Game Plan

Lecture

Biofilms
Review of basic genetics
Bacterial gene structure
Gene regulation
Mutations

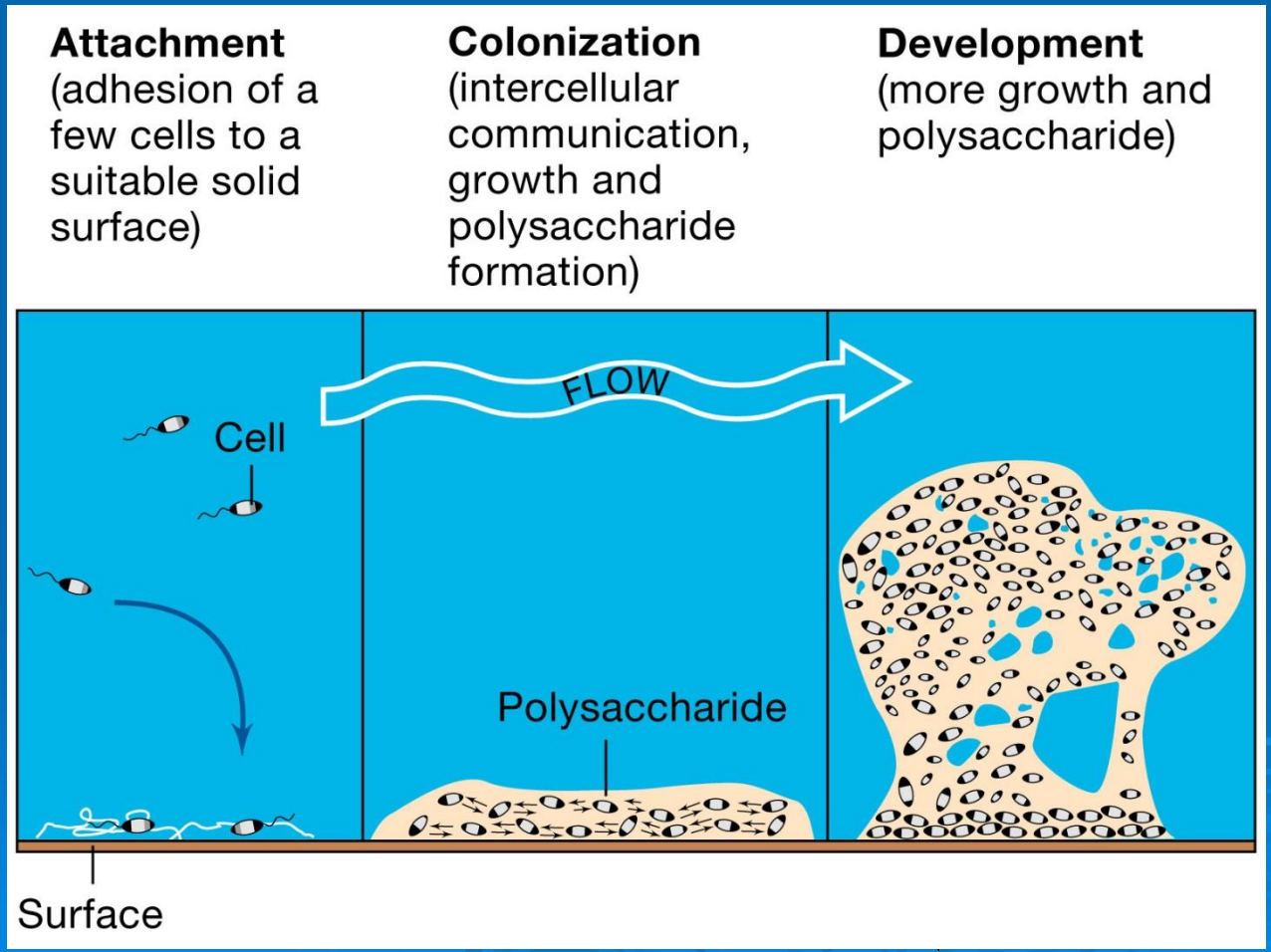
Lab

Growth Curve

Pre-labs
Effects on growth: temp, pH, O2

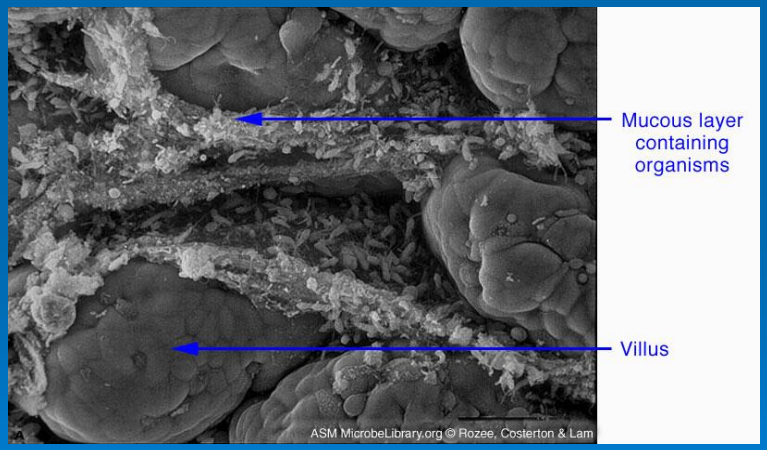
What is a biofilm

Biofilm = an organized system of layers of microbial cells embedded in a polysaccharide matrix associated with surfaces

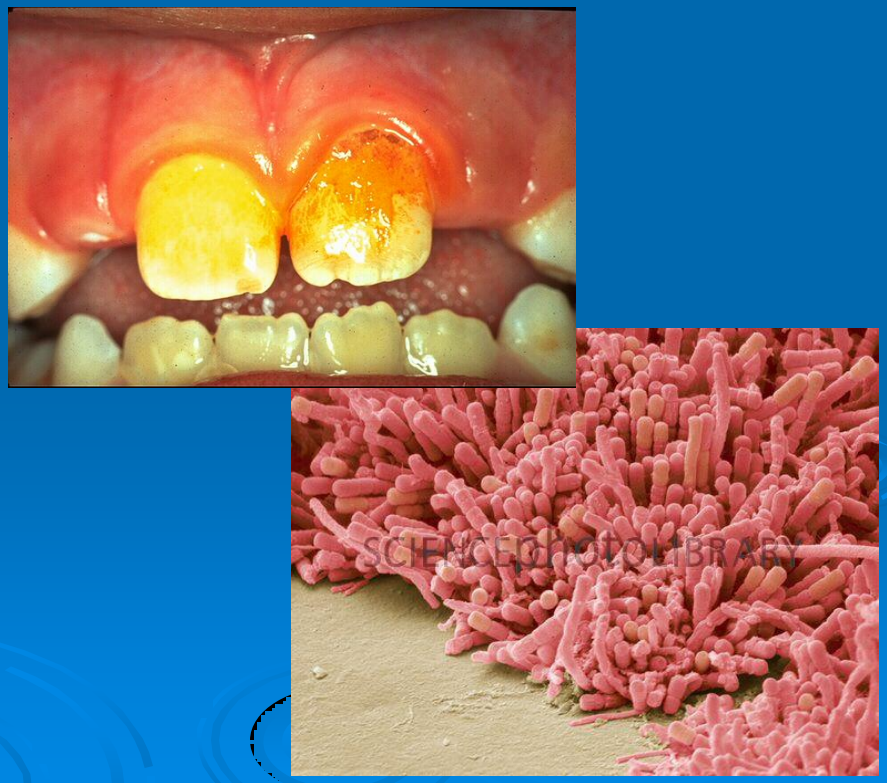


Where do we find biofilms?

- **Humans/medical equipment** - on catheters, teeth, middle ear infections, GI + GU tracts, lungs (cystic fibrosis), biomedical products, implanted devices



Intestinal biofilm



Dental biofilm

Where do we find biofilms?

- **Humans/medical equipment** - on catheters, teeth, middle ear infections, GI, GU tract, lungs (cystic fibrosis), biomedical products, implanted devices
- **Aquatic environments** - on algae, rocks, ships

Aquatic biofilm

Where do we find biofilms?

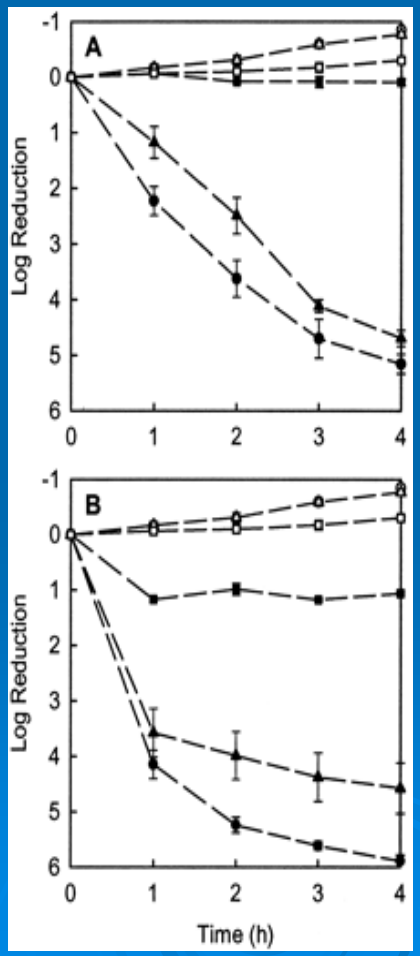
- **Humans/medical equipment** - on catheters, teeth, middle ear infections, GI, GU tract, lungs (cystic fibrosis), biomedical products, implanted devices
- **Aquatic environments** - on algae, rocks, ships
- **Industry** - pipes, air conditioning vents, plastics

(b) A bacterial biofilm that formed on a steel surface over two months in an industrial water system. The large oval bodies are diatoms that became entrapped in the sticky biofilm.

Industrial biofilm

Why form a biofilm?

1. Protection from antibiotics, toxins, and immune cells



Effect of tobramycin (A) and ciprofloxacin (B) on survivial of:

planktonic cells (circle)
resuspended biofilm cells (triangle)
biofilm colony cells (squares)

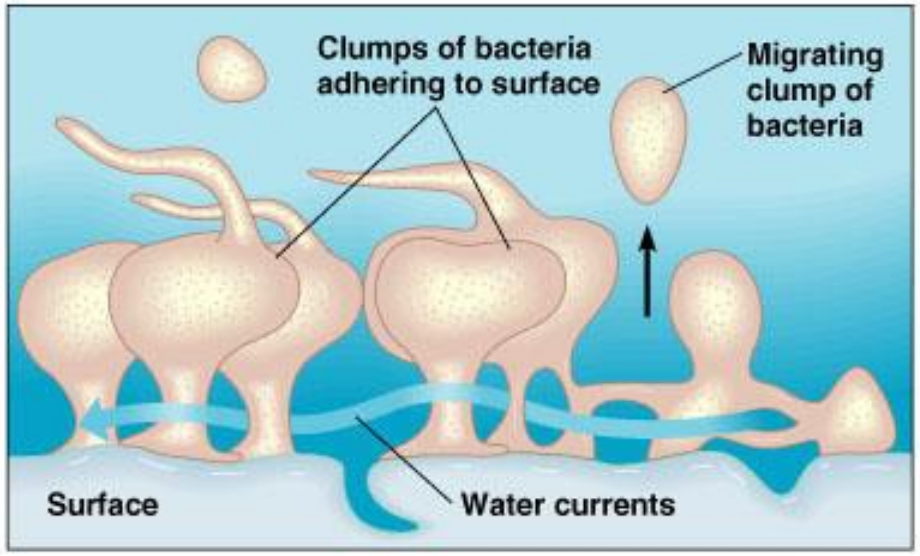
Open symbols are untreated

Why form a biofilm?

1. Protection from antibiotics, toxins, and immune cells

2. Favorable microenvironment

- Source of nutrients
- Highly hydrated
- Low oxygen



(a) Water currents move, as shown by the blue arrow, among pillars of slime formed by the growth of bacteria attached to solid surfaces. This allows efficient access to nutrients and removal of bacterial waste products. Individual slime-forming bacteria or bacteria in clumps of slime detach and move to new locations.

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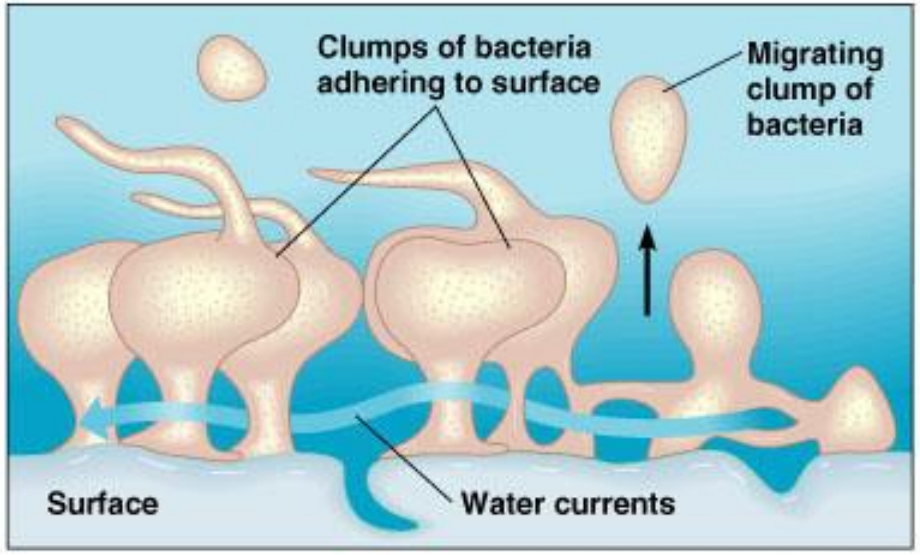
Why form a biofilm?

1. Protection from antibiotics, toxins, and immune cells

2. Favorable microenvironment

- Source of nutrients
- Highly hydrated
- Low oxygen

3. Stability



(a) Water currents move, as shown by the blue arrow, among pillars of slime formed by the growth of bacteria attached to solid surfaces. This allows efficient access to nutrients and removal of bacterial waste products. Individual slime-forming bacteria or bacteria in clumps of slime detach and move to new locations.

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Figure 8.1a

Why form a biofilm?

1. Protection from antibiotics, toxins, and immune cells

2. Favorable microenvironment

- Source of nutrients
- Highly hydrated
- Low oxygen

3. Stability

4. Community

- Gene transfer, signal transduction and quorum sensing

Figure 8.1a

Genetics terminology

Genetics- the study of genes

Genes- a segment of DNA that codes a functional production (protein)

