

Microbiology

Chapter 8

Microbial Genetics

本內容已由授課教師方翠筠修訂

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Structure and Function of Genetic Material

Genetics (遺傳學) : The study of what genes are, how they carry information, how information is expressed, and how genes are replicated

Gene (基因): A segment of DNA that encodes a functional product, usually a protein

Chromosome (染色體): Structure containing DNA that physically carries hereditary information; the chromosomes contain the genes

Genome (基因體): All the genetic information in a cell

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- **Genomics** (基因體學): The molecular study of genomes
- **Genotype**: The genes of an organism
- **Phenotype**: Expression of the genes

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Determine Relatedness

Australia	A	C	C	C	C	G	T	C	C	A	C	C	C	T	T	T	C	A	A	T	T
Egypt	A	A	T	C	G	A	T	C	A	T	C	T	T	C	G	T	C	G	A	T	C
France	A	A	T	C	G	A	T	C	A	T	C	G	T	C	G	T	C	G	A	T	C
Israel	A	T	C	C	A	T	T	C	A	T	C	C	T	C	A	T	C	G	A	T	T
Italy	A	T	C	C	A	C	T	C	A	T	C	C	T	C	G	T	C	G	A	T	T
Kenya	A	T	C	C	A	C	T	C	A	T	C	C	T	C	G	T	C	G	A	T	T
Mexico	A	A	C	C	C	T	T	C	C	T	C	C	C	C	T	T	C	G	A	T	T
United States	A	A	C	C	C	C	T	C	C	T	C	C	C	C	T	T	C	G	A	T	T
Uganda	A	T	A	C	G	A	T	C	A	T	G	C	T	C	G	T	C	C	A	T	C

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Clinical Focus, p. 223

Determine Relatedness

- Which strain is more closely related to the Uganda strain?

Strain	% Similar to Uganda
Kenya	71%
U.S.	51%

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E. coli

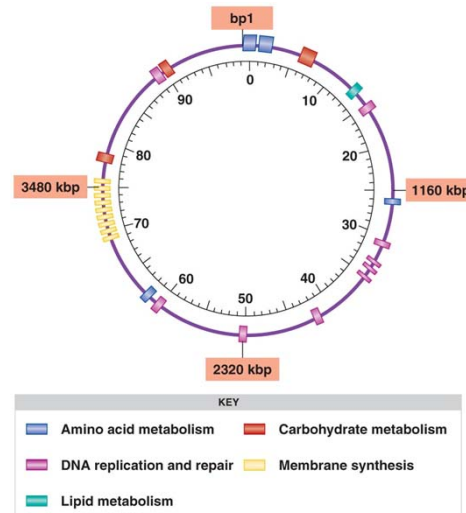


(a) The tangled mass and looping strands of DNA emerging from this disrupted *E. coli* cell are part of its single chromosome.

Figure 8.1a

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Genetic Map of the Chromosome of *E. coli*

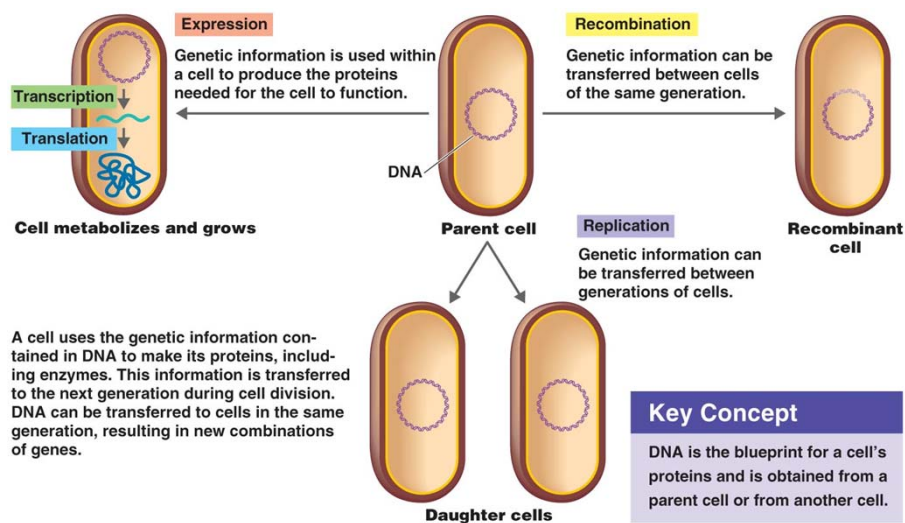


(b) A genetic map of the chromosome of *E. coli*. The numbers inside the circle indicate the number of minutes it takes to transfer the genes during mating between two cells; the numbers in colored boxes indicate the number of base pairs.

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Figure 8.1b

The Flow of Genetic Information

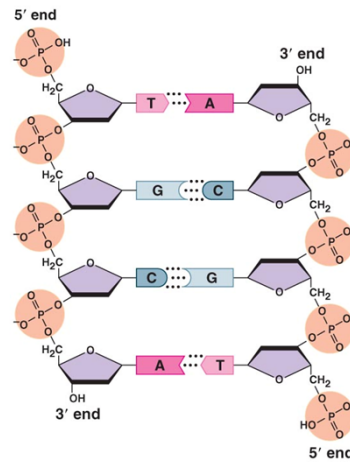


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Figure 8.2

DNA

- Polymer of nucleotides: Adenine, thymine, cytosine, and guanine
- Double helix associated with proteins
- "Backbone" is deoxyribose-phosphate
- Strands are held together by hydrogen bonds between AT and CG
- Strands are antiparallel



(b) The two strands of DNA are antiparallel. The sugar-phosphate backbone of one strand is upside down relative to the backbone of the other strand. Turn the book upside down to demonstrate this.

Figure 8.3b

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Semiconservative Replication

KEY	
T	Thymine
A	Adenine
C	Cytosine
G	Guanine
	Deoxyribose sugar
	Phosphate

- 1 The double helix of the parental DNA separates as weak hydrogen bonds between the nucleotides on opposite strands break in response to the action of replication enzymes.
- 2 Hydrogen bonds form between new complementary nucleotides and each strand of the parental template to form new base pairs.
- 3 Enzymes catalyze the formation of sugar-phosphate bonds between sequential nucleotides on each resulting daughter strand.

(a) The replication fork.

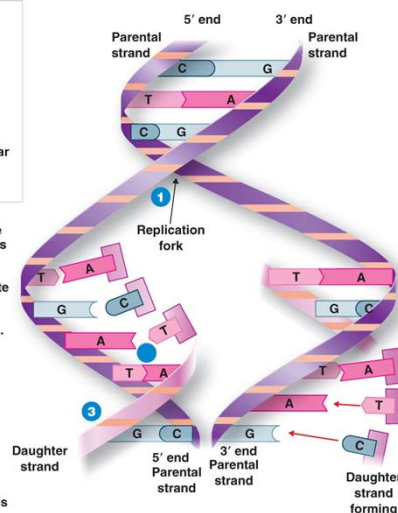


Figure 8.3a

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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

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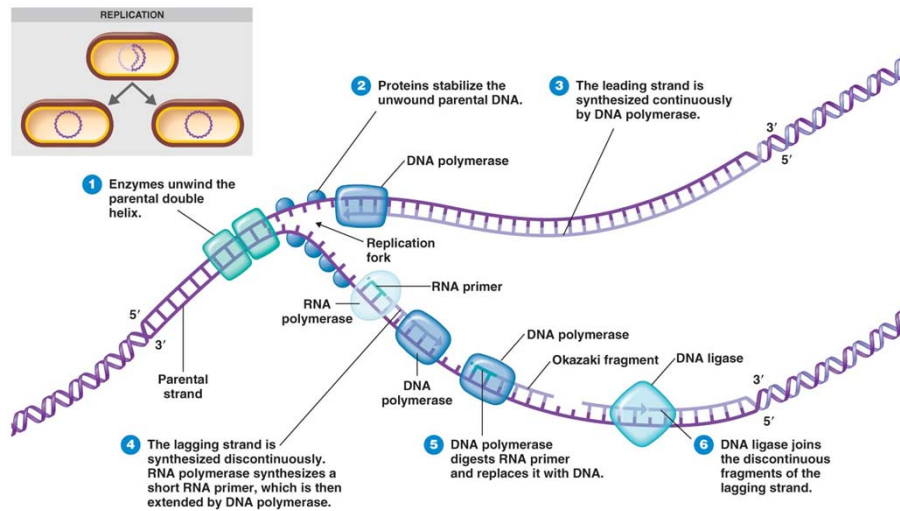
Table 8.1

Table 8.1	Important Enzymes in DNA Replication, Expression, and Repair
Photolyase	Uses 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
snRNP	RNA-protein complex that removes introns and splices exons together
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"

