Genetics
Objectives

- To understand how nucleic acids transport genetic information
- To understand experiments that showed the role of nucleic acids for genetic information
- To learn which enzymes are involved in genetic information flow
- To distinguish mechanisms of genetic exchange
- To understand mutations
- To familiarize with molecular biology tools

1) Structure and Function of Genetic Material

- Genotype: gene
- Phenotype
- Genome: Chromosome, Plasmids

•Genome sizes are expressed in kilobases (1 kb=1000 bp) or megabases (1 Mb=1000000 bp).

•Kilobases are related to other units : 1 µm of linear duplex DNA has approximate a molecular weight of 2 million daltons and contains approximately 3 kb of DNA.

- One megabase of duplex DNA has a mass of 1 fg (10^{-15} g).
- Genome sizes of bacteriophages and viruses range from a few thousand bases to several hundred kilobases.
- Bacterial genomes range from 0.5 Mb to 14 Mb.

•Eukaryotic genomes are diverse, from approximately 10 Mb in some fungi to more than 100000 Mb in certain plants.

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•Genome size in eukaryotes is poorly correlated with organismal complexity. For example, the largest genome known is that of the protozoan *Amoeba dubia*, at 670000 Mb.

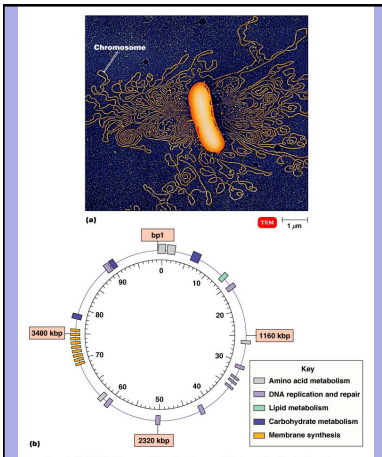


Figure 8.1 - Overview

***E. coli* genome is 4.6 million base pairs= 4.6 Mb;**

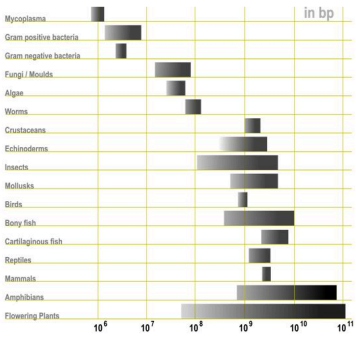
~ 1mm long (1000X cell),

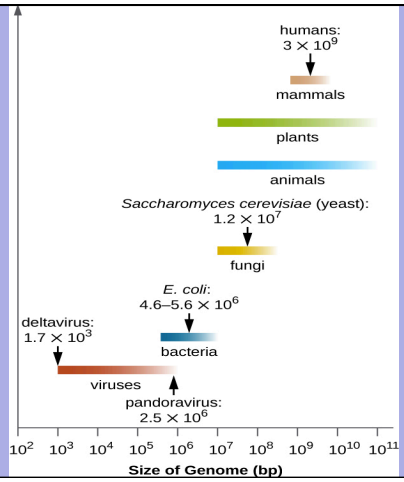
10% cell volume= supercoiled or twisted

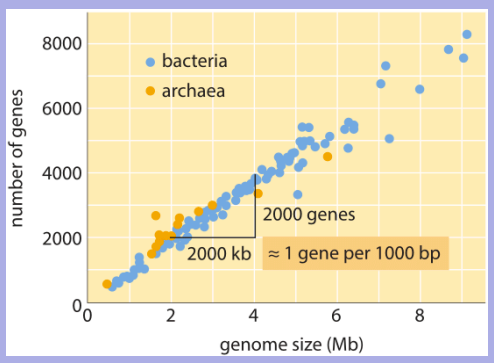
Genome Size Variation

Genome size generally increases as body size increases

In general, larger organisms tend to have larger genomes and greater genome complexity (though there are exceptions)







	Organism	# of protein-coding genes	# of genes naïve estimate: (genome size /1000)	BNID
viruses	HIV 1	9	10	105769
	Influenza A virus	10-11	14	105767
	Bacteriophage λ	66	49	105770
	Epstein Barr virus	80	170	103246
prokaryotes	*Buchnera sp.*	610	640	105757
	T. maritima	1,900	1,900	105766
	S. aureus	2,700	2,900	105500
	V. cholerae	3,900	4,000	105760
	B. subtilis	4,400	4,200	111448
	E. coli	4,300	4,600	105443
eukaryotes	*S. cerevisiae*	6,600	12,000	105444
	C. elegans	20,000	100,000	101364
	A. thaliana	27,000	140,000	111380
	D. melanogaster	14,000	140,000	111379
	F. rubripes	19,000	400,000	111375
	Z. mays	33,000	2,300,000	110565
	M. musculus	20,000	2,800,000	100308
	H. sapiens	21,000	3,200,000	100399, 111378
	T. aestivum (hexaploid)	95,000	16,800,000	105448, 102713

Genomes and Proteomes

Organism	Year Sequenced and Annotated	Genome Size (Base pairs)	Proteome Size (Number of Proteins)
Mycoplasma Genitalium	1995	~588,000	480
Haemophilus Influenzae	1995	~1,500,000	1,709
Escherichia Coli	1997	~4,600,000	4,289
Saccharomyces Cerevisiae	1997	~11,000,000	~6,600
Caenorhabditis Elegans	1998	~86,000,000	~14,300
Drosophila Melanogaster	2000	~137,000,000	~13,500
Homo Sapiens	~Jan 2001	~3,100,000,000	~30,000-60,000

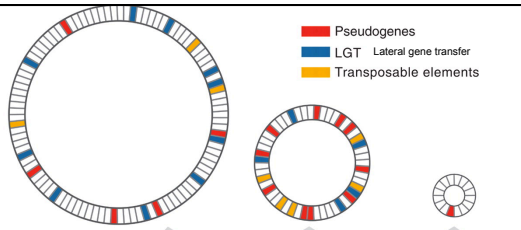
~~32~~ ~~35~~ 37

31 complete microbial genomes (~~87~~ 90 in progress)

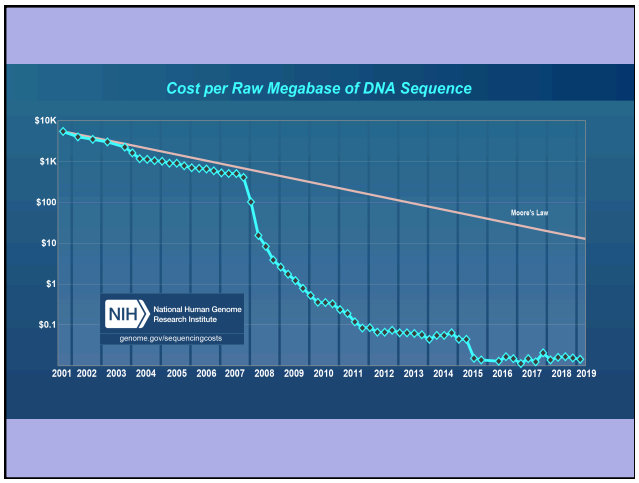
Many new microbial genomes every year

Many other higher organisms' genomes being sequenced

9



	Free-living	Recent or facultative pathogen	Obligate symbiont or pathogen
Genome size	Large (5-10 MB)	Intermediate (2-5 MB)	Small (0.5-1.5 MB)
Number of pseudogenes	Few	Many	Rare
Incidence of LGT	Frequent	Frequent to rare	Rare to none
Selfish genetic elements	Few	Common	Rare
Genome organization	Stable or unstable	Unstable	Stable
Effective population size	Large	Small	Small