Genome- all of the genetic material in a cell

Genomics- molecular study of genomes

Genotype- genes of an organism

Phenotype- physical expression of the genes

Review of DNA processing

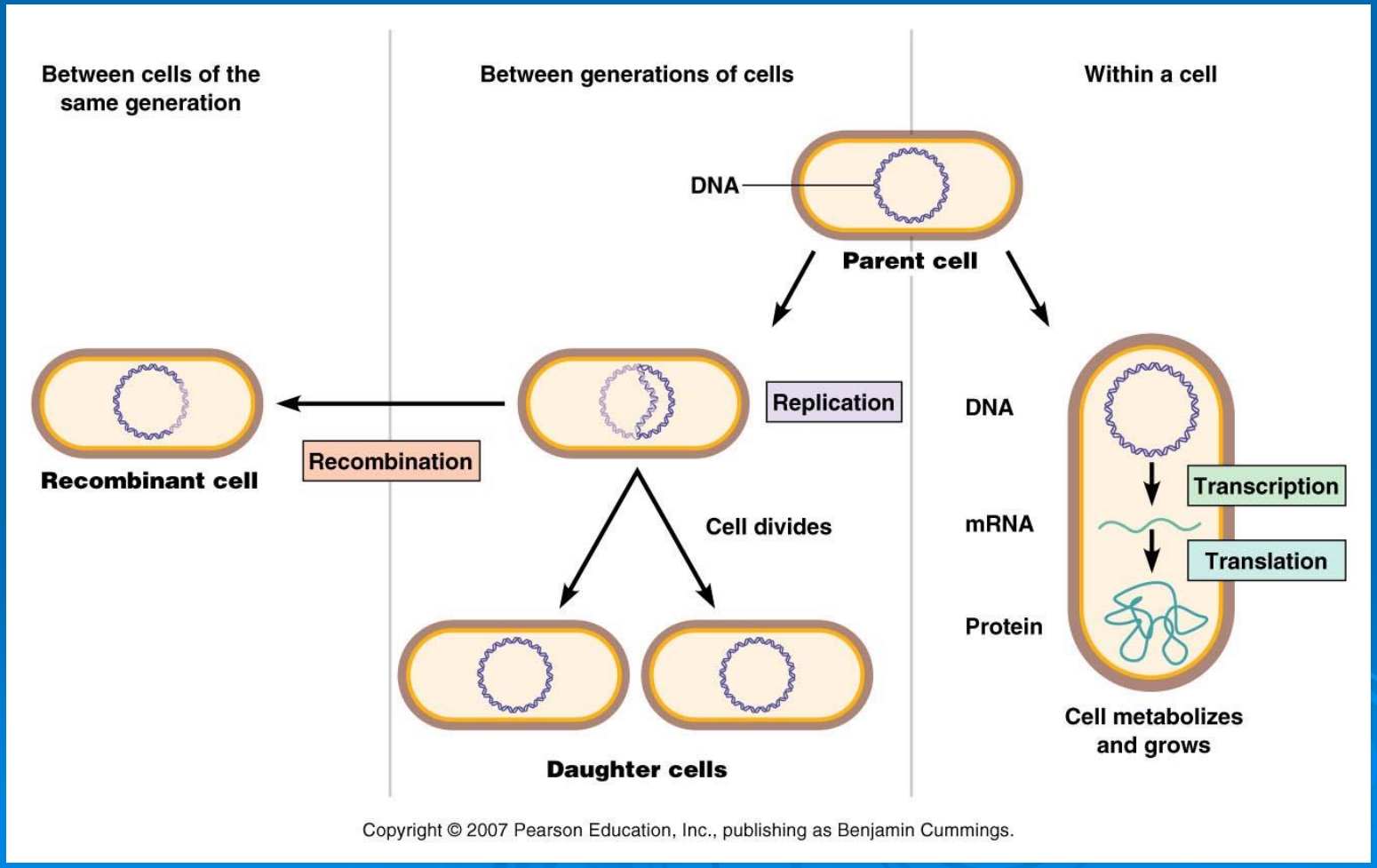

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Figure 8.2

DNA structure

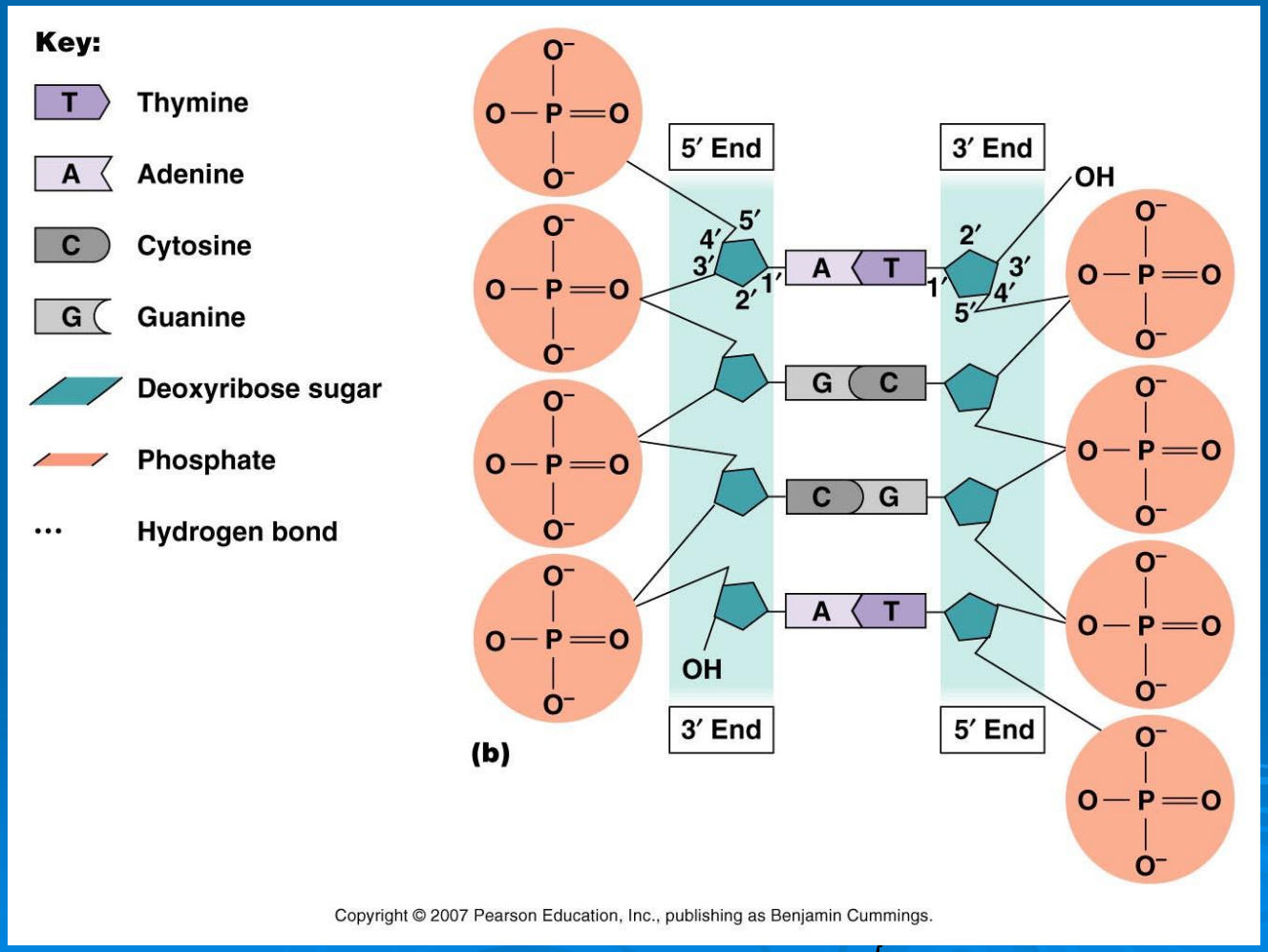

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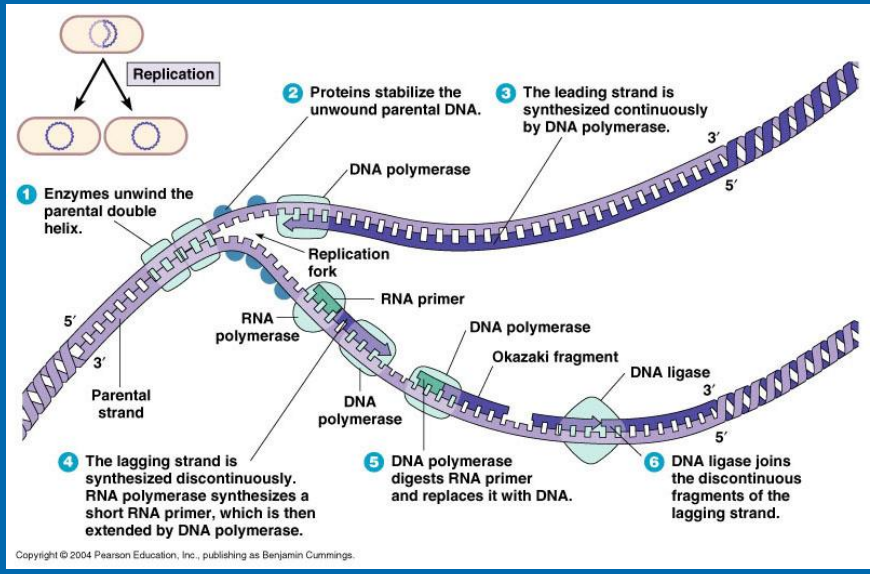
Figure 8.3b

DNA replication

-**Enzymes:** DNA polymerase, DNA ligase primase to make RNA primers, accessory enzymes (topoisomerase, gyrase, helicase)

-Antiparallel 5’ to 3’ synthesis results in leading and lagging strands

-Semi-conservative replication



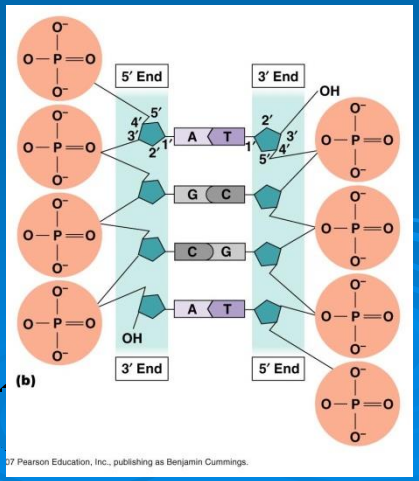


Figure 8.5

Bacterial DNA replication

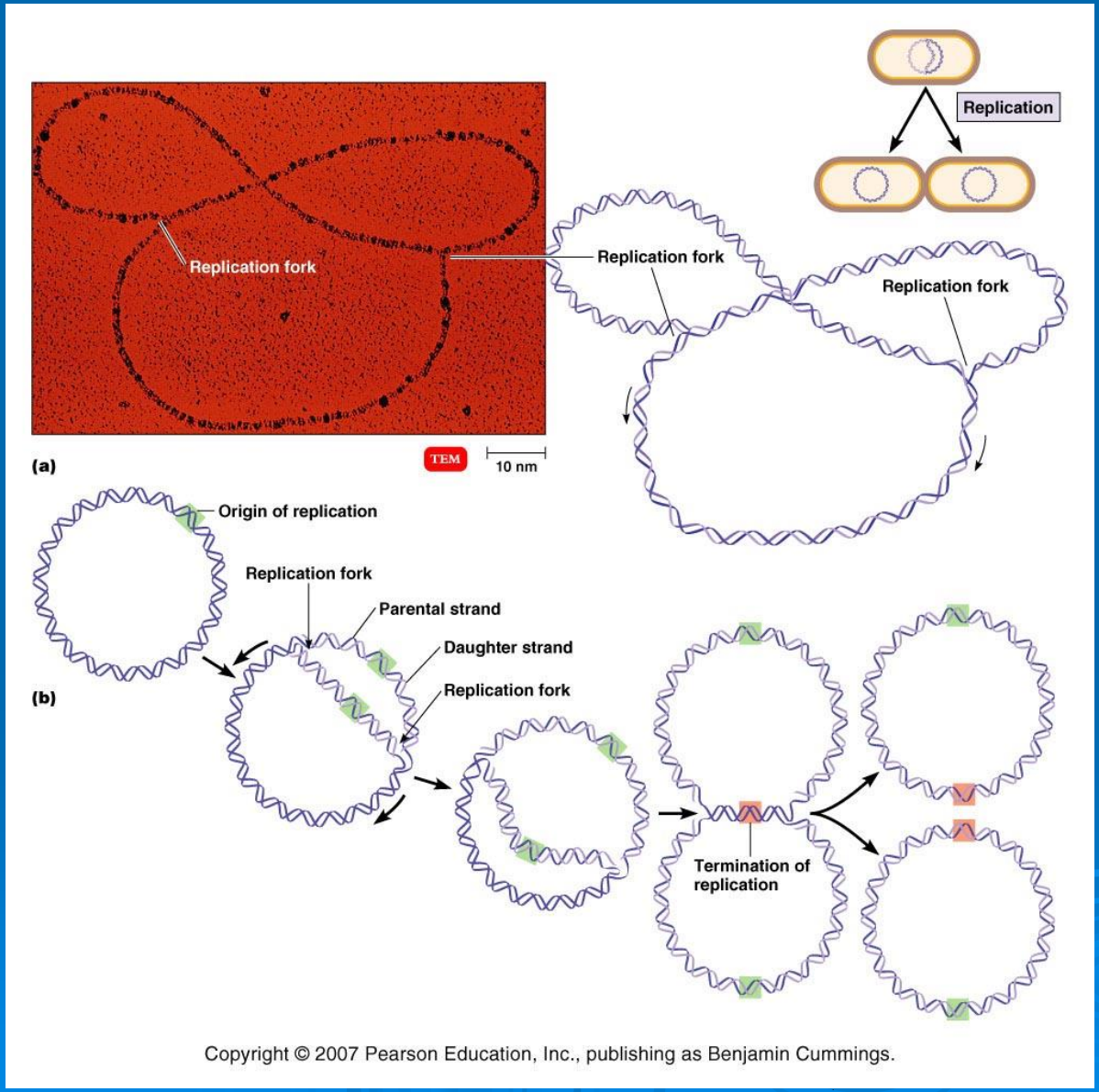


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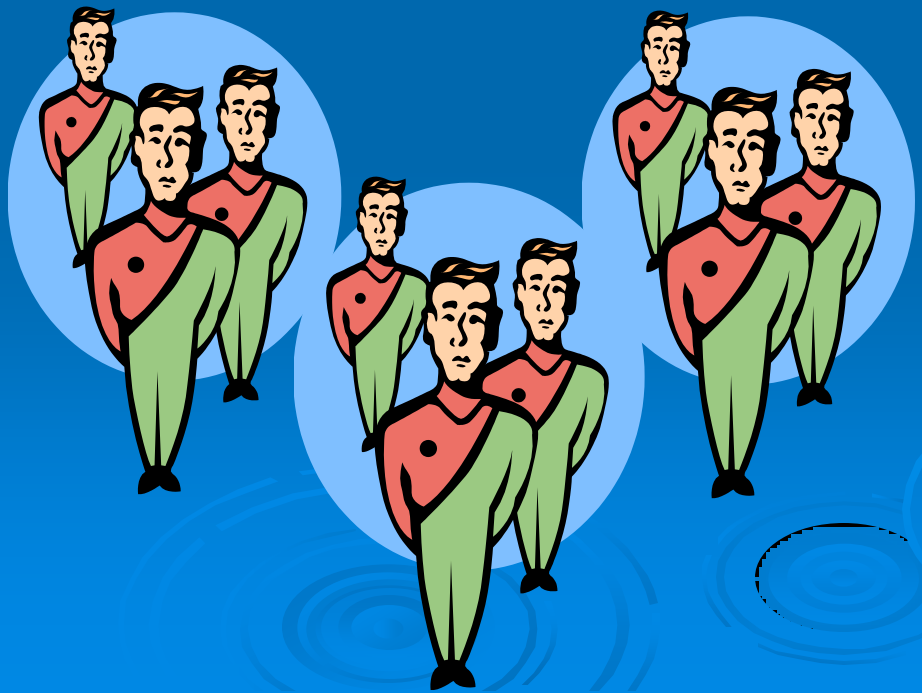
Figure 8.6 - Overview

Pit stop

The *E. coli* genome replicates every 45 minutes, but divides every 26 minutes in ideal conditions. How do you reconcile these two facts?



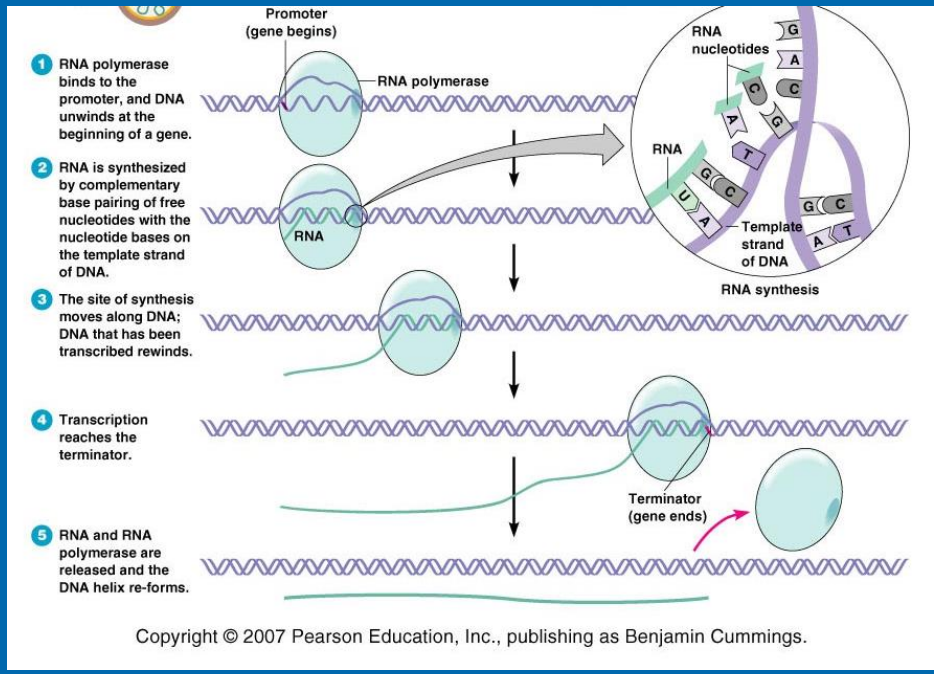
RNA transcription

-**Enzymes:** RNA polymerase

-Promoters and terminators start and end process

-5’ to 3’ synthesis

-Types: tRNA, mRNA, rRNA



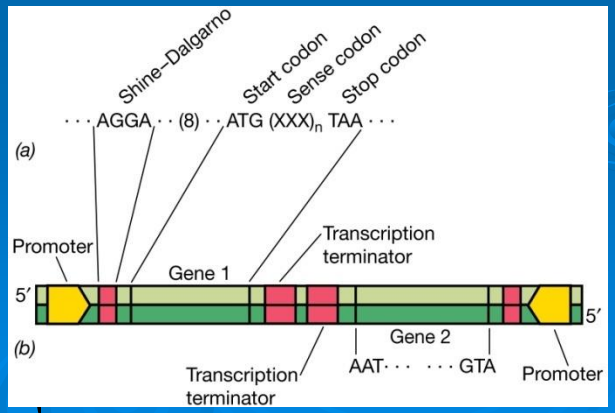


Figure 8.7

Reminder: RNA processing differences in eukaryotes

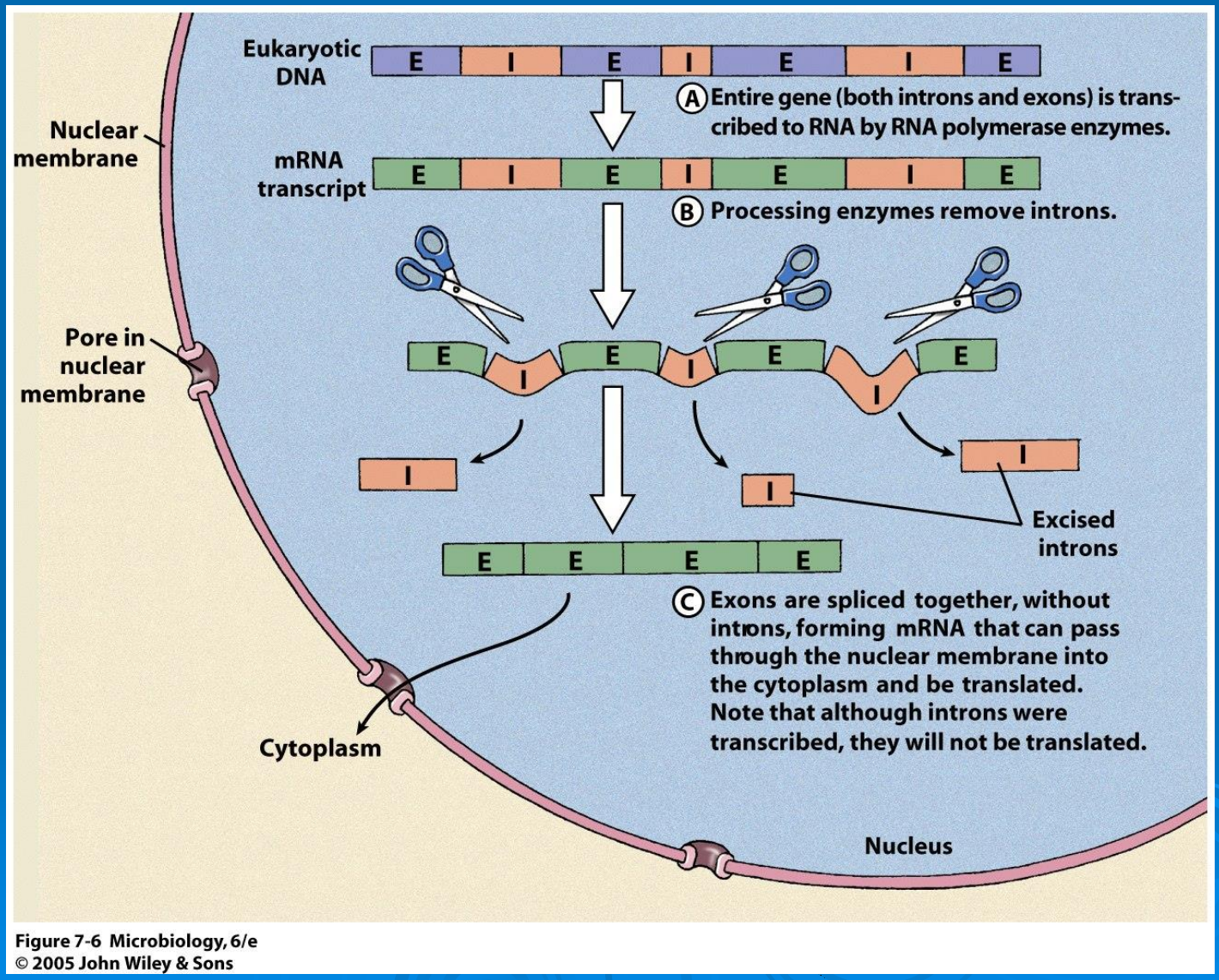


Figure 7-6 Microbiology, 6/e
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Translation and the Genetic Code