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Table 8.1

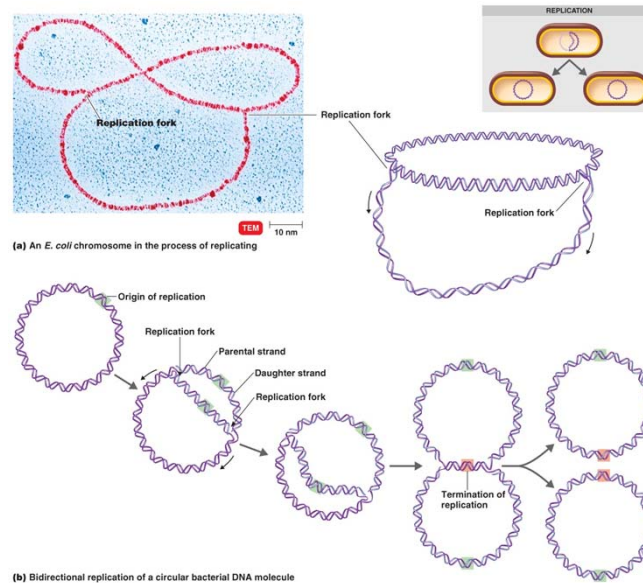
DNA Synthesis



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Figure 8.5

Replication of Bacterial DNA



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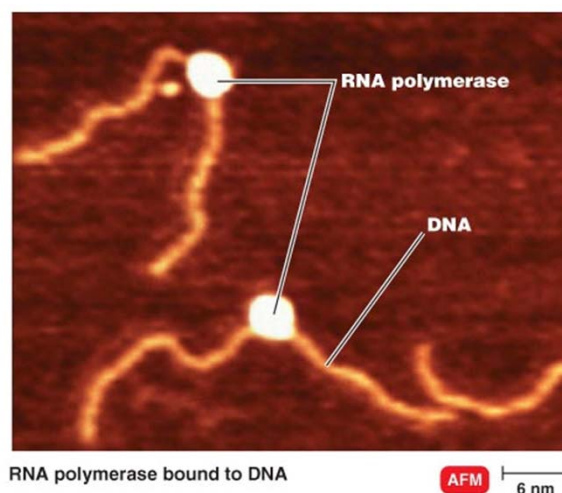
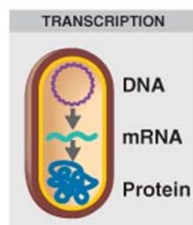
Figure 8.6

Transcription

- DNA is transcribed to make RNA (mRNA, tRNA, and rRNA)
- Transcription begins when RNA polymerase binds to the **promoter** sequence
- Transcription proceeds in the 5' → 3' direction
- Transcription stops when it reaches the **terminator** sequence

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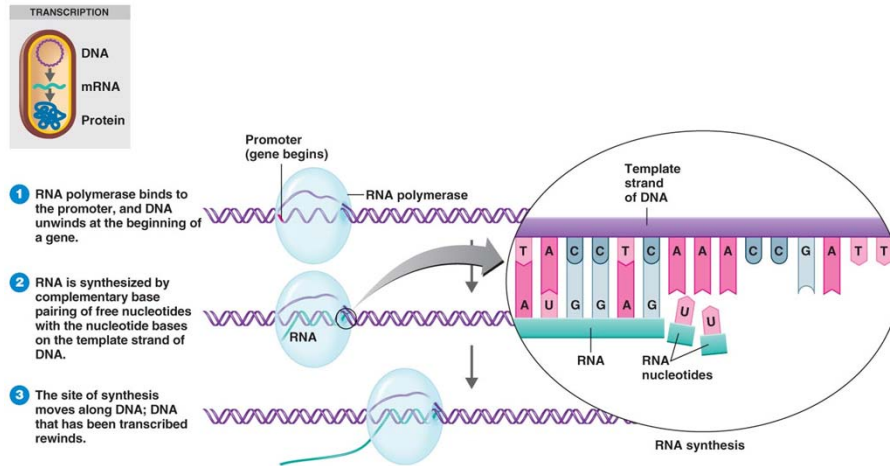
Transcription



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Figure 8.7

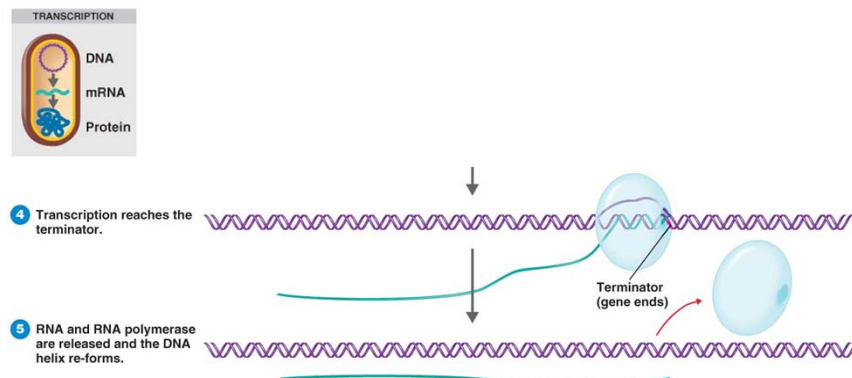
The Process of Transcription



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Figure 8.7

The Process of Transcription

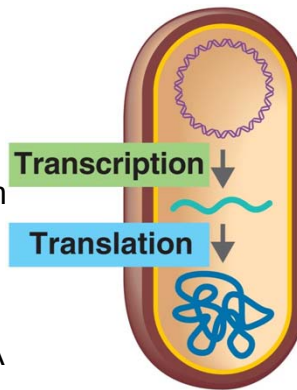


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Figure 8.7

Translation

- Base sequence → amino acid sequence
- mRNA is translated in the form of codons (3 nucleotides)
- Translation of mRNA begins at the start codon: AUG
- Translation ends at a **stop codon (nonsense codons)**: UAA, UAG, UGA
- **Sense codons** code for amino acids



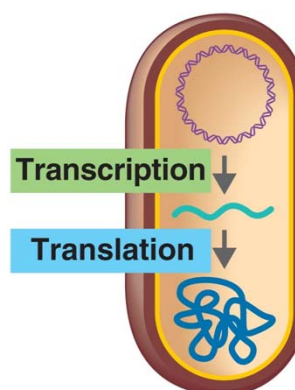
Cell metabolizes and grows

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Figure 8.2

The Genetic Code

- 64 **sense codons** on mRNA encode the 20 amino acids
- The genetic code is **degenerate** → Most amino acids are signaled by several alternative codons → **degeneracy**



Cell metabolizes and grows

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Figure 8.2

The Genetic Code

- Codons are written in terms of their base sequence in mRNA.
- Degeneracy allows for a certain amount of change, or mutation, in the DNA without affecting the protein ultimately produced.

		Second position				
	U	C	A	G		
U	UUU	UCU	UAU	UGU	U	
	UUC	UCC	UAC	UGC	C	
	UUA	UCA	UAA Stop	UGA Stop	A	
	UUG	UCG	UAG Stop	UGG Trp	G	
C	CUU	CCU	CAU	CGU	U	
	CUC	CCC	CAC	CGC	C	
	CUA	CCA	CAA	CGA	A	
	CUG	CCG	CAG	CGG	G	
A	AUU	ACU	AAU	AGU	U	
	AUC	ACC	AAC	AGC	C	
	AUA	ACA	AAA	AGA	A	
	AUG Met/start	ACG	AAG	AGG	G	
G	GUU	GCU	GAU	GGU	U	
	GUC	GCC	GAC	GGC	C	
	GUA	GCA	GAA	GGA	A	
	GUG	GCG	GAG	GGG	G	

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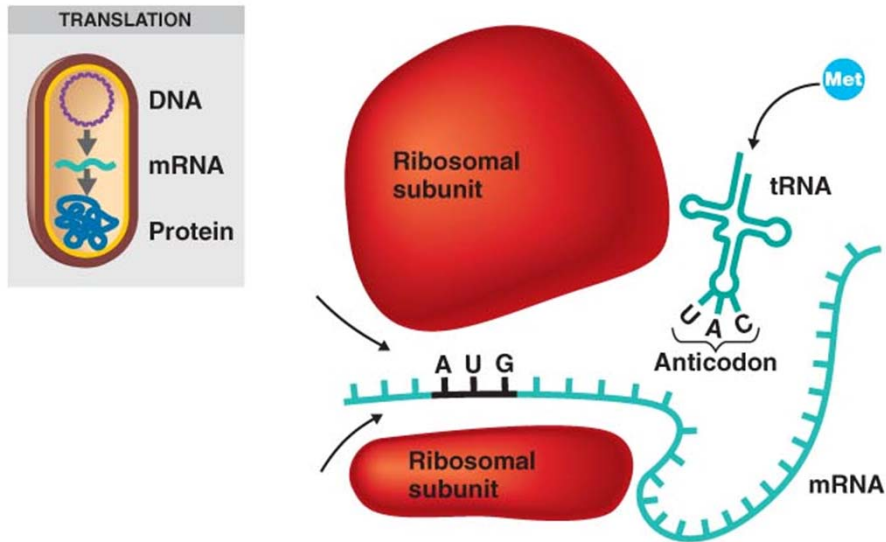
Figure 8.8

Translation

- The mRNA associates with **ribosomes**, which consist of rRNA and protein.
- Specific amino acids are carried by tRNA. tRNA has a base triplet called an **anticodon**.
- The base pairing of codon and anticodon at the ribosome results in specific amino acids being brought to the site of protein synthesis.
- mRNA is read in the 5' → 3' direction
- Translation ends when the ribosome reaches a stop codon on the mRNA.

