Microbiology

Microbial Genetics

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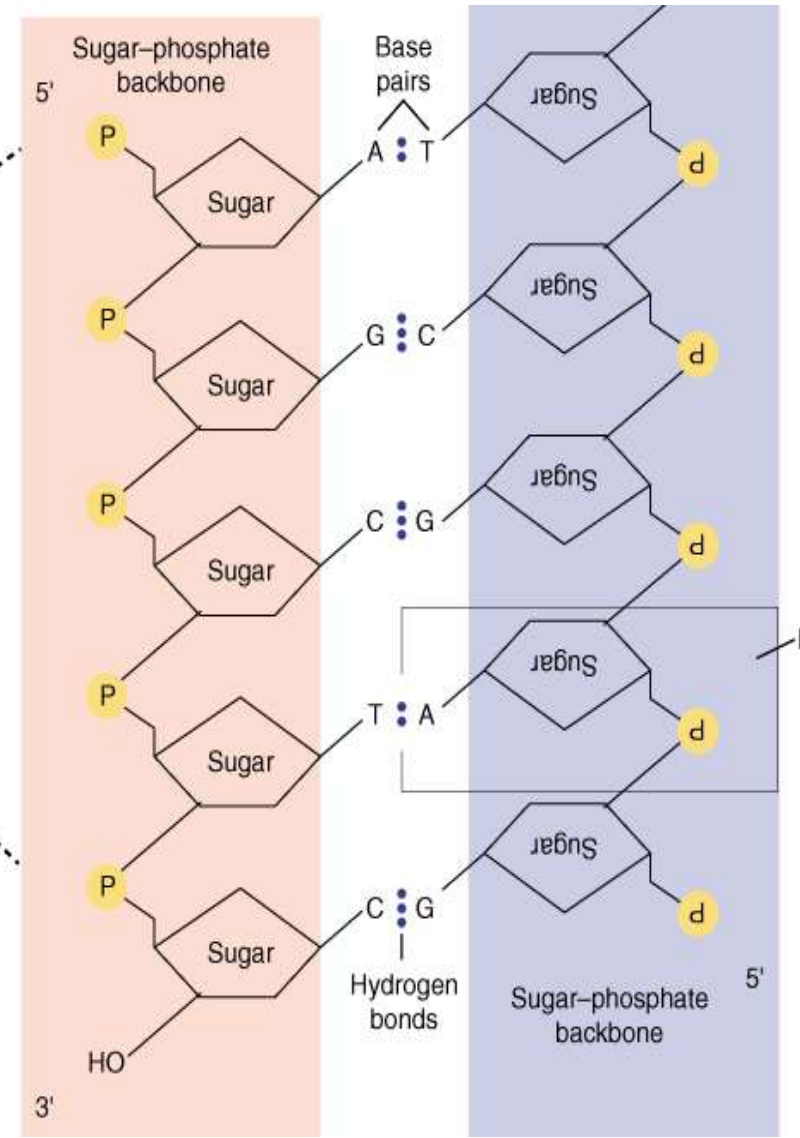
The Structure and Replication of Genomes

- **Genetics**
  - Study of inheritance and inheritable traits as expressed in an organism's genetic material
- **Genome**
  - The entire genetic complement of an organism
  - Includes its genes and nucleotide sequences

Terminology

- Genetics
- Genome
- Gene
- Chromosome
- Base pairs
- Genetic code
- Genomics
- Genotype
- Phenotype

DNA



The structure of nucleic acids

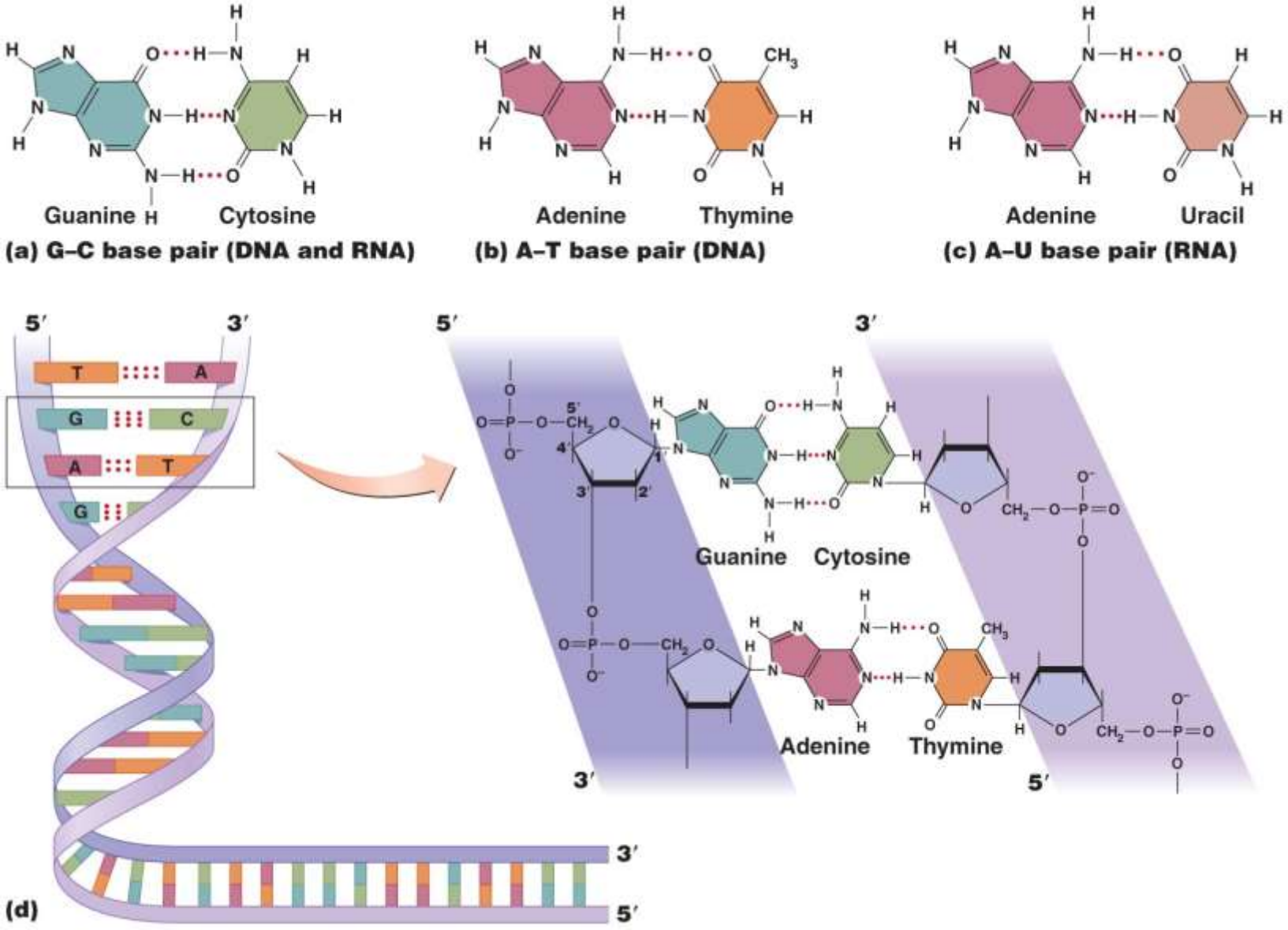


Figure 7.1

The Structure and Replication of Genomes

- **The Structure of Prokaryotic Genomes**
  - Prokaryotic chromosomes
    - Main portion of DNA, along with associated proteins and RNA
    - Prokaryotic cells are haploid (single chromosome copy)
    - Typical chromosome is circular molecule of DNA in nucleoid

Bacterial genome

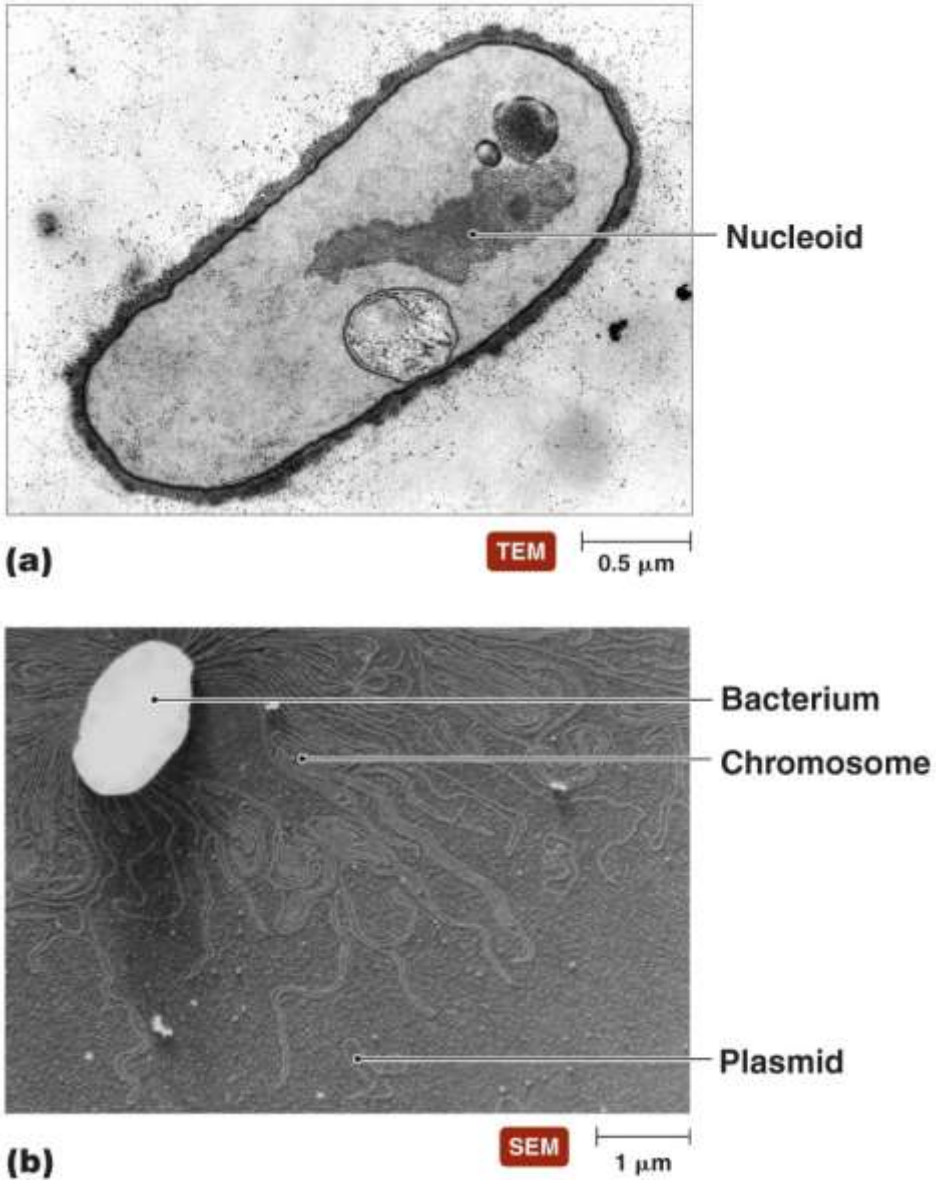


Figure 7.2

The Structure and Replication of Genomes

- **The Structure of Prokaryotic Genomes**
  - Plasmids
    - Small molecules of DNA that replicate independently
    - Not essential for normal metabolism, growth, or reproduction
    - Can confer survival advantages
    - Many types of plasmids
      - Fertility factors
      - Resistance factors
      - Bacteriocin factors
      - Virulence plasmids

The Structure and Replication of Genomes

- **The Structure of Eukaryotic Genomes**
  - Nuclear chromosomes
    - Typically have more than one chromosome per cell
    - Chromosomes are linear and sequestered within nucleus
    - Eukaryotic cells are often diploid (two chromosome copies)

Eukaryotic nuclear chromosomal packaging

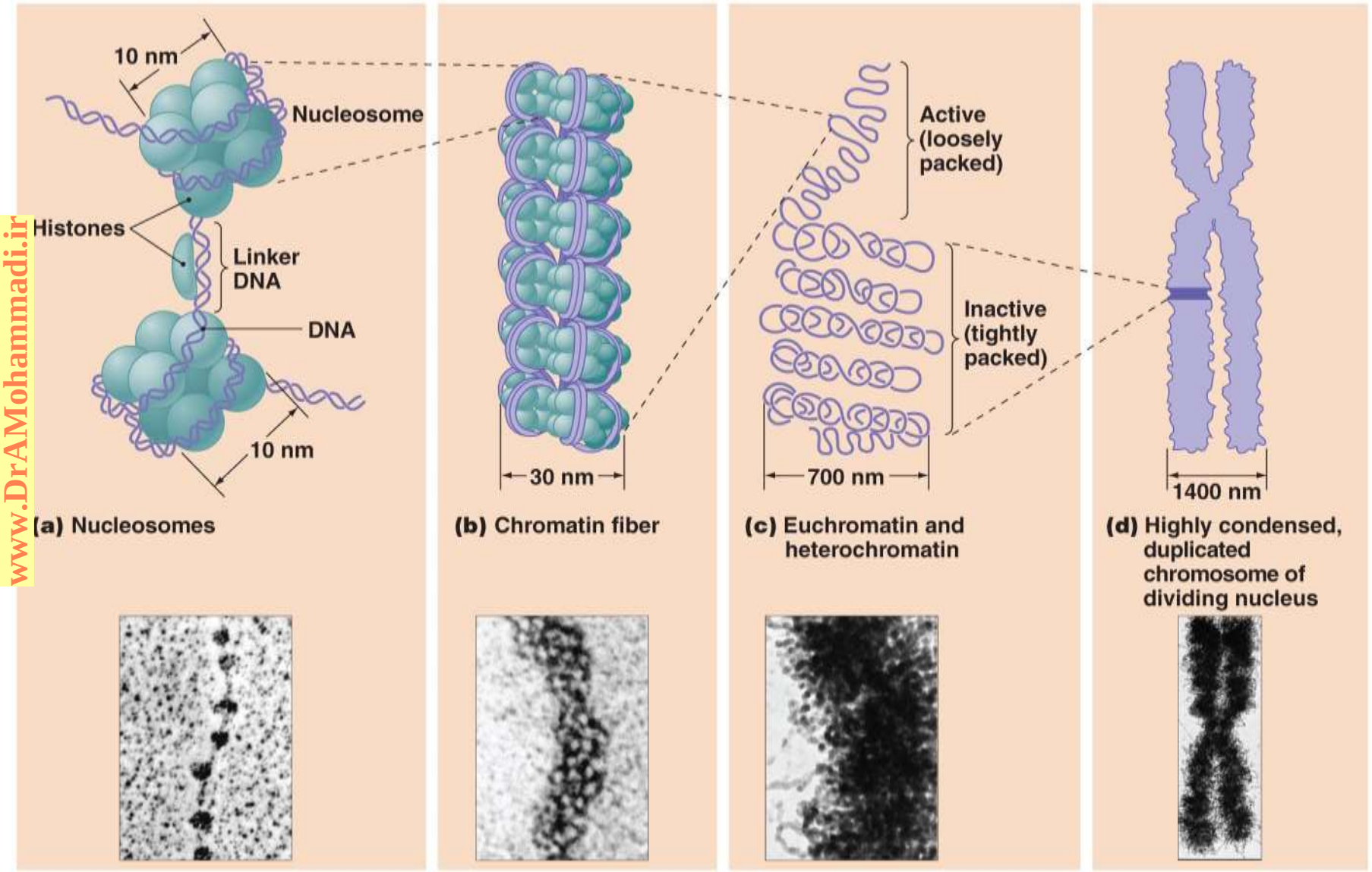


Figure 7.3

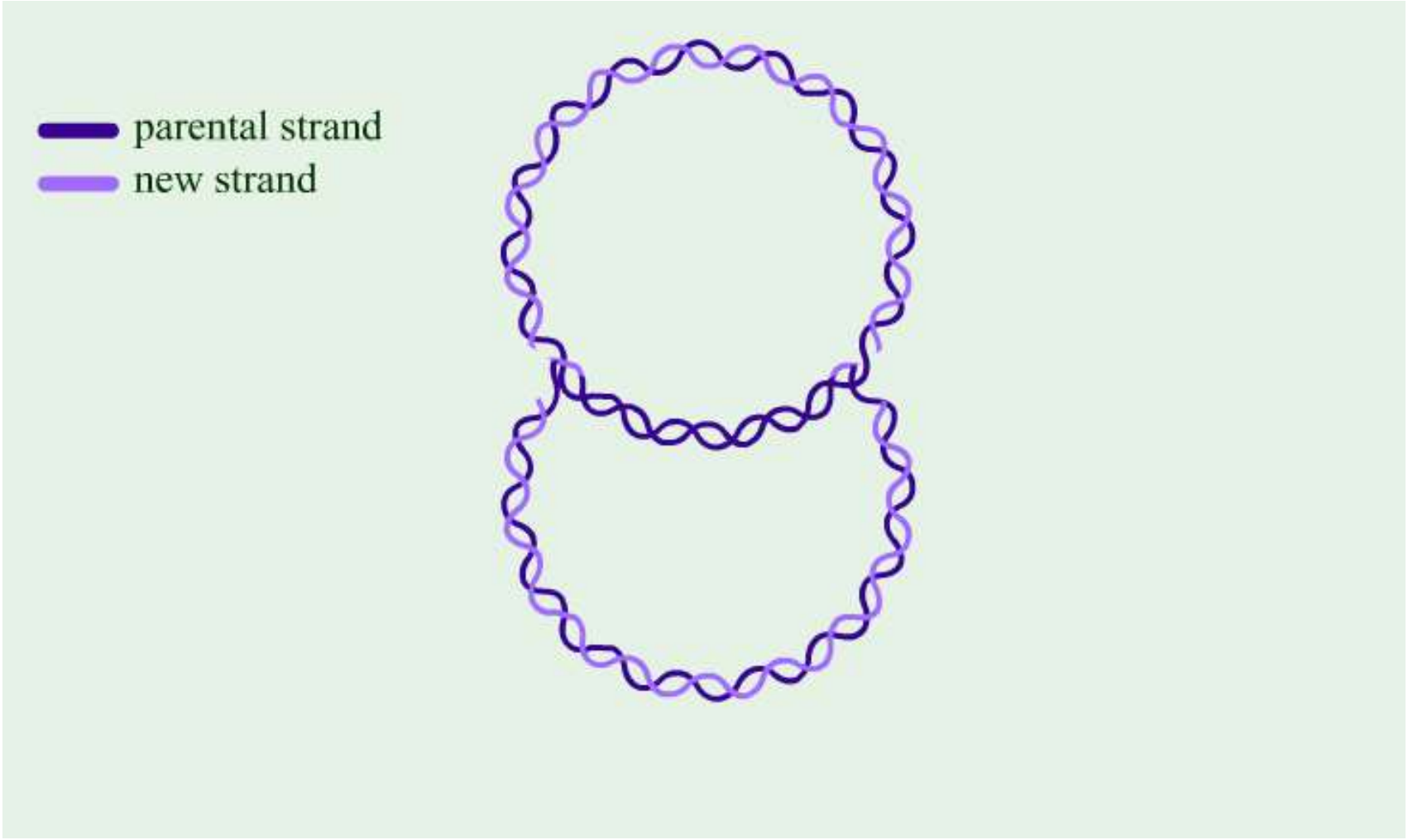
The Structure and Replication of Genomes

- **The Structure of Eukaryotic Genomes**
  - Extranuclear DNA of eukaryotes
    - DNA molecules of mitochondria and chloroplasts
      - Resemble chromosomes of prokaryotes
      - Only code for about 5% of RNA and proteins
    - Some fungi and protozoa carry plasmids

The Structure and Replication of Genomes

- **DNA Replication**
  - Anabolic polymerization process that requires monomers and energy
    - Triphosphate deoxyribonucleotides serve both functions
  - Key to replication is complementary structure of the two strands
  - Replication is semiconservative
    - New DNA composed of one original and one daughter strand

The Structure and Replication of Genomes



PLAY Animation: DNA Replication: Overview

The dual role of triphosphate deoxyribonucleotides

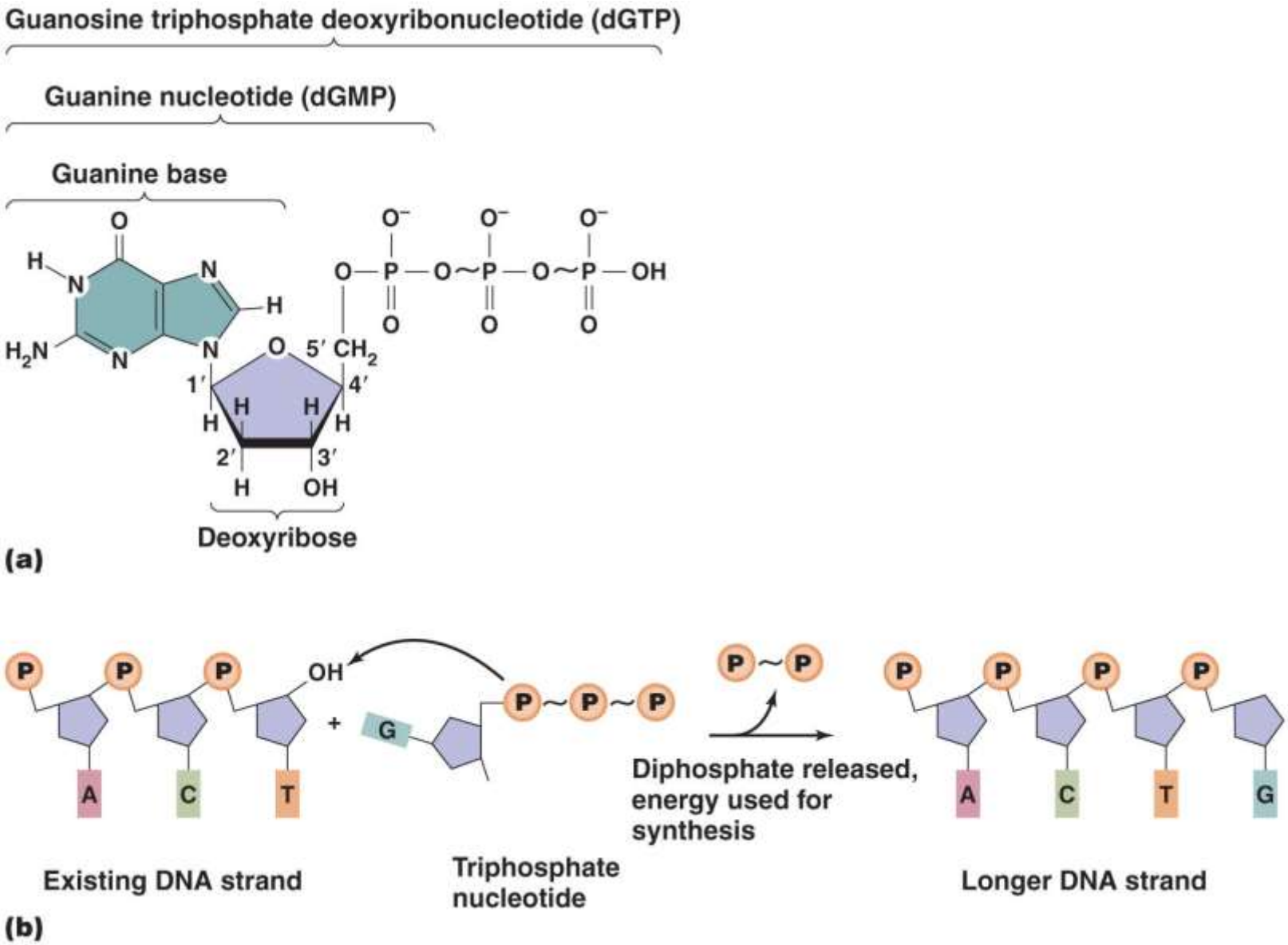
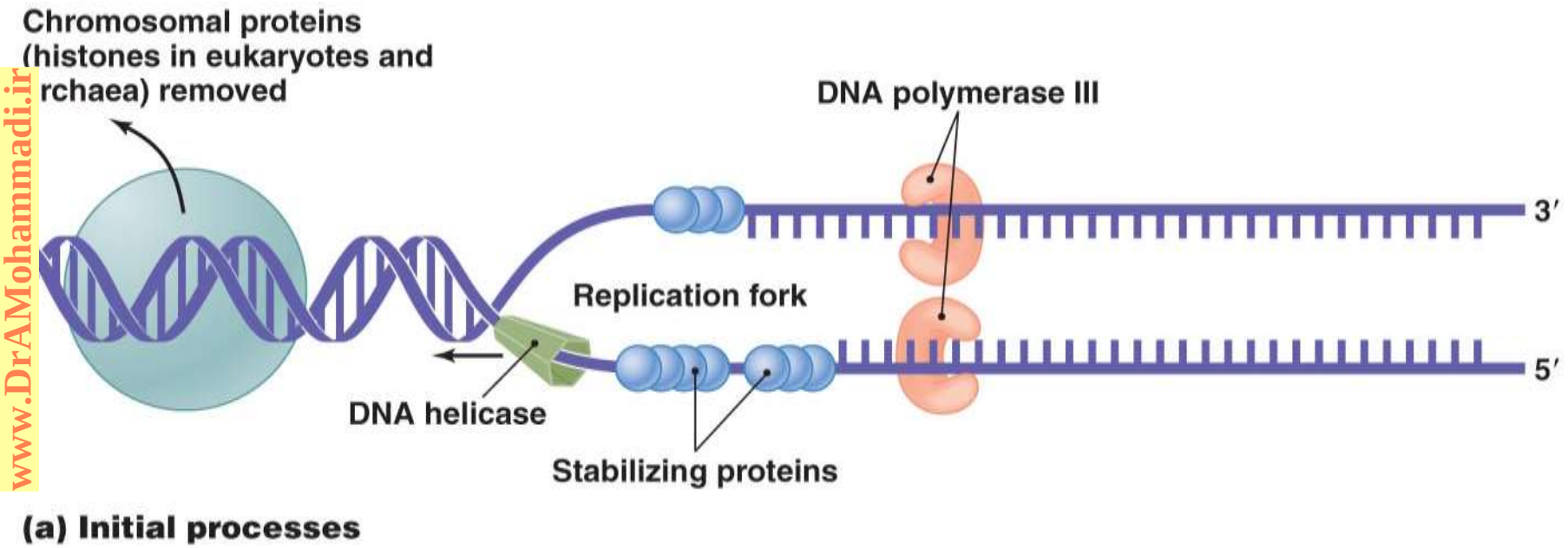


Figure 7.4

The Structure and Replication of Genomes

- **DNA Replication**
  - Initial processes in replication
    - Bacterial DNA replication begins at the origin
    - DNA polymerase replicates DNA only 5' to 3'
    - Because strands are antiparallel, new strands are synthesized differently
      - Leading strand synthesized continuously
      - Lagging strand synthesized discontinuously

DNA replication

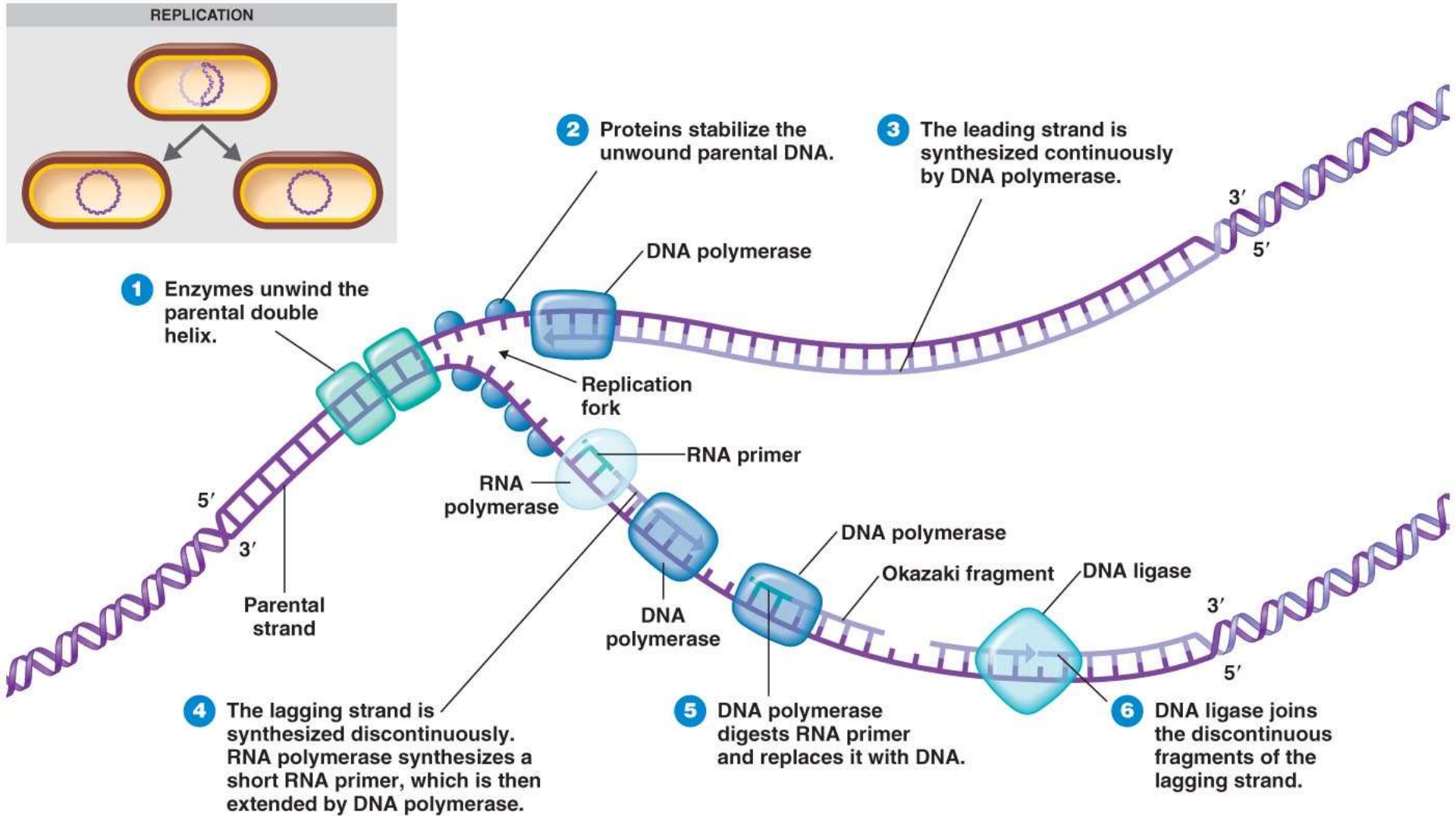


(a) Initial processes

Figure 7.5a

Replication fork

Replication in 5' → 3' direction



DNA replication

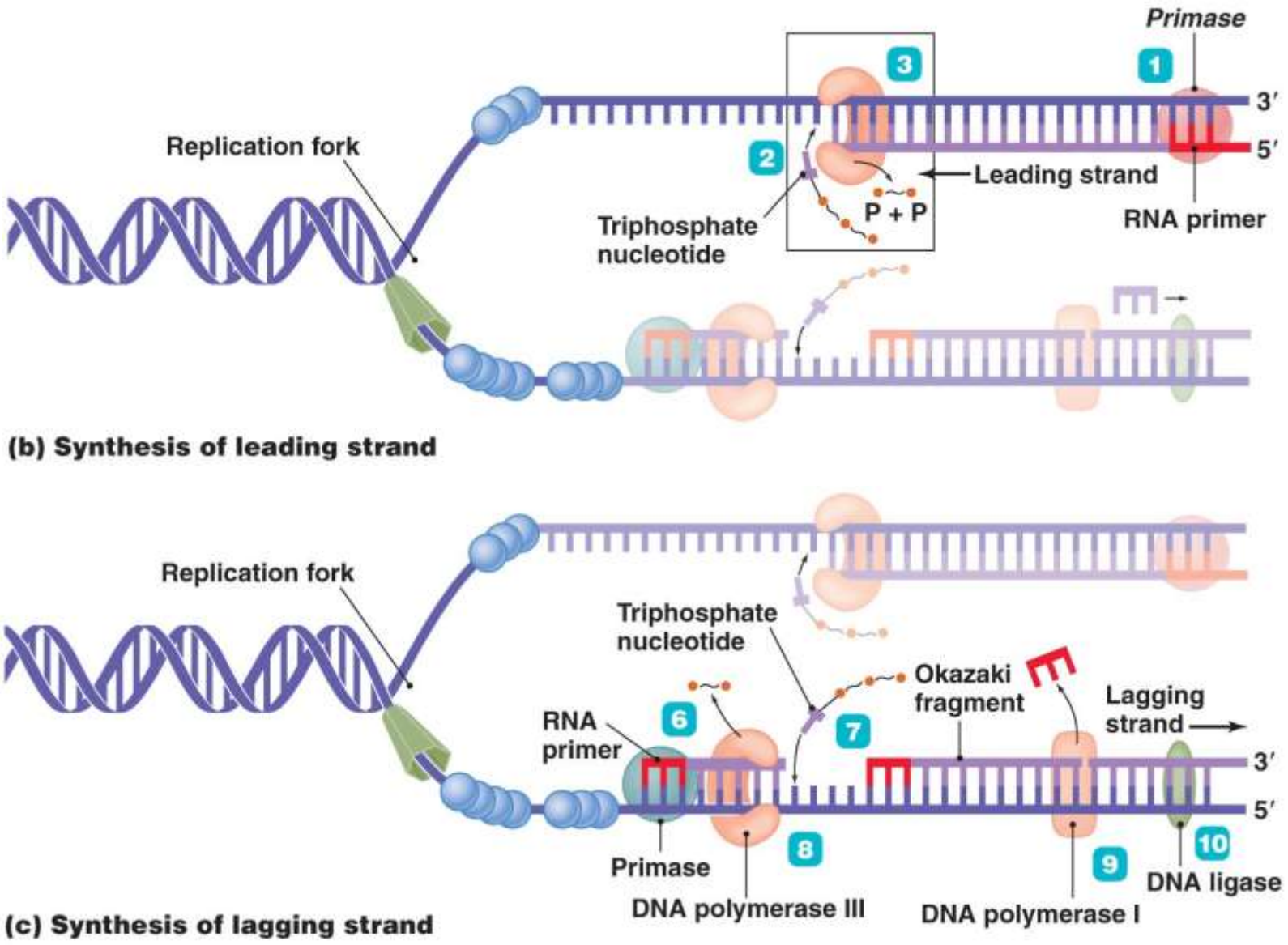


Figure 7.5b/c

The Structure and Replication of Genomes

- **DNA Replication**
  - Other characteristics of bacterial DNA replication
    - Bidirectional
    - Topoisomerases remove supercoils in DNA molecule
    - DNA is methylated
      - Control of genetic expression
      - Initiation of DNA replication
      - Protection against viral infection
      - Repair of DNA

