

Chapter 9

Microbial Genetics



- **Genetic Information**
- **DNA Replication**
- **Gene Expression --Protein Synthesis**
- **Transmission of DNA**
- **Change of Genetic Information --Mutation**

Genetic information

- The blueprint for synthesis of all components of a cell or virus
 - **Encoded in DNA** of cellular organisms and DNA viruses
 - **RNA** of RNA viruses

Figure 9.2

- **Genetics** The study of genetic information, including:

1. How it is **organized**

2. How it is **expressed**

3. How it **changes**

4. How it is **reproduced** and **inherited**

Review of DNA Structure

- Nucleotides linked together by phosphodiester bonds
- Two strands twisted into a helix and held together by hydrogen bonds between bases
 - **A** bonds to **T**
 - **G** bonds to **C**
 - The nucleotide sequences of the 2 strands are **complementary** to each other
 - The sequence of a strand can be predicted if you know the sequence of its complementary strand

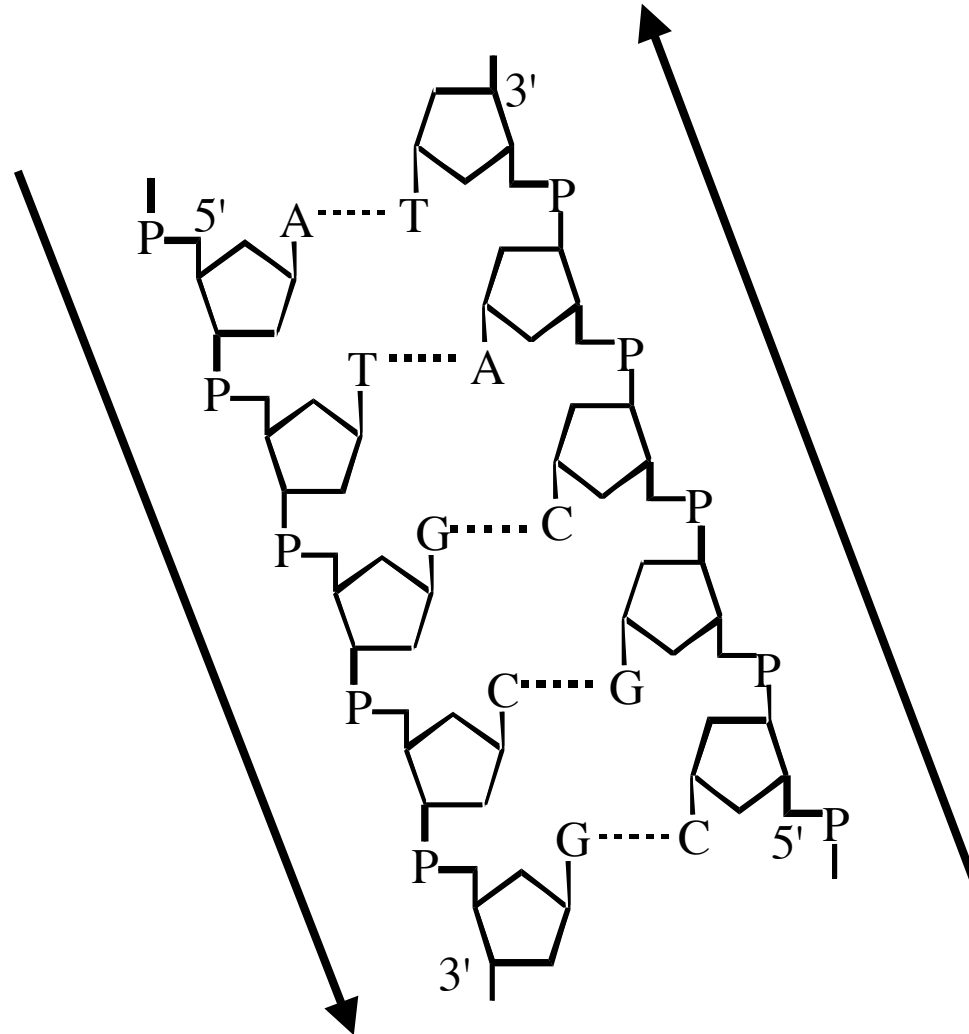
Ex.

5' -----A-T-G-C-T-T-G-C-----3'



3'-----T-A-C-G-A-A-C-G-----5' Complimentary strand

- Strands run in opposite directions $5' \rightarrow 3'$
 $3' \leftarrow 5'$
 - 5' refers to carbon # 5 of deoxyribose
 - 3' refers to carbon # 3 of deoxyribose