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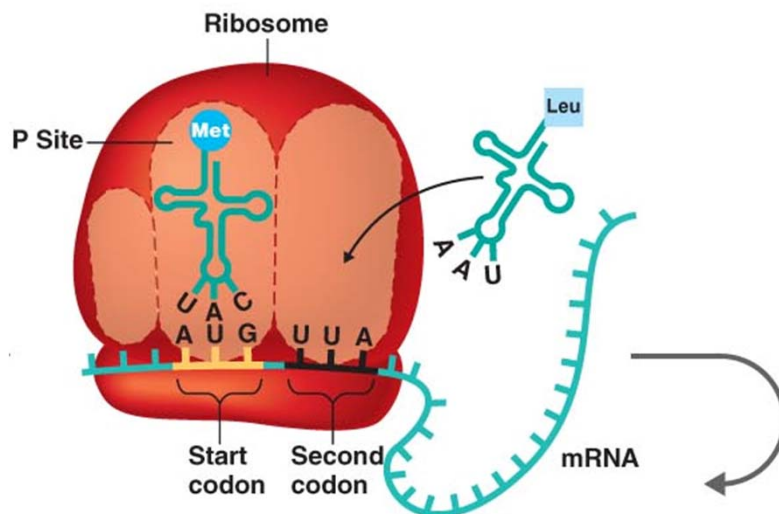
The Process of Translation



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Figure 8.9

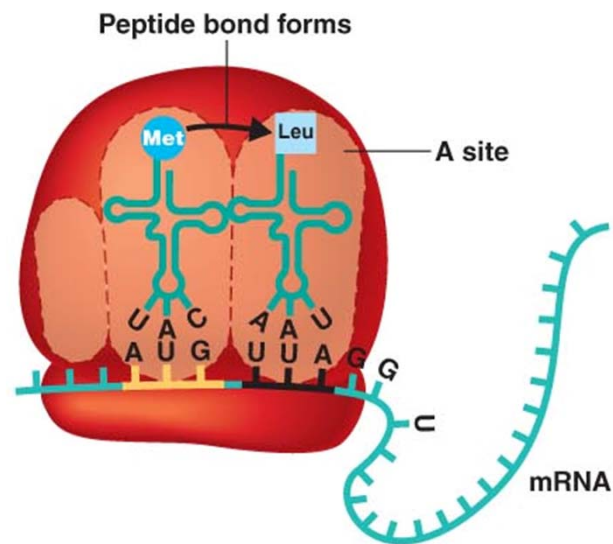
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Figure 8.9

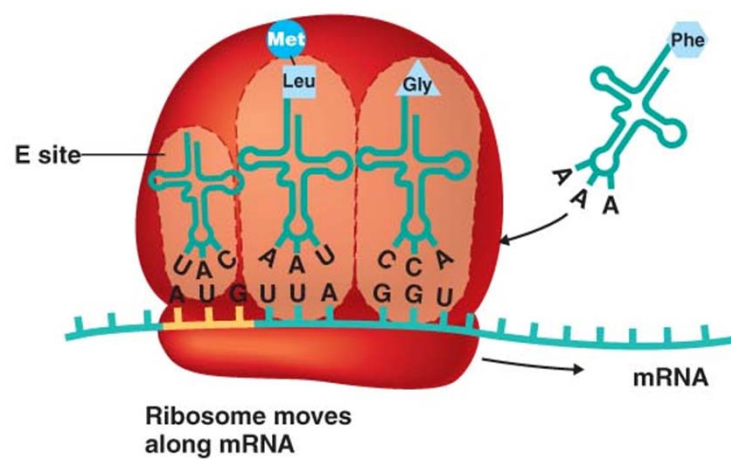
The Process of Translation



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Figure 8.9

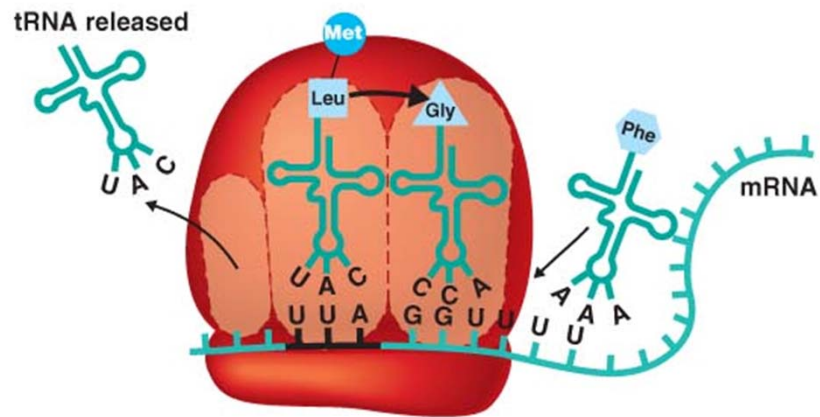
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Figure 8.9

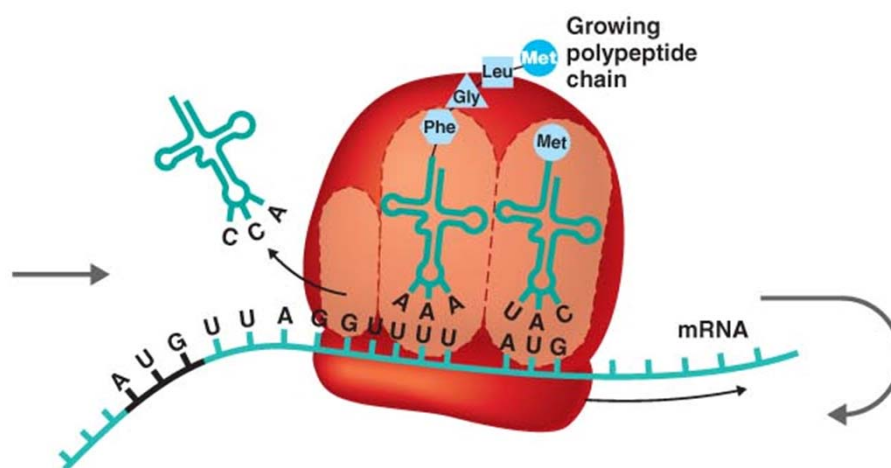
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Figure 8.9

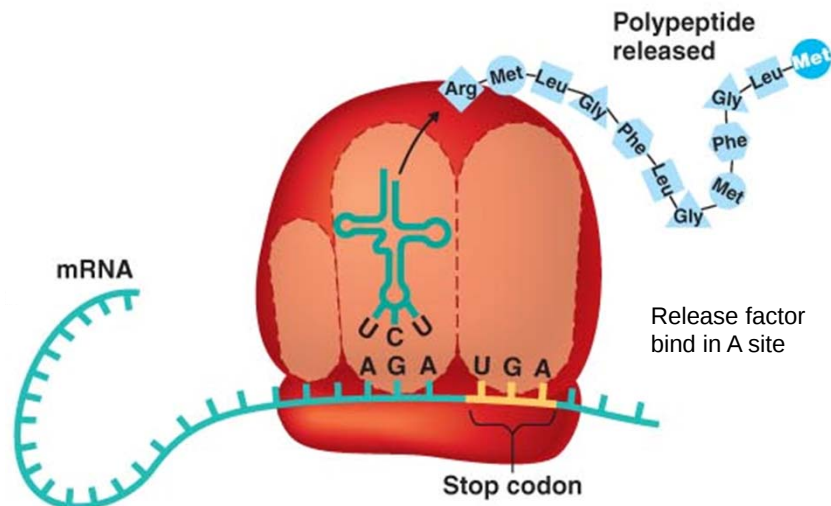
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Figure 8.9

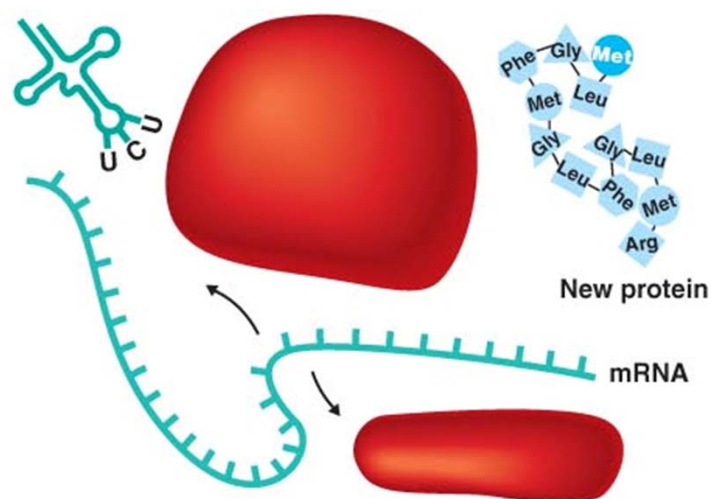
The Process of Translation



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Figure 8.9

The Process of Translation

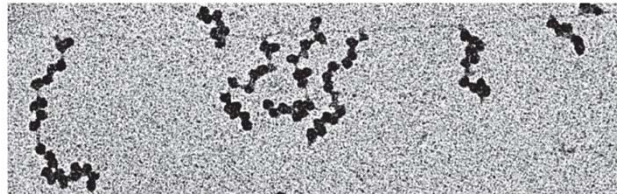
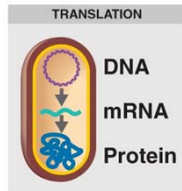


PLAY ANIMATION Translation: Process

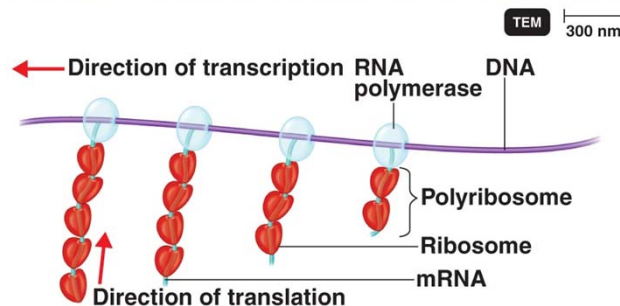
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Figure 8.9

Simultaneous Transcription & Translation



In prokaryotes, translation can begin before transcription is complete.