-**Players:** ribosomes (proteins + rRNA), tRNA, and mRNA

-Codons

-Anticodons

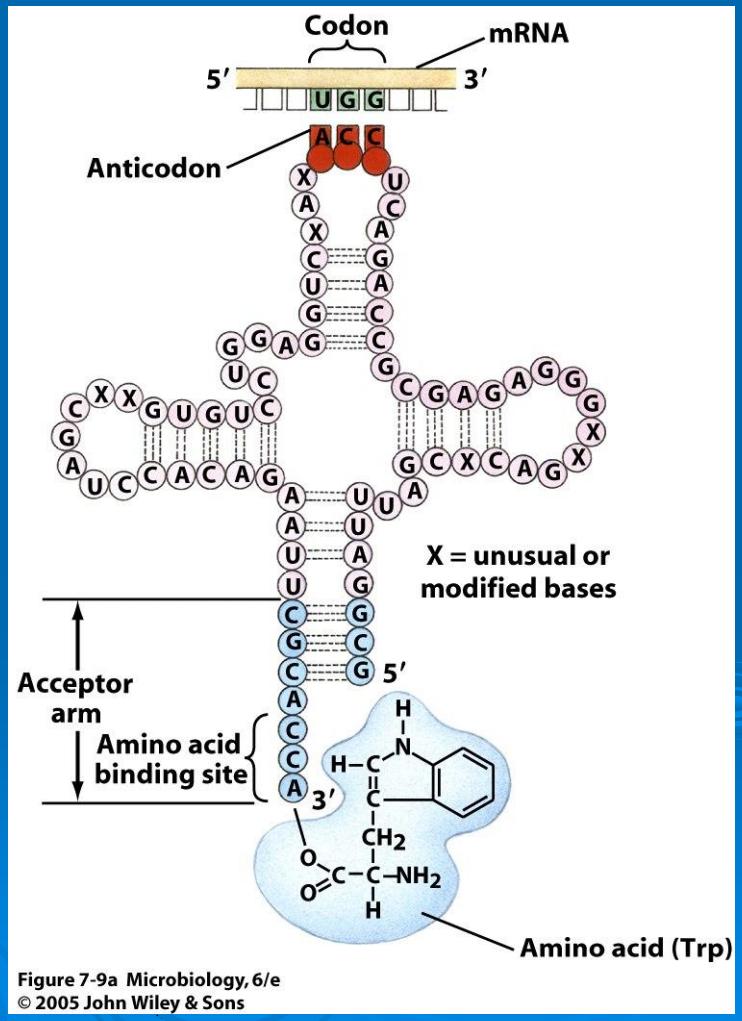

Figure 7-9a Microbiology, 6/e
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Translation and the Genetic Code

-Players: ribosomes (+ rRNA), tRNA, and mRNA

-Codons

-Anticodons

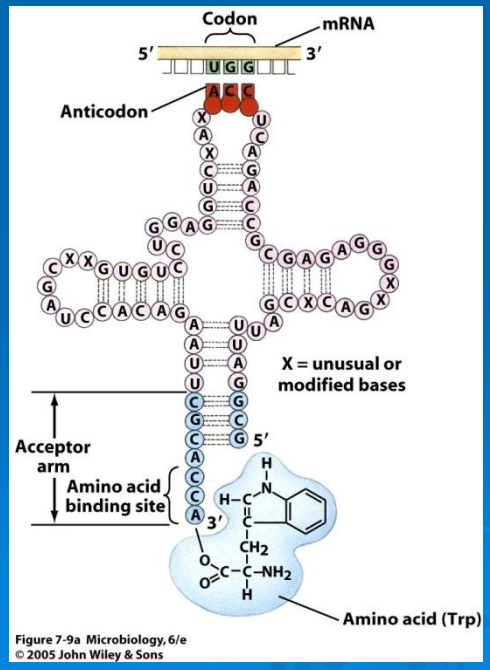

Figure 7-9a Microbiology, 6/e
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First position	Second position: U	Second position: C	Second position: A	Second position: G	Third position
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met/start	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

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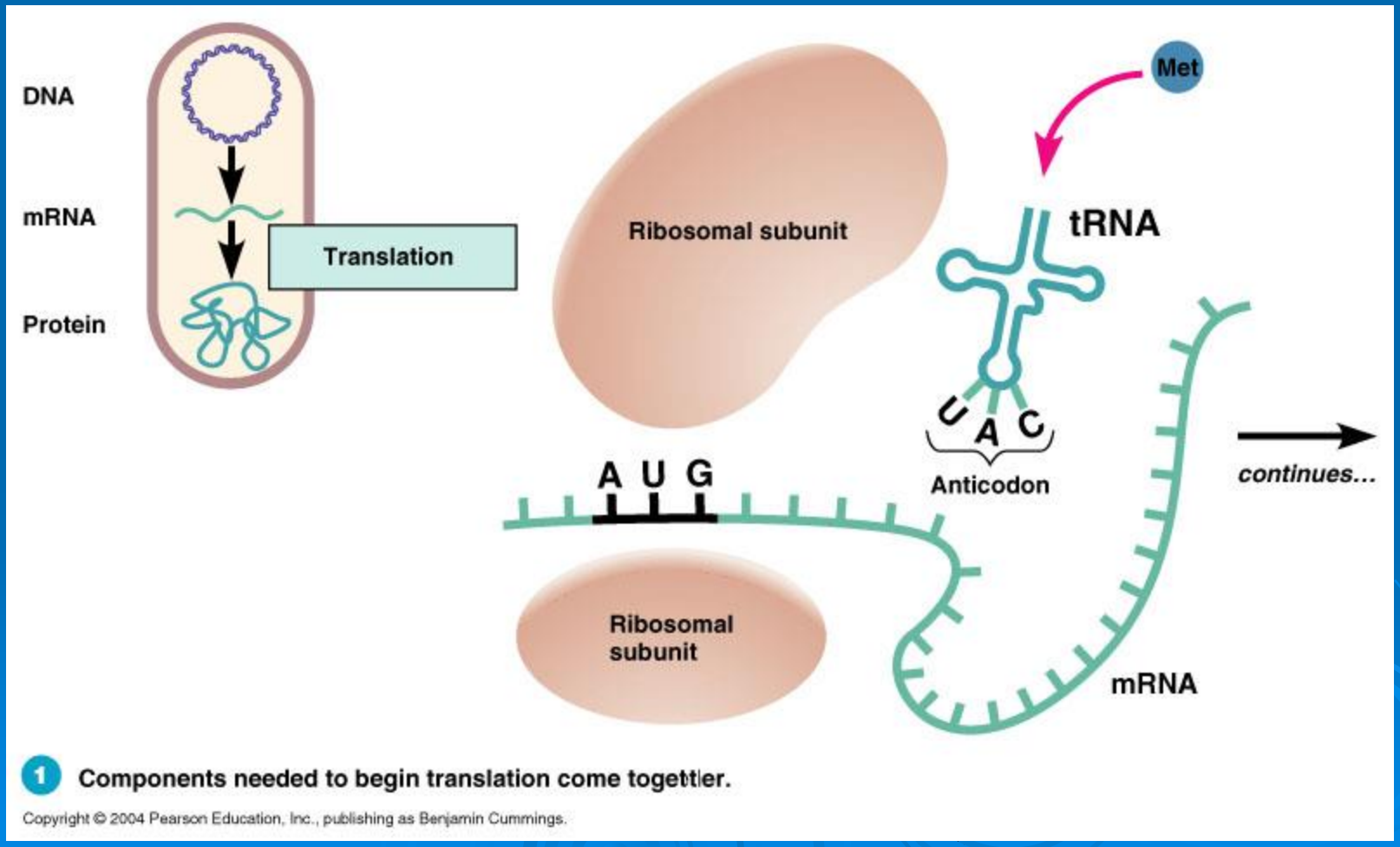
Translation



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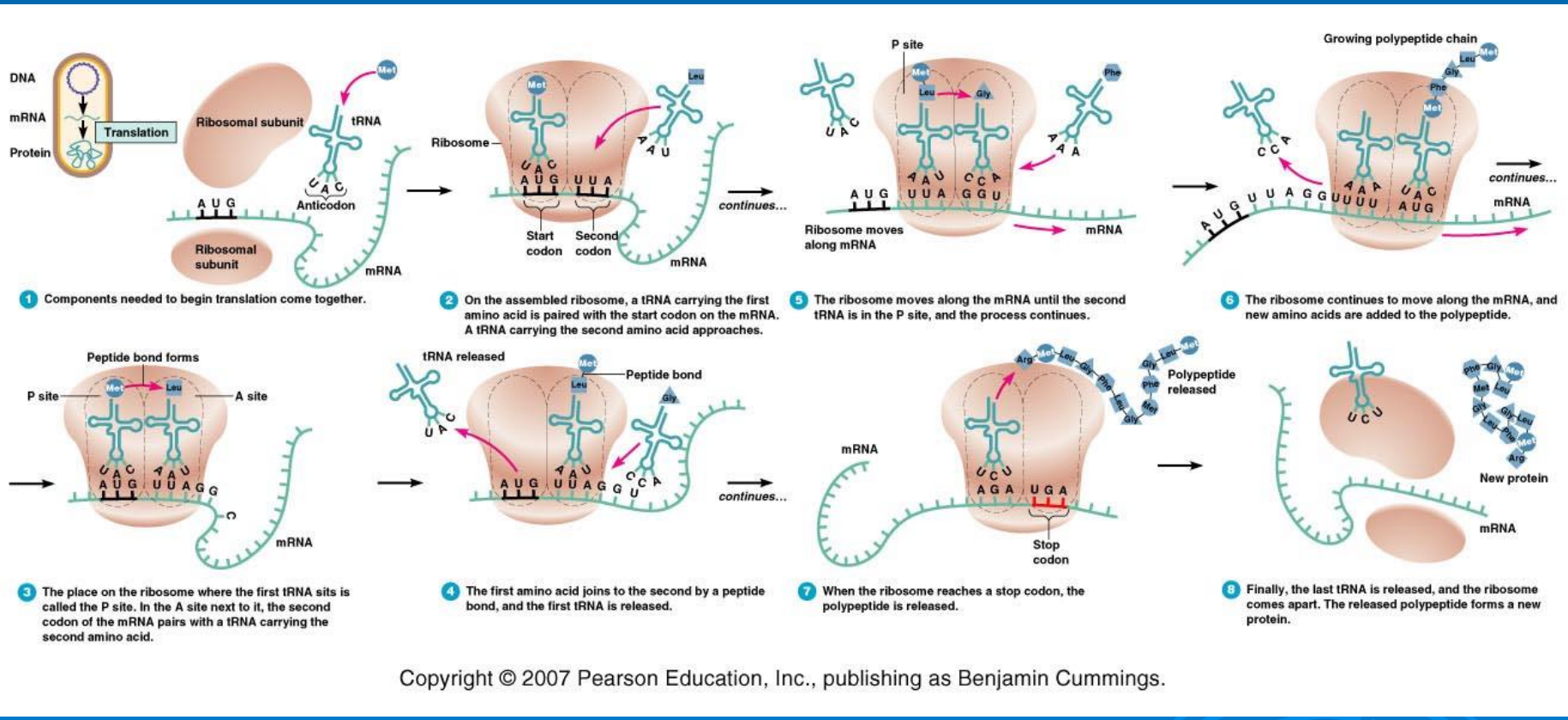
Translation



1 Components needed to begin translation come together.

2 On the assembled ribosome, a tRNA carrying the first amino acid is paired with the start codon on the mRNA. A tRNA carrying the second amino acid approaches.

3 The place on the ribosome where the first tRNA sits is called the P site. In the A site next to it, the second codon of the mRNA pairs with a tRNA carrying the second amino acid.

4 The first amino acid joins to the second by a peptide bond, and the first tRNA is released.

5 The ribosome moves along the mRNA until the second tRNA is in the P site, and the process continues.

6 The ribosome continues to move along the mRNA, and new amino acids are added to the polypeptide.

7 When the ribosome reaches a stop codon, the polypeptide is released.

8 Finally, the last tRNA is released, and the ribosome comes apart. The released polypeptide forms a new protein.

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Universal Genetic Code

Gene 5’ ATG GTC CGA GCC CGC TAA GGC 3’
3’ TAC CAG GCT CGG GCG ATT CCG 5’

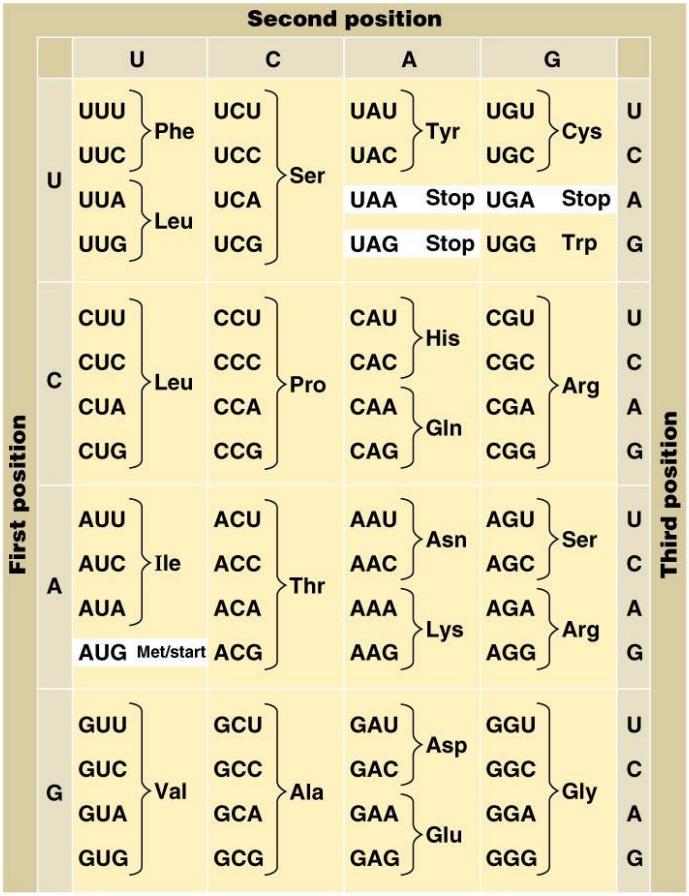

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Figure 8.8

Universal Genetic Code

Gene 5’ ATG GTC CGA GCC CGC TAA GGC 3’
3’ TAC CAG GCT CGG GCG ATT CCG 5’