Nucleotides

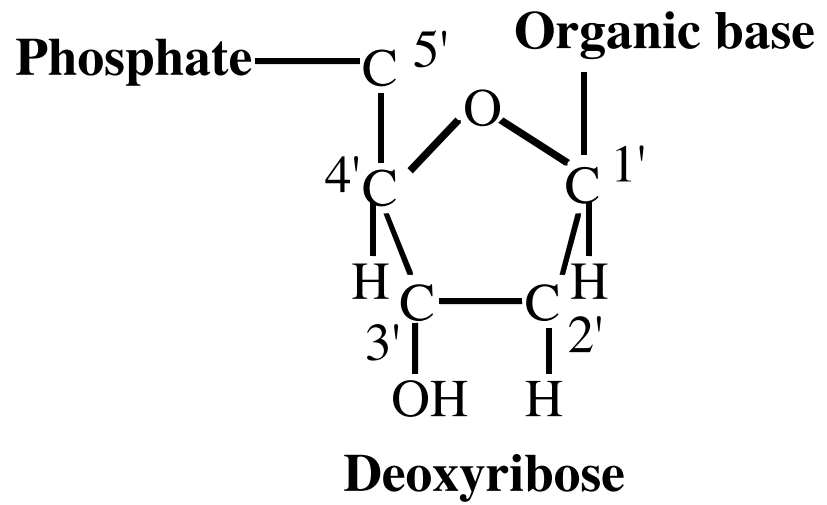


Figure 9.4

DNA as the Genetic Material

- Present in cell as large molecules called **chromosomes**
- Most bacteria have one circular chromosome
- Genetic information is arranged on the chromosome in units called genes
- **Gene** --the basic unit of heredity
 - Contains information for synthesis of a protein
 - Sequence of nucleotide bases specifies the sequence of amino acids of a protein

Ex. **A-T-G-G-T-T-G-T-T-T-C-G-C-C-C---**
 ↓ ↓ ↓ ↓ ↓
 Methionine-Valine-Valine-Serine-Proline---

Haemophilus influenzae chromosome

~1,830,000 nucleotides

~1,800 genes

-An **average gene size** is @ 1000 nucleotides long

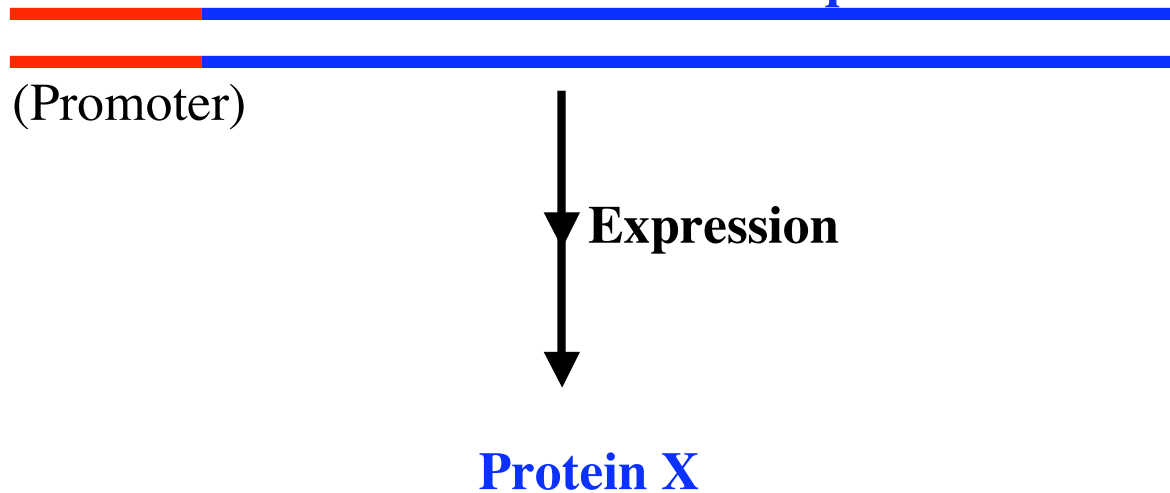
-Contains information specifying the sequence of @ 300 amino acids of a protein

- Genes also contain **noncoding** nucleotide sequences at their beginning that **regulate expression** in the cell

-Ex. When and how often the information is used to make a protein

- **Gene X**

Regulation Coding nucleotides: specify amino acid sequence



◆ How much information is needed by a cell?

○ *Escherichia coli* K-12 has 1 chromosome

~ 4.6 million nucleotides in length

~ 4,400 genes (1 gene ~ 1000 nucleotides long)

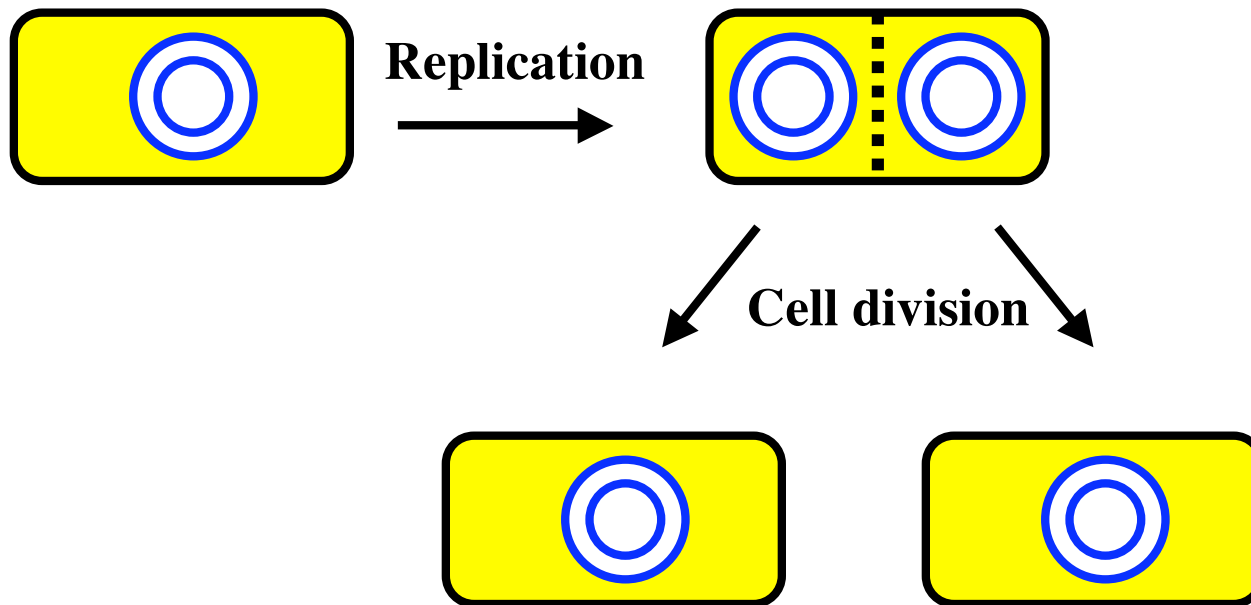
○ Human cell has 23 pairs (46) chromosomes