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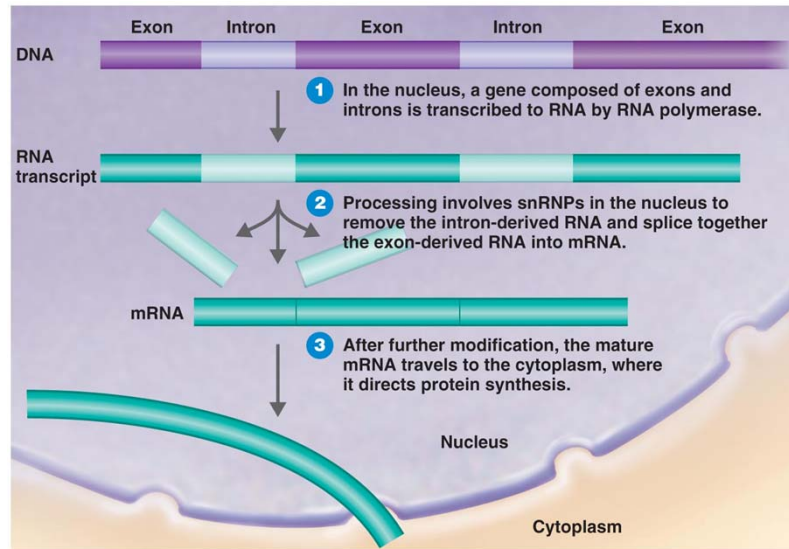
Figure 8.10

Translation in eukaryotes

- In eukaryotes, transcription takes place in the nucleus. The mRNA must be completely synthesized and moved through the nuclear membrane to the cytoplasm before translation can begin.
- In eukaryotic cells the regions of genes that code for proteins are often interrupted by noncoding DNA.
 - Exon: the region of DNA expressed
 - Intron: the intervening region of DNA that do not encode protein
 - The RNA undergoes processing before it leaves the nucleus (Figure 8.11).

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RNA Processing in Eukaryotes



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Figure 8.11

The Regulation of Bacterial Gene Expression

Pre-transcriptional control

Repression and induction

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Pre-transcriptional control

- Regulating protein synthesis at the gene level is energy-efficient because proteins are synthesized only as they are needed.
- **Constitutive genes** are expressed at a fixed rate
- Other genes are expressed only as needed
 - **Repressible** genes
 - **Inducible** genes
 - **Catabolite** repression
- For these gene regulatory mechanisms, the control is aimed at mRNA synthesis.

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Repression and induction

- Repression inhibits the synthesis of one or several (repressible) enzymes.
- When cells are exposed to a particular end product, the synthesis of enzymes related to that product decreases.
- In the presence of certain chemicals (inducers), cells synthesize more enzymes. This process is called induction.
 - The production of β -galactosidase by *E. coli* in the presence of lactose → transported into cells → converted into the inducer allolactose → lactose can be metabolized by β -galactosidase

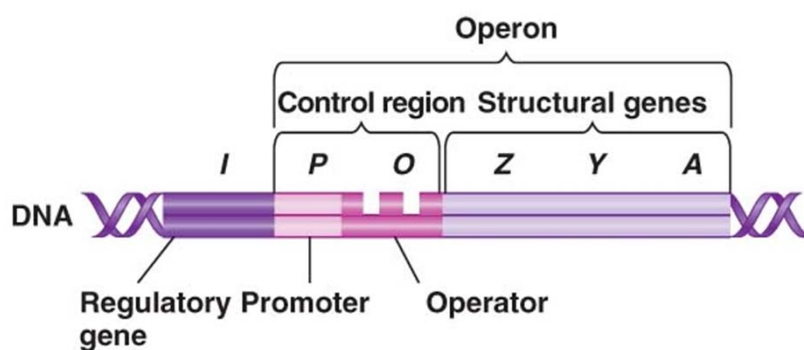
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The operon model of gene expression

- In bacteria, a group of coordinately regulated structural genes (with related metabolic functions) and the promoter and operator sites that control their transcription are called an operon (Figure 8.12.1).
- A regulatory gene codes for the repressor protein (Figure 8.12.1).
- In an inducible system
 - When the inducer is absent, the repressor binds to the operator and no mRNA is synthesized (Figure 8.12.2a).
 - When the inducer is present, it binds to the repressor so that it cannot bind to the operator → mRNA is made → enzyme synthesis is induced (Figure 8.12.3)

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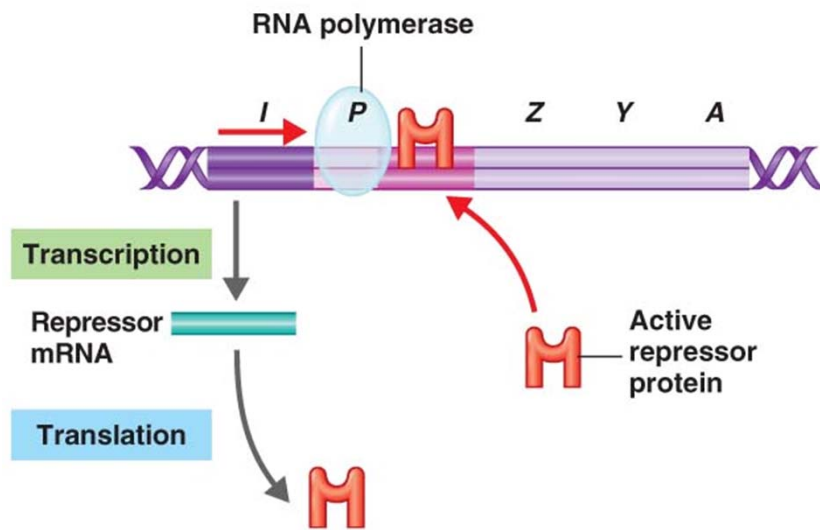
Operon



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Figure 8.12

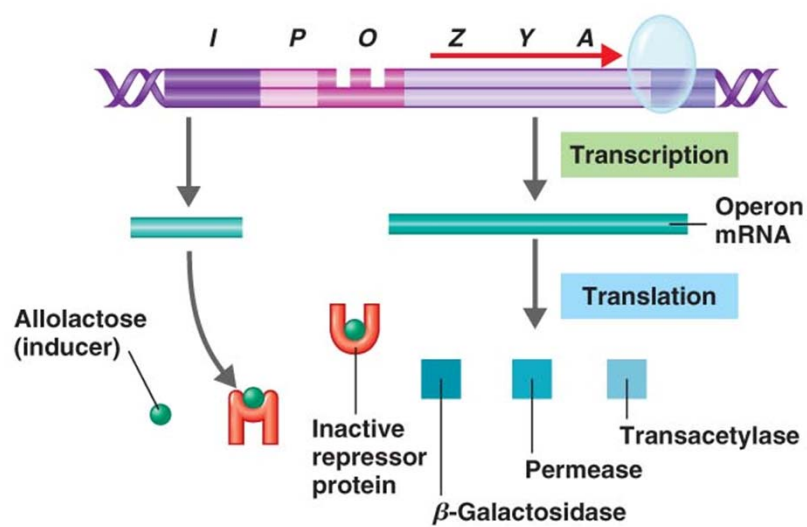
Induction



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Figure 8.12

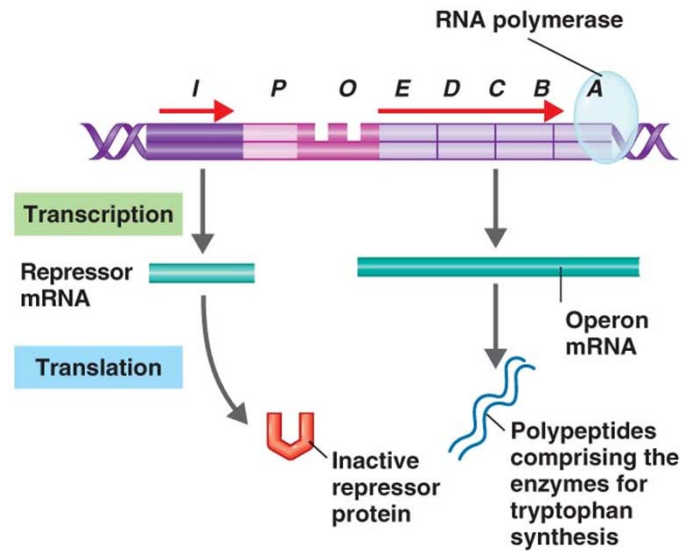
Induction



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Figure 8.12

Repression



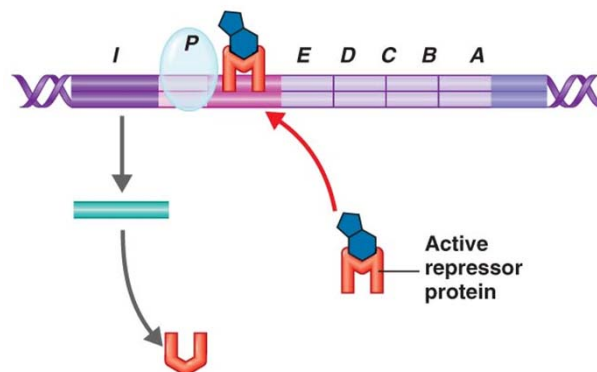
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Figure 8.13

