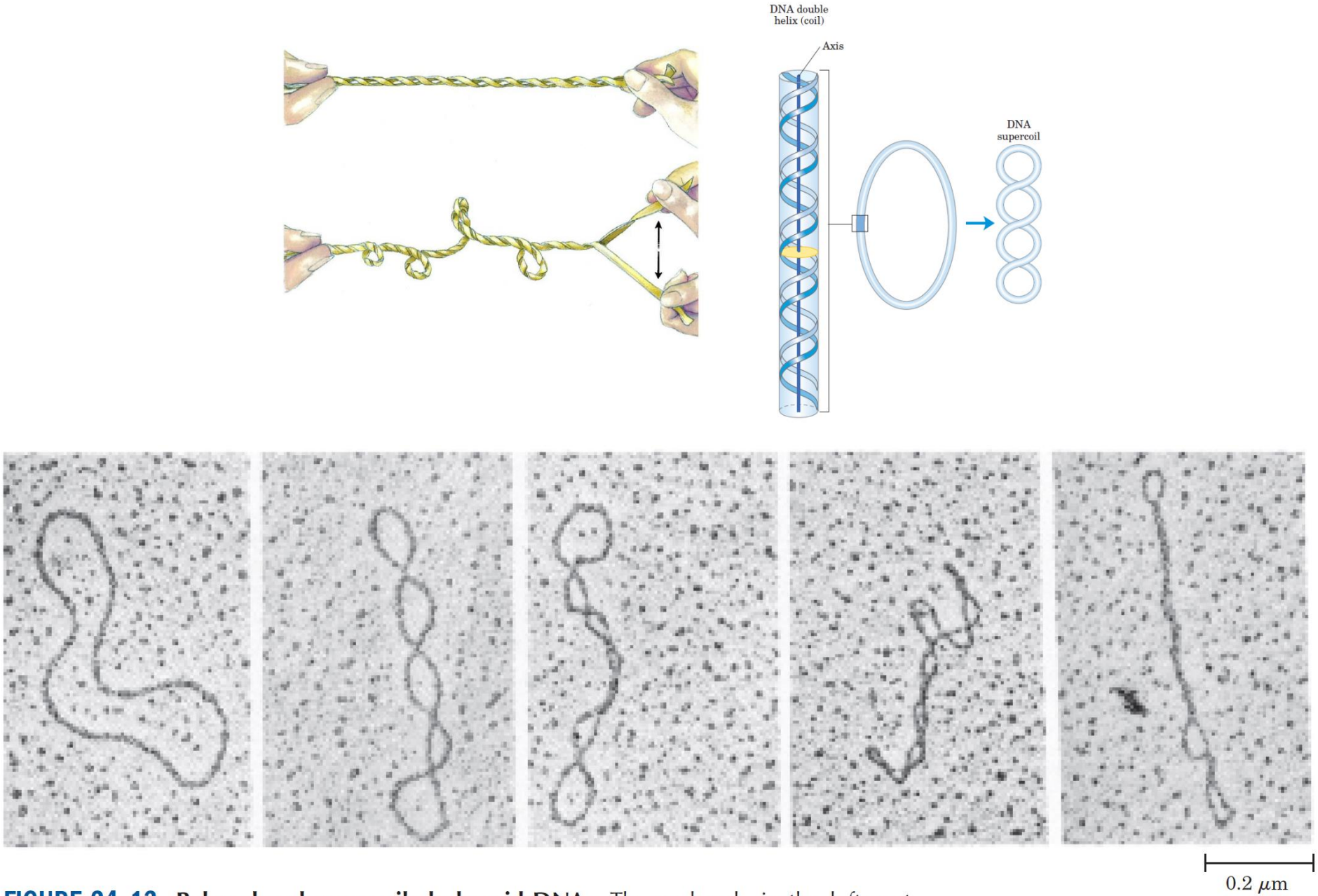
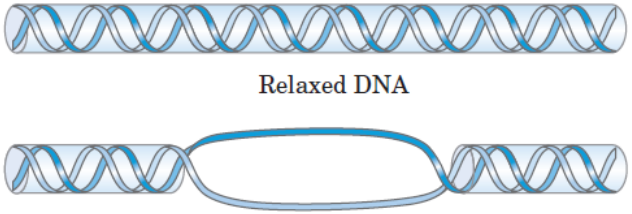


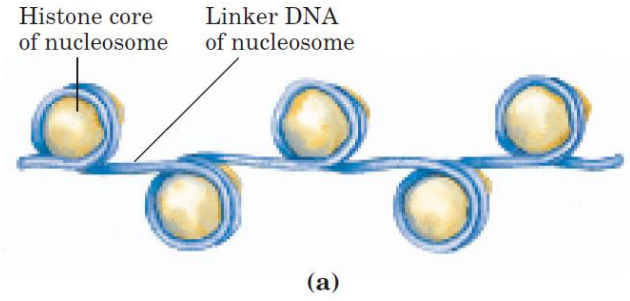
FIGURE 24–13 **Relaxed and supercoiled plasmid DNAs.** The molecule in the leftmost electron micrograph is relaxed; the degree of supercoiling increases from left to right.

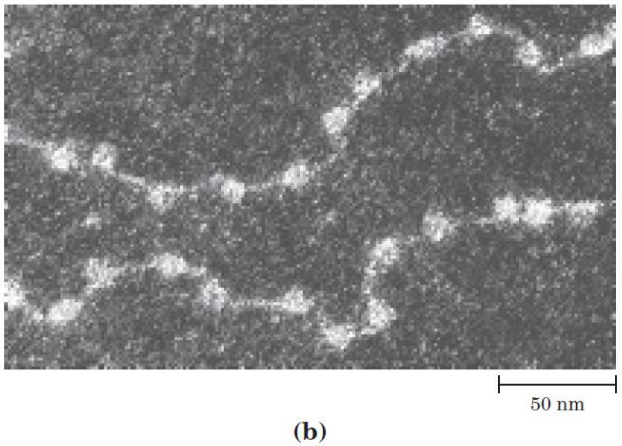
- The enzymes that increase or decrease the extent of DNA underwinding are topoisomerases. These enzymes play an especially important role in processes such as replication and DNA packaging.



- **Chromatin** consists of fibers containing protein and DNA in approximately equal masses, along with a small amount of RNA.

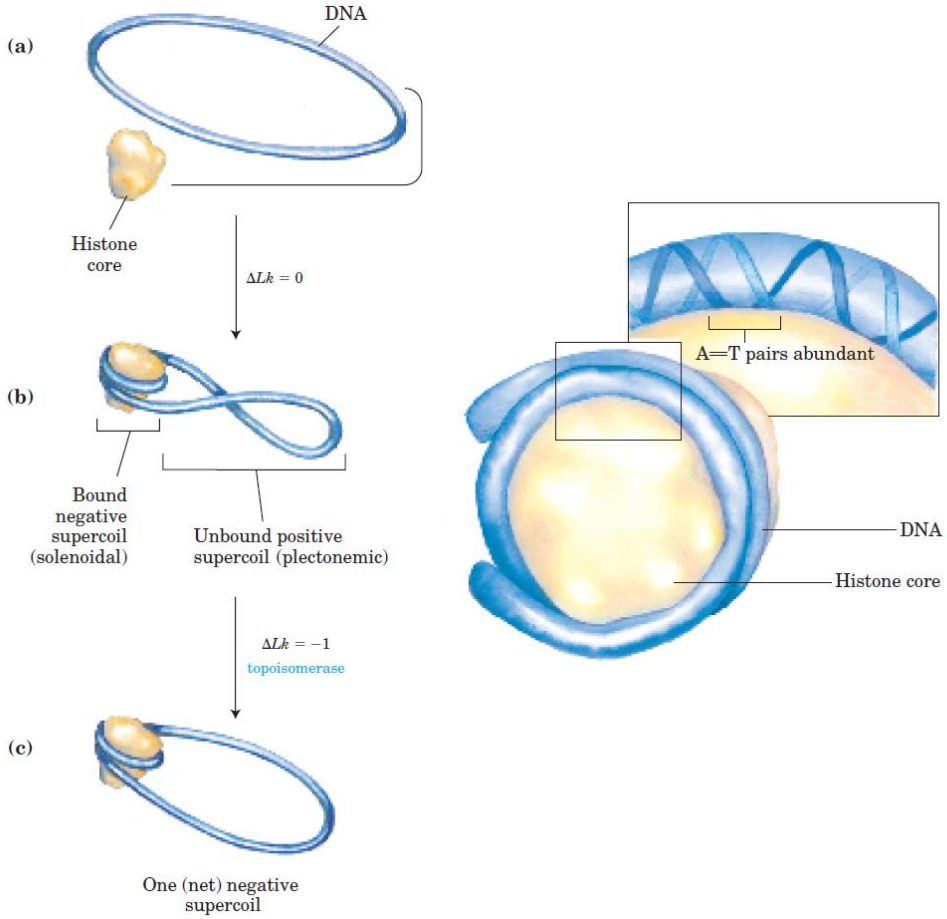
- The DNA in the chromatin is very tightly associated with proteins called histones, which package and order the DNA into structural units called nucleosomes.

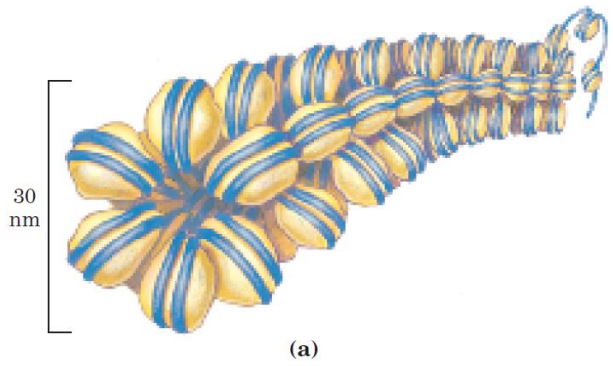


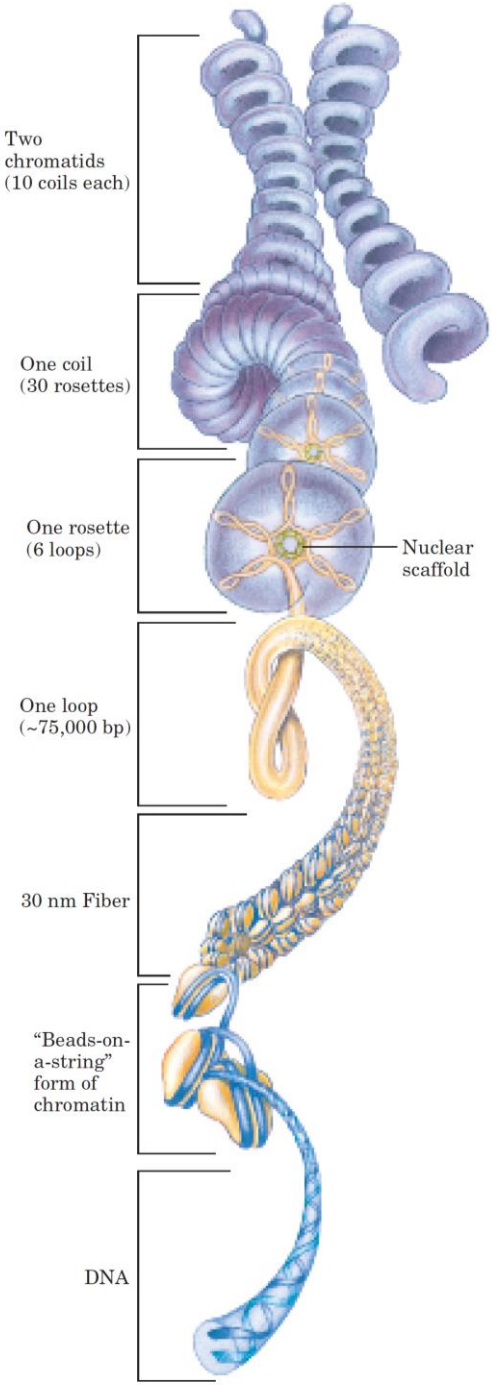


- Histones have molecular weights between 11,000 and 21,000 and are very rich in the basic amino acids arginine and lysine (together these make up about one-fourth of the amino acid residues).

- All eukaryotic cells have five major classes of histones, differing in molecular weight and amino acid composition

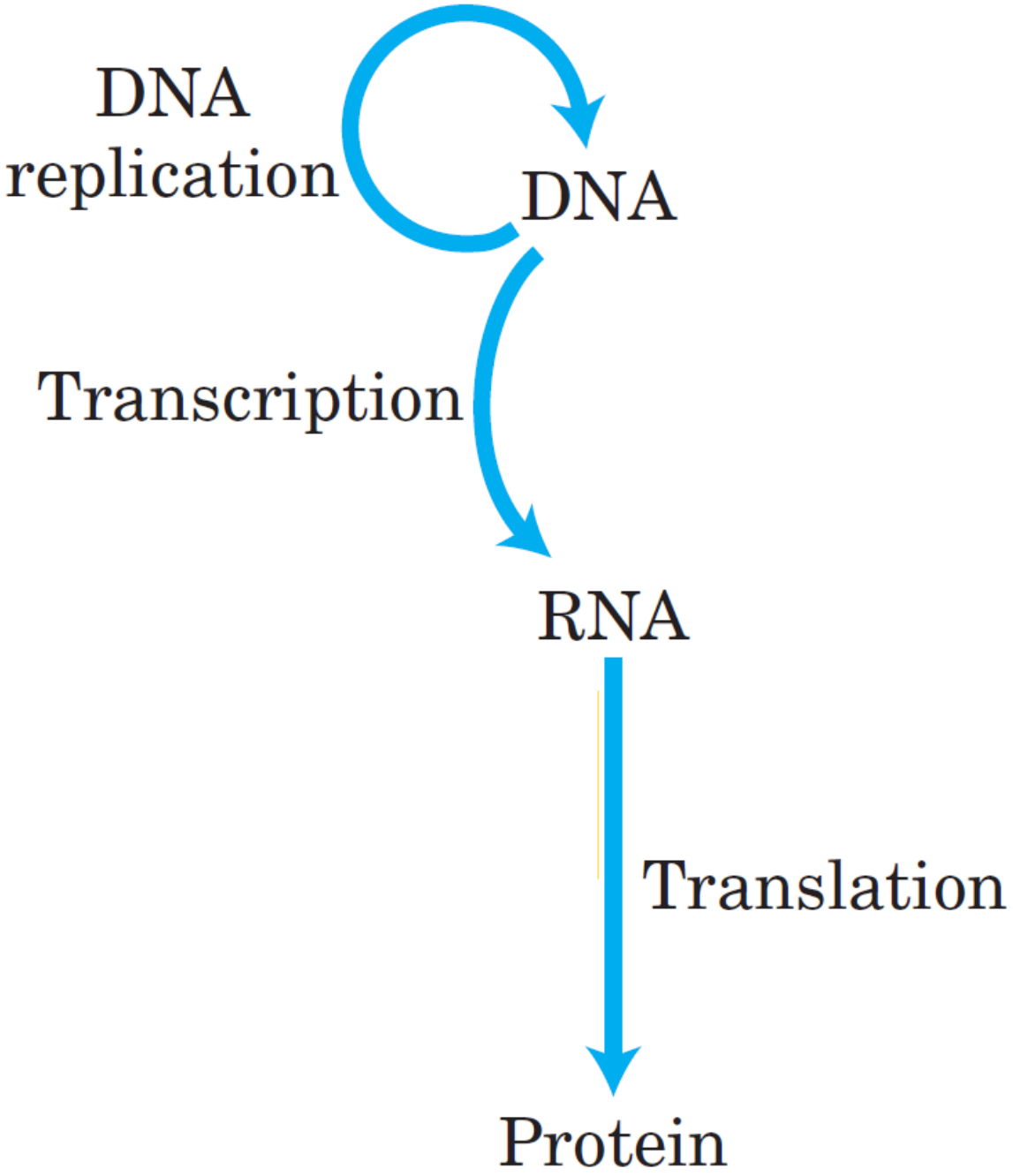






- Wrapping of DNA around a nucleosome core compacts the DNA length about sevenfold.
- The overall compaction in a chromosome, however, is greater than 10,000-fold—ample evidence for even higher orders of structural organization.
- A third major class of chromatin proteins, in addition to the histones and topoisomerases, is the **SMC proteins** (structural maintenance of chromosomes) which helps to compact the chromatin.

DNA and RNA Methabolism


DNA
replication
DNA
Transcription
RNA
Translation
Protein

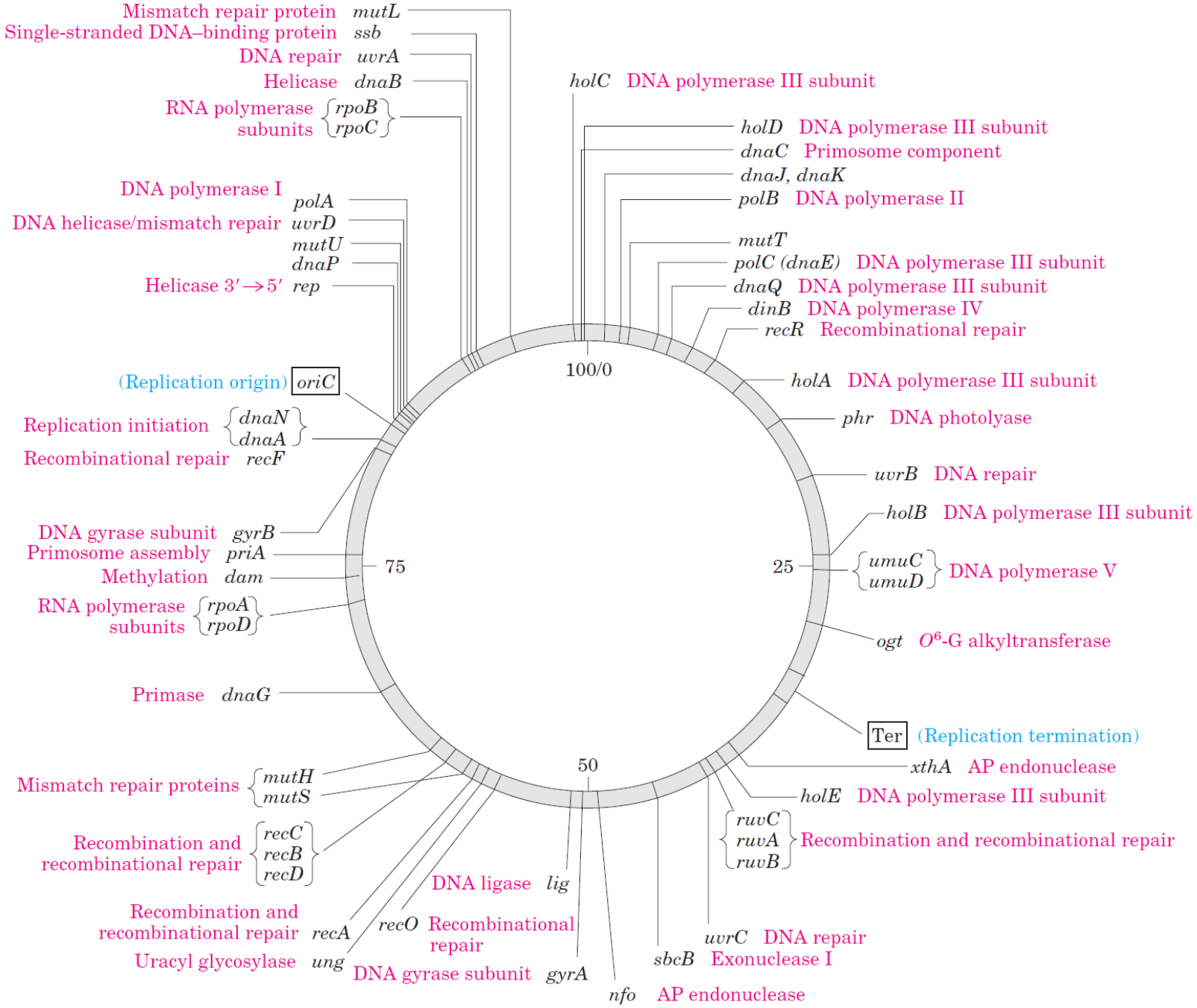
- As the repository of genetic information, DNA occupies a unique and central place among biological macromolecules.

- The nucleotide sequences of DNA encode the primary structures of all cellular RNAs and proteins and, through enzymes, indirectly affect the synthesis of all other cellular constituents.

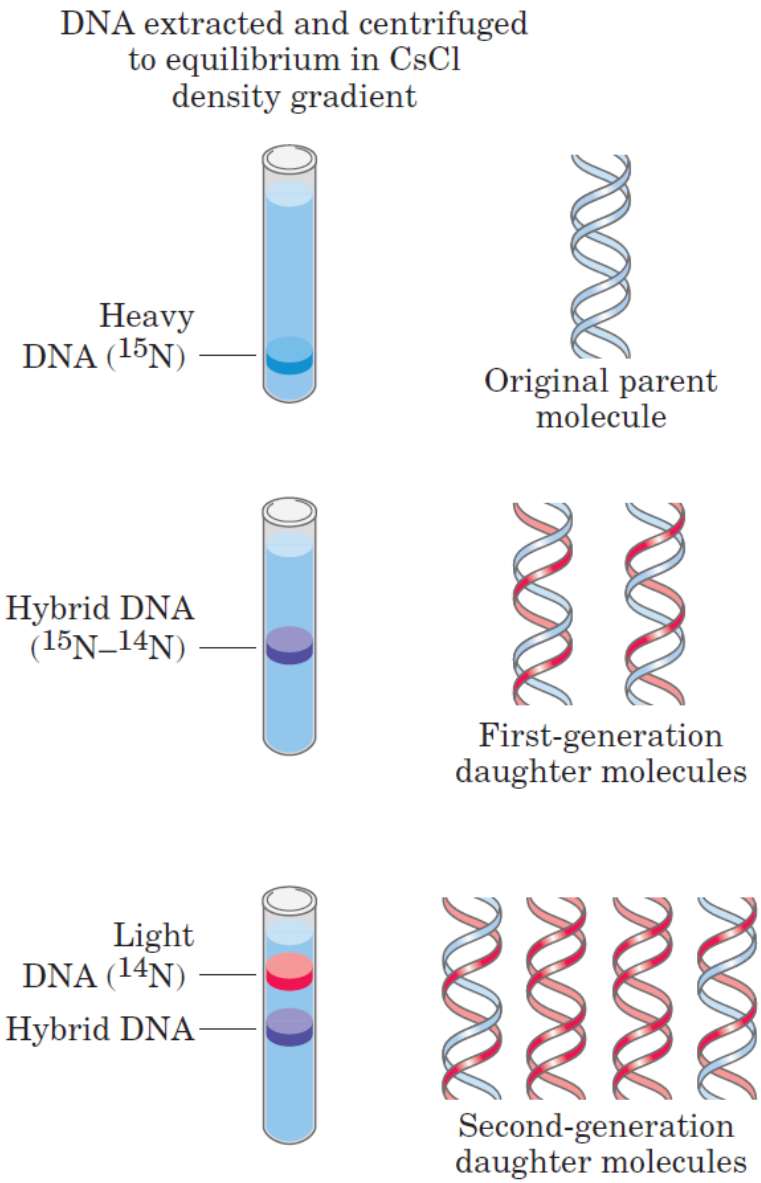
- DNA is a marvelous device for the stable storage of genetic information.

- The phrase “stable storage,” however, conveys a static and misleading picture.

- DNA metabolism comprises both the process that gives rise to faithful copies of DNA molecules (replication) and the processes that affect the inherent structure of the information (repair and recombination).