RNA 5’ AUG

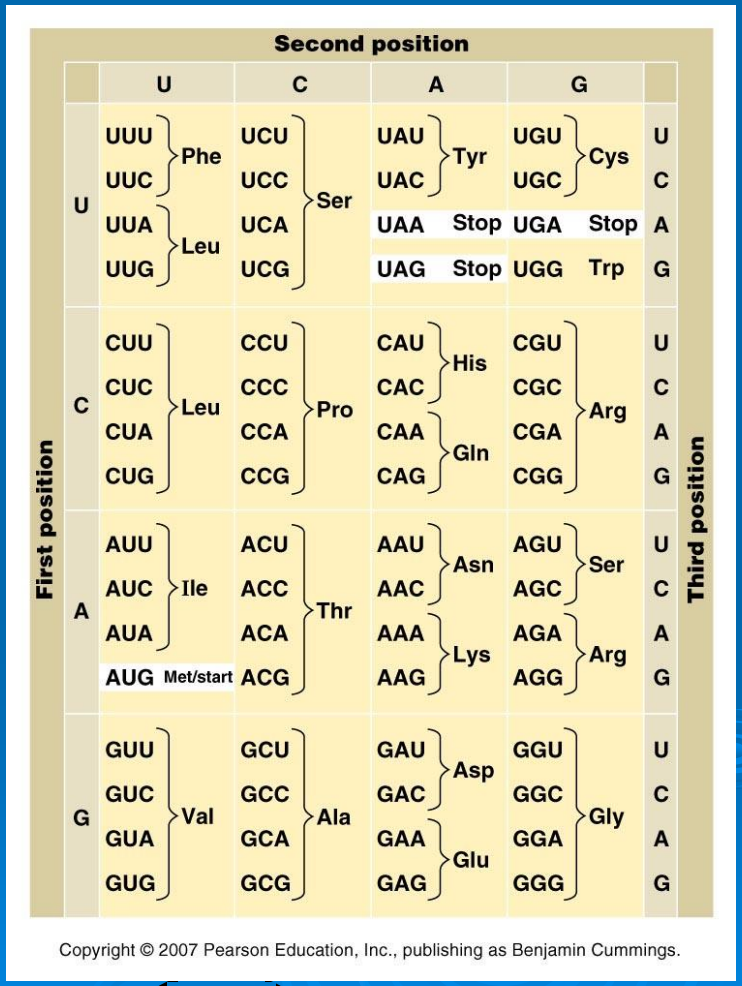

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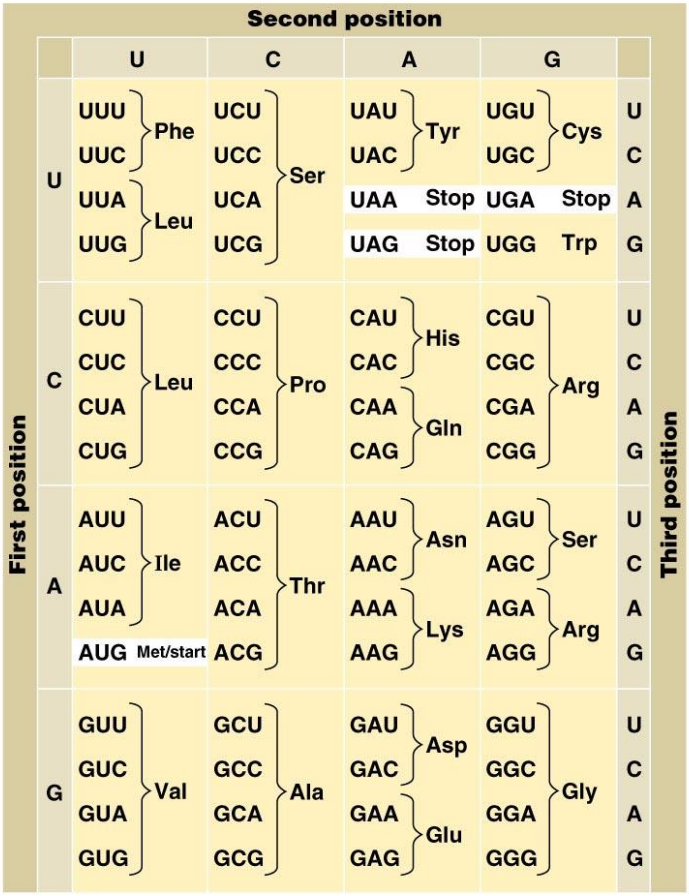
Figure 8.8

Universal Genetic Code

Gene 5’ ATG GTC CGA GCC CGC TAA GGC 3’
3’ TAC CAG GCT CGG GCG ATT CCG 5’

RNA 5’ AUG GUC



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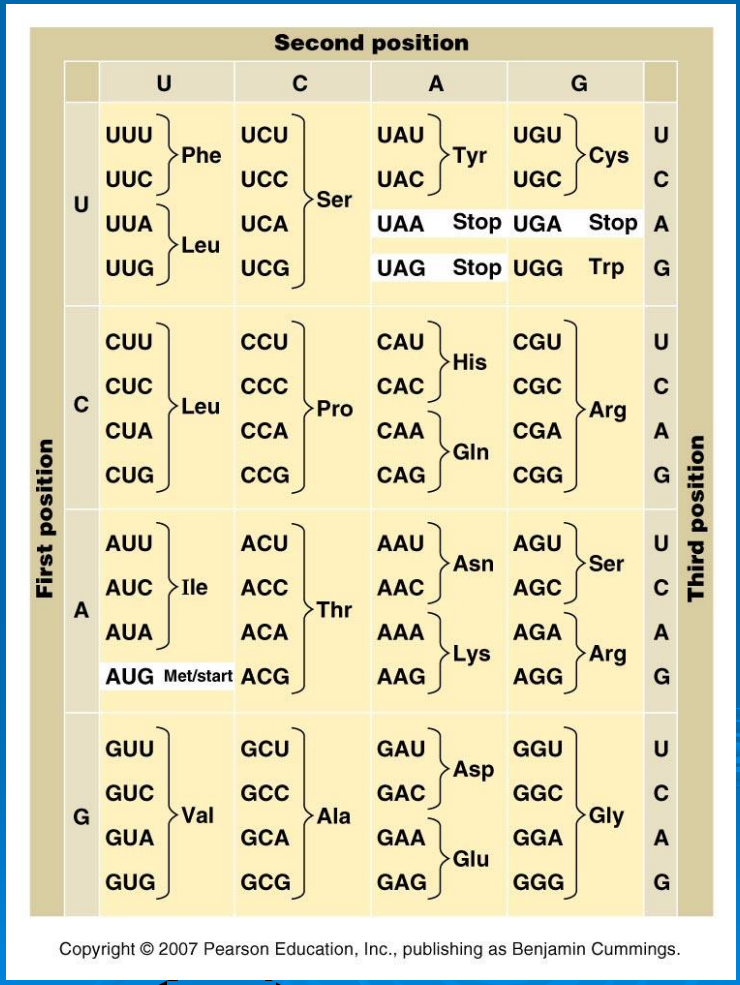
Universal Genetic Code

Gene 5’ ATG GTC CGA GCC CGC TAA GGC 3’
3’ TAC CAG GCT CGG GCG ATT CCG 5’

RNA 5’ AUG GUC CGA



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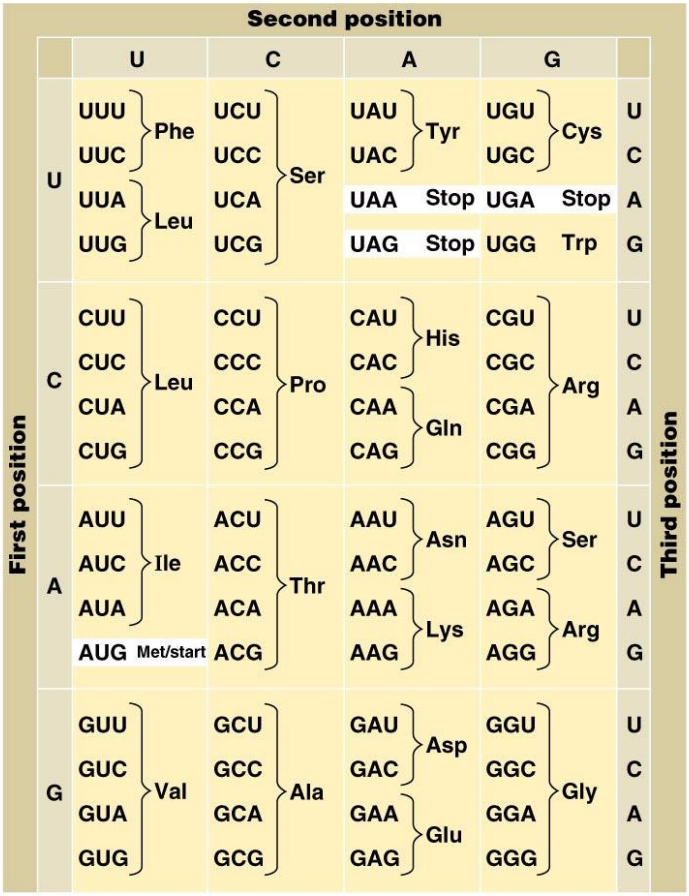
Universal Genetic Code

Gene 5’ ATG GTC CGA GCC CGC TAA GGC 3’
3’ TAC CAG GCT CGG GCG ATT CCG 5’

RNA 5’ AUG GUC CGA GCC



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Universal Genetic Code

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3’ TAC CAG GCT CGG GCG ATT CCG 5’

RNA 5’ AUG GUC CGA GCC CGC

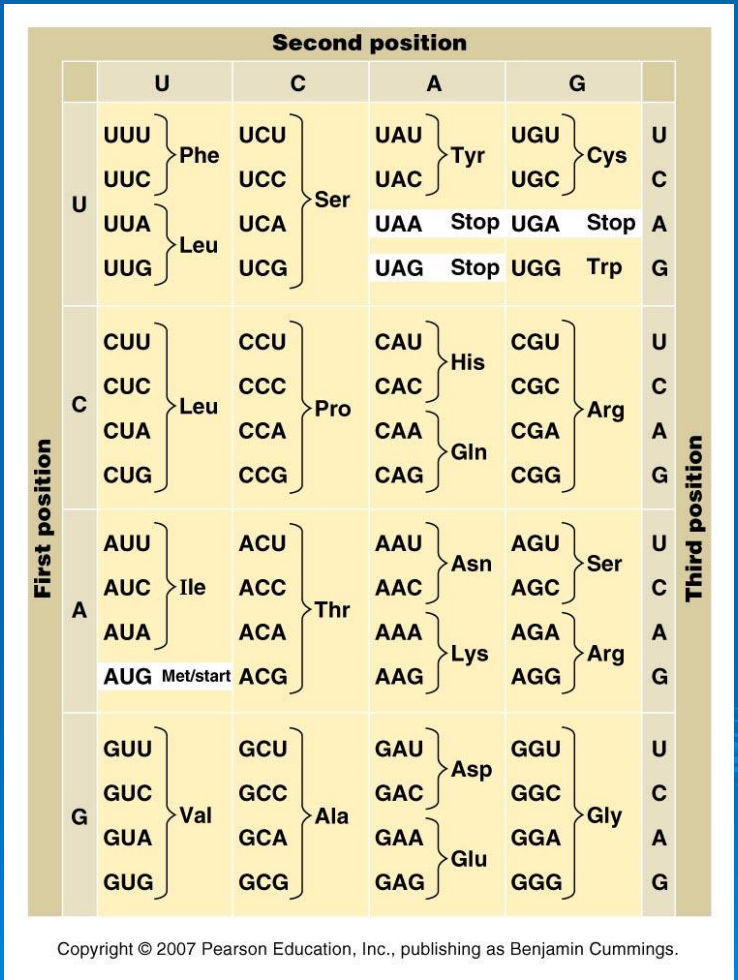

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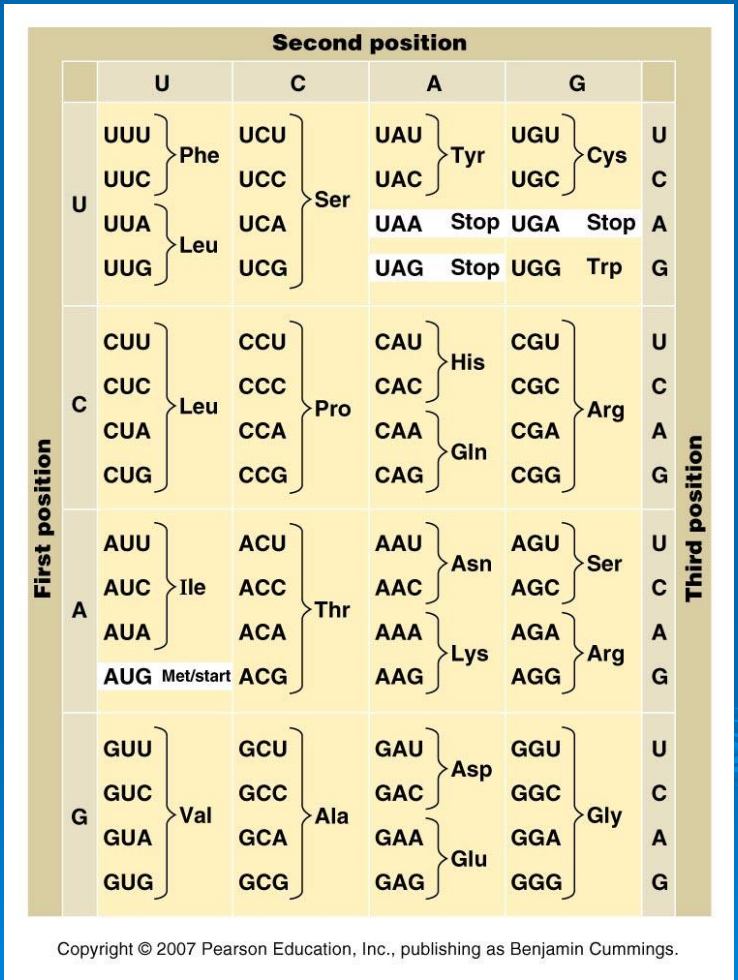
Figure 8.8

Universal Genetic Code

Gene 5’ ATG GTC CGA GCC CGC TAA GGC 3’
3’ TAC CAG GCT CGG GCG ATT CCG 5’

RNA 5’ AUG GUC CGA GCC CGC UAA



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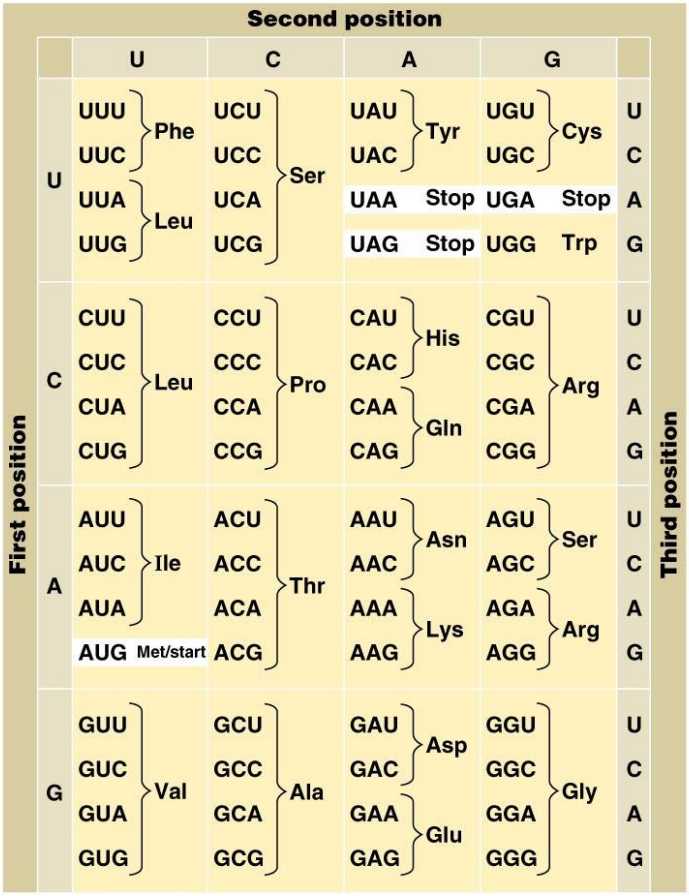
Universal Genetic Code

Gene 5’ ATG GTC CGA GCC CGC TAA GGC 3’
3’ TAC CAG GCT CGG GCG ATT CCG 5’

RNA 5’ AUG GUC CGA GCC CGC UAA GGC 3’

Protein



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Universal Genetic Code

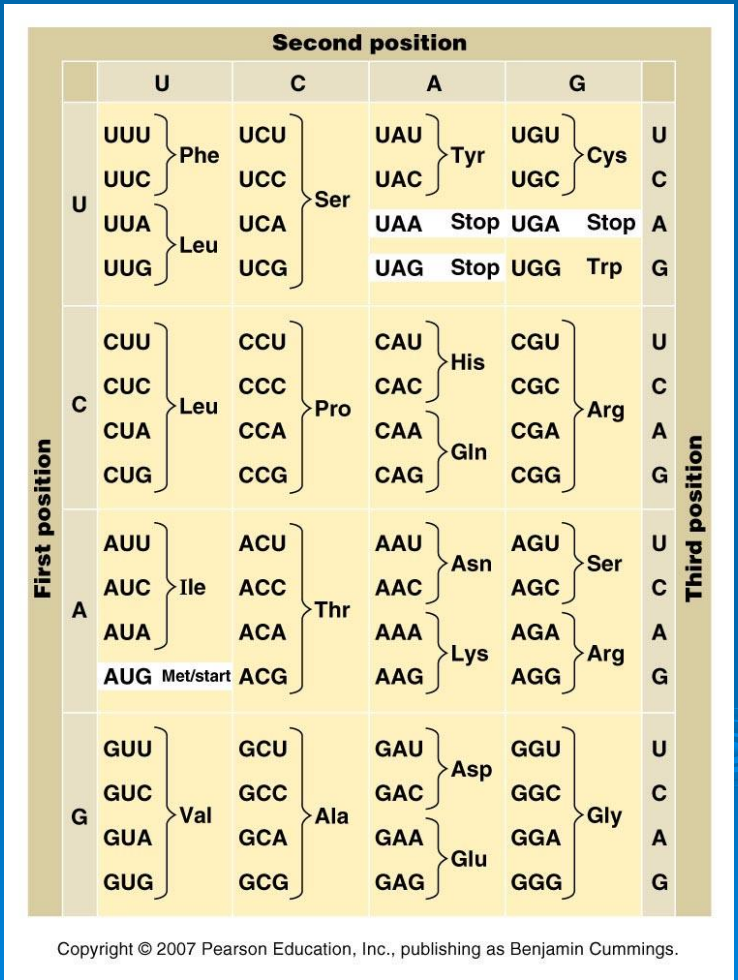
Gene 5’ ATG GTC CGA GCC CGC TAA GGC 3’
3’ TAC CAG GCT CGG GCG ATT CCG 5’

