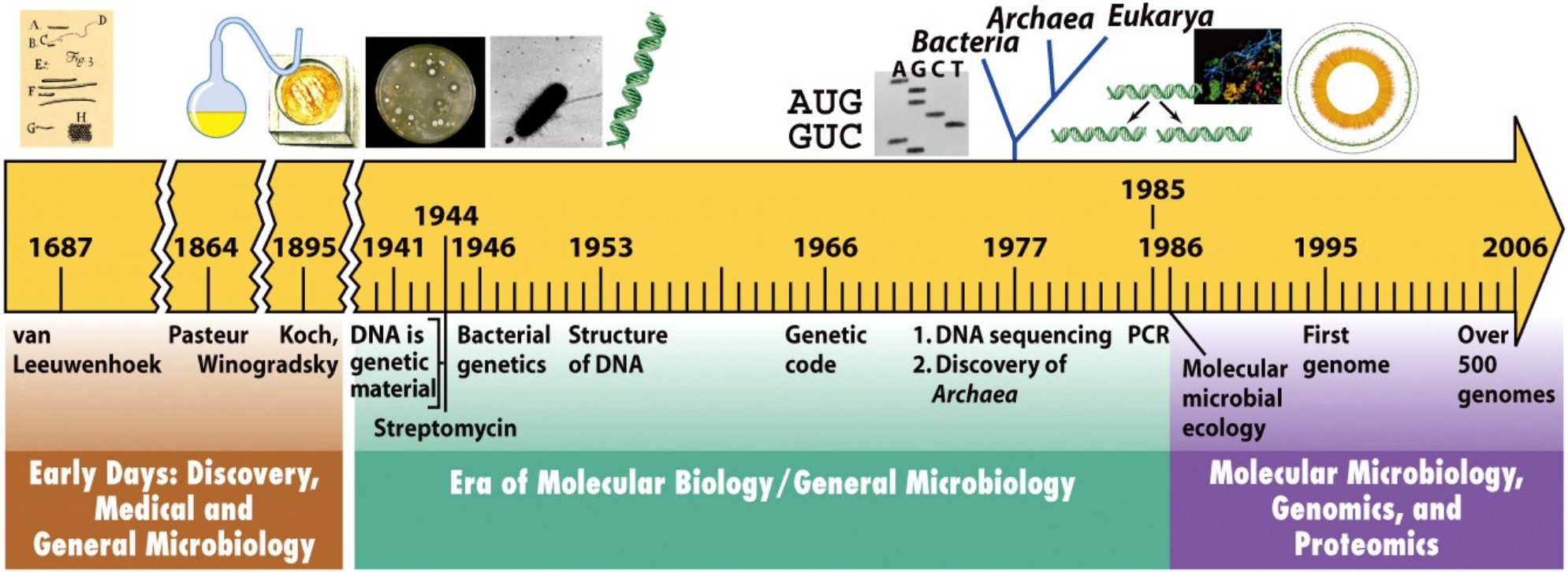
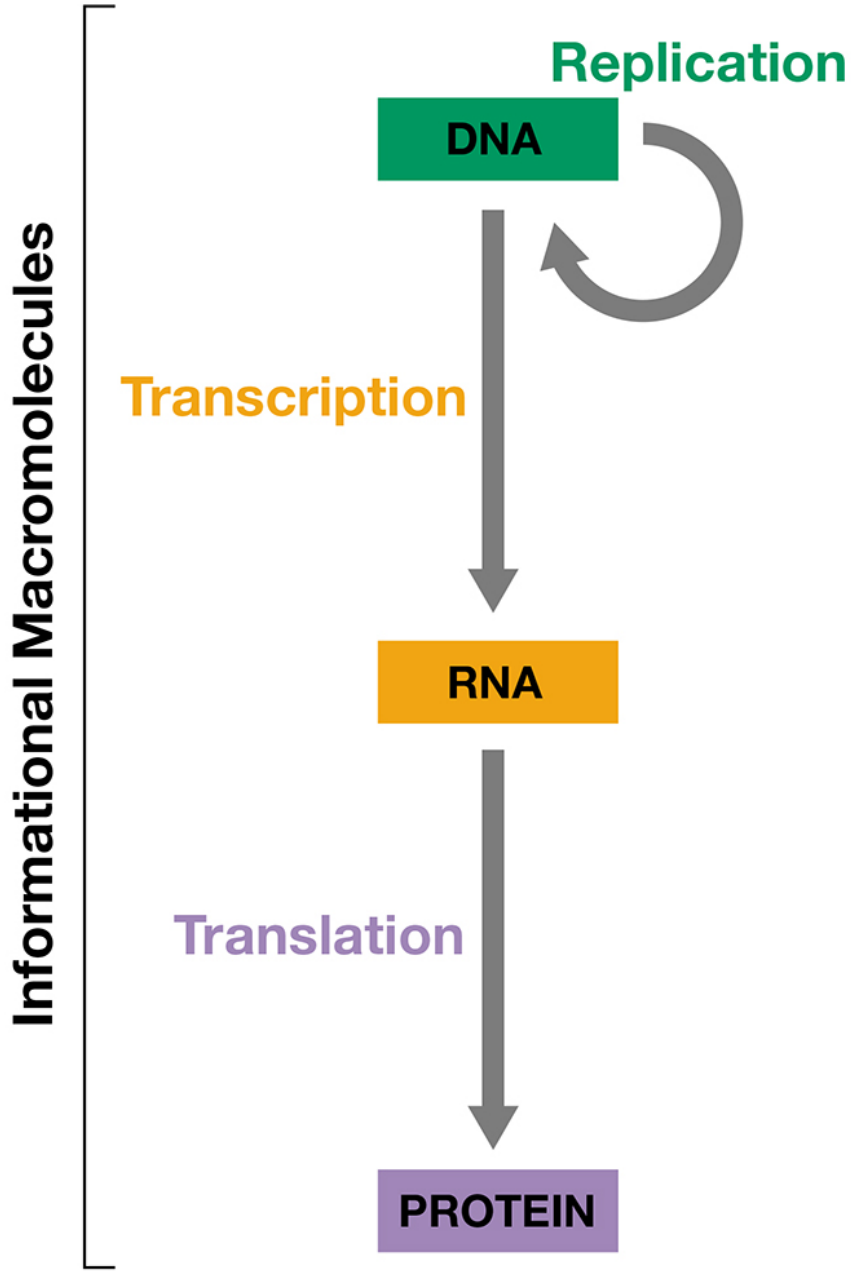


Figure 1-17 Brock Biology of Microorganisms 11/e

The Steps in Information flow

Definition of gene: A segment of DNA specifying a protein (via mRNA), a rRNA or a tRNA

The information in the gene is present as the sequence of bases in the DNA: purines (A & G) and pyrimidines (T & C)



(a)

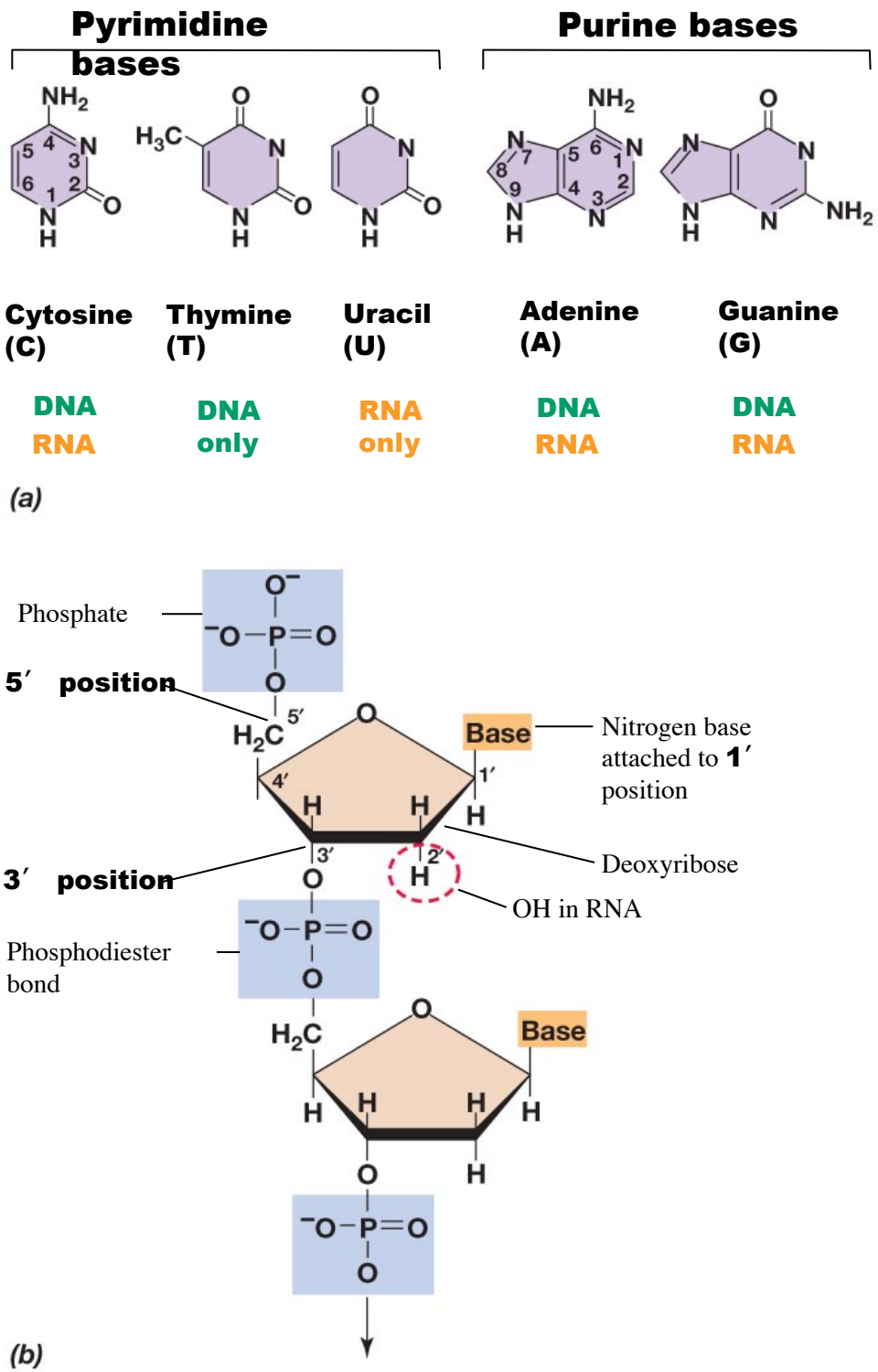
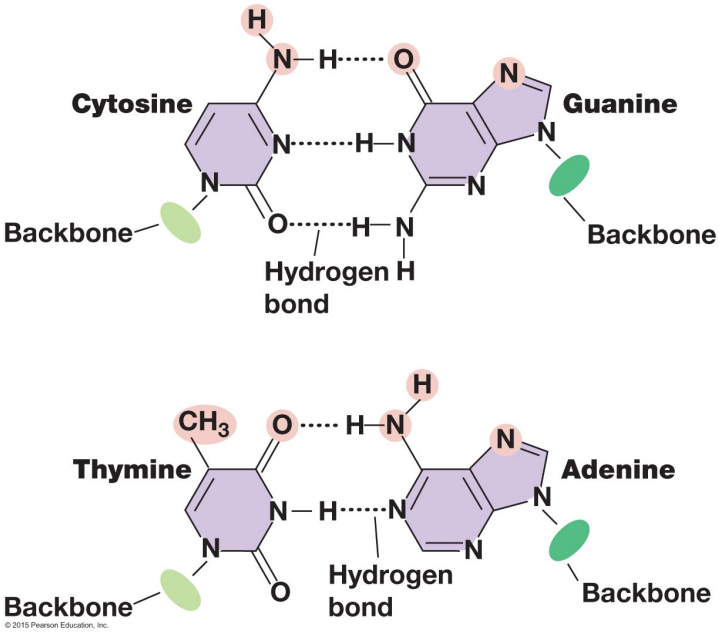


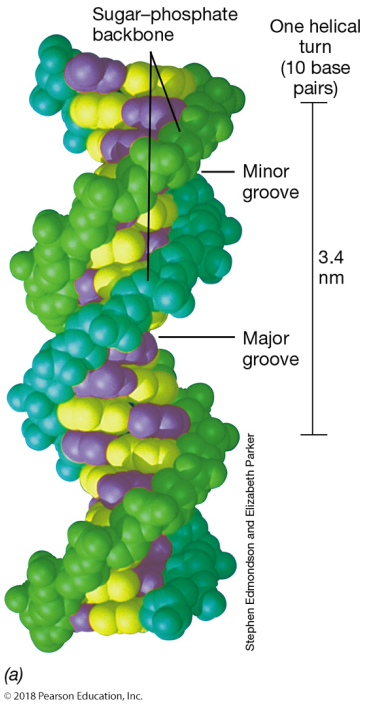
Figure 4.1

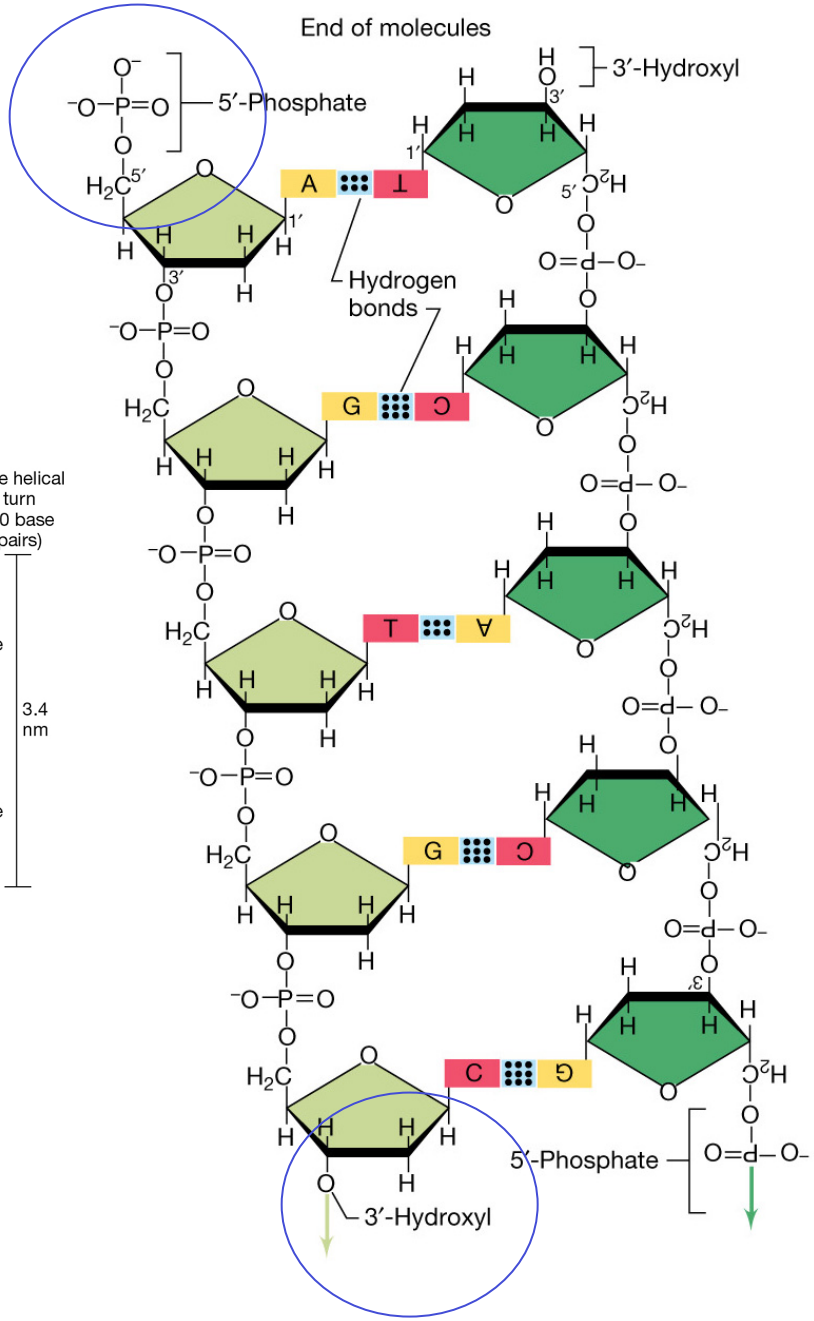
DNA structure

DNA is a double-stranded molecule that forms a helical configuration and is measured in terms of numbers of base pairs. The complementarity of DNA arises from the specific pairing of the purine and pyrimidine bases.