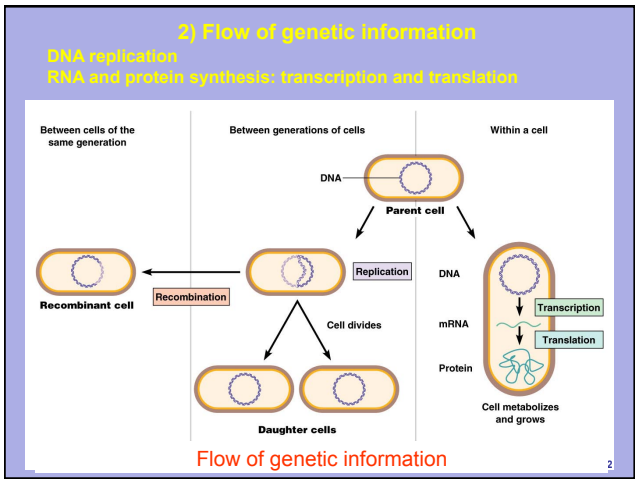


TABLE 8.1	Important Enzymes in DNA Replication, Expression, and Repair
DNA gyrase	Relaxes supercoiling ahead of the replication fork.
DNA ligase	Makes covalent bonds to join DNA strands; joins Okazaki fragments and new segments in excision repair.
DNA polymerase	Synthesizes DNA; proofreads and repairs DNA.
Endonucleases	Cut DNA backbone in a strand of DNA; facilitate repair and insertions.
Exonucleases	Cut DNA from an exposed end of DNA; facilitate repair.
Helicase	Unwinds double-stranded DNA.
Methylase	Adds methyl group to selected bases in newly-made DNA.
Photolyases	Use visible light energy to separate UV-induced pyrimidine dimers.
Primase	Makes RNA primers from a DNA template.
Ribozyme	RNA enzyme that removes introns and splices exons together.
RNA polymerase	Copies RNA from a DNA template.
Topoisomerase	Relaxes supercoiling ahead of the replication fork; separates DNA circles at the end of DNA replication.
Transposase	Cuts DNA backbone leaving single-stranded "sticky ends."

Table 8.1

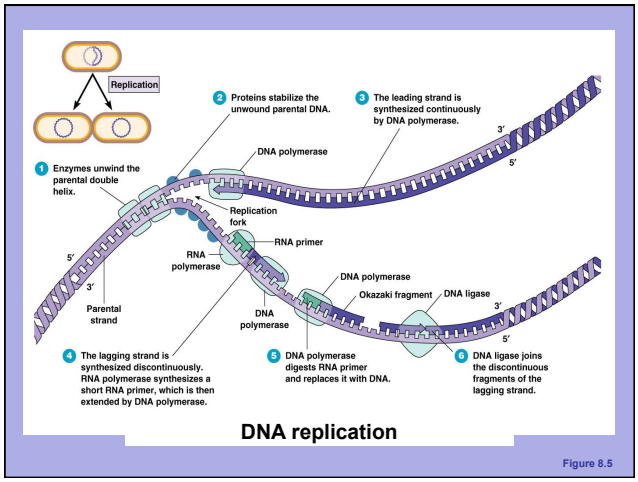


Figure 8.5

Bacterial DNA replication

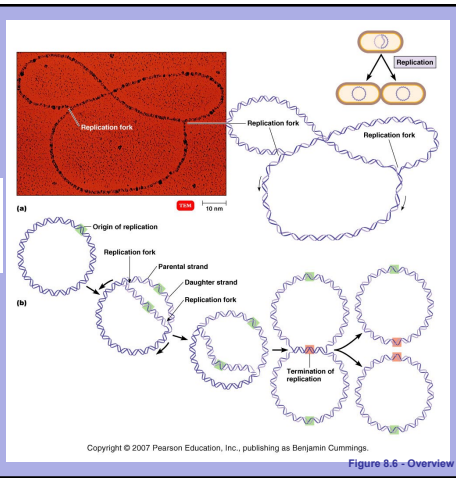


Figure 8.6 - Overview

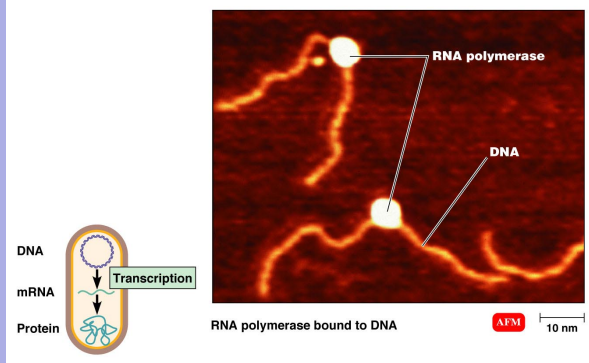


Figure 8.7 (2 of 7)

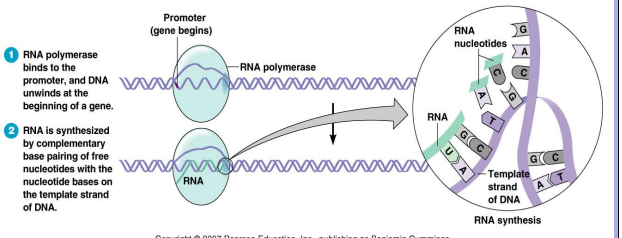


Figure 8.7 (3 of 7)

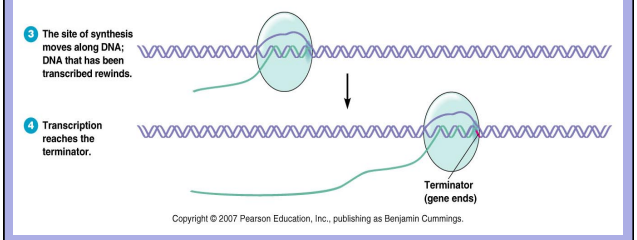
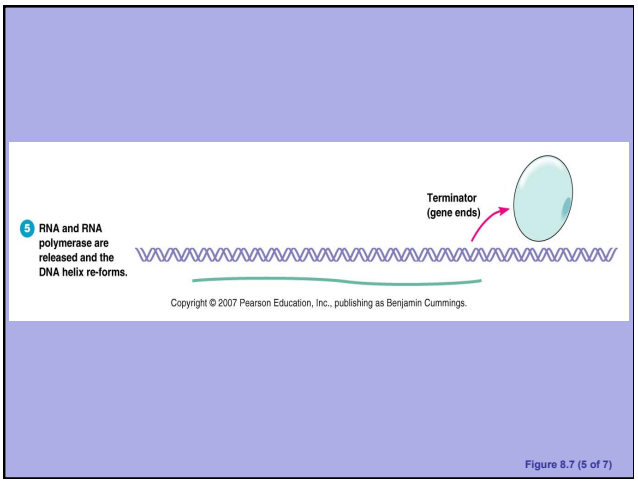


Figure 8.7 (4 of 7)



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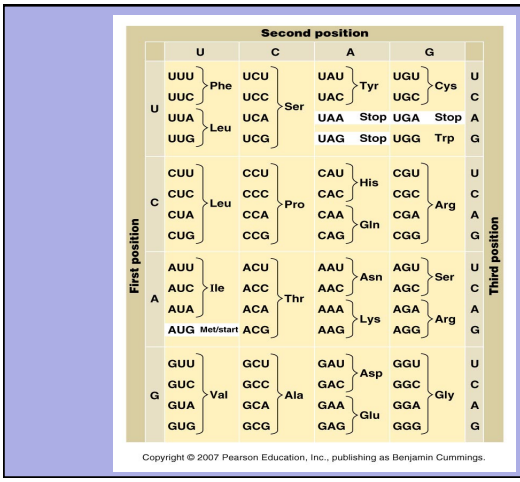