The bidirectionality of DNA replication in prokaryotes

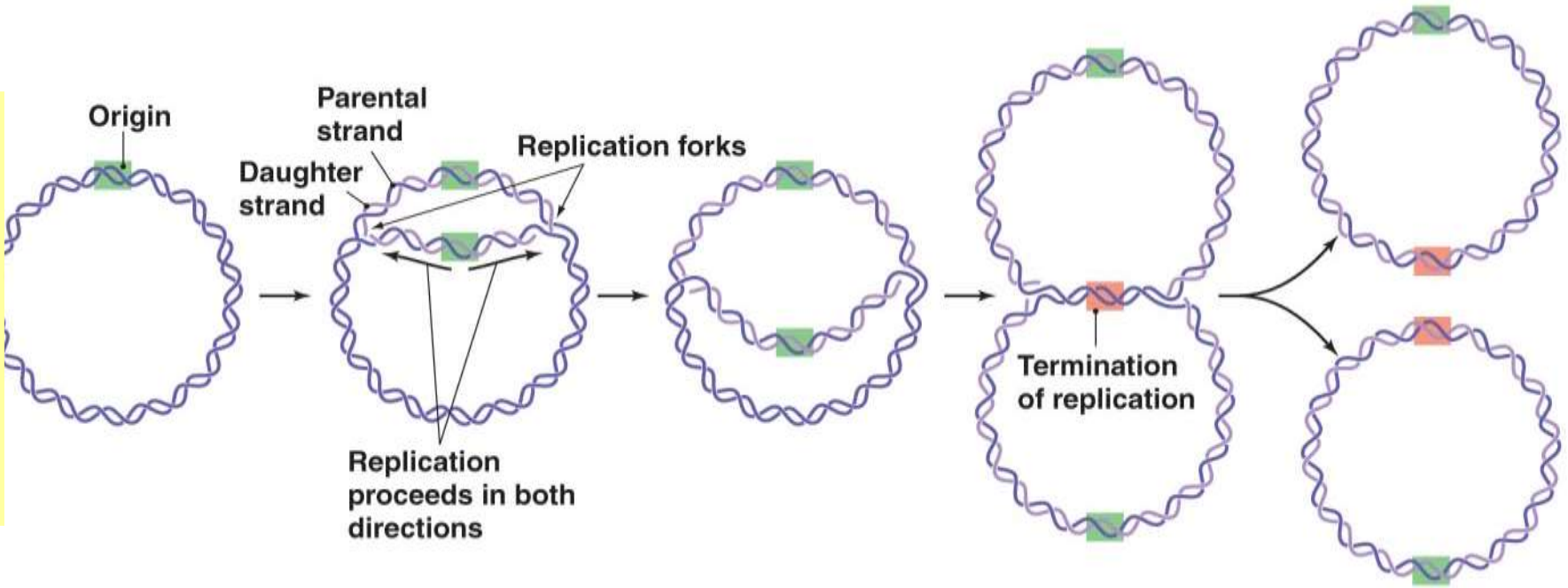
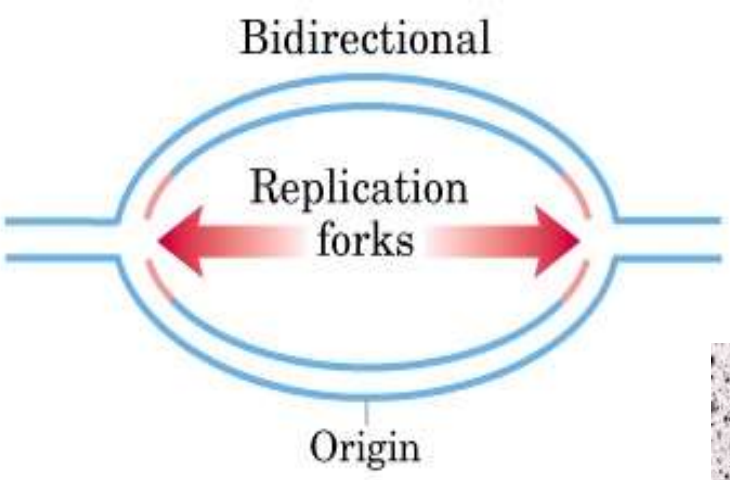
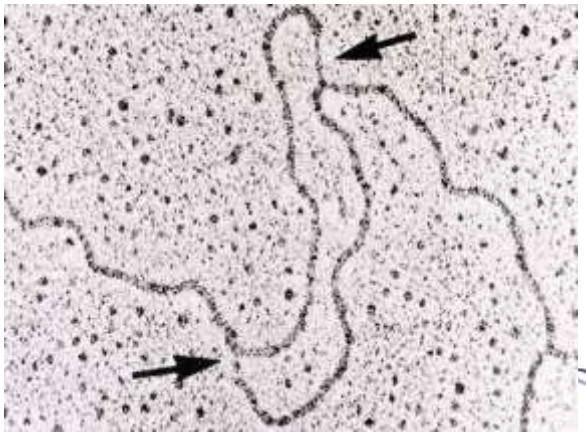


Figure 7.6

Replication 1; 2; 3 of circular bacterial Chromosome





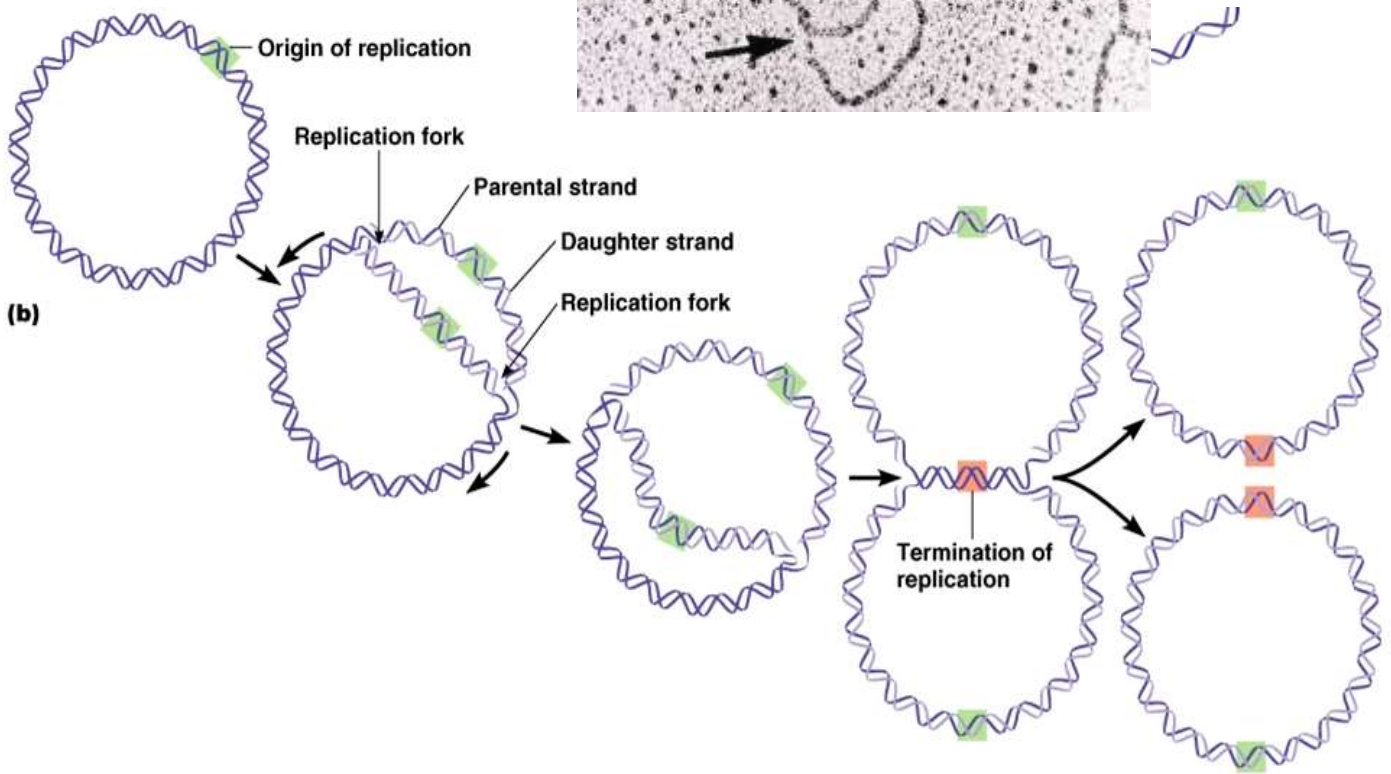


Fig 8.6

The Structure and Replication of Genomes

- **DNA Replication**
  - Replication of eukaryotic DNA
    - Similar to bacterial replication
    - Some differences
      - Uses four DNA polymerases
      - Thousands of replication origins
      - Shorter Okazaki fragments
      - Plant and animal cells methylate only cytosine bases

Gene Function

- **The Relationship Between Genotype and Phenotype**
  - Genotype
    - Set of genes in the genome
  - Phenotype
    - Physical features and functional traits of the organism

Gene Function

- **The Transfer of Genetic Information**
  - Transcription
    - Information in DNA is copied as RNA
  - Translation
    - Polypeptides synthesized from RNA
  - Central dogma of genetics
    - DNA transcribed to RNA
    - RNA translated to form polypeptides

The central dogma of genetics

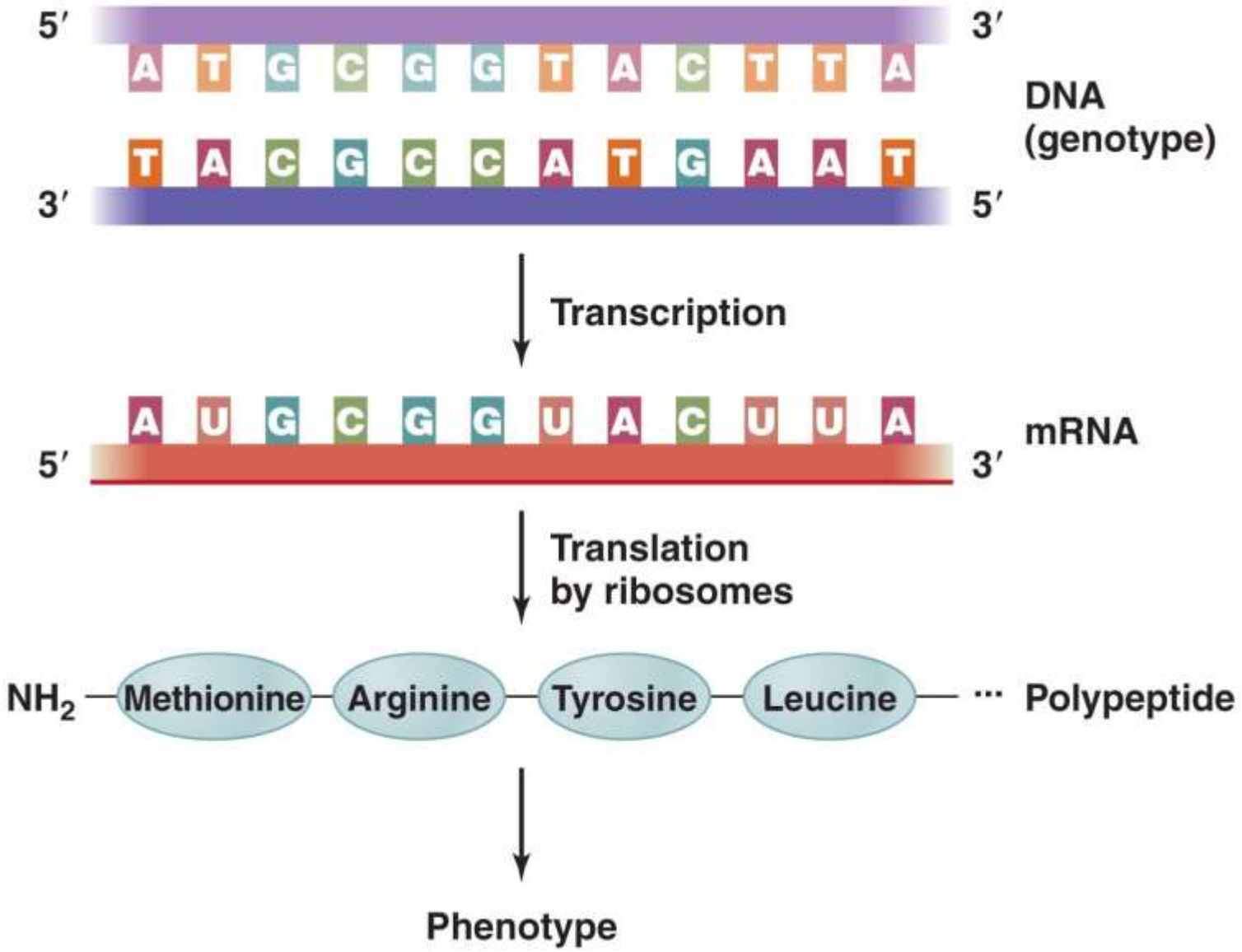
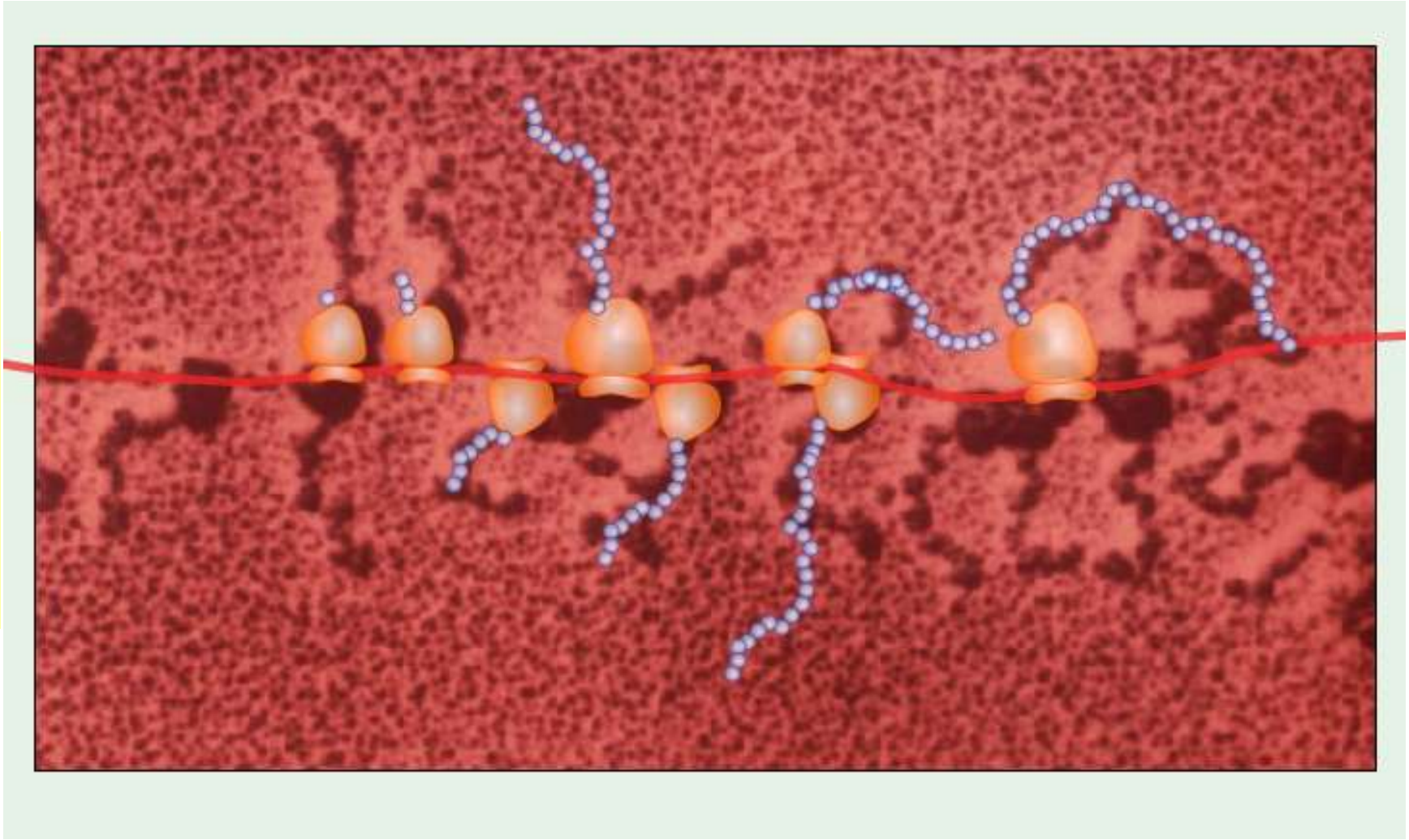


Figure 7.7

Gene Function



PLAY Animation: Translation: Overview

Gene Function

- **The Events in Transcription**
  - Four types of RNA transcribed from DNA
    - RNA primers
    - mRNA
    - rRNA
    - tRNA
  - Occur in nucleoid of prokaryotes

Type of RNA	Functions in	Function
Messenger RNA (mRNA) 	Nucleus, migrates to ribosomes in cytoplasm	Carries DNA sequence information to ribosomes
Transfer RNA (tRNA) 	Cytoplasm	Provides linkage between mRNA and amino acids; transfers amino acids to ribosomes
Ribosomal RNA (rRNA) 	Cytoplasm	Structural component of ribosomes

Transfer RNA

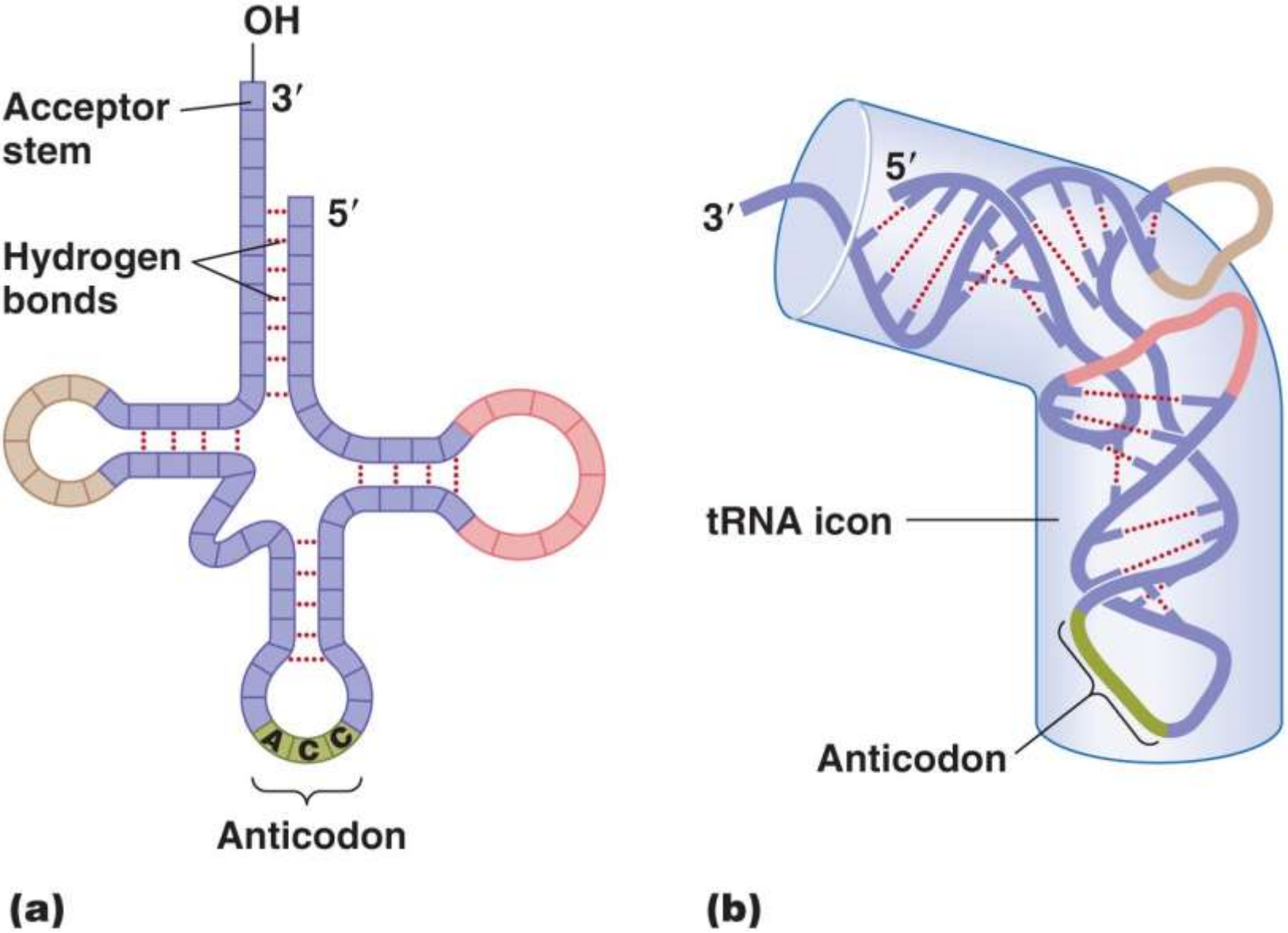


Figure 7.13

Ribosomal structures

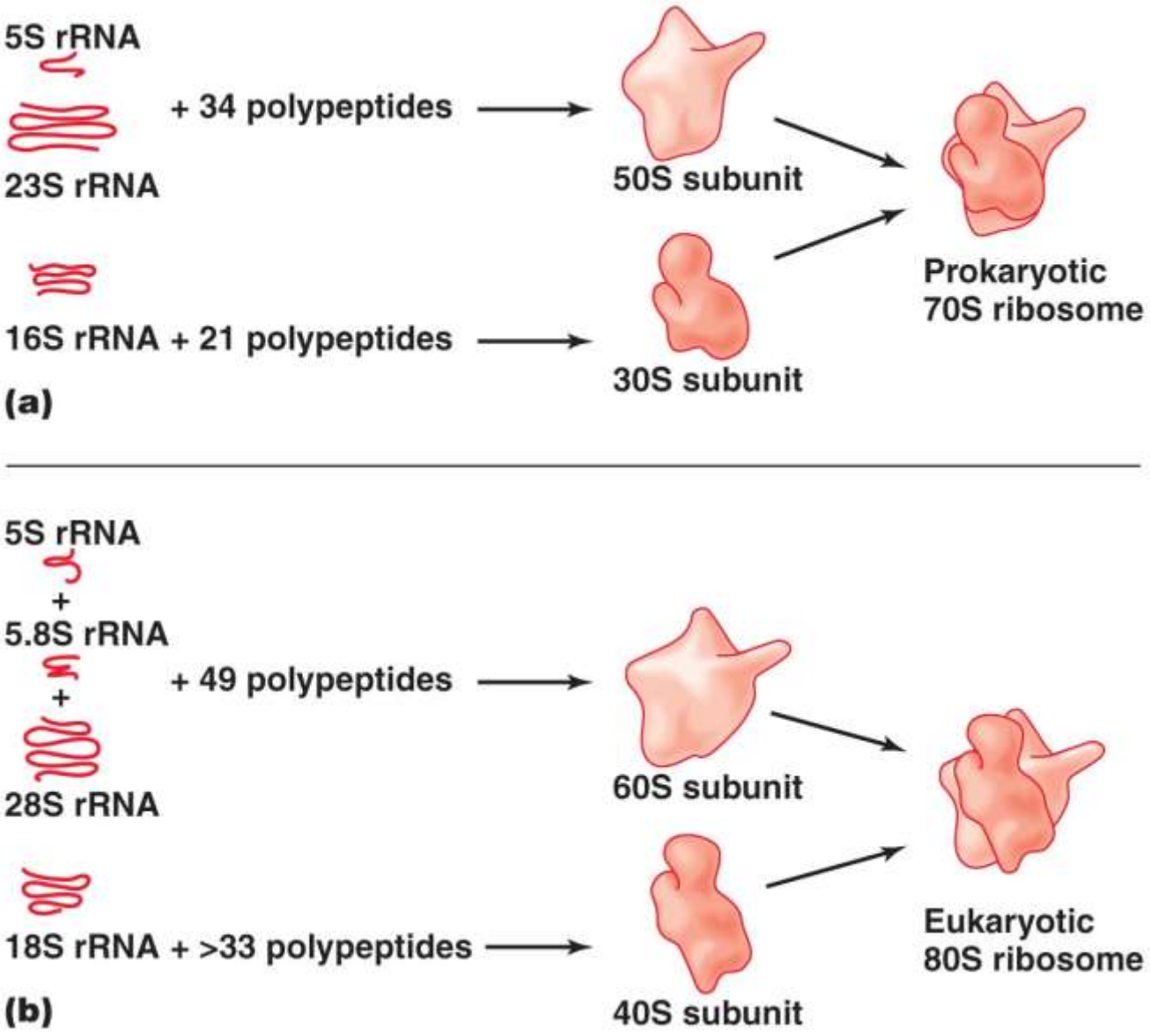
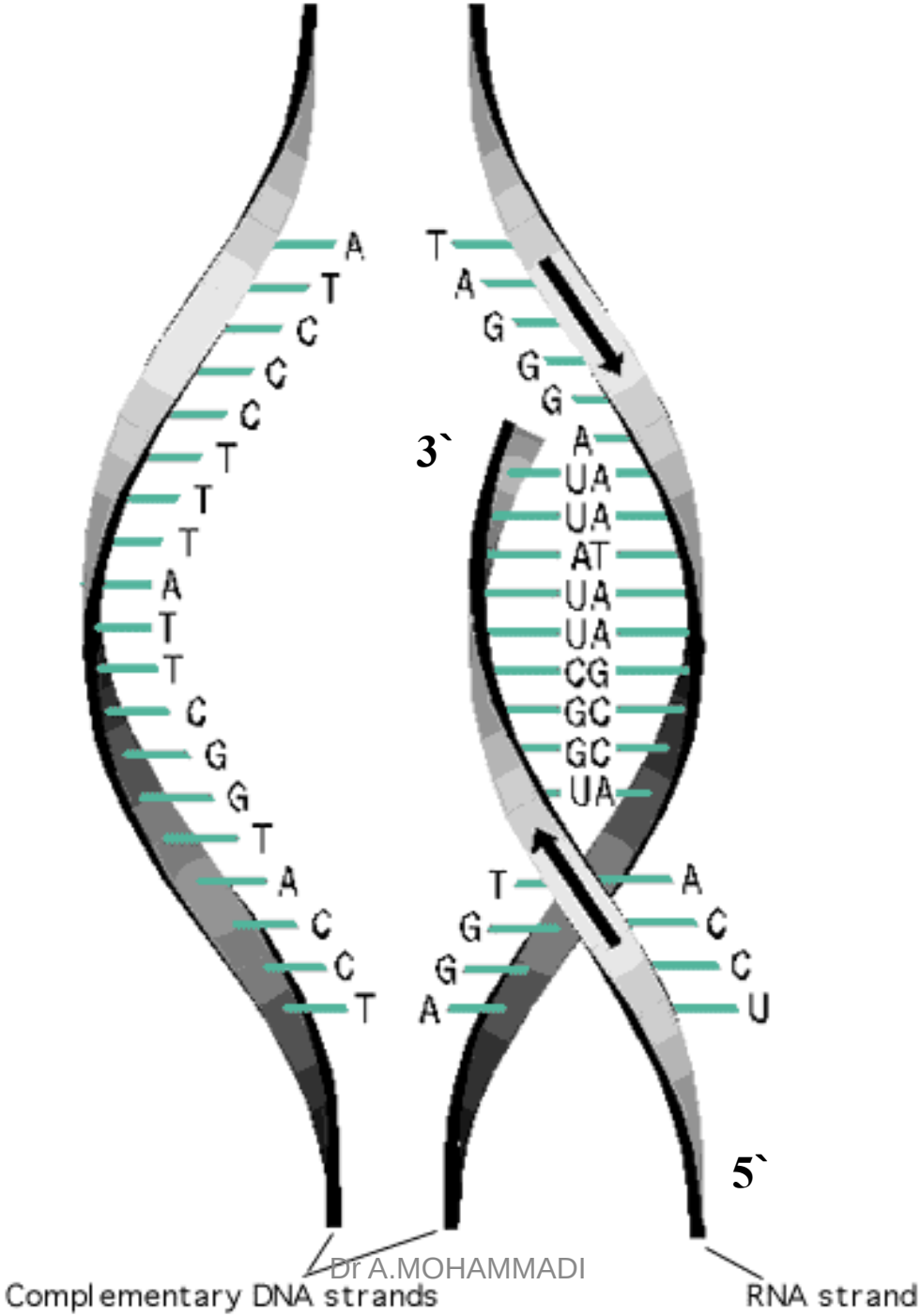