Watson and Crick, 1953
Nobel prize in Physiology and Medicine, 1962







Specific pairing between adenine (A) and thymine (T) and between guanine (G) and cytosine (C) via hydrogen bonds. C and T are pyrimidine bases. A and G are purine bases.

The length of a DNA molecule is usually expressed in number of base pairs. Kb: kilobase (1,000 bases). Mb: megabase (one million bases).
Example: *P. aeruginosa* genome: 6.26 Mb

Complementary and antiparallel nature of DNA. Note that one chain ends in a 5' -phosphate group, whereas the other ends in a free 3' -hydroxyl.

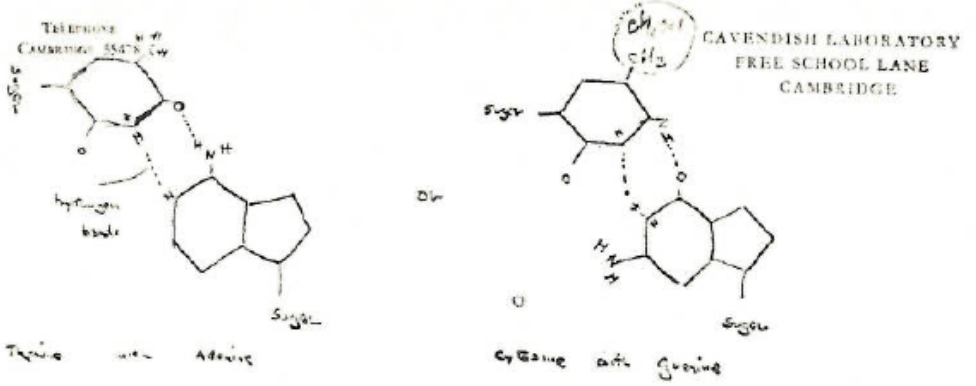
Part of a letter written by Jim Watson to Max Delbruck in which he explains the double helix.

March 12, 1953

From: “The Double Helix” by James Watson, 1968

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF PHYSICS

CAVENDISH LABORATORY
FREE SCHOOL LANE
CAMBRIDGE



Thymine with Adenine

Cytosine with Guanine

While my diagram is crude, in fact these pairs form 2 only wide hydrogen bonds in which all of the angles are exactly right. This pairing is based on the effective existence of only one out of the two possible tautomeric forms - in all cases we prefer the keto form over the enol, over the amino over the imino. This is a definitively an assumption but Jerry Donohue and Bill Cochran tell us that, for all organic molecules so far examined, the keto and amino forms are present in preference to the enol and imino possibilities.

The model has been derived entirely from stereochemical considerations with the only theory consideration being the spacing of the pair of bases 3.4 Å which was originally found by Astbury. It turns & builds itself with approximately 10 residues / per turn ie 34 Å. The screw is right handed.

The X-ray pattern approximately agrees with the model, but since the photographs available now are poor and meagre (we have no photographs of our own and the faculty must use Astbury's photographs) this agreement does in no way constitute a proof of our model. We are certainly a long way from proving its correctness. To do this we must obtain collaboration from the group at Kings College London who possess very excellent photographs of a crystalline phase in addition to rather good photographs of a paracrystalline phase. One model has been made in reference to the paracrystalline form and as yet we have no clear views as to how these helices can

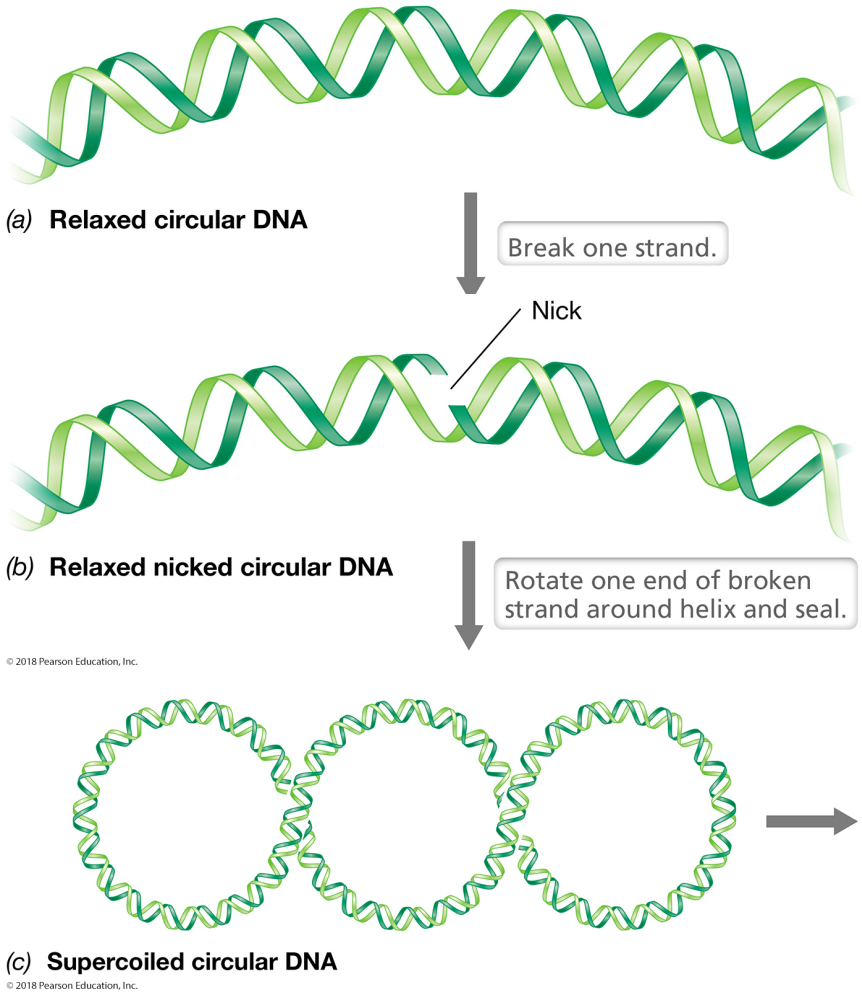
DNA Structure: Supercoiling

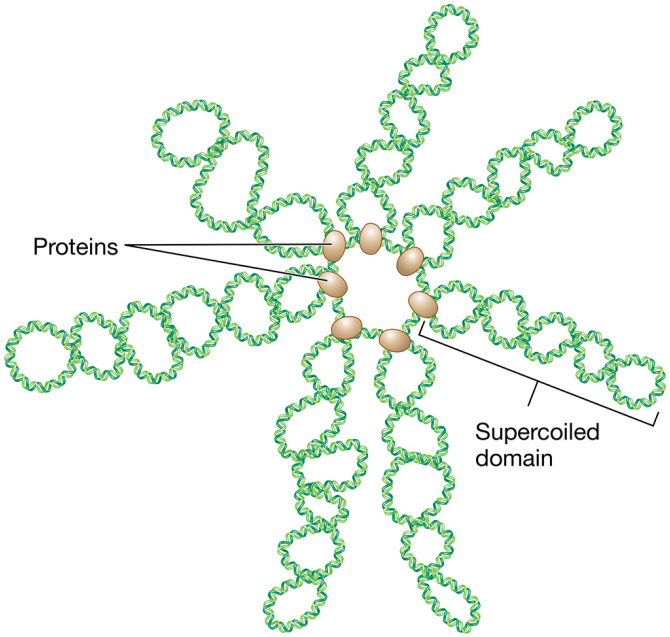
A solution to pack a large amount of DNA into small cells or viral particles

How long is the DNA of *E. coli*?

What is the volume of an *E. coli* cell?

The very long DNA molecule can be packaged into the cell because it is supercoiled

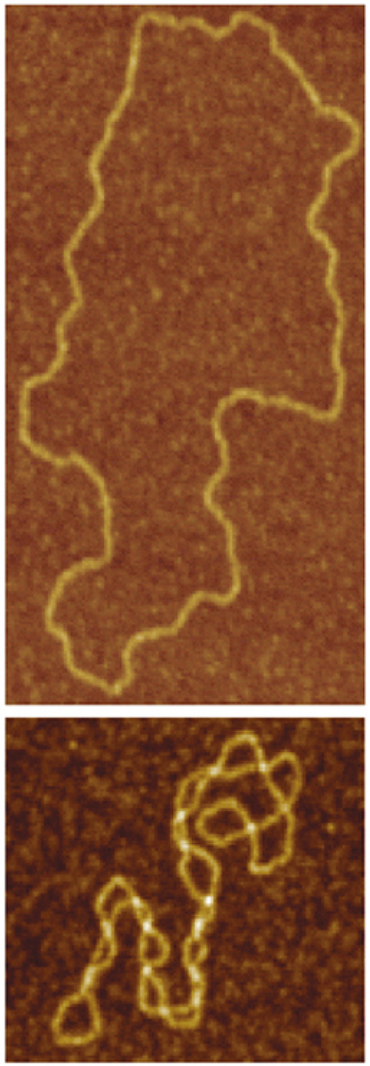




Supercoiled DNA. Parts a, b and c show supercoiled circular DNA and relaxed, nicked circular DNA interconversions. A nick is a break in a phosphodiester bond of one strand. (d) In actuality, the double-stranded DNA in the bacterial chromosome is arranged not in one supercoil but in several supercoiled domains, as shown here.

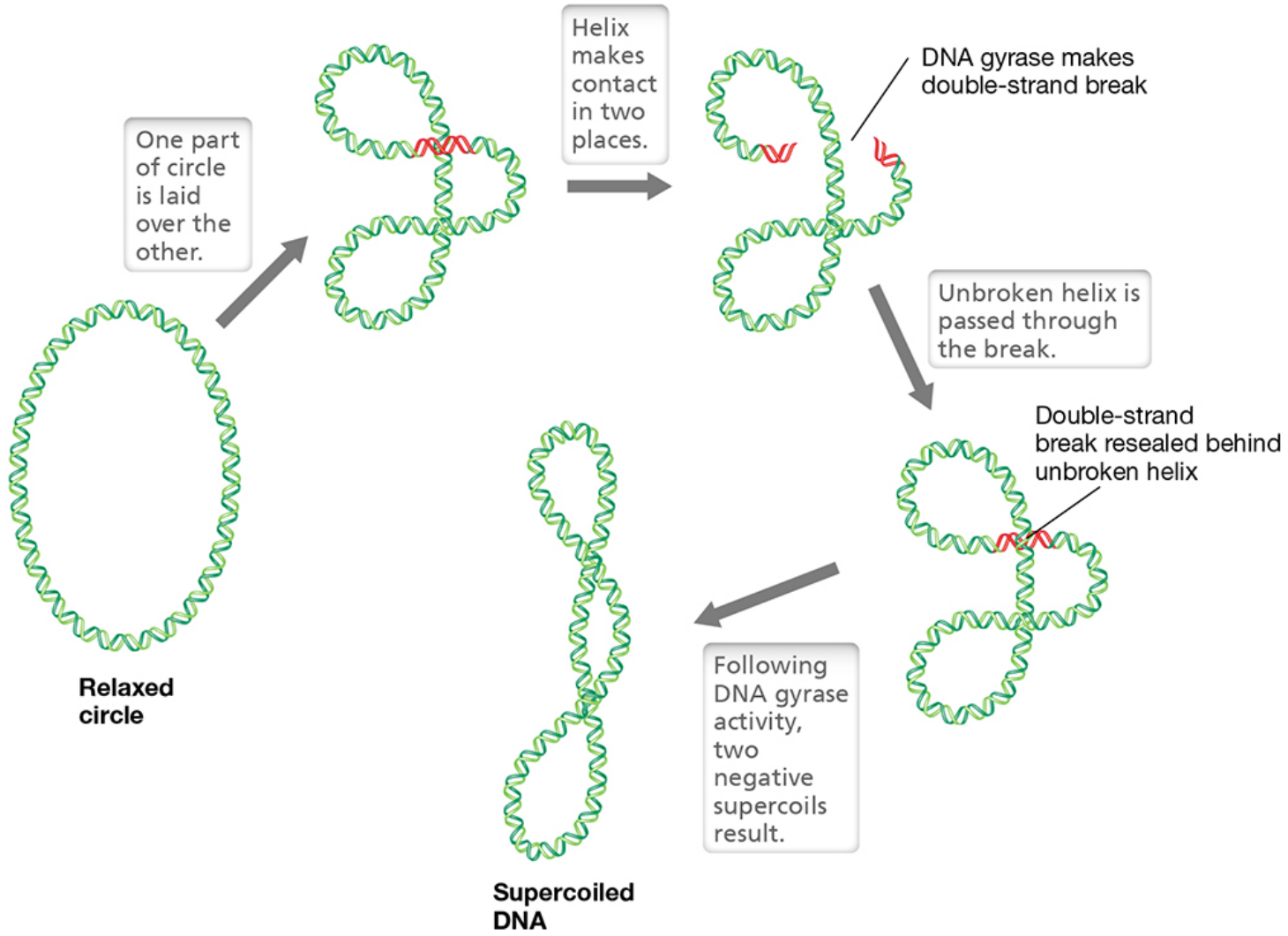
DNA Structure: Supercoiling (cont.)

In prokaryotes, this supercoiling is produced by enzymes called topoisomerases



Andrzej Stasiak. Modified from G. Witz and A. Stasiak. 2010. *Nucleic Acids Research* 38:2119-2133.

(a)

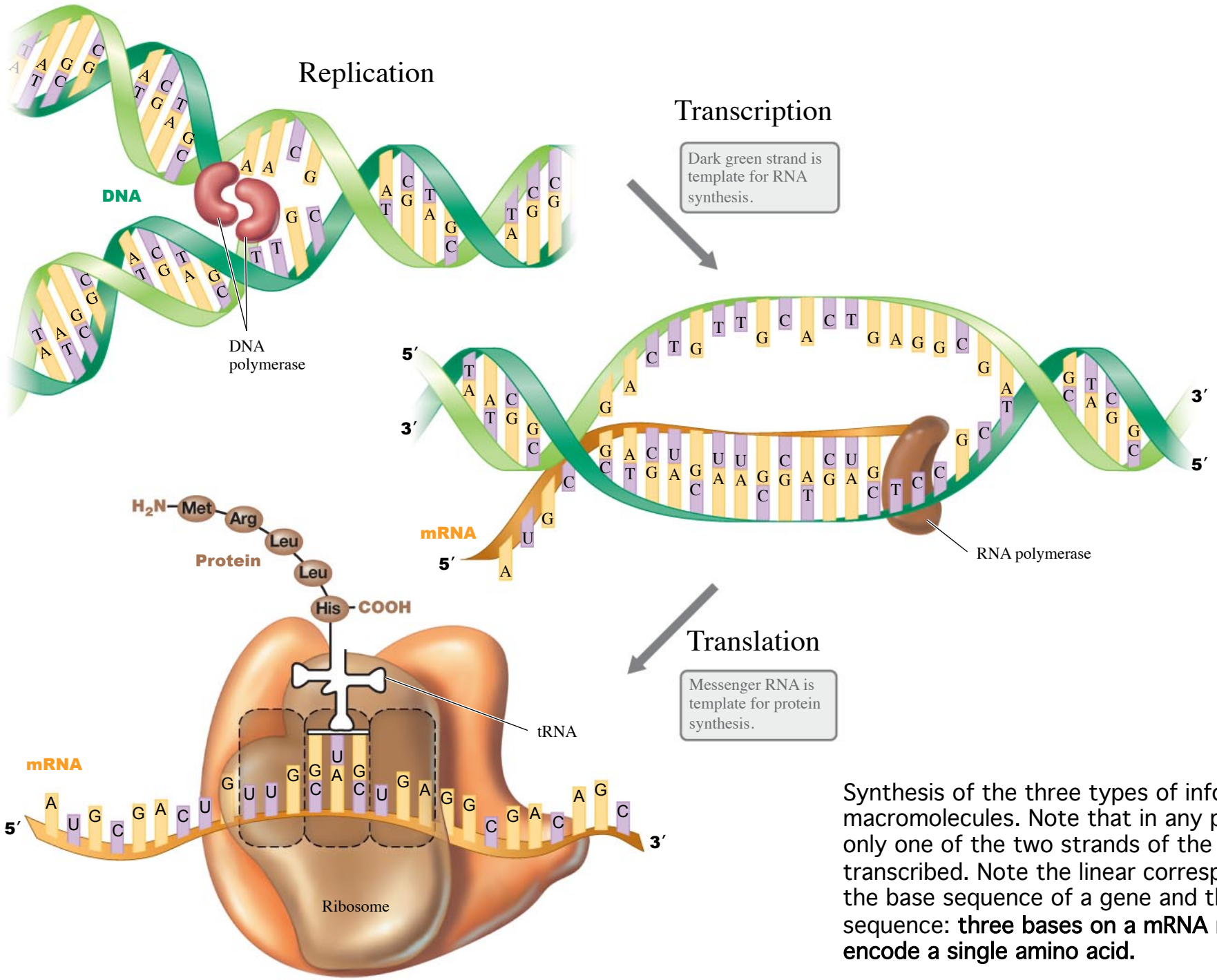


(b)