Repression

- In the repressible systems, the repressor require a **corepressor** in order to bind to the operator

- corepressor inhibits enzyme synthesis
- operon off



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Figure 8.13

Positive Regulation

- Regulation of the lactose operon also depends on **the level of glucose** in the medium. → control the intracellular level of **cyclic AMP (cAMP)**
- Enzymes that metabolize glucose are constitutive, and cells grow at their maximal rate with glucose as their carbon source (Figure 8.14).
- When glucose is no longer available, **cAMP** accumulates in the cells.
 - cAMP binds to **catabolite activator protein (CAP)** → CAP then binds to *lac* promoter → initiate transcription by enhancing the binding of RNA polymerase to promoter
 - The transcription of the *lac* operon requires both the presence of lactose and the absence of glucose (Figure 8.15)

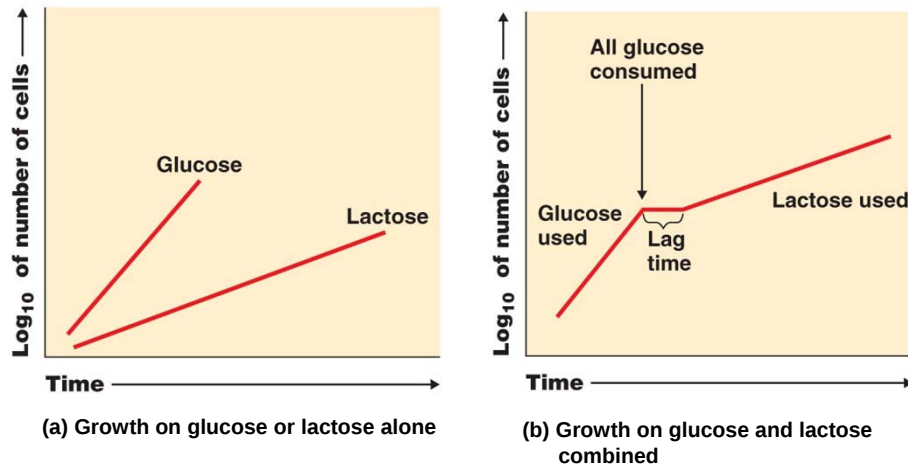
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Positive Regulation

- Cyclic AMP is an alarmone, a cellular alarm signal that promotes a cell's response to environmental or nutritional stress.
- The presence of glucose inhibits metabolism of alternative carbon sources is termed **catabolite repression** (or the glucose effect).

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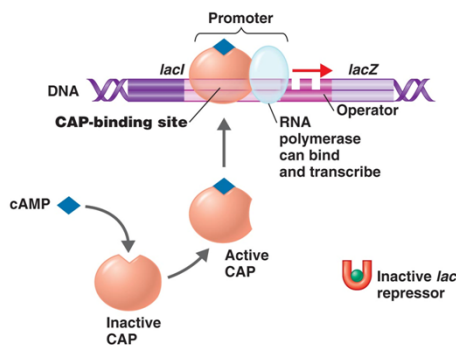
Catabolite Repression



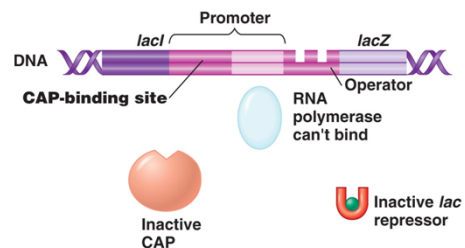
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Figure 8.14

- Lactose present, no glucose



- Lactose + glucose present



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Figure 8.15

Epigenetic Control

- Eukaryotic and bacterial cells can turn genes off by methylating certain nucleotides.
- The methylated (off) genes are passed to offspring cells.
- Unlike mutations, this isn't permanent, and the genes can be turned on in a later generation.
- Epigenetic inheritance (epigenetic = on genes)
 - e.g. bacteria behave differently in a biofilm

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Post-transcriptional control

- Single-stranded RNA molecular of ~22 nucleotides, called **microRNAs (miRNAs)**, inhibit protein production in eukaryotic cells.
- In human, miRNAs produced during development allow different cells (have the same genes) to produce different proteins.
- **MicroRNAs** combine with mRNA; the resulting doubled-stranded RNA is enzymatically destroyed. → the mRNA-encoded protein is not made (Figure 8.16)

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Mutation: Change in the Genetic Material

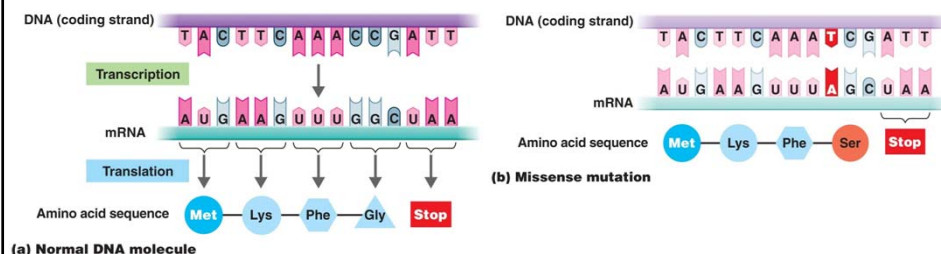
- Mutations may be neutral, beneficial, or harmful
- A **neutral mutation** has no harmful or beneficial effect on the organism.
- A **harmful mutation** is a mutation that decreases the fitness of the organism.
- A **beneficial mutation** is a mutation that increases fitness of the organism, or which promotes traits that are desirable.
- **Mutagen:** Agent that causes mutations
- **Spontaneous mutations:** Occur in the absence of a mutagen

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Mutation

1. Base substitution (point mutation): base was replaced

- Missense mutation: result in change in amino acid
- Silent mutation: no change in amino acid

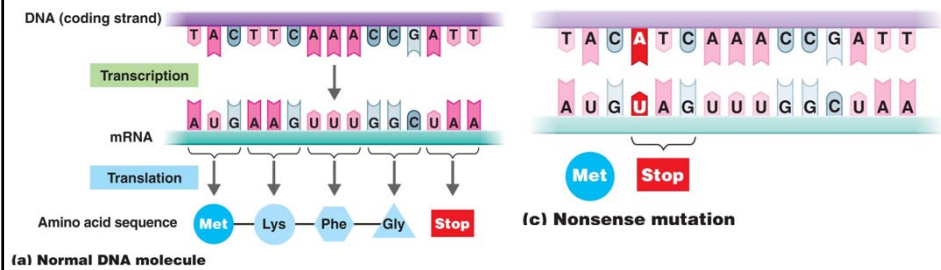


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Figure 8.18a, b

Mutation

c. Nonsense mutation: Results in a nonsense codon

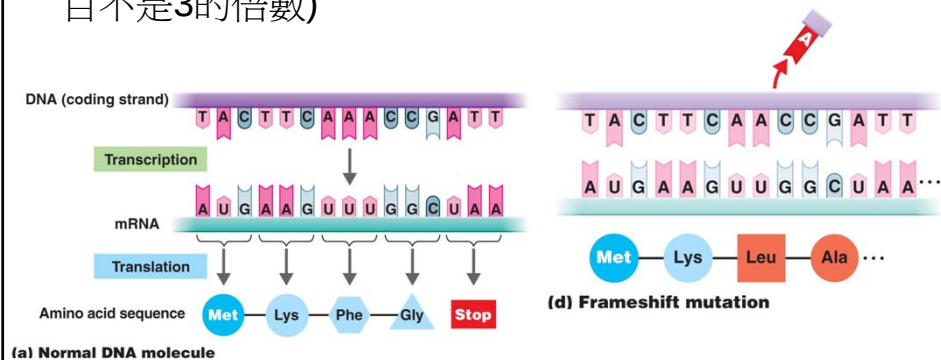


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Figure 8.18a, c

Mutation

2. Frameshift mutation: Insertion or deletion of one or more nucleotide pairs cause the shift of the translational reading frame (插入或缺失的數目不是3的倍數)