~ 3.2 billion nucleotides

~ 30,000 to 40,000 genes

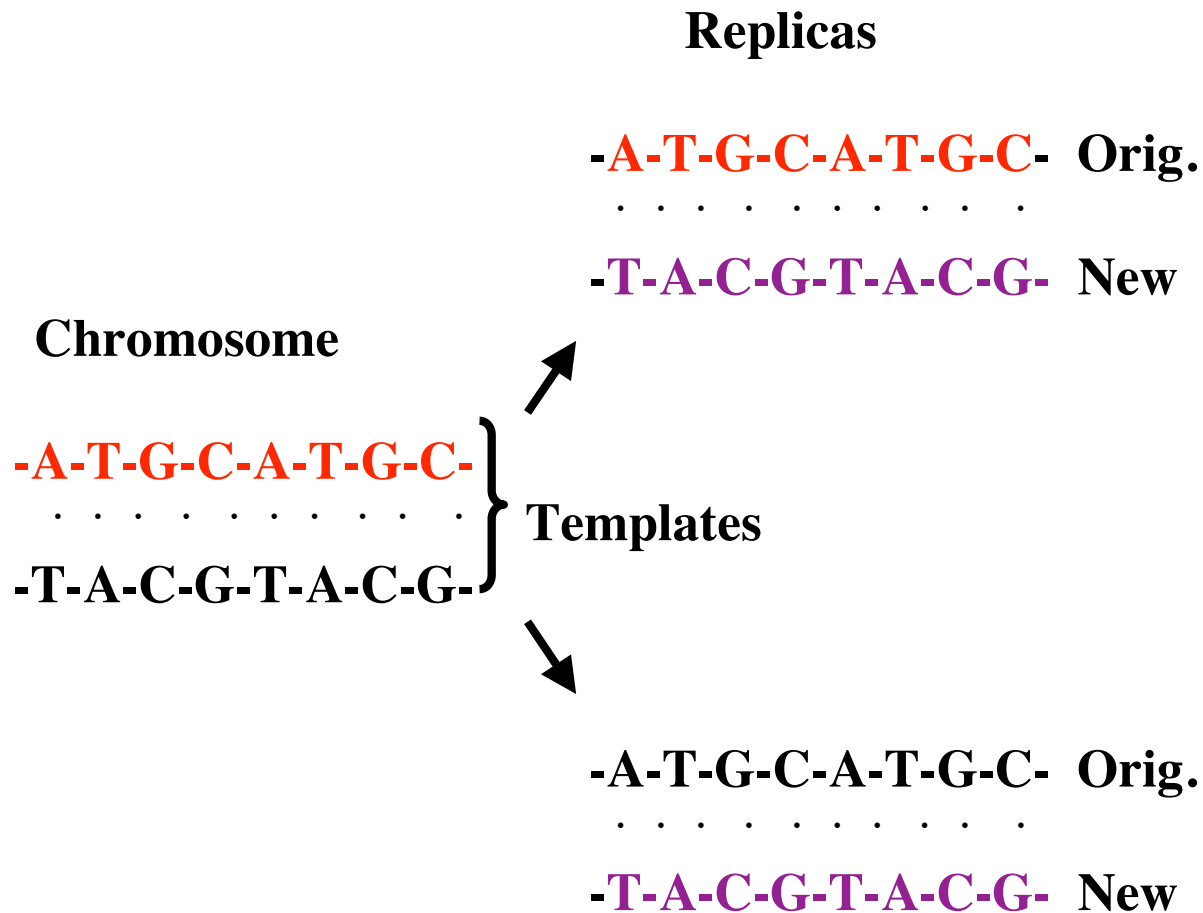
Replication of Chromosomes

- Growing cells reproduce by dividing into two daughter cells
- A growing cell must precisely duplicate (**replicate**) its chromosome before it divides so that each daughter cell will receive a copy
 - **Replica**: an exact copy of an object