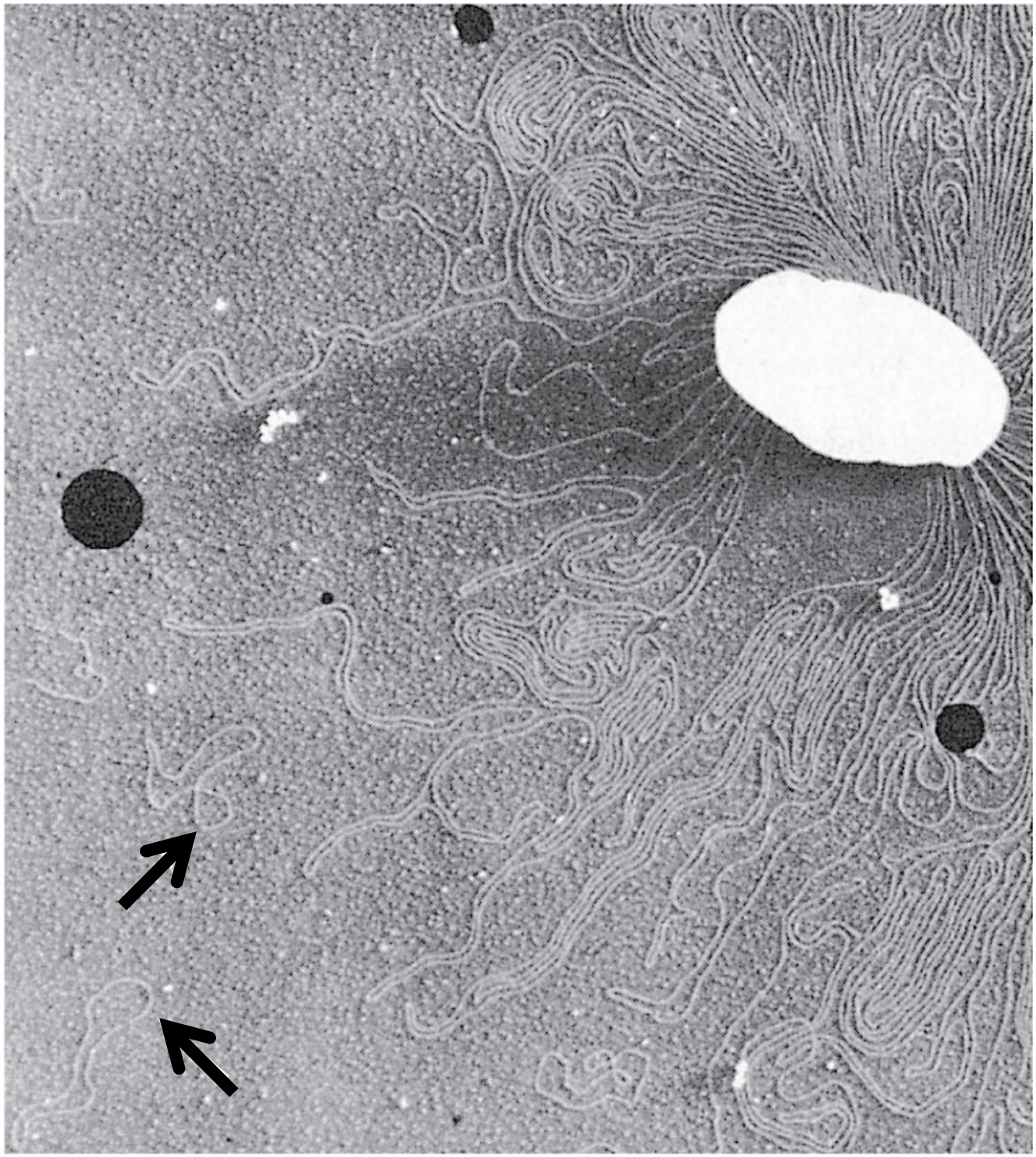
Introduction of supercoiling into a circular DNA by activity of topoisomerase II, a DNA gyrase, which makes double-strand breaks. The enzyme reverse gyrase introduces positive supercoils, and it is found in hyperthermophilic Archaea. Supercoiling is known to affect gene expression.

Figure 4.5

Synthesis of the three types of informational molecules

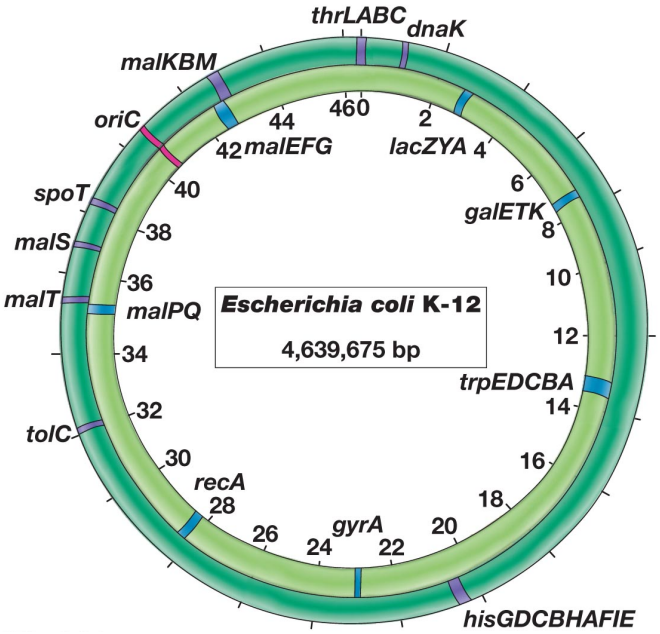


Synthesis of the three types of informational macromolecules. Note that in any particular region, only one of the two strands of the DNA double helix is transcribed. Note the linear correspondence between the base sequence of a gene and the amino acid sequence: **three bases on a mRNA molecule (codon) encode a single amino acid.**



Huntington Potter and David Dressler

The bacterial chromosome and plasmids (arrows)



The chromosome of *Escherichia coli* strain K-12

Figure 4.8

In addition to the **chromosome**, a number of other genetic elements exist in cells

TABLE 4.1 Kinds of genetic elements

Organism	*Element*	*Type of nucleic acid*	*Description*
Virus	Virus genome	Single- or double-stranded DNA or RNA	Relatively short, circular or linear
Bacteria/Archaea	Chromosome	Double-stranded DNA	Extremely long, usually circular
Eukaryote	Chromosome	Double-stranded DNA	Extremely long, linear
Mitochondrion or chloroplast	Organellar genome	Double-stranded DNA	Medium length, usually circular
All organisms	Plasmid[a]	Double-stranded DNA	Relatively short circular or linear, extrachromosomal
All organisms	Transposable element	Double-stranded DNA	Always found inserted into another DNA molecule

[a]Plasmids are uncommon in eukaryotes.

Plasmids are DNA molecules that exist separately from the chromosome of the cell.

Transposable elements are molecules of DNA that can move from one site on a chromosome to another.

Mitochondria and chloroplasts contain their own DNA chromosomes.

Viruses contain a **genome**, either DNA or RNA, that controls their own replication.

TABLE 4.2 Major enzymes that participate in DNA replication in *Bacteria*

Enzyme	*Encoding genes*	*Function*
DNA gyrase	*gyrAB*	Replaces supercoils ahead of replisome
Origin-binding protein	*dnaA*	Binds origin of replication to open double helix
Helicase loader	*dnaC*	Loads helicase at origin
Helicase	*dnaB*	Unwinds double helix at replication fork
Single-strand binding protein	*ssb*	Prevents single strands from annealing
Primase	*dnaG*	Primes new strands of DNA
DNA polymerase III		Main polymerizing enzyme
Sliding clamp	*dnaN*	Holds Pol III on DNA
Clamp loader	*holA–E*	Loads Pol III onto sliding clamp
Dimerization subunit (Tau)	*dnaX*	Holds together the two core enzymes for the leading and lagging strands
Polymerase subunit	*dnaE*	Strand elongation
Proofreading subunit	*dnaQ*	Proofreading
DNA polymerase I	*polA*	Excises RNA primer and fills in gaps
DNA ligase	*ligA, ligB*	Seals nicks in DNA
Tus protein	*tus*	Binds terminus and blocks progress of the replication fork
Topoisomerase IV	*parCE*	Unlinking of interlocked circles

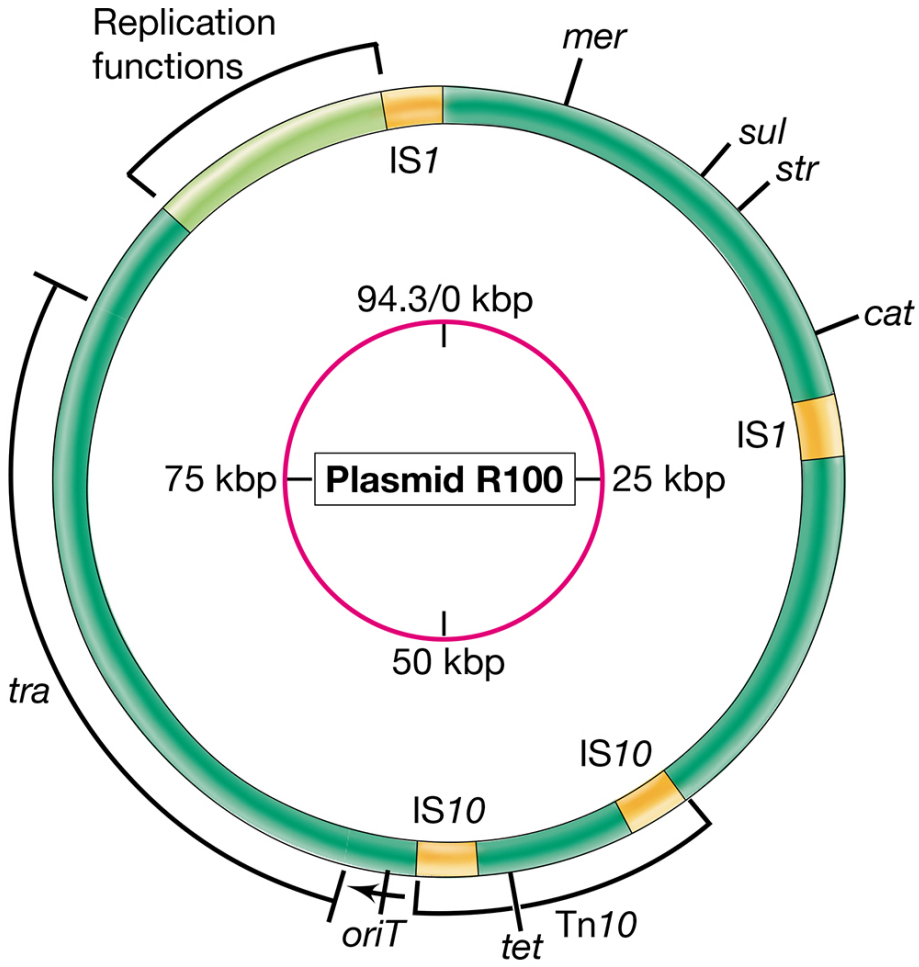
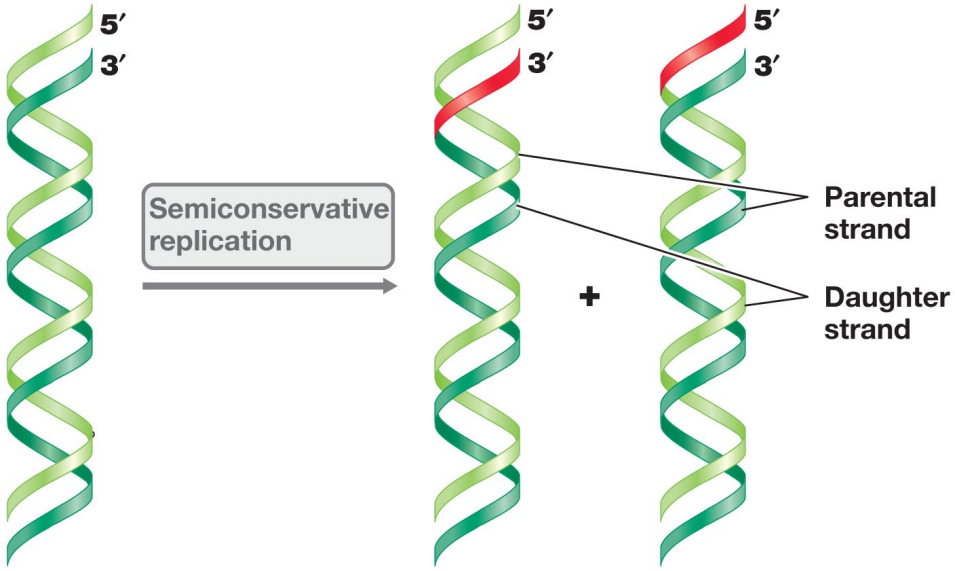


Figure 4.10

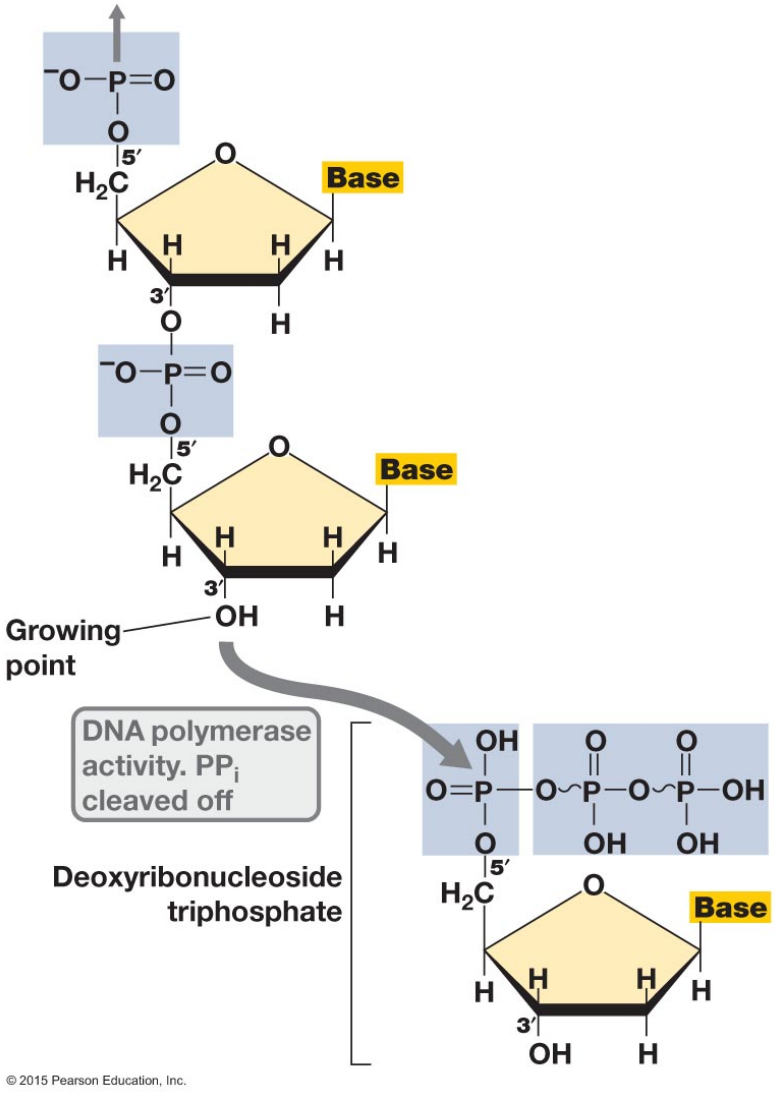
DNA Replication

Both strands of the DNA helix serve as templates for the synthesis of two new strands (**semiconservative replication**)s