RNA 5’ AUG GUC CGA GCC CGC UAA GGC 3’

Protein

Met

(formylmethionine in bacteria)



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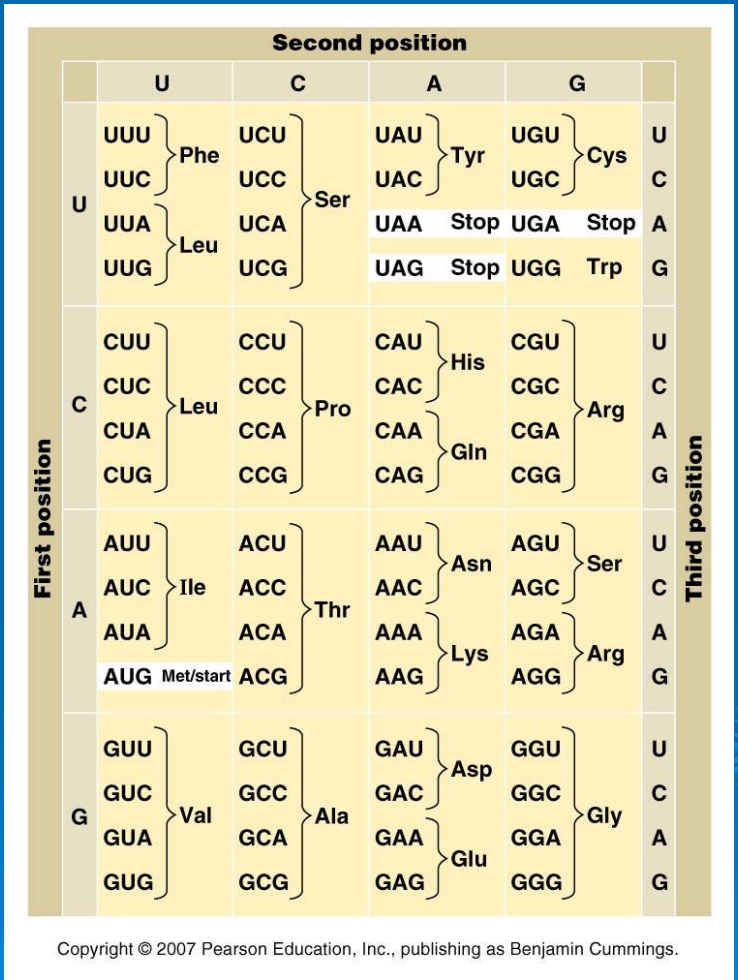
Universal Genetic Code

Gene 5’ ATG GTC CGA GCC CGC TAA GGC 3’
3’ TAC CAG GCT CGG GCG ATT CCG 5’

RNA 5’ AUG GUC CGA GCC CGC UAA GGC 3’

Protein

Met Val



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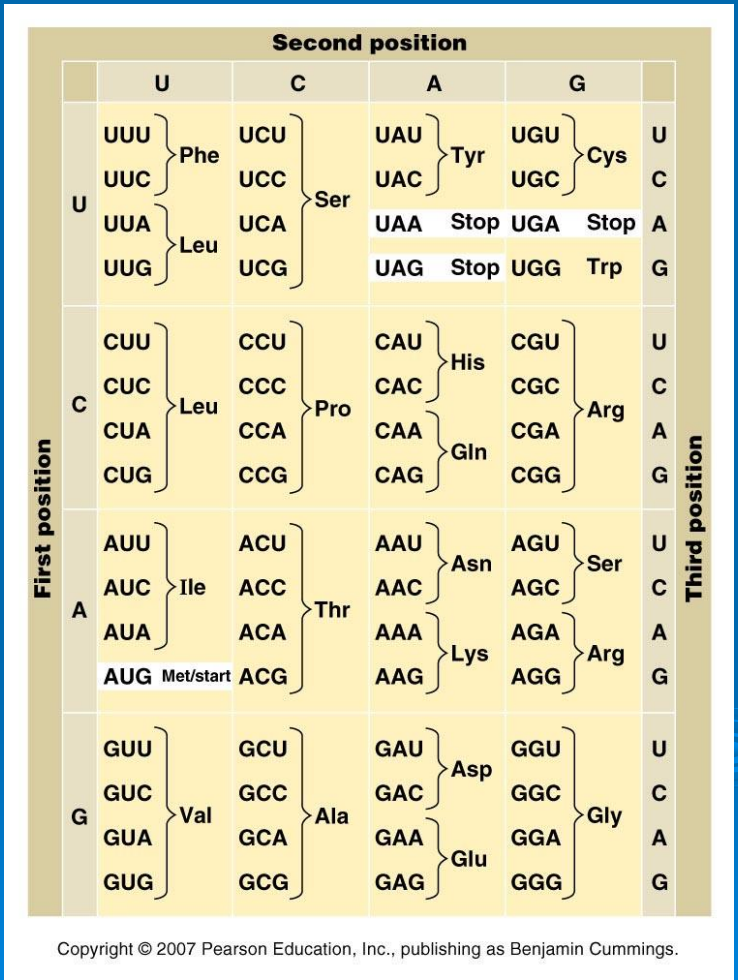
Universal Genetic Code

Gene 5’ ATG GTC CGA GCC CGC TAA GGC 3’
3’ TAC CAG GCT CGG GCG ATT CCG 5’

RNA 5’ AUG GUC CGA GCC CGC UAA GGC 3’

Protein

Met Val Arg



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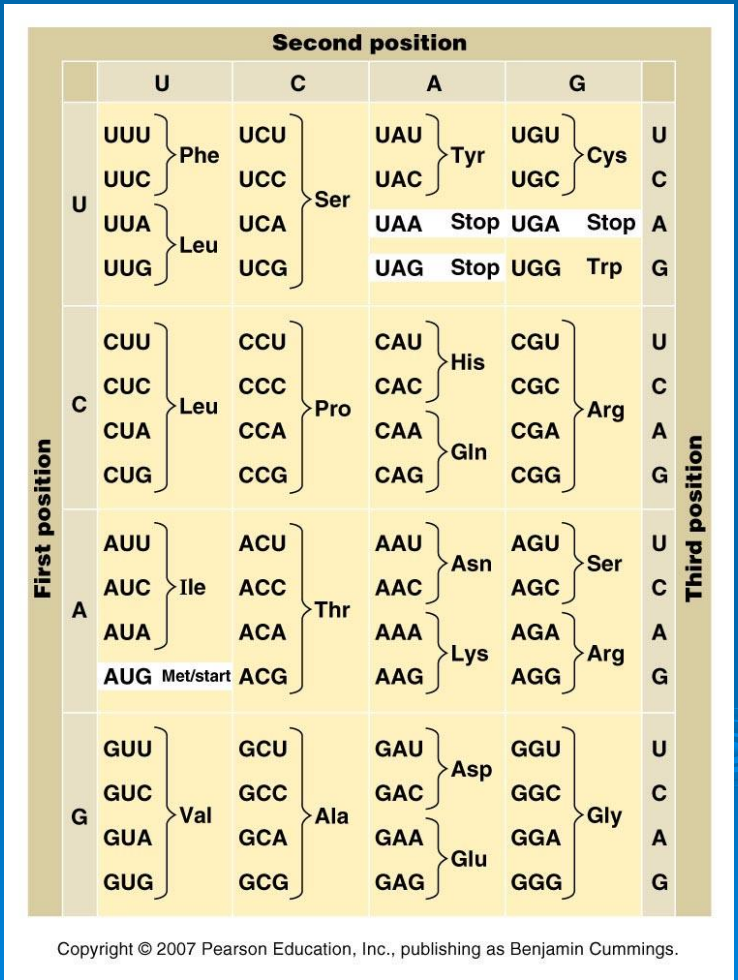
Universal Genetic Code

Gene 5’ ATG GTC CGA GCC CGC TAA GGC 3’
3’ TAC CAG GCT CGG GCG ATT CCG 5’

RNA 5’ AUG GUC CGA GCC CGC UAA GGC 3’

Protein

Met Val Arg Ala



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Gene 5’ ATG GTC CGA GCC CGC TAA GGC 3’
3’ TAC CAG GCT CGG GCG ATT CCG 5’

RNA 5’ AUG GUC CGA GCC CGC UAA GGC 3’

Protein

Met Val Arg Ala Arg

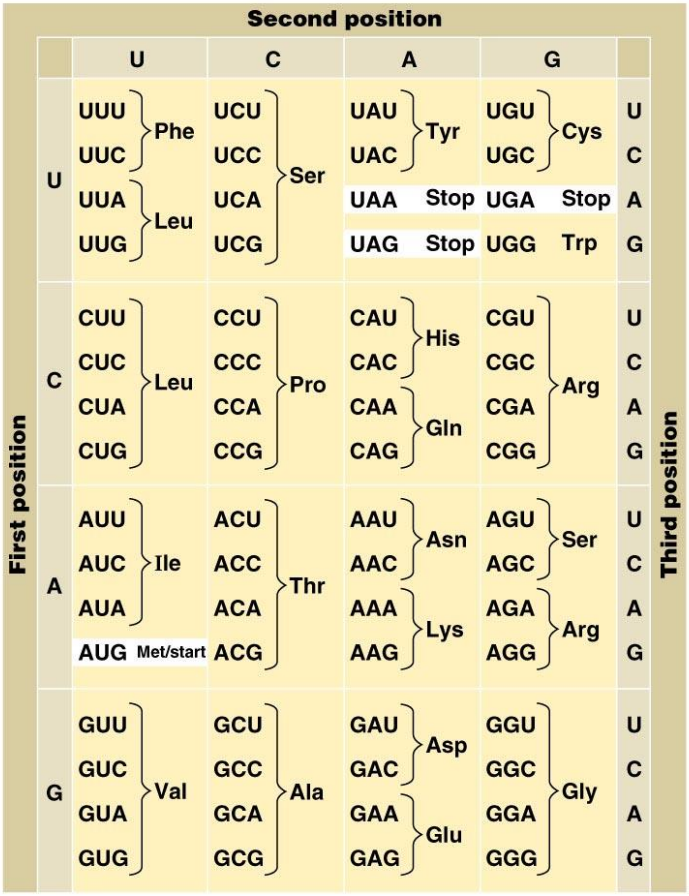

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Figure 8.8

Universal Genetic Code

Gene 5’ ATG GTC CGA GCC CGA TAA GGC 3’
3’ TAC CAG GCT CGG GCT ATT CCG 5’

RNA 5’ AUG GUC CGA GCC CGA UAA GGC 3’

Protein

Met Val Arg Ala Arg Stop

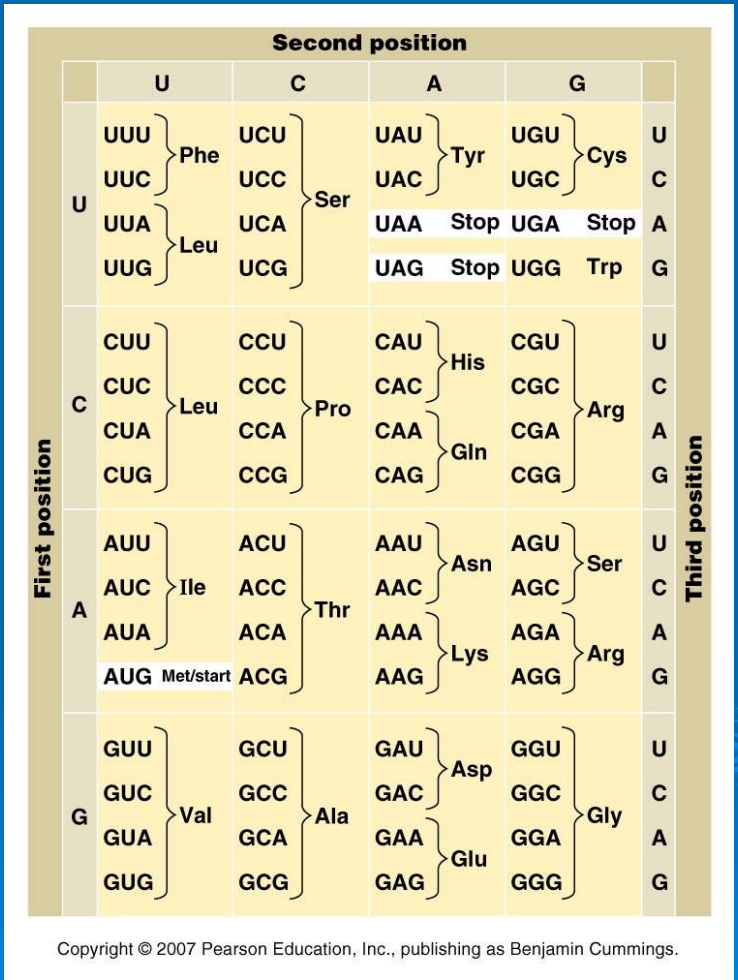

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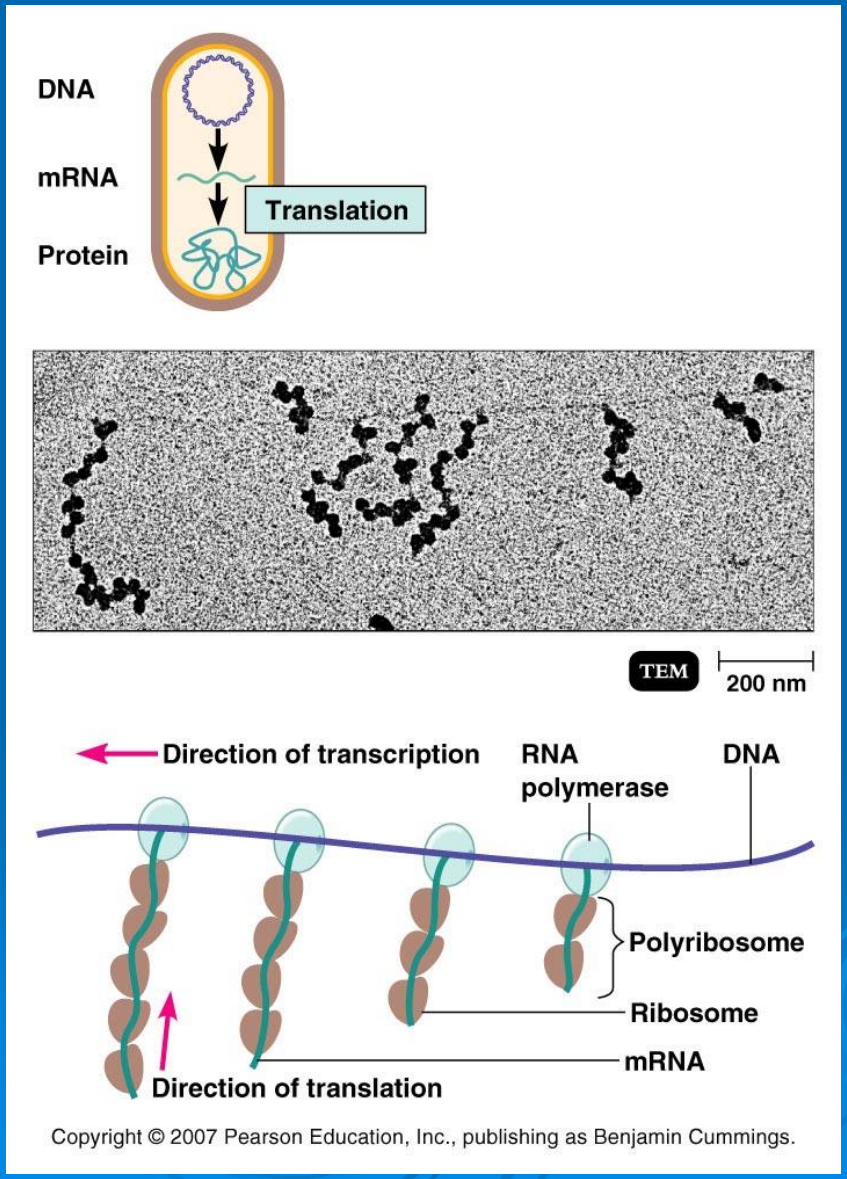
Figure 8.8

DNA processing in bacteria- simultaneous transcription/translation



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Independent study- EXTRA CREDIT

Clinical focus: Tracking viral infections

Clinical Applications and Evaluations questions 2 & 3 (pg 237 in 12th Ed; pg 241 in 13th Ed) deal with strains of HIV and human herpesvirus. Answer the questions as instructed below:

For question 2: Answer questions as asked
For question 3: Transcribe and translate the DNA **and** then answer questions as asked