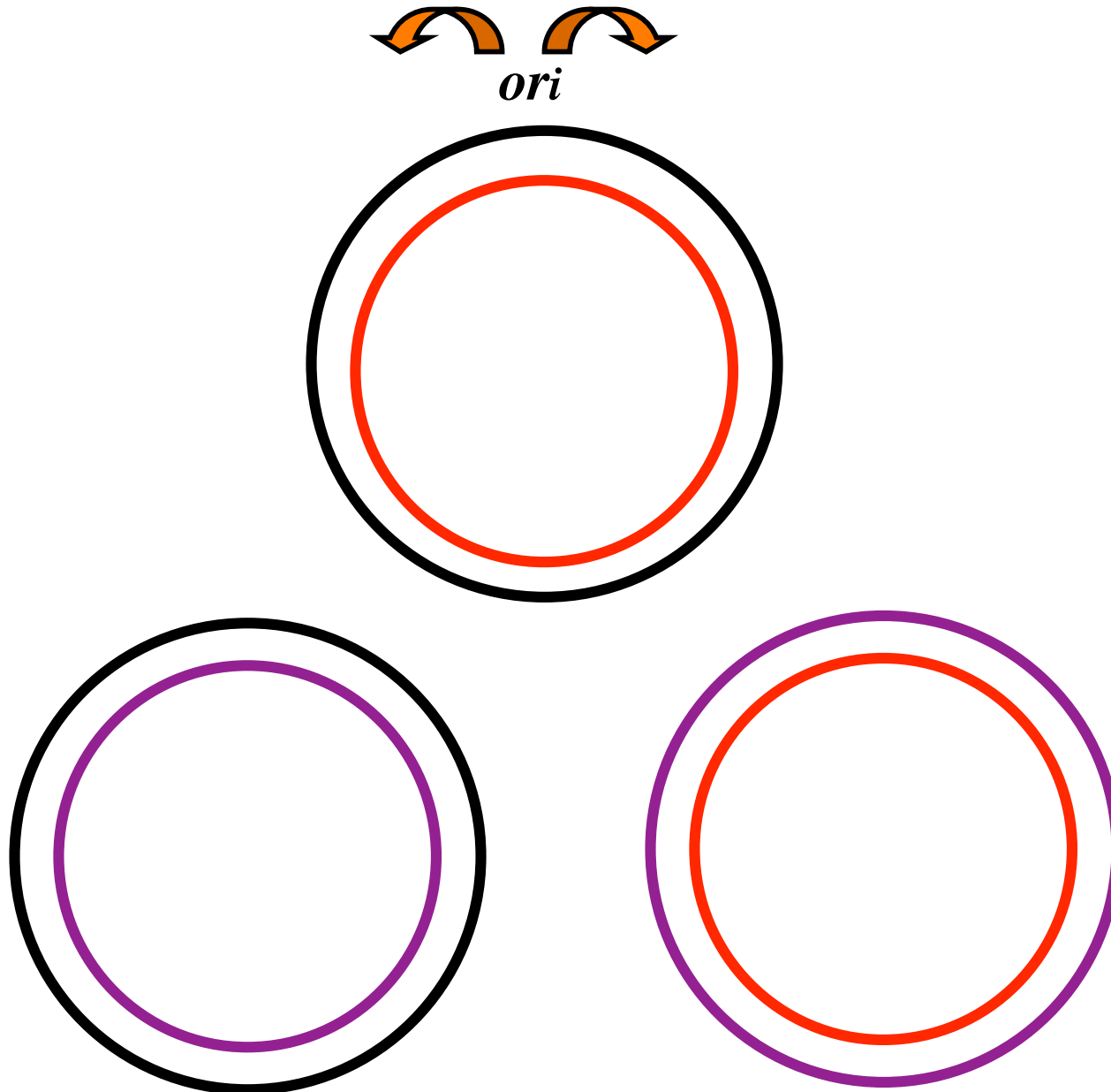
Mechanism of DNA Replication

- Each strand serves as a **template** for synthesis of a new strand
 - The **specificity of base pairing** allows the nucleotide sequence of a chromosome to be precisely copied



- Replication is **semiconservative**
- Both replicas have one original and one new strand
- **DNA polymerase** Enzyme that catalyzes synthesis of new DNA strands

- Replication begins at the *ori* (**origin of replication**) and proceeds in opposite directions around the chromosome



Expression of genetic information

- Most **genes** contain information that specifies the sequence of amino acids of a protein
 - Expression of genetic information = protein synthesis
- Protein synthesis involves two steps.



1. Transcription: Nucleotide (**base**) **sequence of gene** is copied as a sequence of **messenger RNA (mRNA)**

2. Translation: **Protein** is **synthesized** using the mRNA sequence to specify the amino acid sequence

Figure 9.8

Table 9.2

Mechanisms of Gene expression

1. Transcription

- 1 DNA strand of a gene is used as a **template** for mRNA synthesis
- **Base pairing rules** result in mRNA with a sequence of bases that is complimentary to that of template DNA

Ex.