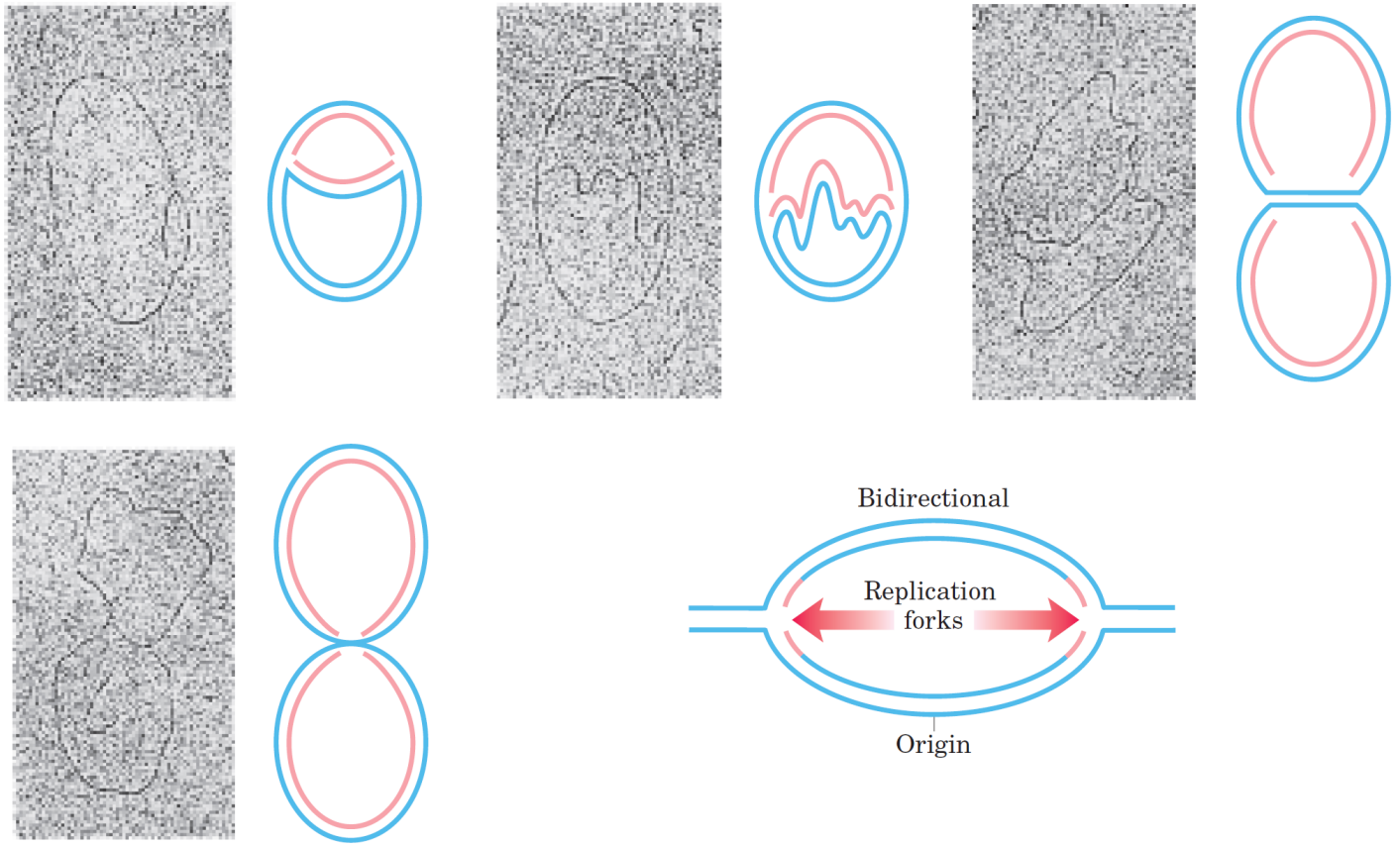

Mismatch repair protein mutL
Single-stranded DNA–binding protein ssb
DNA repair uvrA
Helicase dnaB
RNA polymerase subunits rpoB rpoC
DNA polymerase I polA
DNA helicase/mismatch repair uvrD
mutU
dnaP
Helicase 3′→5′ rep
(Replication origin) oriC
Replication initiation dnaN dnaA
Recombinational repair recF
DNA gyrase subunit gyrB
Primosome assembly priA
Methylation dam
RNA polymerase subunits rpoA rpoD
Primase dnaG
Mismatch repair proteins mutH mutS
Recombination and recombinational repair recC recB recD
Recombination and recombinational repair recA
Uracyl glycosylase ung
recO Recombinational repair
DNA ligase lig
DNA gyrase subunit gyrA
nfo AP endonuclease
sbcB Exonuclease I
uvrC DNA repair
ruvC ruvA ruvB Recombination and recombinational repair
holE DNA polymerase III subunit
xthA AP endonuclease
Ter (Replication termination)
ogt O6-G alkyltransferase
umuC umuD DNA polymerase V
holB DNA polymerase III subunit
uvrB DNA repair
phr DNA photolyase
holA DNA polymerase III subunit
recR Recombinational repair
dinB DNA polymerase IV
dnaQ DNA polymerase III subunit
polC (dnaE) DNA polymerase III subunit
mutT
polB DNA polymerase II
dnaJ, dnaK
dnaC Primosome component
holD DNA polymerase III subunit
holC DNA polymerase III subunit
100/0
25
50
75

- Organisms create faithful copies of themselves by replication.
- The fundamental properties of the DNA replication process and the mechanisms used by the enzymes that catalyze it have proved to be essentially identical in all species.
- Each DNA strand serves as a template for the synthesis of a new strand, producing two new DNA molecules, each with one new strand and one old strand.
- This is semiconservative replication.



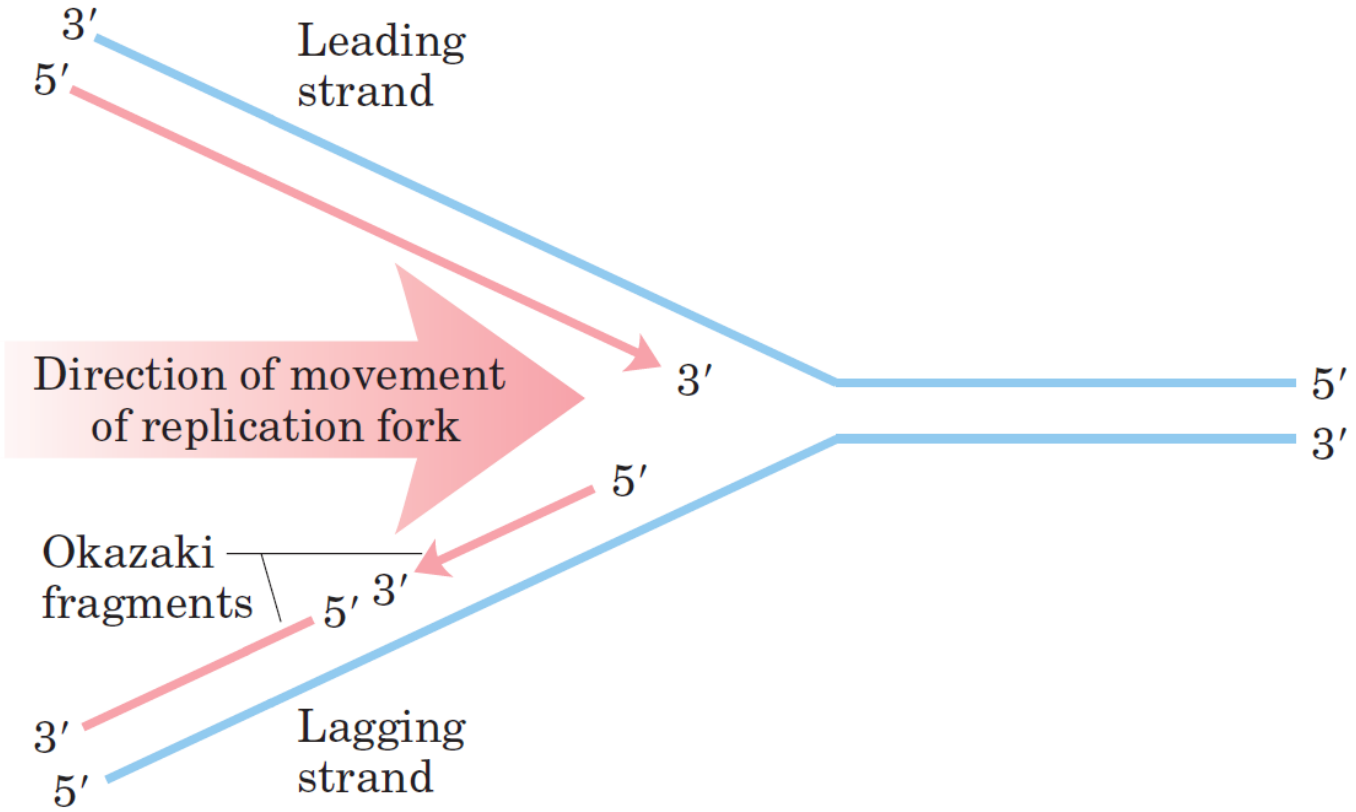
- Replication begins at an origin and usually proceeds bidirectionally.

- One or both ends of the loop are dynamic points, termed **replication forks**, where parent DNA is being unwound and the separated strands quickly replicated.



- For circular DNA molecules, the two replication forks meet at a point on the side of the circle opposite to the origin.

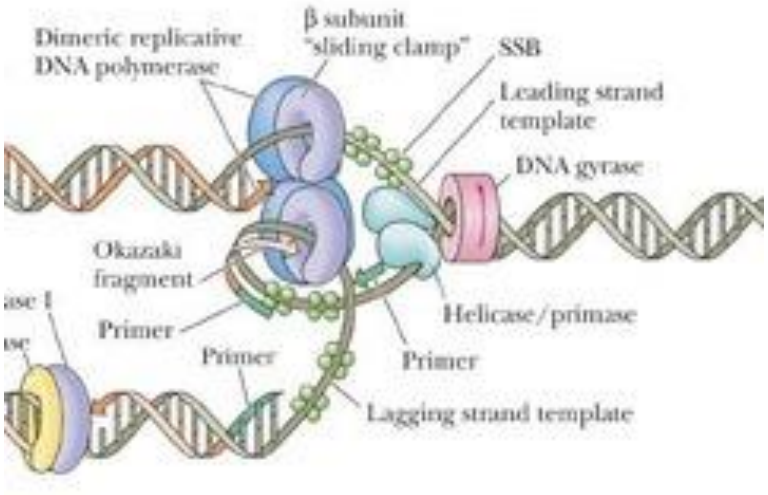
- DNA synthesis proceeds in a 5'→3' direction and is semidiscontinuous, which means one chain continously replicated (leading strand)while the other chain (lagging strand) replicate discontinously.



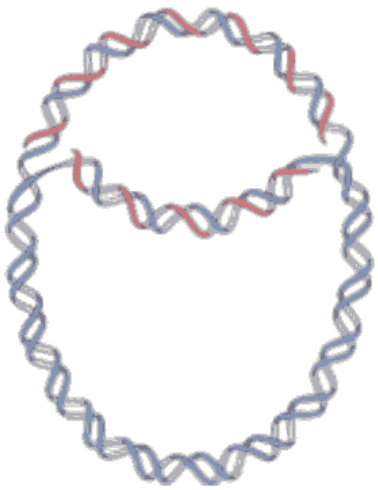
- Since a new strand of DNA is always synthesized in the 5'→3' direction, the continuous strand, or leading strand, is the one in which 5'→3' synthesis proceeds in the same direction as replication fork movement.

- The discontinuous strand, or lagging strand, is the one in which is the one in which 5'→3' synthesis proceeds in the direction opposite to the direction of fork movement.

- Therefore the new DNA strands in the lagging strand is synthesized in short pieces, called Okazaki fragments.



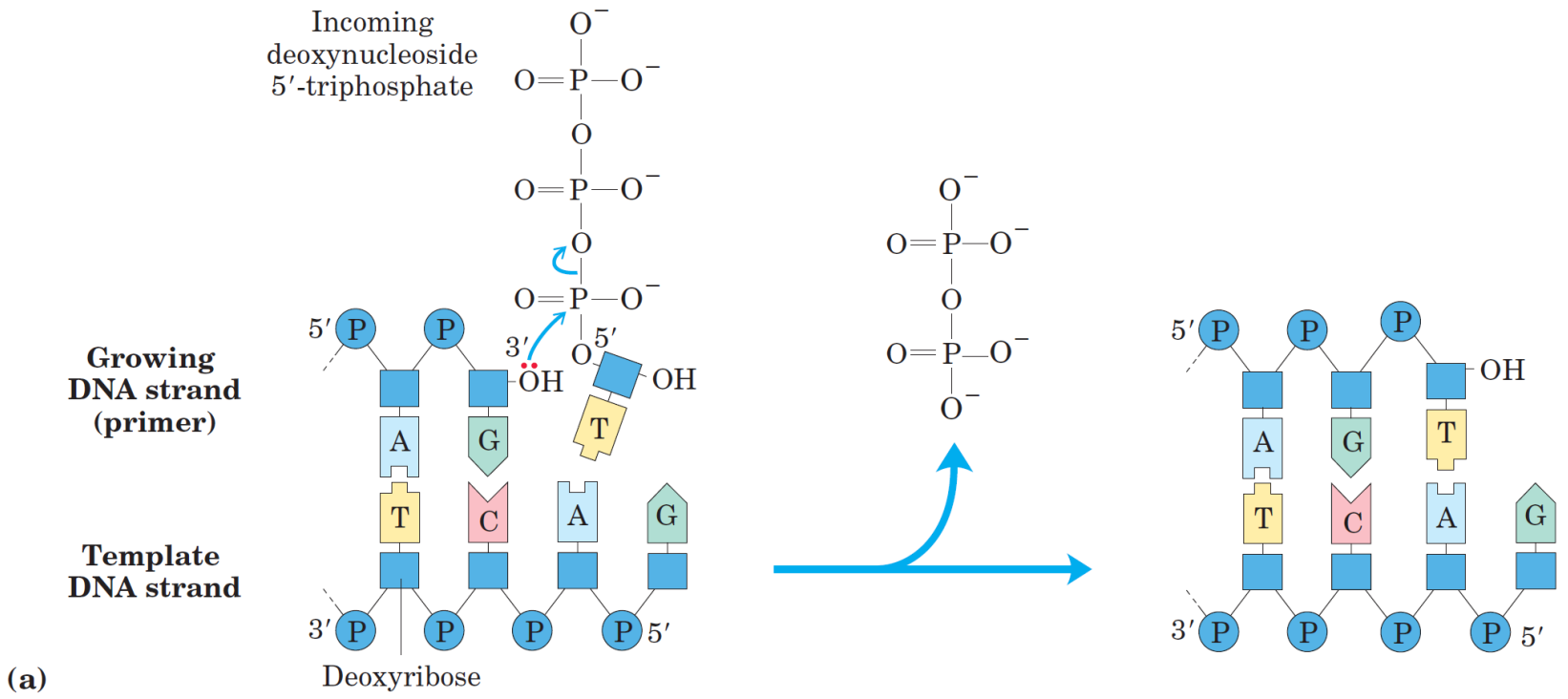
- *Circular E. Coli chromosome replicated in five stages;*
- 1) Unwinding of parental double helix
- 2) Synthesis of a RNA primer.
- 3) DNA growth at 5'→3' direction.
- 4) Removal of primer.
- 5) Ligation of newly synthesized DNA
- DNA is synthesized by DNA polymerases.
- First discovered polymerase is DNA polymerase I of *E. Coli.*
- *E. Coli contains at least four other distinct DNA polymerases.*



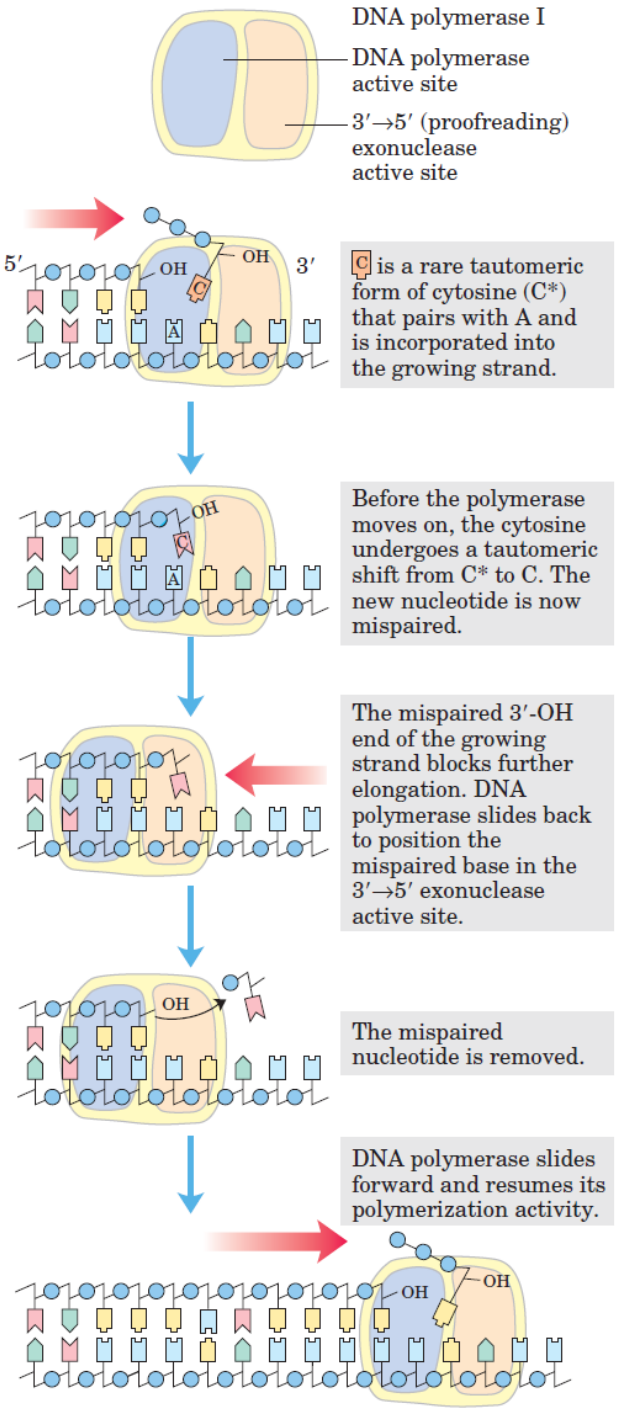
❖The fundamental reaction is a phosphoryl group transfer where dNMP and dNTP are deoxynucleoside 5’-monophosphate and 5’-triphosphate, respectively.

$$(\text{dNMP})_n + \text{dNTP} \longrightarrow (\text{dNMP})_{n+1} + \text{PP}_\text{i}$$

DNA → Lengthened DNA



- All DNA polymerases require a template. The polymerization reaction is guided by a template DNA strand according to the base-pairing rules predicted by Watson and Crick.
- DNA polymerase I require a primer. A primer is a strand segment (complementary to the template, generally RNA) with a free 3'-hydroxyl group to which a nucleotide can be added.
- Replication is very accurate. In E. coli, a mistake is made only once for every 10^{10} bp.
- DNA polymerase synthesize DNA at 5'→3' direction and has proofreading ability at 3'→5' direction.
- The 5'→3' exonuclease activity removes the mispaired nucleotide, and then correct one added.



- *E. coli* has at least five DNA polymerases.

- DNA polymerase III is the principal replication enzyme in E. coli. It is much faster than DNA polymerase I.

- DNA polymerase I, then, is not the primary enzyme of replication; instead it performs a host of clean-up functions during replication, recombination, and repair. The polymerase's special functions are enhanced by its 5'→3' exonuclease activity.

- DNA polimeraz III don't have 5'→3' exonuclease activity.

- Replication in E. coli requires not just a single DNA polymerase but 20 or more different enzymes and proteins, each performing a specific task.

- The entire complex has been termed the DNA replicase system or replisome.

- Access to the DNA strands that are to act as templates requires separation of the two parent strands. This is generally accomplished by **helicases**, enzymes that move along the DNA and separate the strands, using chemical energy from ATP.

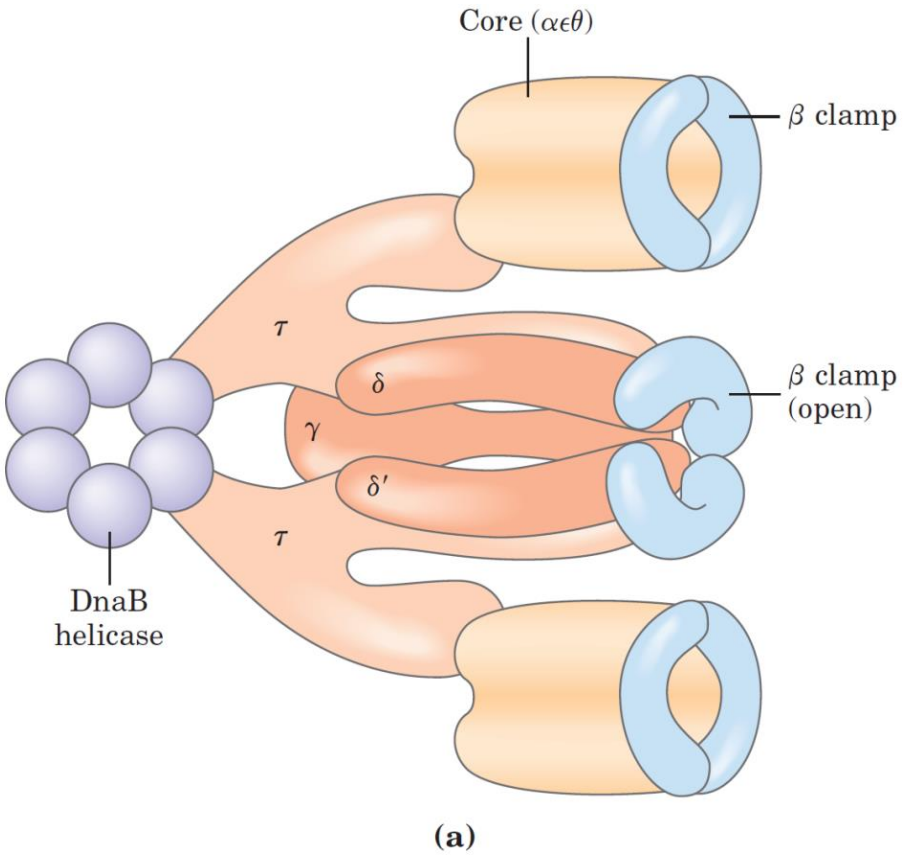
- Strand separation creates topological stress in the helical DNA structure, which is relieved by the action of **topoisomerases**.

- The separated strands are stabilized by **DNA-binding proteins**.