Meselson and Stahl, 1958

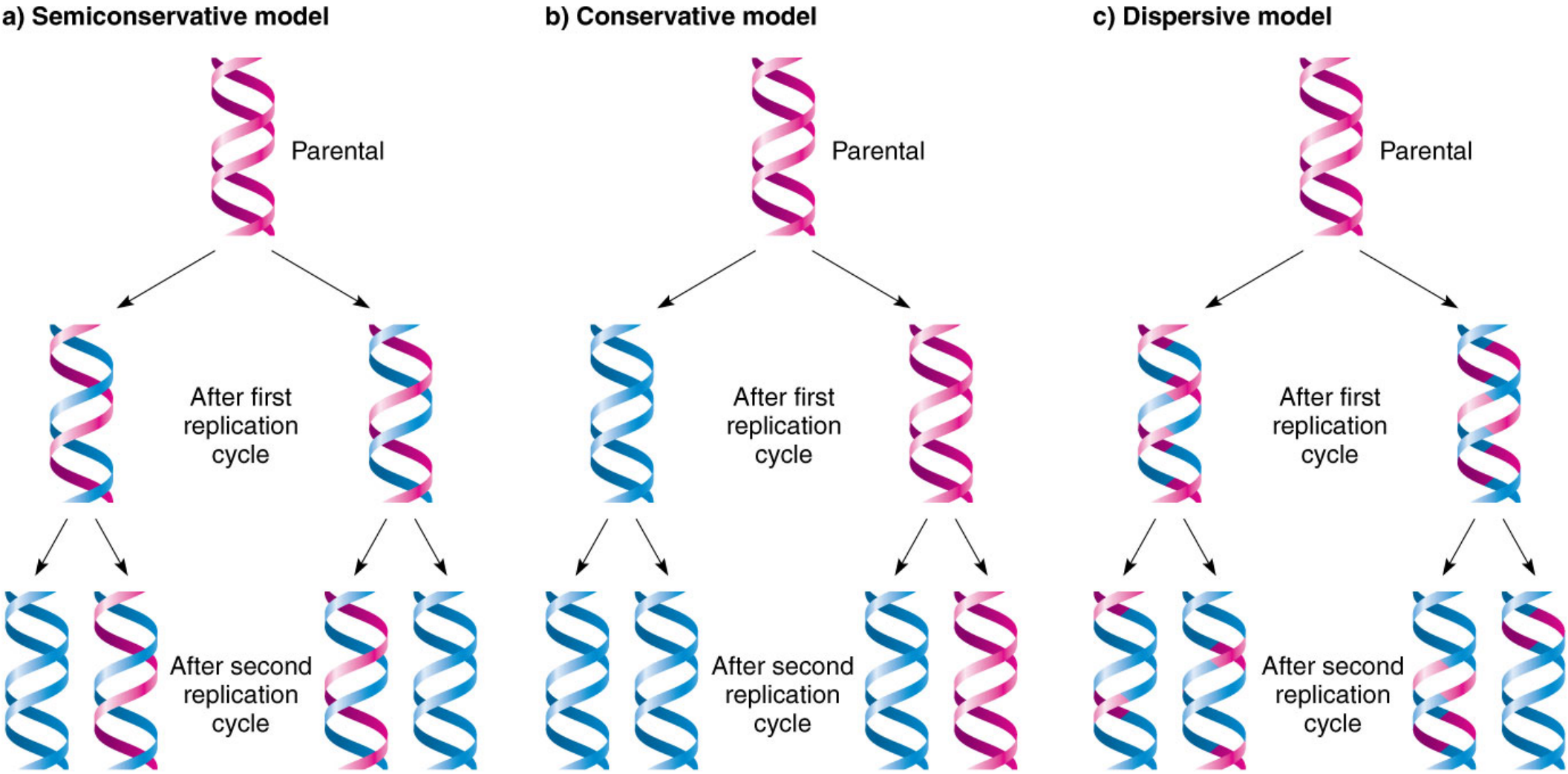


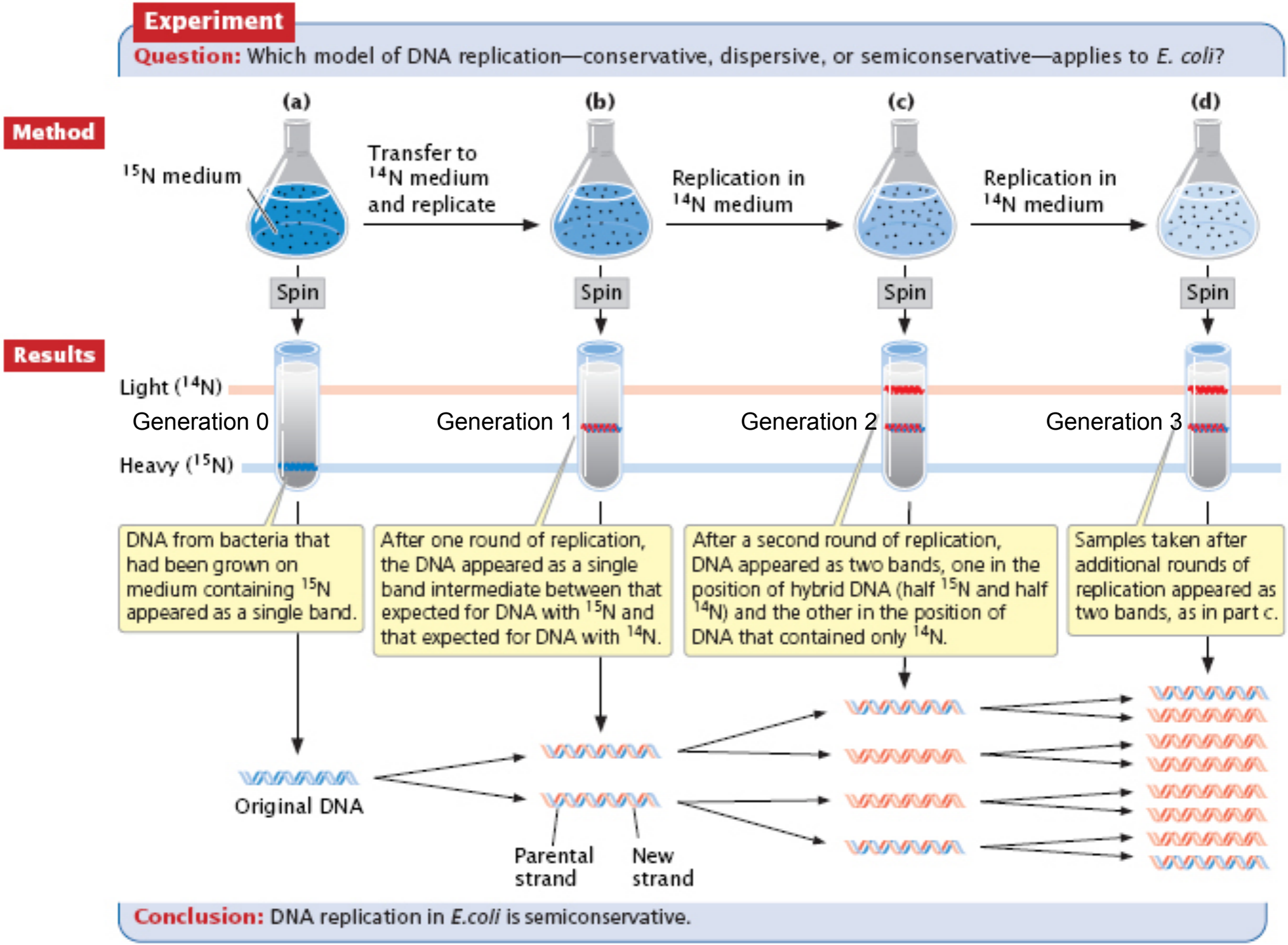
The two progeny double helices each contain one parental strand and one new strand (semiconservative). The new strands are elongated by addition to the 3' end



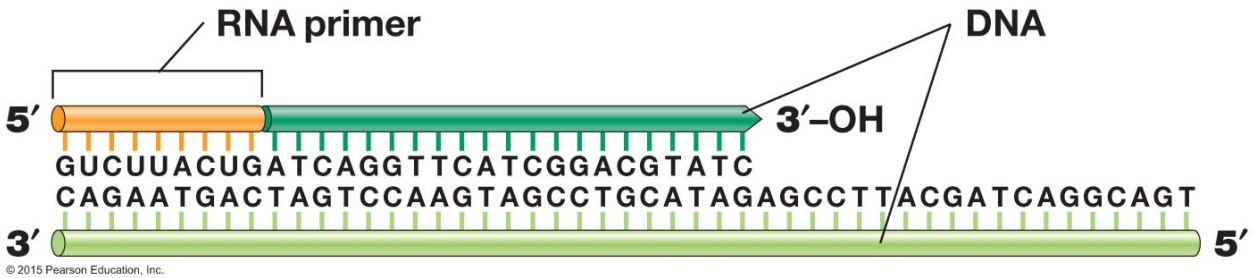
(a) DNA replication is a semiconservative process in both prokaryotes and eukaryotes. Note that the new double helices each contain one new and one old strand. (b) Structure of the DNA chain and mechanism of growth by addition from a deoxyribonucleoside triphosphate at the 3´-end of the chain. Growth always proceeds from the 5´-phosphate to the 3´-hydroxyl end, the 5'-P of the incoming nucleotide being attached to the 3'-OH of the previously added nucleotide. The enzyme DNA polymerase catalyzes the addition reaction.

Models of DNA Replication



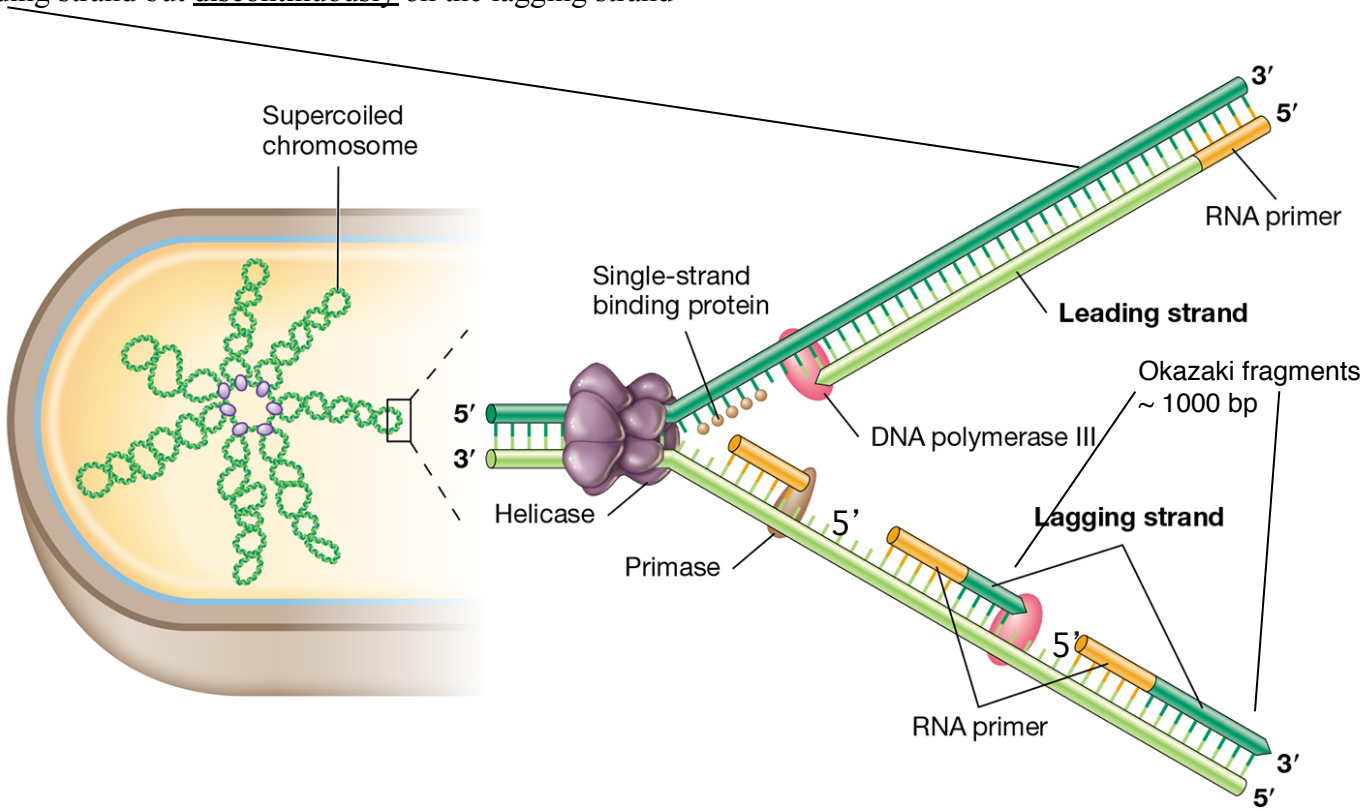

Experiment
Question: Which model of DNA replication—conservative, dispersive, or semiconservative—applies to E. coli?
Method
(a)
(b)
(c)
(d)
15N medium
Transfer to 14N medium and replicate
Replication in 14N medium
Replication in 14N medium
Spin
Spin
Spin
Spin
Results
Light (14N)
Heavy (15N)
Generation 0
Generation 1
Generation 2
Generation 3
DNA from bacteria that had been grown on medium containing 15N appeared as a single band.
After one round of replication, the DNA appeared as a single band intermediate between that expected for DNA with 15N and that expected for DNA with 14N.
After a second round of replication, DNA appeared as two bands, one in the position of hybrid DNA (half 15N and half 14N) and the other in the position of DNA that contained only 14N.
Samples taken after additional rounds of replication appeared as two bands, as in part c.
Original DNA
Parental strand
New strand
Conclusion: DNA replication in E.coli is semiconservative.

DNA Replication (cont.)



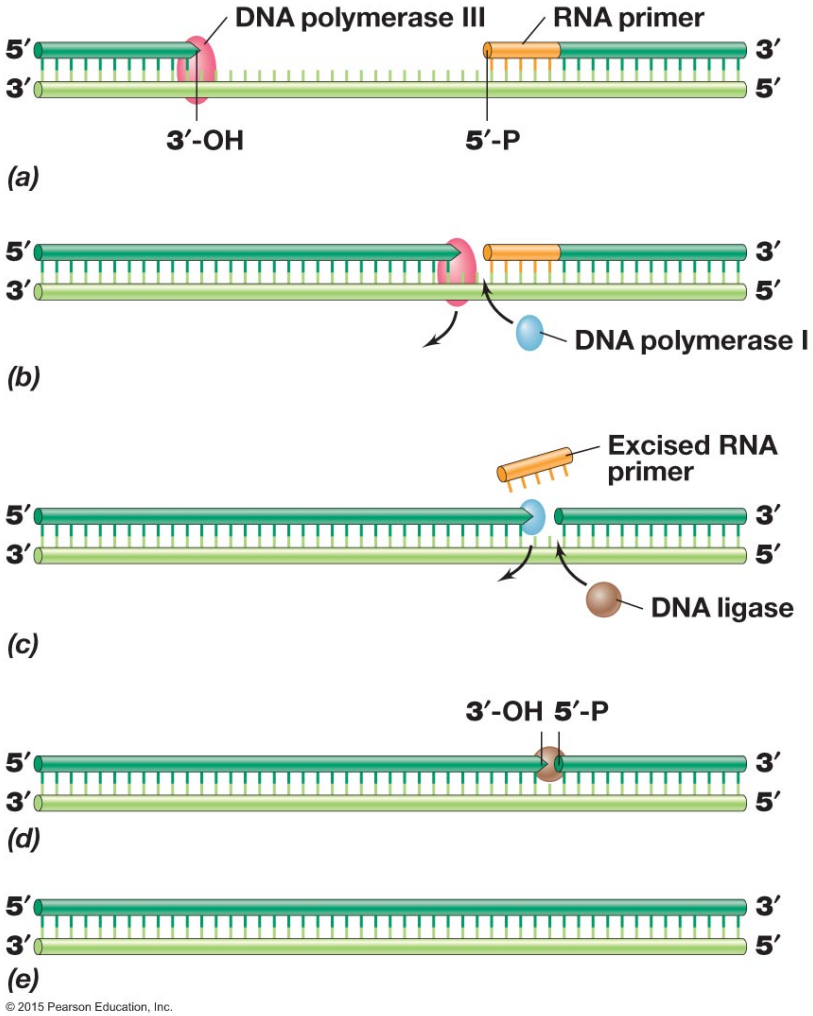
DNA polymerases require a **primer**, which is composed of RNA. This short segment is synthesized by the enzyme **primase**. No known DNA polymerase can begin a new chain without a primer. All these enzymes can only add a nucleotide to a preexisting 3'-OH group. Hence, the necessity for a primer.