Figure 7.14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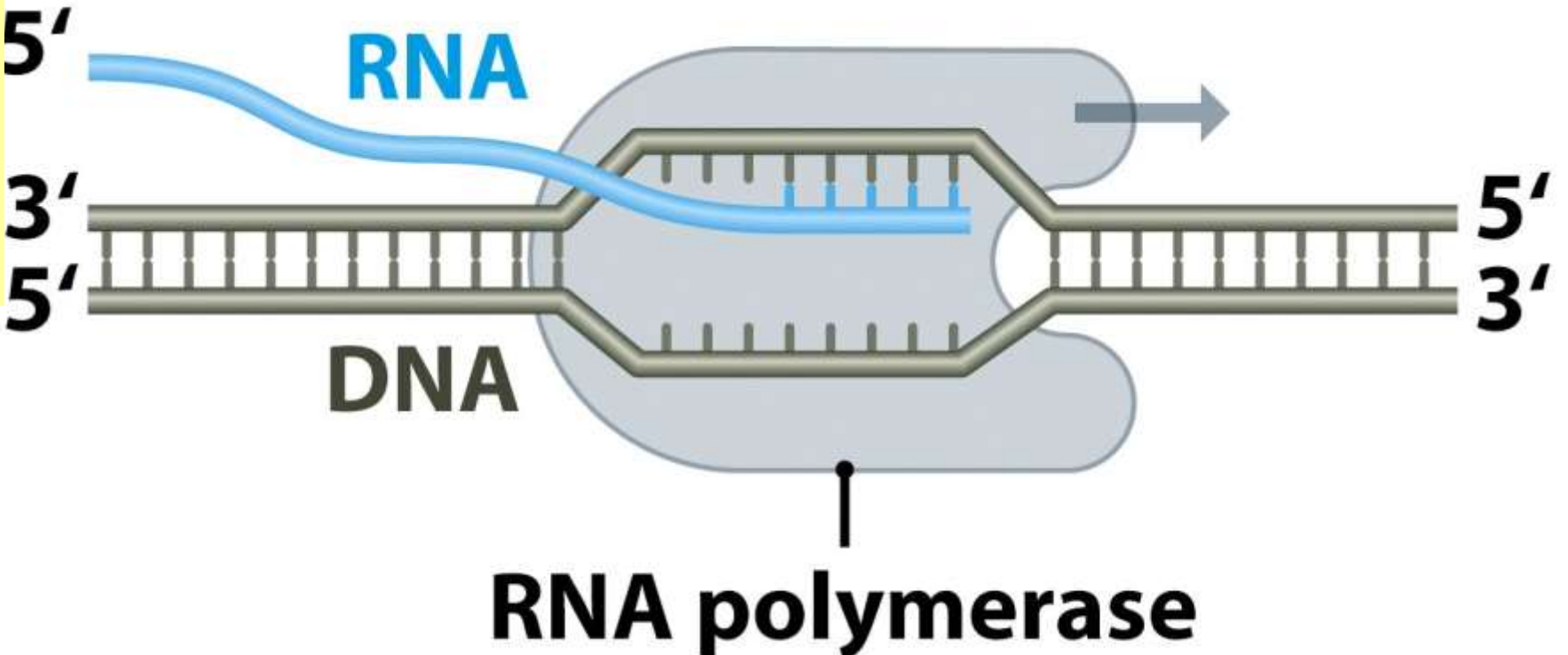
A
C
C
U
3`
5`
Complementary DNA strands
RNA strand

Transcription

ساخت رشته RNA جدید (transcript)به کمک انزیم RNA پلیمراز طی پروسه transcription (رونویسی یا نسخه برداری) بر اساس تناسب نوکلئوتیدی از روی رشته DNA تمپلیت از جهت `5 به `3

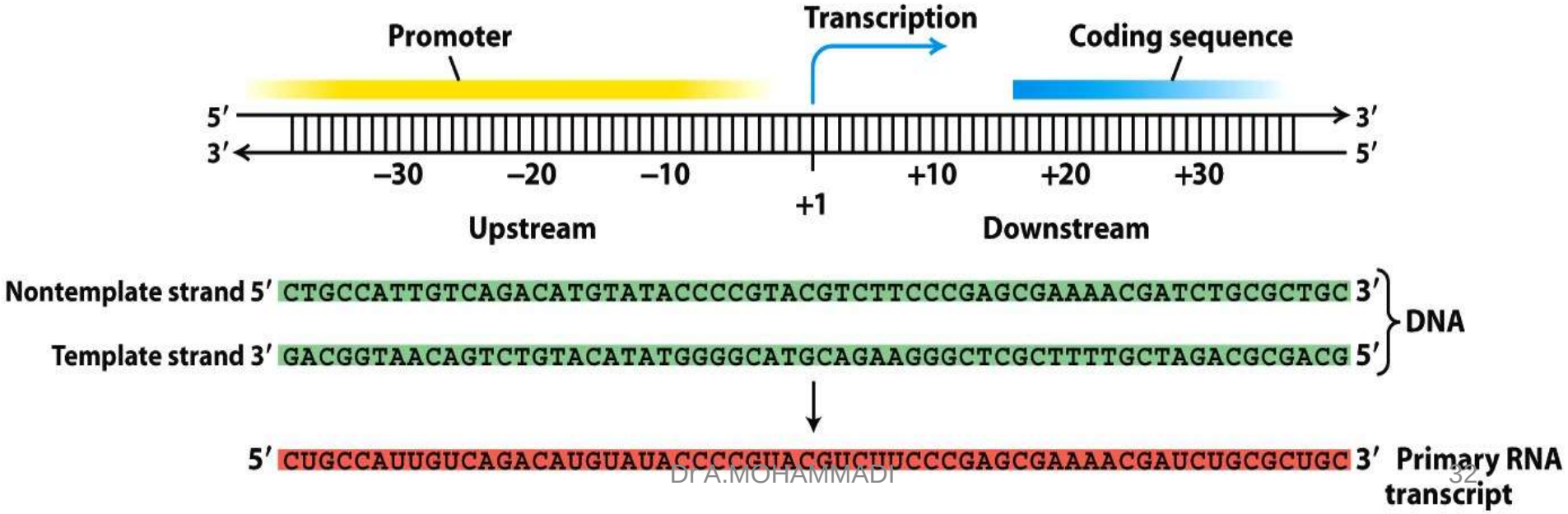


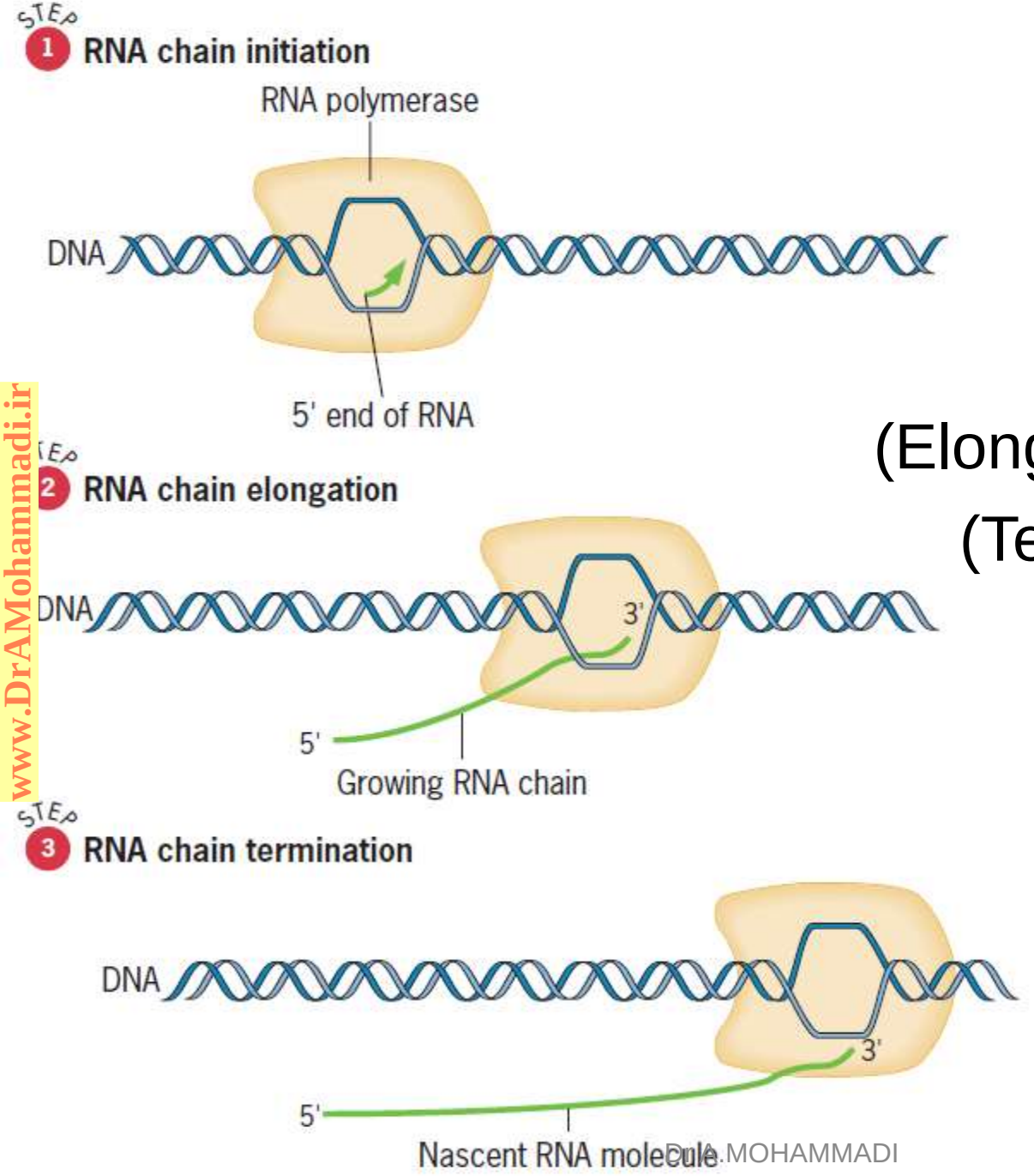
Transcription

پروموتر: (Promoter) توالی (مجموعه ای از نوکلئوتیدهای) ویژه ای که انزیم RNAپلیمراز به آن قسمت متصل میشود

پایین دست (Downstream): جهتی که رشته الگوی DNA در آن جهت رونویسی می شود

بالادست (Upstream): جهت مخالف رونویسی





رونویسی سه مرحله دارد

۱ ـ شروع (Initiation)

۲ ـ طویل سازی (Elongation)

۳ ـ خاتمه (Termination)

Concurrent RNA transcription

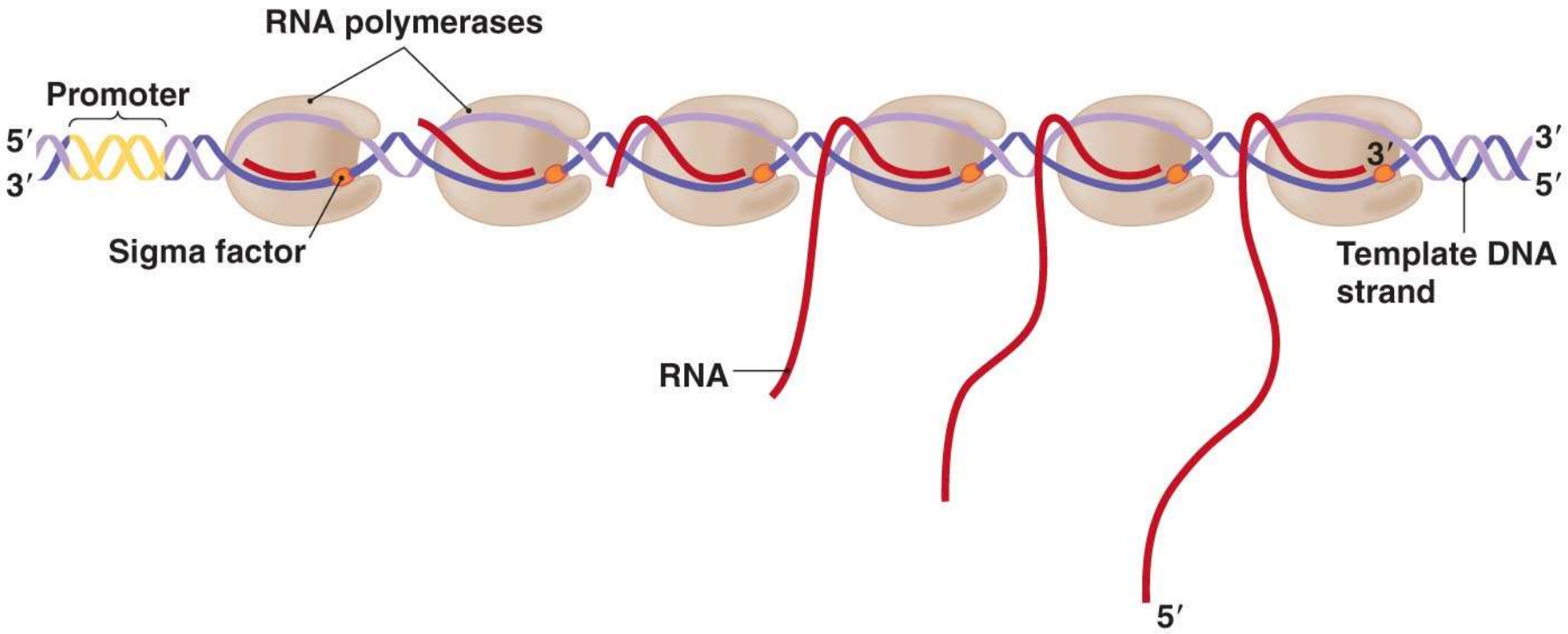


Figure 7.9

Processing eukaryotic mRNA

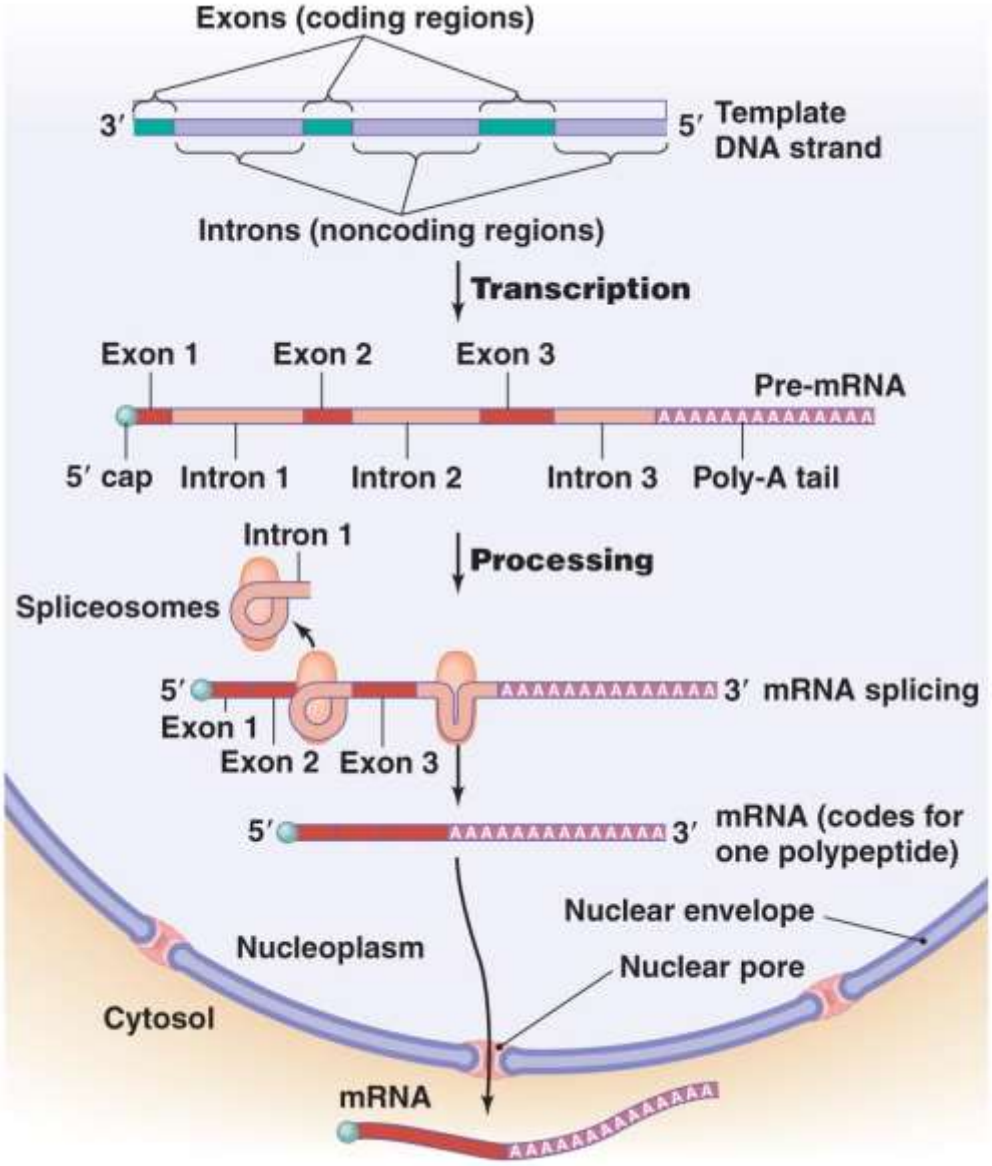


Figure 7.10

Gene Function

- **Translation**
  - Process where ribosomes use genetic information of nucleotide sequences to synthesize polypeptides

Genetic code

۴ نوع باز داریم ولی ۲۰ نوع آمینواسید داریم.

در طی ترجمه، زبان ۴ بازی DNA و RNA تبدیل به زبان ۲۰ آمینواسیدی پروتئین میشود.

اگر هر نوکلئوتیدی کدکننده یک امینو اسید باشد: $4^1=4$

یعنی ۴ آمینواسید خواهیم داشت.

اگر دو نوکلئوتید متصل شده به یکدیگر کدکننده یک امینو اسید باشد: $4^2=16$

یعنی ۱۶آمینواسید خواهیم داشت.

اگر سه نوکلئوتید متصل شده به یکدیگر کدکننده امینو اسید باشد: $4^3=64$

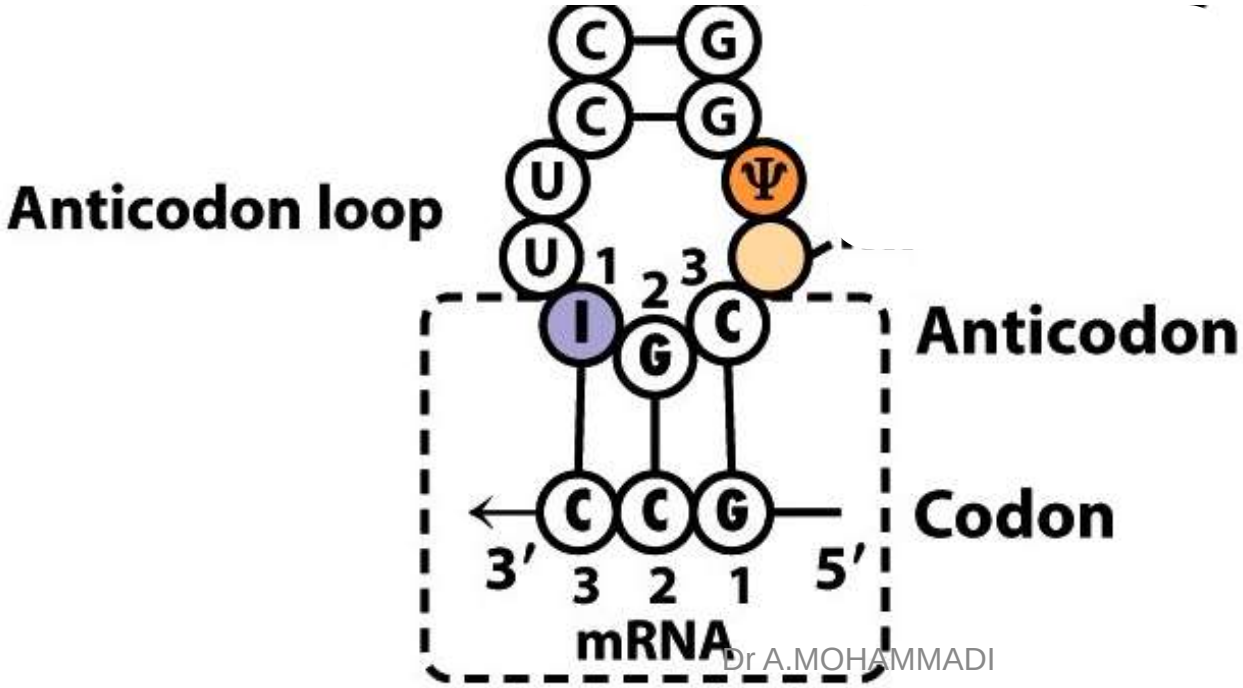
یعنی ۶۴ آمینواسید خواهیم داشت.

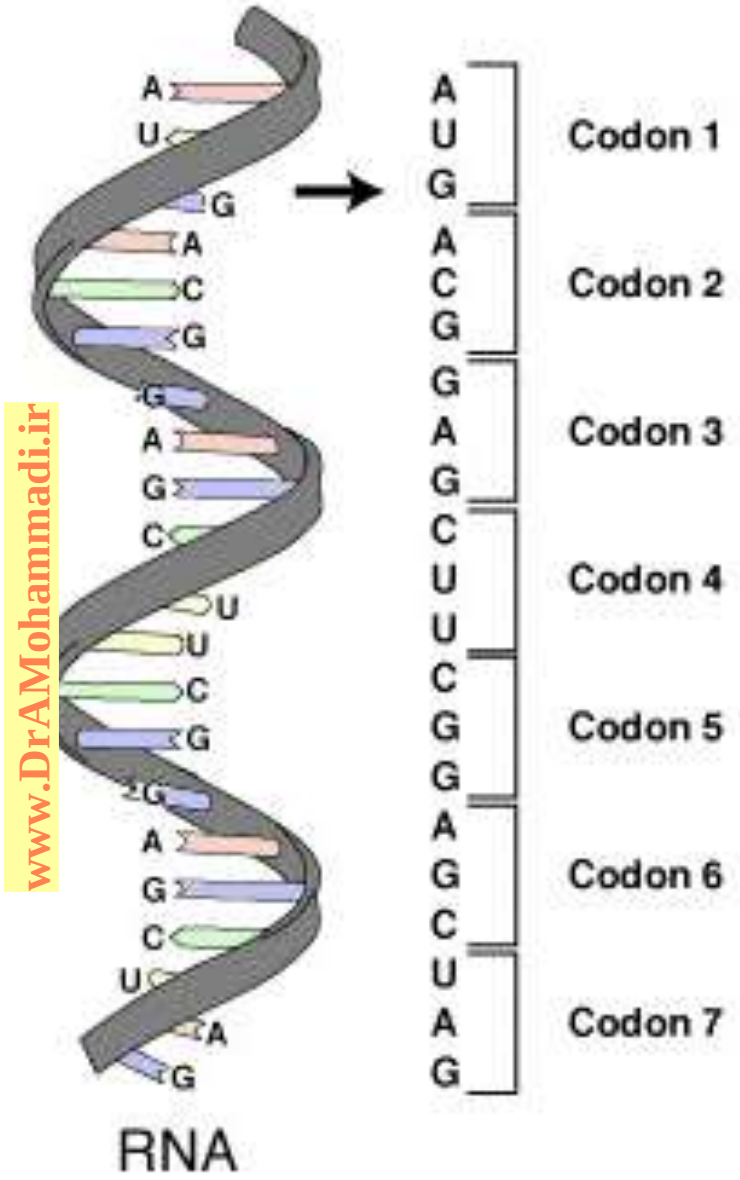
کد ژنتیکی ما باید سه نوکلئوتید (Triplet) باشد.

Genetic code

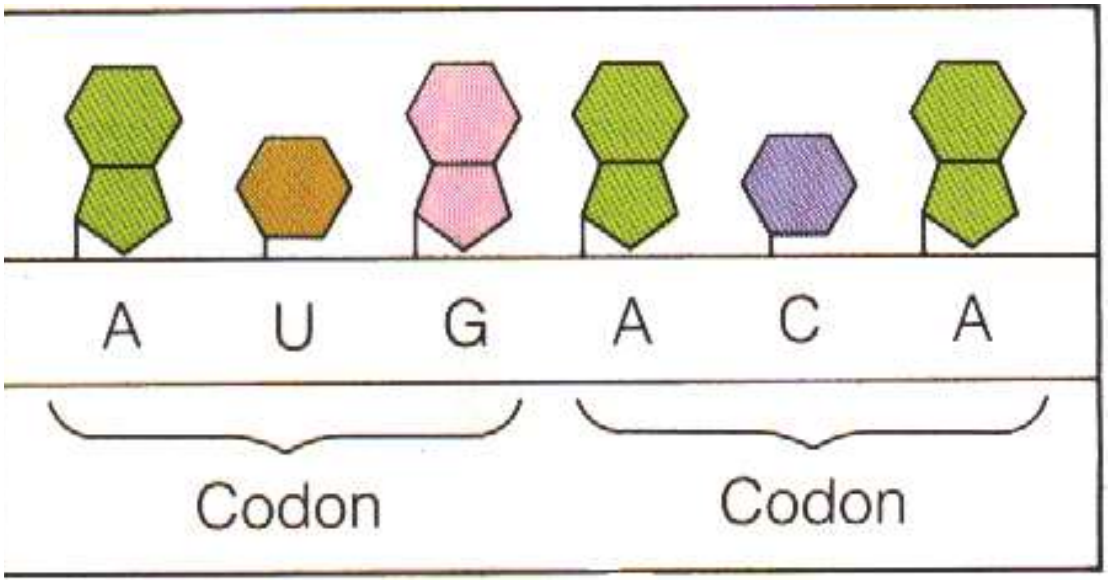
کد ژنتیکی یا کد سه تایی (کدون codon) : قرار گرفتن ۳ نوکلئوتید در کنار همدیگر در ساختمان mRNA ، یک کدون را تشکیل می دهد.

هر کدونی قدرت اتصال به یک tRNA را دارد. کدون به قسمت آنتی کدون tRNA در بازوی آنتی کدونی tRNA متصل می شود.

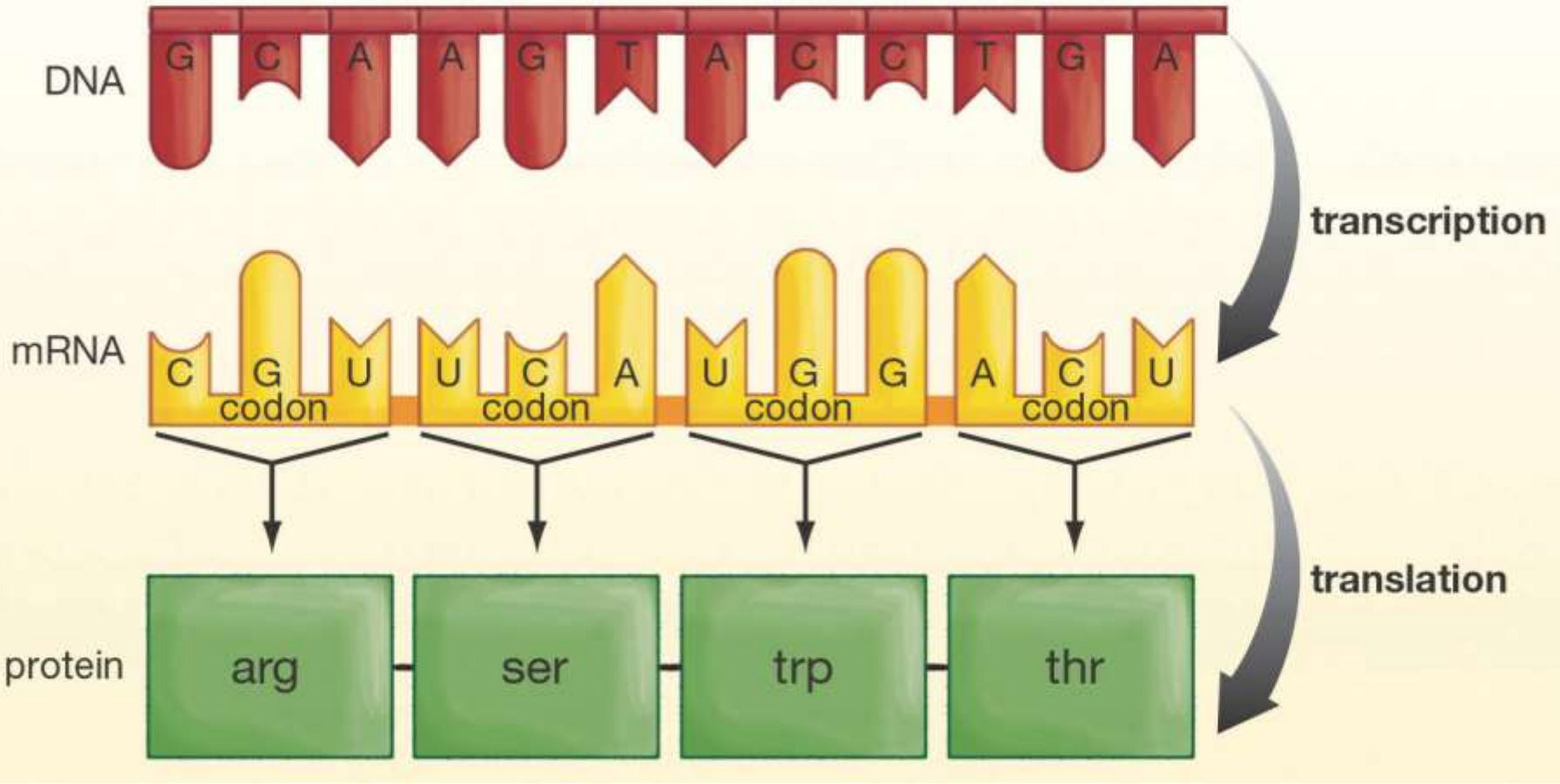




Ribonucleic acid



The Triplet Code



Genetic code

۶۴ کد مختلف داریم ولی ۲۰ آمینواسید داریم. پس هر آمینواسیدی می تواند چند کد داشته باشد. یعنی چند tRNA اختصاصی دارد که به آن آمینواسید متصل می شود. آمینواسیدها کد مشترک ندارند یعنی هر کدی برای هر tRNA اختصاصی است.

تنها دو آمینواسید تریپتوفان(UGG) و متیونین (AUG) هستند که فقط یک کد دارند.