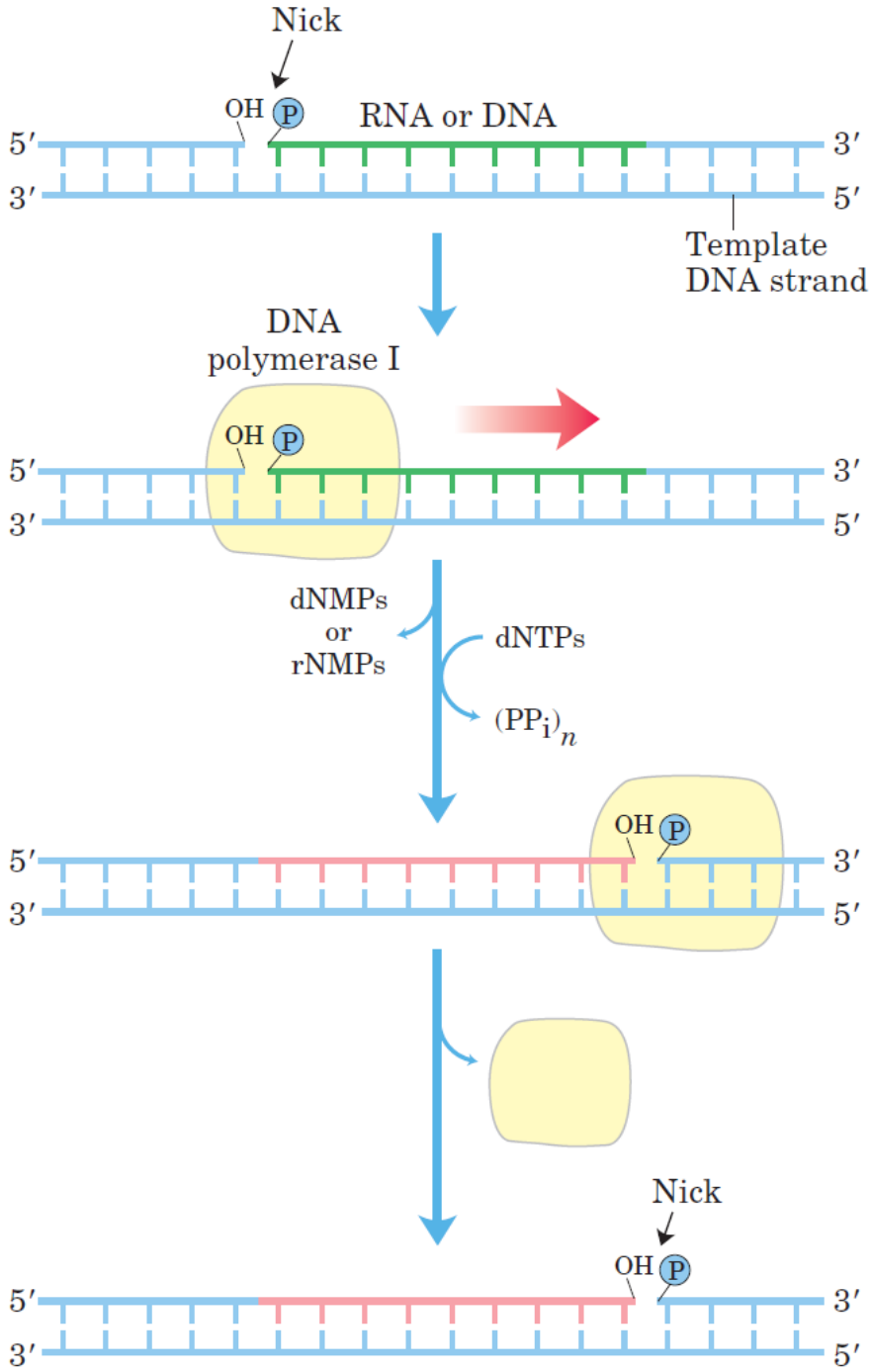
- Before DNA polymerases can begin synthesizing DNA, primers must be present on the template—generally short segments of RNA synthesized by enzymes known as **primases**.

- DNA synthesized by **DNA polymerase III**, and the RNA primers are removed and replaced by DNA; in E. coli, this is one of the many functions of **DNA polymerase I**.

- After an RNA primer is removed and the gap is filled in with DNA, a nick remains in the DNA backbone in the form of a broken phosphodiester bond. These nicks are sealed by **DNA ligases**.


Core ($\alpha\epsilon\theta$)
β clamp
τ
δ
γ
δ'
τ
β clamp
(open)
DnaB
helicase

(a)

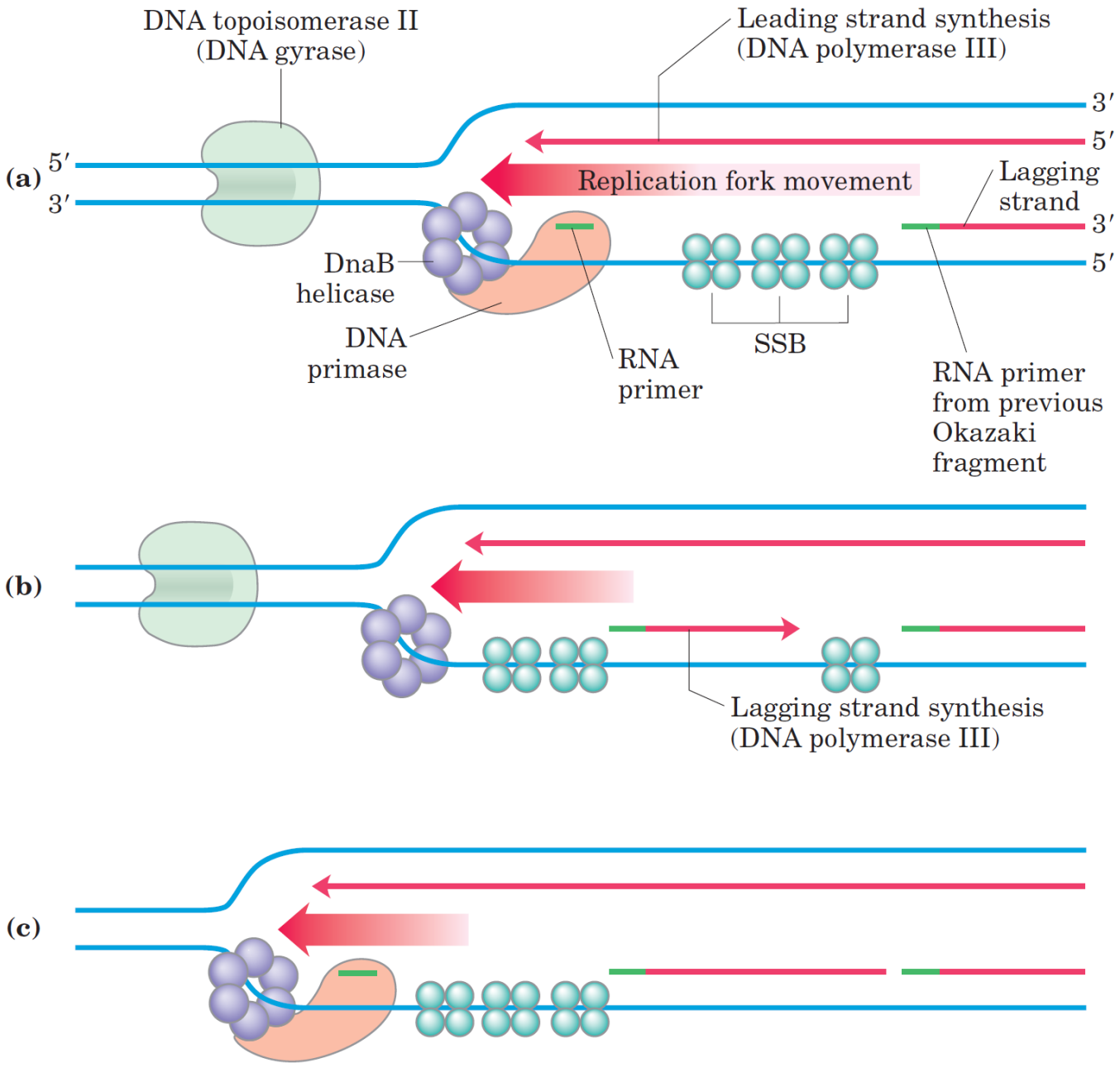

Nick
OH
P
RNA or DNA
5′
3′
3′
5′
Template
DNA strand
DNA
polymerase I
dNMPs
or
rNMPs
dNTPs
$(PP_i)_n$
Nick

- The synthesis of a DNA molecule can be divided into three stages: initiation, elongation, and termination, distinguished both by the reactions taking place and by the enzymes required.

- Initiation The E. coli replication origin, oriC, consists of 245 bp; it bears DNA sequence elements that are highly conserved among bacterial replication origins.

TABLE 25–3 Proteins Required to Initiate Replication at the *E. coli* Origin

Protein	M_r	*Number of subunits*	*Function*
DnaA protein	52,000	1	Recognizes ori sequence; opens duplex at specific sites in origin
DnaB protein (helicase)	300,000	6*	Unwinds DNA
DnaC protein	29,000	1	Required for DnaB binding at origin
HU	19,000	2	Histonelike protein; DNA-binding protein; stimulates initiation
Primase (DnaG protein)	60,000	1	Synthesizes RNA primers
Single-stranded DNA–binding protein (SSB)	75,600	4*	Binds single-stranded DNA
RNA polymerase	454,000	5	Facilitates DnaA activity
DNA gyrase (DNA topoisomerase II)	400,000	4	Relieves torsional strain generated by DNA unwinding
Dam methylase	32,000	1	Methylates (5′)GATC sequences at *oriC*


DNA topoisomerase II
(DNA gyrase)
Leading strand synthesis
(DNA polymerase III)
(a)
5′
3′
3′
5′
Replication fork movement
Lagging
strand
3′
5′
DnaB
helicase
DNA
primase
RNA
primer
SSB
RNA primer
from previous
Okazaki
fragment
(b)
Lagging strand synthesis
(DNA polymerase III)
(c)

- The elongation phase of replication includes two distinct but related operations: leading strand synthesis and lagging strand synthesis.

- Parent DNA is first unwound by DNA helicases, and the resulting topological stress is relieved by topoisomerases. Each separated strand is then stabilized by SSB.

- Leading strand synthesis, the more straightforward of the two, begins with the synthesis by primase of a short RNA primer at the replication origin. Deoxyribonucleotides are added to this primer by DNA polymerase III. Leading strand synthesis then proceeds continuously.

- Lagging strand synthesis, is accomplished in short Okazaki fragments and replication done with the same way as the leading strand, however since DNA polymerase III only synthesize DNA at 5'→3' direction some conformational changes occurs in DNA chain.

- Once an Okazaki fragment has been completed, its RNA primer is removed and replaced with DNA by DNA polymerase I, and the remaining nick is sealed by DNA ligase.

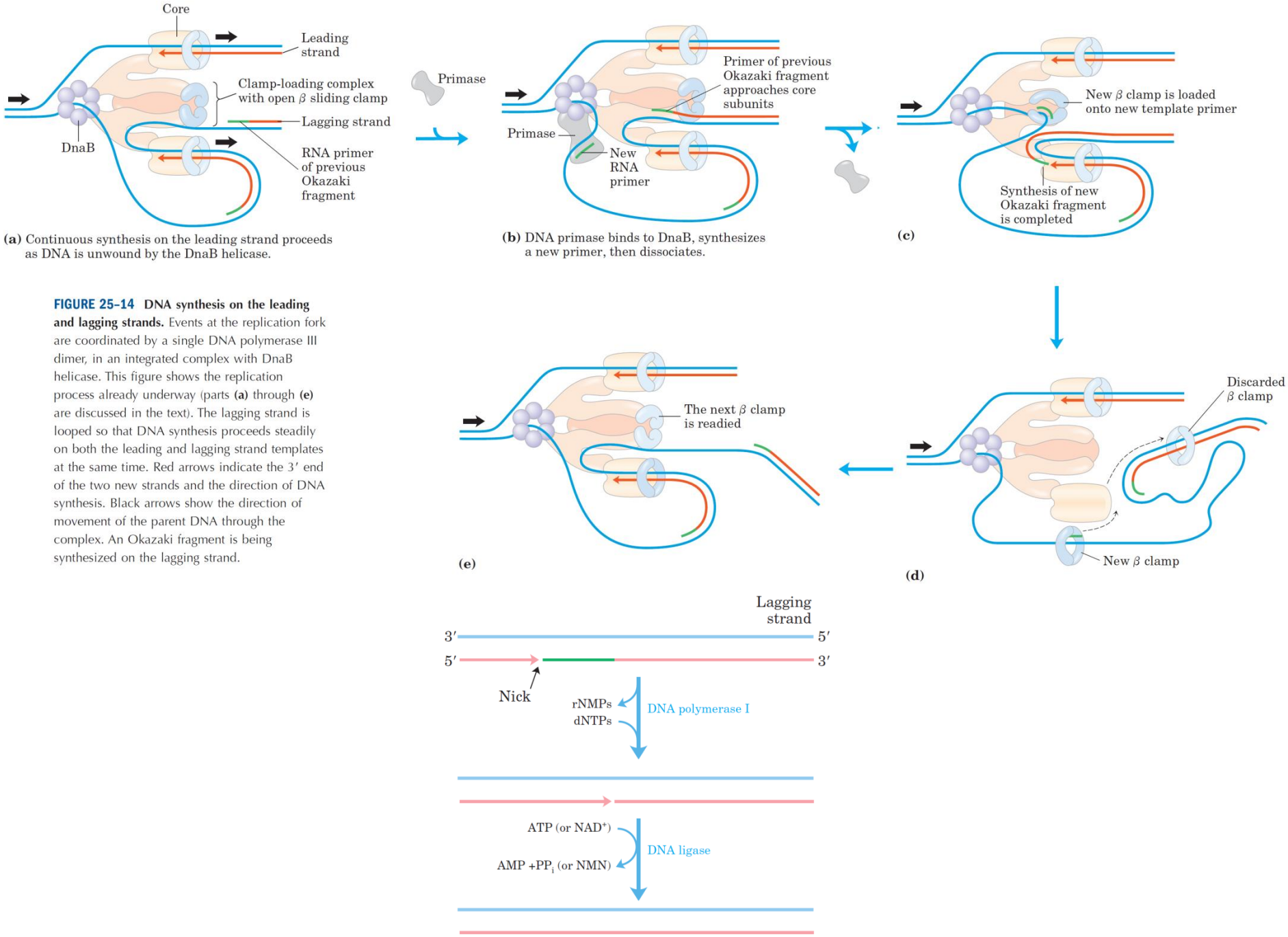
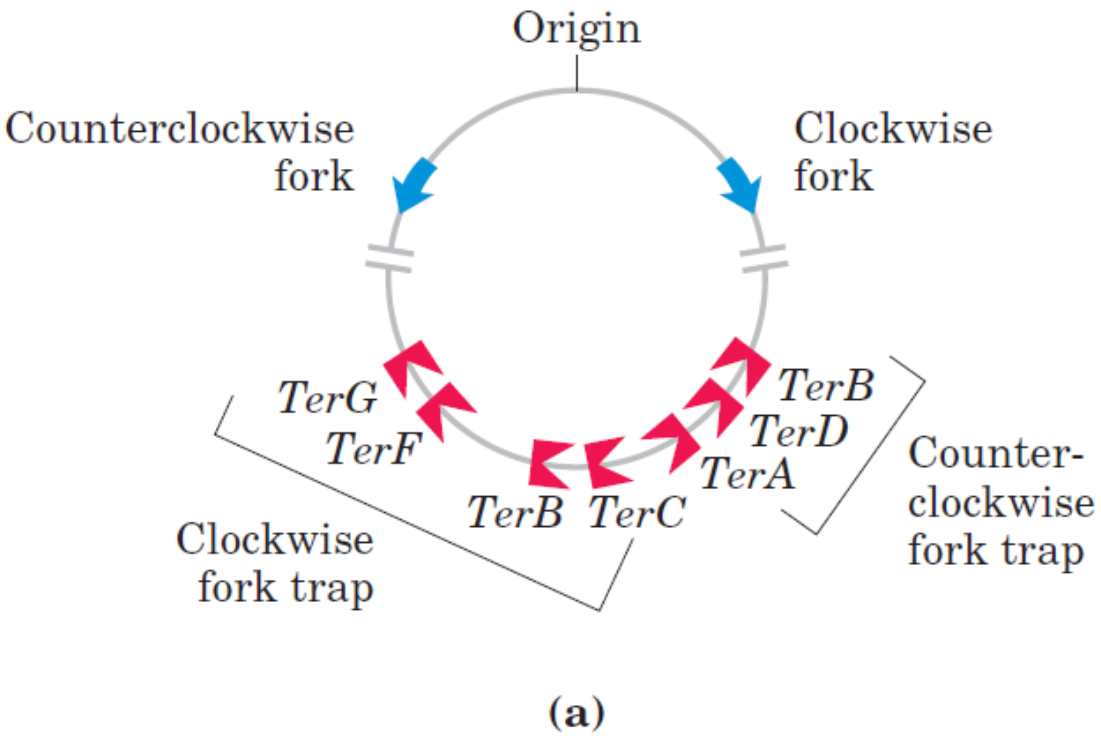


FIGURE 25–14 DNA synthesis on the leading and lagging strands. Events at the replication fork are coordinated by a single DNA polymerase III dimer, in an integrated complex with DnaB helicase. This figure shows the replication process already underway (parts **(a)** through **(e)** are discussed in the text). The lagging strand is looped so that DNA synthesis proceeds steadily on both the leading and lagging strand templates at the same time. Red arrows indicate the 3′ end of the two new strands and the direction of DNA synthesis. Black arrows show the direction of movement of the parent DNA through the complex. An Okazaki fragment is being synthesized on the lagging strand.

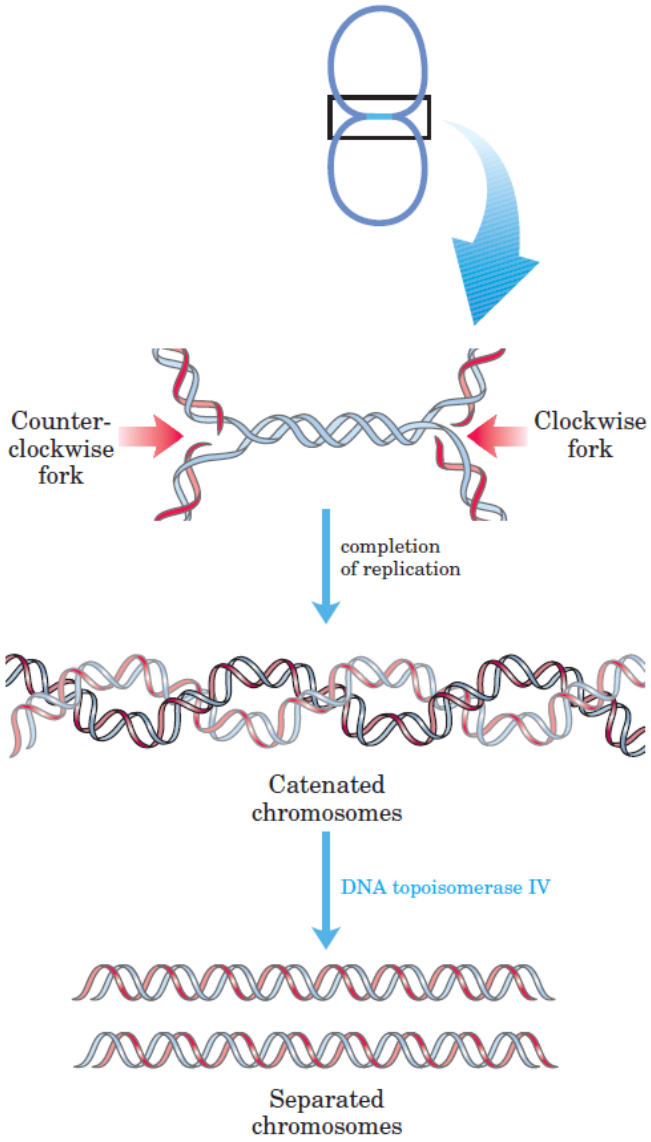
TABLE 25–4 Proteins at the *E. coli* Replication Fork

Protein	M_r	*Number of subunits*	*Function*
SSB	75,600	4	Binding to single-stranded DNA
DnaB protein (helicase)	300,000	6	DNA unwinding; primosome constituent
Primase (DnaG protein)	60,000	1	RNA primer synthesis; primosome constituent
DNA polymerase III	791,500	17	New strand elongation
DNA polymerase I	103,000	1	Filling of gaps; excision of primers
DNA ligase	74,000	1	Ligation
DNA gyrase (DNA topoisomerase II)	400,000	4	Supercoiling

- Termination Eventually, the two replication forks of the circular E. coli chromosome meet at a terminus region.
- The terminus sequences are arranged on the chromosome to create a sort of trap that a replication fork can enter but cannot leave.
- Separation of the catenated circles in E. coli requires topoisomerase IV (a type II topoisomerase).
- The separated chromosomes then segregate into daughter cells at cell division.


Origin
Counterclockwise fork
Clockwise fork
TerG
TerF
TerB
TerC
TerA
TerD
TerB
Clockwise fork trap
Counter-clockwise fork trap

(a)


Counter-clockwise fork
Clockwise fork
completion of replication
Catenated chromosomes
DNA topoisomerase IV
Separated chromosomes

(b)

- The DNA molecules in eukaryotic cells are considerably larger than those in bacteria and are organized into complex nucleoprotein structures.

- The essential features of DNA replication are the same in eukaryotes and prokaryotes, and many of the protein complexes are functionally and structurally conserved.

- Eukaryotes contain many origins of replication, called autonomously replicating sequences (ARS) or replicators, therefore replication start at different points.

- The rate of replication fork movement in eukaryotes (~50 nucleotides/s) is only one-twentieth that observed in E. Coli.

- Like bacteria, eukaryotes have several types of DNA polymerases.

Table 7-4 DNA polymerase in eukaryotic cells

Property	α	β	γ	δ	ε
Intracellular compartment	Nucleus	Nucleus	Mitochondria	Nucleus	Nucleus
Primase binding	Present	Absent	Absent	Absent	Absent
Biological function	Replication initiation	DNA repair	Mitochondrial DNA replication	Lagging strand replication	Leading strand replication
Subunits	4	1	4 (identical)	2	?
Molecular weight of catalytic site (X10^3)	160–185	40	125	125	210–230 or 125–140
Km against dNTP (μM)	2–5	10	0.5	2–4	?
3′ exonuclease activity	Absent	Absent	Present	Present	Present
Sensitivity to arabinosyl-CTP	High	Low	Low	High	?
Sensitivity to aphidicolin	High	Low	Low	High	High

- A cell generally has only one or two sets of genomic DNA. Damaged proteins and RNA molecules can be quickly replaced by using information encoded in the DNA, but DNA molecules themselves are irreplaceable.

- Maintaining the integrity of the information in DNA is a cellular imperative, supported by an elaborate set of DNA repair systems. DNA can become damaged by a variety of processes, some spontaneous, others catalyzed by environmental agents.

- The most serious outcome is a change in the base sequence of the DNA, which, if replicated and transmitted to future cell generations, becomes permanent.

- A permanent change in the nucleotide sequence of DNA is called a mutation.

- In mammals there is a strong correlation between the accumulation of mutations and cancer.

- A simple test developed by Bruce Ames measures the potential of a given chemical compound to promote certain easily detected mutations in a specialized bacterial strain.

- Hücrelerde farklı tamir sistemleri mevcuttur, farklı DNA hasarlarında farklı sistemler devreye girerek hasarlı DNA'yı tamir ederler.