Figure 8.8

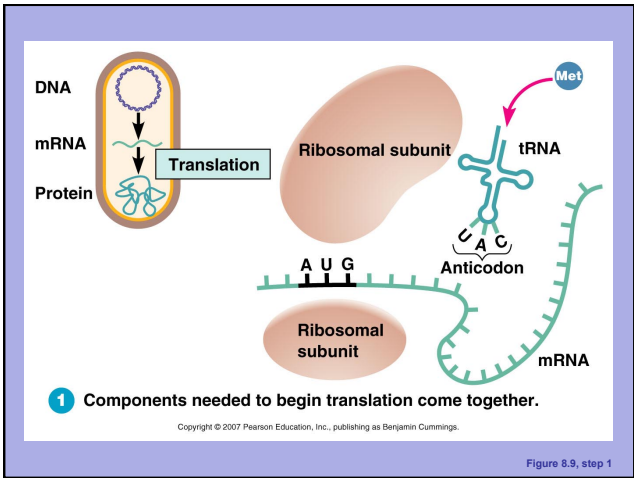

Figure 8.9, step 1

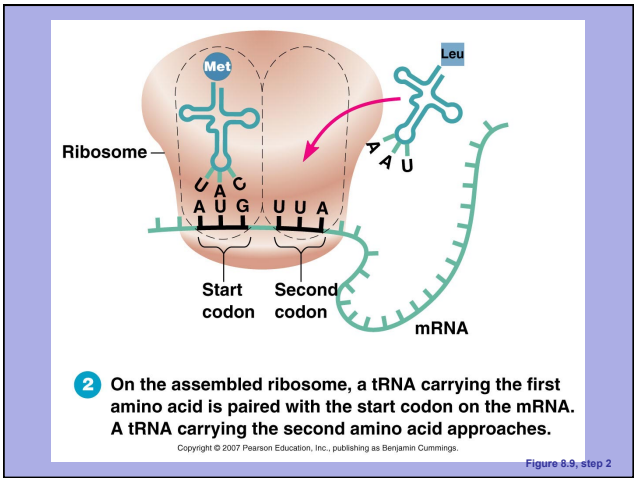

Figure 8.9, step 2

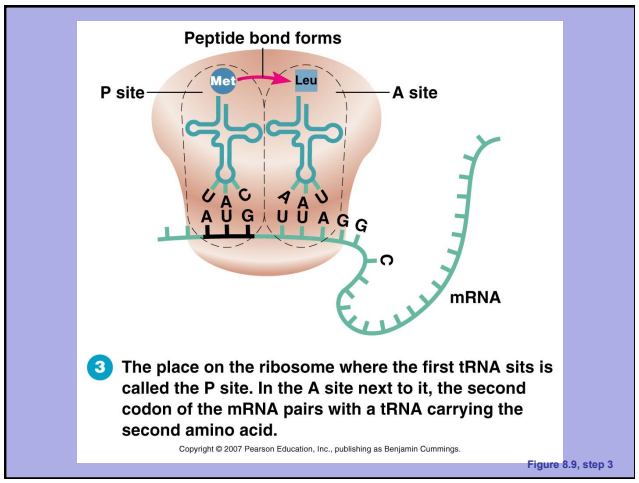
Peptide bond forms
P site
Met
Leu
A site
mRNA
3 The place on the ribosome where the first tRNA sits is called the P site. In the A site next to it, the second codon of the mRNA pairs with a tRNA carrying the second amino acid.
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Figure 8.9, step 3

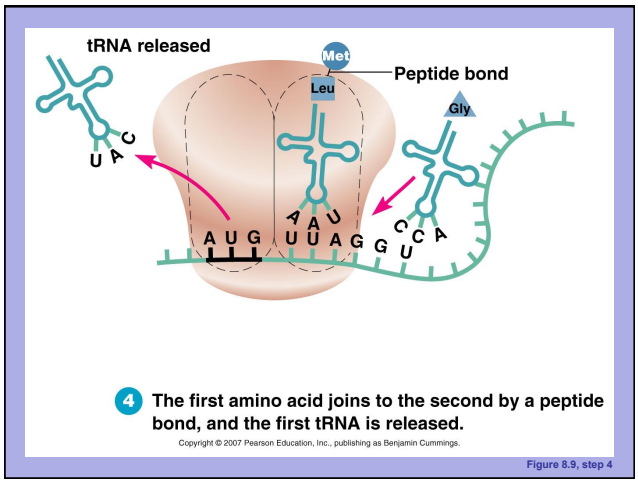
tRNA released
Met
Leu
Peptide bond
Gly
4 The first amino acid joins to the second by a peptide bond, and the first tRNA is released.
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Figure 8.9, step 4

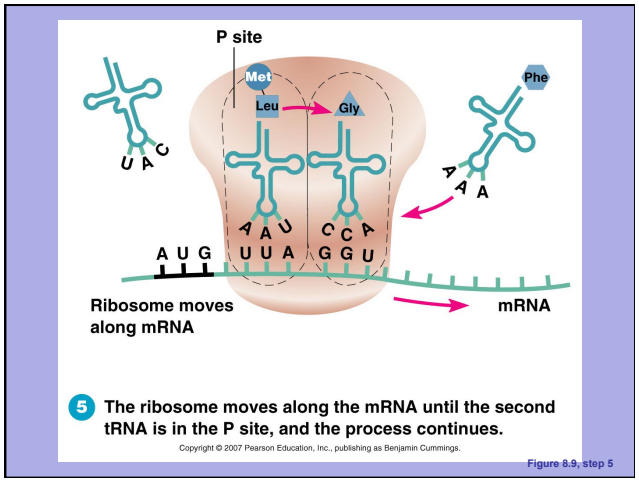
P site
Met
Leu
Gly
Phe
Ribosome moves along mRNA
mRNA
5 The ribosome moves along the mRNA until the second tRNA is in the P site, and the process continues.
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Figure 8.9, step 5

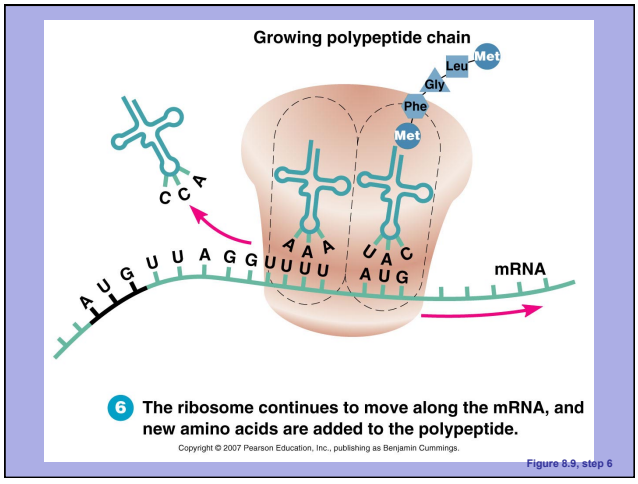
Growing polypeptide chain
Met
Leu
Gly
Phe
Met
mRNA
6 The ribosome continues to move along the mRNA, and new amino acids are added to the polypeptide.
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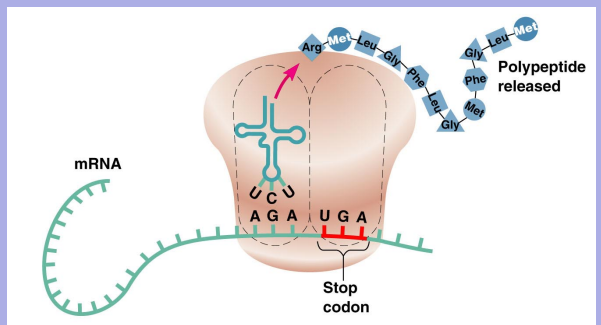
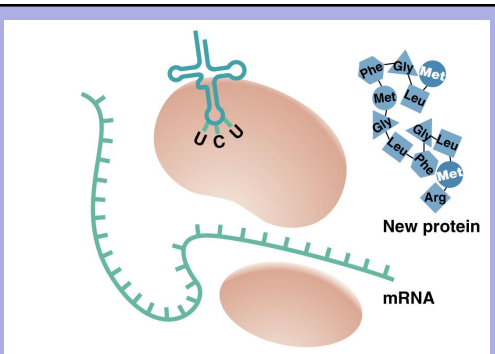
Figure 8.9, step 6



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

Figure 8.9, step 7



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

Figure 8.9, step 8

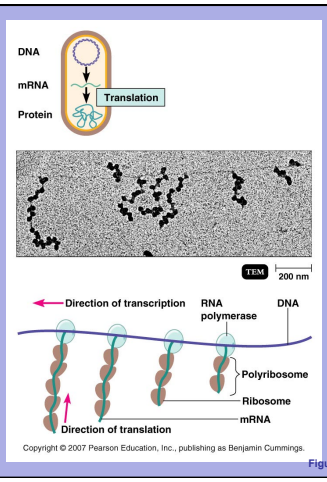


Figure 8.10 - Overview (1 of 4)

L3 . Genetics 2.