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Figure 8.18a, d

The Frequency of Mutation

- **Spontaneous mutation** rate = 1 in 10^9 replicated base pairs or 1 in 10^6 replicated genes
- **Mutagens** increase to 10^{-5} or 10^{-3} per replicated gene

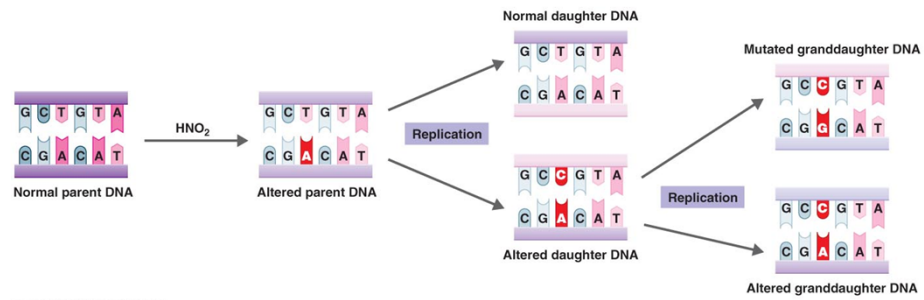
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Chemical Mutagens

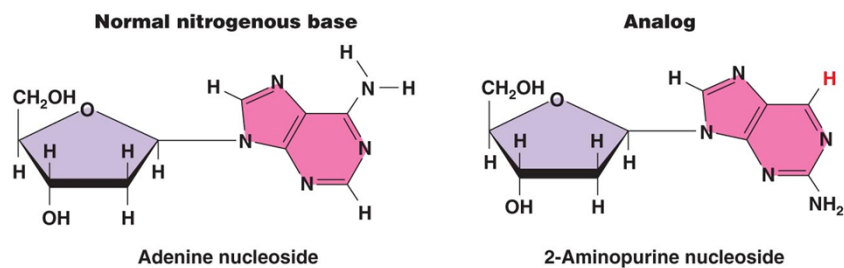
1. Base-pair mutagens: e.g. Nitrous acid converts the base adenine to a form that no longer pairs with thymine (T) but instead pairs with cytosine (C) (Figure 8.18). → point mutation
2. Nucleotide analog: structurally similar to normal nitrogenous bases, but have slightly altered base-pairing properties (Figure 8.19).
3. Frameshift mutagens: cause small deletions or insertions, e.g. benzpyrene, aflatoxin, and acridine dyes.
 - Frameshift mutagens usually have the right size and chemical properties to slip between the stacked base pairs of the DNA double helix. → offsetting DS DNA → gap → insertion or deletion during DNA replication.

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Nitrous acid as a mutagen (Fig. 8.19)

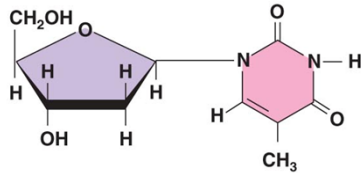


Chemical Mutagens: nucleoside analogs

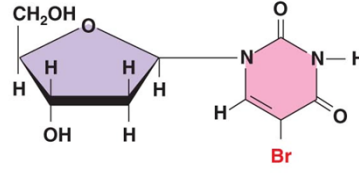


(a) The 2-aminopurine is incorporated into DNA in place of adenine but can pair with cytosine, so an AT pair becomes a CG pair.

Chemical Mutagens



Thymine nucleoside



5-Bromouracil nucleoside

(b) The 5-bromouracil is used as an anticancer drug because it is mistaken for thymine by cellular enzymes but pairs with **guanine**. In the next DNA replication, an AT pair becomes a GC pair.

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Figure 8.19b

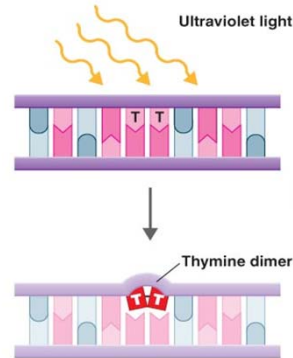
Radiation

- **Ionizing radiation** (X rays and gamma rays) causes the formation of ions that can react with nucleotides and the deoxyribose-phosphate backbone
- Results in **base substitutions** or **breakage of sugar-phosphate backbone**
- Nucleotide excision repairs mutations

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Radiation

- UV radiation causes thymine dimers



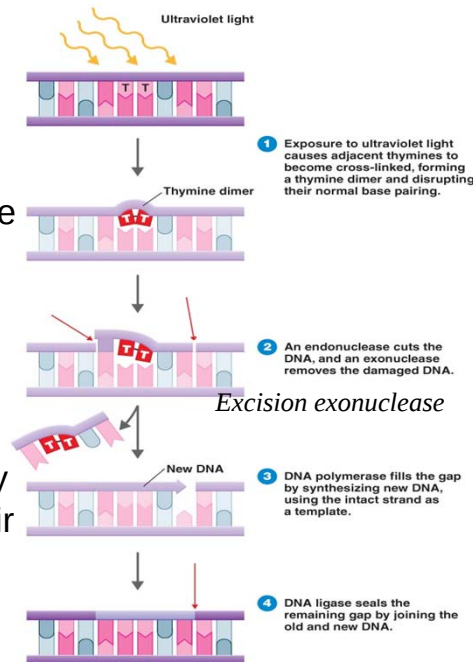
- 1 Exposure to ultraviolet light causes adjacent thymines to become cross-linked, forming a thymine dimer and disrupting their normal base pairing.

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Figure 8.20

Repair

1. **Photolyase** (light-repair enzymes) use visible light energy to **separate the thymine dimer** back to the original two thymines
2. **Nucleotide excision repair** (Figure 8.20) (not restricted to UV-induced damage)
3. **Mismatch-repair** (for newly synthesized DNA): a repair endonuclease cuts the nonmethylated strand



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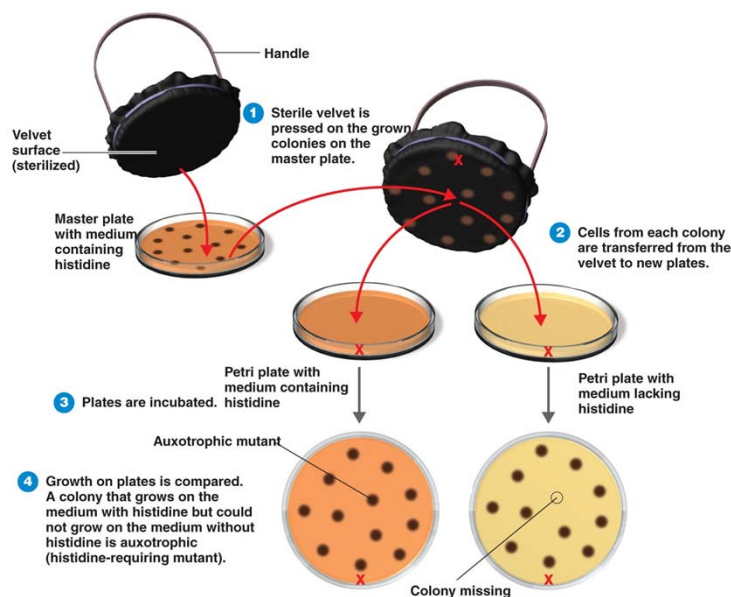
Figure 8.20

Identifying mutants

- Mutants can be detected by selecting or testing for an altered phenotype.
- **Positive (direct) selection** detects mutant cells because they grow or appear different.
- **Negative (indirect) selection** detects mutant cells because they do not grow.
- Replica plating (Figure 8.21) is used for negative selection to detect, for example, auxotrophs that have nutritional requirements not possessed by the parent (nonmutated) cell.

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Replica Plating



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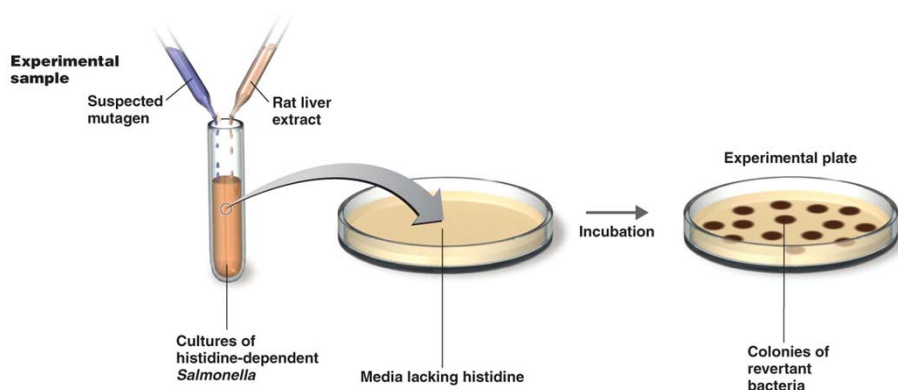
Figure 8.21

Identifying chemical carcinogens

- The Ames test is a relatively inexpensive and rapid test for identifying possible chemical carcinogens.
- The test assumes that **a mutant cell can convert to a normal cell in the presence of a mutagen** and that **many mutagens are carcinogens**
 - Many chemical must be **activated by animal enzymes** for mutagenic or carcinogenic activity to appear.
 - The chemical to be test and the mutant bacteria usually incubated with **rat liver extract**, a rich source of activation enzymes.
- **Histidine auxotrophs of *Salmonella*** are exposed to a potential carcinogen, and reversions to the histidine-synthesizing state are measured.
- **The number of observed revertants** provides an indication of the degree to which a substance is mutagenic and therefore possibly carcinogenic.