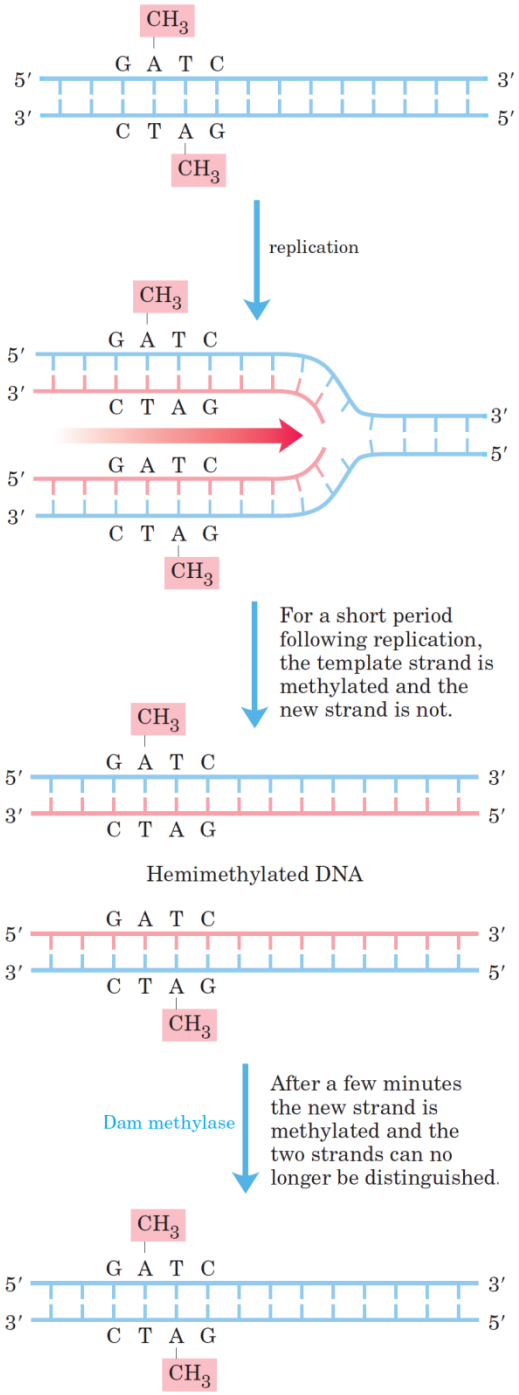
TABLE 25–5 Types of DNA Repair Systems in *E. coli*

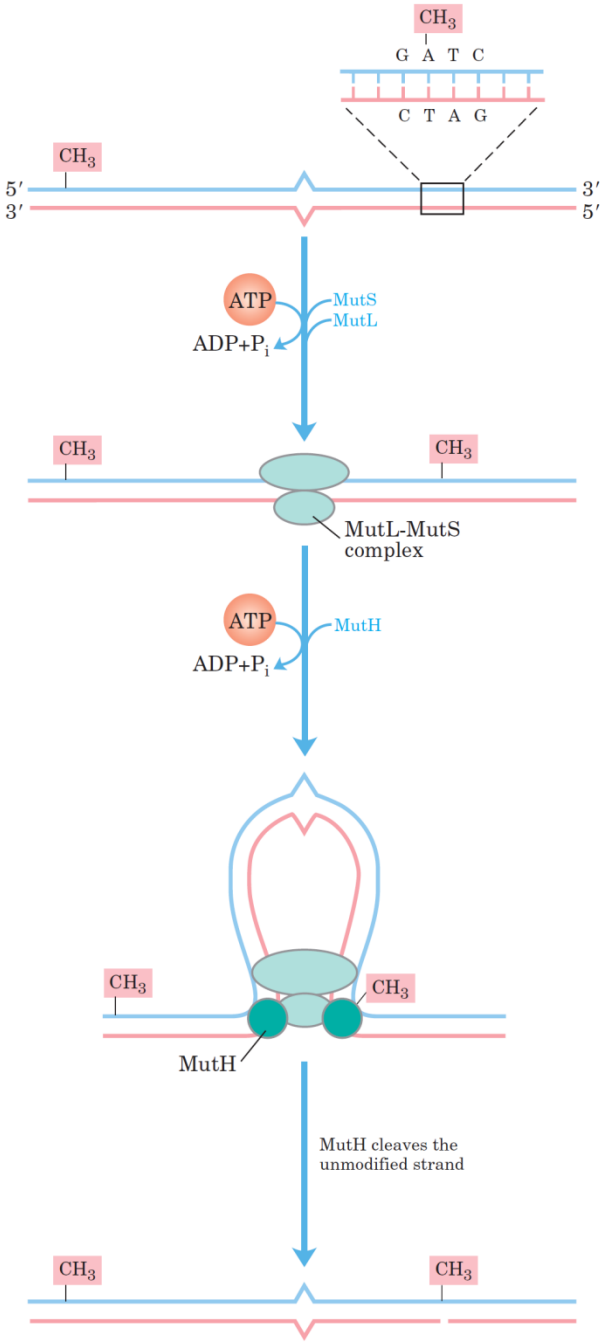
Enzymes/proteins	*Type of damage*
Mismatch repair	
Dam methylase	Mismatches
MutH, MutL, MutS proteins	
DNA helicase II	
SSB	
DNA polymerase III	
Exonuclease I	
Exonuclease VII	
RecJ nuclease	
Exonuclease X	
DNA ligase	
Base-excision repair	
DNA glycosylases	Abnormal bases (uracil, hypoxanthine, xanthine); alkylated bases; in some other organisms, pyrimidine dimers
AP endonucleases	
DNA polymerase I	
DNA ligase	
Nucleotide-excision repair	
ABC excinuclease	DNA lesions that cause large structural changes (e.g., pyrimidine dimers)
DNA polymerase I	
DNA ligase	
Direct repair	
DNA photolyases	Pyrimidine dimers
O^6-Methylguanine-DNA methyltransferase	O^6-Methylguanine
AlkB protein	1-Methylguanine, 3-methylcytosine

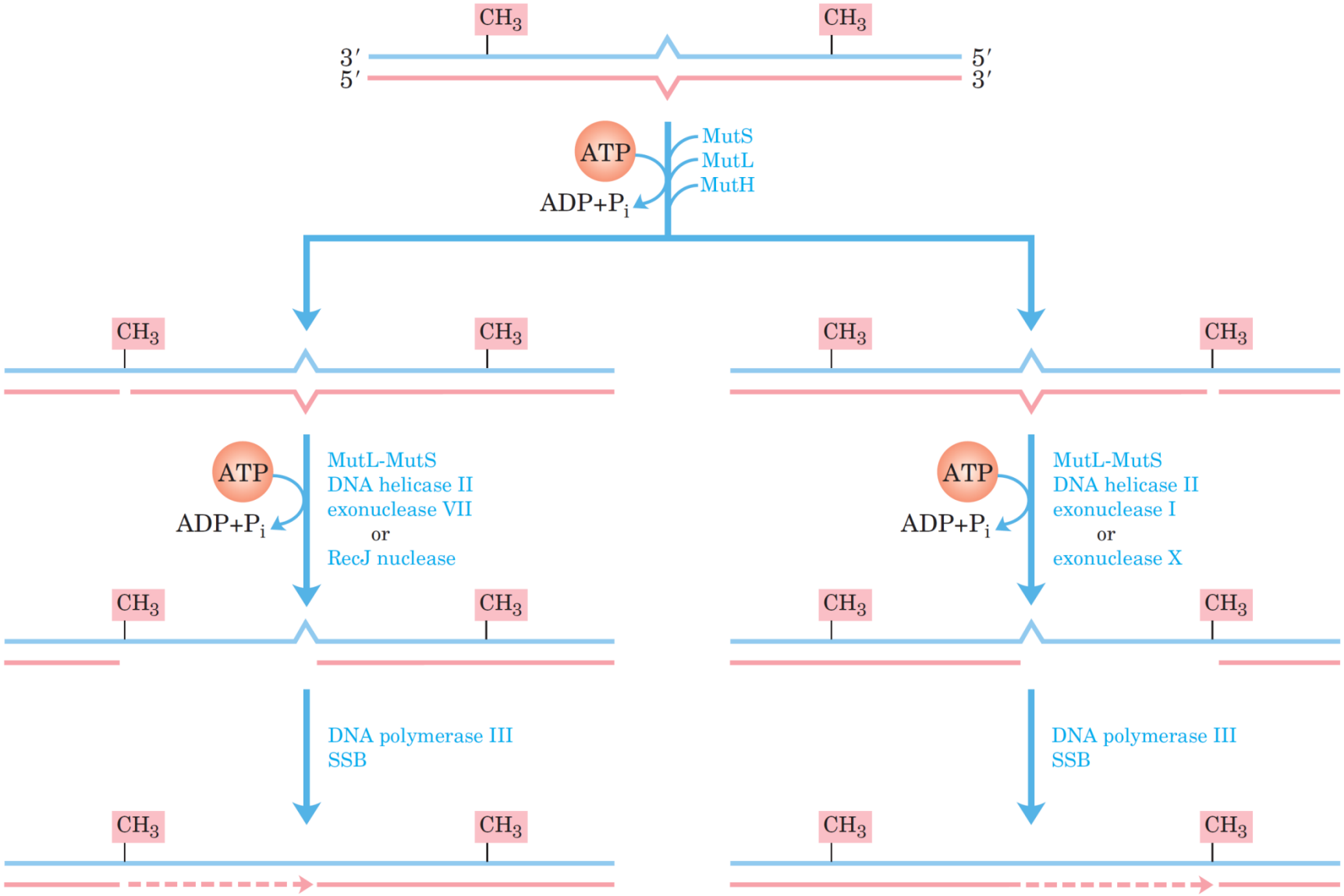
- **Mismatch Repair** correction of the rare mismatches left after replication in E. coli improves the overall fidelity of replication

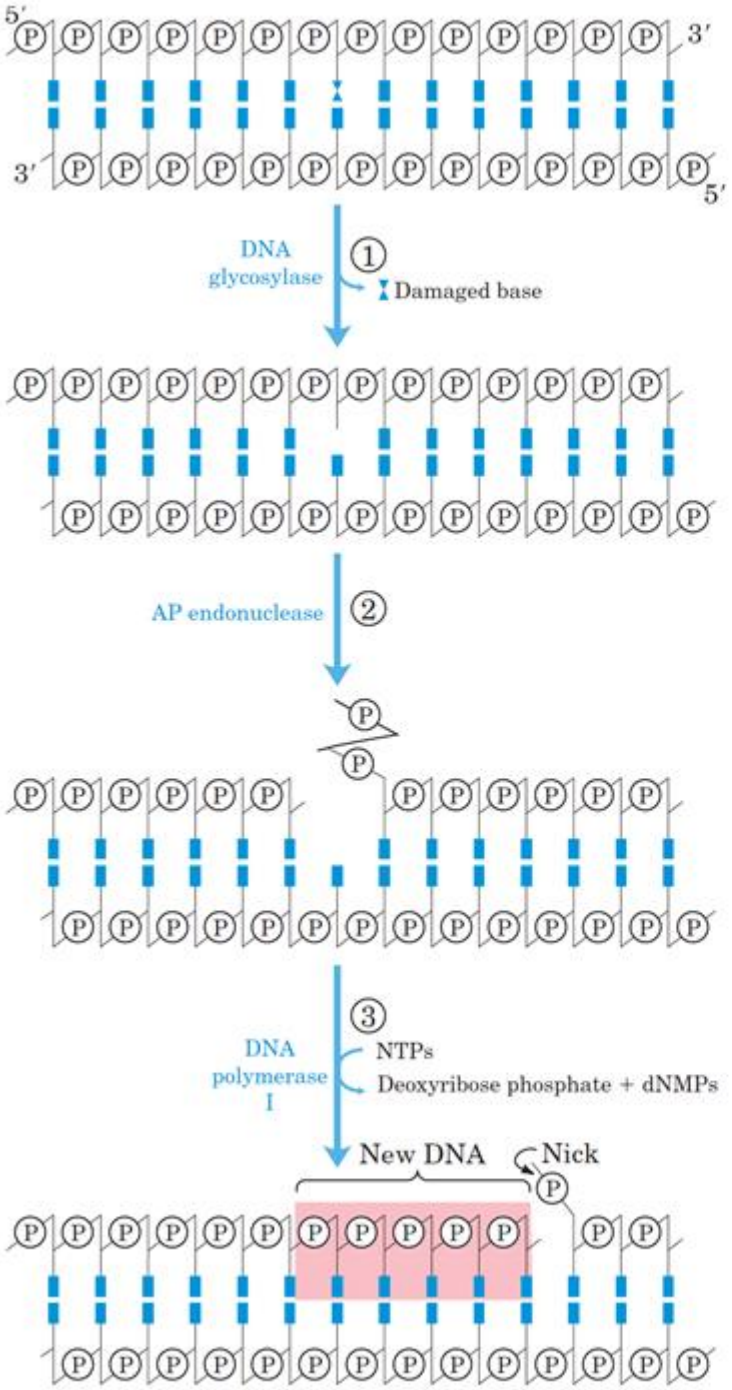
- Old (correct strand) determined by methylation in E. Coli.

- Özel proteinler metillenmiş GTAC sekuansını tanıyarak eski zincirin yenisinden ayırt edilmesini sağlarlar.






CH3
CH3
3′
5′
5′
3′
ATP
ADP+Pi
MutS
MutL
MutH
CH3
CH3
CH3
CH3
ATP
ADP+Pi
MutL-MutS
DNA helicase II
exonuclease VII
or
RecJ nuclease
ATP
ADP+Pi
MutL-MutS
DNA helicase II
exonuclease I
or
exonuclease X
CH3
CH3
CH3
CH3
DNA polymerase III
SSB
DNA polymerase III
SSB
CH3
CH3
CH3
CH3



❖ **Base-Excision Repair** Every cell has a class of enzymes called DNA glycosylases that recognize particularly common DNA lesions (such as the products of cytosine and adenine deamination) and remove the affected base by cleaving the N-glycosyl bond.

FIGURE 25–23 DNA repair by the base-excision repair pathway. ① A DNA glycosylase recognizes a damaged base and cleaves between the base and deoxyribose in the backbone. ② An AP endonuclease cleaves the phosphodiester backbone near the AP site. ③ DNA polymerase I initiates repair synthesis from the free 3′ hydroxyl at the nick, removing (with its 5′→3′ exonuclease activity) a portion of the damaged strand and replacing it with undamaged DNA. ④ The nick remaining after DNA polymerase I has dissociated is sealed by DNA ligase.

- DNA lesions that cause large distortions in the helical structure of DNA generally are repaired by the **nucleotide-excision repair** system

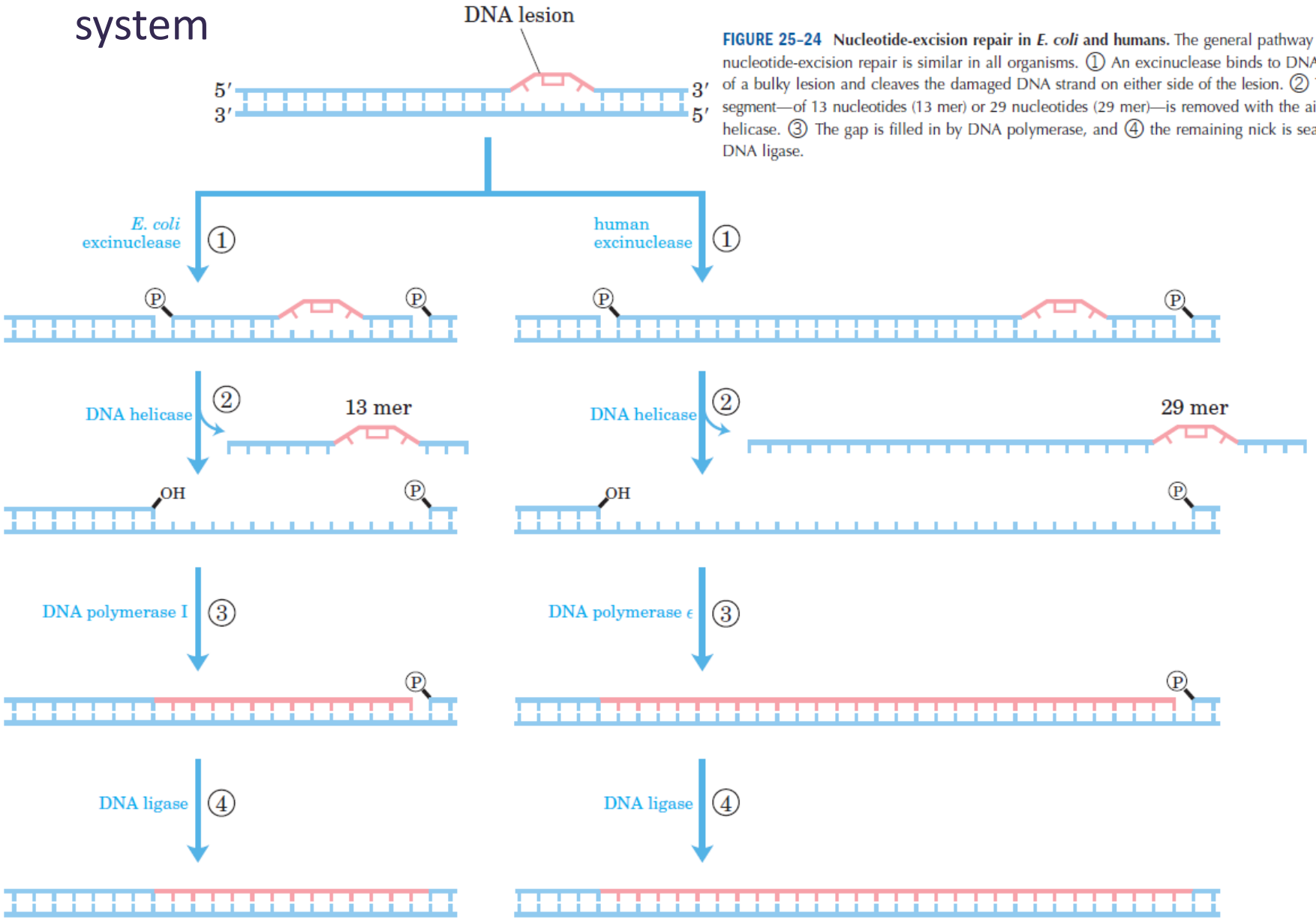
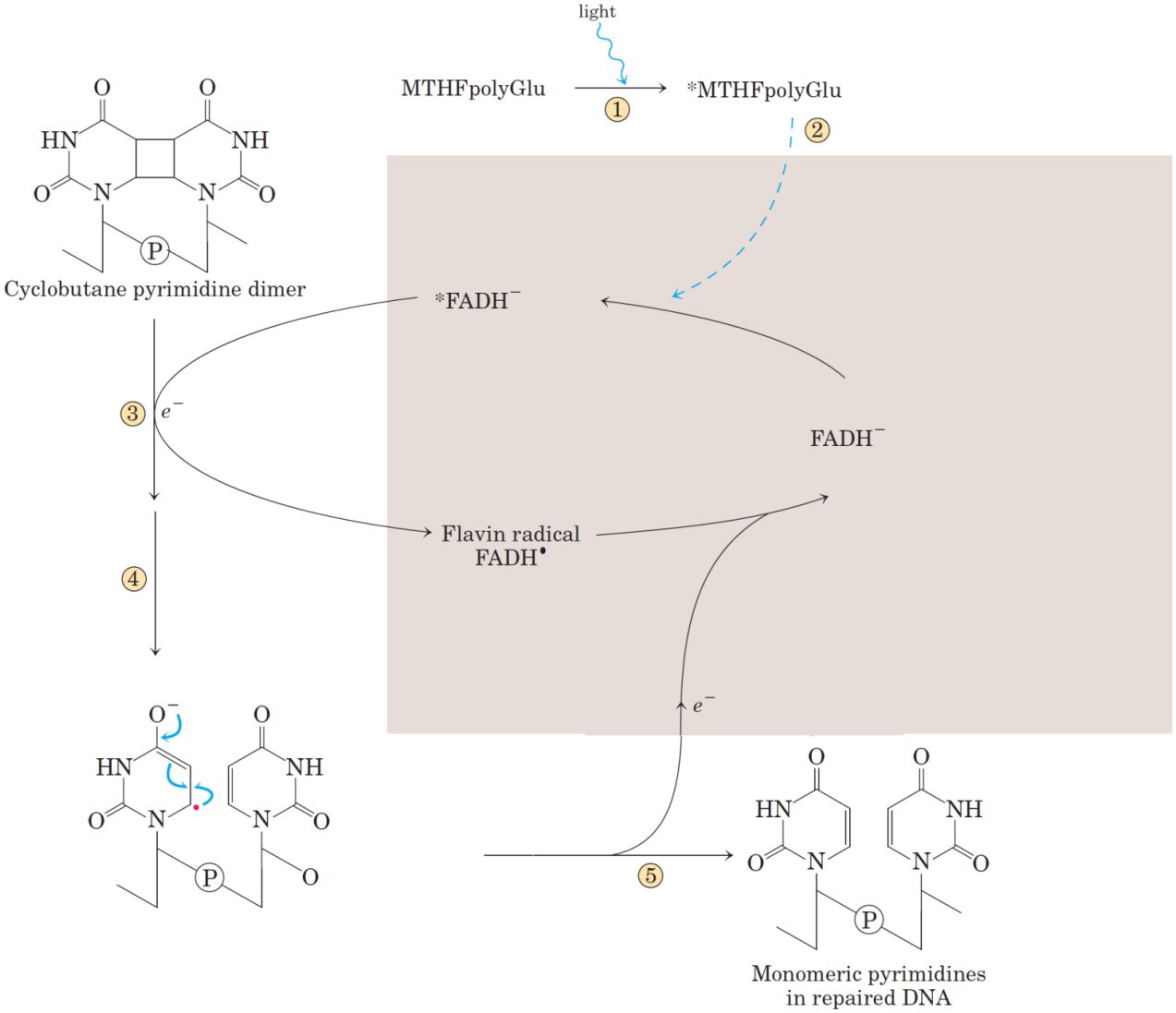


FIGURE 25-24 **Nucleotide-excision repair in *E. coli* and humans.** The general pathway of nucleotide-excision repair is similar in all organisms. ① An excinuclease binds to DNA at the site of a bulky lesion and cleaves the damaged DNA strand on either side of the lesion. ② The DNA segment—of 13 nucleotides (13 mer) or 29 nucleotides (29 mer)—is removed with the aid of a helicase. ③ The gap is filled in by DNA polymerase, and ④ the remaining nick is sealed with DNA ligase.

❖ **Direct Repair** Several types of damage are repaired without removing a base or nucleotide. The best-characterized example is direct photoreactivation of cyclobutane pyrimidine dimers, a reaction promoted by DNA photolyases.



❖Transposable elements, or **transposons** are segments of DNA, found in virtually all cells, move, or "jump," from one place on a chromosome to another on the same or a different chromosome.

❖DNA sequence homology is not usually required for this movement, called transposition; the new location is determined more or less randomly.

❖Insertion of a transposon in an essential gene could kill the cell, so transposition is tightly regulated and usually very infrequent. Transposons are perhaps the simplest of molecular parasites, adapted to replicate passively within the chromosomes of host cells.

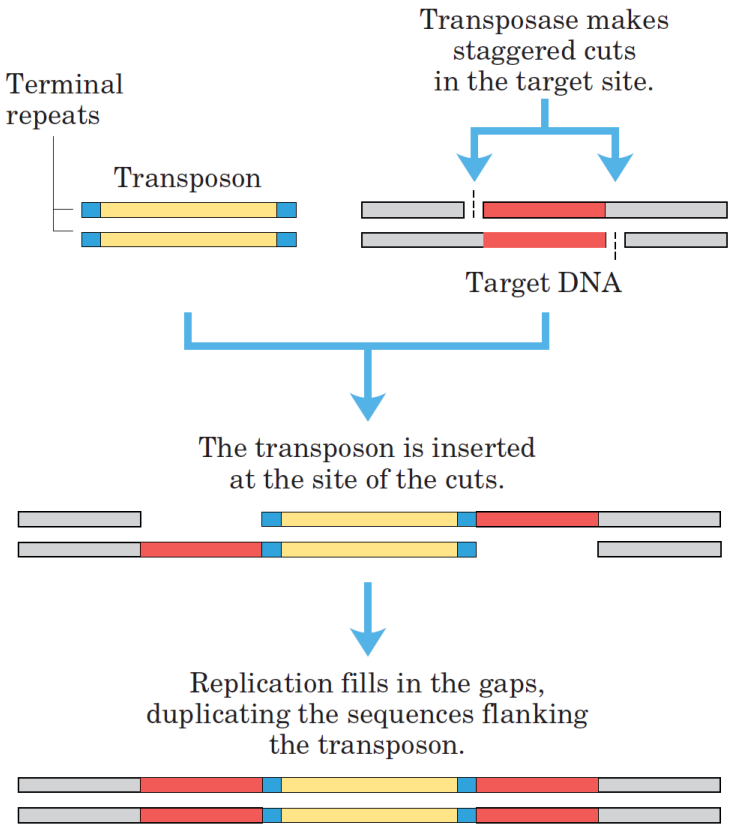


FIGURE 25–42 Duplication of the DNA sequence at a target site when a transposon is inserted. The duplicated sequences are shown in red. These sequences are generally only a few base pairs long, so their size (compared with that of a typical transposon) is greatly exaggerated in this drawing.