Regulation of bacterial gene expression

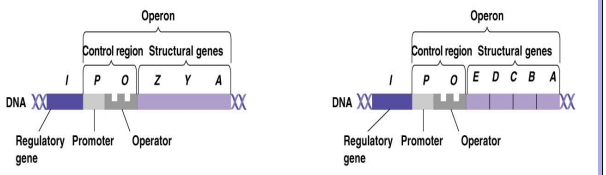
Mutations

Genetic Exchange in Prokaryotes

3) Regulation of bacterial gene expression

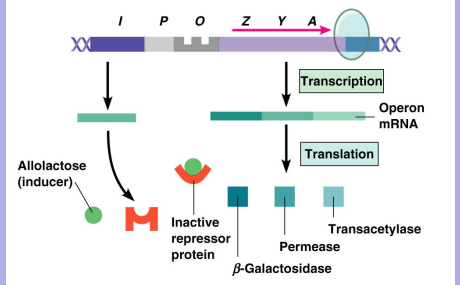
Repression and Induction

The operon model



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

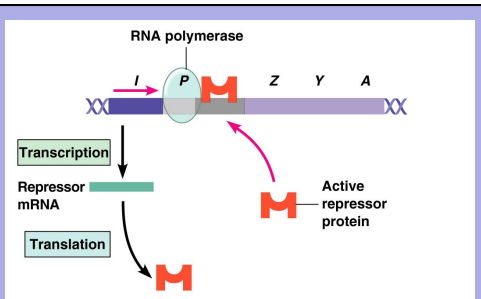
Figure 8.12, step 1



3 Repressor inactive, operon on. When the inducer allolactose binds to the repressor protein, the inactivated repressor can no longer block transcription. The structural genes are transcribed, ultimately resulting in the production of the enzymes needed for lactose catabolism.

(a) An inducible operon

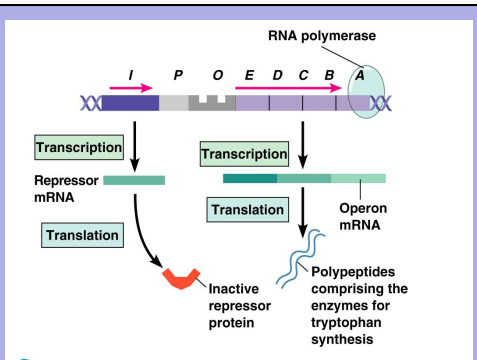
Figure 8.12a, step 3



2 Repressor active, operon off. The repressor protein binds with the operator, preventing transcription from the operon.

(a) An inducible operon

Figure 8.12a, step 2



2 Repressor inactive, operon on. The repressor is inactive, and transcription and translation proceed, leading to the synthesis of tryptophan.

(b) A repressible operon

Figure 8.12b, step 2

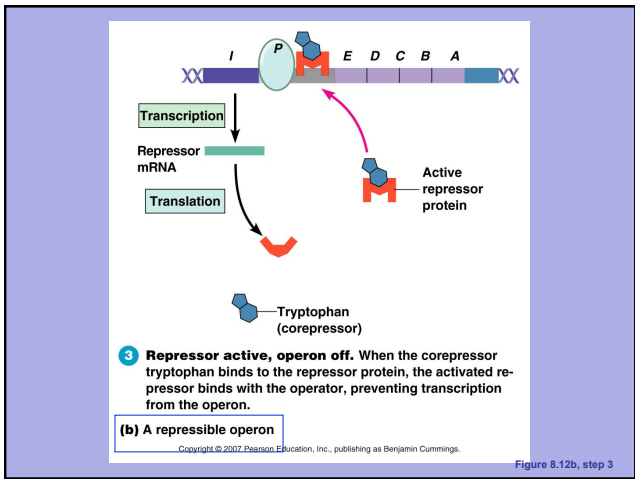
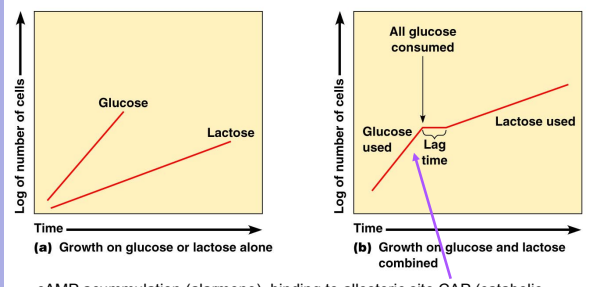


Figure 8.12b, step 3

Positive Regulation



cAMP acummulation (alarmone), binding to allosteric site CAP (catabolic activator protein), followed by binding to *lac* promoter

Figure 8.13 - Overview

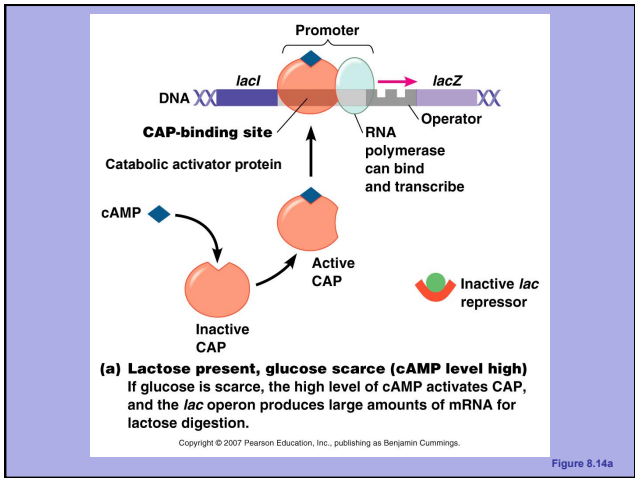


Figure 8.14a

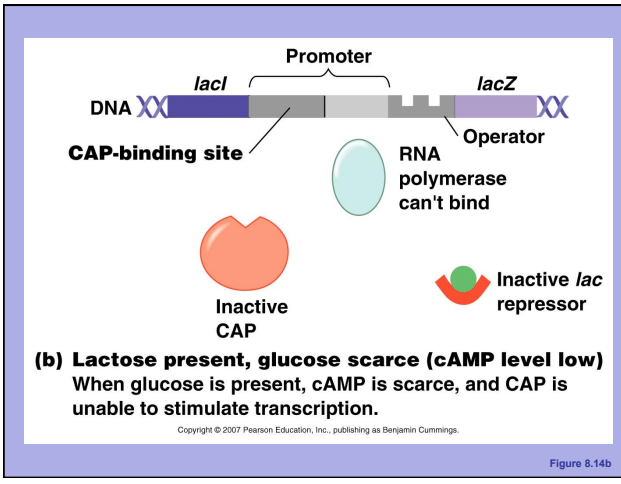
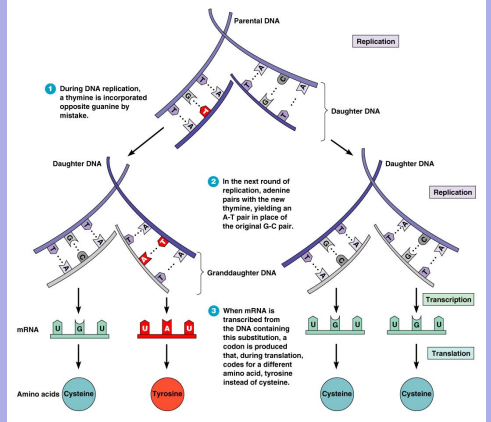