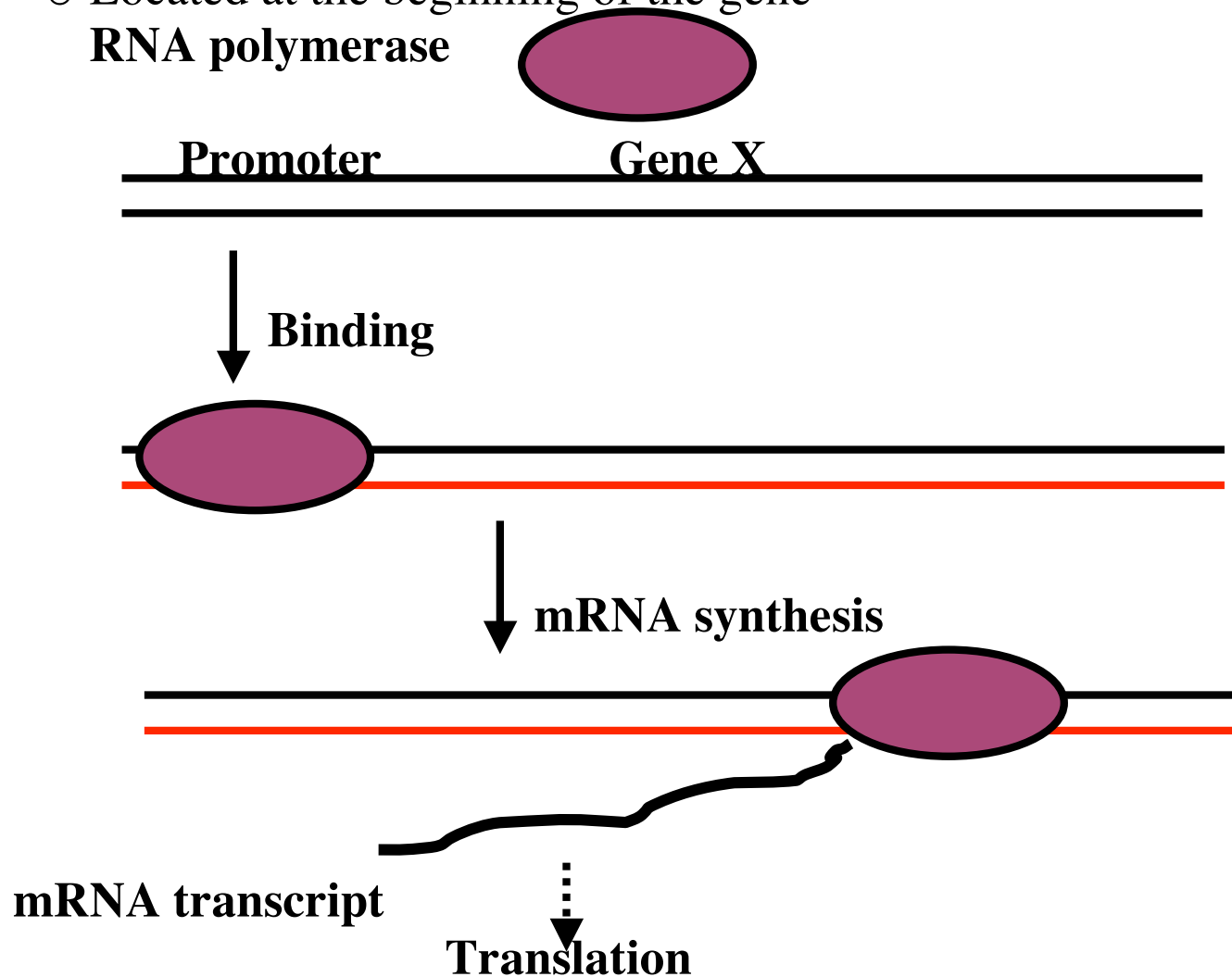
DNA { --ATG CAT GCG-- Sense or coding strand
--TAC GTA CGC-- **Template strand**



Transcription

mRNA --AUG CAU GCG-- Transcript
(U substitutes for T in RNA)

- **RNA polymerase** is the enzyme that catalyzes synthesis of mRNA
- Transcription of a gene starts when RNA polymerase binds to DNA at a sequence called the **promoter**
 - Located at the beginning of the gene



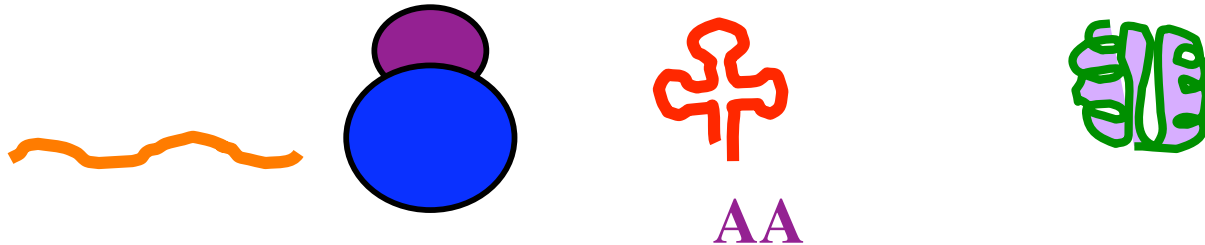
2. Translation: Message is decoded and protein is synthesized

mRNA $\xrightarrow{\text{Translation}}$ Protein

Ex. AUG CAU GCG ... $\xrightarrow{\hspace{1cm}}$ Met-His-Ala ...
(Nucleotide sequence) (Amino acid sequence)

- **Ribosome** is site of translation (protein synthesis)
 - Composed of proteins and ribosomal RNA (rRNA)
- **Transfer RNA** (tRNA) brings each amino acid to the ribosome as needed

mRNA + Ribosome + tRNA_{AA} \longrightarrow Protein



➤ How does mRNA specify which of the 20 amino acids to incorporate into a particular protein?