Due next class period

Game Plan

Lecture

Gene regulation and the operon
Mutations

Lab

Review results from Growth Curve
Effects on growth: temp, pH, O2

Pre-labs
Growth Control: temp and UV

Review of DNA processing

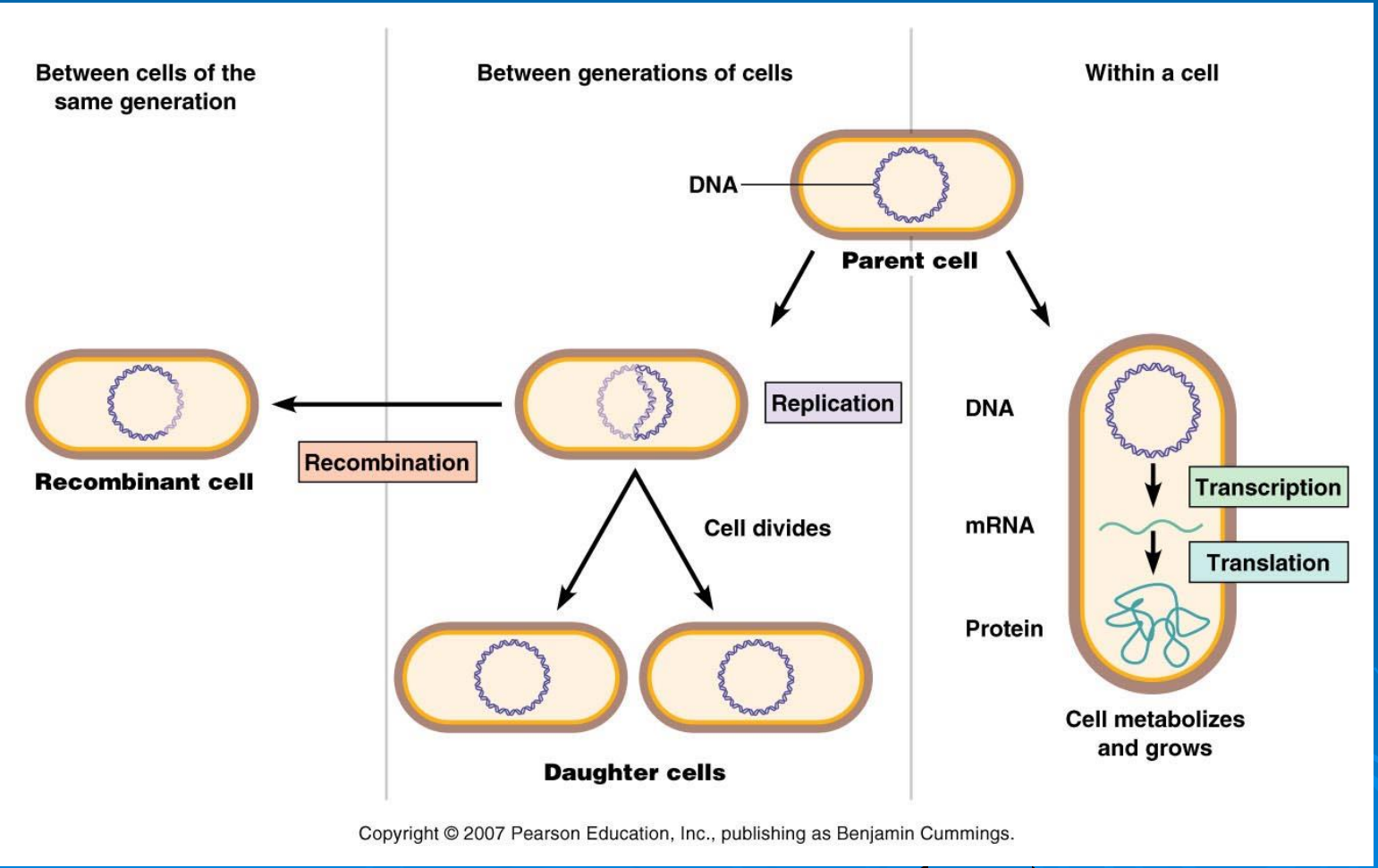

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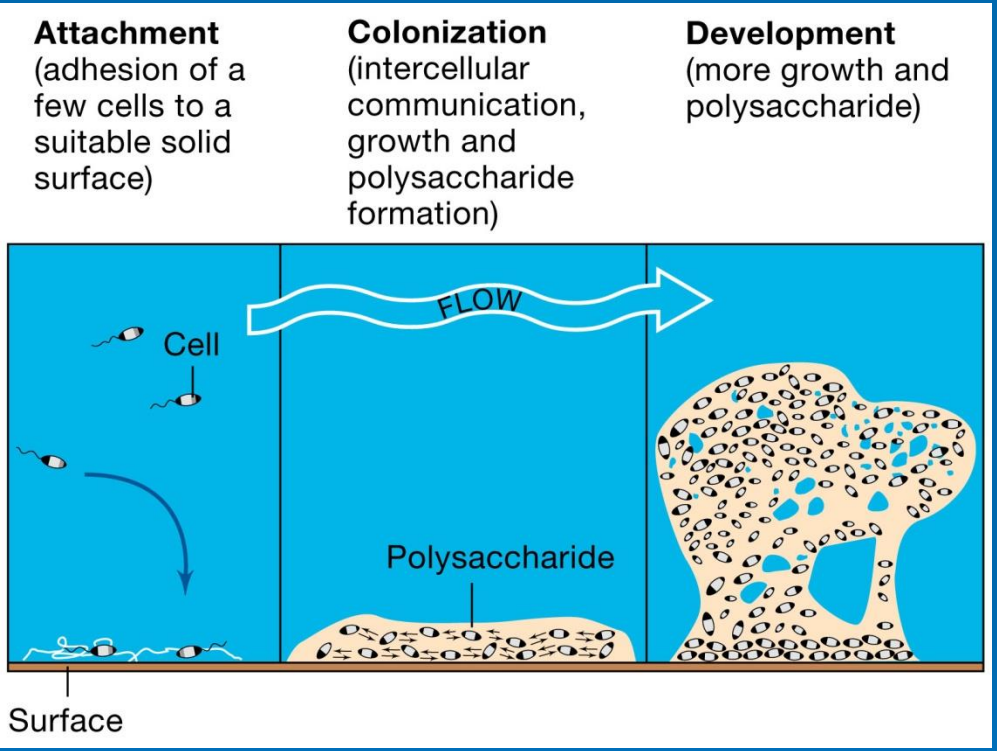
Figure 8.2

What changes a bacterium from planktonic to sessile (i.e. what causes biofilm formation)?



Genes being turned on and off!

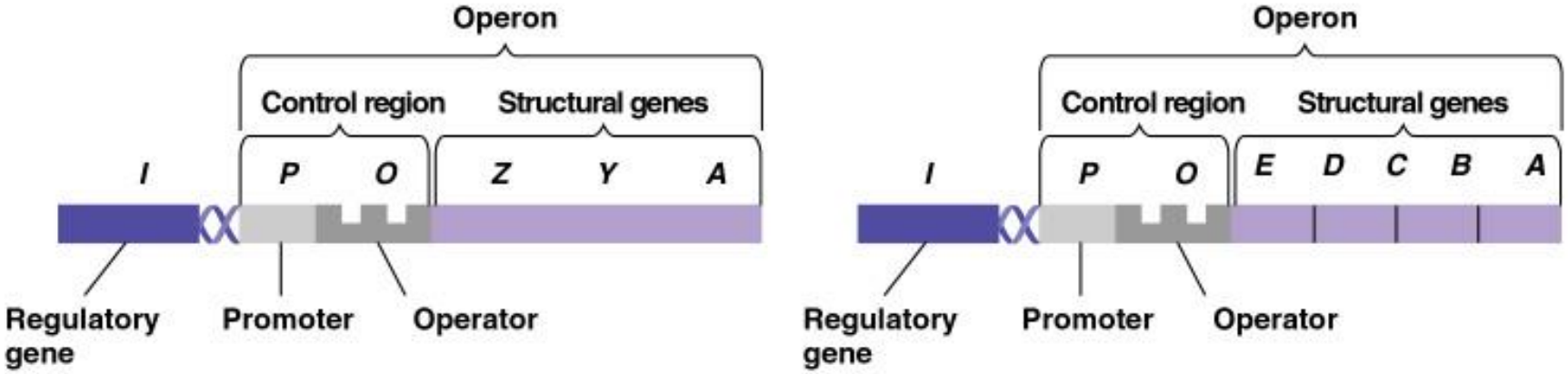
Example:
Differential expression of 2.9-17% *P. aeruginosa* genes between planktonic and biofilm cells

When are genes on and off?

-**Constitutive genes:** genes are constantly "on" (60-80%)

-**Regulated genes:** can be repressed and induced

The Jacob and Monad Operon Model

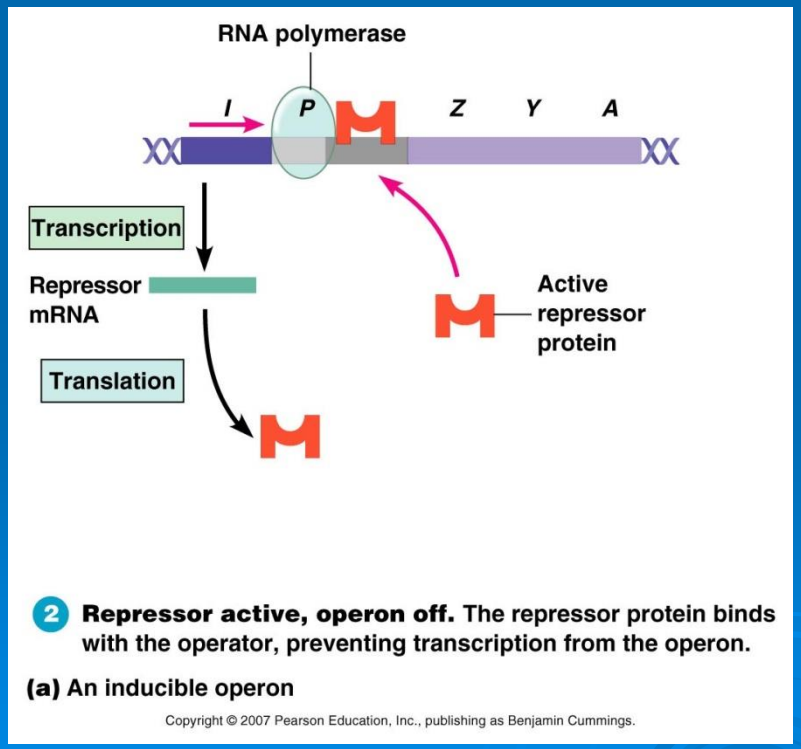


1 **Structure of the operon.** The operon consists of the promoter (*P*), and operator (*O*) sites, and structural genes which code for the protein. The operon is regulated by the product of the regulatory gene (*I*).

Regulation of bacterial genes

1. **Response to nutrients in the environment**

   Example: <u>Inducible genes</u>
   Lactose operon



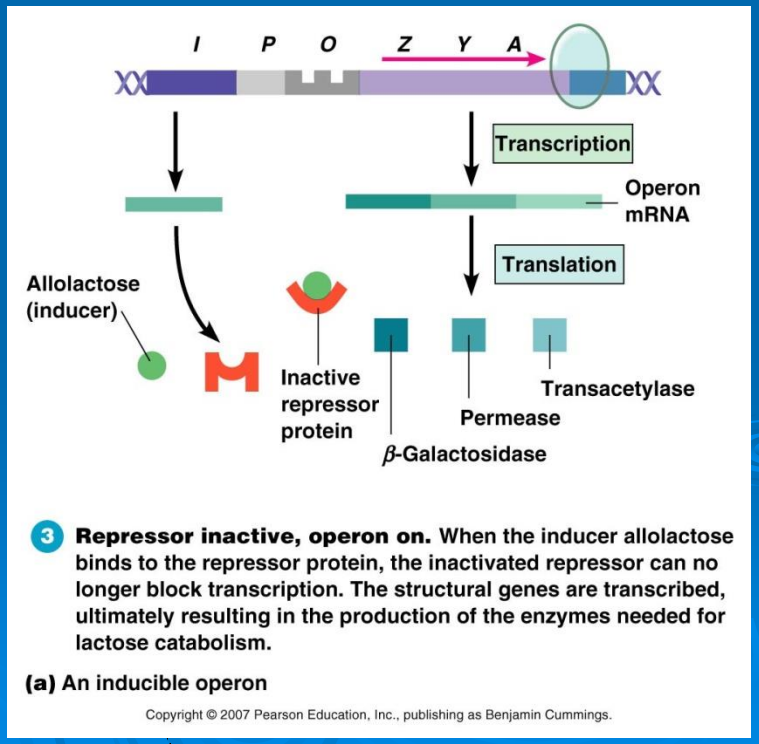


Figure 8.12a

Regulation of bacterial genes

1. **Response to nutrients in the environment**

Example: Inducible genes

Lactose operon

Lac operon induction needs lactose AND low glucose

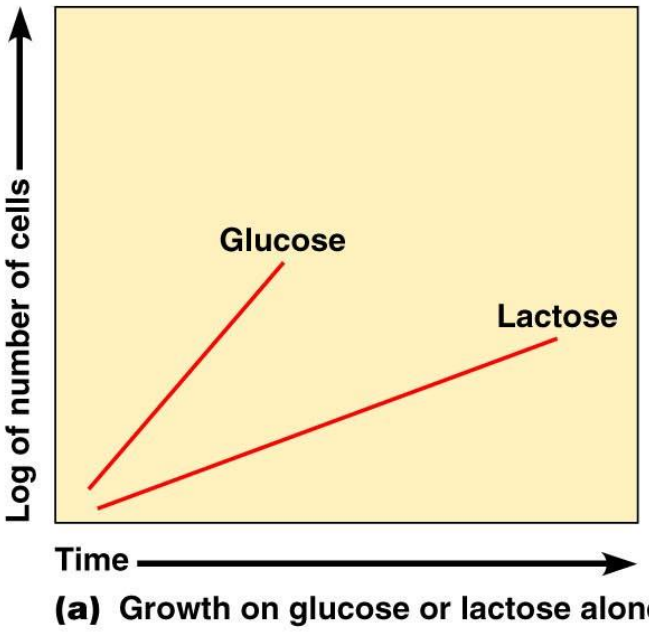


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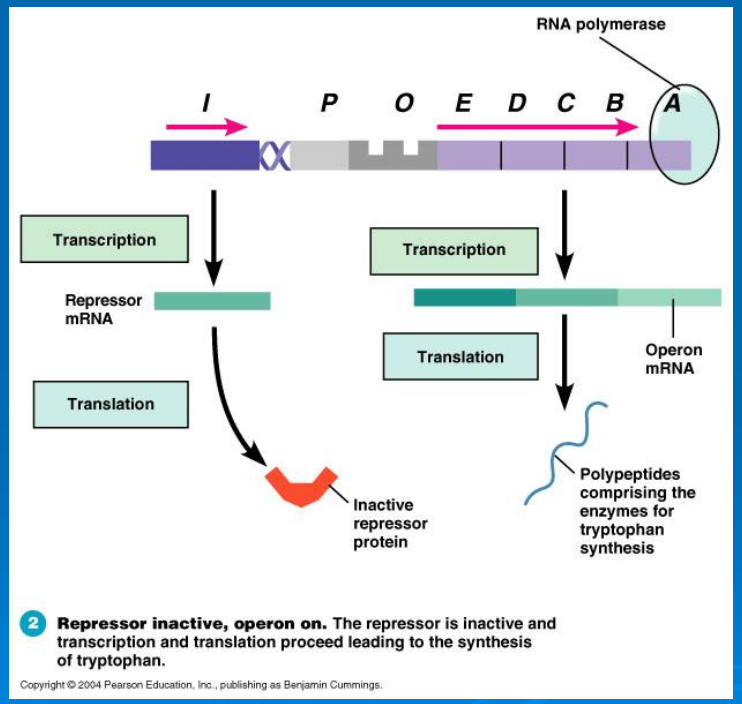
Figure 8.13 - Overview

Regulation of bacterial genes

1. **Response to nutrients in the environment**

Example: <u>Repressible genes</u>
Tryptophan operon



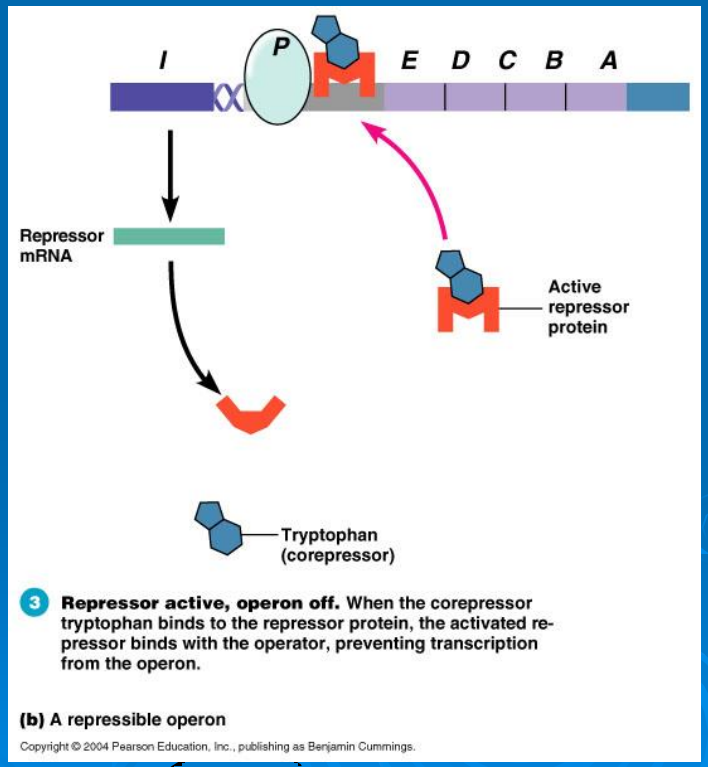


Figure 8.12b

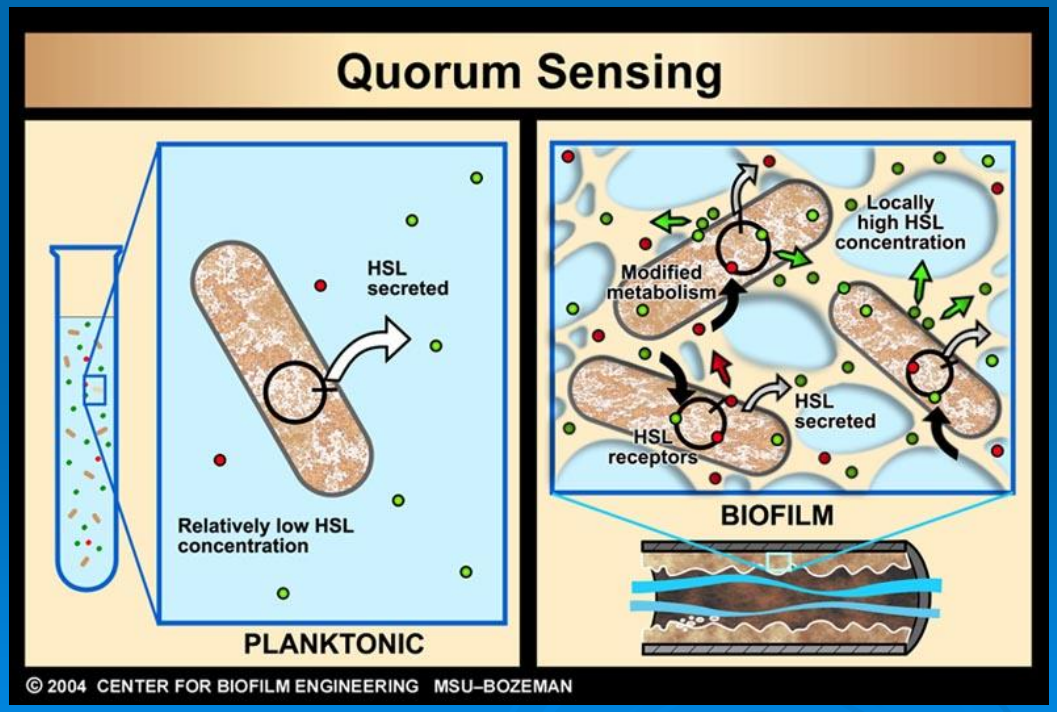
Regulation of bacterial genes

2. **Quorum sensing of the environment=** ability of bacteria to communicate and coordinate behavior (through gene expression) by release of signaling molecules (phermones)