Extension of the DNA occurs <u>continuously</u> on the leading strand but <u>discontinuously</u> on the lagging strand



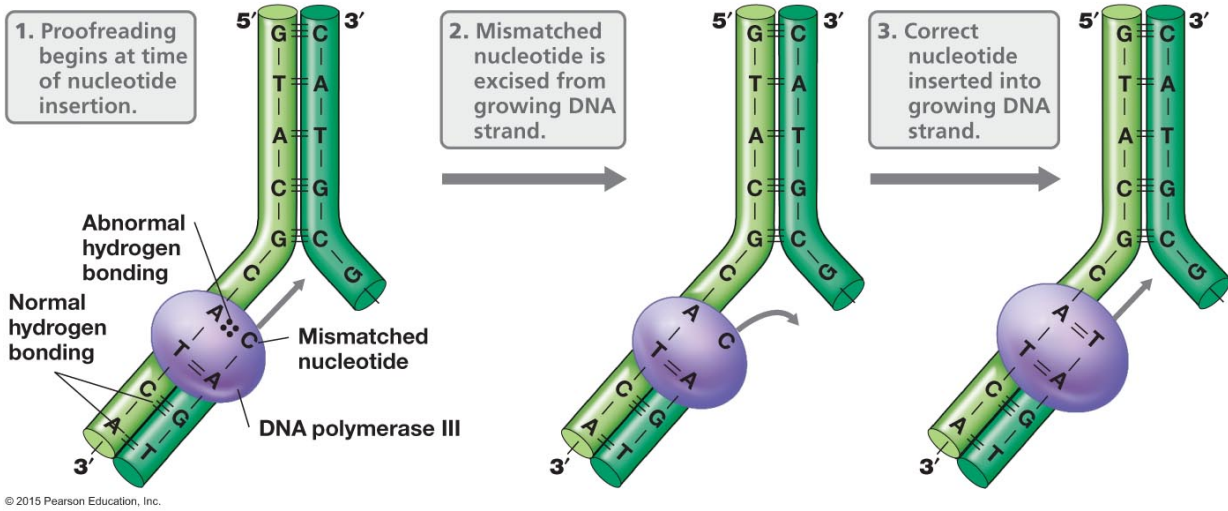
Events at the DNA <u>replication fork</u>. Note the polarity and antiparallel nature of the DNA strands. The substrates for <u>primase</u> are ribonucleotide triphosphates, while for <u>DNA polymerase</u>, they are deoxyribonucleotide triphosphates.

DNA Replication (cont.)



Sealing two fragments on the lagging strand. (a) DNA polymerase III is synthesizing DNA in the 5´ → 3´ direction toward the RNA primer of a previously synthesized fragment on the lagging strand. (b) On reaching the fragment, DNA polymerase I replaces III. (c) **DNA polymerase I continues synthesizing DNA while removing the RNA primer from the previous fragment.** (d) DNA ligase replaces DNA polymerase I after the primer has been removed. (e) DNA ligase seals the two fragments together.

During DNA replication the mutation rate is low (10^{-8}-10^{-11} errors per base pair inserted)
Proofreading: mismatch repair



Proofreading by the 3´ → 5´ exonuclease activity of DNA polymerase III. (a) A mismatch in base pairing at the terminal base pair causes the polymerase to pause briefly. This is a signal for the proofreading activity (b) to excise the mismatched nucleotide, after which the correct base is incorporated (c) by polymerase activity.

TABLE 4.2 Major enzymes that participate in DNA replication in *Bacteria*

Enzyme	*Encoding genes*	*Function*
DNA gyrase	*gyrAB*	Replaces supercoils ahead of replisome
Origin-binding protein	*dnaA*	Binds origin of replication to open double helix
Helicase loader	*dnaC*	Loads helicase at origin
Helicase	*dnaB*	Unwinds double helix at replication fork
Single-strand binding protein	*ssb*	Prevents single strands from annealing
Primase	*dnaG*	Primes new strands of DNA
DNA polymerase III		Main polymerizing enzyme
Sliding clamp	*dnaN*	Holds Pol III on DNA
Clamp loader	*holA–E*	Loads Pol III onto sliding clamp
Dimerization subunit (Tau)	*dnaX*	Holds together the two core enzymes for the leading and lagging strands
Polymerase subunit	*dnaE*	Strand elongation
Proofreading subunit	*dnaQ*	Proofreading
DNA polymerase I	*polA*	Excises RNA primer and fills in gaps
DNA ligase	*ligA, ligB*	Seals nicks in DNA
Tus protein	*tus*	Binds terminus and blocks progress of the replication fork
Topoisomerase IV	*parCE*	Unlinking of interlocked circles

RNA synthesis: Transcription

The three major types of RNA:

Messenger RNA (mRNA): contains the genetic information to encode one or more polypeptides. Its role is at the genetic level, by carrying the genetic information from the DNA.

Transfer RNA (tRNA): an adaptor molecule used in translation that has specificity for both a particular amino acid and for one or more codons. Its role is at the functional and structural levels.

Ribosomal RNA (rRNA): types of RNA found in the ribosome (functional and structural roles).

Transcription

Transcription of RNA from DNA involves the enzyme **RNA polymerase**, which adds bases onto the 3' ends of growing chains.

Unlike DNA polymerase, RNA polymerase **needs no primer** and recognizes a specific start site on the DNA called the **promoter**

Steps in RNA synthesis.

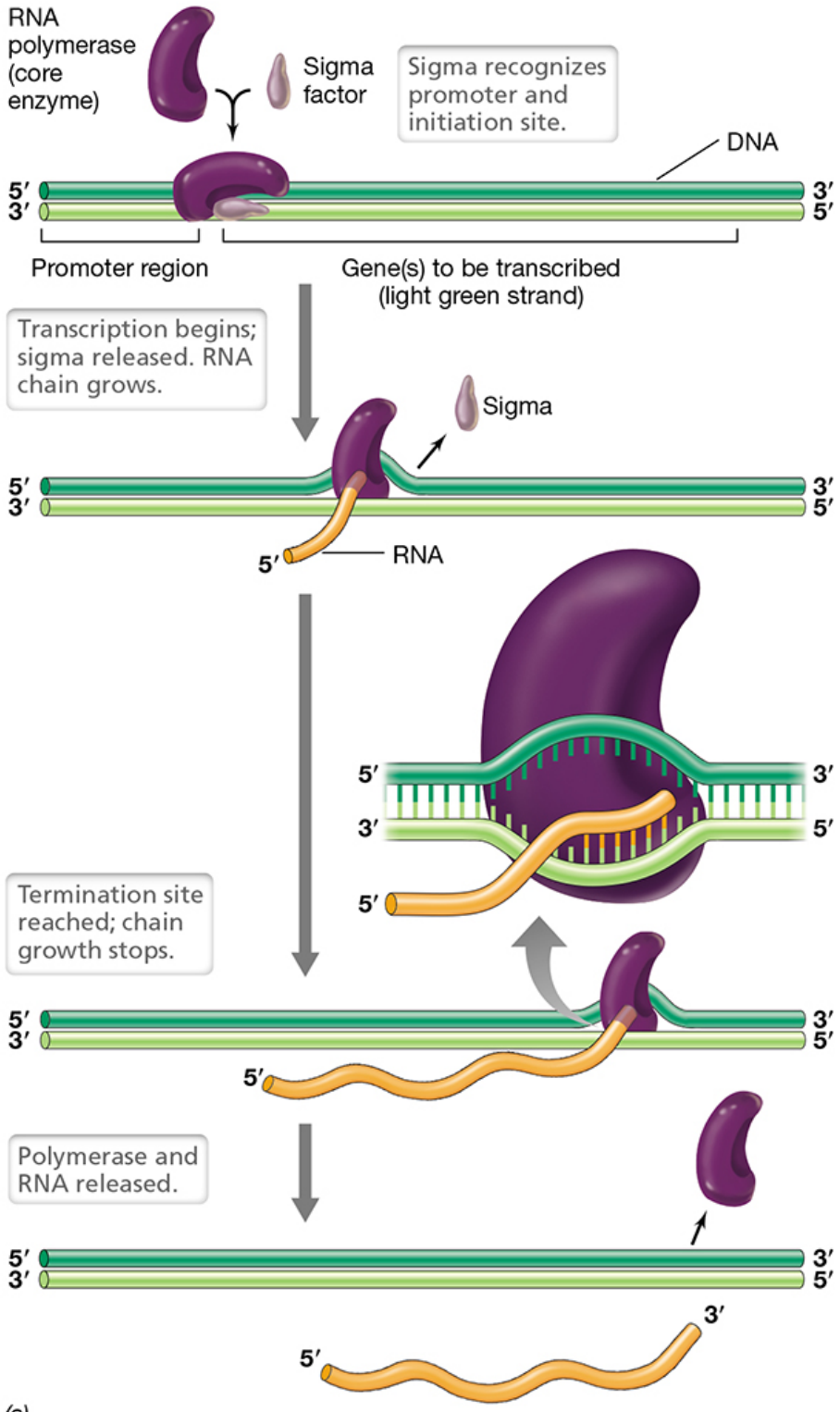
The initiation and termination sites are specific nucleotide sequences on the DNA.

The sigma factor allows RNA polymerase to recognize the initiation site (the promoter).

Which strand of the DNA is transcribed is determined by the orientation of the promoter sequence.

The sigma factor is released during elongation.

RNA polymerase moves down the DNA chain, causing temporary opening of the double helix and transcription of one of the DNA strands. When a termination site is reached, chain growth stops, and the mRNA and polymerase are released.



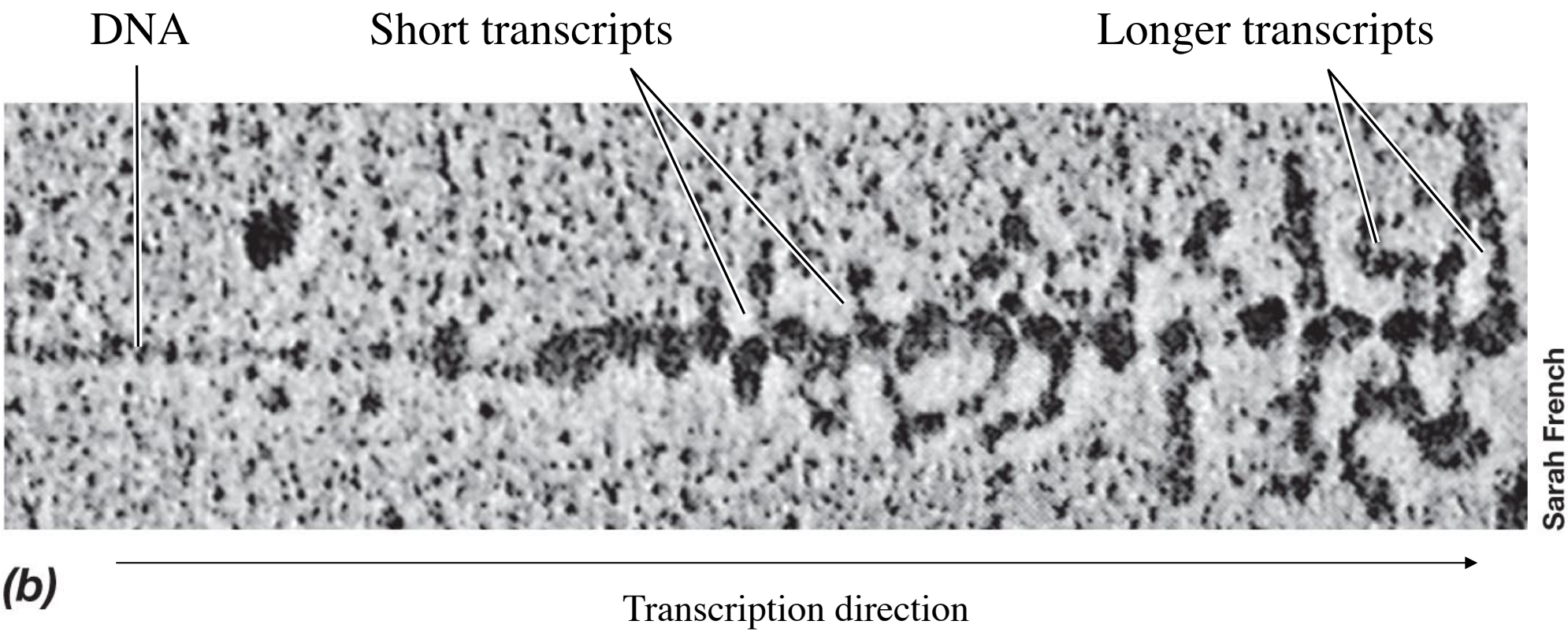
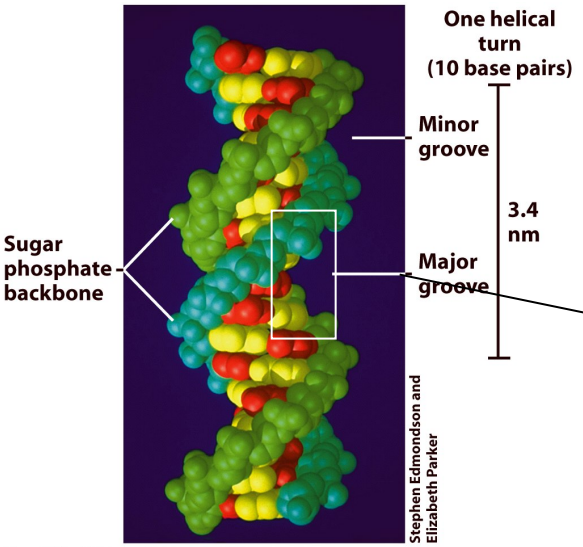

DNA
Short transcripts
Longer transcripts
Sarah French
(b)
Transcription direction

Figure 4.19b

The interaction of RNA polymerase with the promoter

In *Bacteria,* promoters are recognized by the sigma subunit of RNA polymerase. Promoters recognized by a specific sigma factor have very similar sequences.



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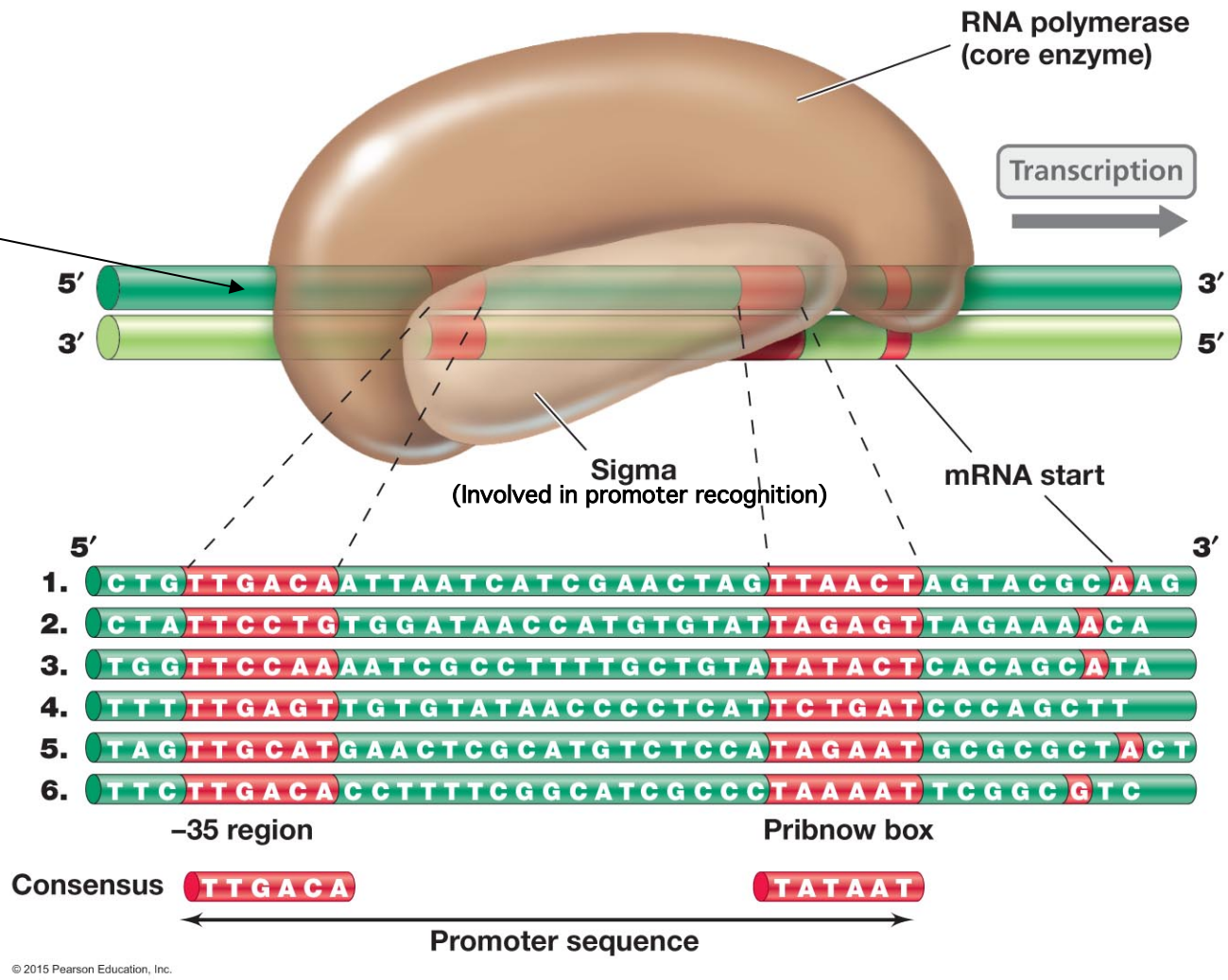


Any given bacterium synthesizes several sigma factors, which are involved in the transcription of different genes. Hence, sigma factors control gene expression

Shown below the diagram are six different promoter sequences identified in *Escherichia coli* , a species of *Bacteria*. The contacts of the RNA polymerase with the –35 sequence and the Pribnow box (–10 sequence) are shown. Transcription begins at a unique base just downstream from the Pribnow box. Strong promoters are most like the consensus and are more effective in binding the RNA polymerase.