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Ames Test for Chemical Carcinogens

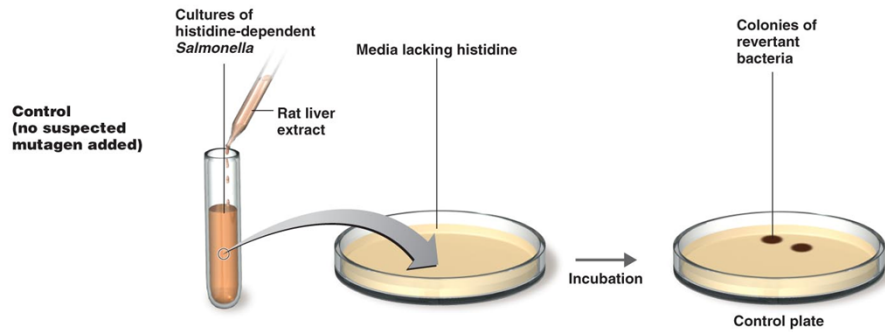


The principle of Ames test is based on the hypothesis that the application of mutagen leads mutations in many genes including the defective gene and some of those mutations cause the reversal of ability to synthesize histidine (reverse mutations).

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Figure 8.22

Ames Test for Chemical Carcinogens

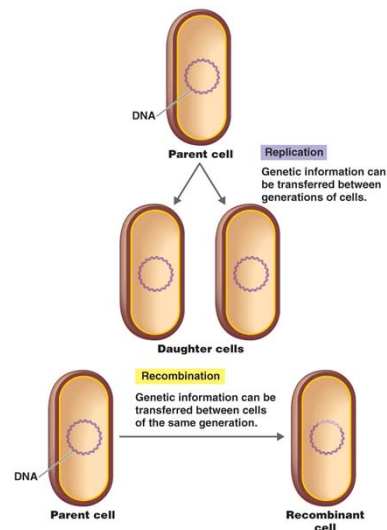


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Figure 8.22

Genetic Recombination

- **Vertical gene transfer:** Occurs during reproduction between generations of cells.
- **Horizontal gene transfer:** The transfer of genes between cells of the same generation.



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Genetic Recombination

- Exchange of genes between two DNA molecules
 - Crossing over occurs when two chromosomes break and rejoin

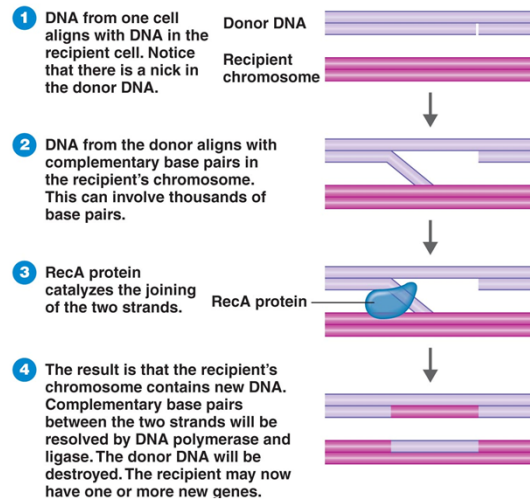


Figure 8.23

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Specific types of genetic transfer in bacteria

1. Transformation (轉形作用): genes are transferred from one bacterium to another by using a “naked” DNA in solution .

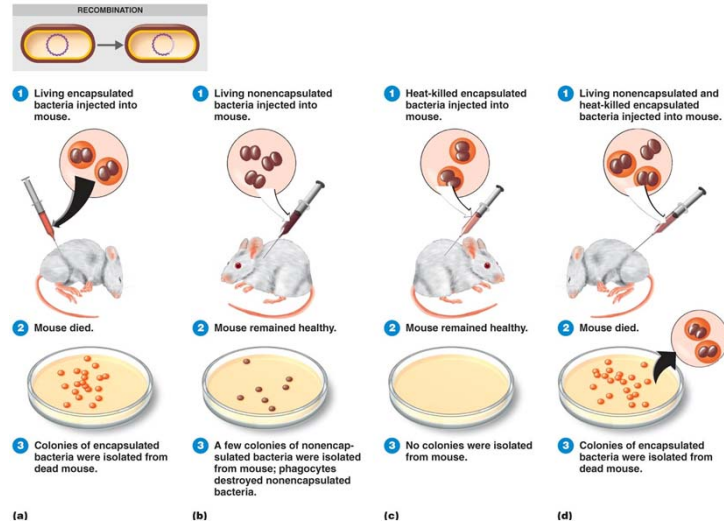
- First demonstrated in *Streptococcus pneumoniae* (Figure 8.24), and occurs naturally among a few genera of bacteria
- A recipient cell takes up donor DNA and integrate them into their own chromosomes by recombination (Figure 8.25).
- When a recipient is in a physical state in which it can take up the donor DNA, it is said to be **competent**.
- Competence results from alterations in the cell wall that make it permeable to large DNA molecules.

2. Conjugation (接合作用): requires contact between living cells

3. Transduction (轉導作用): requires a bacteriophage

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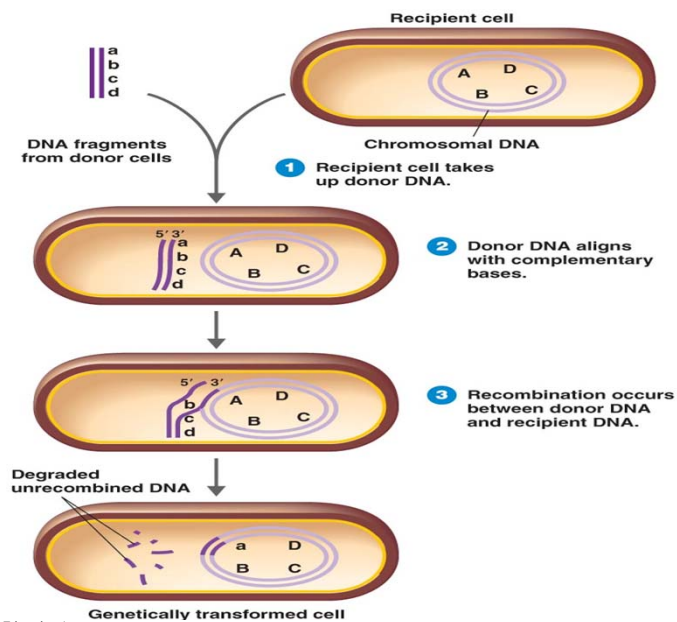
Genetic Transformation