❖Expression of the information in a gene generally involves production of an RNA molecule transcribed from a DNA template.

❖Strands of RNA and DNA may seem quite similar at first glance, differing only in that RNA has a hydroxyl group at the 2’ position of the aldopentose and uracil instead of thymine.

❖However, unlike DNA, most RNAs carry out their functions as single strands, strands that fold back on themselves and have the potential for much greater structural diversity than DNA.

❖RNA is thus suited to a variety of cellular functions.

❖Bazı virüslerde yer alan RNA genomlarının dışında kalan bütün RNA molekülleri bir DNA kalıbı üzerinden sentezlenmişlerdir.

❖All RNA molecules except the RNA genomes of certain viruses are derived from information permanently stored in DNA with a mechanism called as **transcription.**

❖During transcription, an enzyme system converts the genetic information in a segment of double-stranded DNA into an RNA strand with a base sequence complementary to one of the DNA strands.

❖Three major kinds of RNA are produced.

❖**Messenger RNAs (mRNAs)** encode the amino acid sequence of one or more polypeptides specified by a gene or set of genes.

❖**Transfer RNAs (tRNAs)** read the information encoded in the mRNA and transfer the appropriate amino acid to a growing polypeptide chain during protein synthesis.

❖**Ribosomal RNAs (rRNAs)** are constituents of ribosomes, the intricate cellular machines that synthesize proteins.

❖Many additional specialized RNAs have regulatory or catalytic functions or are precursors to the three main classes of RNA.

❖During replication the entire chromosome is usually copied, but transcription is more selective.

❖Only particular genes or groups of genes are transcribed at any one time, and some portions of the DNA genome are never transcribed.

❖Transcription resembles replication in its fundamental chemical mechanism, its polarity (direction of synthesis), and its use of a template. And like replication, transcription has initiation, elongation, and termination phases.

❖Transcription differs from replication in that it does not require a primer and, generally, involves only limited segments of a DNA molecule.

❖Additionally, within transcribed segments only one DNA strand serves as a template.

❖RNA Is synthesized by RNA polymerases and the chemistry and mechanism of RNA synthesis closely resemble those used by DNA polymerases (with the exception of primer dependance).

❖Initiation occurs when RNA polymerase binds at specific DNA sequences called promoters, newly synthesized RNA remain attached to the DNA until the end of the transcription.

❖During the elongation phase of transcription, the growing end of the new RNA strand base-pairs temporarily with the DNA template to form a short hybrid RNA-DNA double helix.

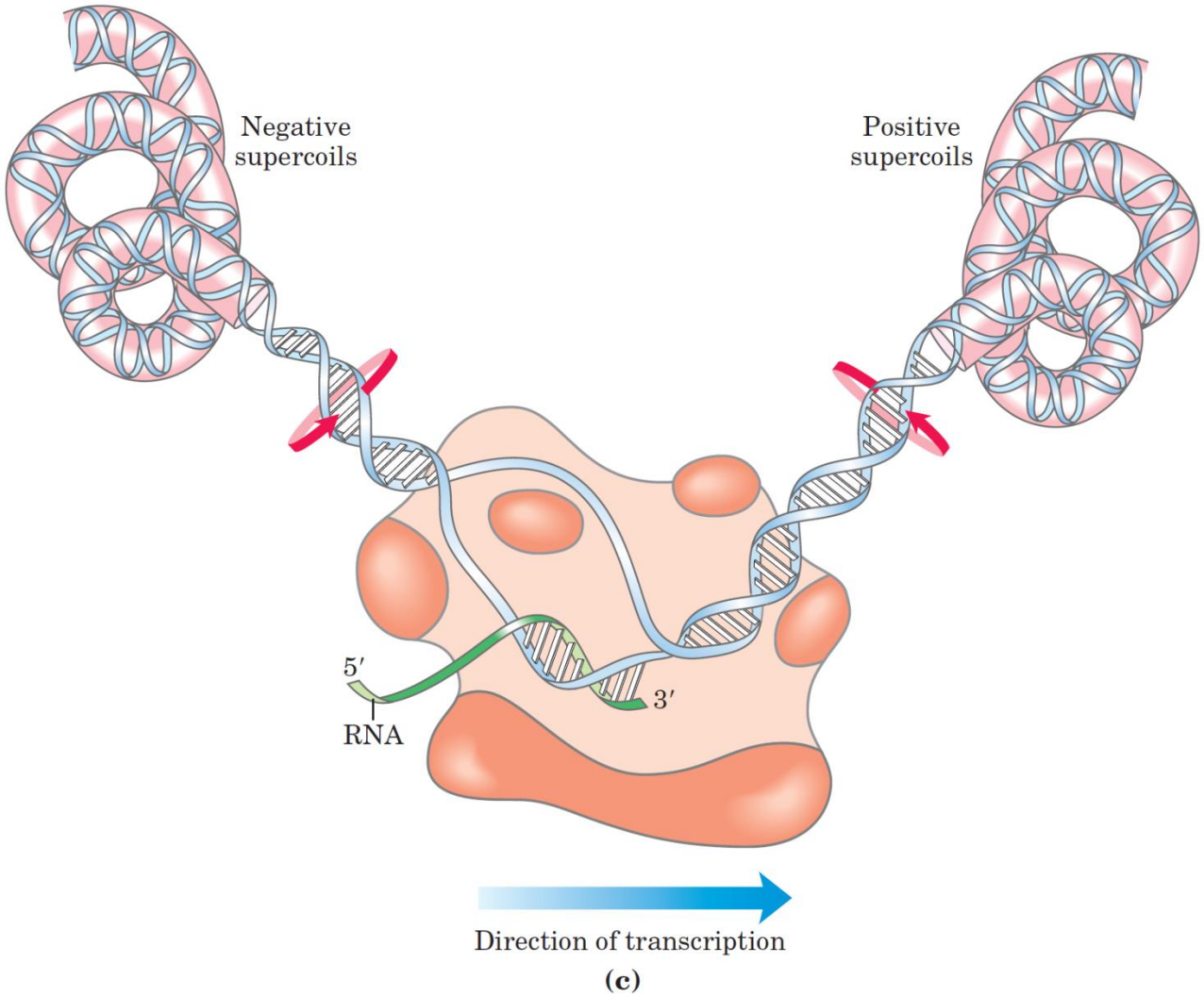
❖To enable RNA polymerase to synthesize an RNA strand complementary to one of the DNA strands, the DNA duplex must unwind over a short distance, forming a transcription “bubble.”

❖The strand that serves as template for RNA synthesis is called the template strand and the DNA strand complementary to the template called as the nontemplate strand, or coding strand.

(5′) CGCTATAGCGTTT(3′) DNA nontemplate (coding) strand

(3′) GCGATATCGCAAA(5′) DNA template strand

(5′) CGCUAUAGCGUUU(3′) RNA transcript

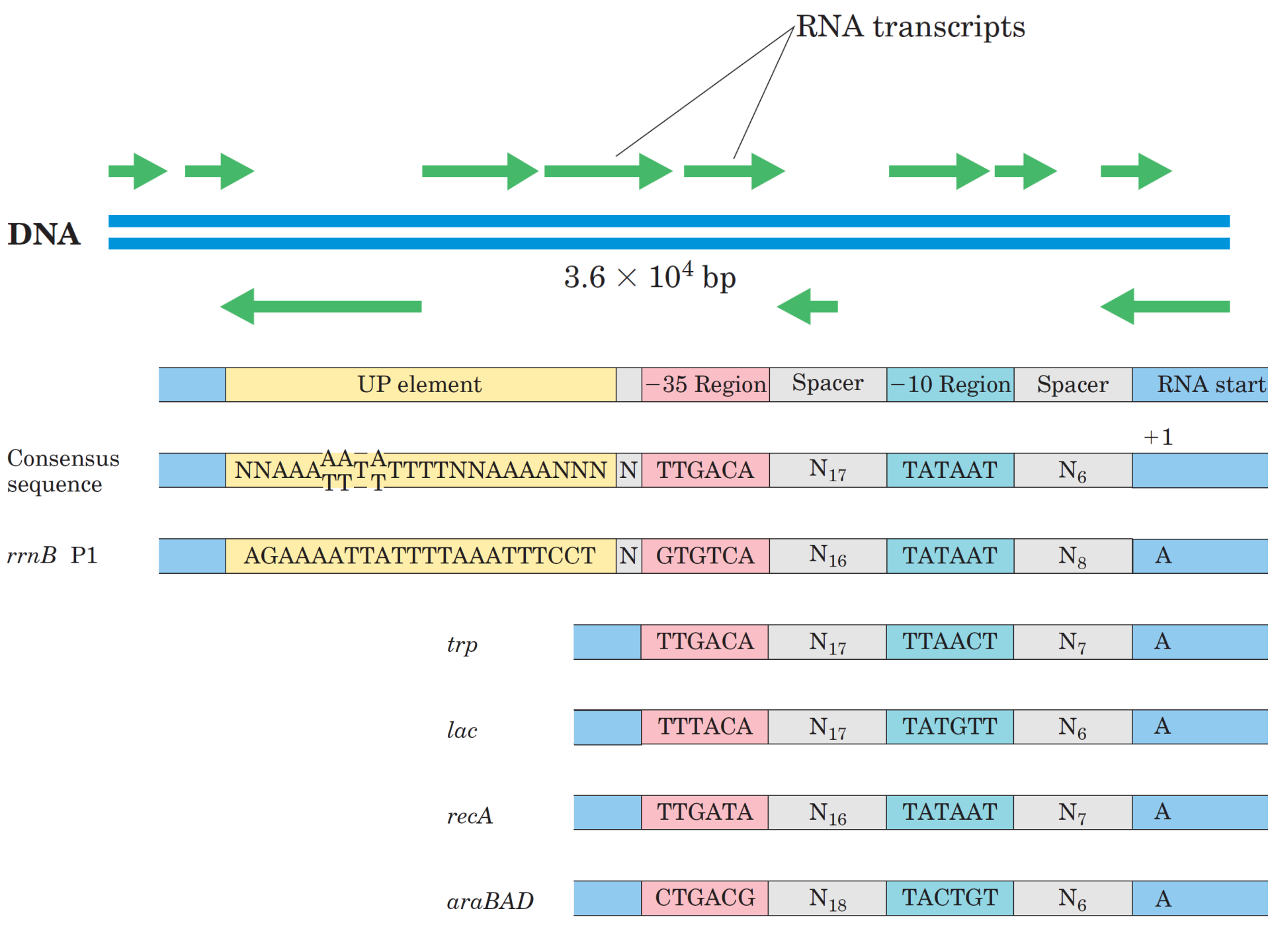


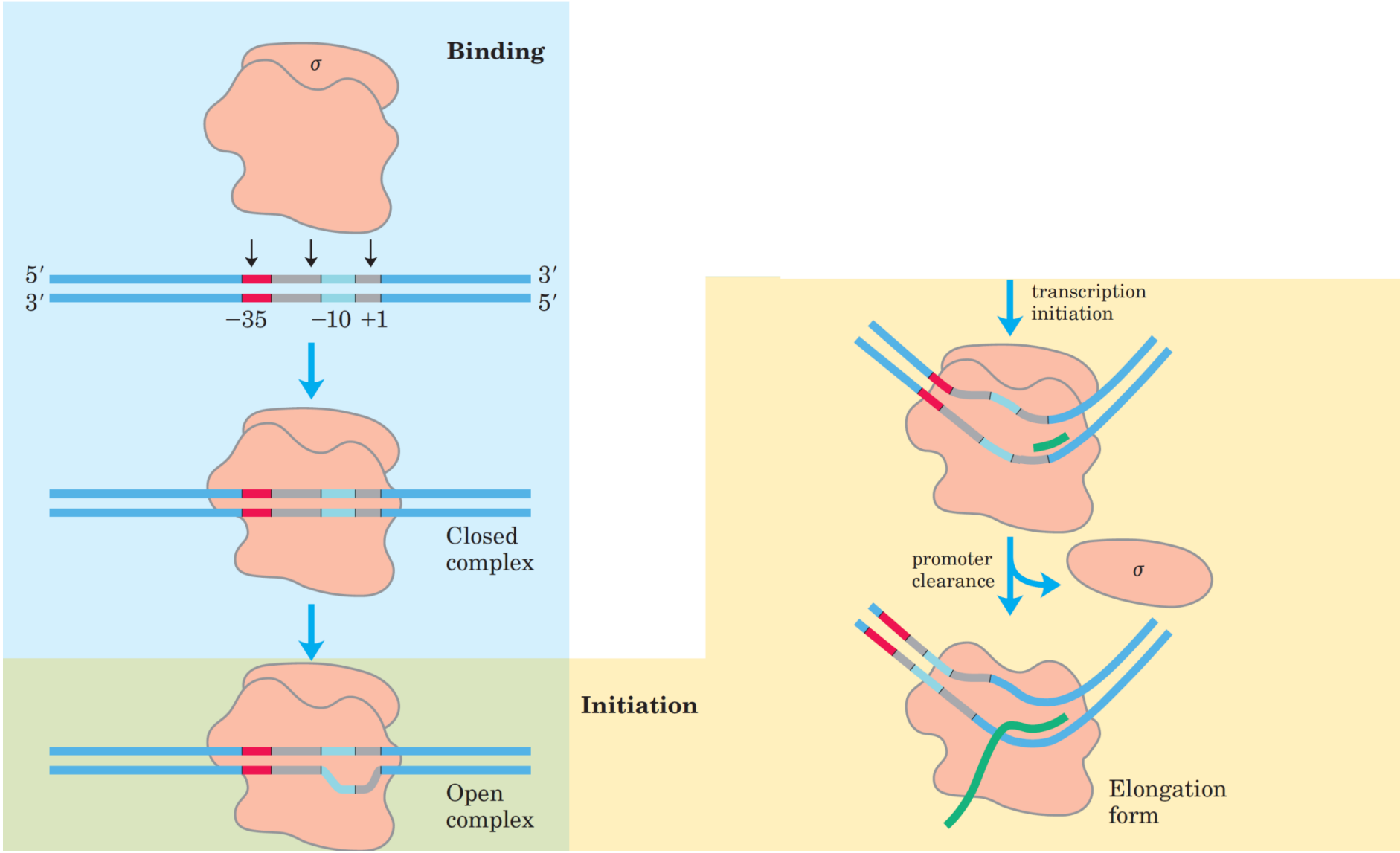
❖RNA polymerases lack a separate proofreading 3'→5' exonuclease active site (such as that of many DNA polymerases), and the error rate for transcription is higher than that for chromosomal DNA replication.

❖RNA polimeraz ekzonükleaz aktivitesine sahip değildir bu nedenle hata kontrol edici özelliği yoktur ve transkripsiyonun hata oranı replikasyondan yüksektir.

❖RNA polymerase binds to specific sequences in the DNA called promoters, which direct the transcription of adjacent segments of DNA (genes).

❖The sequences where RNA polymerases bind can be quite variable however certain nucleotides that are particularly common at each position form a **consensus sequence**.


RNA transcripts
DNA
3.6 × 10^4 bp
UP element
−35 Region
Spacer
−10 Region
Spacer
RNA start
+1
Consensus sequence
NNAAA(AA/TT)(A/T)TTTTNNAAAANNN
N
TTGACA
N17
TATAAT
N6
rrnB P1
AGAAAATTATTTTAAATTTCCT
N
GTGTCA
N16
TATAAT
N8
A
trp
TTGACA
N17
TTAACT
N7
A
lac
TTTACA
N17
TATGTT
N6
A
recA
TTGATA
N16
TATAAT
N7
A
araBAD
CTGACG
N18
TACTGT
N6
A


Binding
σ
5′
3′
3′
5′
−35
−10
+1
Closed complex
Open complex
Initiation
transcription initiation
promoter clearance
σ
Elongation form

❖Specific sequences signal termination of RNA synthesis.

❖The process of termination is not yet well understood in eukaryotes, in E. coli has at least two classes of termination signals: one class relies on a protein factor called *ρ* (rho) and the other is *ρ*-independent.

❖The transcriptional machinery in the nucleus of a eukaryotic cell is much more complex than that in bacteria.

❖Eukaryotes have three RNA polymerases, designated I, II, and III, which are distinct complexes but have certain subunits in common.

❖Each polymerase has a specific function and is recruited to a specific promoter sequence.

Table 29.2 **Eukaryotic RNA polymerases**

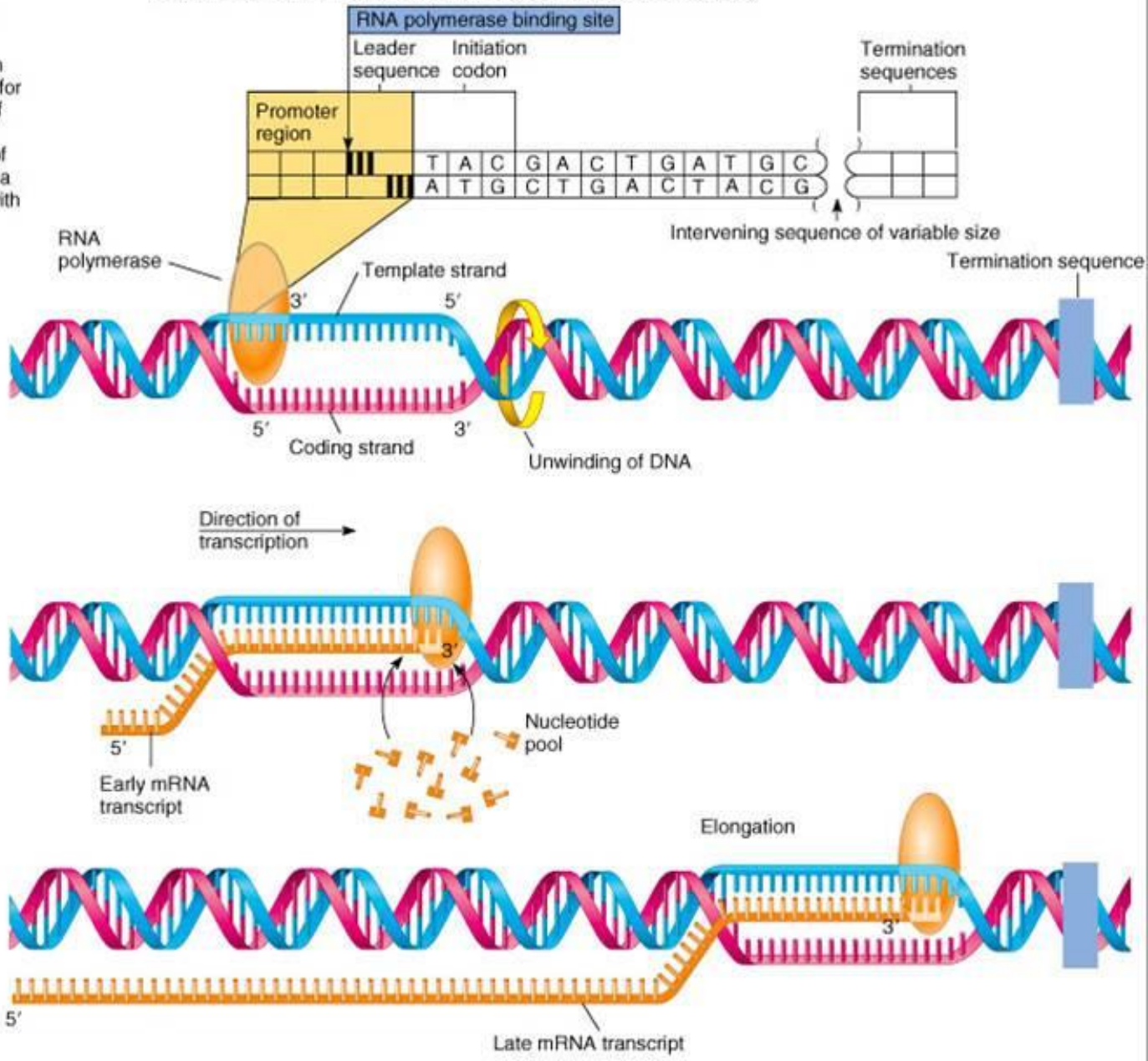
Type	Location	Cellular transcripts	Effects of α-amanitin
I	Nucleolus	18S, 5.8S, and 28S rRNA	Insensitive
II	Nucleoplasm	mRNA precursors and snRNA	Strongly inhibited
III	Nucleoplasm	tRNA and 5S rRNA	Inhibited by high concentrations

TABLE 26–1 Proteins Required for Initiation of Transcription at the RNA Polymerase II (Pol II) Promoters of Eukaryotes

Transcription protein	*Number of subunits*	*Subunit(s) M_r*	*Function(s)*
Initiation			
Pol II	12	10,000–220,000	Catalyzes RNA synthesis
TBP (TATA-binding protein)	1	38,000	Specifically recognizes the TATA box
TFIIA	3	12,000, 19,000, 35,000	Stabilizes binding of TFIIB and TBP to the promoter
TFIIB	1	35,000	Binds to TBP; recruits Pol II–TFIIF complex
TFIIE	2	34,000, 57,000	Recruits TFIIH; has ATPase and helicase activities
TFIIF	2	30,000, 74,000	Binds tightly to Pol II; binds to TFIIB and prevents binding of Pol II to nonspecific DNA sequences
TFIIH	12	35,000–89,000	Unwinds DNA at promoter (helicase activity); phosphorylates Pol II (within the CTD); recruits nucleotide-excision repair proteins
Elongation*			
ELL†	1	80,000	
p-TEFb	2	43,000, 124,000	Phosphorylates Pol II (within the CTD)
SII (TFIIS)	1	38,000	
Elongin (SIII)	3	15,000, 18,000, 110,000	

*The function of all elongation factors is to suppress the pausing or arrest of transcription by the Pol II–TFIIF complex.

†Name derived from *e*leven-nineteen *l*ysine-rich *l*eukemia. The gene for ELL is the site of chromosomal recombination events frequently associated with acute myeloid leukemia.



(a) Overall view of a gene. Each gene contains a specific promoter region and a leader sequence for guiding the beginning of transcription. This is followed by the region of the gene that codes for a polypeptide and ends with a series of terminal sequences that stop translation.

(b) DNA is unwound at the promoter by RNA polymerase. Only one strand of DNA, called the template strand, is copied by the RNA polymerase. This strand runs in the 3′ to 5′ direction.

(c) As the RNA polymerase moves along the strand, it adds complementary nucleotides as dictated by the DNA template, forming the single-stranded mRNA that reads in the 5′ to 3′ direction.

(d) The polymerase continues transcribing until it reaches a termination site and the mRNA transcript is released for translation. Note that the section of the DNA that has been transcribed is rewound into its original configuration.