TABLE 4.3 Sigma factors in *Escherichia coli*

Name[a]	*Upstream recognition sequence*[b]	*Function*
σ^{70} RpoD	TTGACA	For most genes, major sigma factor for normal growth
σ^{54} RpoN	TTGGCACA	Nitrogen assimilation
σ^{38} RpoS	CCGGCG	Stationary phase, plus oxidative and osmotic stress
σ^{32} RpoH	TNTCNCCTTGAA	Heat shock response
σ^{28} FliA	TAAA	For genes involved in flagella synthesis
σ^{24} RpoE	GAACTT	Response to misfolded proteins in periplasm
σ^{19} FecI	AAGGAAAAT	For certain genes in iron transport

[a]Superscript number indicates size of protein in kilodaltons. Many factors also have other names, for example, σ^{70} is also called σ^{D}.

[b]N = any nucleotide.

RNA polymerase from the three domains

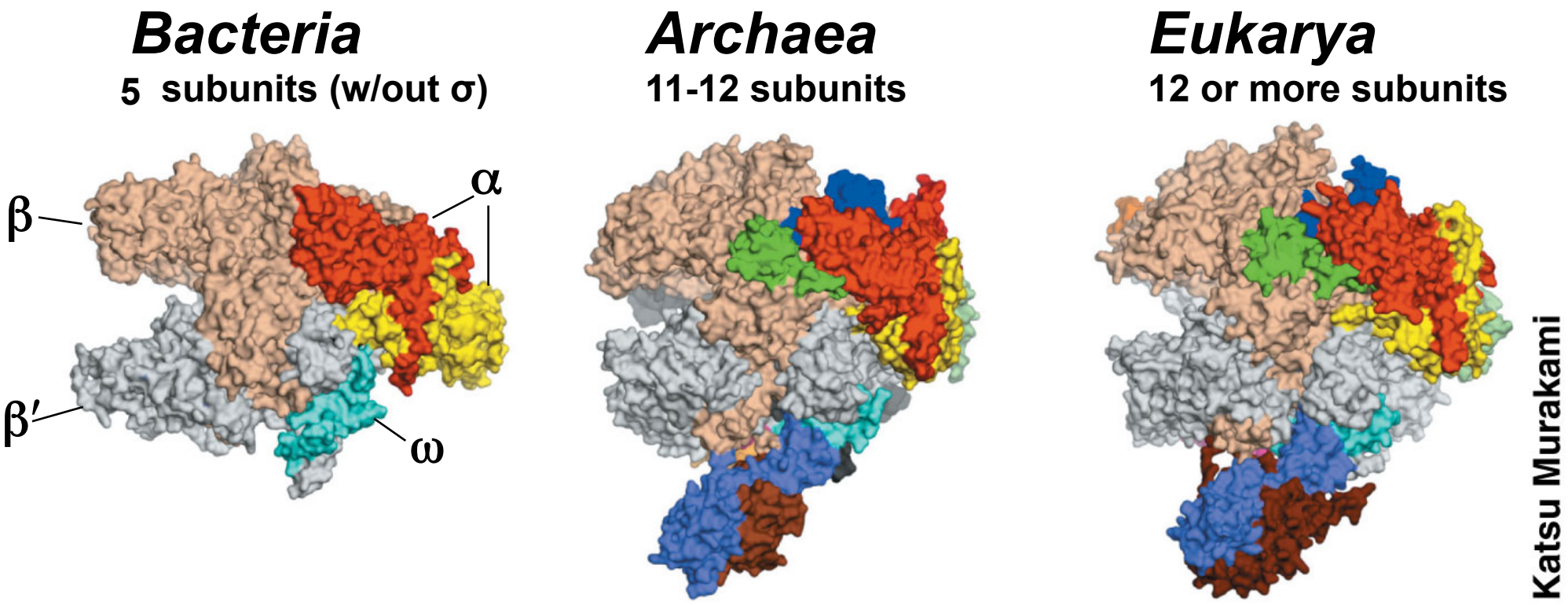
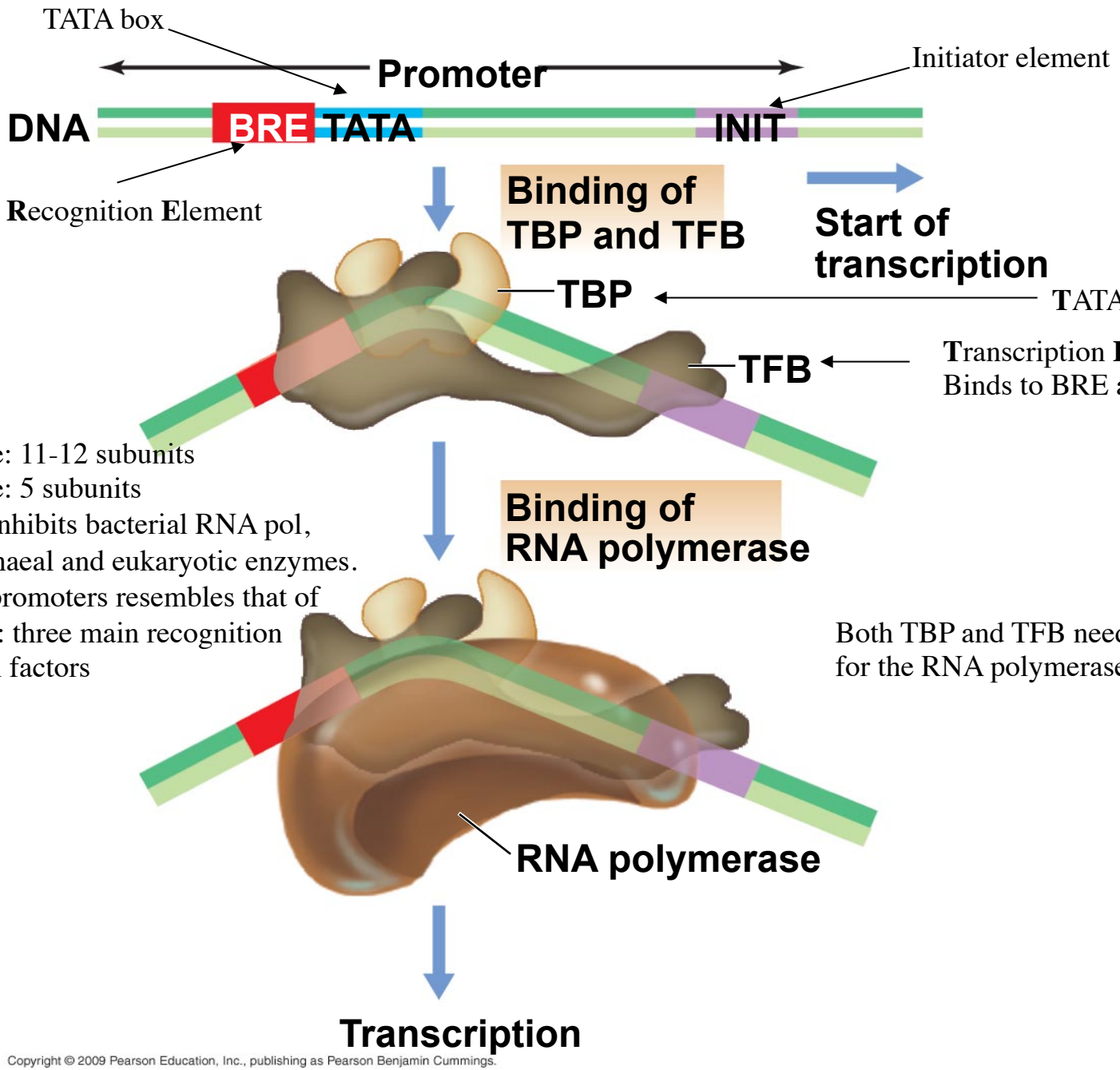


Figure 4.20

Promoter Architecture and Transcription in Archaea

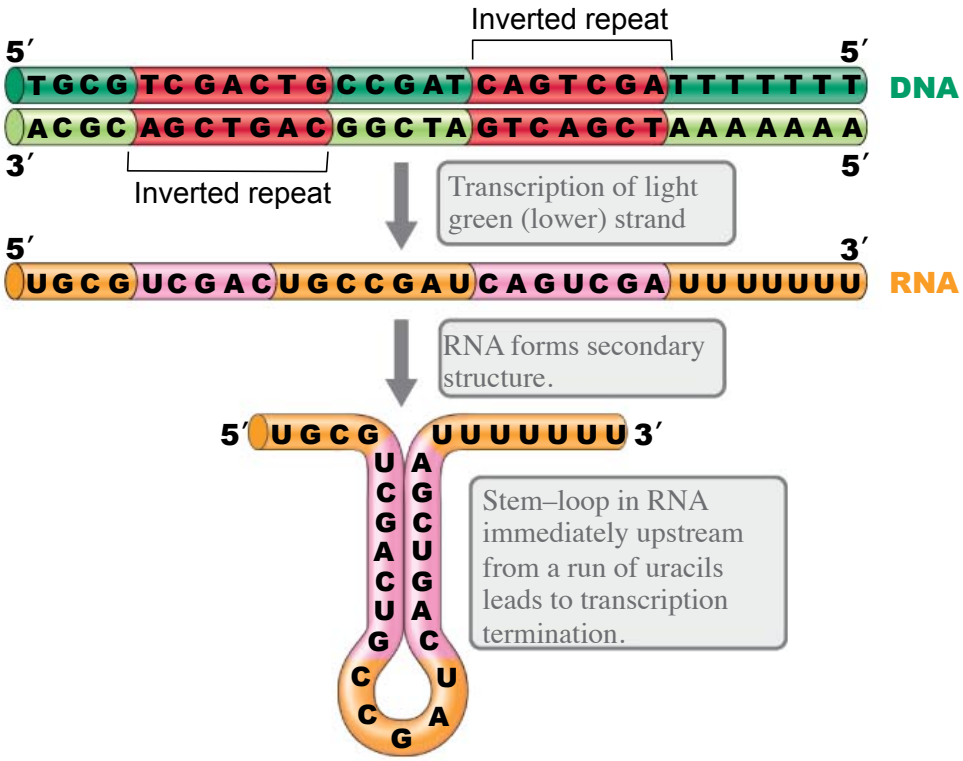


- Archaeal RNA polymerase: 11-12 subunits
- Bacterial RNA polymerase: 5 subunits
- The antibiotic rifampicin inhibits bacterial RNA pol, but does not inhibit the archaeal and eukaryotic enzymes.
- The structure of archaeal promoters resembles that of some eukaryotic promoters: three main recognition sequences and transcription factors

Both TBP and TFB need to be in place for the RNA polymerase to bind

Transcription Termination

RNA polymerase stops transcription at specific sites called transcription terminators. Inverted repeats in transcribed DNA lead to formation of a stem-loop structure in the RNA, which can result in termination of transcription.



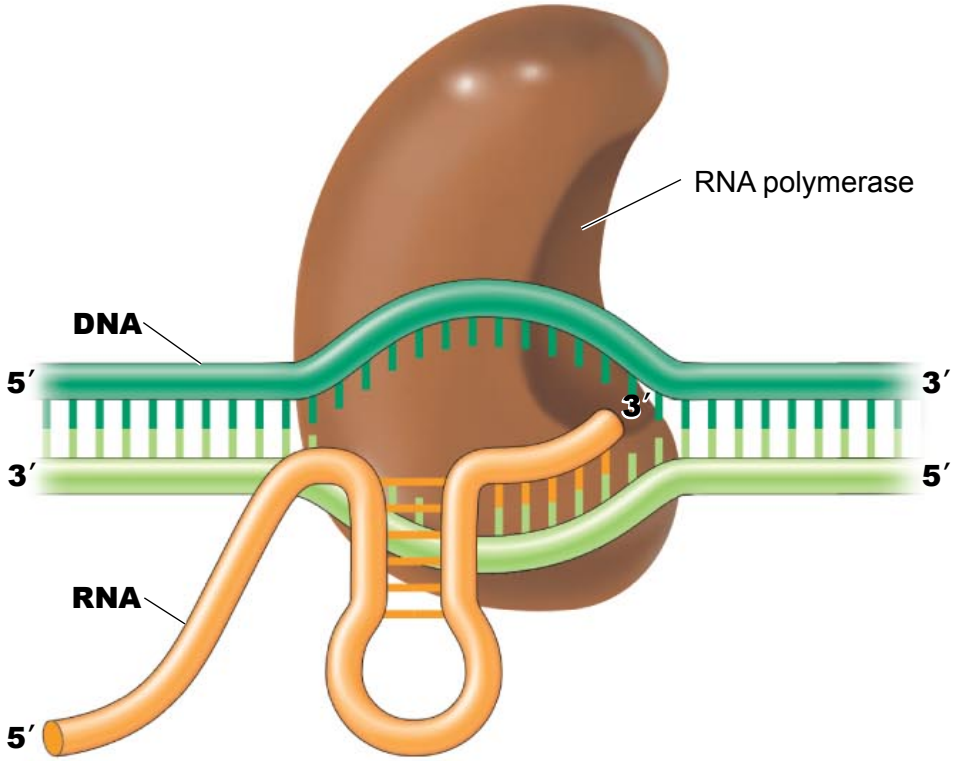


Figure 4.24

Unit of Transcription: The Operon

The unit of transcription often contains more than a single **gene**. Transcription of several genes into a single mRNA molecule may occur in prokaryotes, and so the mRNA may contain the information for more than one polypeptide

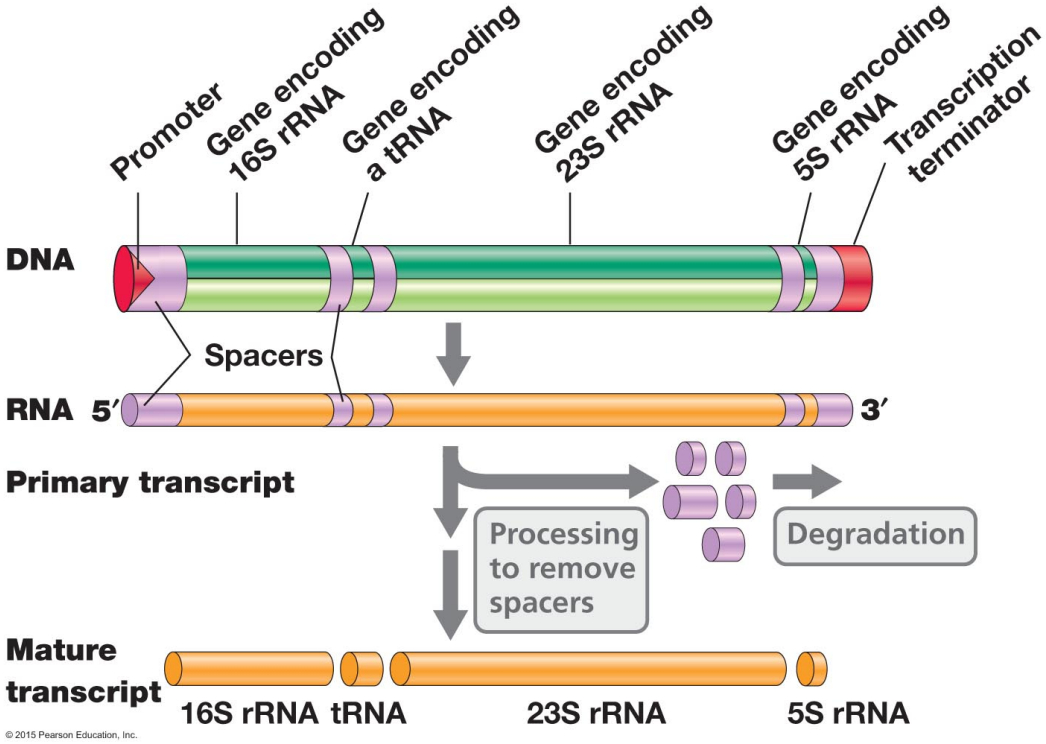


Fig. 4.22 A ribosomal **rRNA** transcription unit **(rRNA operon)** from *Bacteria* and its subsequent processing