Regulation of bacterial genes

2. Quorum sensing of the environment

Example: Biofilm genes



Gram negative:

homoserine lactones (HSL), a signaling molecule, results in loss of flagella, induction of virulence genes

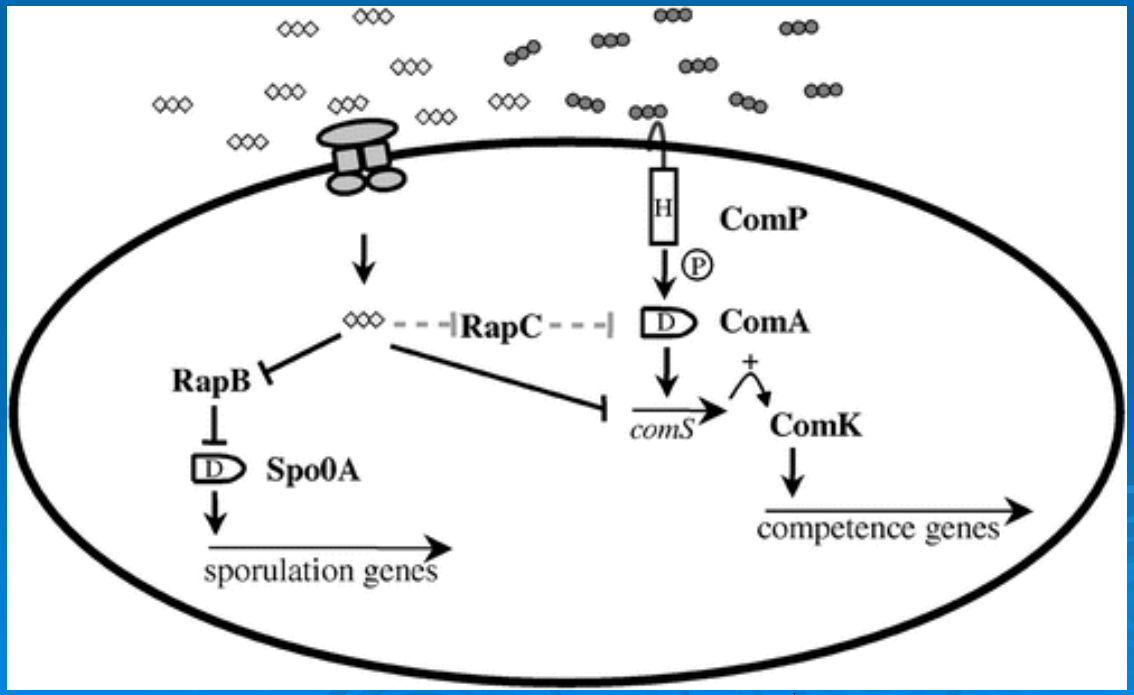
Gram positives:

peptides

Regulation of bacterial genes

2. Quorum sensing of the environment

Example: Sporulation genes- low nutrients and QS induce sporulation genes and shut down competence genes

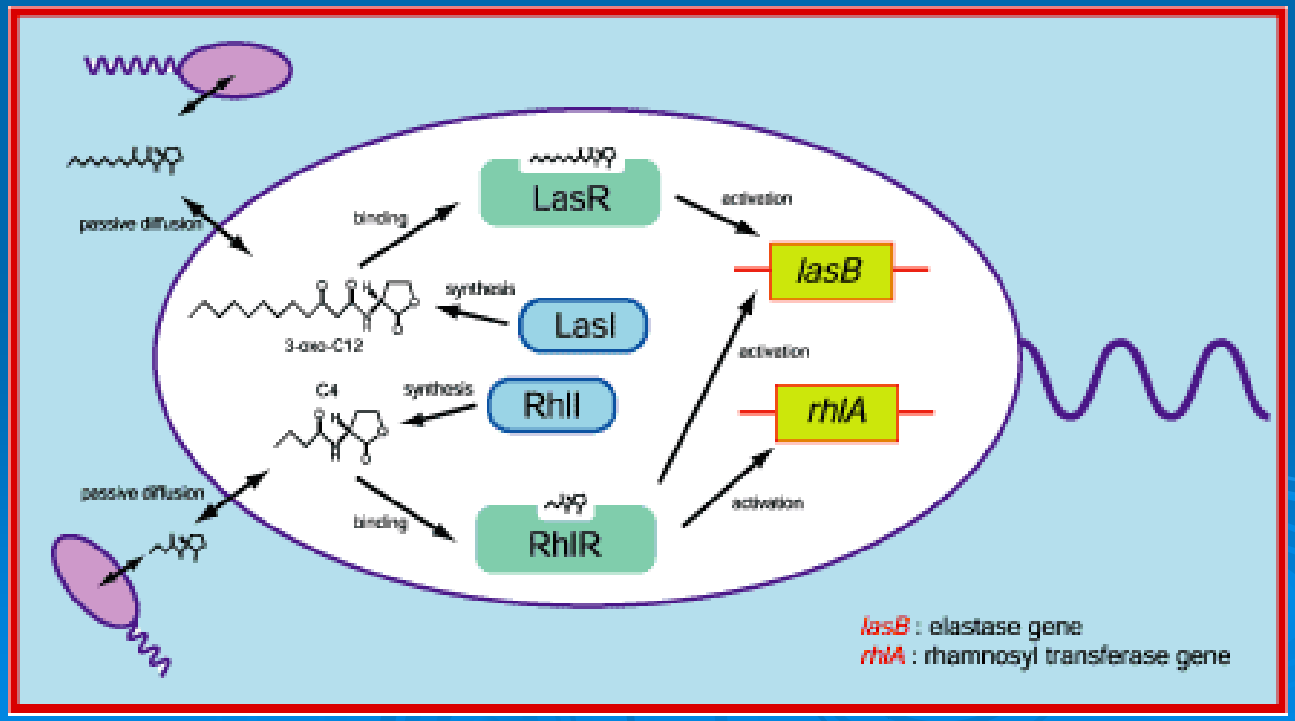


Bacillus subtilis sporulation

Regulation of bacterial genes

2. Quorum sensing of the environment

Example: Virulence genes- *P. aeruginosa* uses QS to sense high cell density and activate virulence genes to cause disease



If you inoculate a flask of TSB with a single *E. coli*, after 24 hours will all the cells be identical?

Why or why not?

Mutations:
base pair substitutions

1. **Missense and nonsense mutations**

   Effect on protein:

   No change, altered function, loss of function

   Examples:

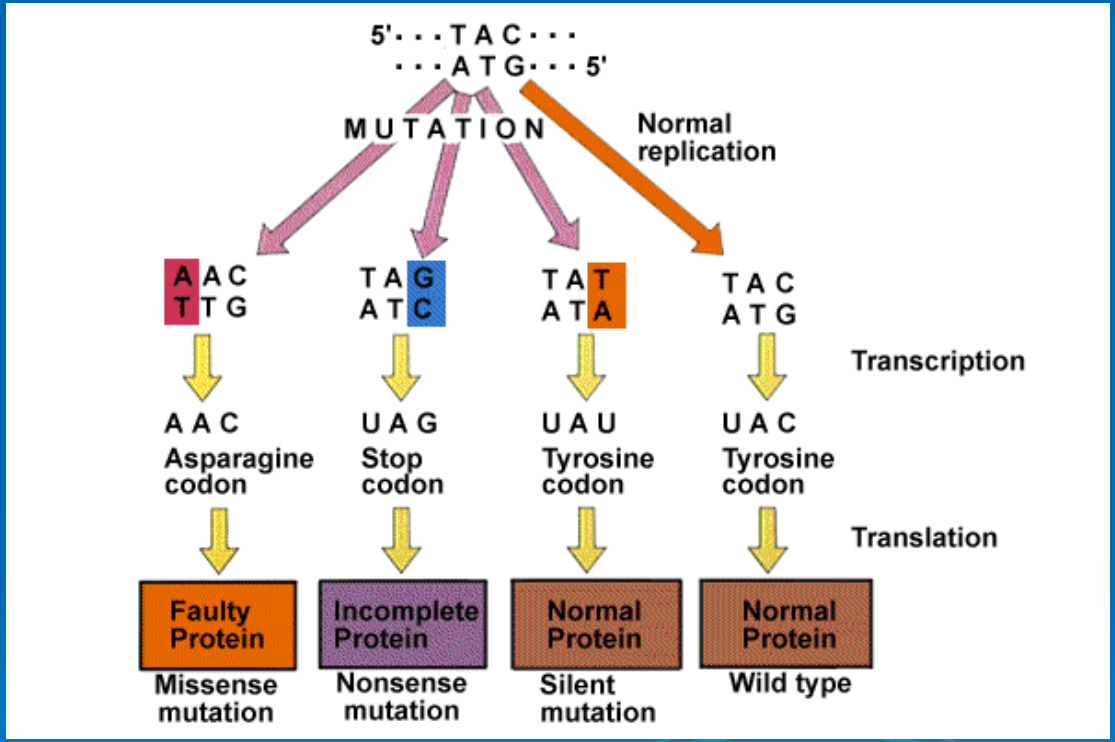
   Sickle cell disease (mis)

   Cystic fibrosis (non)

2. **Silent mutations**

   Effect on protein:

   No change in protein



Mutations: frameshifts

3. Frameshift mutations

Effect on protein:

Many possible outcomes

Rarely no change

Examples (trinucleotide repeat diseases):

Fragile X Syndrome

Huntington's Disease

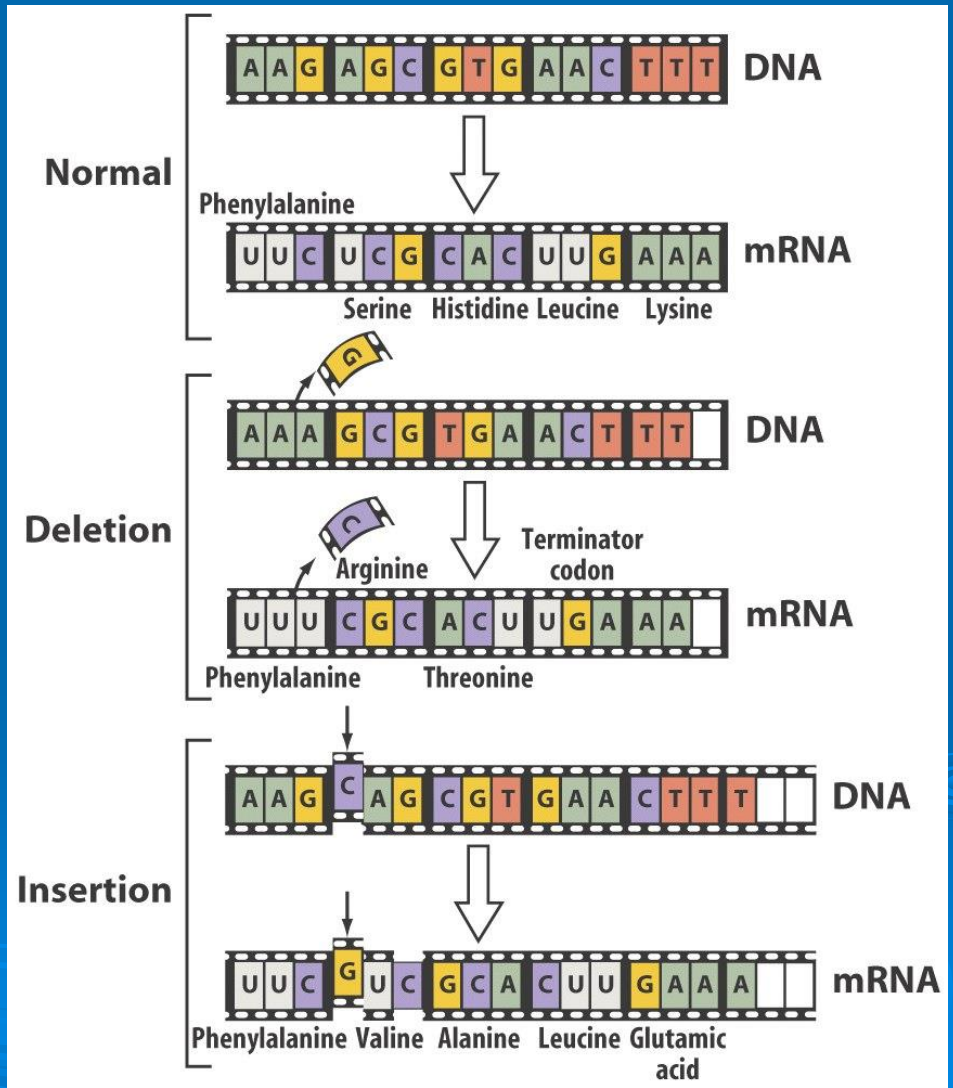


Figure 7-17 Microbiology, 6/e
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Mutagens

TABLE 7.4

Some Mutagens and Their Effects

Mutagen	Effects
Chemical Agents	
Base analog *Examples:* caffeine, 5-bromouracil	Substitutes "look-alike" molecule for the normal nitrogenous base during DNA replication → point mutation.
Alkylating agent *Example:* nitrosoguanidine	Adds an alkyl group, such as methyl group ($—CH_3$), to nitrogenous base, resulting in incorrect pairing → point mutation.
Deaminating agent *Example:* nitrous acid, nitrates, nitrites	Removes an amino group ($—NH_2$) from a nitrogenous base → point mutation.
Acridine derivative *Example:* acridine dyes, quinacrine	Inserts into DNA ladder between backbones to form a new rung, distorting the helix → frameshift mutation.
Radiation	
Ultraviolet	Links adjacent pyrimidines to each other, as in thymine dimer formation, and thereby impairs replication.
X-ray and gamma ray	Ionize and break molecules in cells to form free radicals, which in turn break DNA.