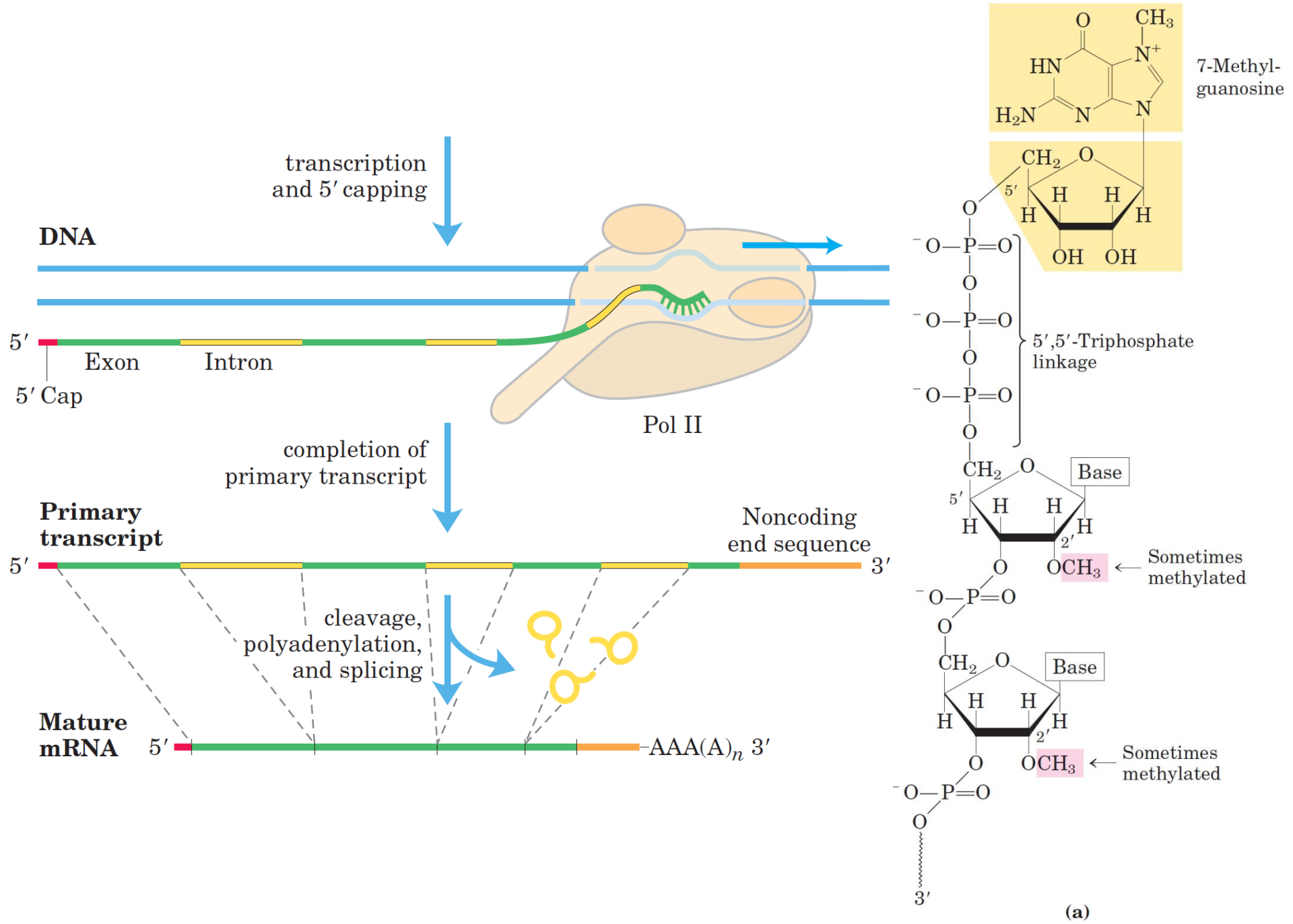
❖Many of the RNA molecules in bacteria and virtually all RNA molecules in eukaryotes are processed to some degree after synthesis.

❖Some of the most interesting molecular events in RNA metabolism occur during this postsynthetic processing.

❖Intriguingly, several of the enzymes that catalyze these reactions consist of RNA rather than protein.

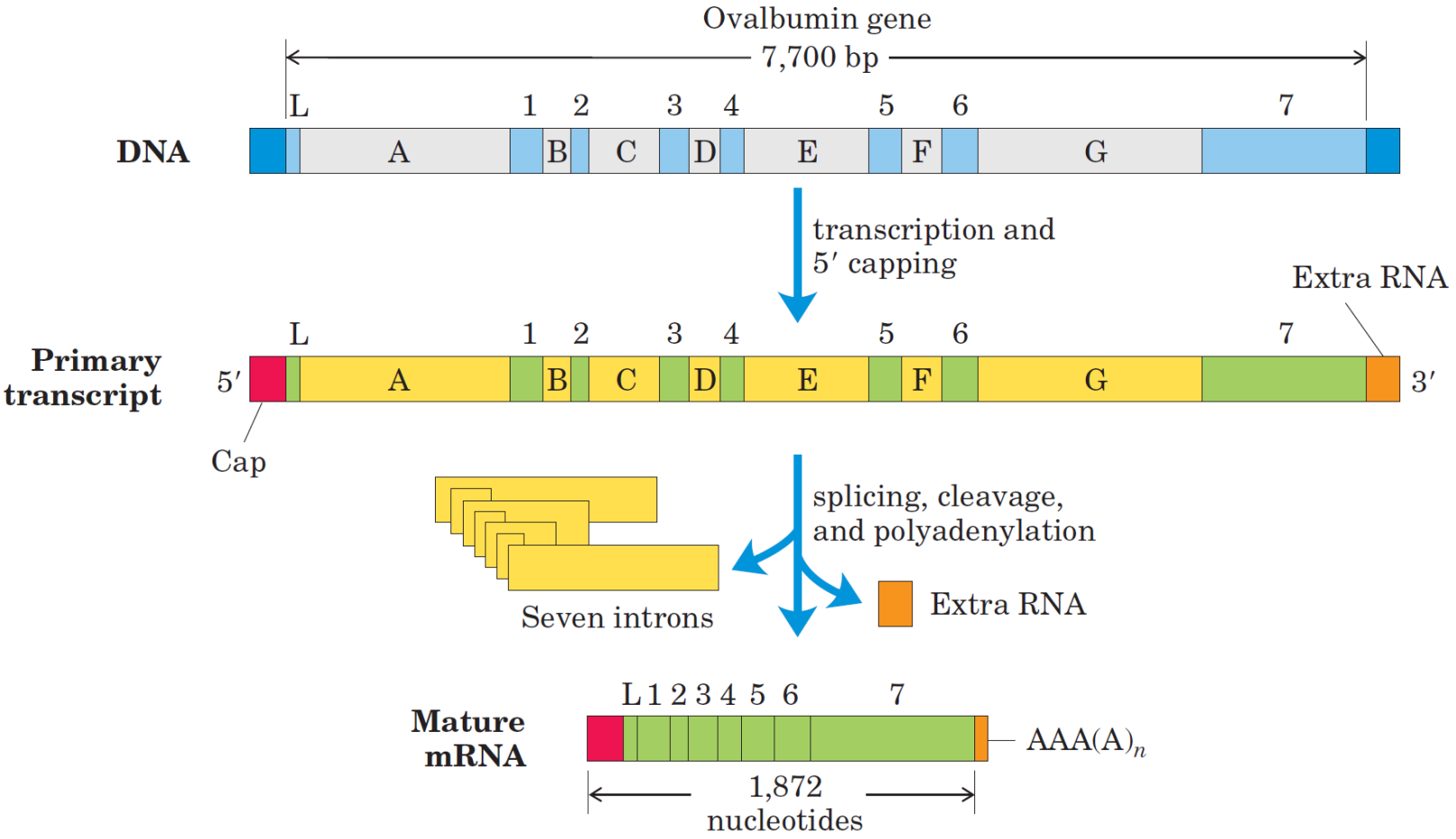
❖The discovery of these catalytic RNAs, or **ribozymes**, has brought a revolution in thinking about RNA function and about the origin of life.

❖A newly synthesized RNA molecule is called a **primary transcript**. Perhaps the most extensive processing of primary transcripts occurs in eukaryotic mRNAs and in tRNAs of both bacteria and eukaryotes.


transcription
and 5′ capping
DNA
5′
Exon
Intron
5′ Cap
Pol II
completion of
primary transcript
Primary
transcript
Noncoding
end sequence
5′
3′
cleavage,
polyadenylation,
and splicing
Mature
mRNA
5′
–AAA(A)n 3′
7-Methyl-
guanosine
5′,5′-Triphosphate
linkage
Base
Sometimes
methylated
Base
Sometimes
methylated
3′
(a)

❖In a process called splicing, the introns are removed from the primary transcript and the exons are joined to form a continuous sequence that specifies a functional polypeptide.

❖Spliceosomes contains small nuclear RNA (snRNA) and proteins.



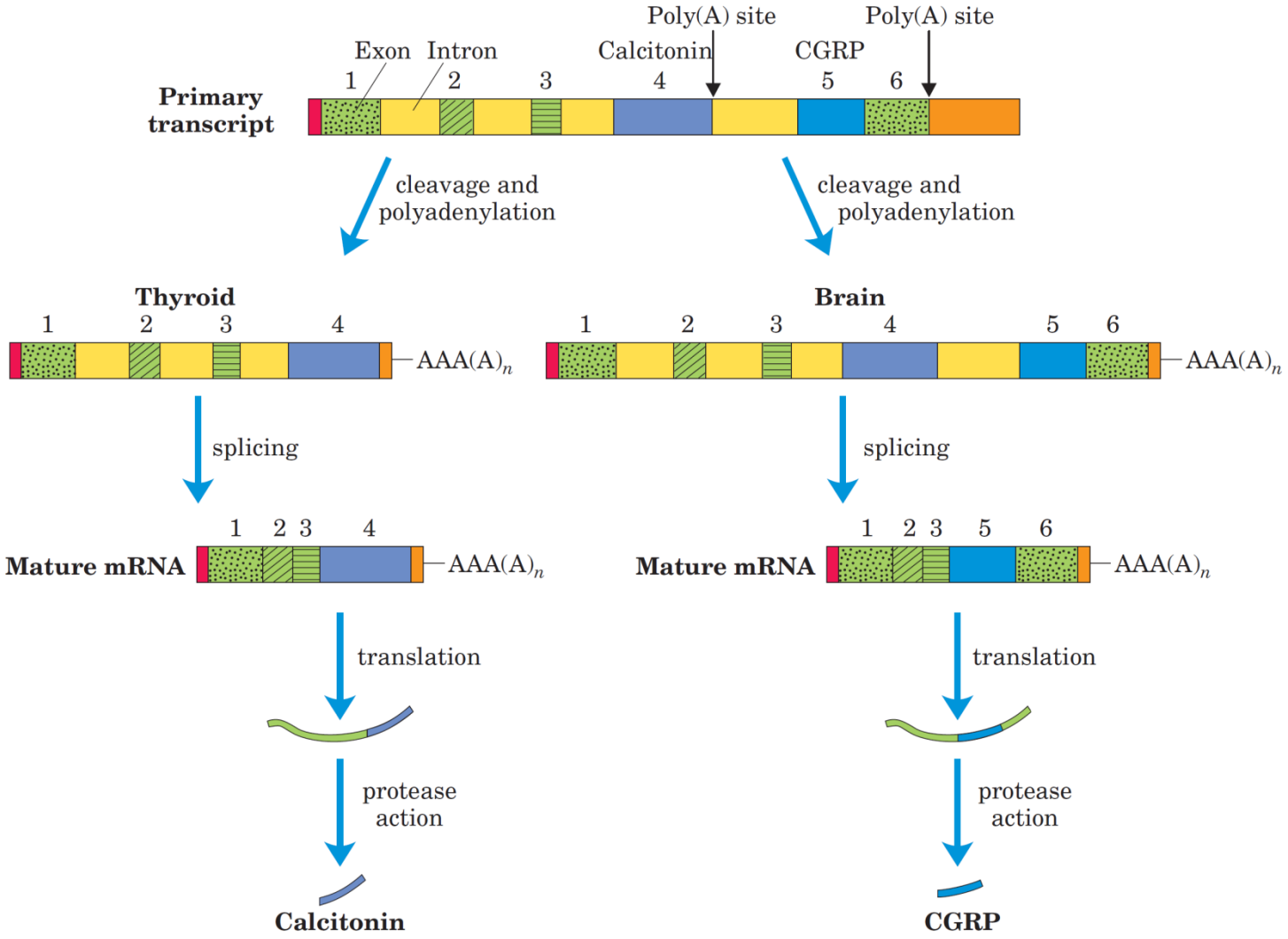
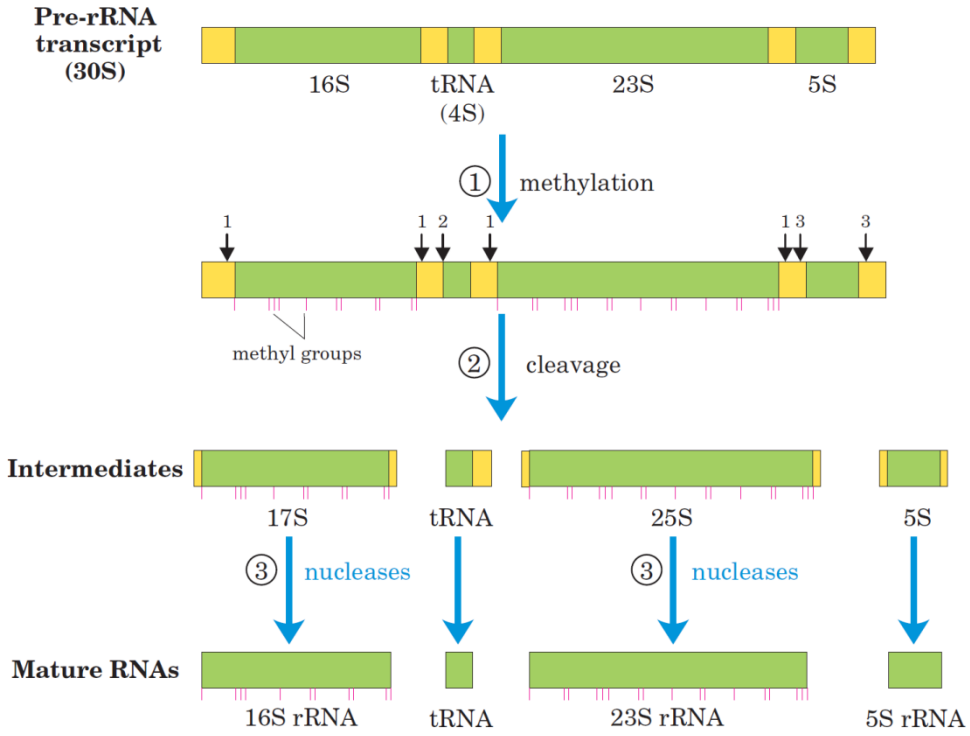
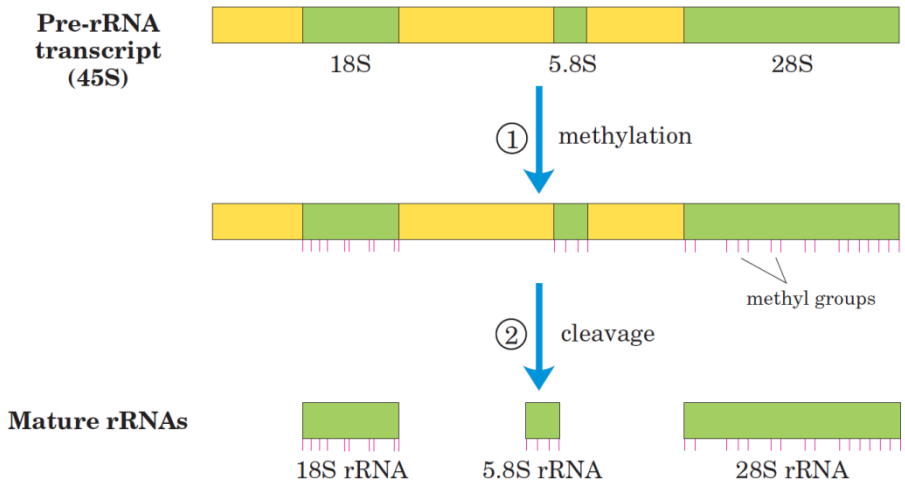


FIGURE 26–20 Alternative processing of the calcitonin gene transcript in rats. The primary transcript has two poly(A) sites; one predominates in the brain, the other in the thyroid. In the brain, splicing eliminates the calcitonin exon (exon 4); in the thyroid, this exon is retained. The resulting peptides are processed further to yield the final hormone products: calcitonin-gene-related peptide (CGRP) in the brain and calcitonin in the thyroid.

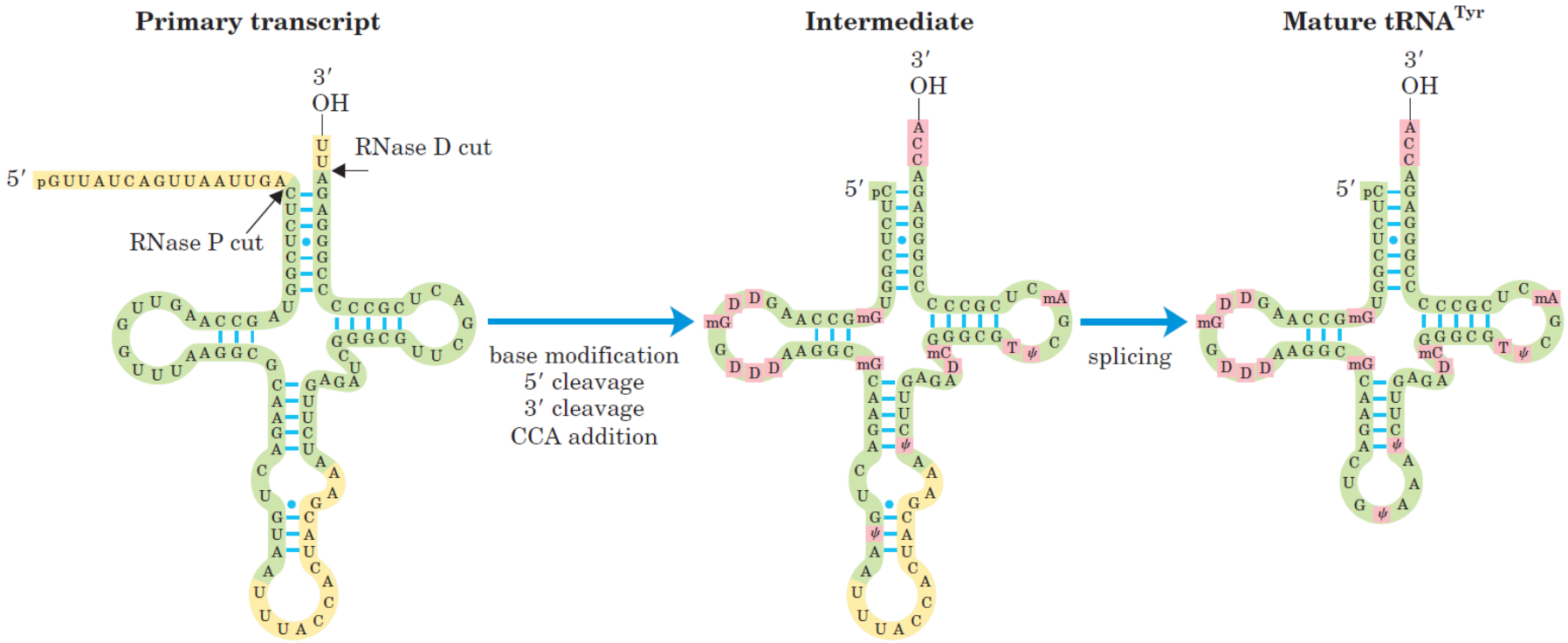
❖Not only mRNAs, rRNAs and tRNAs also modified after their transcription.



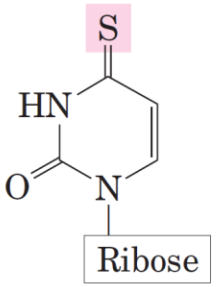


❖Most cells contains 40 - 50 different tRNAs.

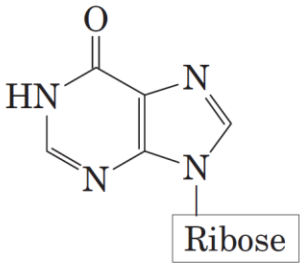
❖They generally synthesize as longer RNA chains and modified after their transcription.



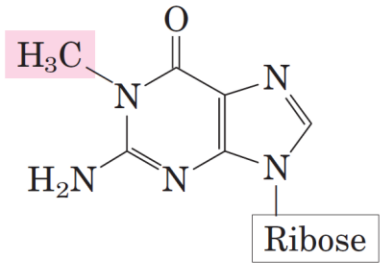
❖Also some bases modified after transcription.