یک کد شروع ترجمه داریم

AUG

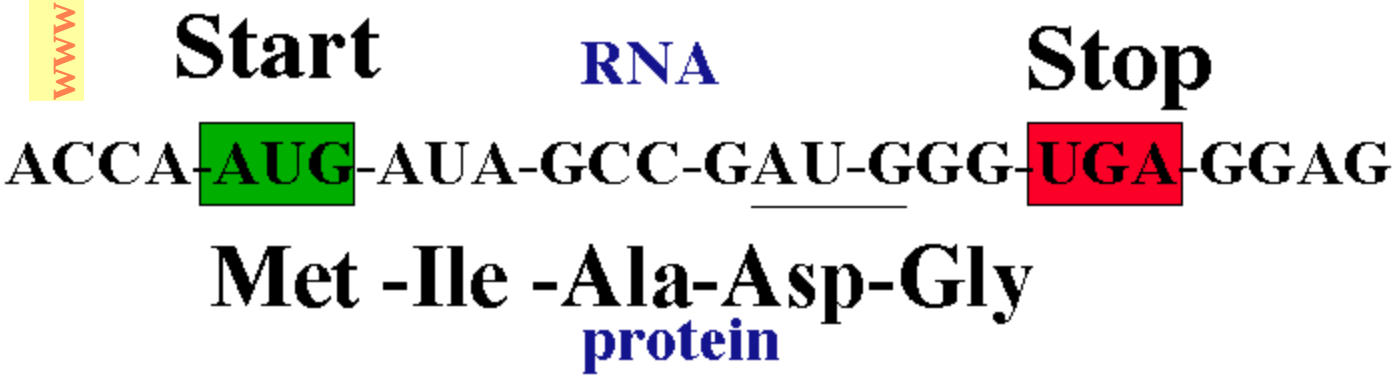
سه کد خاتمه

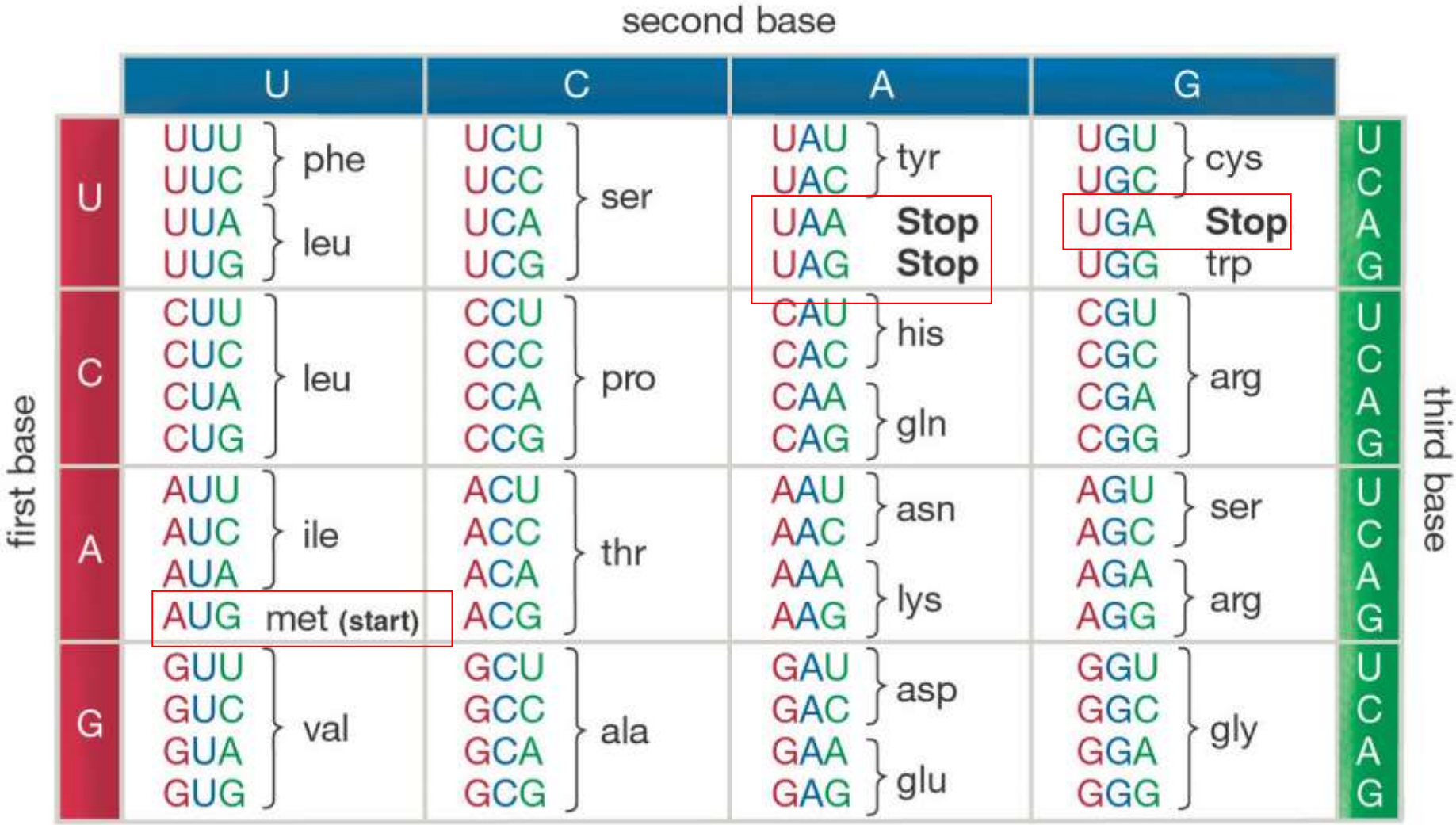
ترجمه هم داریم:

UGA

UAA

UAG




second base
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third base
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UUU phe
UUC
UUA leu
UUG
UCU ser
UCC
UCA
UCG
UAU tyr
UAC
UAA Stop
UAG Stop
UGU cys
UGC
UGA Stop
UGG trp
C
CUU leu
CUC
CUA
CUG
CCU pro
CCC
CCA
CCG
CAU his
CAC
CAA gln
CAG
CGU arg
CGC
CGA
CGG
A
AUU ile
AUC
AUA
AUG met (start)
ACU thr
ACC
ACA
ACG
AAU asn
AAC
AAA lys
AAG
AGU ser
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GAA glu
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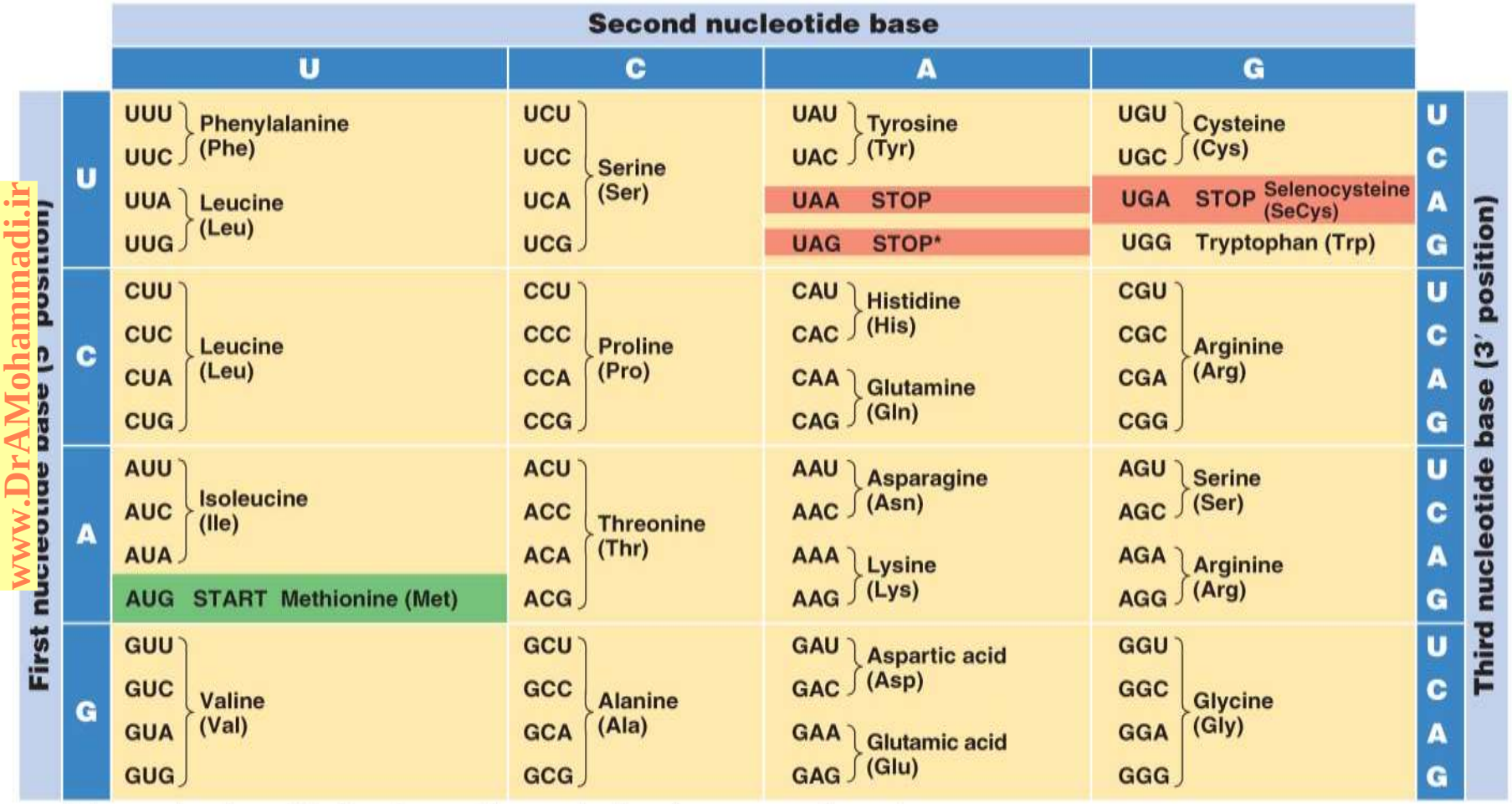
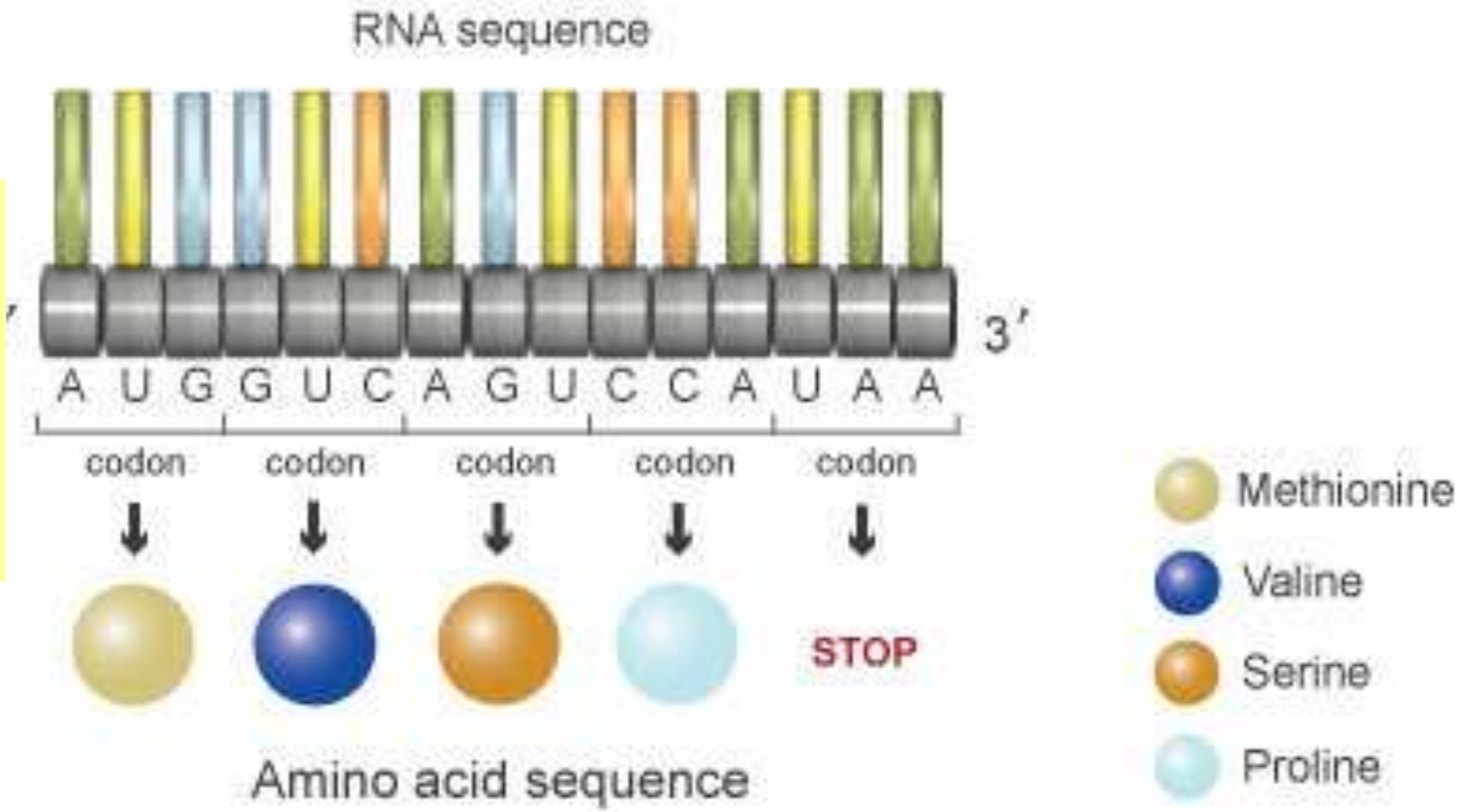
The genetic code

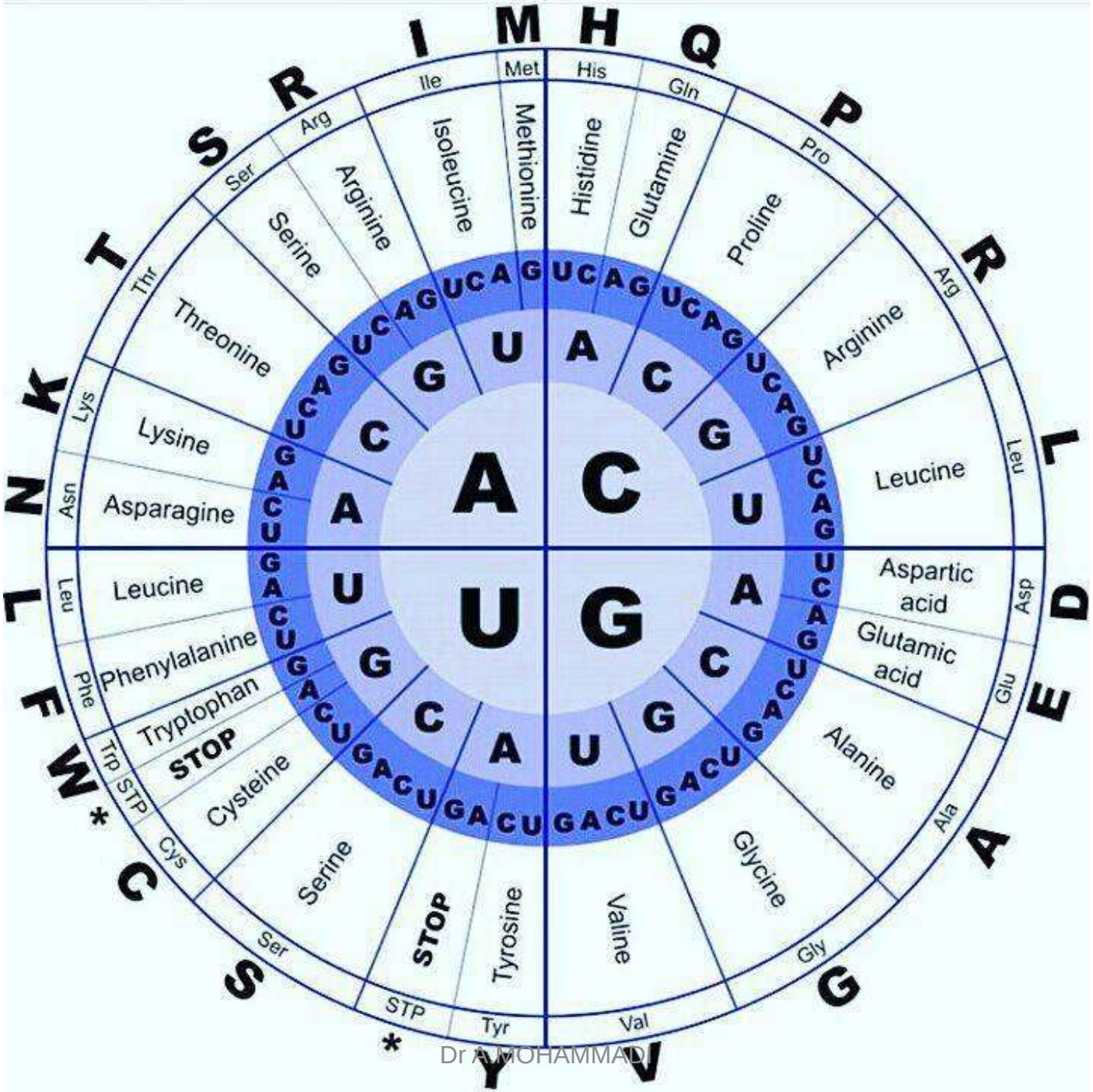


*also codes for a 22nd amino acid, pyrrolysine, in some prokaryotes.

Figure 7.11

Amino acid	Number of codons	Amino acid	Number of codons
Met	1	Tyr	2
Trp	1	Ile	3
Asn	2	Ala	4
Asp	2	Gly	4
Cys	2	Pro	4
Gln	2	Thr	4
Glu	2	Val	4
His	2	Arg	6
Lys	2	Leu	6
Phe	2	Ser	6

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Isoleucine
Arginine
Serine
Histidine
Glutamine
Proline
Arginine
Threonine
Lysine
Asparagine
Leucine
Phenylalanine
Leucine
Tryptophan
STOP
Cysteine
Serine
STOP
Tyrosine
Valine
Glycine
Alanine
Aspartic acid
Glutamic acid
Leucine
Arginine
Proline
Glutamine
Histidine
Met
Ile
Arg
Ser
Thr
Lys
Asn
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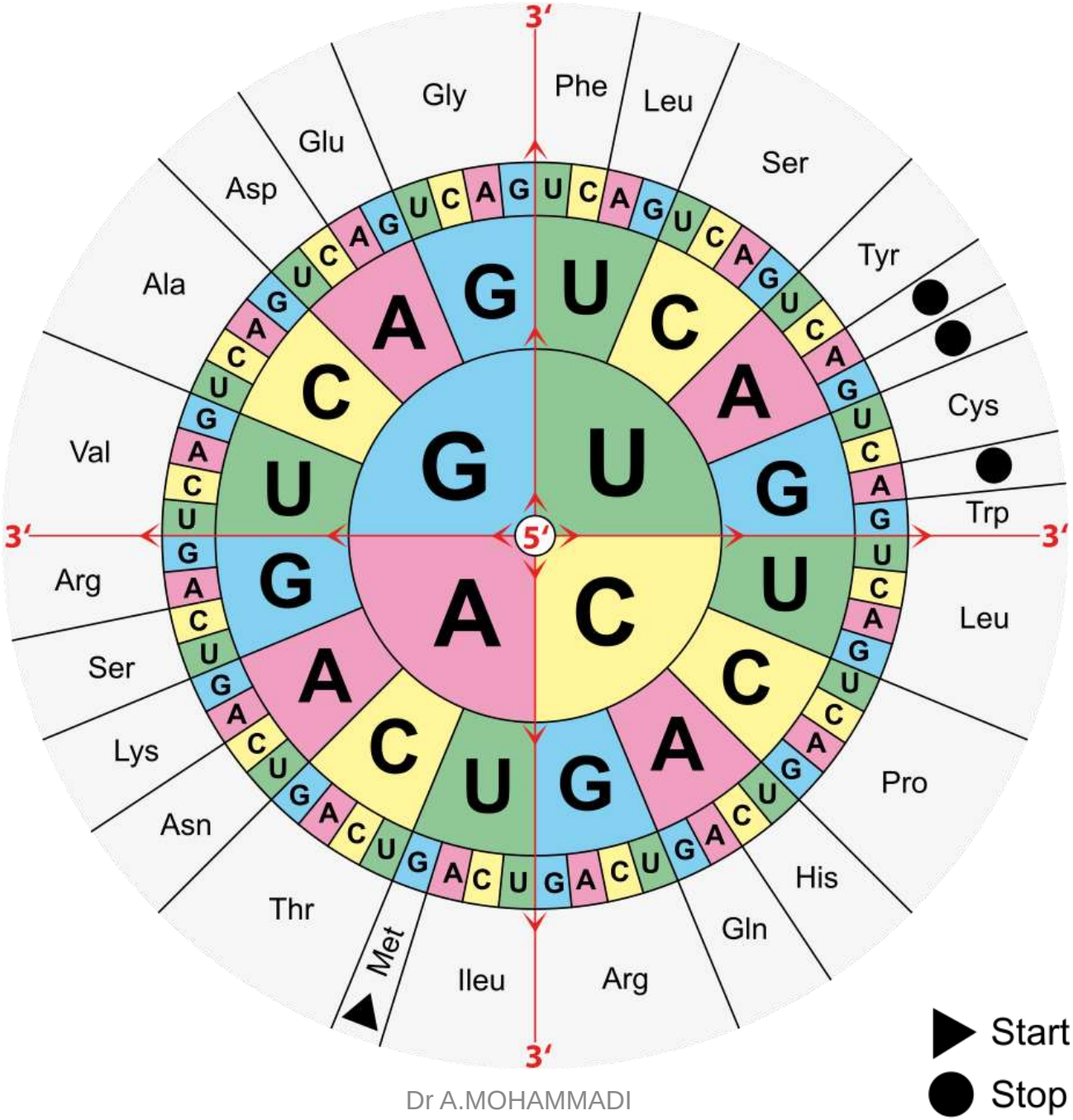


Gene Function

- **Translation**
  - Participants in translation
    - Messenger RNA
    - Transfer RNA
    - Ribosomes and ribosomal RNA

Prokaryotic mRNA can code for several polypeptides

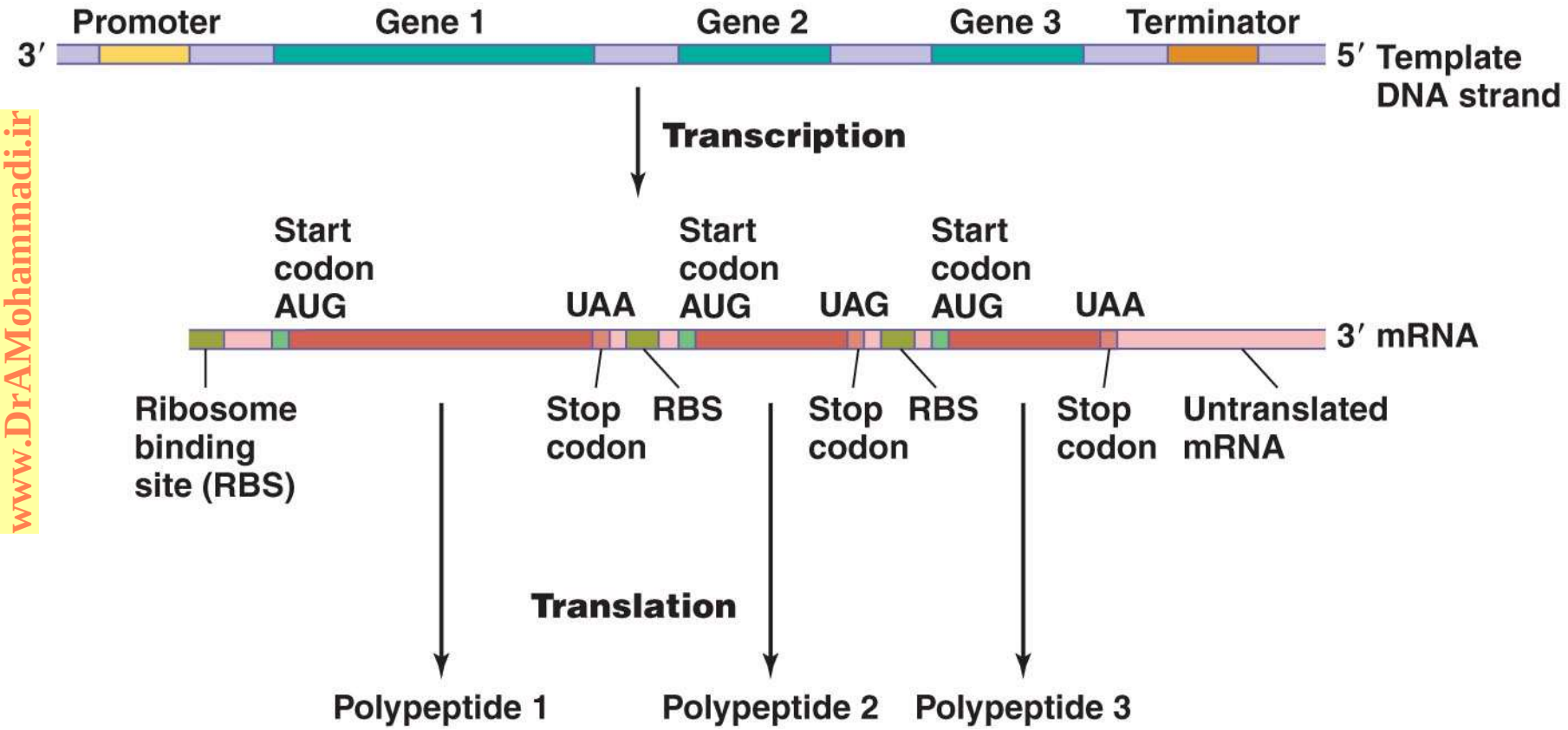
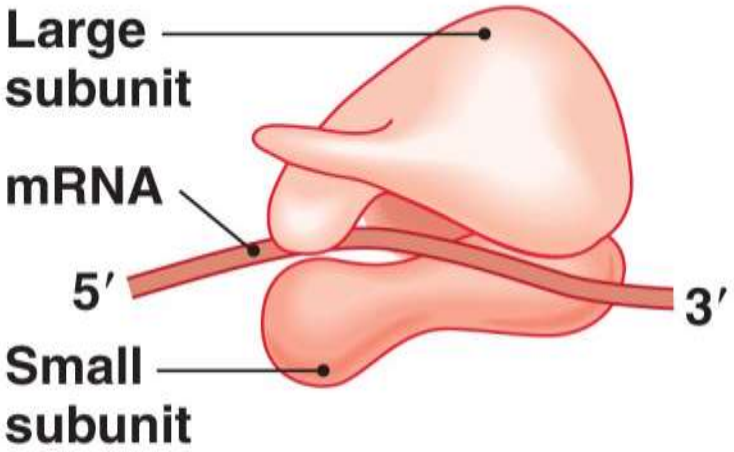
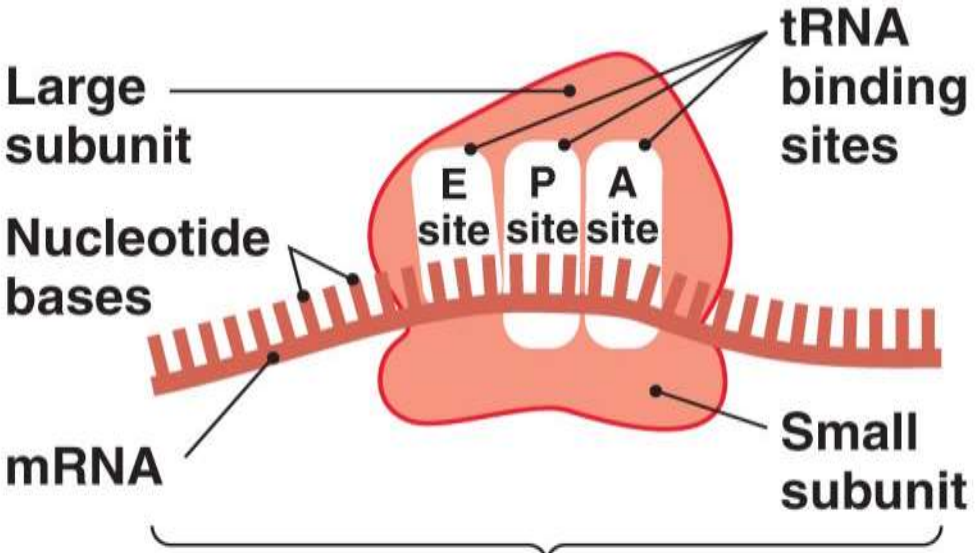


Figure 7.12

Assembled ribosome and its tRNA-binding sites



Prokaryotic ribosome (angled view) attached to mRNA



Prokaryotic ribosome (schematic view) showing tRNA binding sites

Figure 7.15

Gene Function

- **Translation**
  - Three stages of translation
    - Initiation
    - Elongation
    - Termination
  - All stages require additional protein factors
  - Initiation and elongation require energy (GTP)

The initiation of translation in prokaryotes

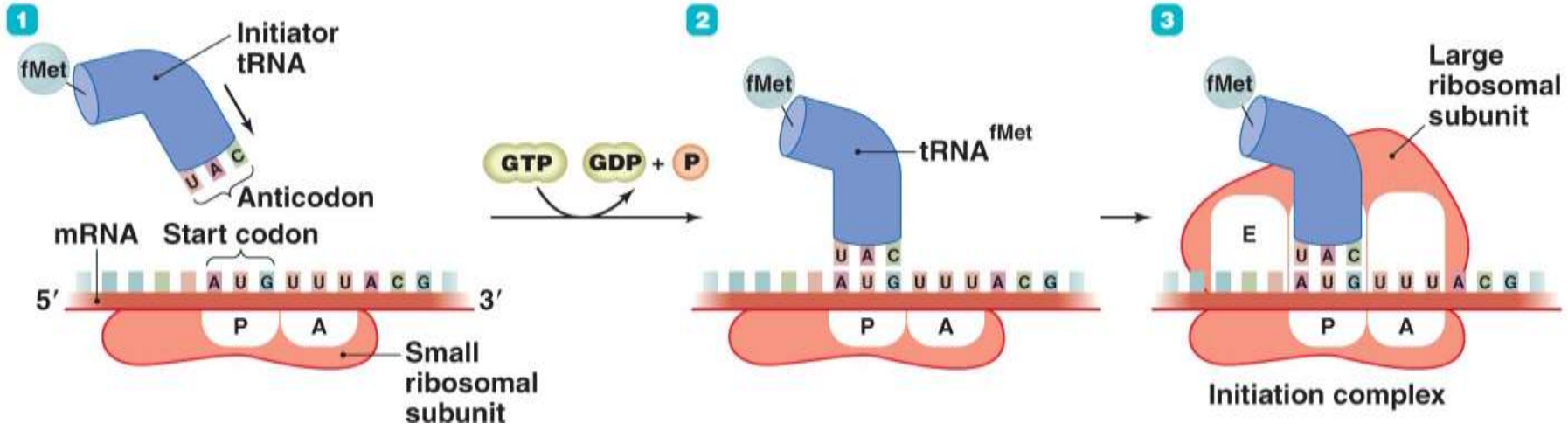


Figure 7.16

The elongation stages of translation

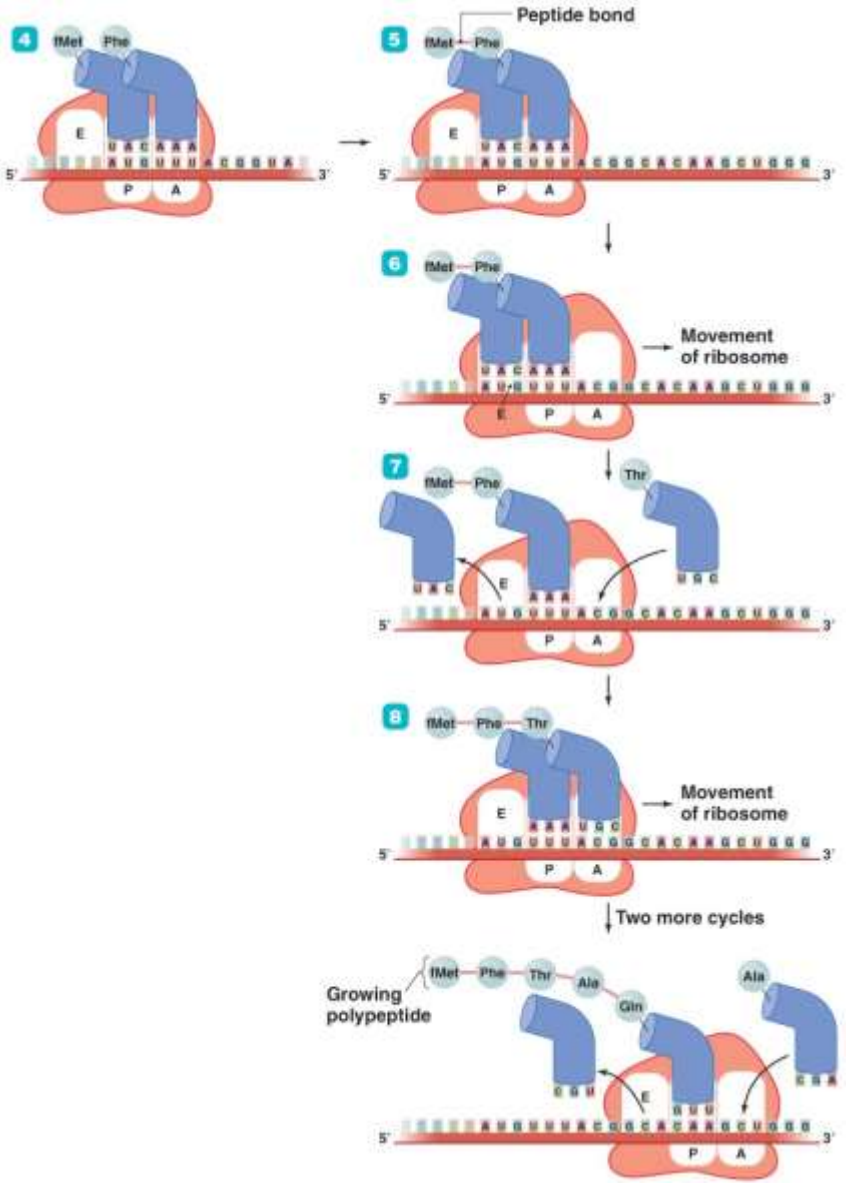
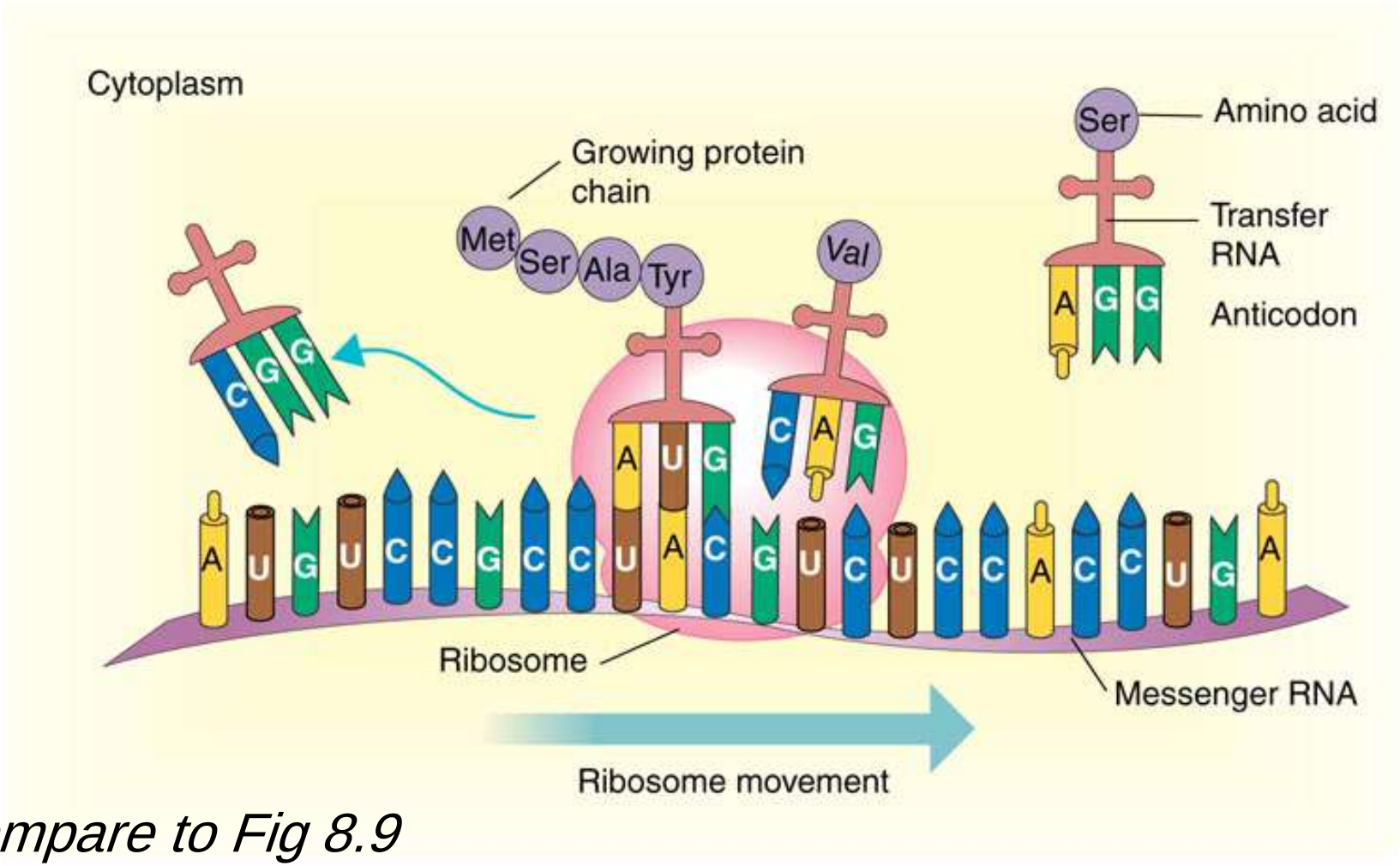