Figure 8.14b

Mutations



Base substitution

– Overview

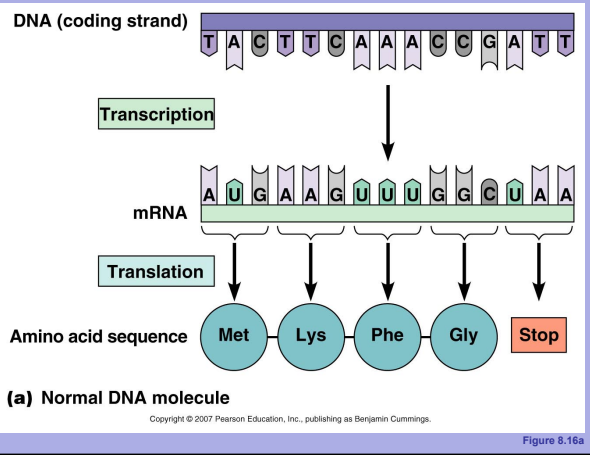


Figure 8.16a

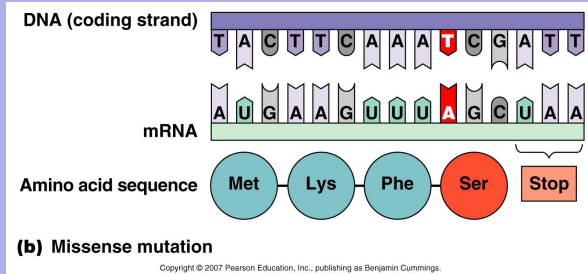
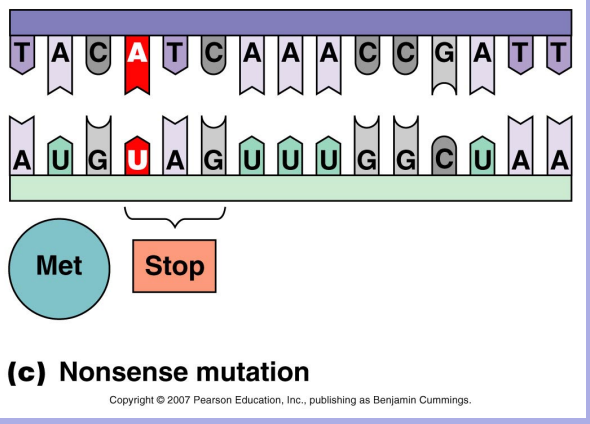
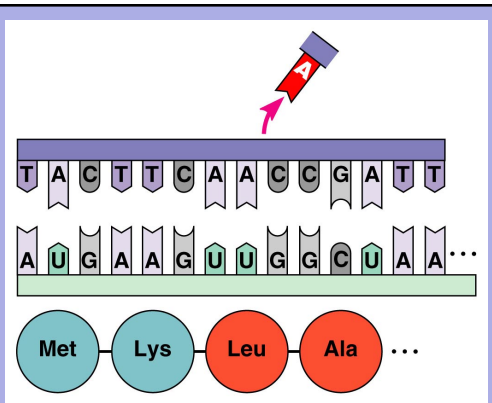


Figure 8.16b



(c) Nonsense mutation

Figure 8.16c



(d) Frameshift mutation

Figure 8.16d

Mutagens are chemical, physical, or biological agents that increase the mutation rate.

Mutagens can alter DNA in many different ways. However, alterations in DNA are not mutations unless they can be inherited.

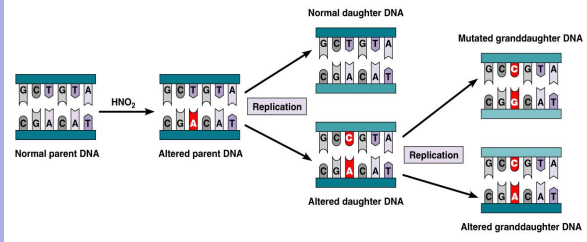
Some DNA damage can lead to cell death if not repaired, and both error-prone as well as high-fidelity DNA repair systems exist.

Table 10.3 Types of mutant strains

Designation	Phenotype
Auxotroph	Requires an exogenous growth factor, e.g., an amino acid or vitamin
Carbon source	Unable to use a particular compound as a source of carbon
Nitrogen source	Unable to use a particular compound as a source of nitrogen
Phosphorus source	Unable to use a particular compound as a source of phosphorus
Sulfur source	Unable to use a particular compound as a source of sulfur
Temperature sensitive	Loses a particular function at a high or low temperature
Heat sensitive	Loses a particular function at a high temperature
Cold sensitive	Loses a particular function at a low temperature
Osmotic sensitive	Loses a particular function at high or low osmolarity
Conditional lethal	Unable to grow in a particular environment (e.g., high temperature) in any medium

Table 10.7 Some physical and chemical mutagens

Agent	Mutagenic action
Physical agents	
X rays	Cause double-strand breaks in DNA, the repair of which leads to macrolesions
UV light	Cause adjacent pyrimidines in DNA to join at positions 4 and 5, forming dimers, which in the process of their repair result mostly in transversions, but also in frameshifts and transitions
Chemical agents	
Base analogs	Become incorporated in DNA and then, owing to their ambiguous pairing on subsequent replication, cause transitions
2-Aminopurine	Can pair with either thymine or cytosine
5-Bromouracil	Can pair with either adenine or guanine
DNA modifiers	
Nitrous acid	Deaminates bases; deamination of cytosine produces uracil and then a CG-to-TA transition
Hydroxylamine	Hydroxylates 6 amino group of cytosine, causing CG-to-TA transition
Alkylating agents (e.g., nitrosoguanidine and ethyl methane sulfonate)	Alkylate DNA bases, distorting DNA structure and resulting in a variety of types of mutations
Intercalating agents (e.g., acridine orange and ethidium bromide)	Intercalate between stacked bases in DNA; replication results in frameshift mutations



Nitrous acid (HNO_2) mutation

Figure 8.17 - Overview (1 of 3)