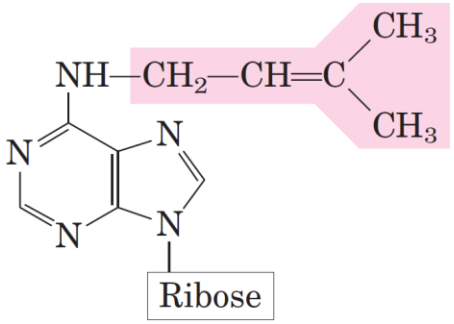
4-Thiouridine (S^4U)



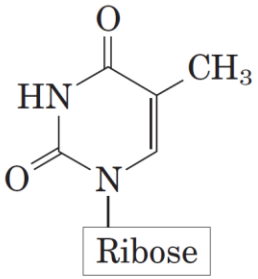
Inosine (I)



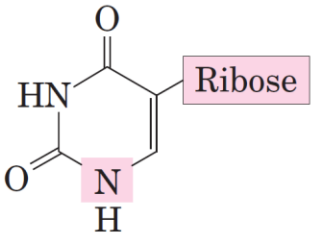
1-Methylguanosine (m^1G)



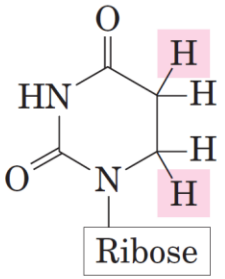
N^6-Isopentenyladenosine (i^6A)



Ribothymidine (T)

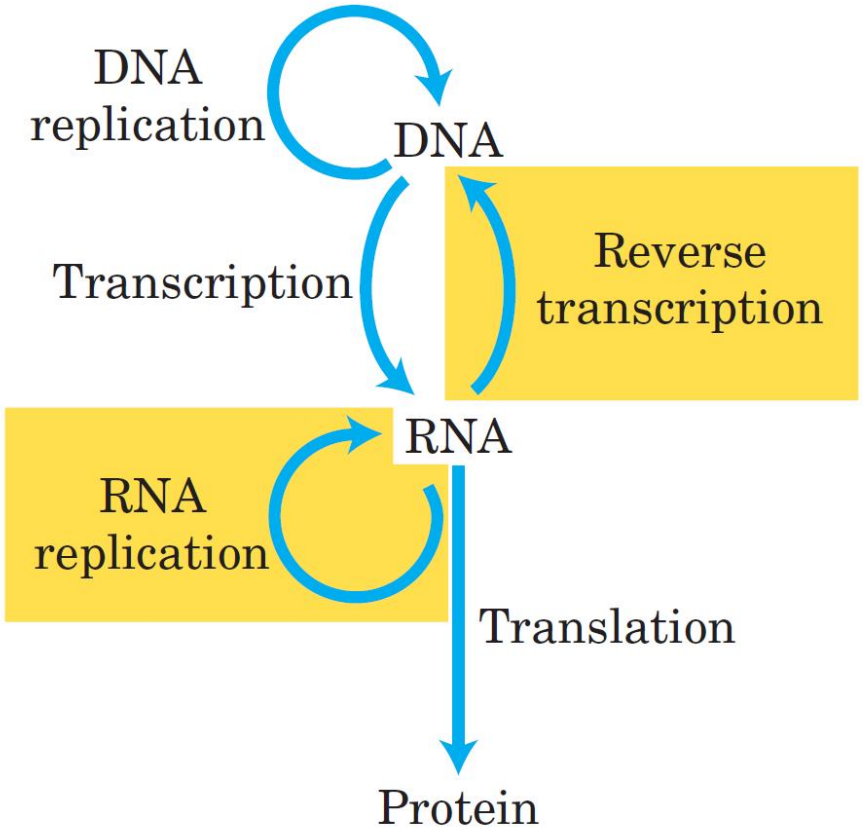


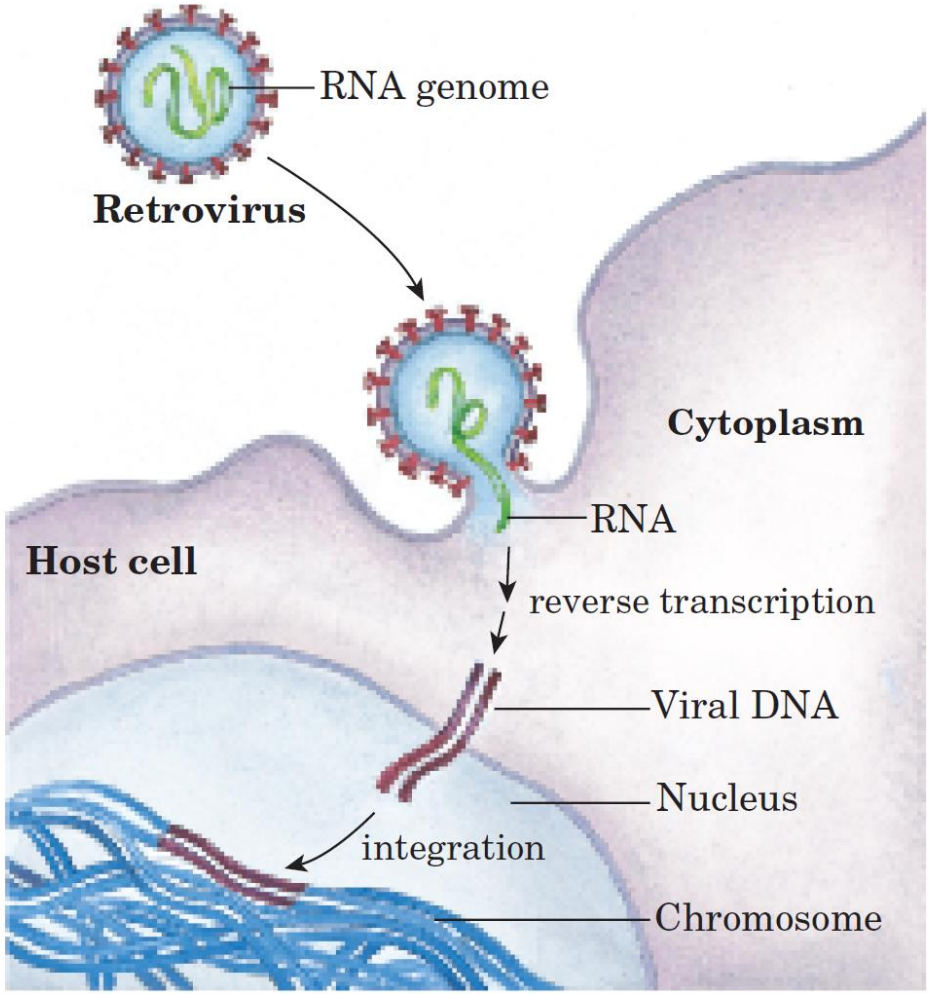
Pseudouridine (ψ)



Dihydrouridine (D)

❖Retroviruses contains RNA as genetic material.





❖Proteins are the end products of most information pathways. A typical cell requires thousands of different proteins at any given moment.

❖These must be synthesized in response to the cell's current needs, transported (targeted) to their appropriate cellular locations, and degraded when no longer needed.

❖A **codon** is a triplet of nucleotides that codes for a specific amino acid. Translation occurs in such a way that these nucleotide triplets are read in a successive, nonoverlapping fashion.

❖Several codons serve special functions.

❖The initiation codon AUG is the most common signal for the beginning of a polypeptide in all cells, in addition to coding for Met residues in internal positions of polypeptides.

❖The termination codons (UAA, UAG, and UGA), also called stop codons or nonsense codons, normally signal the end of polypeptide synthesis and do not code for any known amino acids.

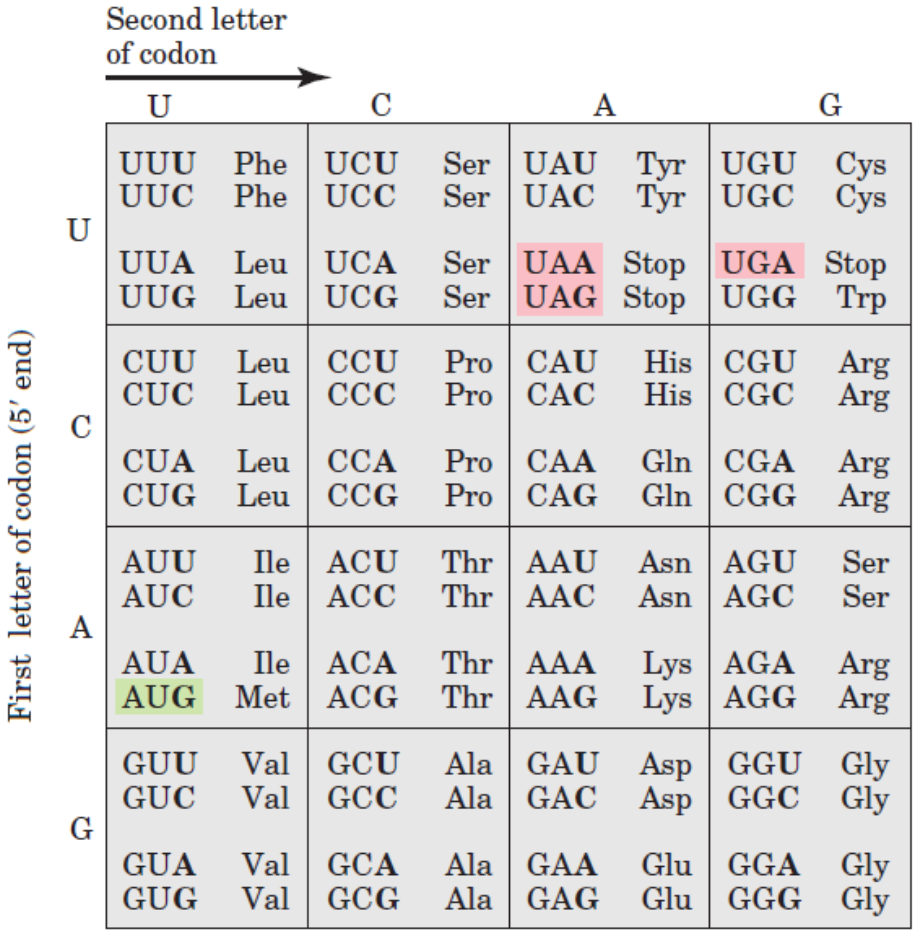


FIGURE 27–7 "Dictionary" of amino acid code words in mRNAs. The codons are written in the 5′⟶3′ direction. The third base of each codon (in bold type) plays a lesser role in specifying an amino acid than the first two. The three termination codons are shaded in pink, the initiation codon AUG in green. All the amino acids except methionine and tryptophan have more than one codon. In most cases, codons that specify the same amino acid differ only at the third base.

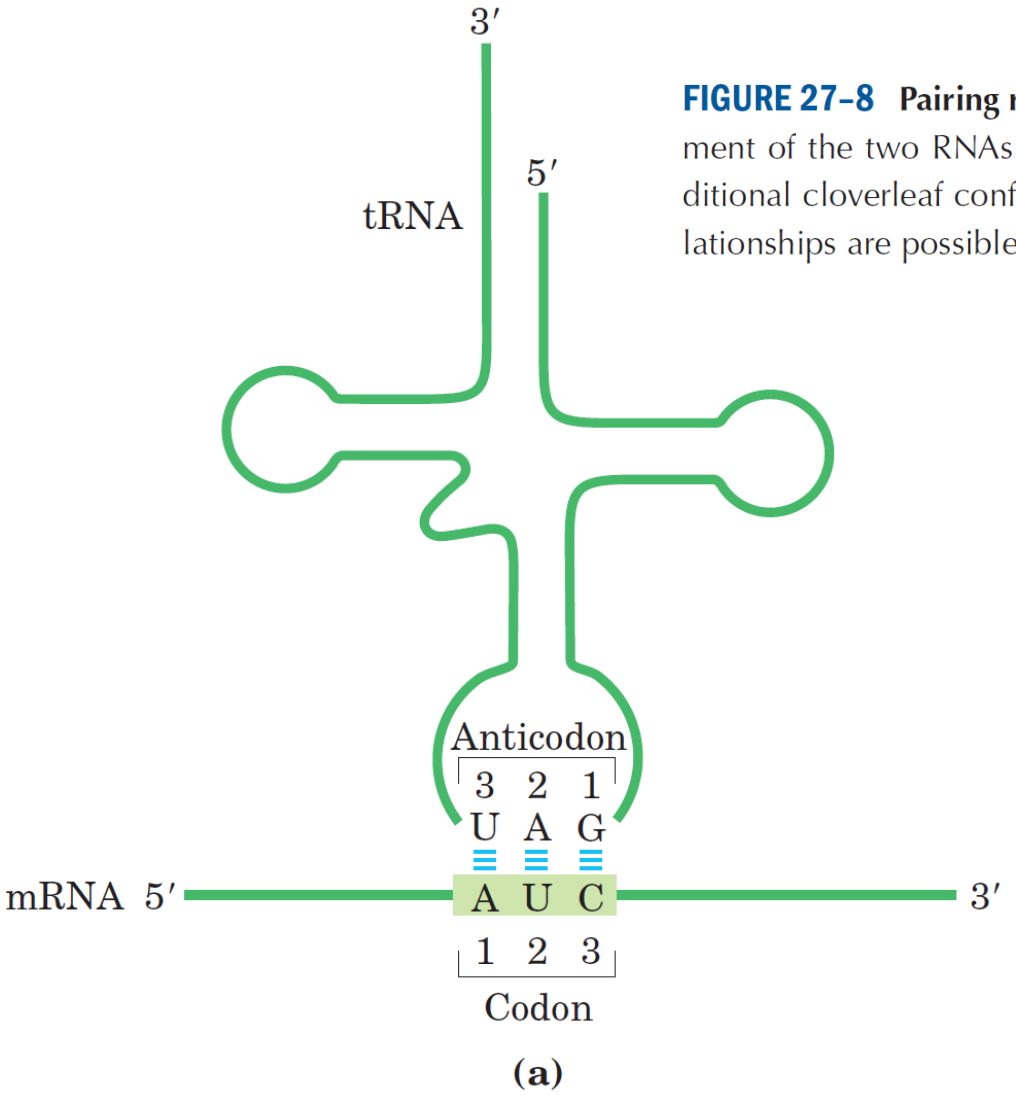
❖When several different codons specify one amino acid, the difference between them usually lies at the third base position (at the 3’ end).

❖For example, alanine is coded by the triplets GCU, GCC, GCA, and GCG.

❖Transfer RNAs base-pair with mRNA codons at a three-base sequence on the tRNA called the anticodon.

❖Some tRNAs recognise more than one codons.

❖Third base of this codon (wobble) make weak hydrogen bonds



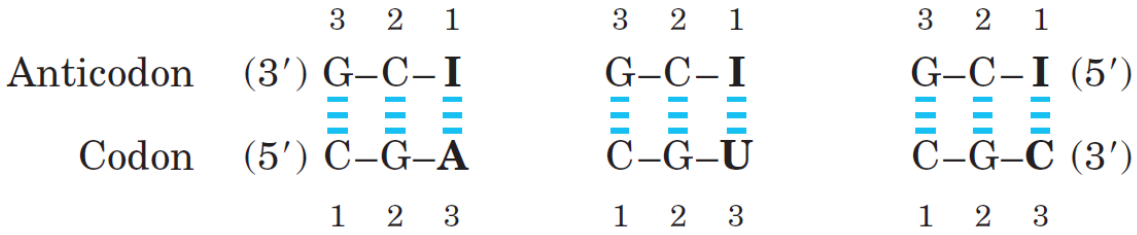


FIGURE 27–8 Pairing relationship of codon and anticodon. **(a)** Alignment of the two RNAs is antiparallel. The tRNA is shown in the traditional cloverleaf configuration. **(b)** Three different codon pairing relationships are possible when the tRNA anticodon contains inosinate.

❖As we have seen for DNA and RNA, the synthesis of polymeric biomolecules can be considered in terms of initiation, elongation, and termination stages.

❖Protein biosynthesis takes place in five stages:

❖**Stage 1: Activation of Amino Acids** For the synthesis of a polypeptide with a defined sequence, two fundamental chemical requirements must be met: (1) the carboxyl group of each amino acid must be activated to facilitate formation of a peptide bond, and (2) a link must be established between each new amino acid and the information in the mRNA that encodes it.

❖Both these requirements are met by attaching the amino acid to a tRNA in the first stage of protein synthesis.

❖**Stage 2: Initiation** The mRNA bearing the code for the polypeptide to be made binds to the smaller of two ribosomal subunits and to the initiating aminoacyl-tRNA. The large ribosomal subunit then binds to form an initiation complex.

❖**Stage 3: Elongation** The nascent polypeptide is lengthened by covalent attachment of successive amino acid units, each carried to the ribosome and correctly positioned by its tRNA, which base-pairs to its corresponding codon in the mRNA.

❖**Stage 4: Termination and Release** Completion of the polypeptide chain is signaled by a termination codon in the mRNA. The new polypeptide is released from the ribosome, aided by proteins called release factors.

❖**Stage 5: Folding and Posttranslational Modification** In order to achieve its biologically active form, the new polypeptide must fold into its proper three-dimensional conformation.

❖Before or after folding, the new polypeptide may undergo enzymatic processing, including removal of one or more amino acids (usually from the amino terminus); addition of acetyl, phosphoryl, methyl, carboxyl, or other groups to certain amino acid residues; proteolytic cleavage; and/or attachment of oligosaccharides or prosthetic groups.

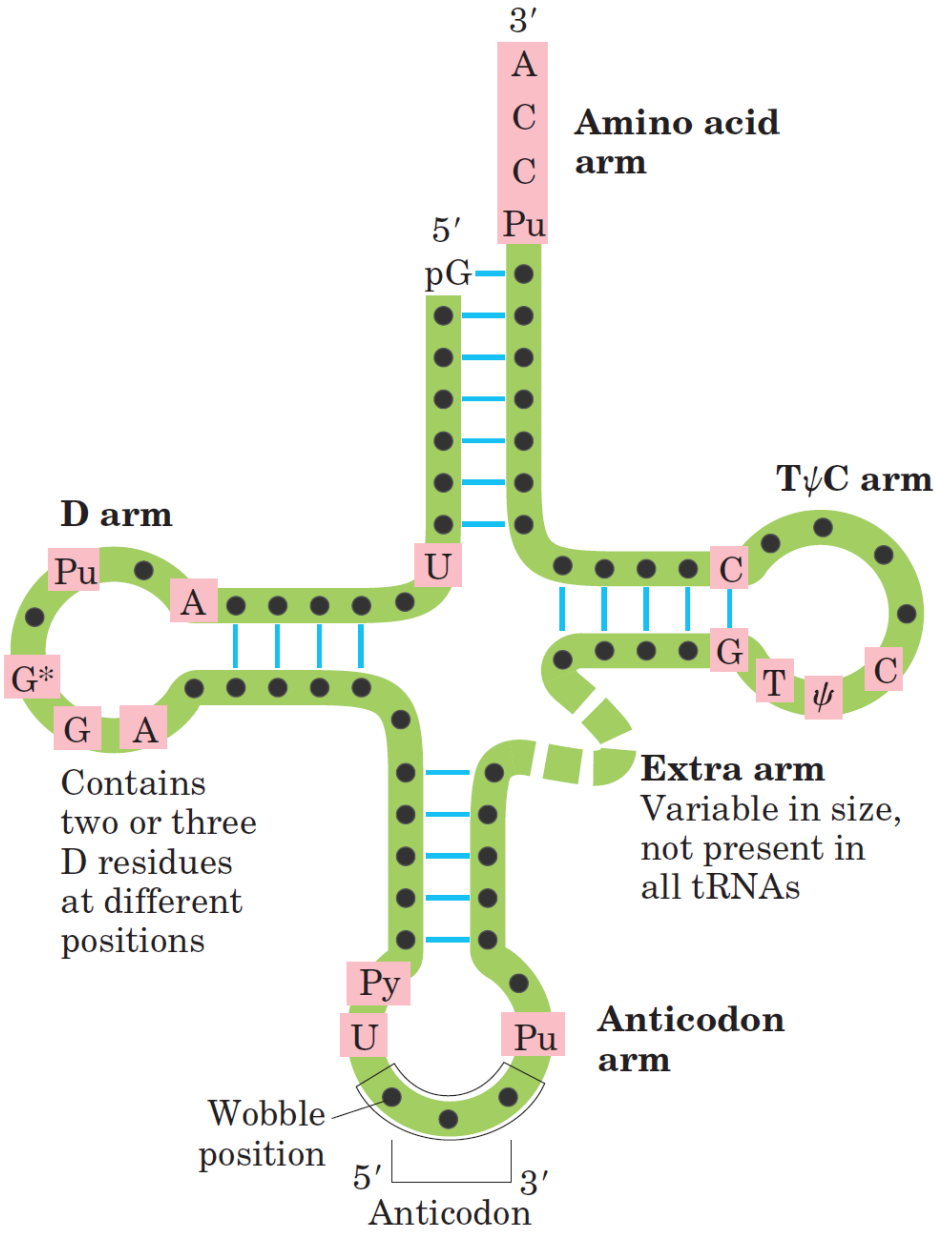
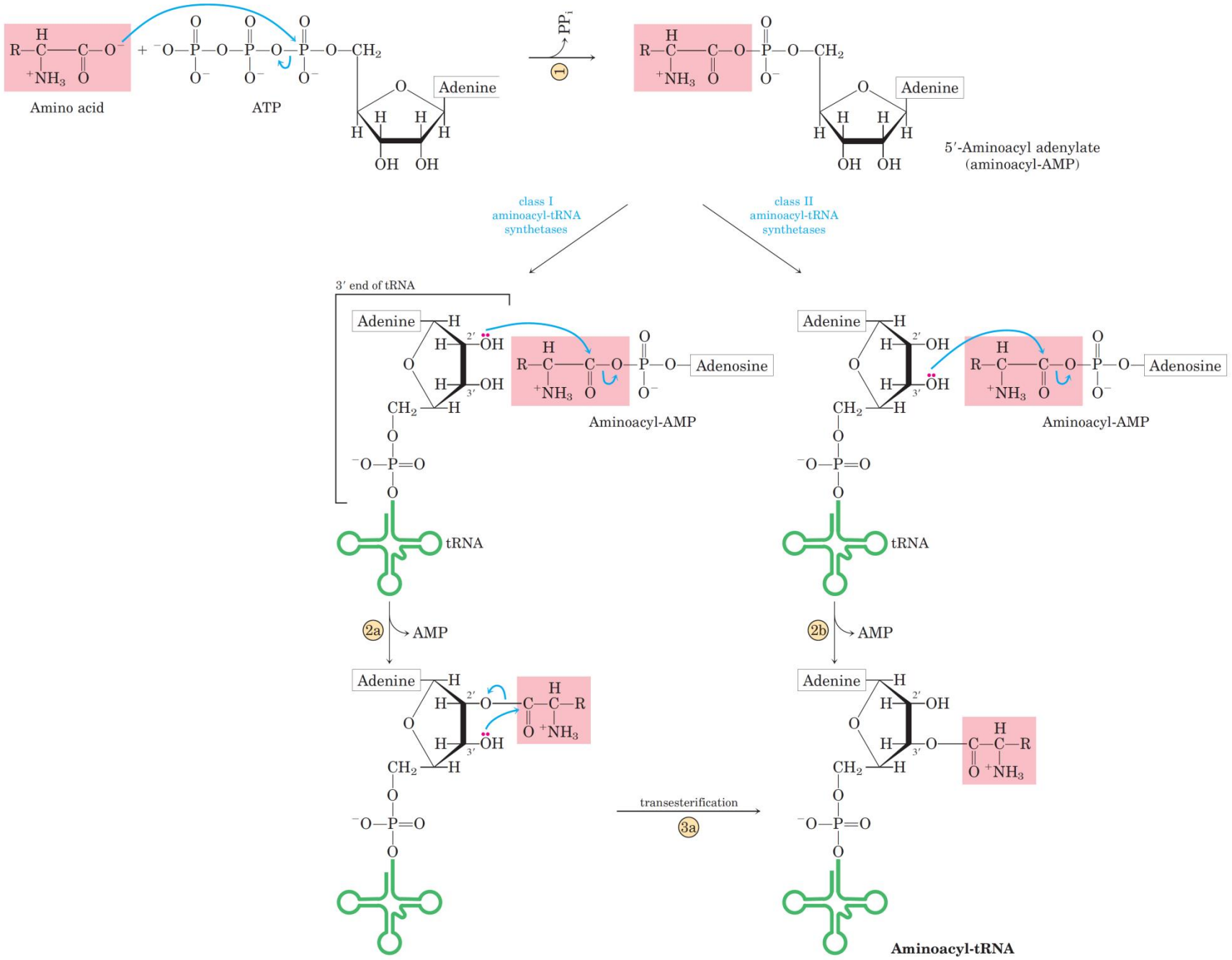
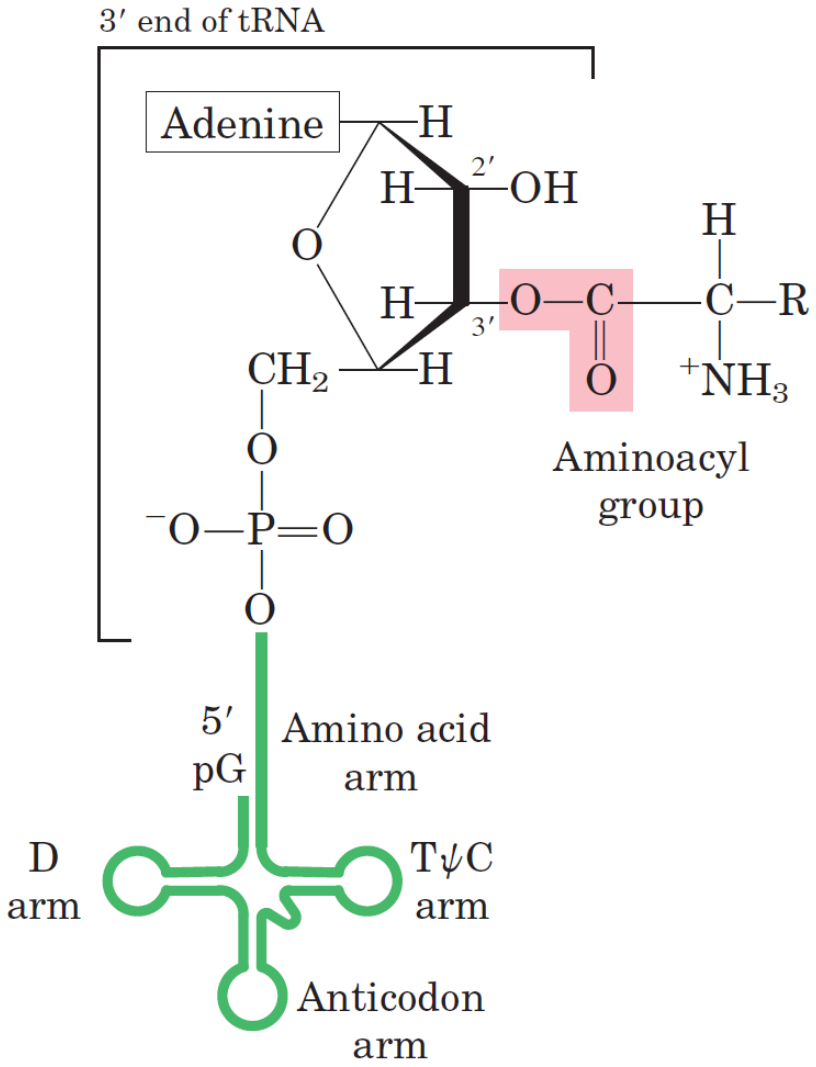