Protein Synthesis (Translation)

- Peptide bonds are formed between amino acids brought to the ribosome by tRNA

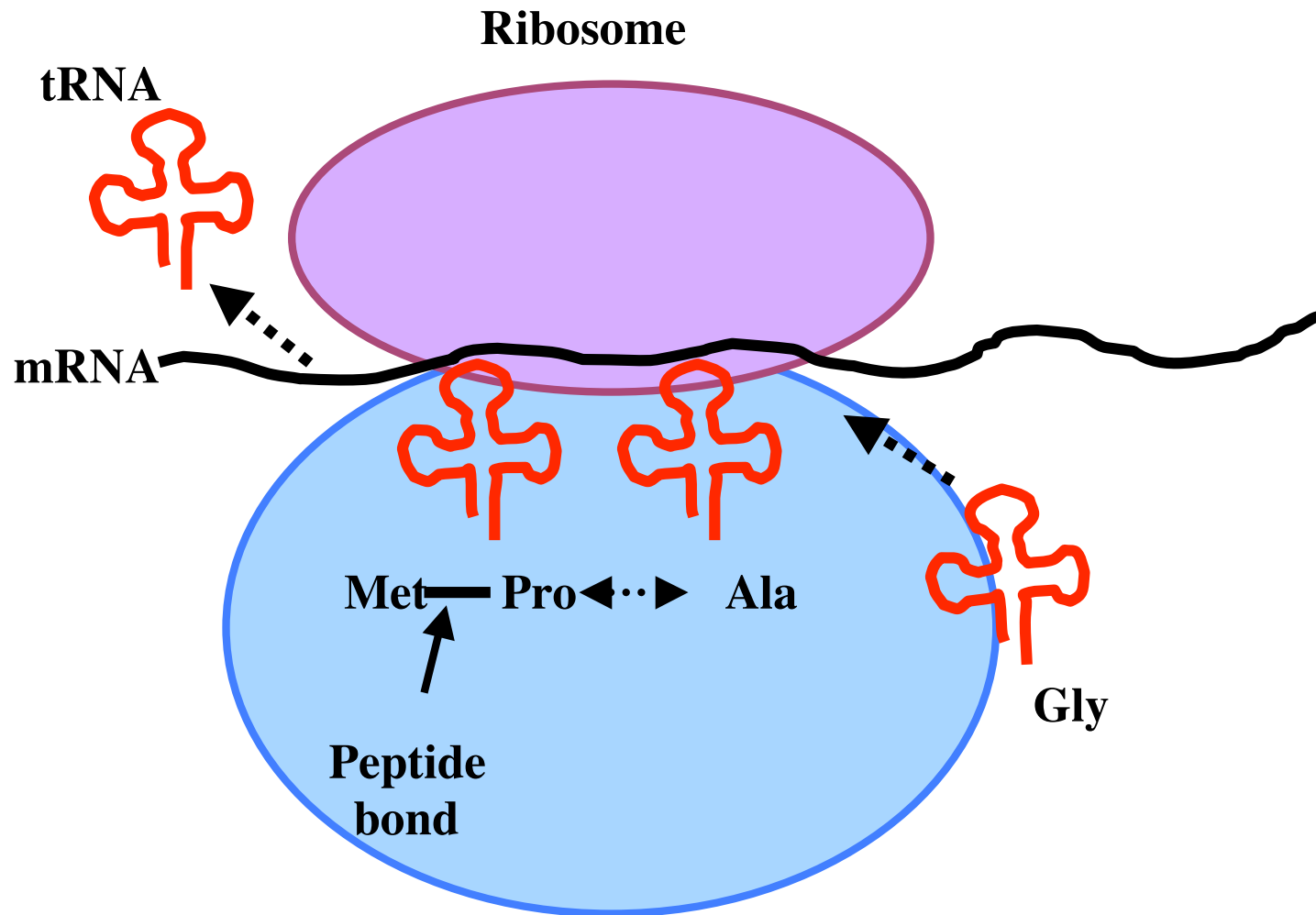


Figure 9.12

The Genetic Code

Codon A sequence of **3 nucleotide bases** in mRNA that specifies a particular amino acid of proteins (or signals the end of protein synthesis)

Ex. AGC = Serine

UGC = Cysteine

- **4** bases are **possible** in a codon: A, U, G or C
 - There are 64 different codons ($4^3 = 64$)
- **61** codons **specify** (encode) the 20 different **amino acids** present in proteins
- Code is **redundant**: most amino acids have more than 1 codon

Ex.

-Phenylalanine	UUU	UUC		
-Isoleucine	AUU	AUC	AUA	
-Alanine	GCU	GCC	GCA	GCG

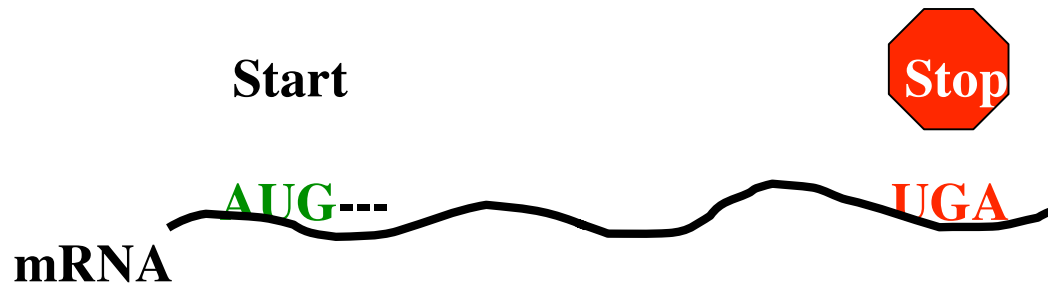
Figure 9.13

Special codons

Start codon (1)