Table 7-4 Microbiology, 6/e

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Base analogs

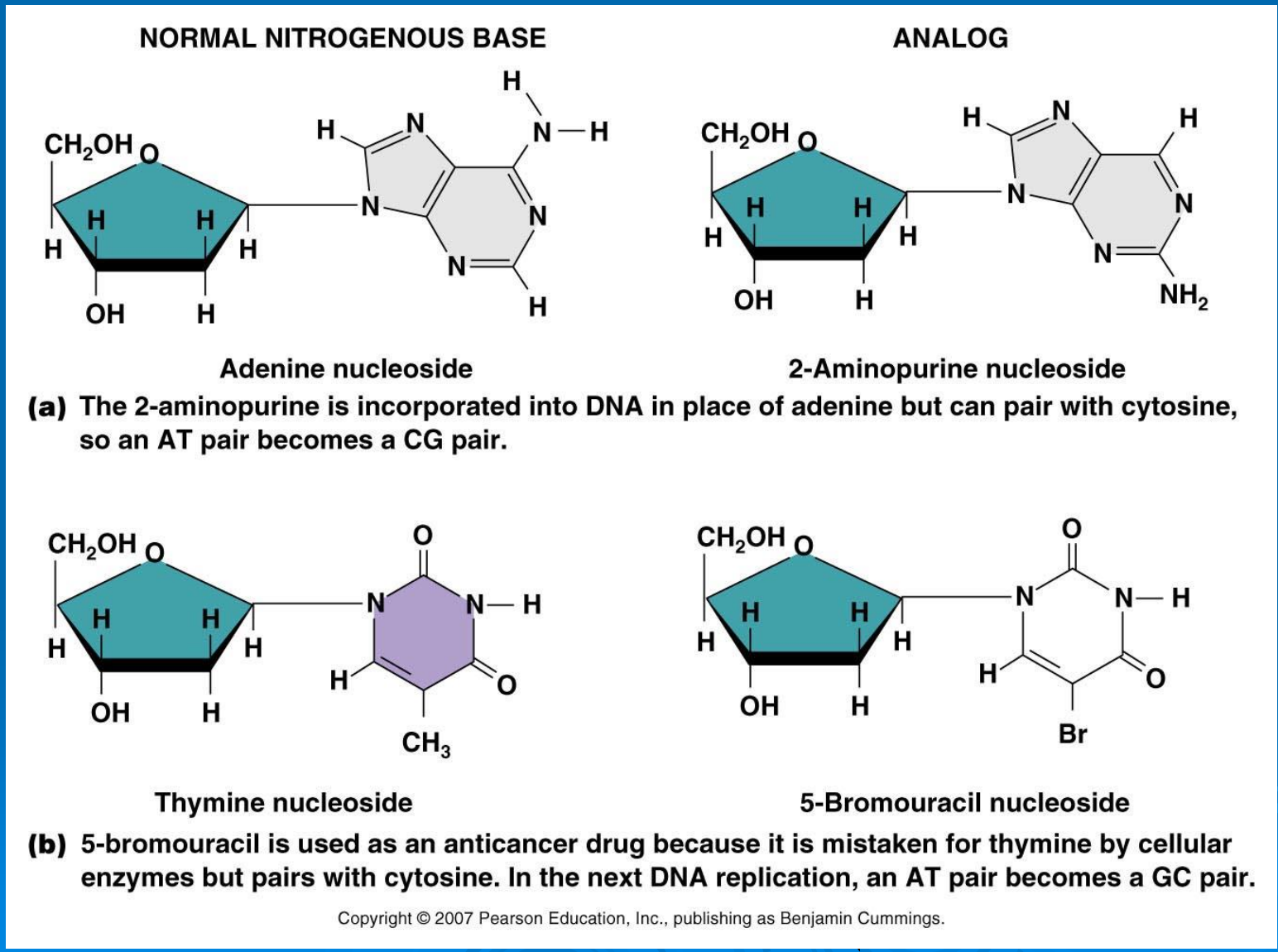

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Figure 8.18 - Overview

Mutations: radiation

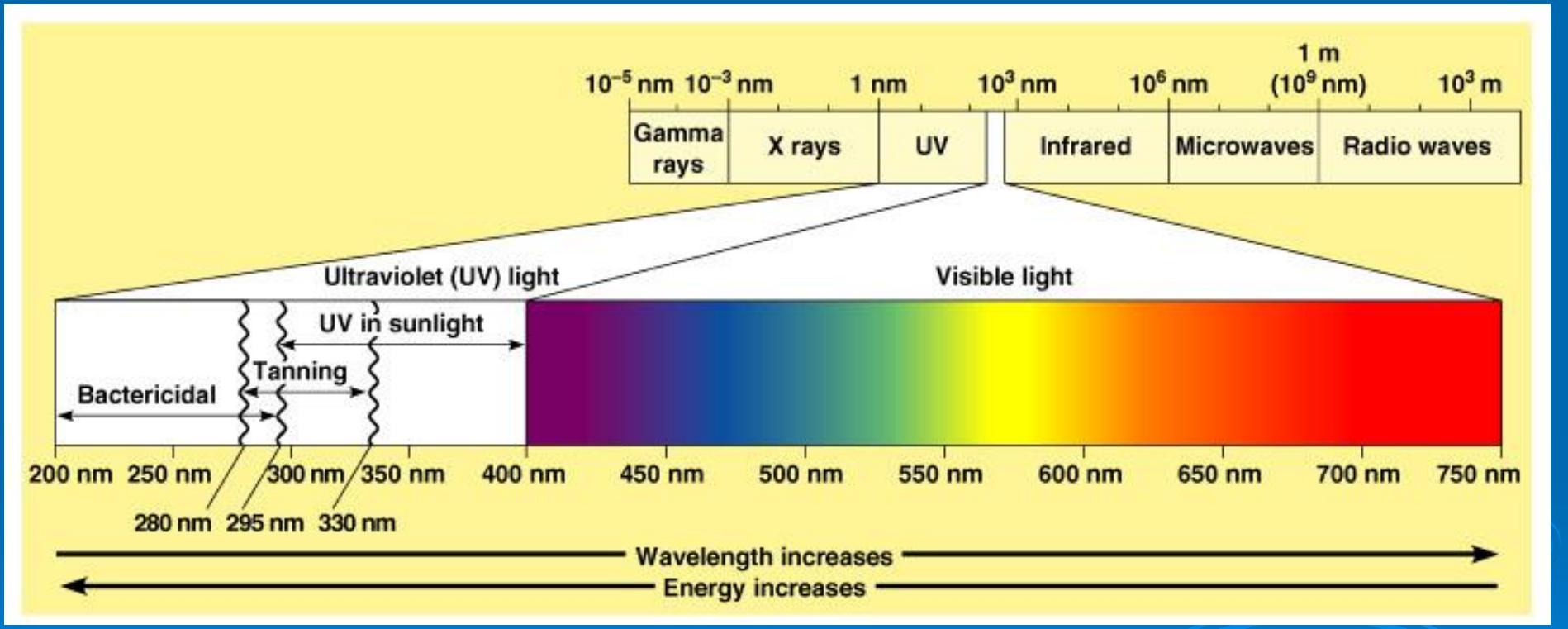
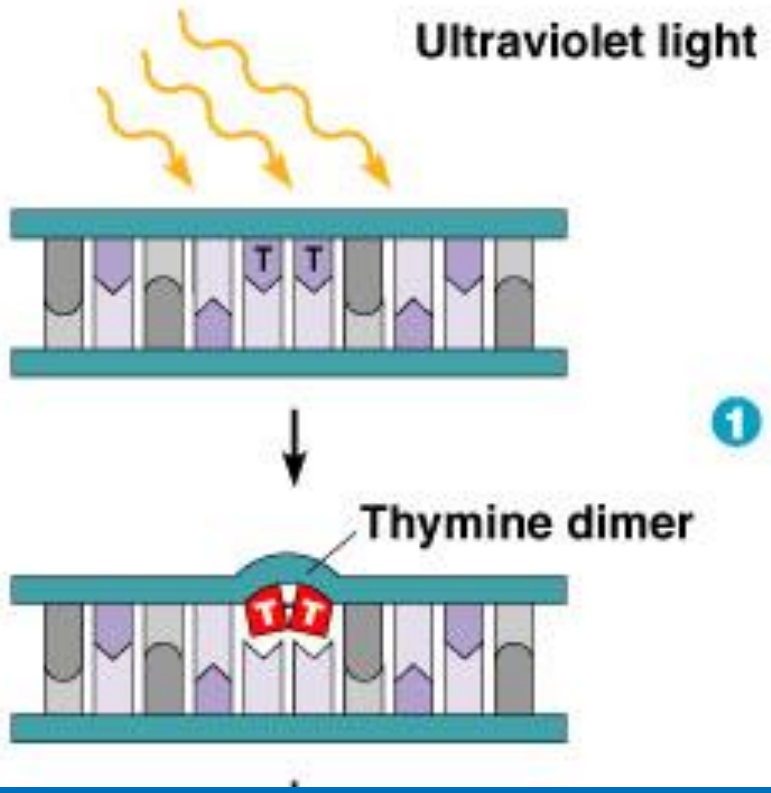


Figure 7.5

Mutations: UV pyrimidine dimers



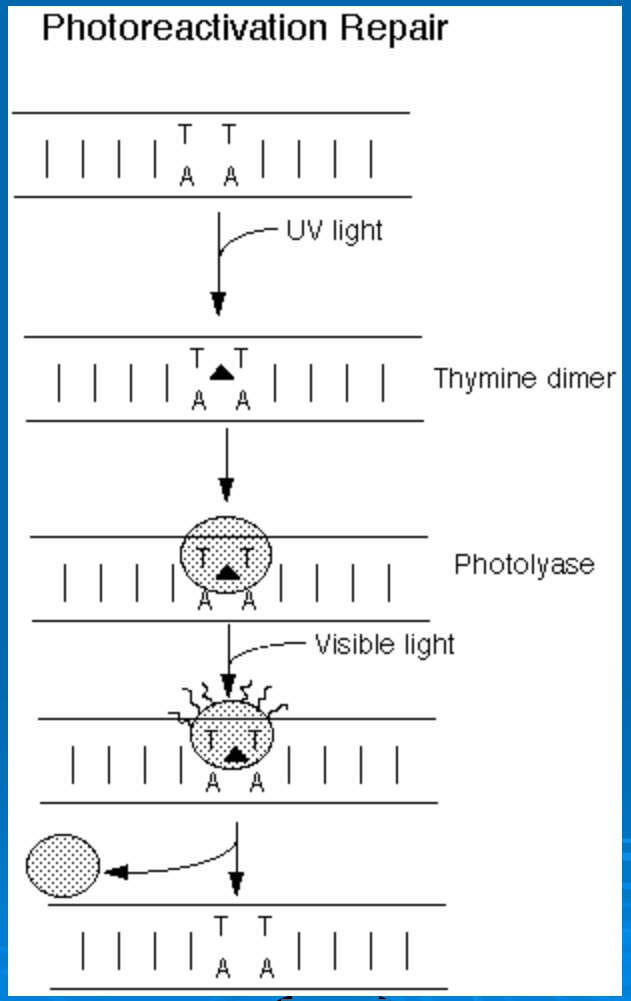
1 Exposure to ultraviolet lighcauses adjacent thymines to become cross-linked, forming a thymine dimer and disrupting their normal base pairing.

Figure 7.5

Mutations: UV pyrimidine dimers

Solutions:

1. **Photoreactivation**
   - Photolyases remove dimers

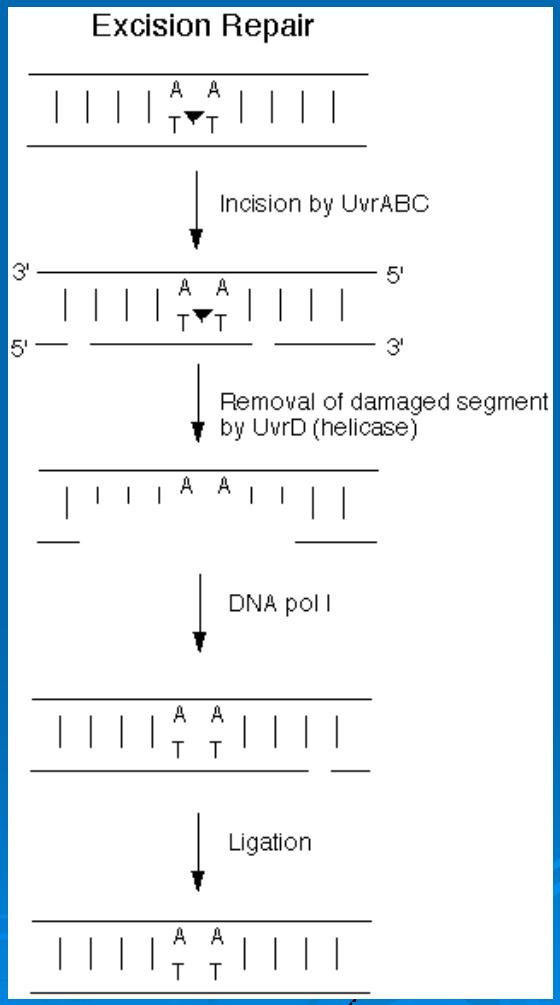


Mutations: UV pyrimidine dimers

Solutions:

1. **Photoreactivation**
   - Photolyases remove dimers

2. **Nucleotide excision repair**
   - Can repair other mutation damage (not just radiation)



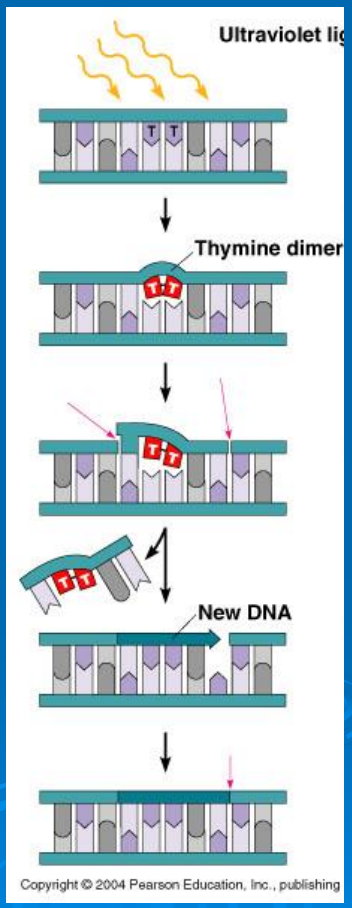


Figure 7.5

Mutations: UV pyrimidine dimers

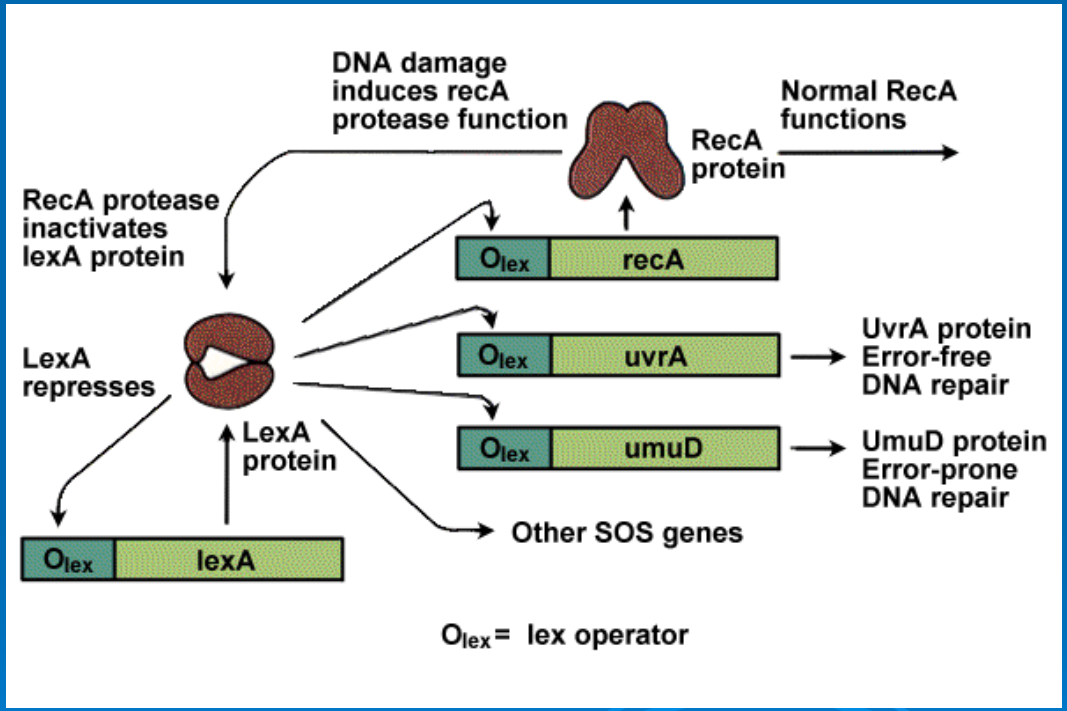
Solutions:

1. **Photoreactivation**
   - Photolyases remove dimers

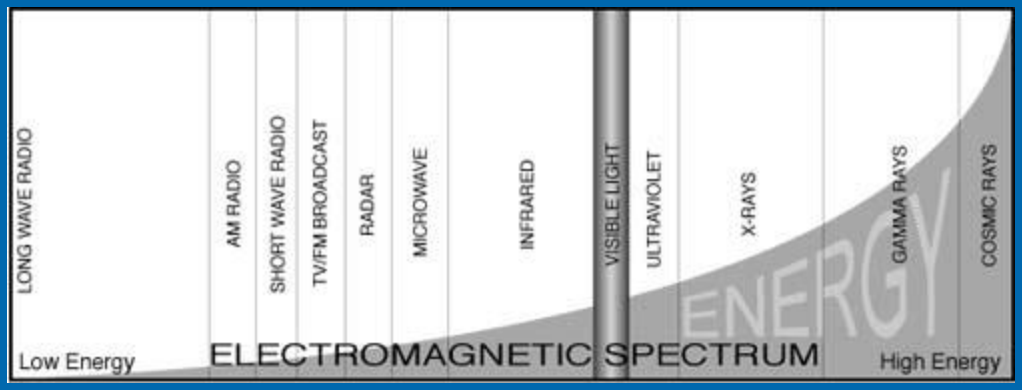
2. **Nucleotide excision repair**
   - Can repair other damage

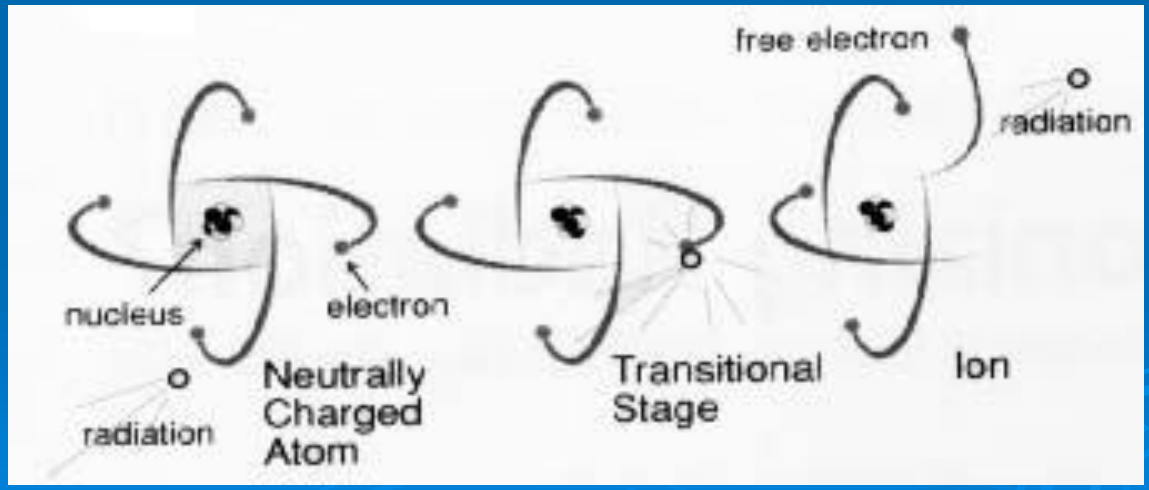
When all else fails…

3. **SOS! repair system**
   - Slows cell division, but results in mutations



Mutations: ionizing radiation





Independent Study

1. Review material!

2. Preview the mechanisms of genetic transfer in microbes: transformation, conjugation and transduction. (see Figures 8.27, 8.28, and 8.30)