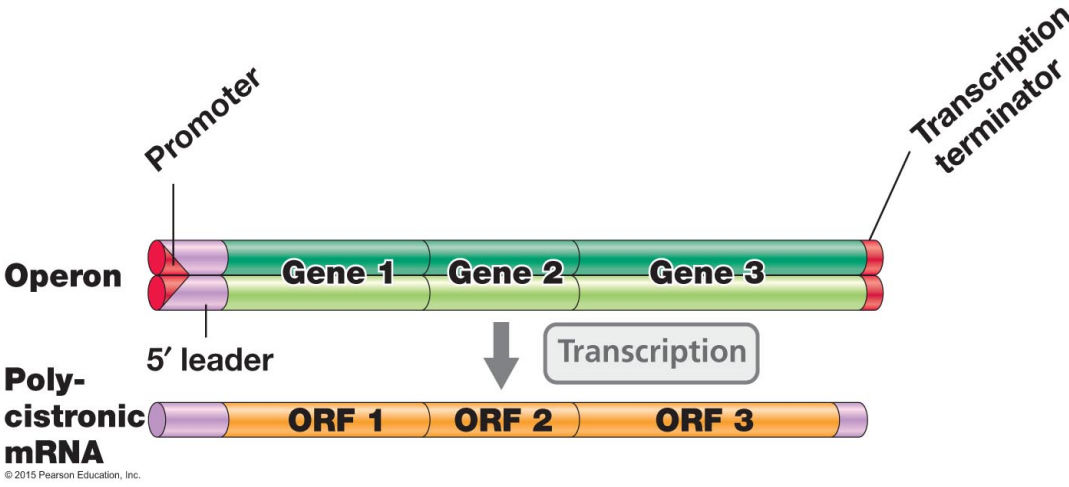
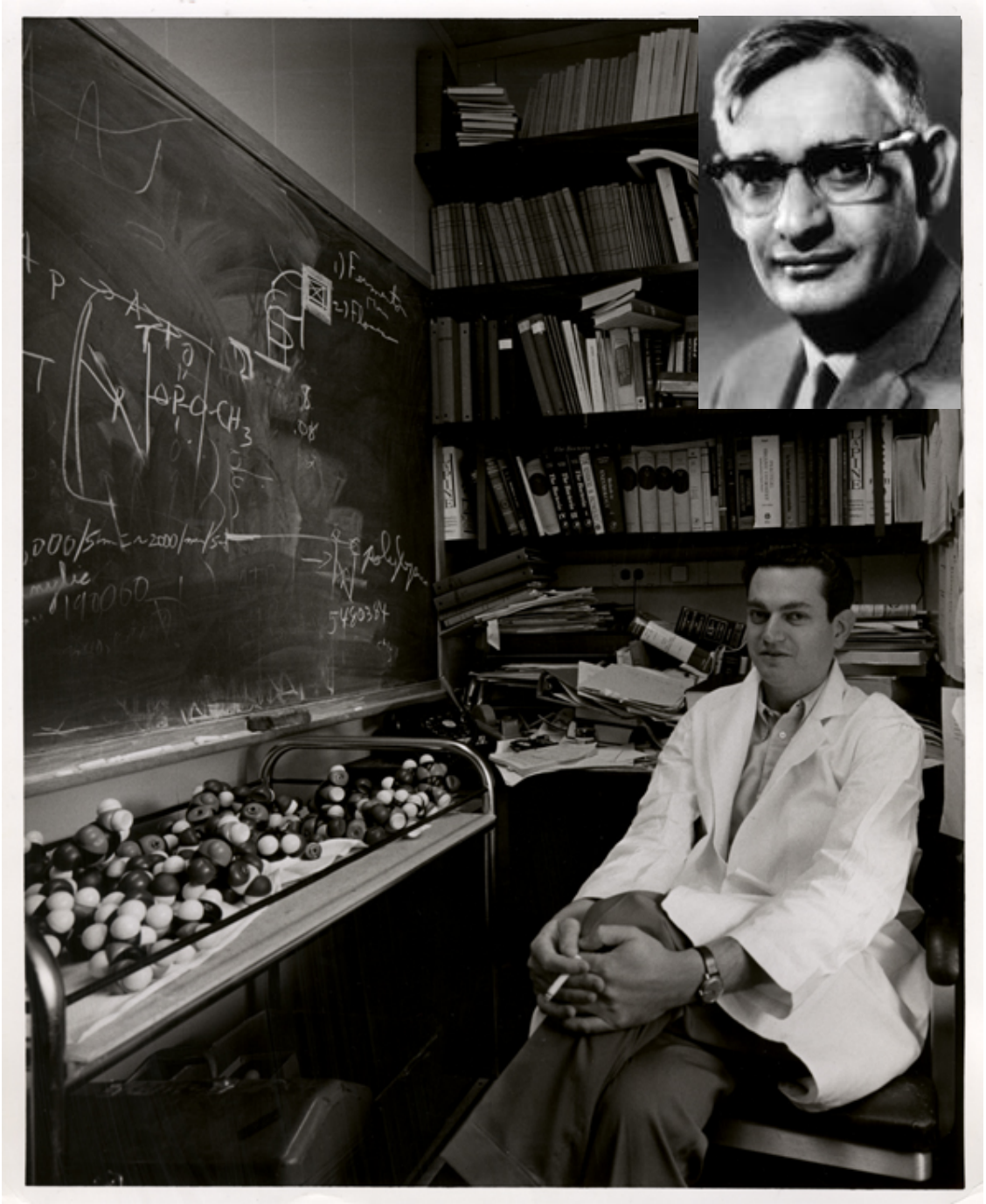


Fig. 4.35 Operon and polycistronic **mRNA** structure in prokaryotes

Cracking the Genetic Code



Marshall Nirenberg and Gobind Khorana, 1966
Nobel prize in Physiology and Medicine, 1968

The genetic code is expressed in terms of RNA, and a single amino acid may be encoded by several different but related **codons.** A codon is recognized following specific base-pairing with a sequence of three bases on a tRNA called the **anticodon**.

A few codons, called **nonsense codons**, do not encode an amino acid. In addition to the nonsense codons, there is also a specific start codon that signals where the translation process should begin.

Some tRNAs can recognize more than one codon.

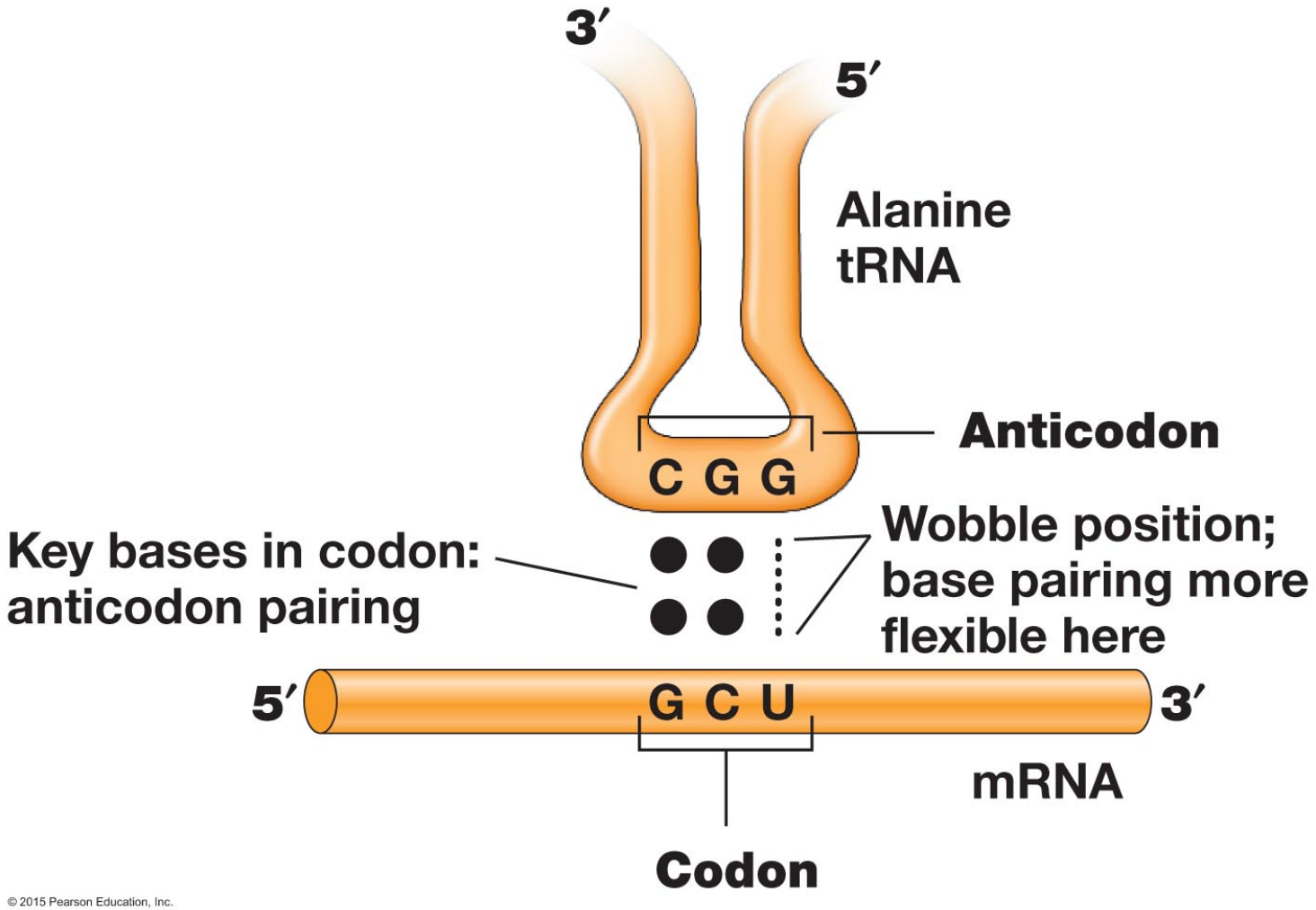
TABLE 4.4 The genetic code as expressed by triplet base sequences of mRNA

Codon	*Amino acid*	*Codon*	*Amino acid*	*Codon*	*Amino acid*	*Codon*	*Amino acid*
UUU	Phenylalanine	UCU	Serine	UAU	Tyrosine	UGU	Cysteine
UUC	Phenylalanine	UCC	Serine	UAC	Tyrosine	UGC	Cysteine
UUA	Leucine	UCA	Serine	UAA	None (stop signal)	UGA	None (stop signal)
UUG	Leucine	UCG	Serine	UAG	None (stop signal)	UGG	Tryptophan
CUU	Leucine	CCU	Proline	CAU	Histidine	CGU	Arginine
CUC	Leucine	CCC	Proline	CAC	Histidine	CGC	Arginine
CUA	Leucine	CCA	Proline	CAA	Glutamine	CGA	Arginine
CUG	Leucine	CCG	Proline	CAG	Glutamine	CGG	Arginine
AUU	Isoleucine	ACU	Threonine	AAU	Asparagine	AGU	Serine
AUC	Isoleucine	ACC	Threonine	AAC	Asparagine	AGC	Serine
AUA	Isoleucine	ACA	Threonine	AAA	Lysine	AGA	Arginine
AUG (start)[a]	Methionine	ACG	Threonine	AAG	Lysine	AGG	Arginine
GUU	Valine	GCU	Alanine	GAU	Aspartic acid	GGU	Glycine
GUC	Valine	GCC	Alanine	GAC	Aspartic acid	GGC	Glycine
GUA	Valine	GCA	Alanine	GAA	Glutamic acid	GGA	Glycine
GUG	Valine	GCG	Alanine	GAG	Glutamic acid	GGG	Glycine

[a]AUG encodes *N*-formylmethionine at the beginning of polypeptide chains of *Bacteria*.

Base pairing between codon and anticodon.

When tRNAs can recognize more than one codon, tRNA molecules form standard base pairs only at the first two positions of the codon, while tolerating irregular base pairing at the third position. This apparent mismatch phenomenon is called **wobble.**

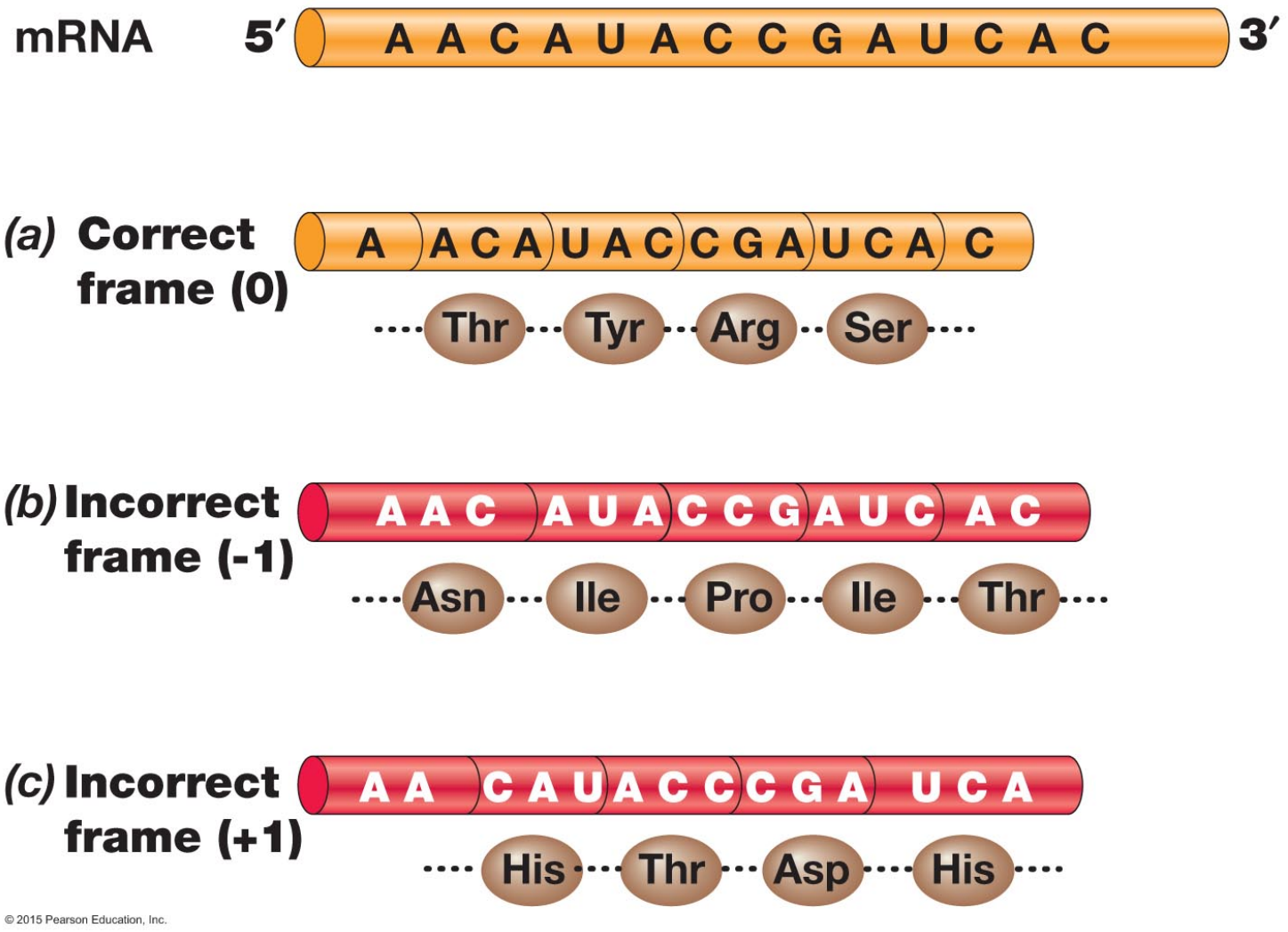


The wobble concept: base pairing is more flexible for the third base of the codon. Only a portion of the tRNA is shown

Figure 4.34

Possible reading frames in a mRNA

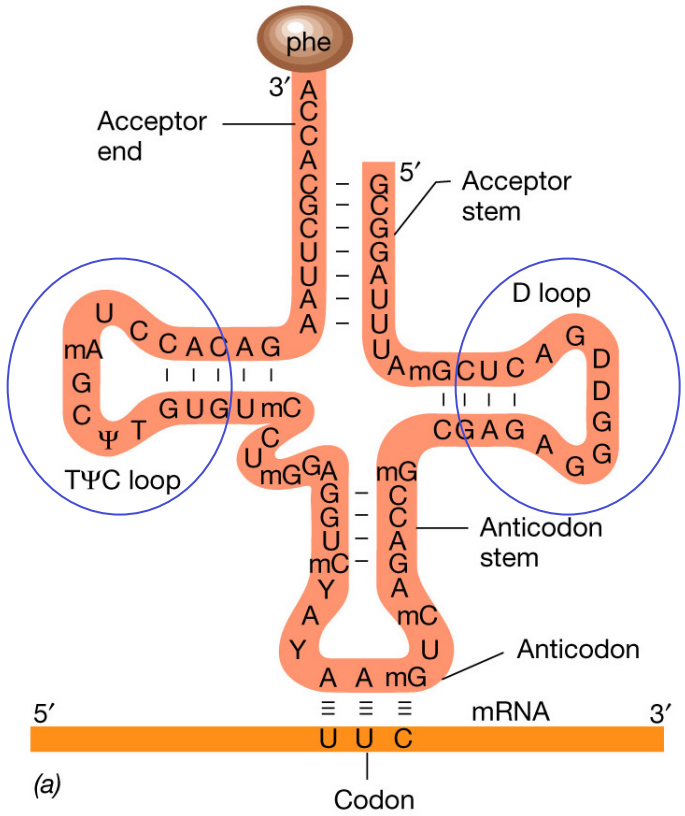
It is important to have a precise starting point because with a triplet code, it is critical that translation begin at the correct location. If it does not, the whole reading frame will be shifted and an entirely different protein (or no protein at all) will be formed



An interior sequence of an mRNA is shown. The "correct" (or 0) reading frame is determined by the start codon of the mRNA. (a) The amino acids that would be encoded by this region of the mRNA if the ribosome were in the –1 reading frame. (b) The amino acids that would be encoded if the ribosome were in the correct reading frame. (c) The amino acids that would be encoded if the ribosome were in the +1 reading frame.