Figure 7.17

The Translation Process in Protein Synthesis



Compare to Fig 8.9

One prokaryotic mRNA, many ribosomes and polypeptides

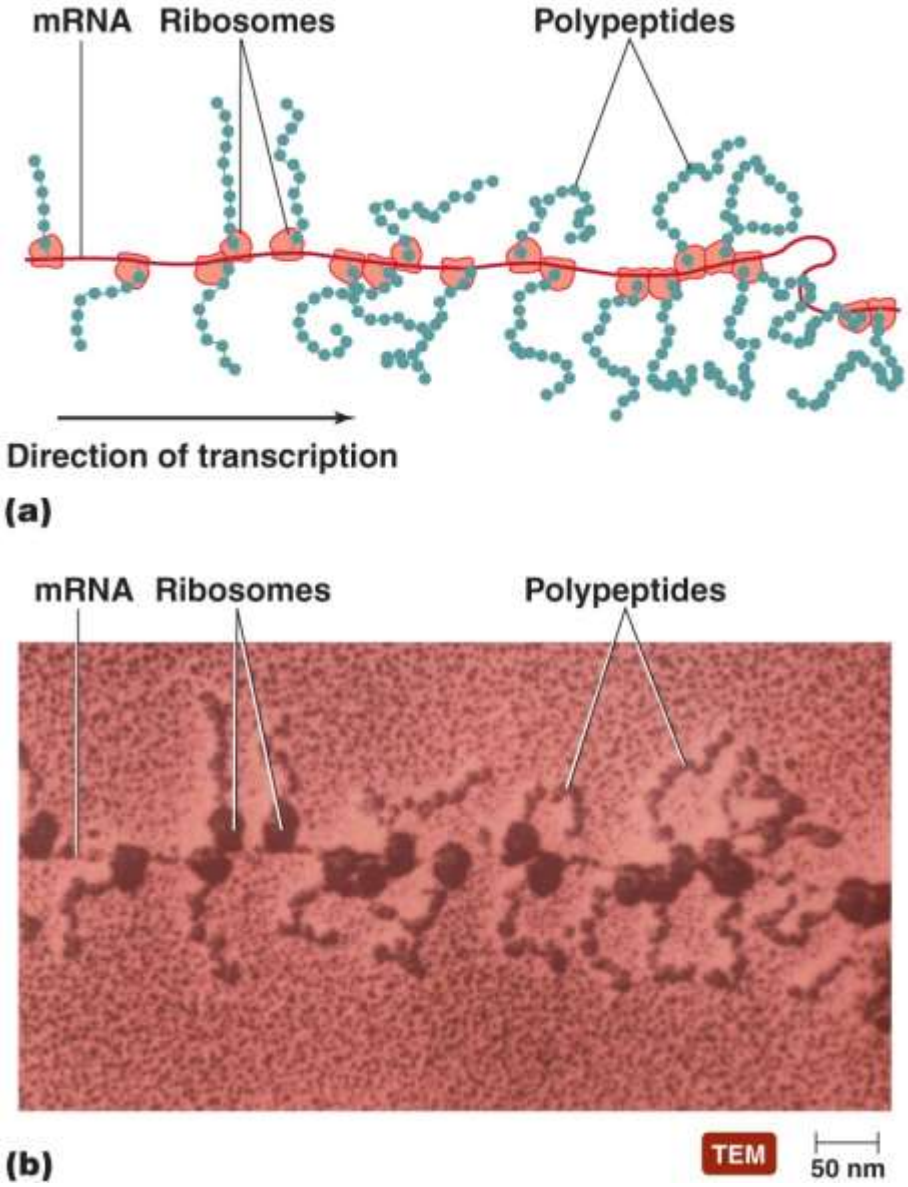
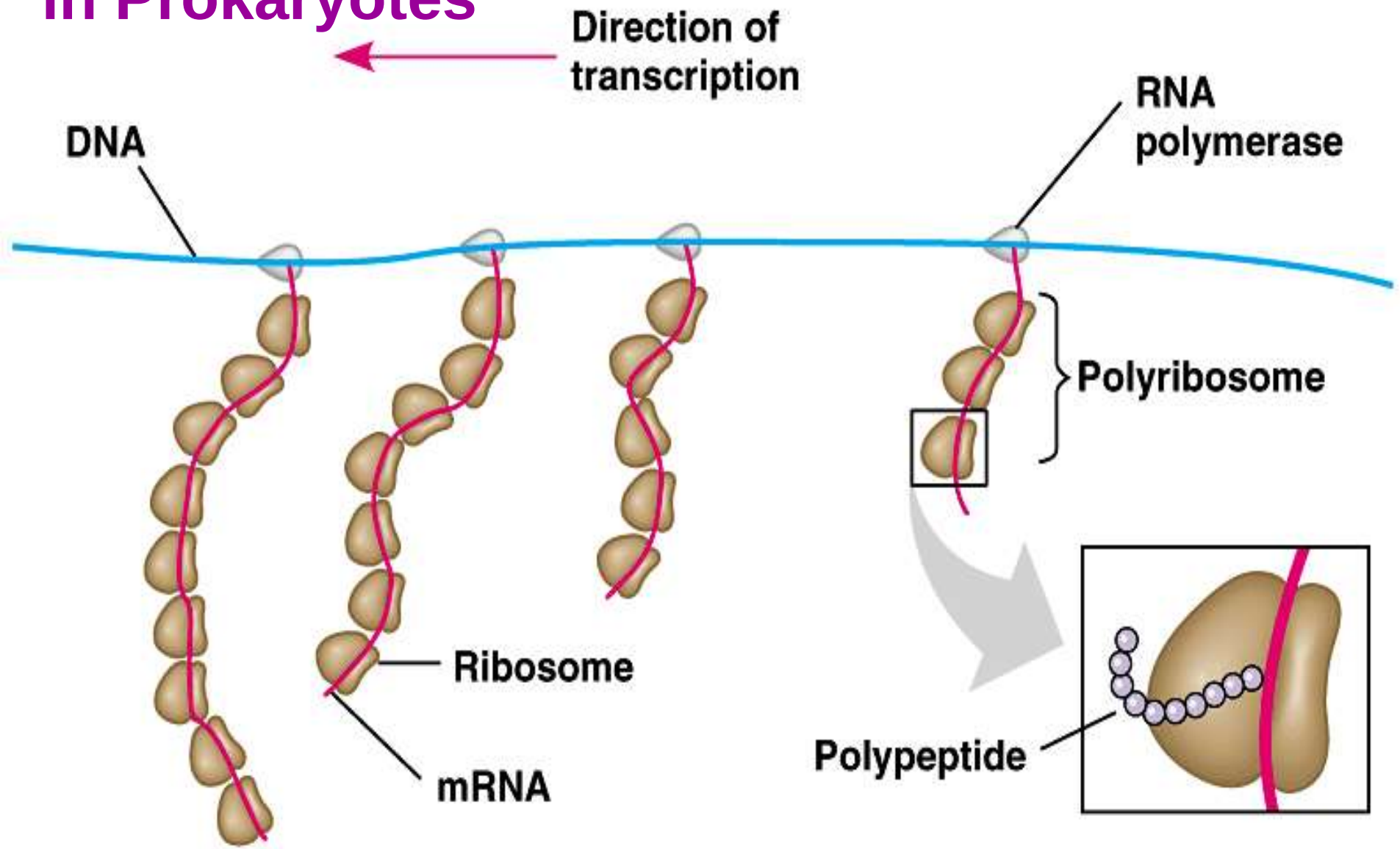


Figure 7.18

Simultaneous Transcription and Translation in Prokaryotes



Compare to Fig 8.10

Gene Function

- **Translation**
  - Stages of translation
    - Termination
      - Release factors (RF) recognize stop codons
        - Modify ribosome to activate ribozymes
      - Ribosome dissociates into subunits
      - Polypeptides released at termination may function alone or together

Gene Function

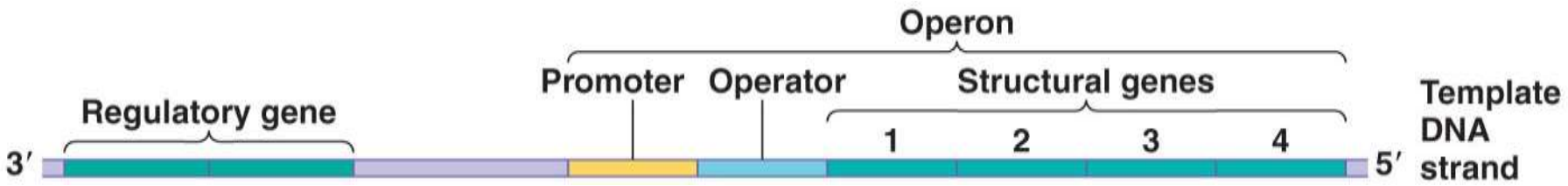
- **Translation**
  - Translation differences in eukaryotes
    - Initiation occurs when ribosomal subunit binds to 5' guanine cap
    - First amino acid is methionine rather than f-methionine

Gene Function

- **Regulation of Genetic Expression**
  - 75% of genes are expressed at all times
  - Other genes transcribed and translated when cells need them
    - Allows cell to conserve energy
  - Regulation of protein synthesis
    - Typically halts transcription
    - Can stop translation directly

Gene Function

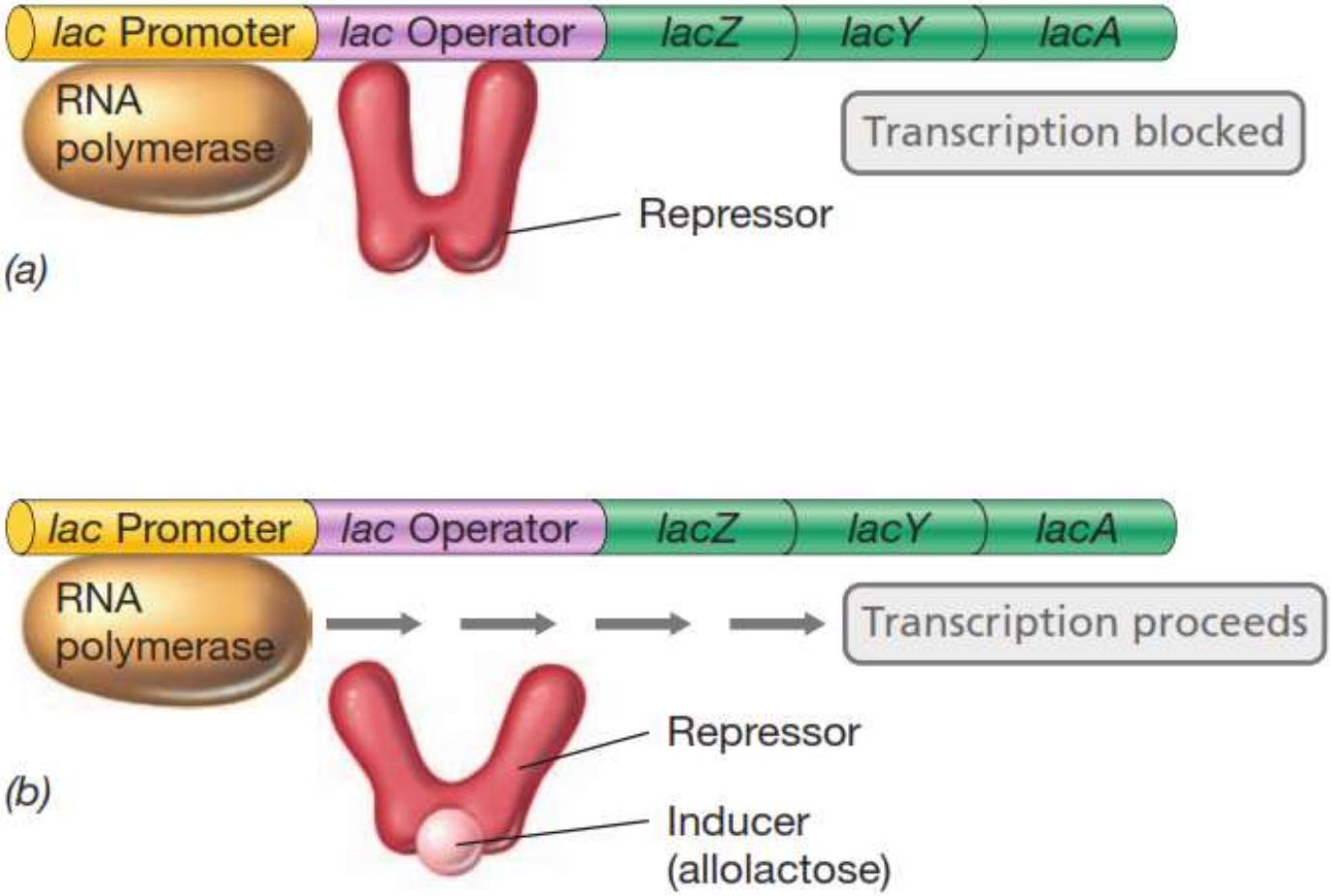
- **Regulation of Genetic Expression**
  - Nature of prokaryotic operons
    - An operon consists of a promoter and a series of genes
    - Some operons are controlled by a regulatory element called an operator



Gene Function

- **Regulation of Genetic Expression**
  - Nature of prokaryotic operons
    - Inducible operons must be activated by inducers
      - Lactose operon
    - Repressible operons are transcribed continually until deactivated by repressors
      - Tryptophan operon

The *lac* operon, an inducible operon



The *lac* operon, an inducible operon

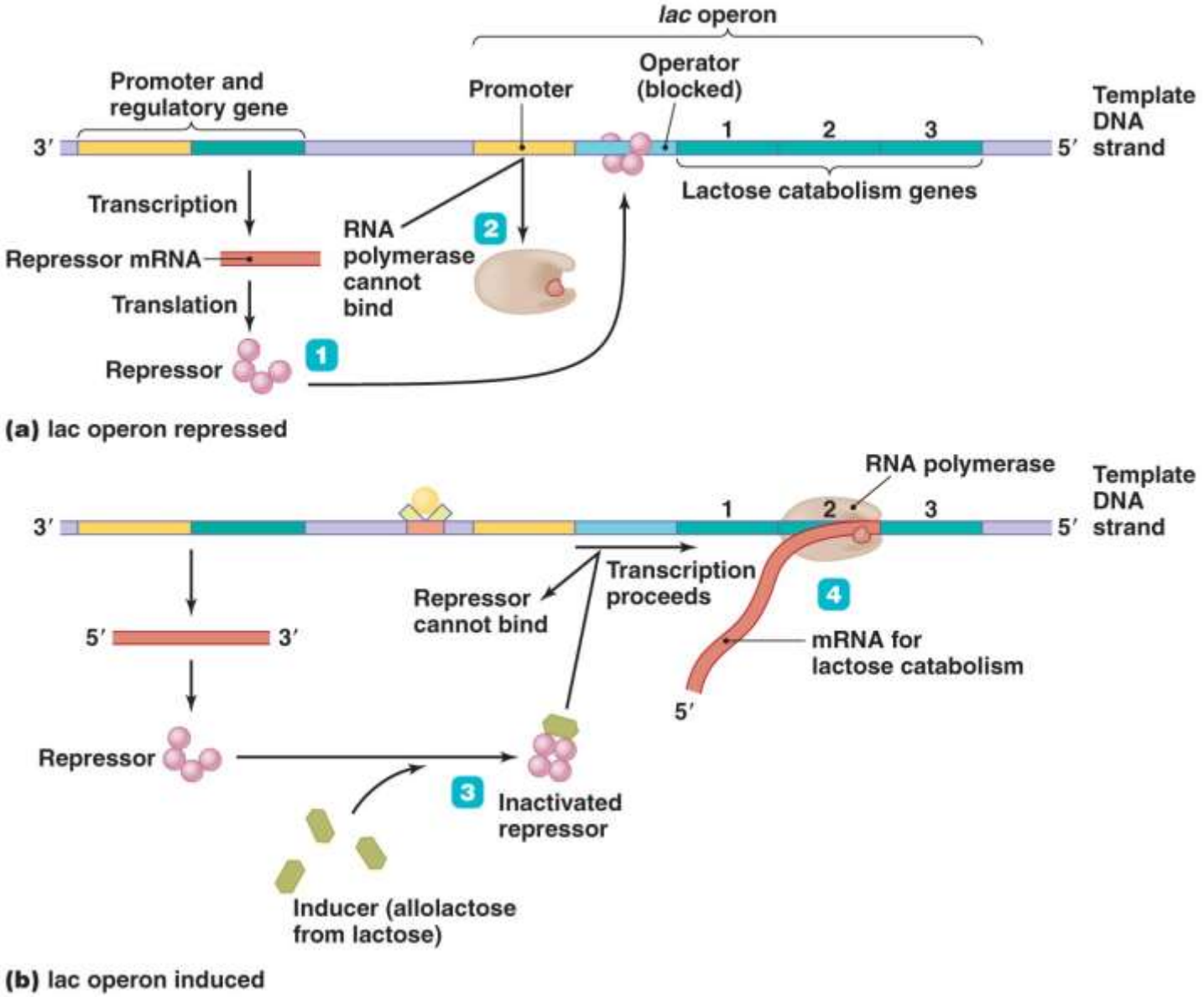


Figure 7.20

The *trp* operon, a repressible operon

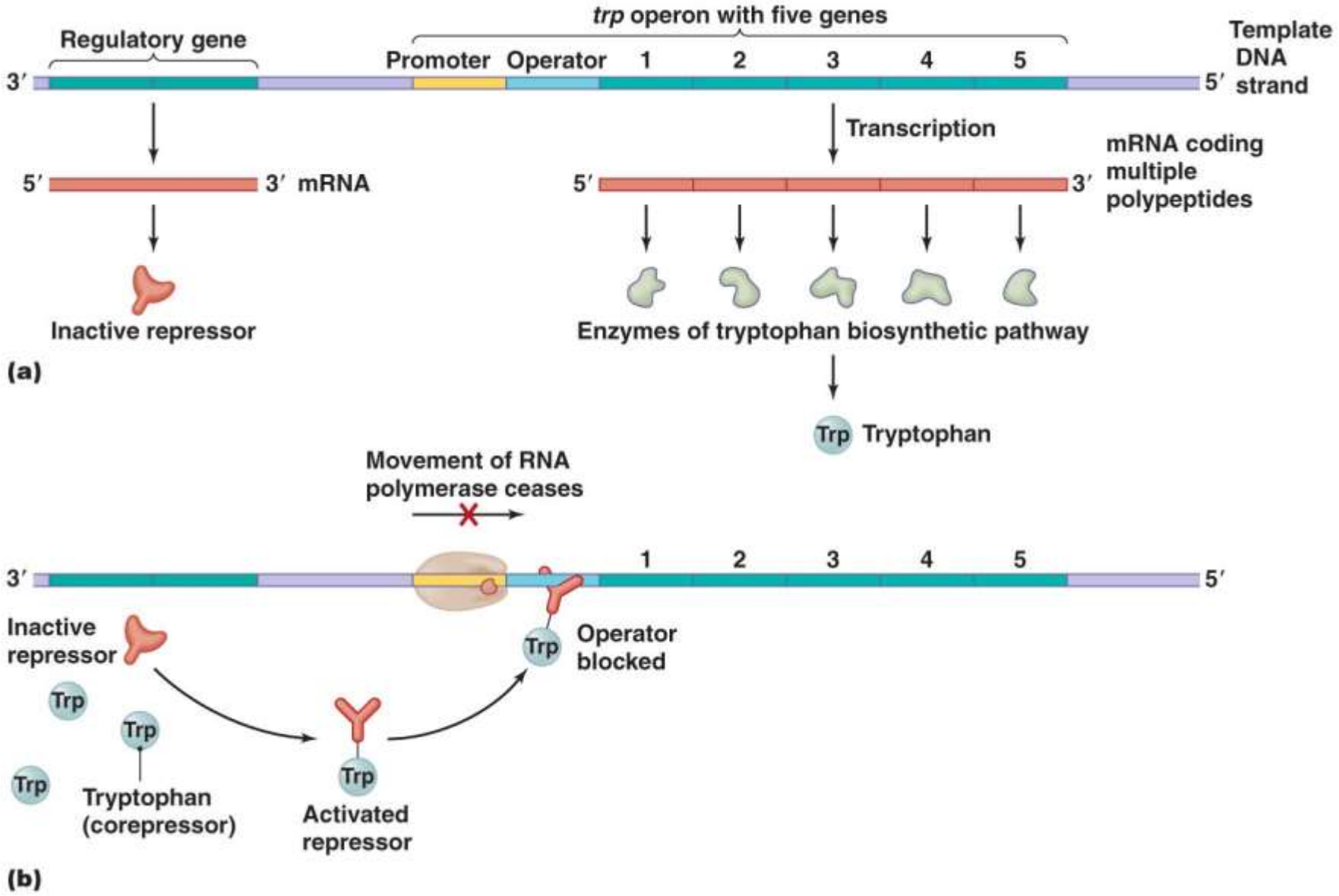


Figure 7.22

Positive regulation: CAP-cAMP enhances *lac* transcription

Regulation of the lactose operon also depends on the level of glucose in the medium, which in turn controls the intracellular level of the small molecule **cyclic AMP (cAMP).** It is an example of an alarmone, a chemical alarm signal that promotes a cell's response to environmental or nutritional stress.

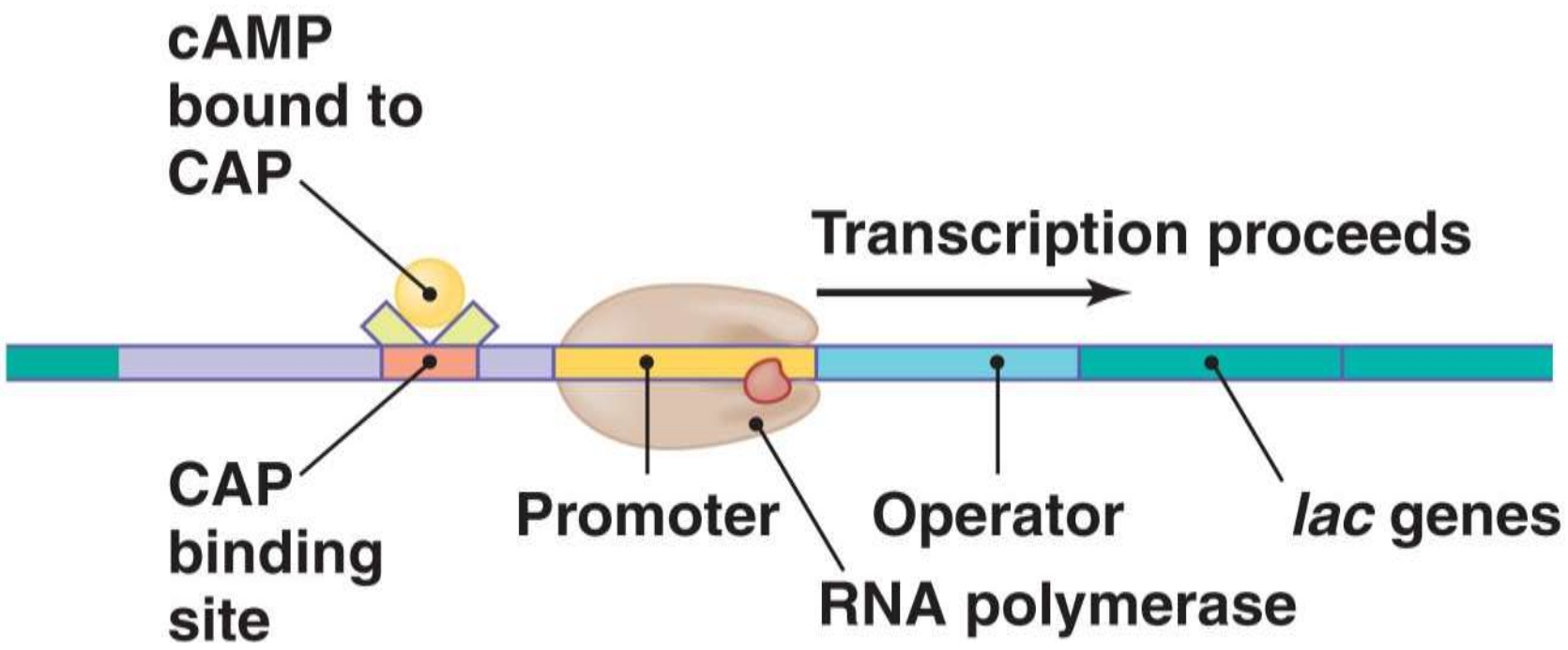
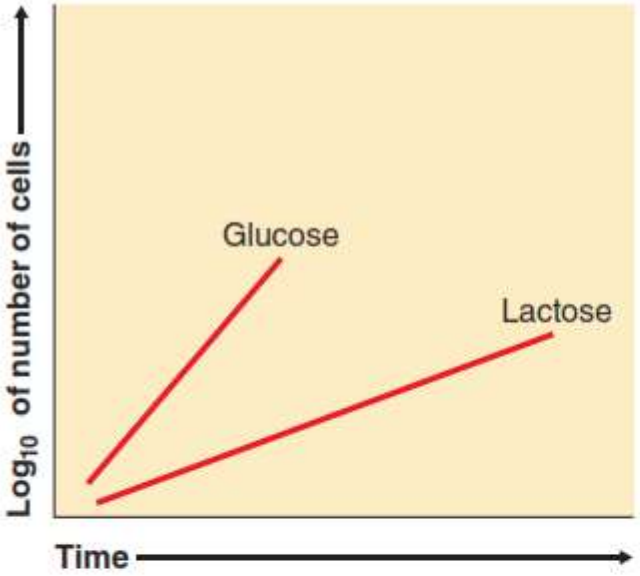
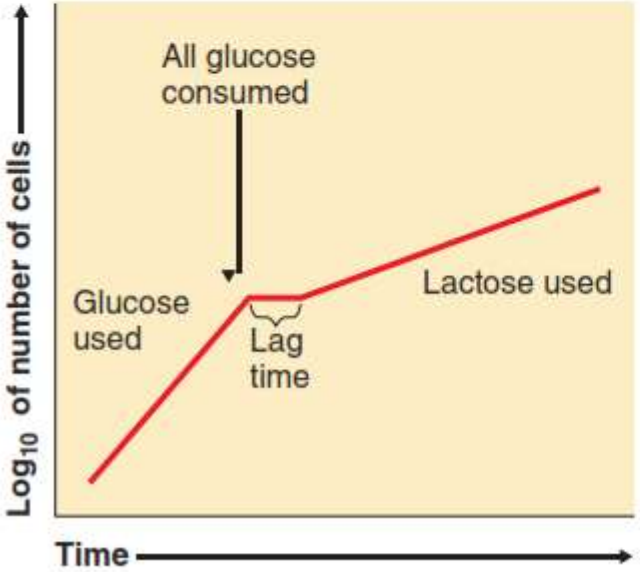


Figure 7.21

Inhibition of the metabolism of alternative carbon sources by glucose is termed **catabolite repression** (or the glucose effect).