Nucleoside analogs

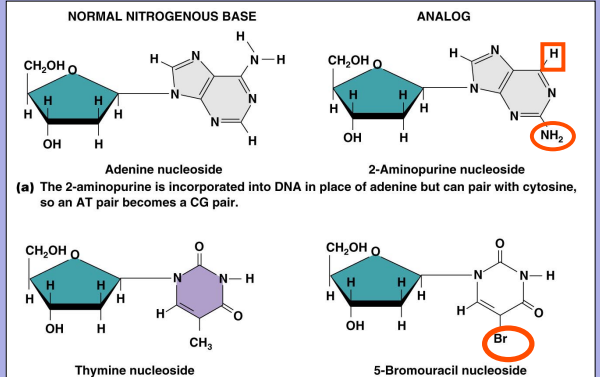


Figure 8.18 - Overview

Thymine dimers

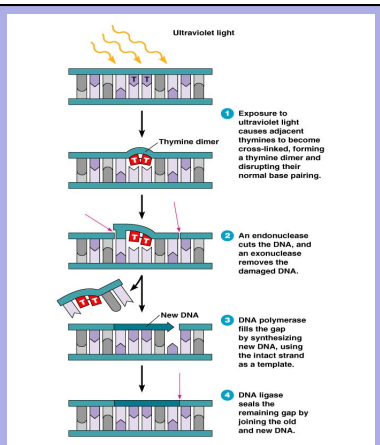


Figure 8.19 - Overview

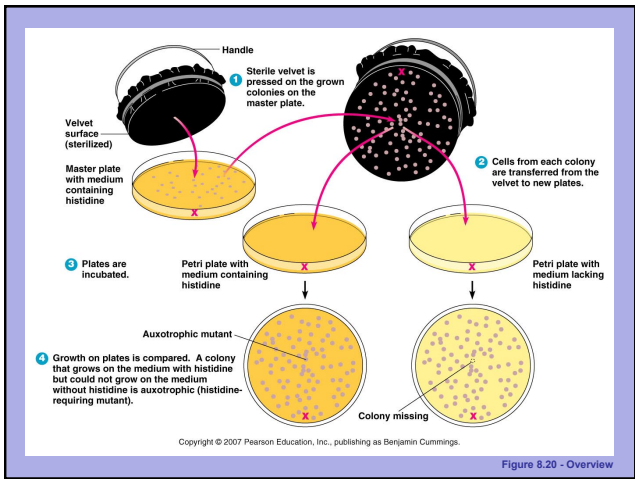


Figure 8.20 - Overview

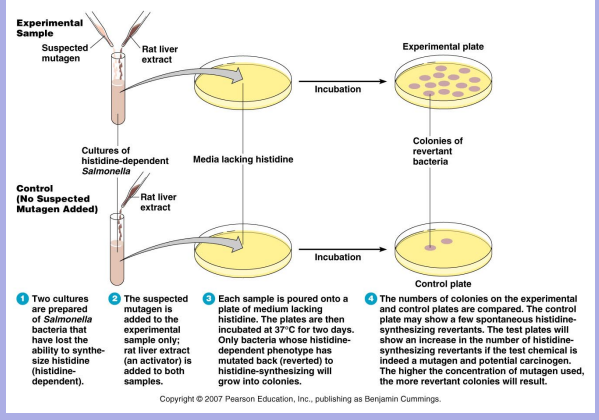
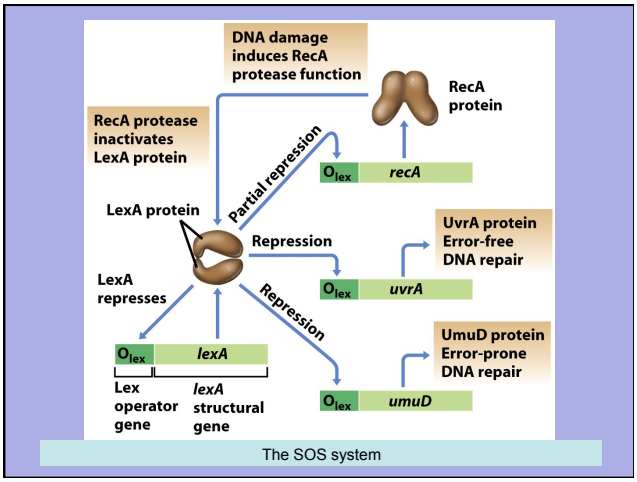
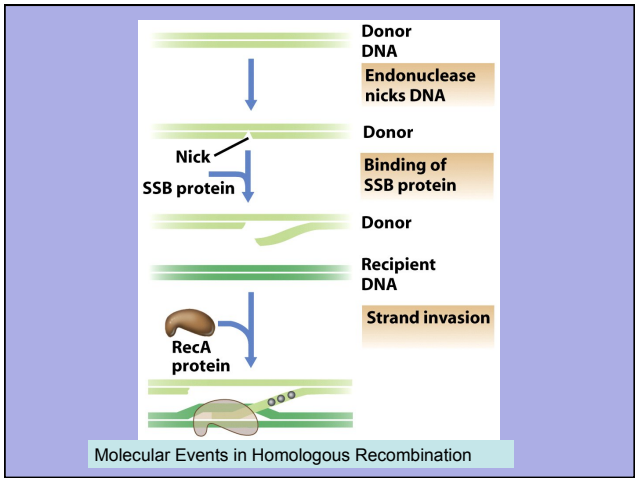


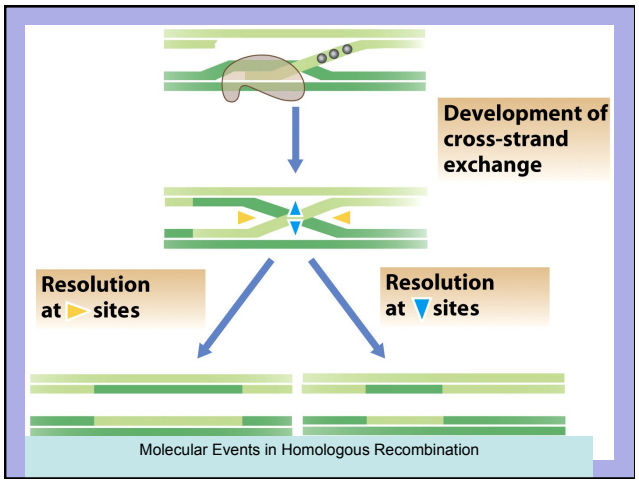
Figure 8.21 - Overview (1 of 3)



The SOS system



Molecular Events in Homologous Recombination



Molecular Events in Homologous Recombination

Genetic Exchange in Prokaryotes

Griffith's experiment

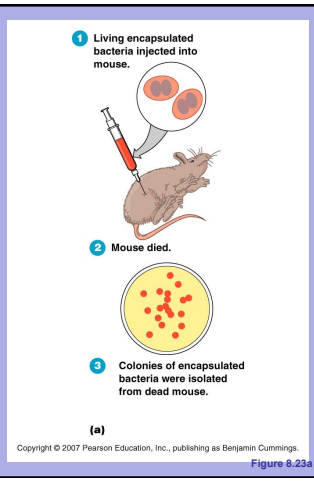


Figure 8.23a

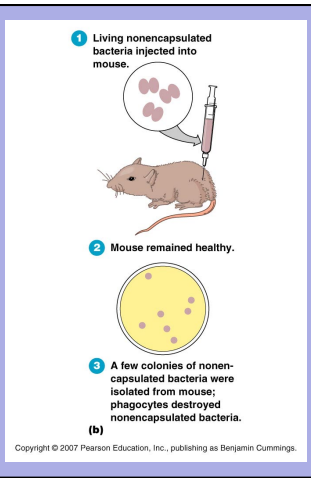


Figure 8.23b

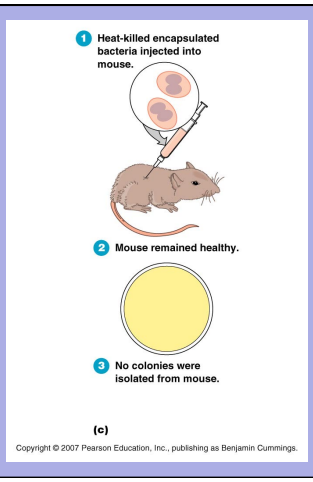


Figure 8.23c

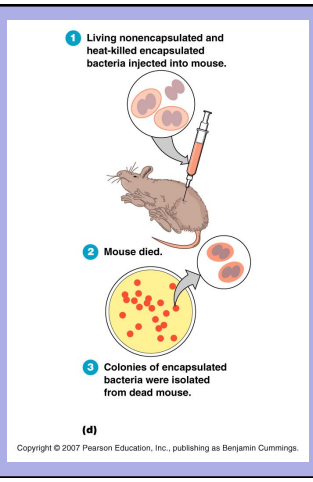
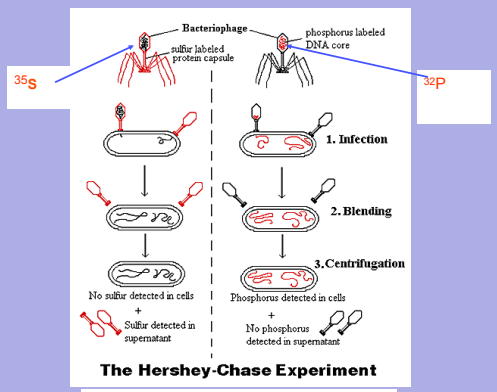