AUG = Methionine

- Located near the beginning of a transcript
- Specifies 1st amino acid of all proteins
(Met is sometimes removed after synthesis of the protein is completed)



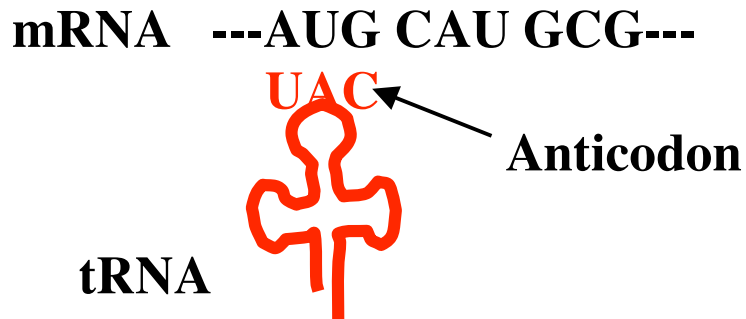
Stop codons (3)

UAA UAG UGA

- Located at the end of a transcript
- Doesn't specify any amino acid
- Signals the end of translation (synthesis of the protein)

Anticodon

- Present in tRNA (transfer RNA)
- Also a sequence of three bases
- Complementary to and base pairs with a codon in mRNA



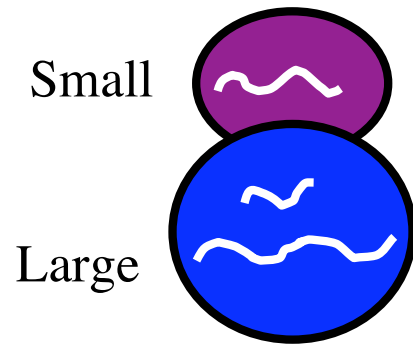
Methionine

- Each tRNA is attached to the amino acid specified by its complementary mRNA codon
- For every codon of mRNA that specifies an amino acid, there is a matching tRNA with a complementary anticodon
- tRNAs bring amino acids to the ribosome during protein synthesis

Figure 9.14

Ribosome

- **Site of protein synthesis**
- Composed of a large and a small subunit
- Subunits contain proteins and **ribosomal RNA (rRNA)**



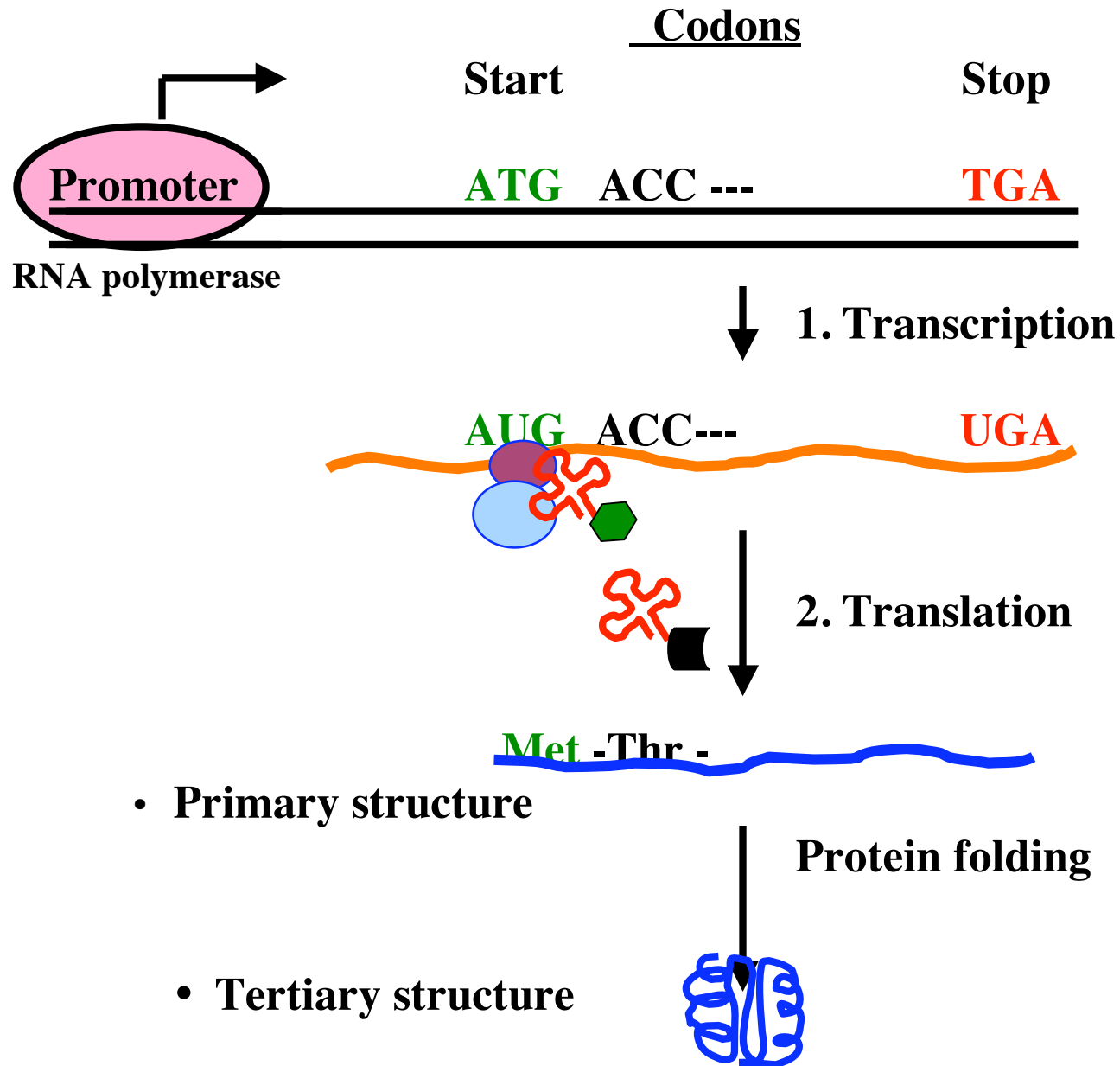
- Interacts with **mRNA** and **tRNA-aa** during protein synthesis
- Where amino acids are joined together by **peptide bonds**
- Actively growing cells have many ribosomes that synthesize enzymes needed to catalyze metabolic reactions