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Figure 8.24

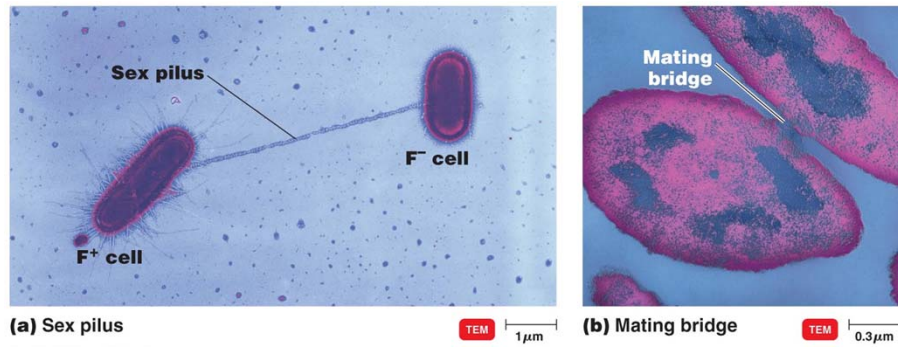
Genetic Recombination



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Figure 8.25

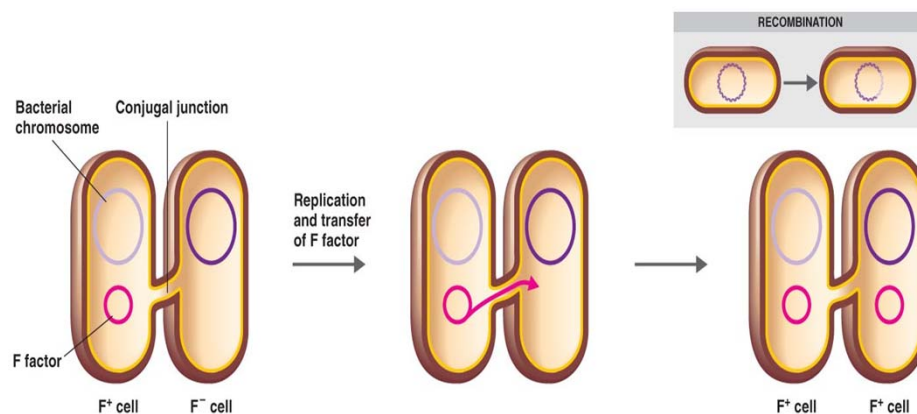
Bacterial Conjugation



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Figure 8.26

Conjugation in *E. coli*



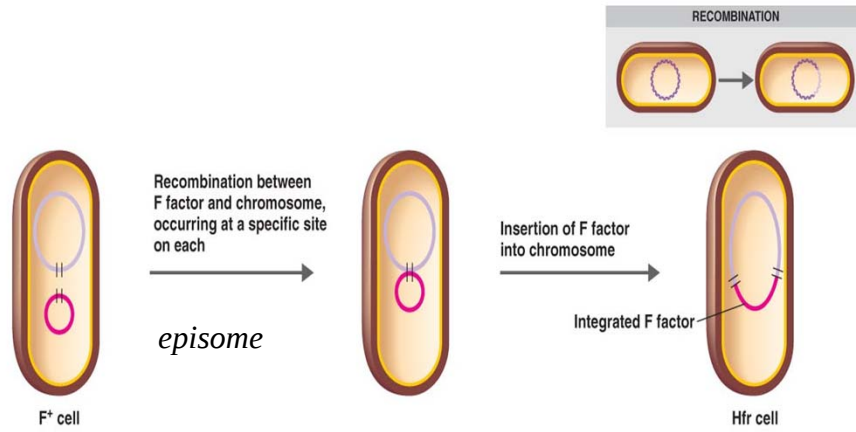
(a) When an F factor (a plasmid) is transferred from a donor (F⁺) to a recipient (F⁻), the F⁻ cell is converted to an F⁺ cell.

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Figure 8.27a

Conjugation in *E. coli*



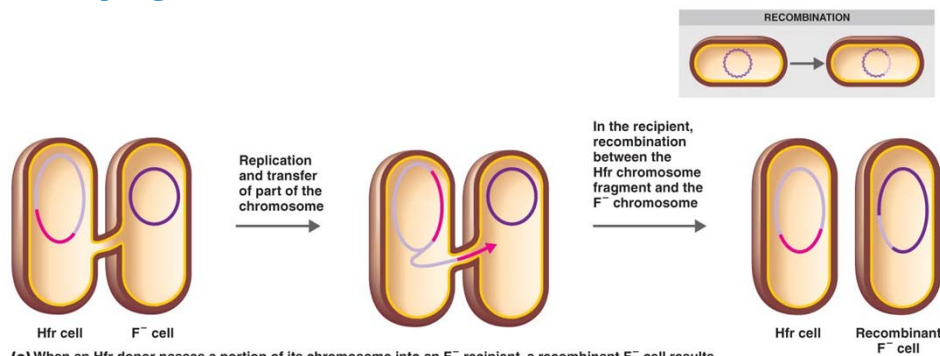
(b) When an F factor becomes integrated into the chromosome of an F⁺ cell, it makes the cell a high frequency of recombination (Hfr) cell.

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Figure 8.27b

Conjugation in *E. coli*



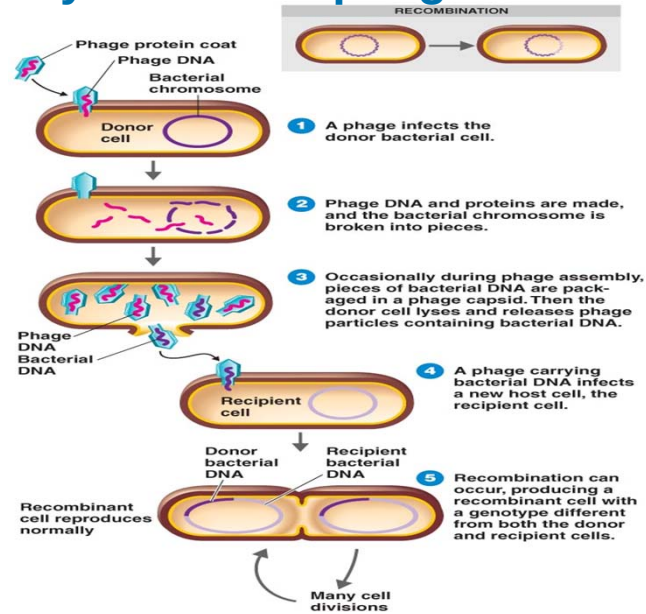
(c) When an Hfr donor passes a portion of its chromosome into an F⁻ recipient, a recombinant F⁻ cell results.

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Figure 8.27c

Transduction by a Bacteriophage



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Figure 8.28

Transduction in Bacteria

- **Generalized transduction:** any bacteria DNA can be transferred from one cell to another.
- **Specialized transduction:** only certain bacteria genes are transferred.
 - e.g. Shiga toxin for *E. coli* O157:H7
 - Chapter 13 will discuss more detail.

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Q&A



- *E. coli* is found naturally in the human large intestine, and there it is beneficial. However, the strain designated *E. coli* O157:H7 produces Shiga toxin. How did *E. coli* acquire this gene from *Shigella*?

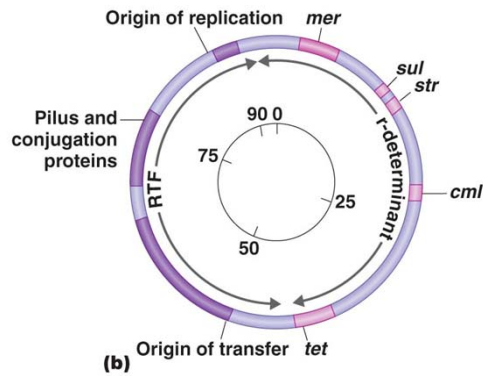
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Plasmids

- Plasmids are self-replicating, gene-containing circular pieces of DNA about 1-5% the size of the bacterial chromosomes.
- **Conjugative plasmid:** carries genes for sex pili and transfer of the plasmid
- **Dissimilation (異化) plasmids:** encode enzymes for catabolism of unusual compounds
- **Resistance factors (R factors):** encode antibiotic resistance (Figure 8.28)

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R Factor, a Type of Plasmid

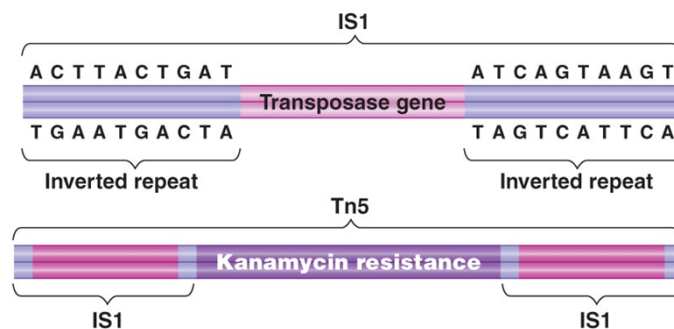


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Figure 8.29

Transposons

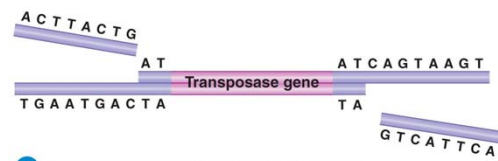
- Segments of DNA that can move from one region of DNA to another
- Contain insertion sequences for cutting and resealing DNA (transposase)
- Complex transposons carry other genes



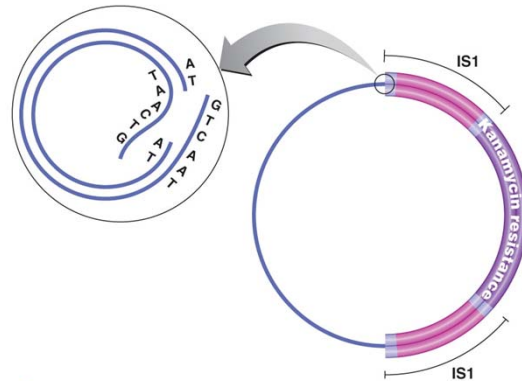
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Figure 8.30a, b

Transposons



1 Transposase cuts DNA, leaving sticky ends.



2 Sticky ends of transposon and target DNA anneal.

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Figure 8.30c

Genes and Evolution

- Mutations and recombination provide diversity
- Fittest organisms for an environment are selected by natural selection

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Evolution

Australia	A	C	C	C	C	G	T	C	C	A	C	C	C	T	T	T	C	A	A	T	T
Egypt	A	A	T	C	G	A	T	C	A	T	C	T	T	C	G	T	C	G	A	T	C
France	A	A	T	C	G	A	T	C	A	T	C	G	T	C	G	T	C	G	A	T	C
Israel	A	T	C	C	A	T	T	C	A	T	C	C	T	C	A	T	C	G	A	T	T
Italy	A	T	C	C	A	C	T	C	A	T	C	C	T	C	G	T	C	G	A	T	T
Kenya	A	T	C	C	A	C	T	C	A	T	C	C	T	C	G	T	C	G	A	T	T
Mexico	A	A	C	C	C	T	T	C	C	T	C	C	C	C	T	T	C	G	A	T	T
United States	A	A	C	C	C	C	T	C	C	T	C	C	C	C	T	T	C	G	A	T	T
Uganda	A	T	A	C	G	A	T	C	A	T	G	C	T	C	G	T	C	C	A	T	C

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Evolution

- Which strain is more closely related to the Uganda strain?
- How did the virus change?

Strain	% Similar to Uganda
Kenya	71%
U.S.	51%

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