Figure 8.23d



Only DNA component carries the genetic information

Genetic Exchange of Information

- Conjugation
- Transformation
- Transduction

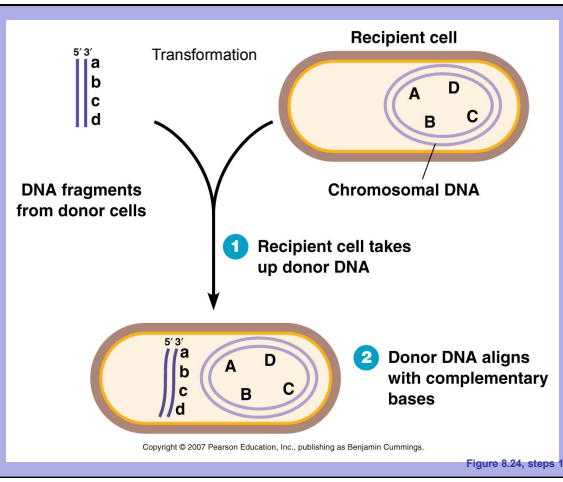


Figure 8.24, steps 1–2

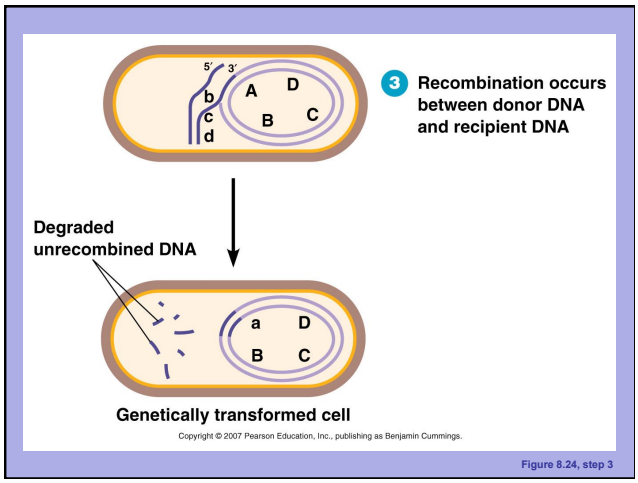


Figure 8.24, step 3

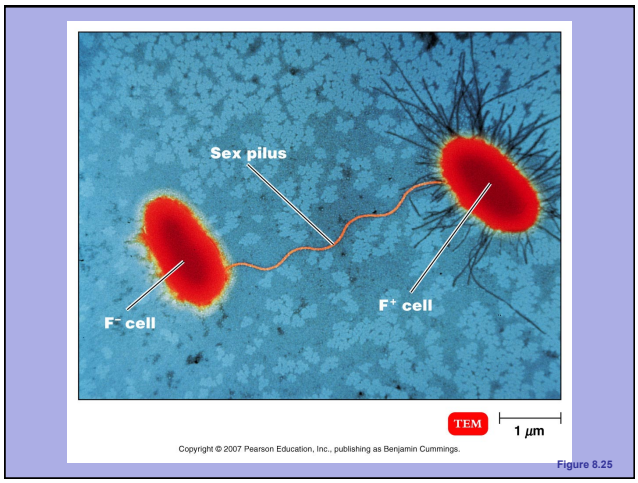


Figure 8.25

Conjugation

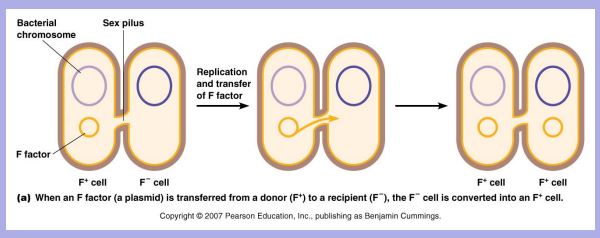


Figure 8.26a

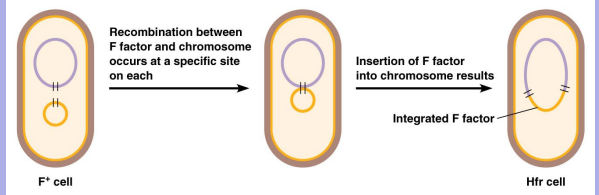
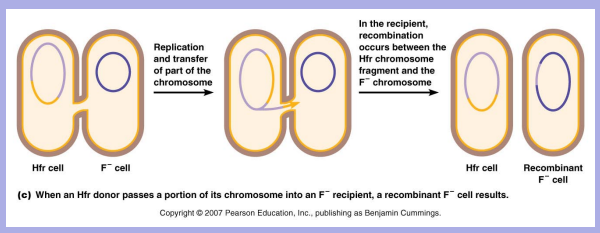
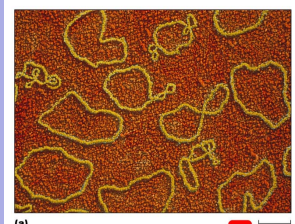


Figure 8.26b



(c) When an Hfr donor passes a portion of its chromosome into an F⁻ recipient, a recombinant F⁻ cell results.

Figure 8.26c



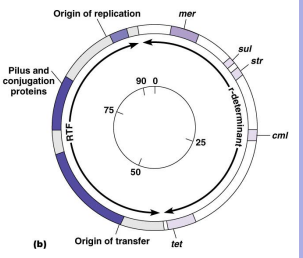
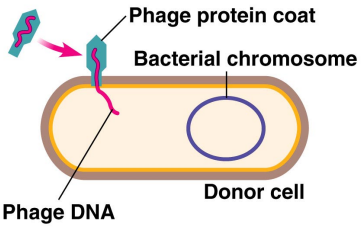


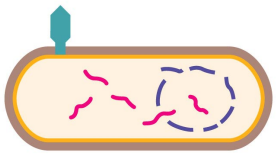
Figure 8.28 - Overview

Transduction



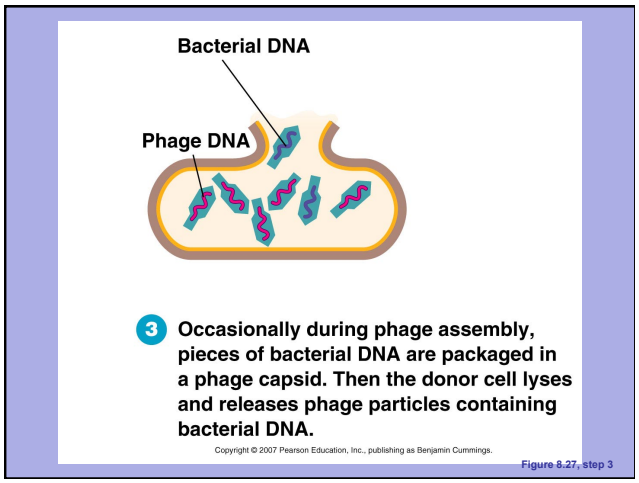
1 A phage infects the donor bacterial cell.