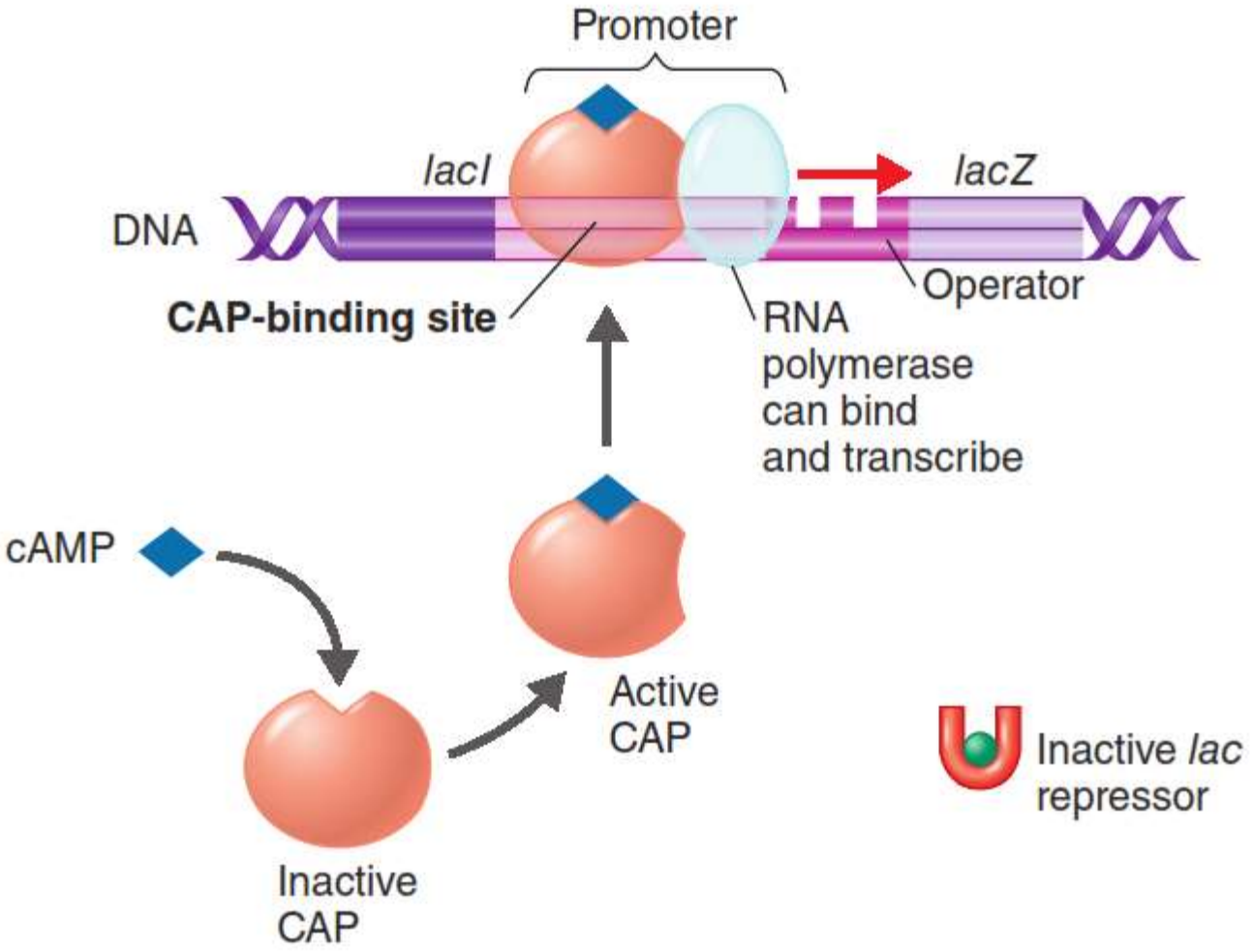


(a) Bacteria growing on glucose as the sole carbon source grow faster than on lactose.

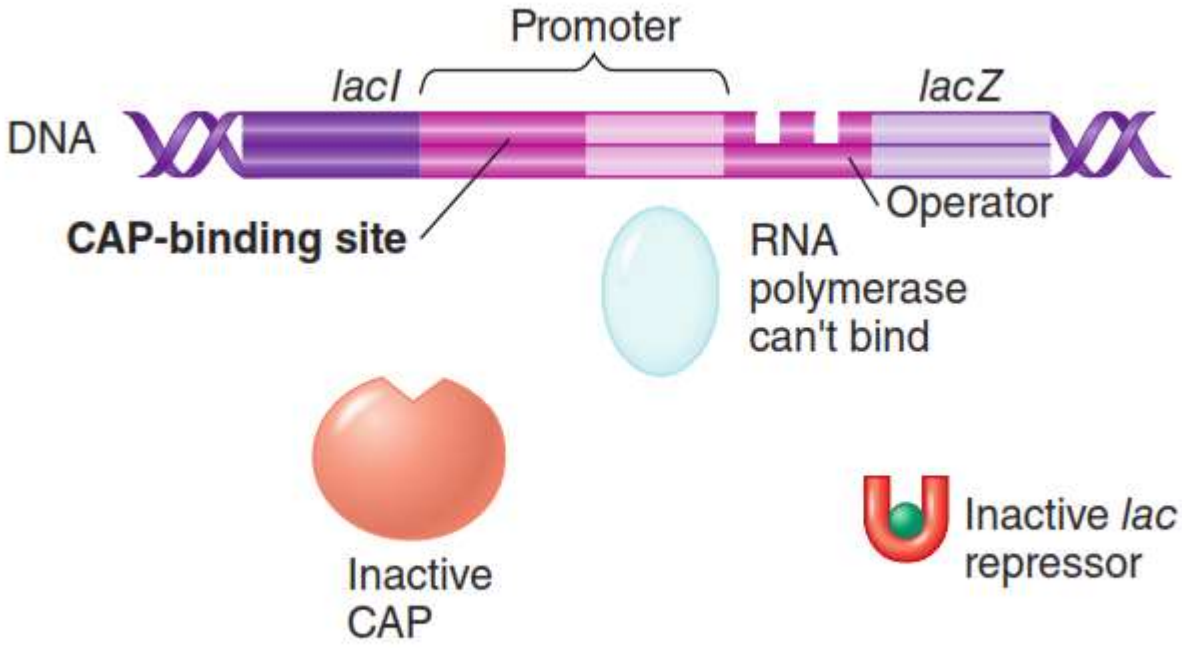


(b) Bacteria growing in a medium containing glucose and lactose first consume the glucose and then, after a short lag time, the lactose. During the lag time, intracellular cAMP increases, the *lac* operon is transcribed, more lactose is transported into the cell, and β-galactosidase is synthesized to break down lactose.

Figure 8.14 The growth rate of *E. coli* on glucose and lactose.



(a) Lactose present, glucose scarce (cAMP level high). If glucose is scarce, the high level of cAMP activates CAP, and the *lac* operon produces large amounts of mRNA for lactose digestion.



(b) Lactose present, glucose present (cAMP level low). When glucose is present, cAMP is scarce, and CAP is unable to stimulate transcription.

Mutations of Genes

- **Mutation**
  - Change in the nucleotide base sequence of a genome
  - Rare event
  - Almost always deleterious
  - Rarely leads to a protein that improves ability of organism to survive

Mutations of Genes

- **Types of Mutations**
  - Point mutations
    - Most common
    - One base pair is affected
    - Insertions, deletions, and substitutions
  - Frameshift mutations
    - Nucleotide triplets after the mutation are displaced
    - Insertions and deletions

The effects of the various types of point mutations

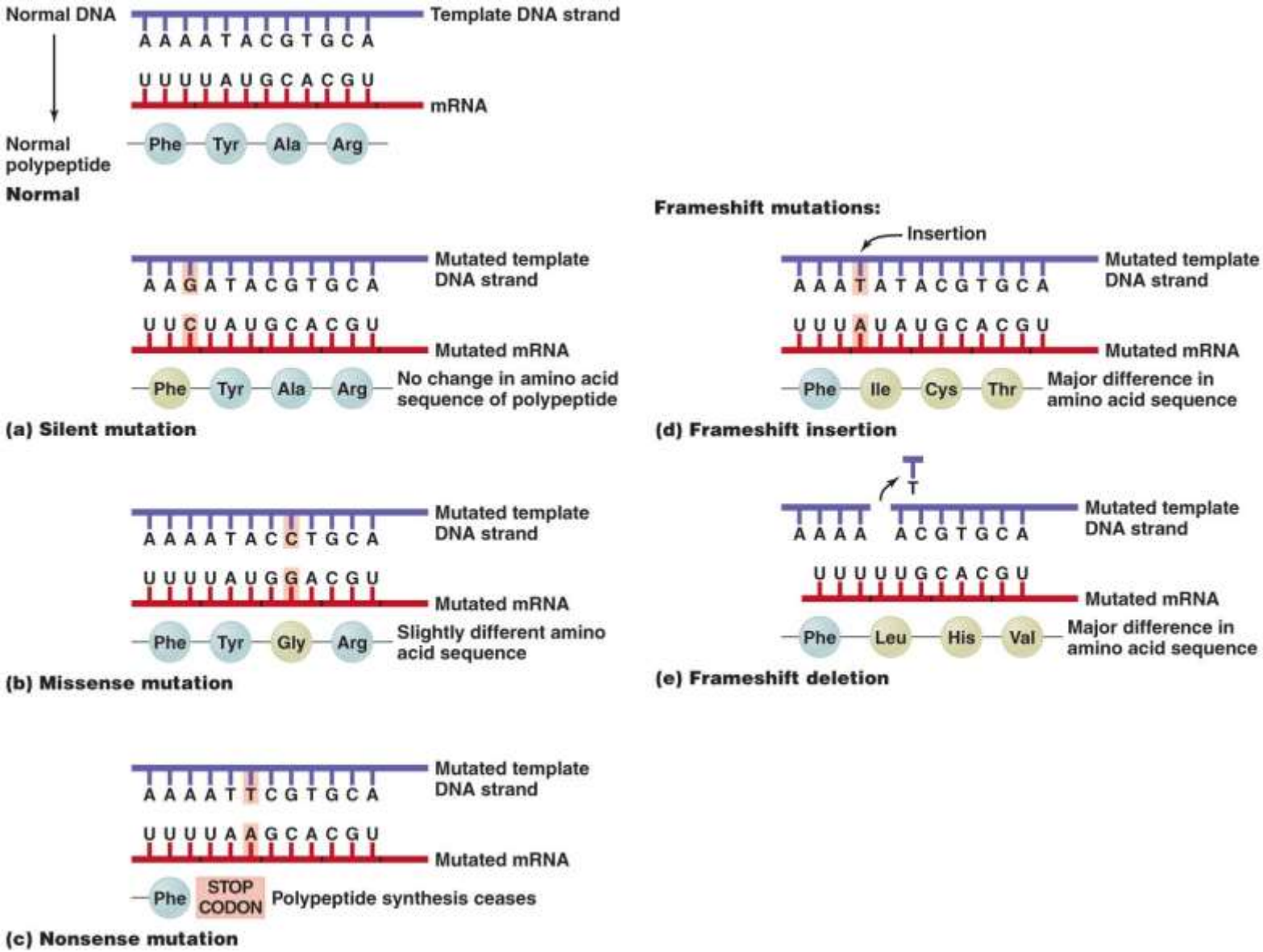


Figure 7.23

Mutations of Genes

- **Mutagens**
  - Radiation
    - Ionizing radiation
    - Nonionizing radiation
  - Chemical mutagens
    - Nucleotide analogs
      - Disrupt DNA and RNA replication
    - Nucleotide-altering chemicals
      - Result in base-pair substitutions and missense mutations
    - Frameshift mutagens
      - Result in nonsense mutations

A pyrimidine dimer

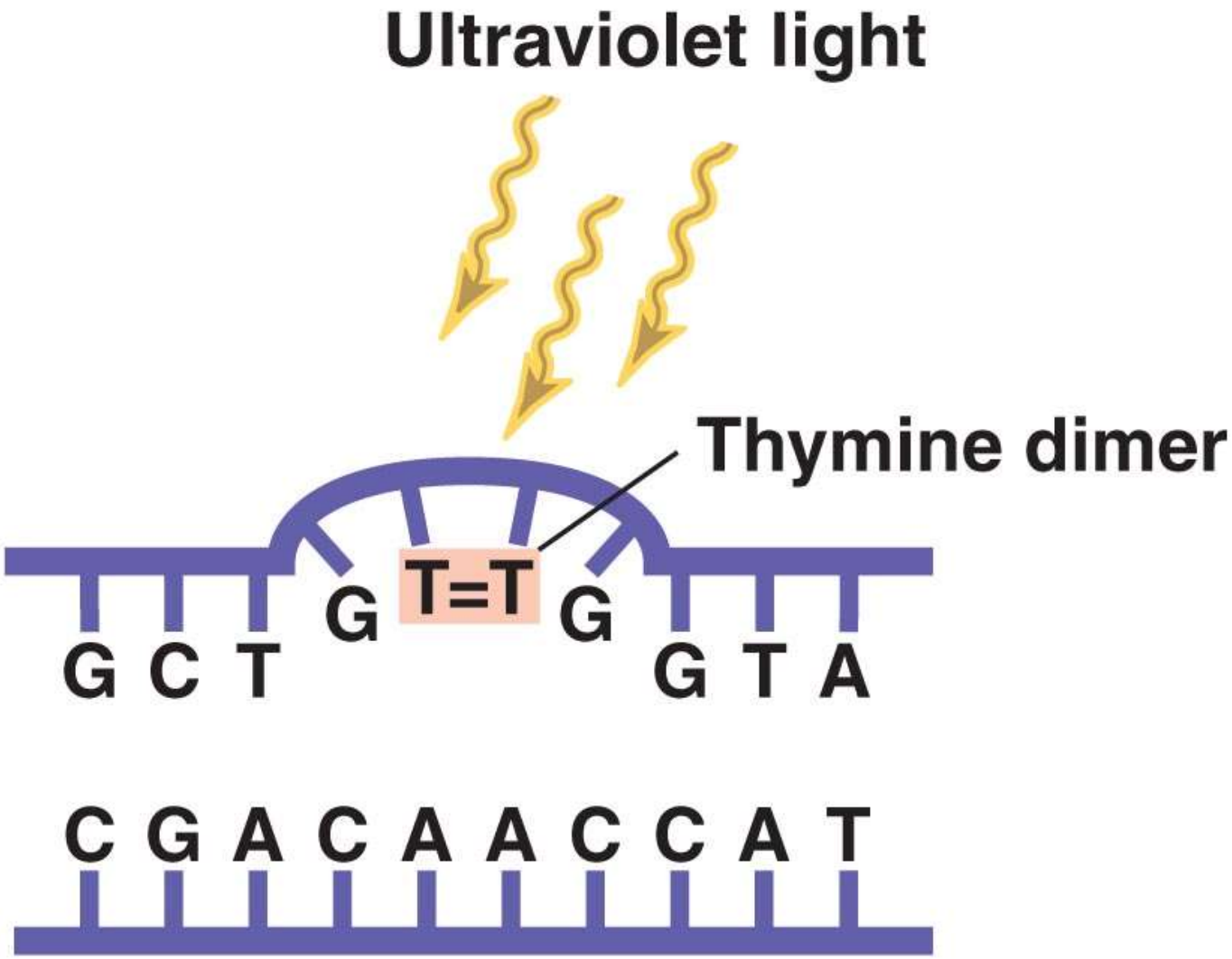


Figure 7.24

The structure and effects of a nucleotide analog

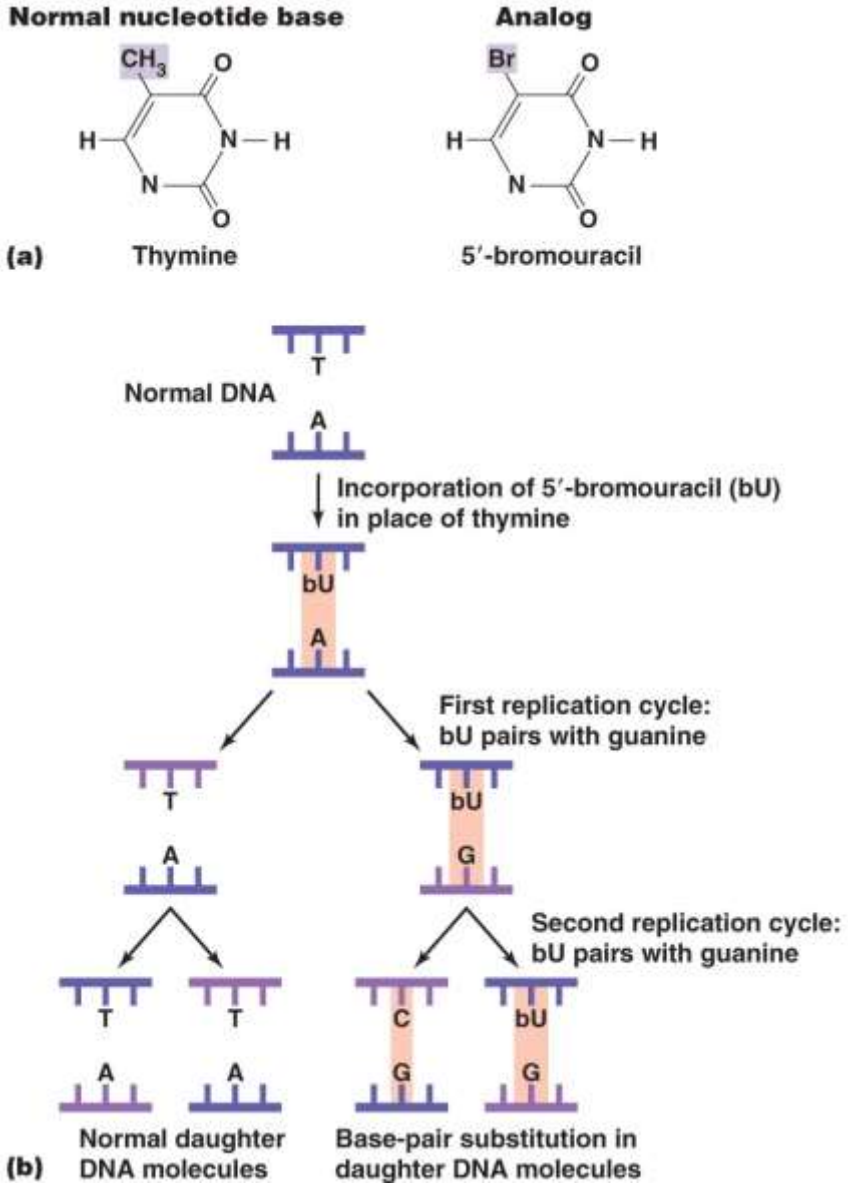
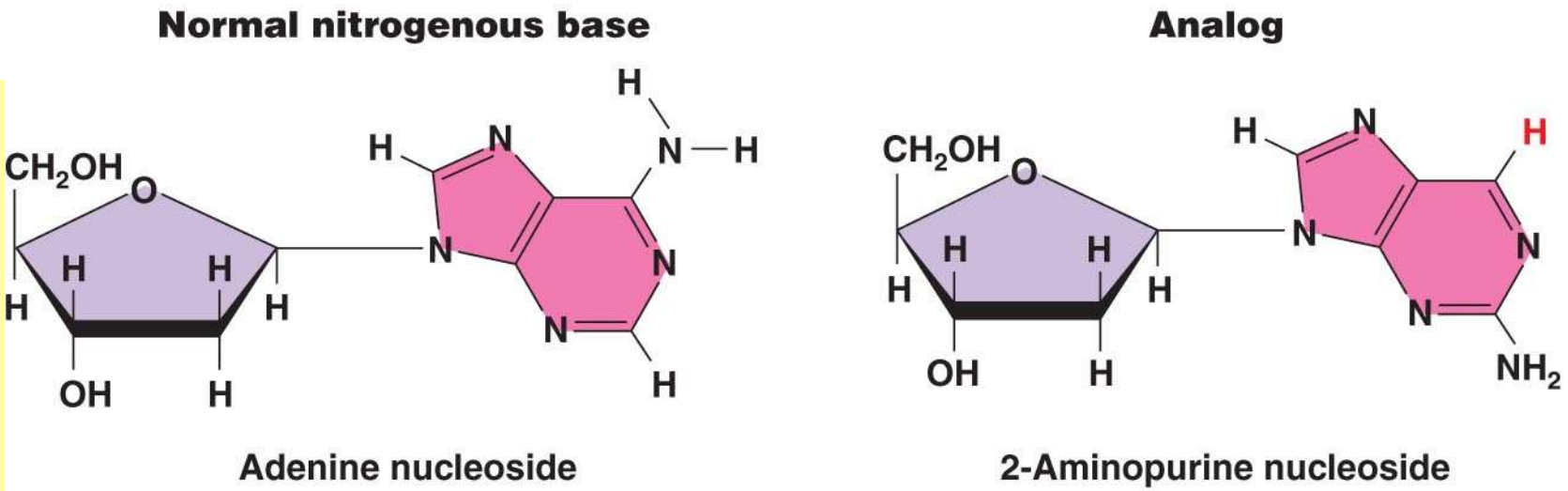


Figure 7.25

Fig 8.19a



Adenine nucleoside

2-Aminopurine nucleoside

a) The 2-aminopurine is incorporated into DNA in place of adenine but can pair with cytosine, so an AT pair becomes a CG pair.

The action of a frameshift mutagen

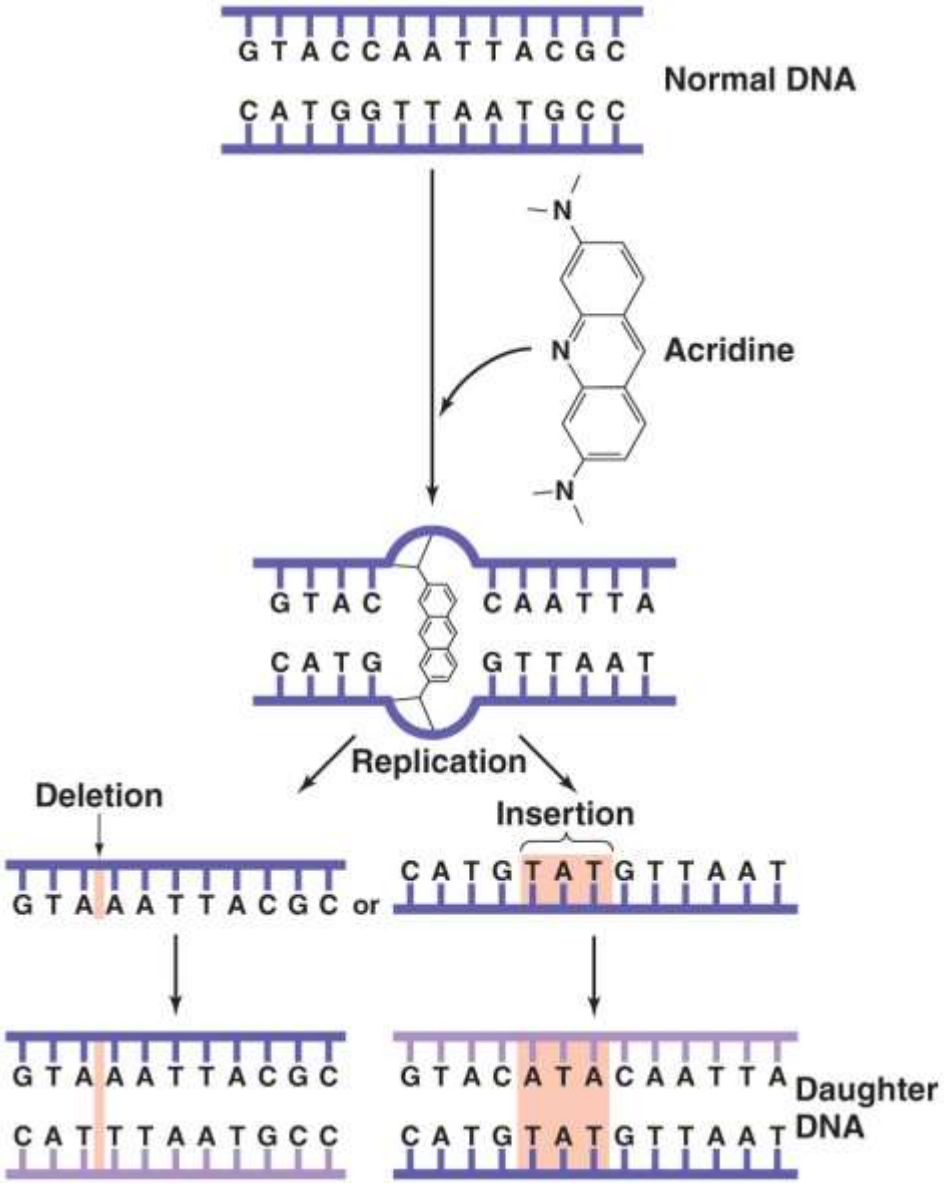
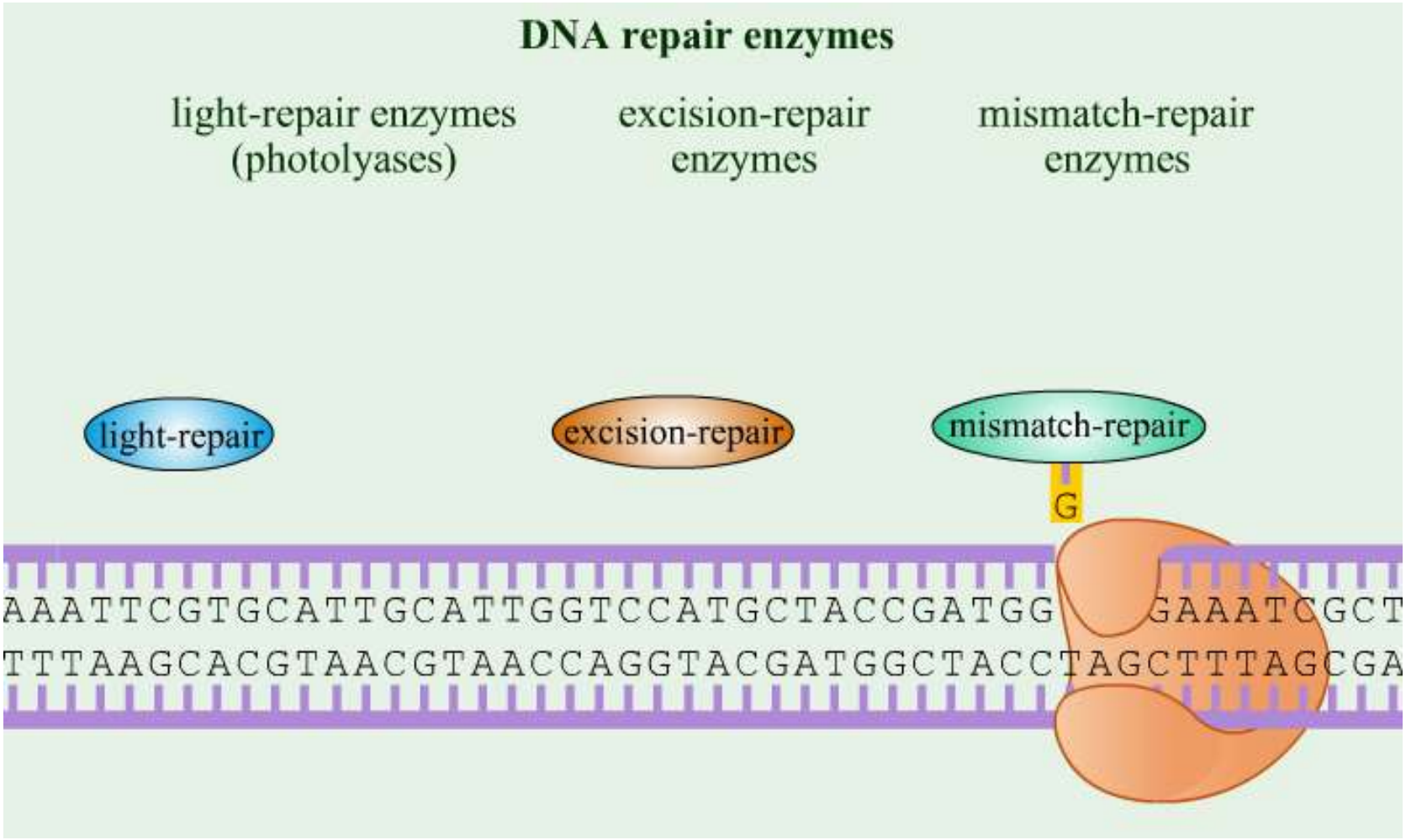


Figure 7.26

Mutations of Genes

- **Frequency of Mutation**
  - Mutations are rare events
    - Otherwise organisms could not effectively reproduce
  - Mutagens increase the mutation rate by a factor of 10 to 1000 times

Mutations of Genes



PLAY Animation: Mutations: Repair

DNA repair mechanisms

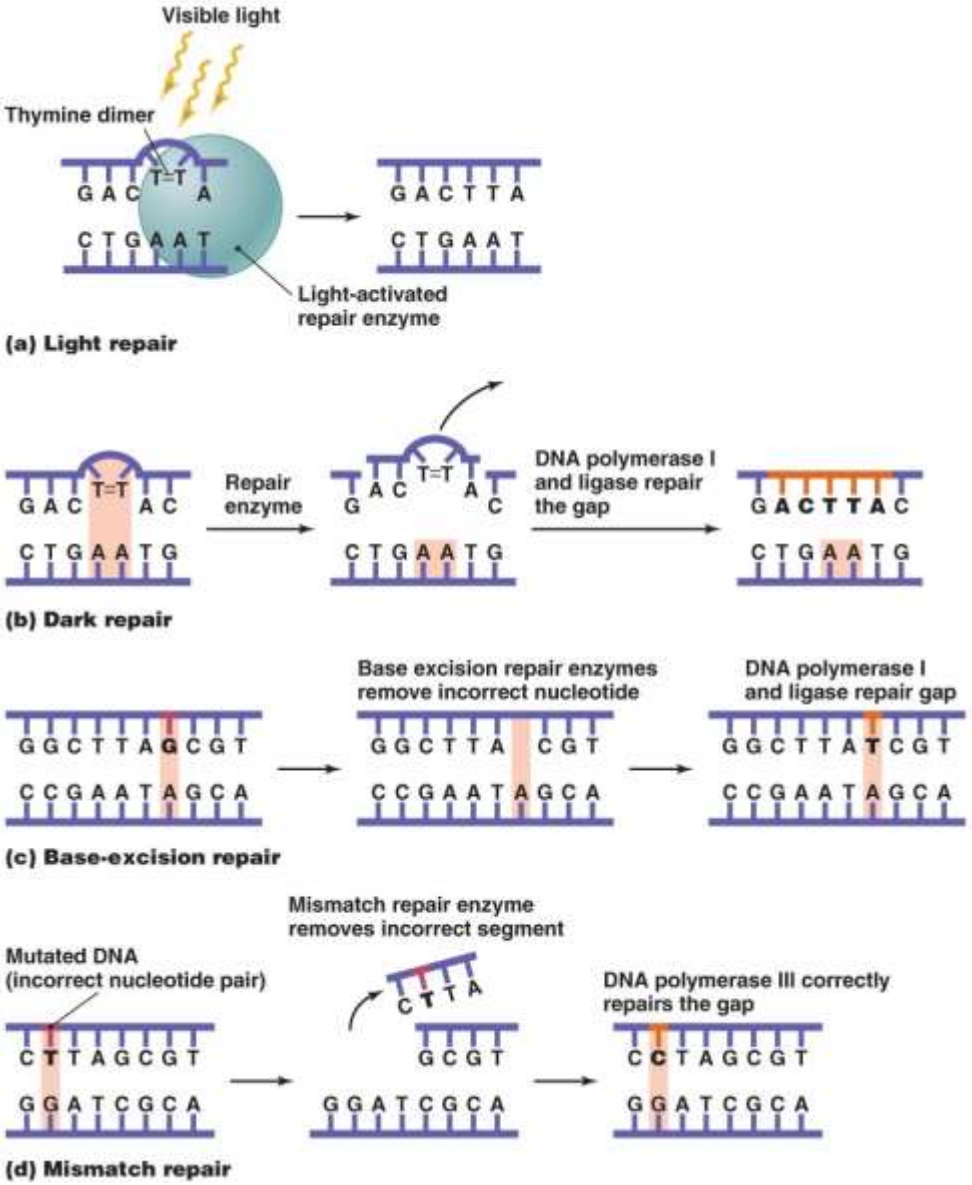


Figure 7.27

Mutations of Genes

- **Identifying Mutants, Mutagens, and Carcinogens**
  - **Methods to recognize mutants**
    1) Positive selection
    2) Negative (indirect) selection
    3) Ames test