Structure of a transfer RNA (tRNA)

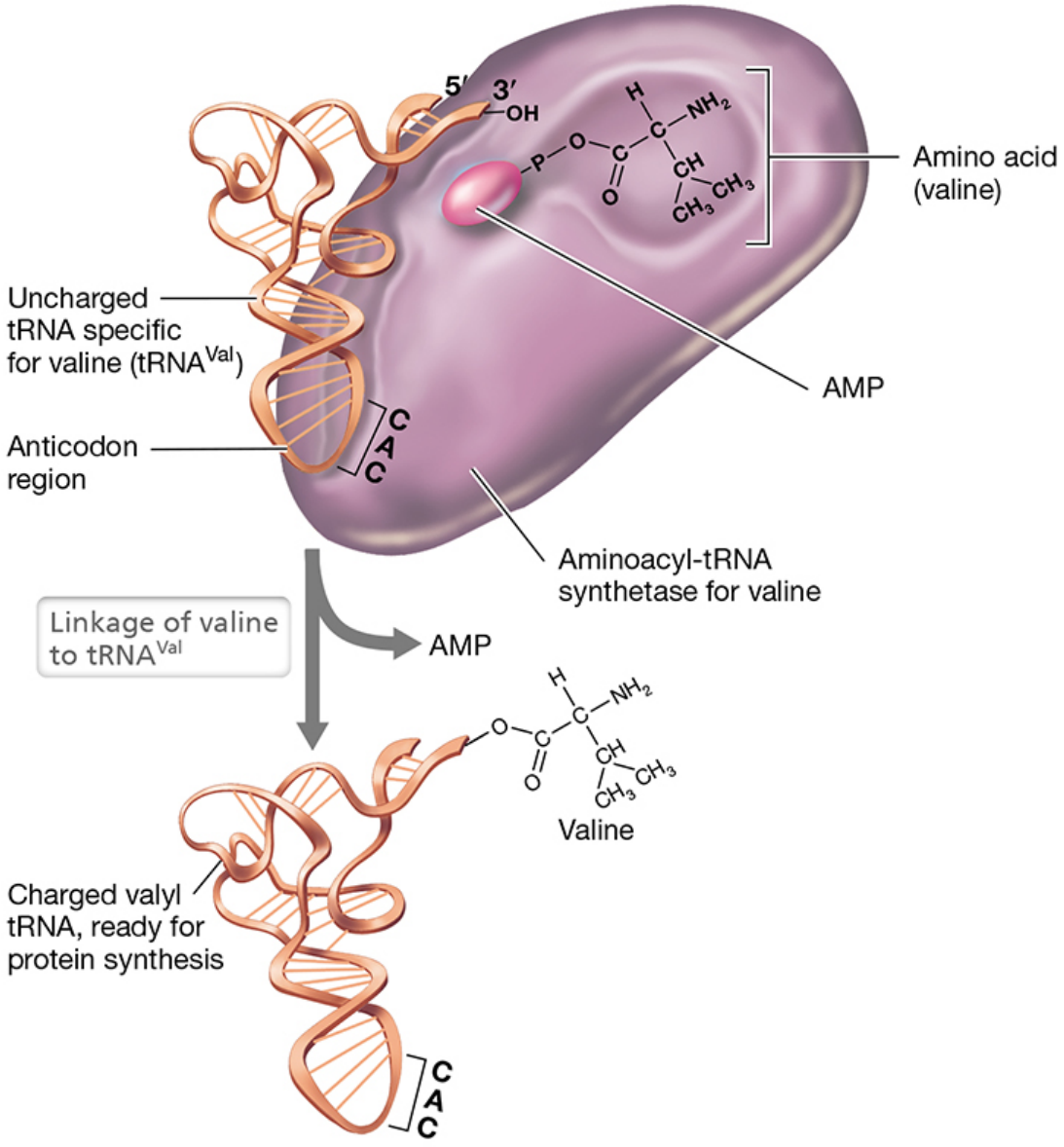
An adaptor molecule used in translation that has specificity for both a particular amino acid and for one or more codons

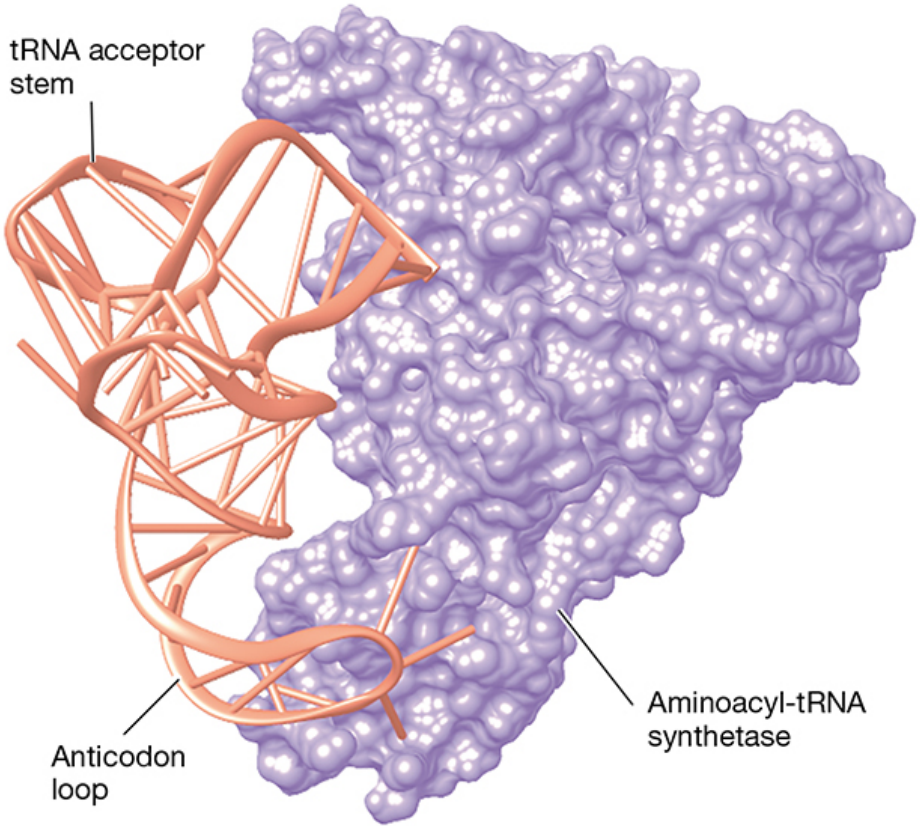


Yeast phenylalanine tRNA. (a) The conventional cloverleaf structure. The amino acid is attached to the ribose of the terminal A at the acceptor end. A, adenine; C, cytosine; U, uracil; G, guanine; ψ, pseudouracil; D, dihydrouracil; m, methyl; Y, a modified purine. (b) In actuality the molecule folds so that the D loop and TψC loops are close together and associate by hydrophobic interactions.

Charging of a tRNA: Aminoacyl-tRNA synthetases

Enzymes called **aminoacyl-tRNA synthetases** attach an amino acid to a tRNA





Modified from G. Caetano-Anollés and A. Nasir. 2012. *Frontiers in Genetics* 3:00172.

(a) Action of an aminoacyl-tRNA synthetase. Recognition of the correct tRNA by a particular synthetase involves contacts between specific nucleic acid sequences and specific amino acids of the synthetase. In this diagram, valyl-tRNA synthetase is shown catalyzing the final step of the reaction, where the valine in valyl-AMP is transferred to tRNA.

The **ribosome** plays a key role in the translation process, bringing together mRNA and aminoacyl tRNAs

TABLE 7.4 Ribosome structure[a]

Property	Prokaryote	Eukaryote
Overall size	70S	80S
Small subunit	30S	40S
Number of proteins	~21	~30
RNA size (number of bases)	16S (1500)	18S (2300)
Large subunit	50S	60S
Number of proteins	~34	~50
RNA size (number of bases)	23S (2900) 5S (120)	28S (4200) 5.8S (160) 5S (120)

[a]Ribosomes of mitochondria and chloroplasts of eukaryotes are similar to prokaryotic ribosomes (Section 14.4).

Translation: Protein Synthesis

Shine-Dalgarno sequence: Involved in binding of the mRNA to the ribosome (ribosome binding site)

Located at the 5' -end of the mRNA, it is complementary to the 3' -end of the 16S rRNA of the ribosome

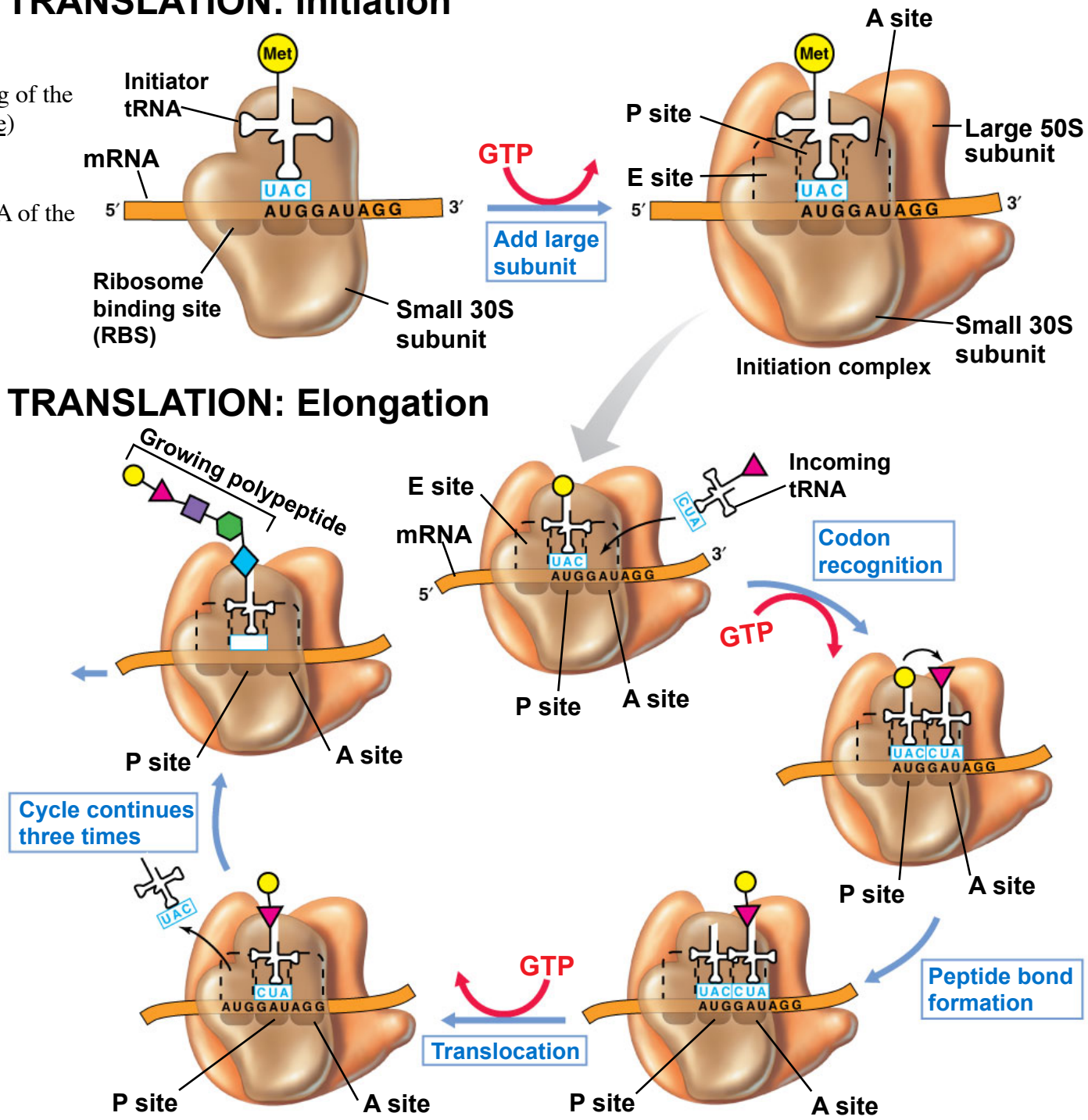
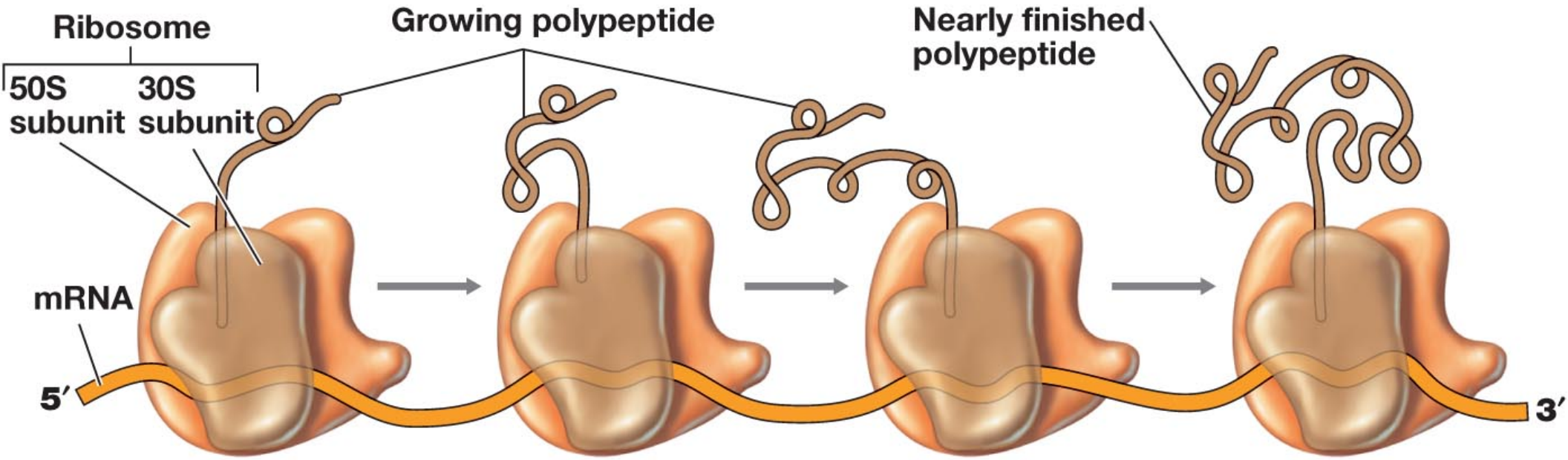


Figure 4.37

Translation: Protein Synthesis (cont.)

Several ribosomes can translate a single mRNA molecule simultaneously, forming a complex called a polysome



Translation by several ribosomes on a single messenger RNA (polysome). Note how the ribosomes nearest the 5´-end of the message are at an earlier stage in the translation process.

Antibiotics that inhibit protein synthesis: Streptomycin (initiation)

Puromycin, Chloramphenicol, Cycloheximide, Tetracycline (elongation)

Figure 4.38

Distinguish between DNA synthesis on a leading and on a lagging strand, addressing the different mechanisms of initiation and elongation of the DNA strand.