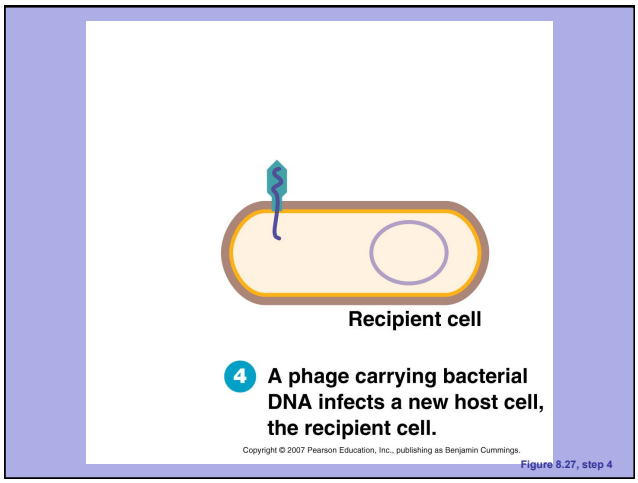
Figure 8.27, step 1



2 Phage DNA and proteins are made, and the bacterial chromosome is broken into pieces.

Figure 8.27, step 2





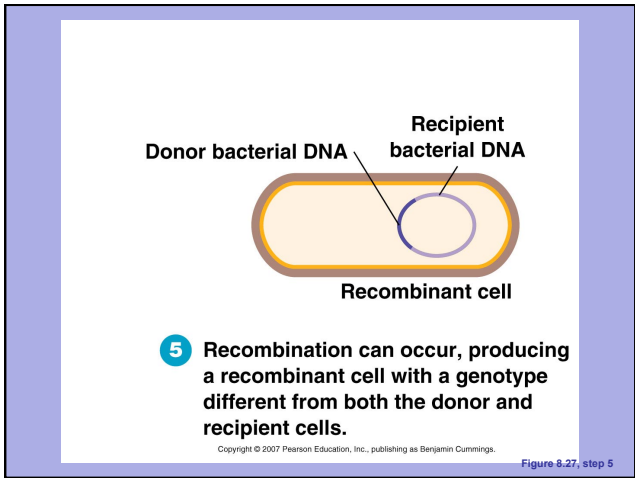
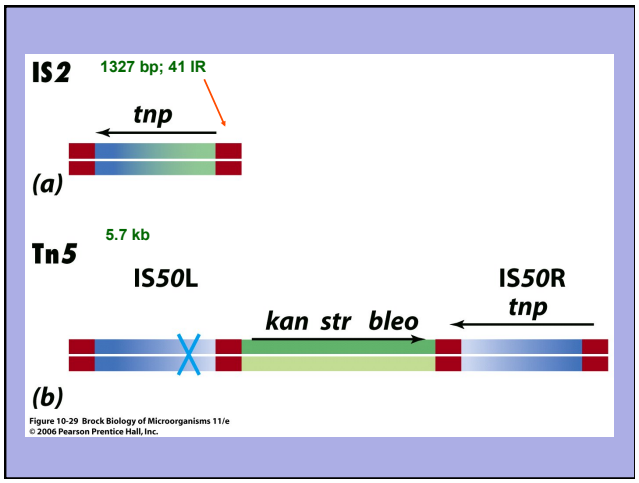


Table 10.6 Properties of transposons

Designation	Characteristics
Kinds of transposons	
Insertion sequences	Relatively short pieces of DNA, 750 to 2,000 bp long, that encode only a transposase; designated IS followed by an italicized number, e.g., IS*1*, IS2, IS*3*
Composite transposons	One or more genes flanked by matching insertion sequences; designated Tn followed by an italicized number, e.g., Tn*5*, Tn*10*
Mechanism of transposition	
Cut and paste	The transposon is cut out of the DNA where it resides and is inserted in a new location.
Replicative	The transposon is replicated; one copy remains at its original location, and the other is located at a new one.


IS2
1327 bp; 41 IR
tnp
(a)
5.7 kb
Tn5
IS50L
IS50R
tnp
kan str bleo
(b)
Figure 10-29 Brock Biology of Microorganisms 11/e
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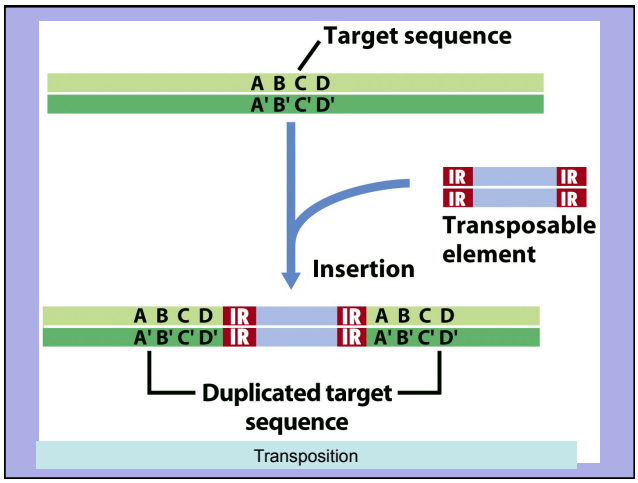
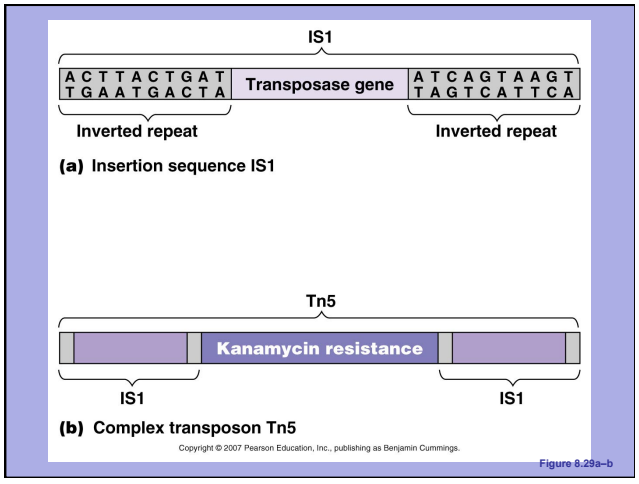

Target sequence
A B C D
A' B' C' D'
IR
IR
IR
IR
Transposable element
Insertion
A B C D IR IR A B C D
A' B' C' D' IR IR A' B' C' D'
Duplicated target sequence
Transposition


IS1
A C T T A C T G A T
T G A A T G A C T A
Transposase gene
A T C A G T A A G T
T A G T C A T T C A
Inverted repeat
Inverted repeat
(a) Insertion sequence IS1
Tn5
Kanamycin resistance
IS1
IS1
(b) Complex transposon Tn5
Copyright © 2007 Pearson Education, Inc., publishing as Benjamin Cummings.
Figure 8.29a–b

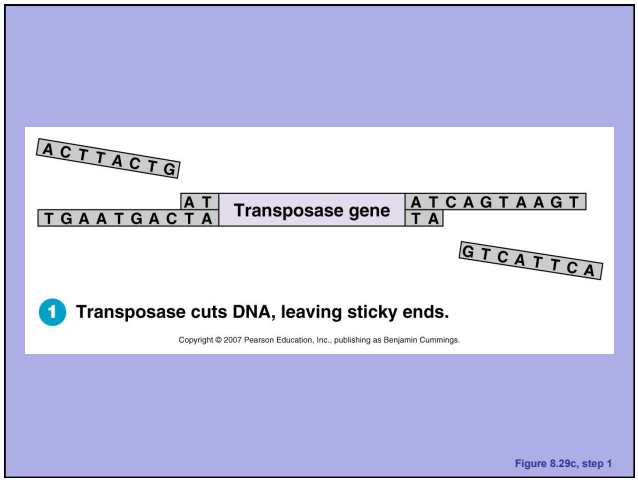

A C T T A C T G
A T
T G A A T G A C T A
Transposase gene
A T C A G T A A G T
T A
G T C A T T C A
1 Transposase cuts DNA, leaving sticky ends.
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Figure 8.29c, step 1

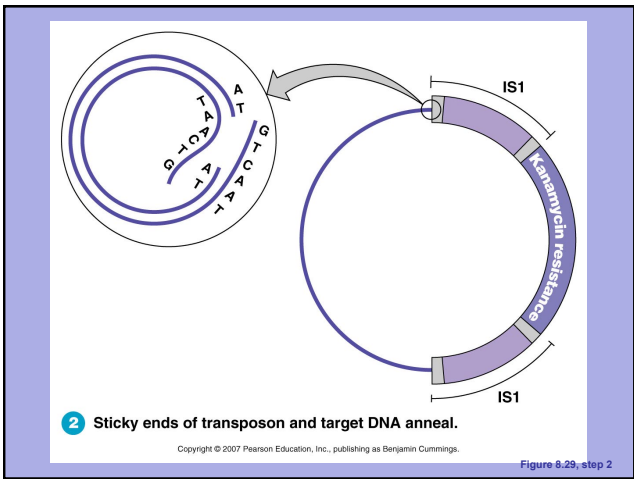

Figure 8.29, step 2