Positive selection of mutants

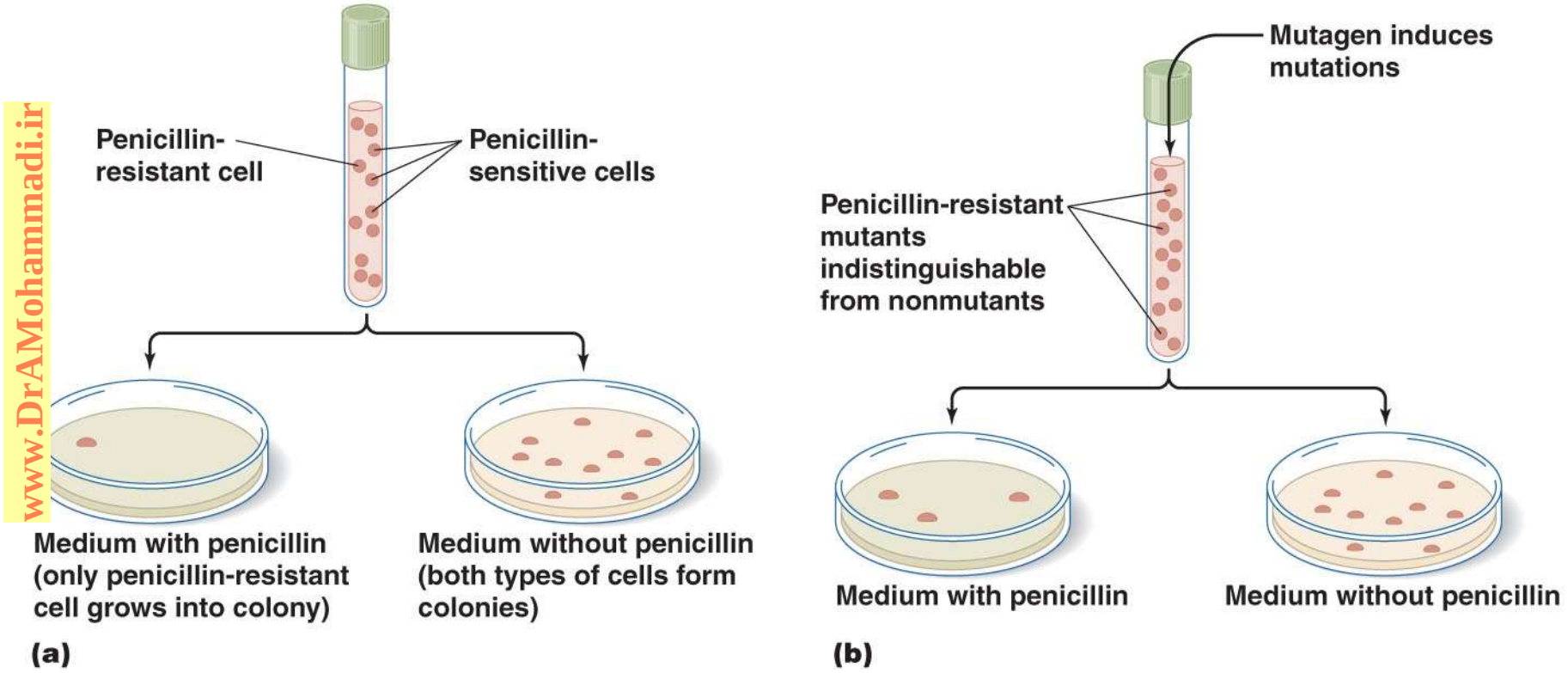


Figure 7.28

The use of negative (indirect) selection

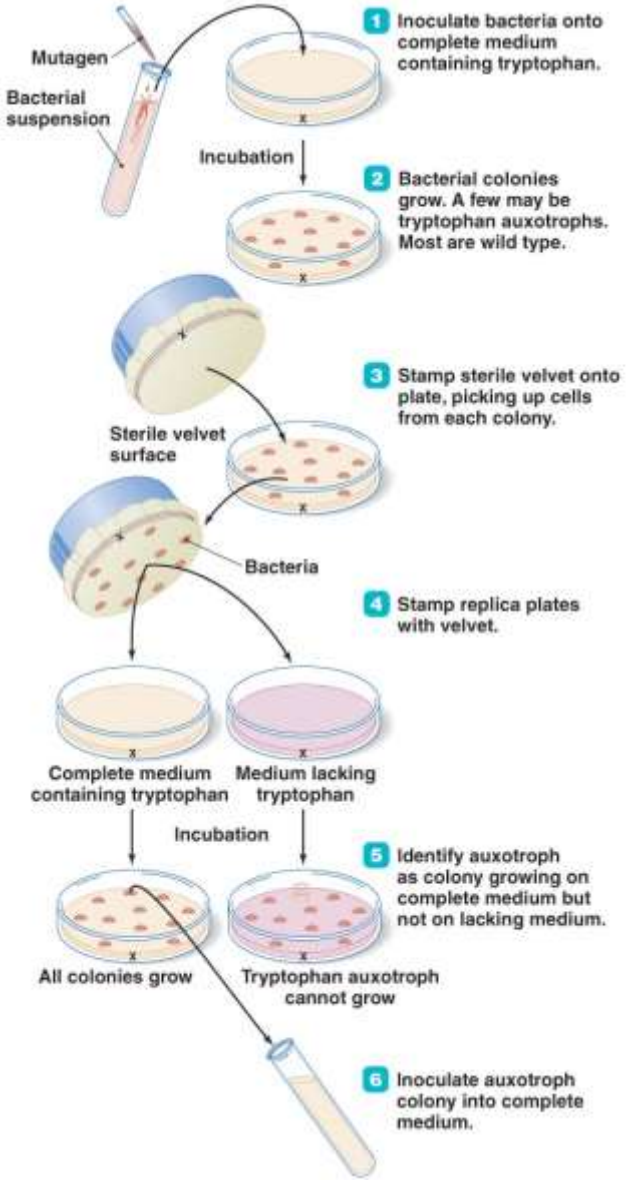


Figure 7.29

The Ames test

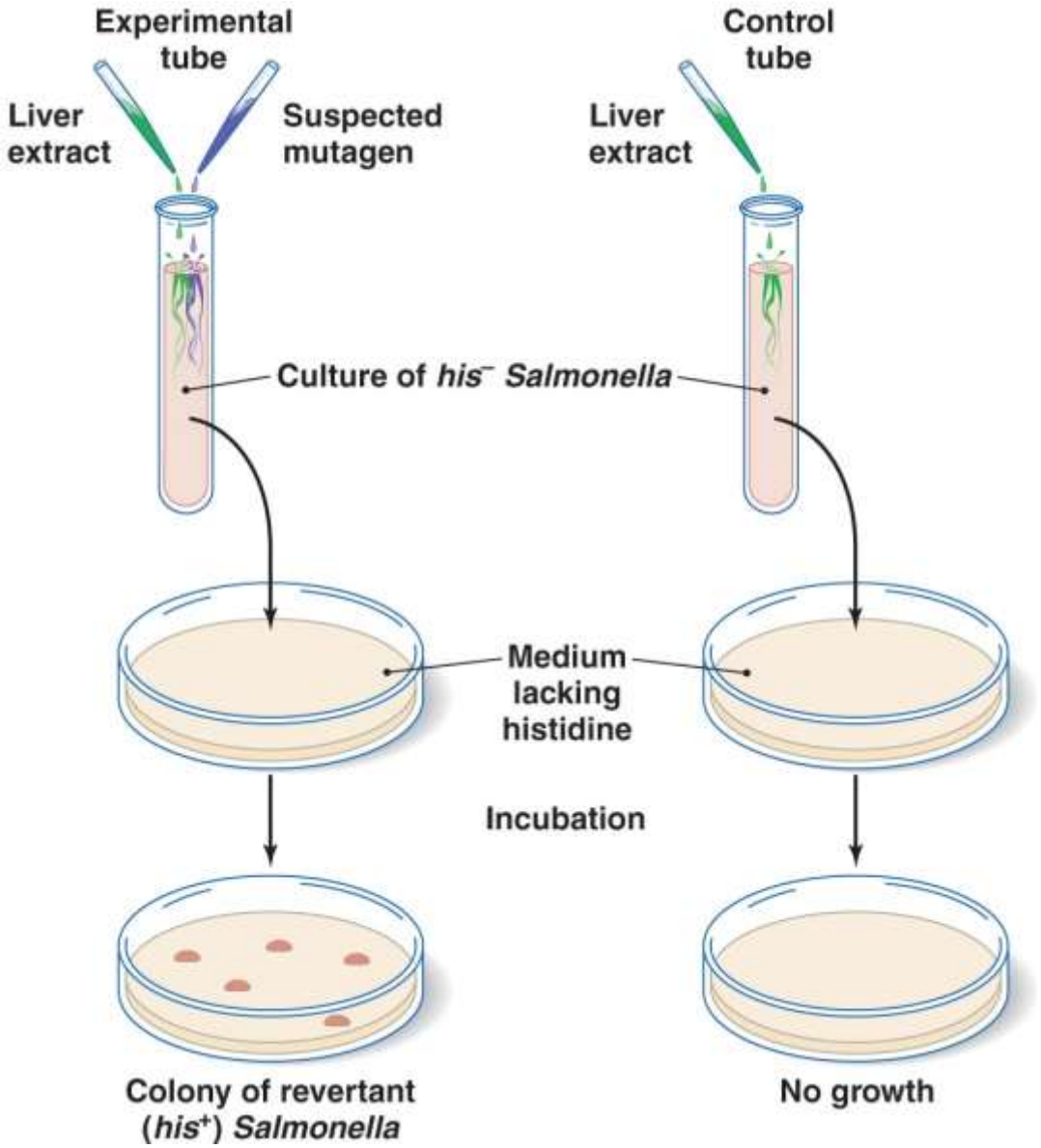


Figure 7.30

Genetic Recombination and Transfer

- Exchange of nucleotide sequences often mediated by homologous sequences
- Recombinants
  - Cells with DNA molecules that contain new nucleotide sequences

Genetic recombination

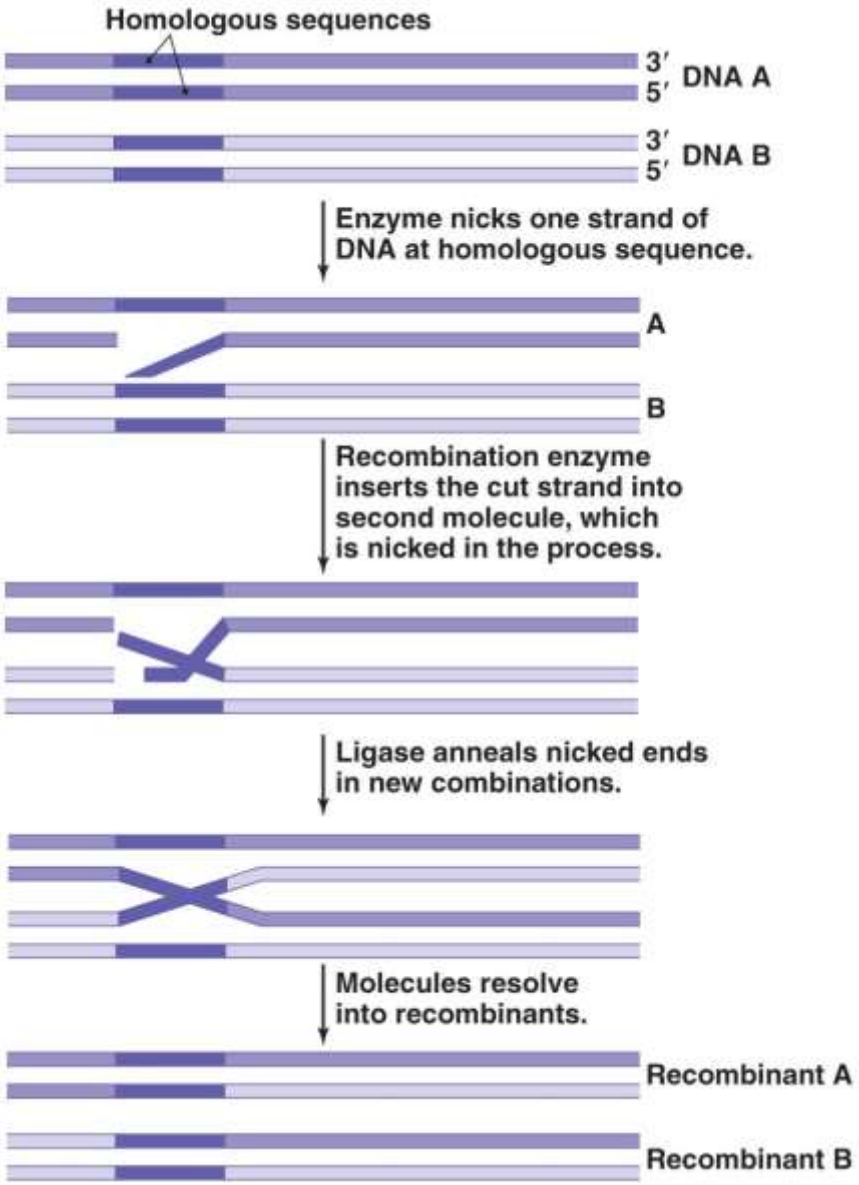


Figure 7.31

Genetic Recombination and Transfer

- **Vertical gene transfer**
  - Organisms replicate their genomes and provide copies to descendants

- **Horizontal Gene Transfer Among Prokaryotes**
  - Horizontal gene transfer
    - Donor cell contributes part of genome to recipient cell
  - Three types
    - Transformation
    - Transduction
    - Bacterial conjugation

Genetic Recombination and Transfer

- **Horizontal Gene Transfer Among Prokaryotes**
  - **Transformation**
    - One of conclusive pieces of proof that DNA is genetic material
    - Cells that take up DNA are **competent**
      - Results from alterations in cell wall and cytoplasmic membrane that allow DNA to enter cell

Transformation of *Streptococcus pneumoniae*

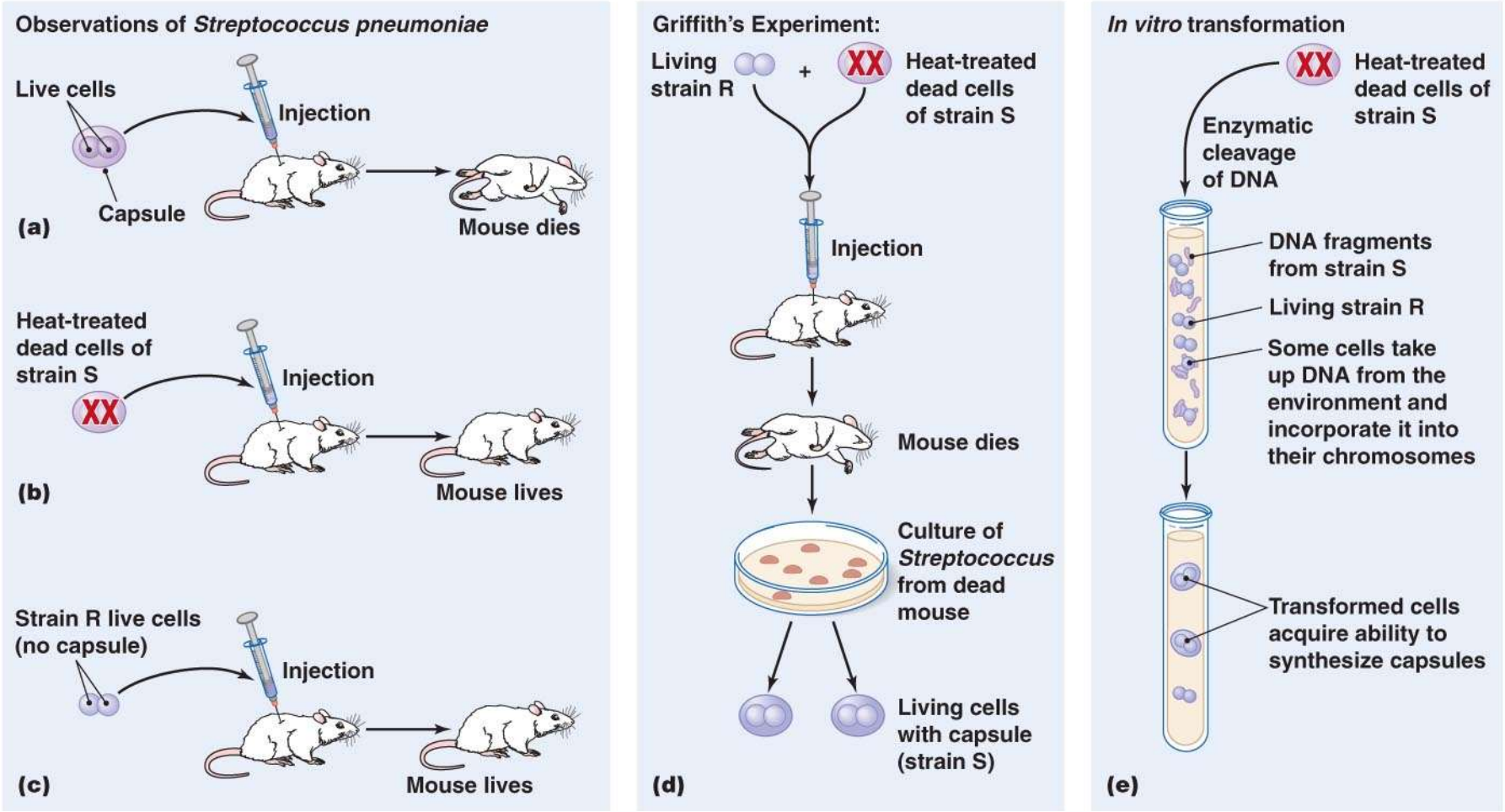


Figure 7.32

Transduction

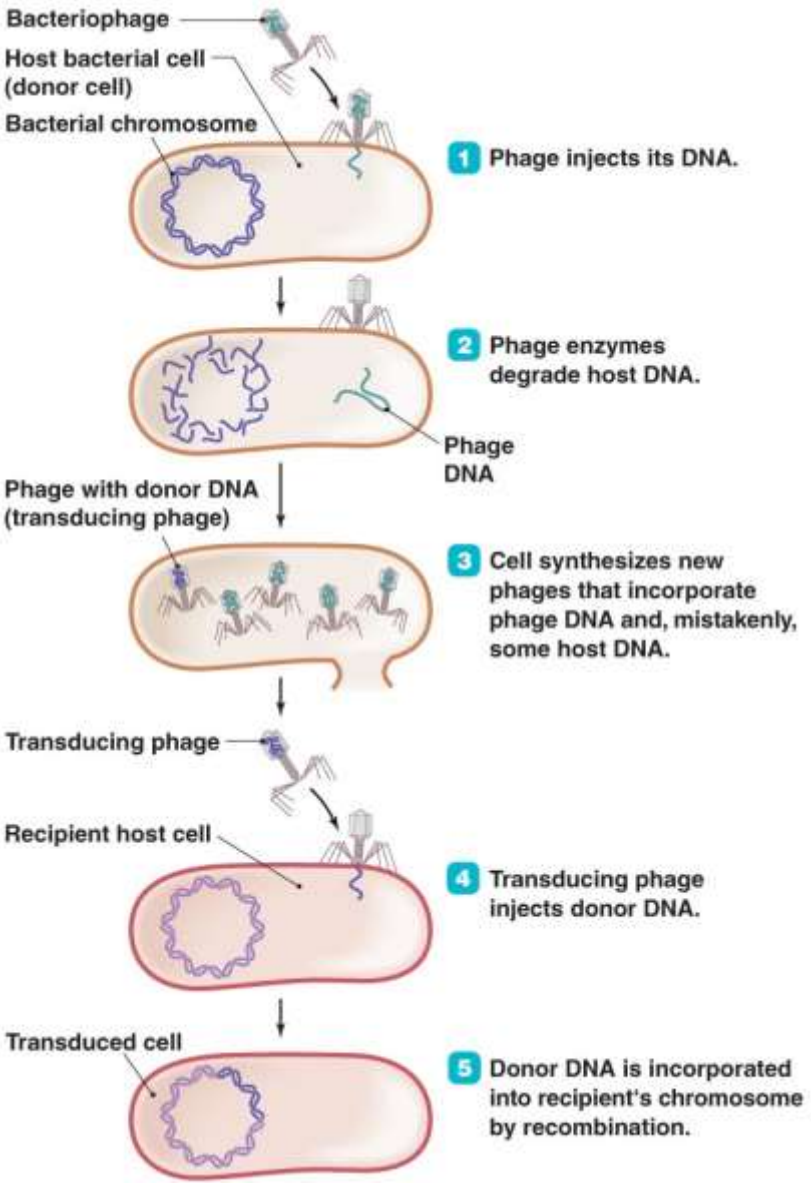
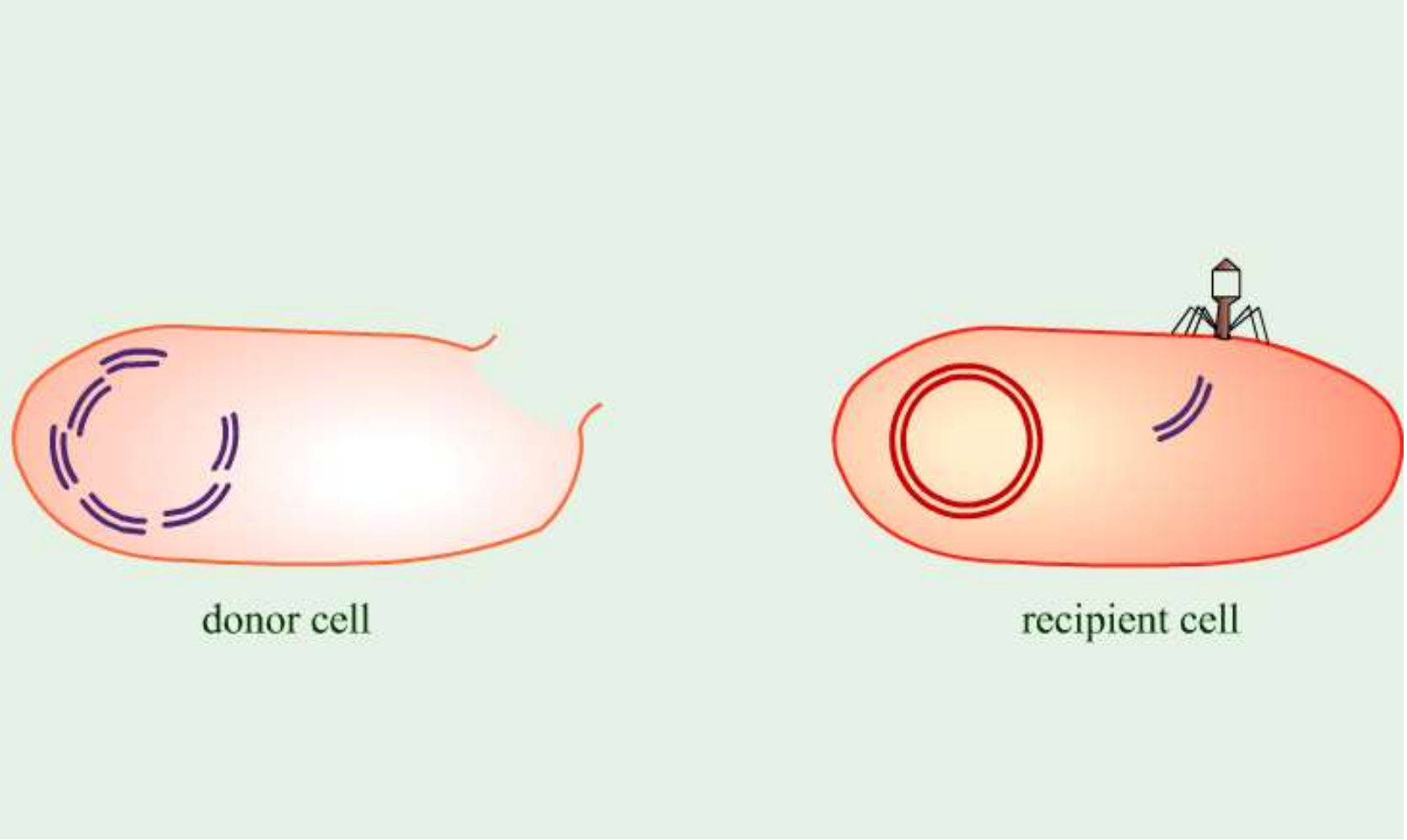


Figure 7.33

Genetic Recombination and Transfer

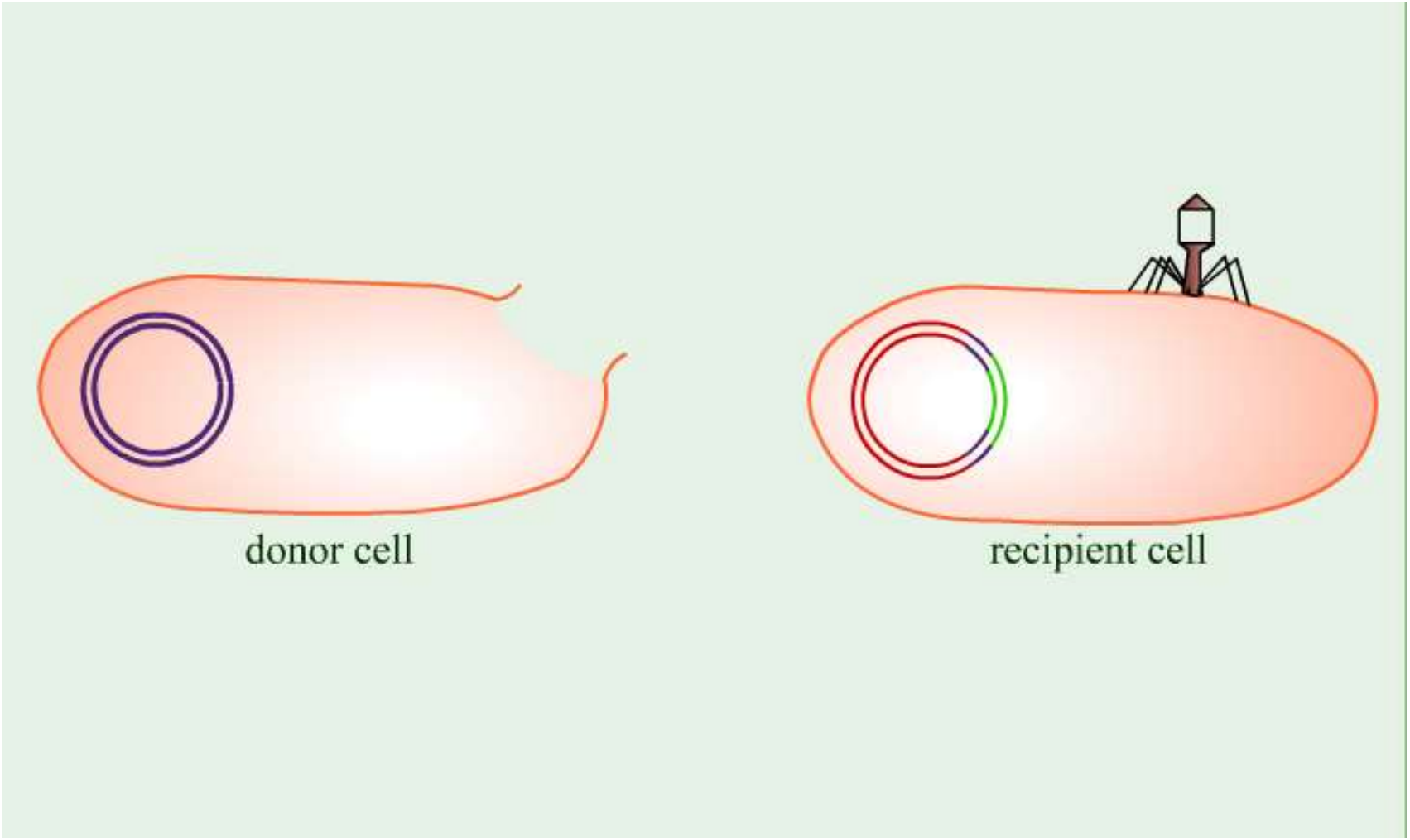
- **Horizontal Gene Transfer Among Prokaryotes**
  - **Transduction**
    - **Generalized transduction**
      - Transducing phage carries random DNA segment from donor to recipient
    - **Specialized transduction**
      - Only certain donor DNA sequences are transferred