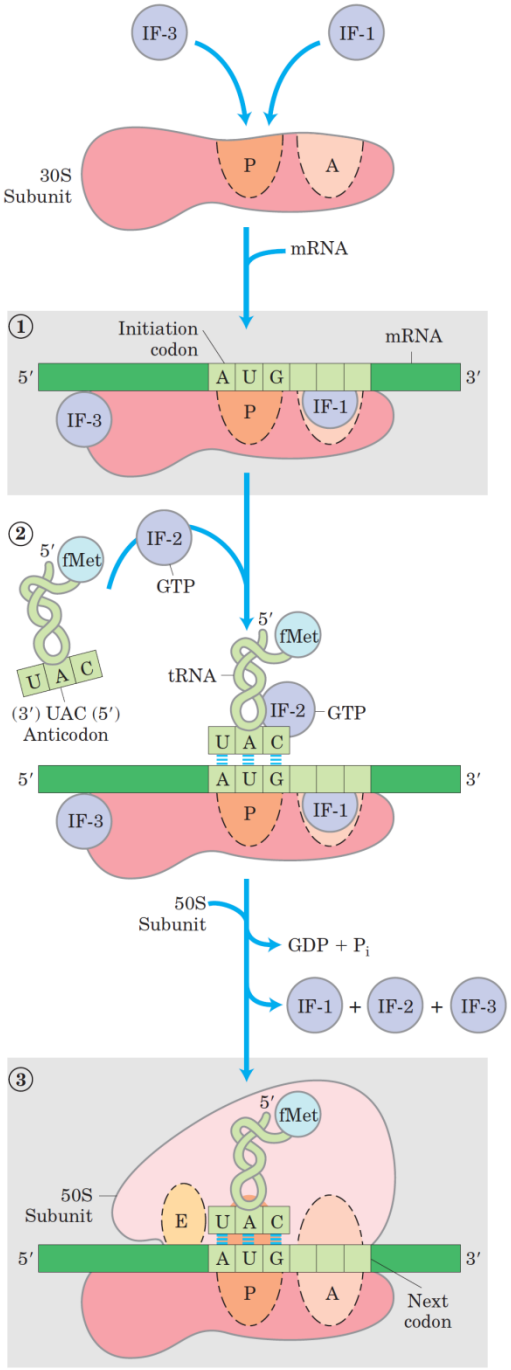
FIGURE 27–12 General cloverleaf secondary structure of tRNAs. The large dots on the backbone represent nucleotide residues; the blue lines represent base pairs. Characteristic and/or invariant residues common to all tRNAs are shaded in pink. Transfer RNAs vary in length from 73 to 93 nucleotides. Extra nucleotides occur in the extra arm or in the D arm. At the end of the anticodon arm is the anticodon loop, which always contains seven unpaired nucleotides. The D arm contains two or three D (5,6-dihydrouridine) residues, depending on the tRNA. In some tRNAs, the D arm has only three hydrogen-bonded base pairs. In addition to the symbols explained in Figure 27–11: Pu, purine nucleotide; Py, pyrimidine nucleotide; G*, guanylate *or* 2′-*O*-methylguanylate.

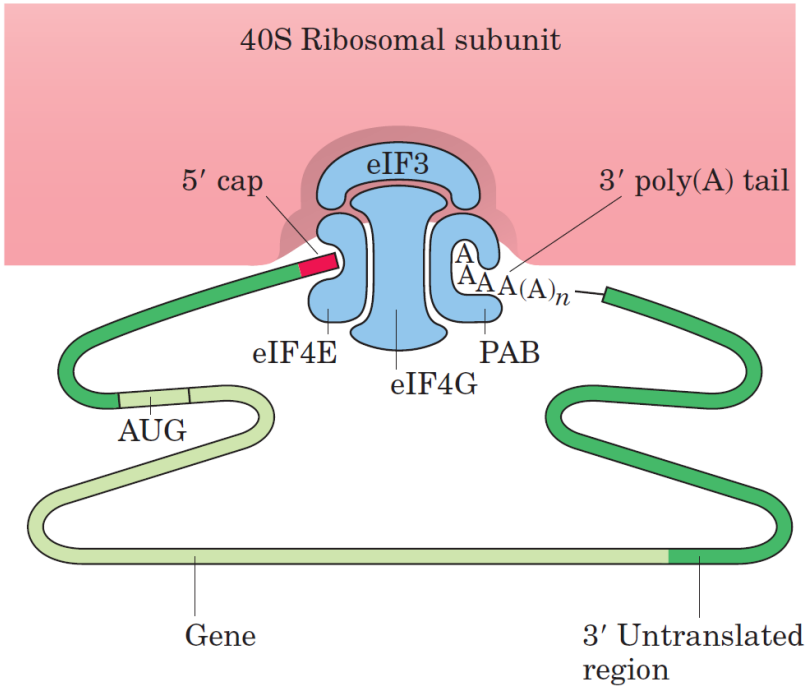

Amino acid
ATP
Adenine
PPi
1
5′-Aminoacyl adenylate
(aminoacyl-AMP)
class I
aminoacyl-tRNA
synthetases
class II
aminoacyl-tRNA
synthetases
3′ end of tRNA
Adenosine
Aminoacyl-AMP
tRNA
2a
AMP
2b
transesterification
3a
Aminoacyl-tRNA

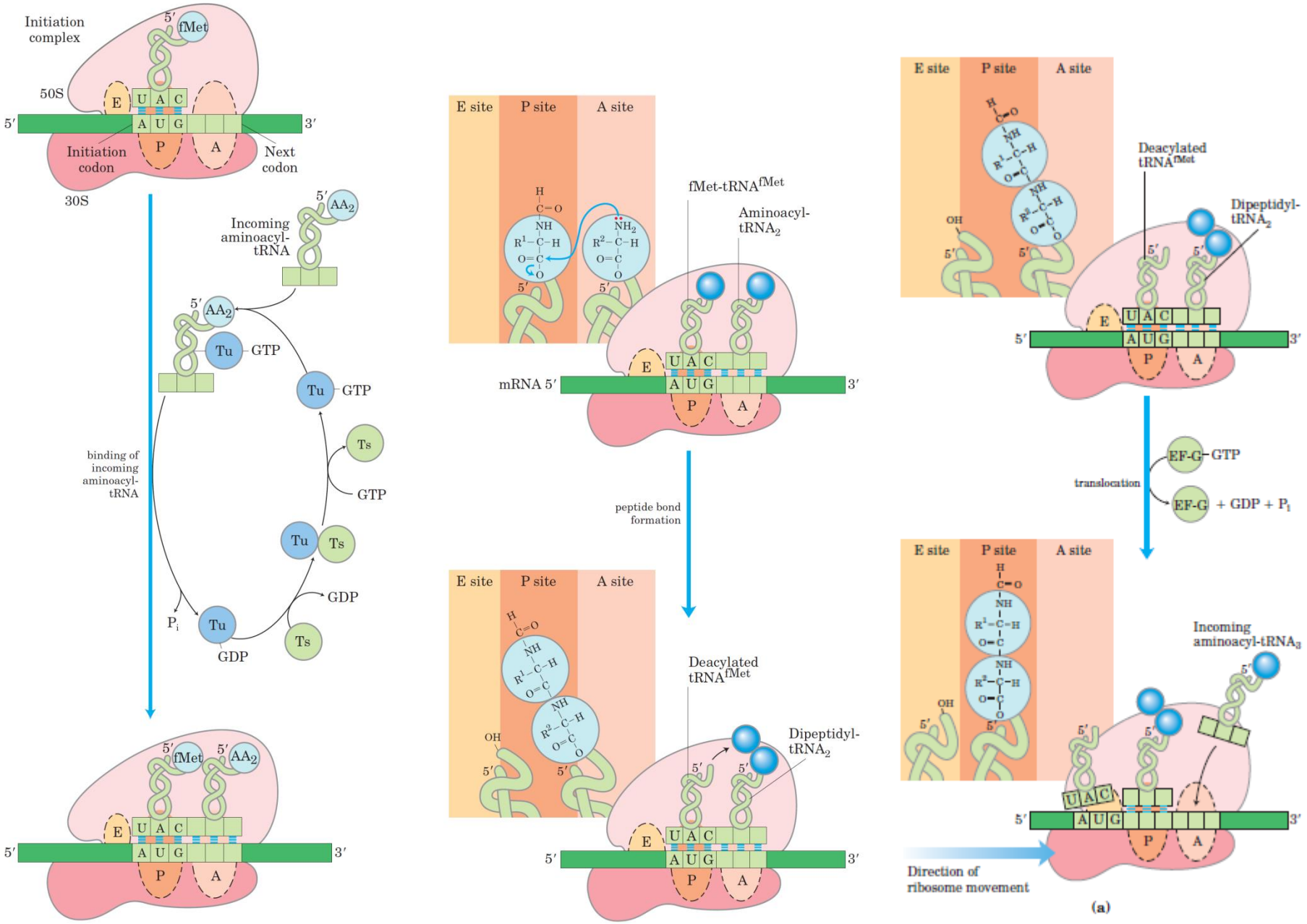
$$\text{Amino acid} + \text{tRNA} + \text{ATP} \xrightleftharpoons{Mg^{2+}} \text{aminoacyl-tRNA} + \text{AMP} + PP_i$$

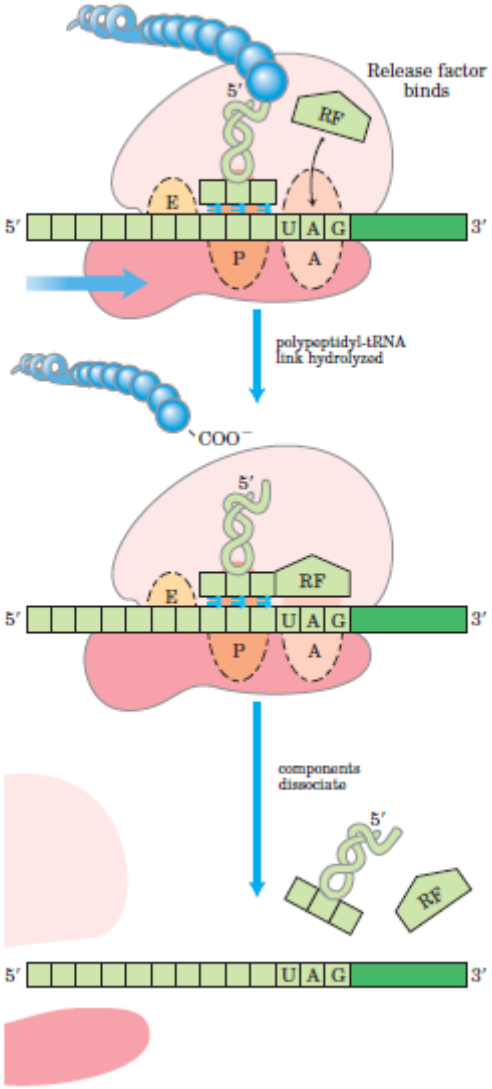
aminoacyl-tRNA synthetase

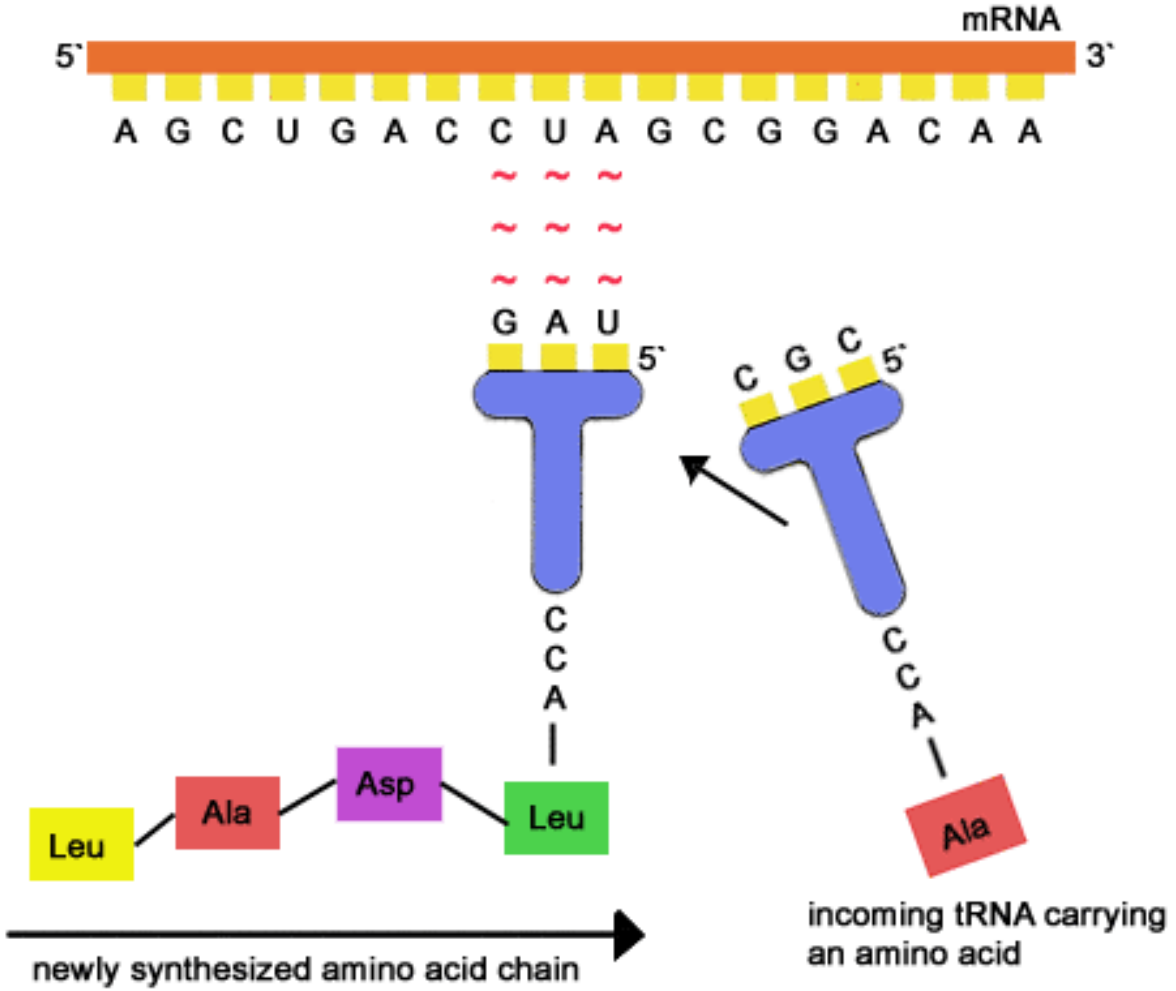



IF-3
IF-1
30S Subunit
P
A
mRNA
1
Initiation codon
mRNA
5′
A U G
3′
IF-3
P
IF-1
2
5′ fMet
IF-2
GTP
U A C
(3′) UAC (5′) Anticodon
5′ fMet
tRNA
IF-2 GTP
U A C
5′
A U G
3′
IF-3
P
IF-1
50S Subunit
GDP + P_i
IF-1 + IF-2 + IF-3
3
5′ fMet
50S Subunit
E
U A C
5′
A U G
3′
P
A
Next codon

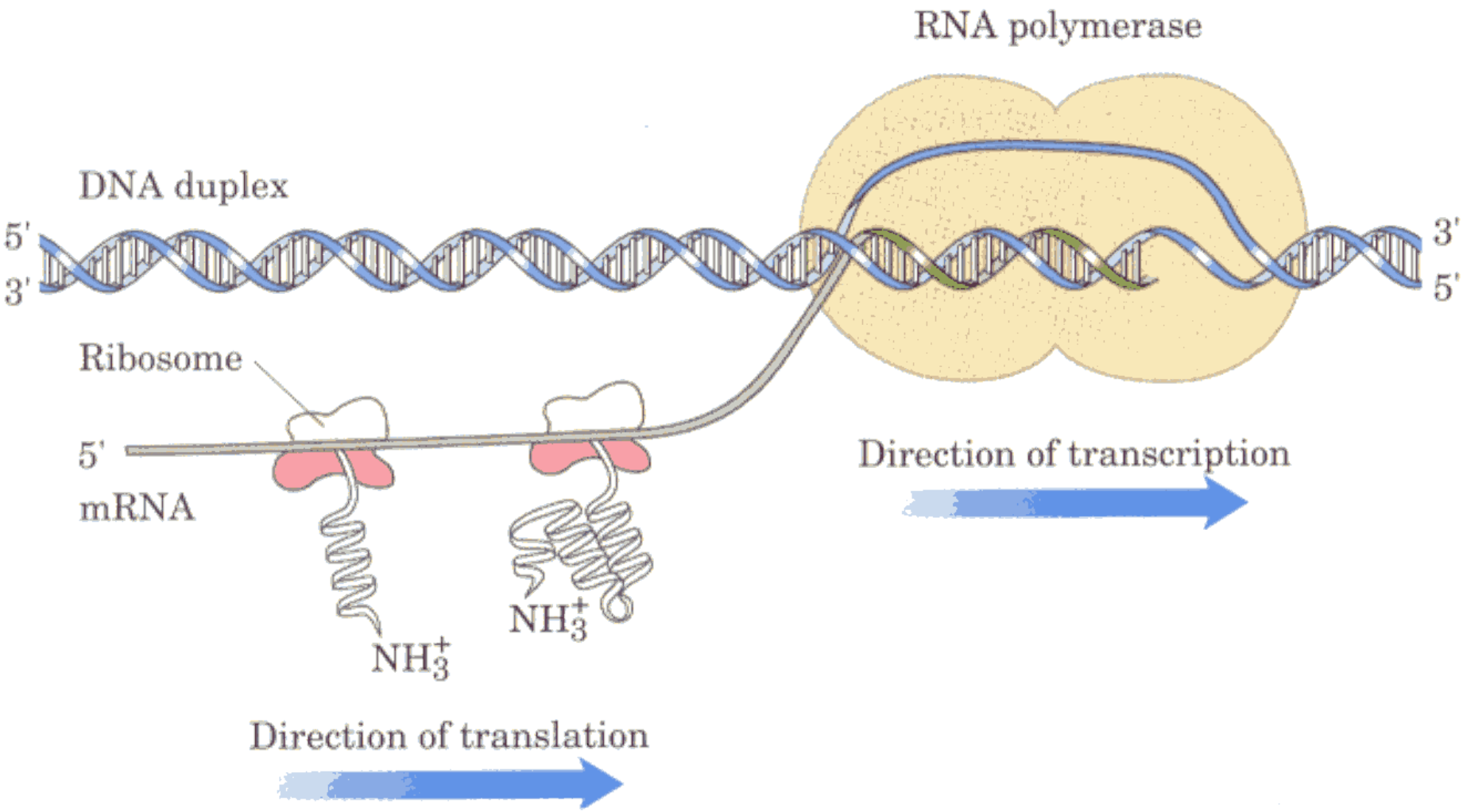

40S Ribosomal subunit
5′ cap
eIF3
3′ poly(A) tail
A
A A A(A)$_n$
eIF4E
eIF4G
PAB
AUG
Gene
3′ Untranslated region


Initiation complex
5′
fMet
50S
E
U A C
A U G
5′
3′
Initiation codon
P
A
Next codon
30S
Incoming aminoacyl-tRNA
5′
AA2
Tu
GTP
Ts
GDP
Pi
binding of incoming aminoacyl-tRNA
E site
P site
A site
fMet-tRNAfMet
Aminoacyl-tRNA2
mRNA 5′
peptide bond formation
Deacylated tRNAfMet
Dipeptidyl-tRNA2
translocation
EF-G
GTP
EF-G + GDP + Pi
Incoming aminoacyl-tRNA3
Direction of ribosome movement
(a)


Release factor binds
RF
5′
E
P
A
U A G
5′
3′
polypeptidyl-tRNA link hydrolyzed
COO⁻
components dissociate


mRNA
5`
3`
A G C U G A C C U A G C G G A C A A
G A U
5`
C C A
Leu
Ala
Asp
Leu
newly synthesized amino acid chain
C G C
5`
C C A
Ala
incoming tRNA carrying an amino acid

❖In prokaryotes transcription and translation occurs at the same time.



Posttranslational modificatios

❖In the final stage of protein synthesis, the nascent polypeptide chain is folded and processed into its biologically active form

- 1) ***Amino-Terminal and Carboxyl-Terminal Modifications***
- 2) ***Loss of Signal Sequences***
- 3) ***Modification of Individual Amino Acids***
- 4) ***Attachment of Carbohydrate Side Chains***
- 5) ***Addition of Isoprenyl Groups***
- 6) ***Addition of Prosthetic Groups***
- 7) ***Proteolytic Processing***
- 8) ***Formation of Disulfide Cross-Links***

- **Amino-Terminal and Carboxyl-Terminal Modifications**

- The first residue inserted in all polypeptides is N-formylmethionine (in bacteria) or methionine (in eukaryotes).

- However, the formyl group, the amino-terminal Met residue, and often additional amino-terminal (and, in some cases, carboxyl-terminal) residues may be removed enzymatically in formation of the final functional protein.

- In as many as 50% of eukaryotic proteins, the amino group of the amino-terminal residue is N-acetylated after translation.

- Carboxyl-terminal residues are also sometimes modified

❖**Loss of Signal Sequences** The 15 to 30 residues at the amino-terminal end of some proteins play a role in directing the protein to its ultimate destination in the cell. Such signal sequences are ultimately removed by specific peptidases.

❖**Modification of Individual Amino Acids** The hydroxyl groups of certain Ser, Thr, and Tyr residues of some proteins are enzymatically phosphorylated by ATP the phosphate groups add negative charges to these polypeptides.

❖The functional significance of this modification varies from one protein to the next. For example, the milk protein casein has many phosphoserine groups that bind Calcium

❖Extra carboxyl groups may be added to Glu residues of some proteins. For example, the blood-clotting protein prothrombin.

❖**Attachment of Carbohydrate Side Chains** The carbohydrate side chains of glycoproteins are attached covalently during or after synthesis of the polypeptide.

❖In some glycoproteins, the carbohydrate side chain is attached enzymatically to Asn residues (N-linked oligosaccharides), in others to Ser or Thr residues (O-linked oligosaccharides).

❖Many proteins that function extracellularly, as well as the lubricating proteoglycans that coat mucous membranes, contain oligosaccharide side chains.

❖**Addition of Isoprenyl Groups** A number of eukaryotic proteins are modified by the addition of groups derived from isoprene (isoprenyl groups). A thioether bond is formed between the isoprenyl group and a Cys residue of the protein.

❖**Addition of Prosthetic Groups** Many prokaryotic and eukaryotic proteins require for their activity covalently bound prosthetic groups. Two examples are the biotinmolecule of acetyl-CoA carboxylase and the heme group of hemoglobin or cytochrome c.

❖**Proteolytic Processing** Many proteins are initially synthesized as large, inactive precursor polypeptides that are proteolytically trimmed to form their smaller, active forms. Examples include proinsulin, some viral proteins, and proteases such as chymotrypsinogen and trypsinogen

❖**Formation of Disulfide Cross-Links** After folding into their native conformations, some proteins form intrachain or interchain disulfide bridges between Cys residues. In eukaryotes, disulfide bonds are common in proteins to be exported from cells.