Genetic Recombination and Transfer



PLAY Animation: Transduction: Generalized Transduction

Genetic Recombination and Transfer



PLAY Animation: Transduction: Specialized Transduction

Bacterial Conjugation

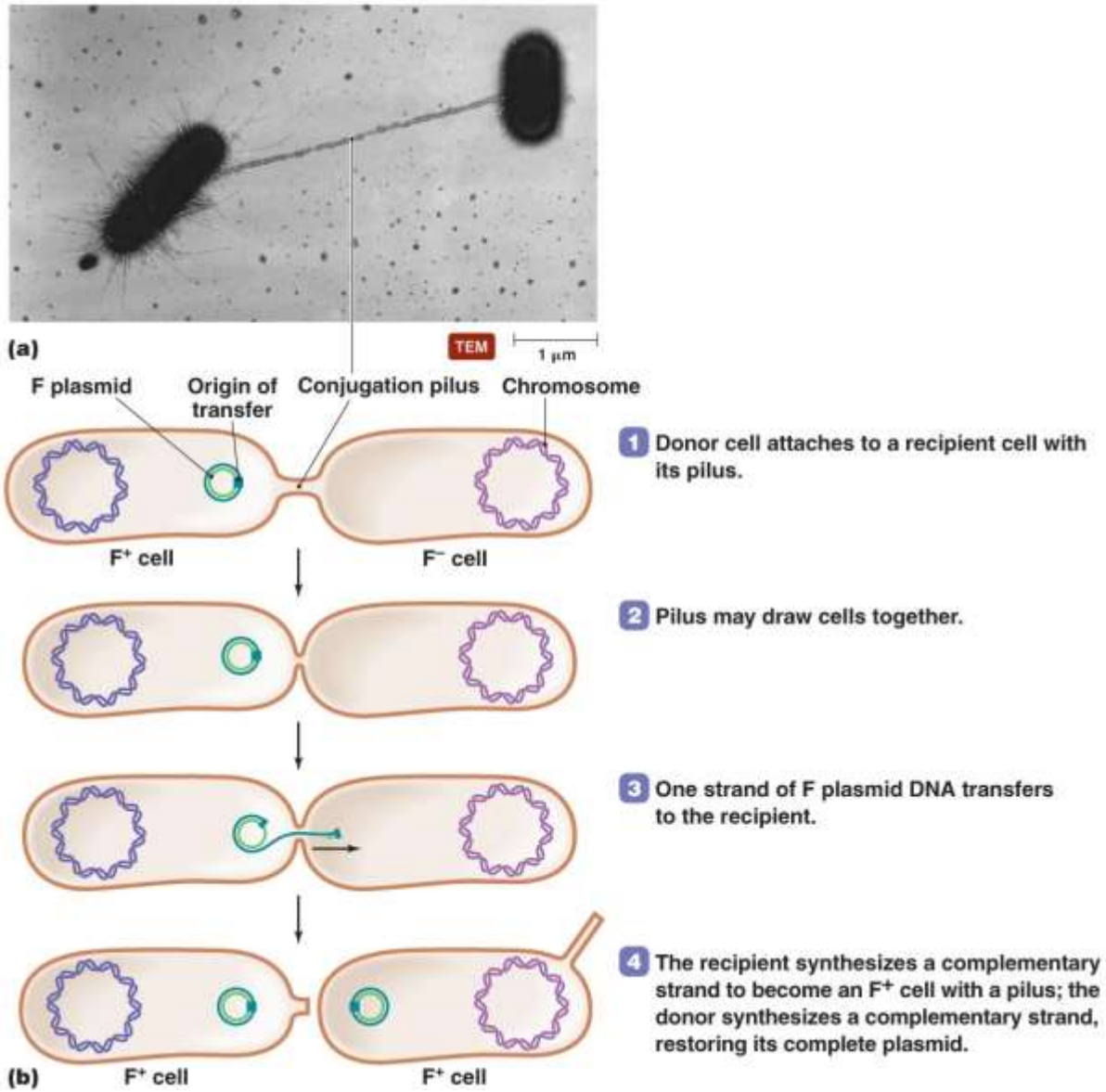
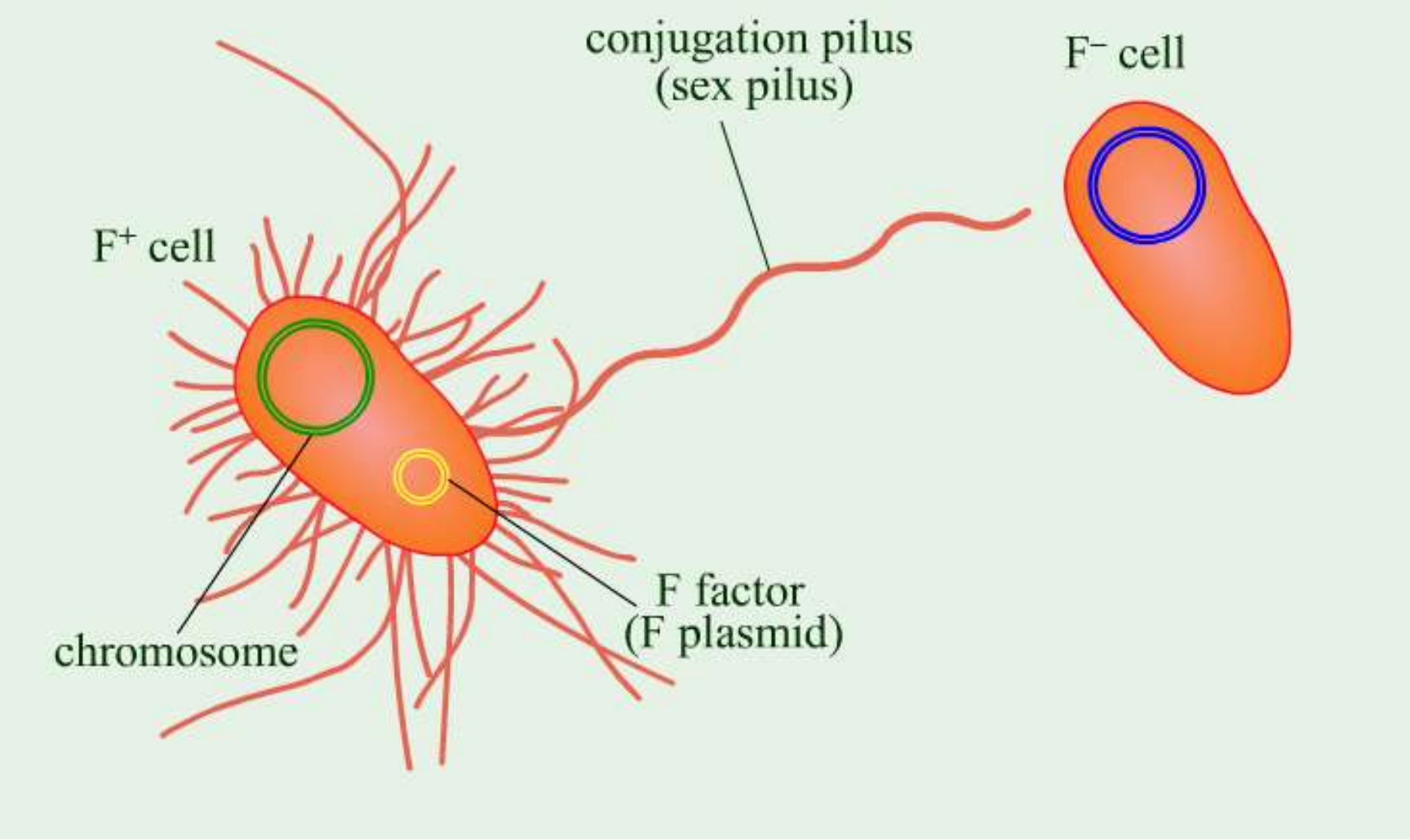


Figure 7.34

Genetic Recombination and Transfer



PLAY Animation: Conjugation: F Factor

Conjugation involving an Hfr cell

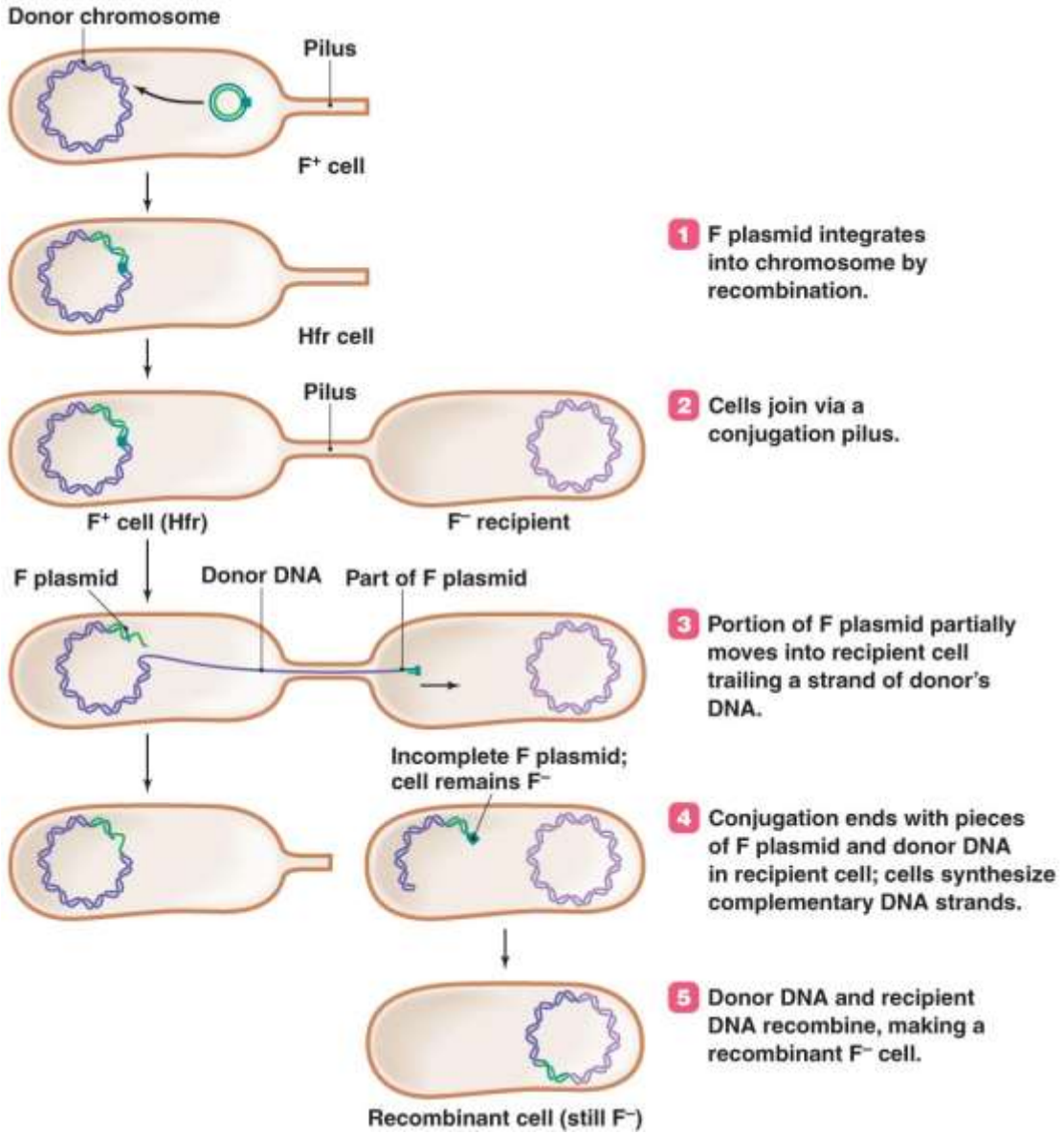
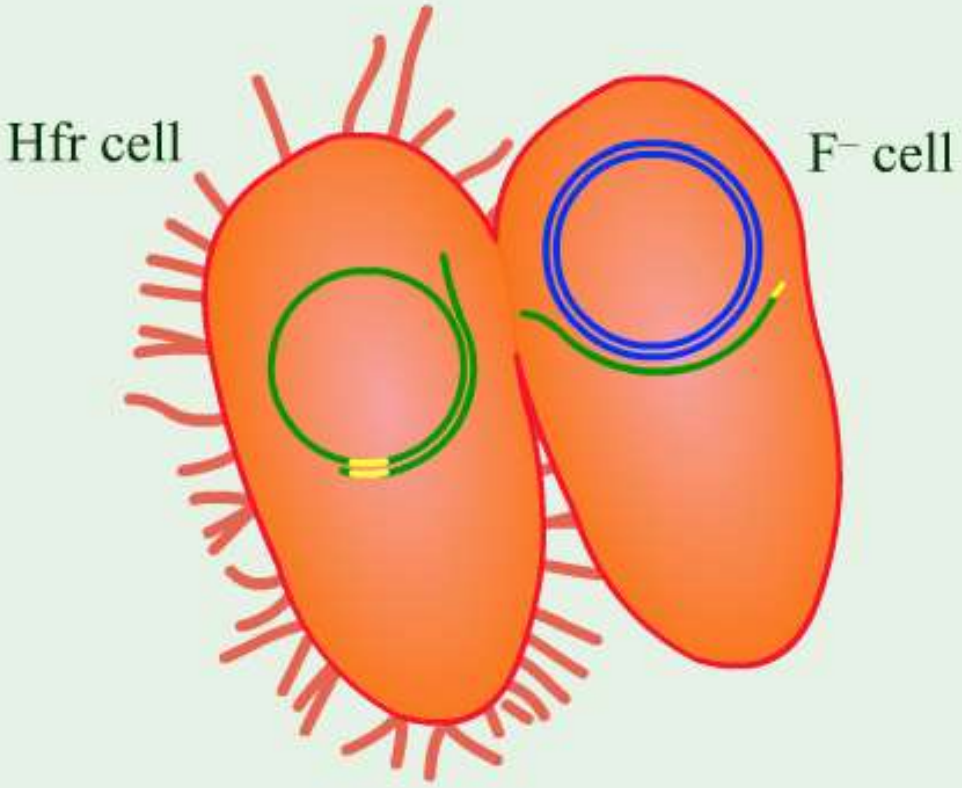


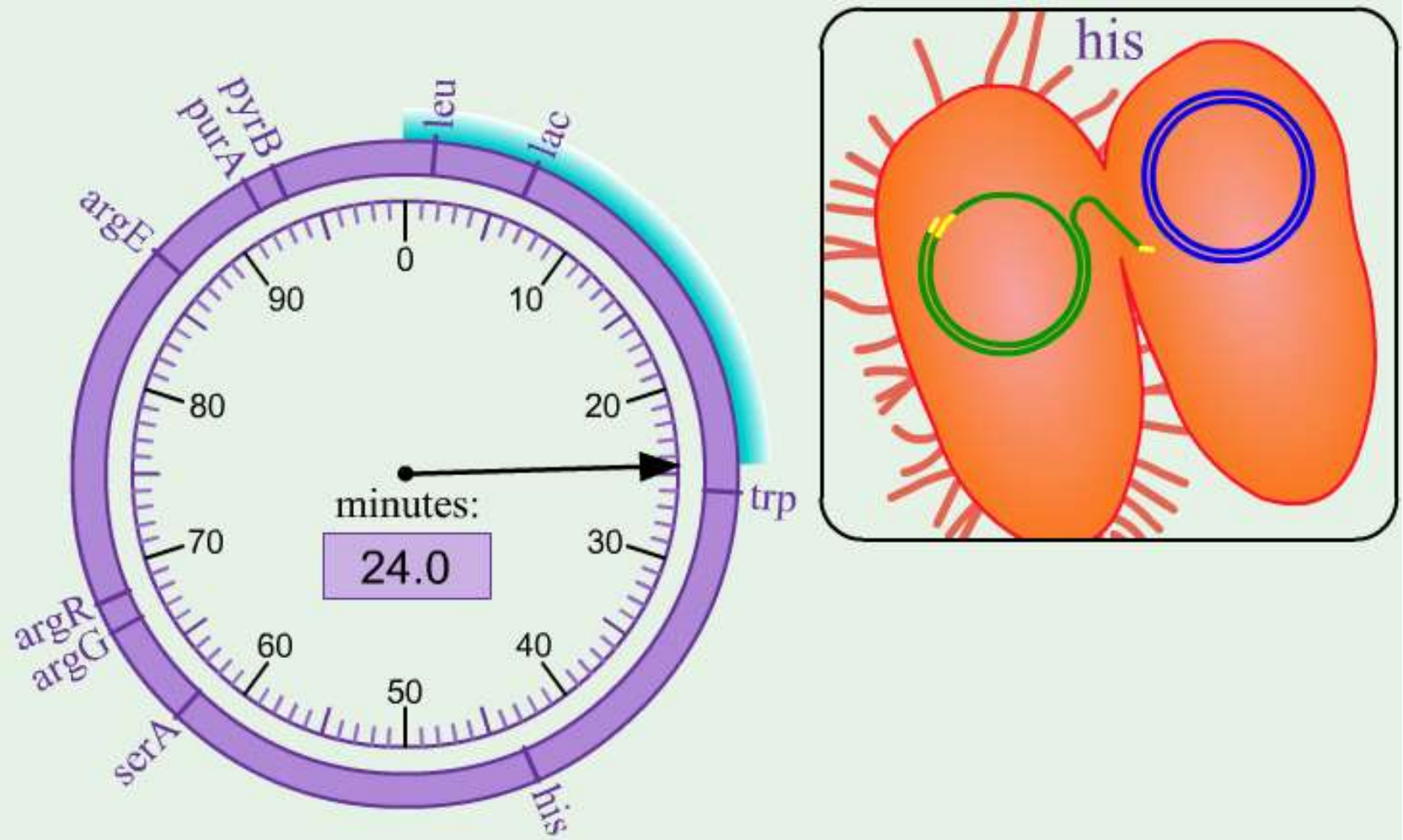
Figure 7.35

Genetic Recombination and Transfer



PLAY Animation: Conjugation: Hfr Conjugation

Genetic Recombination and Transfer

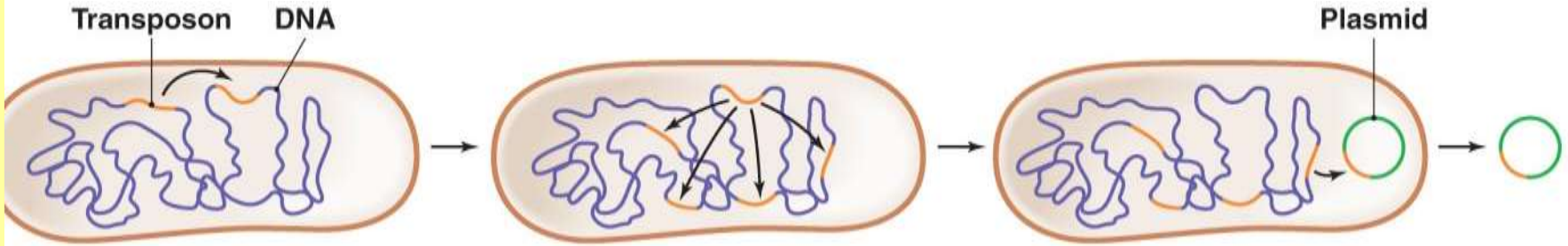


PLAY Animation: Conjugation: Chromosome Mapping

Genetic Recombination and Transfer

- **Transposons and Transposition**
  - Transposons
    - Segments of DNA that move from one location to another in the same or different molecule
  - Result is a kind of frameshift insertion (transpositions)
  - Transposons all contain palindromic sequences at each end

Transposition



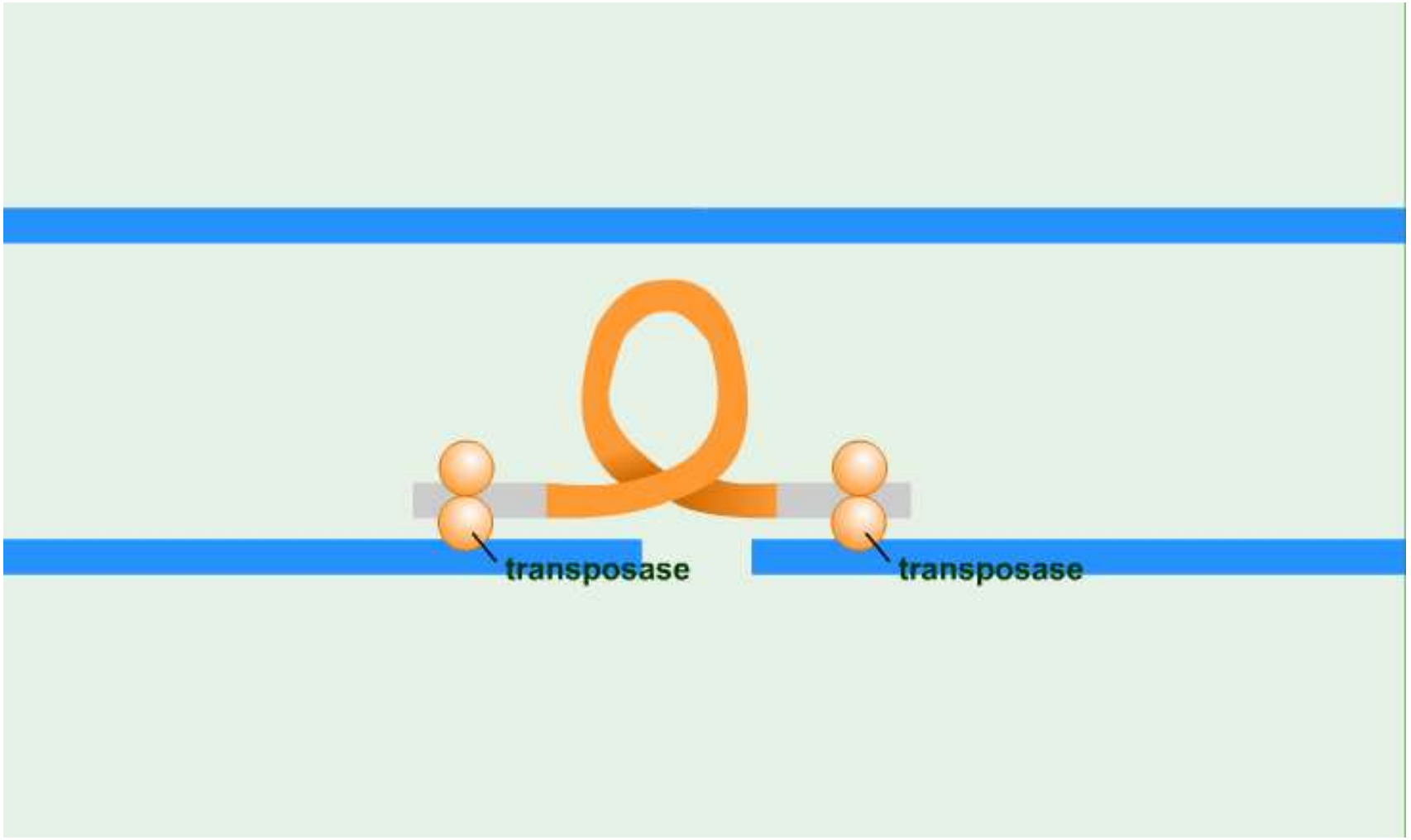
(a) Transposons can jump from one place to another on a DNA molecule.

(b) Transposons may replicate while moving, resulting in a greater number of transposons in the cell.

(c) Transposons can jump to plasmids and be transferred to another cell.

Figure 7.36

Genetic Recombination and Transfer



PLAY Animation: Transposons: Overview

Genetic Recombination and Transfer

- **Transposons and Transposition**
  - Simplest transposons
    - Insertion sequences **(IS)**
    - Have no more than two inverted repeats and a gene for transposase
  - Complex transposons
    - Contain one or more genes not connected with transposition

Transposons

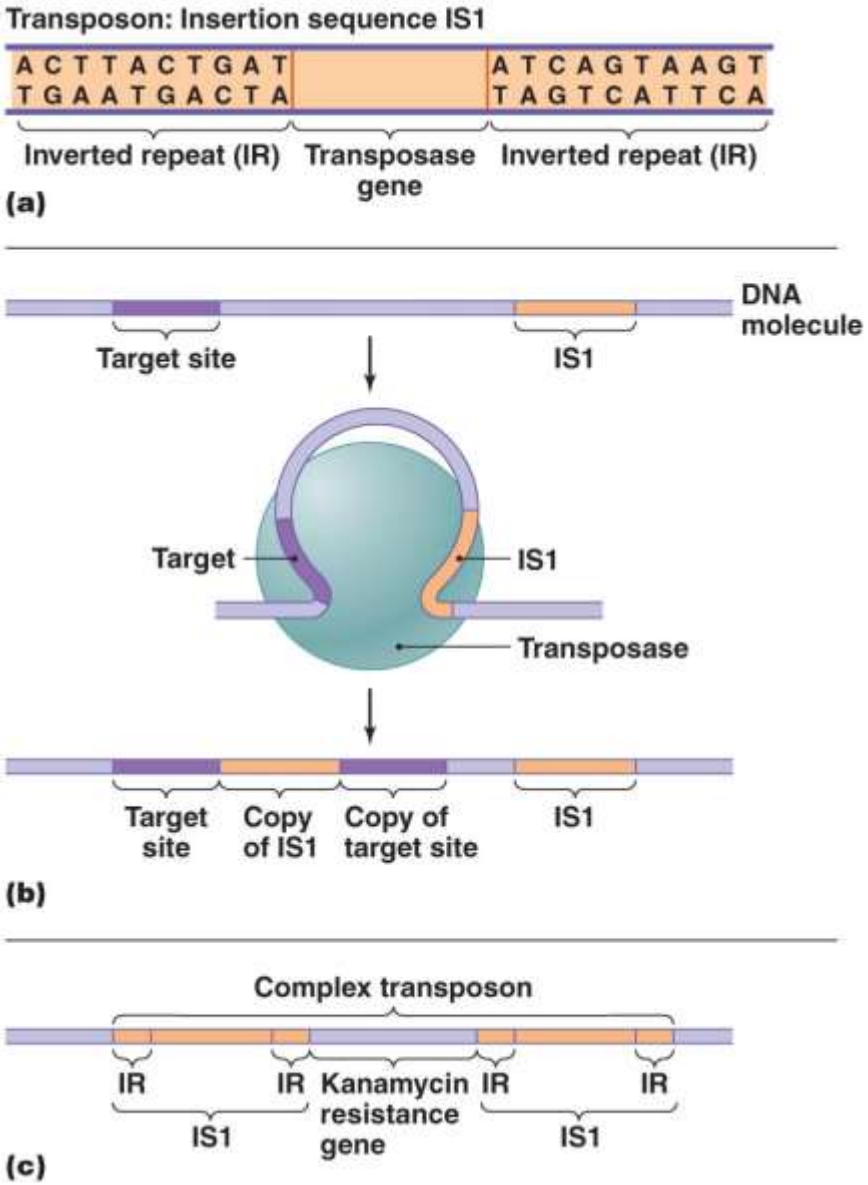
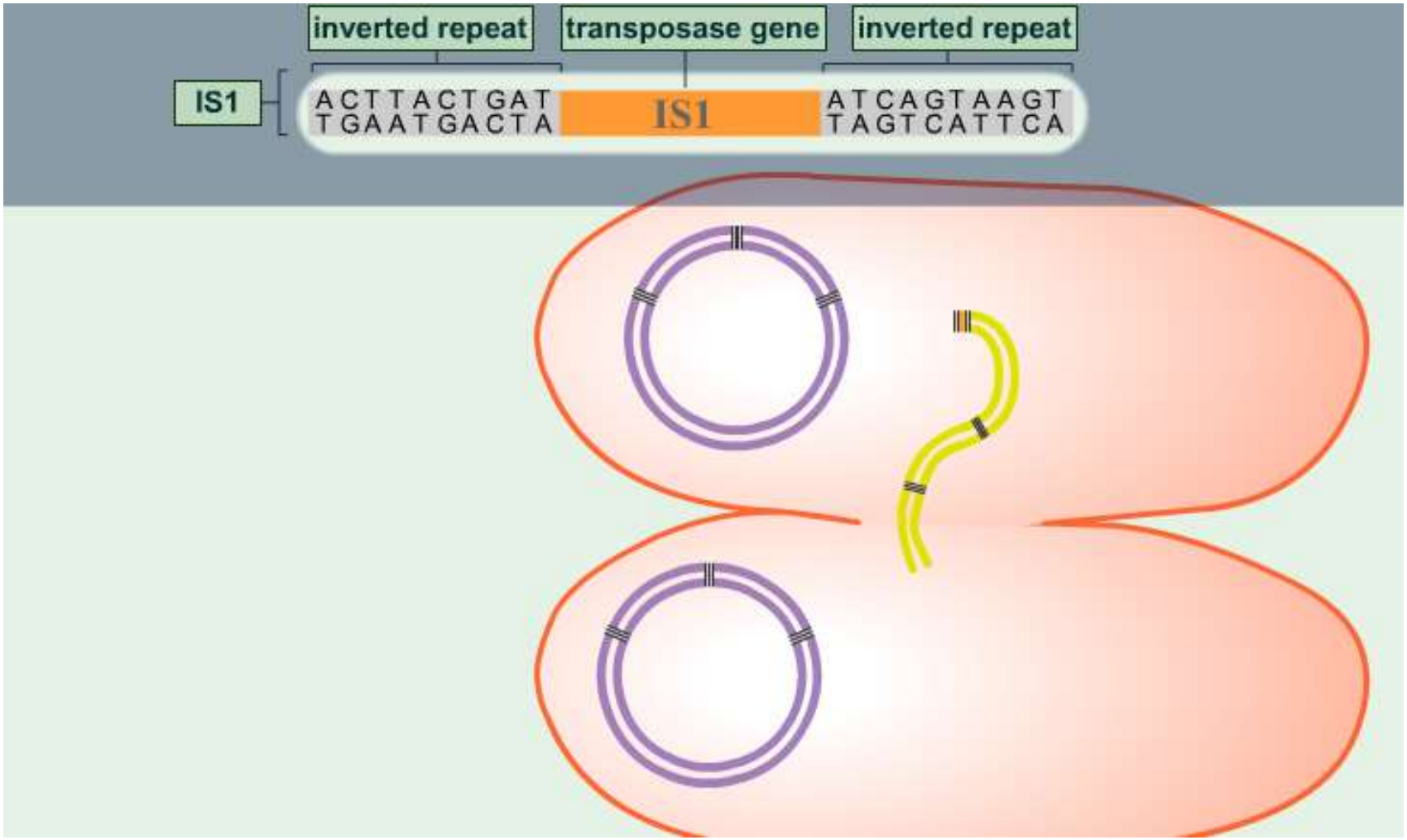


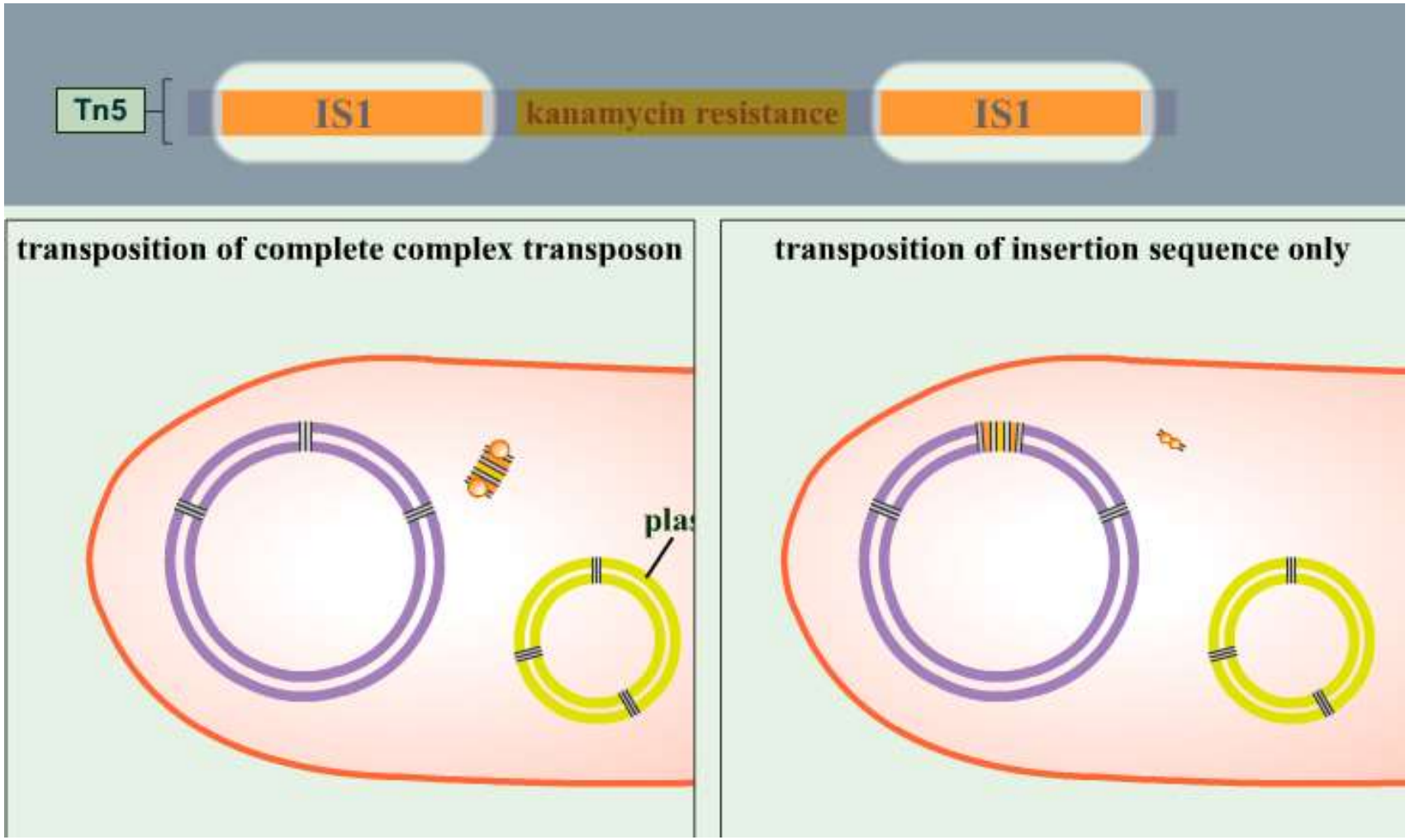
Figure 7.37

Genetic Recombination and Transfer



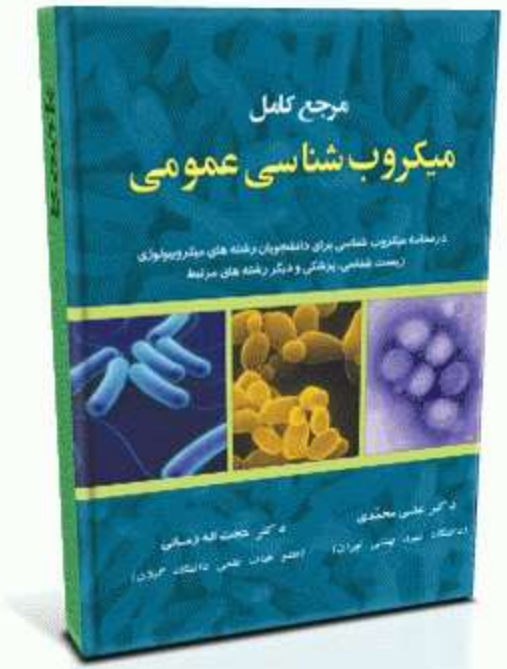
PLAY Animation: Transposons: Insertion Sequences

Genetic Recombination and Transfer



PLAY Animation: Transposons: Complex Transposons

منبع:



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