Some antibiotics kill bacteria by inhibiting protein synthesis

Ex. Erythromycin, Streptomycin, Chloramphenicol, Neomycin

Gene Expression Summary

- RNA polymerase initiates transcription by binding to the promoter region of a gene



Gene expression: transcription and translation of a typical bacterial gene

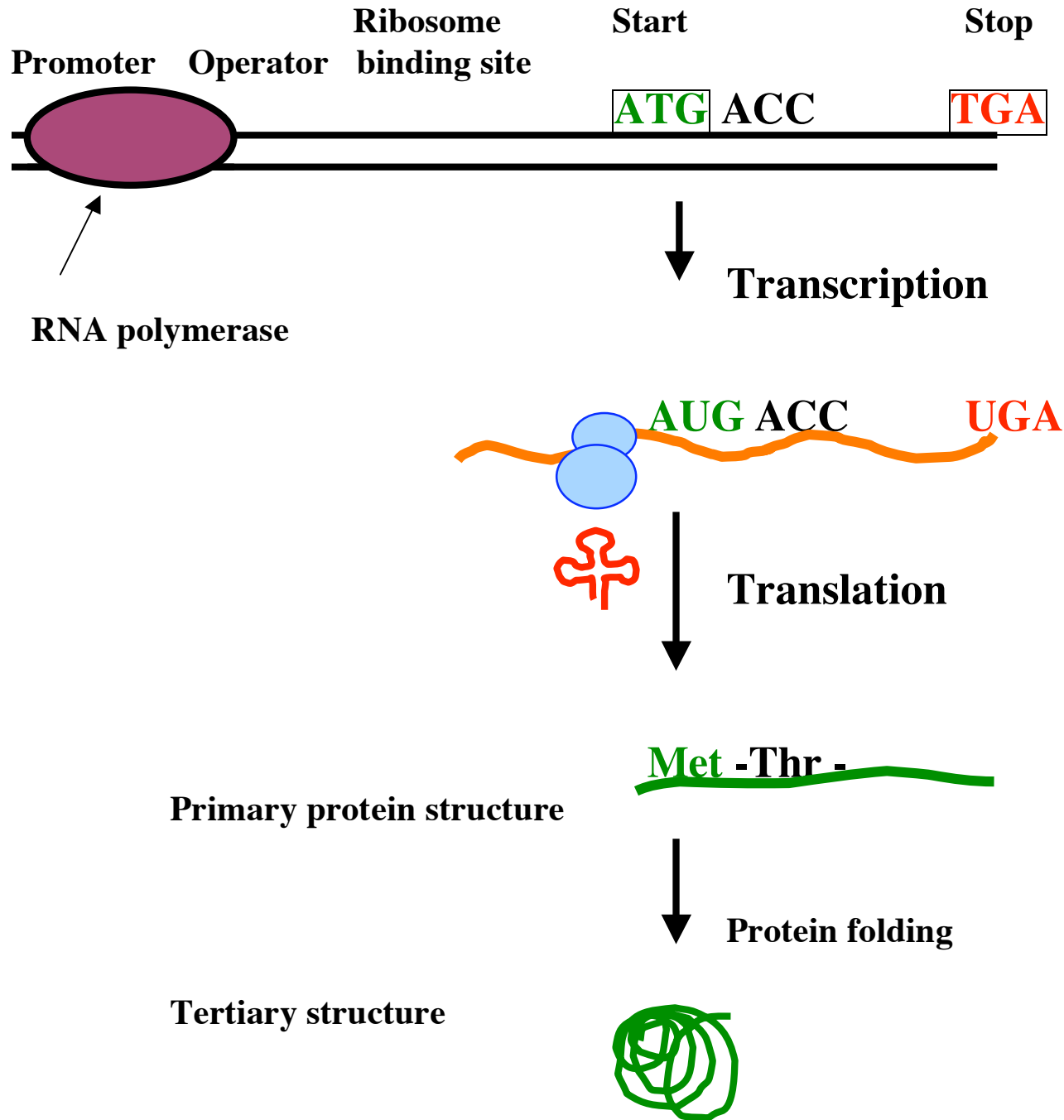


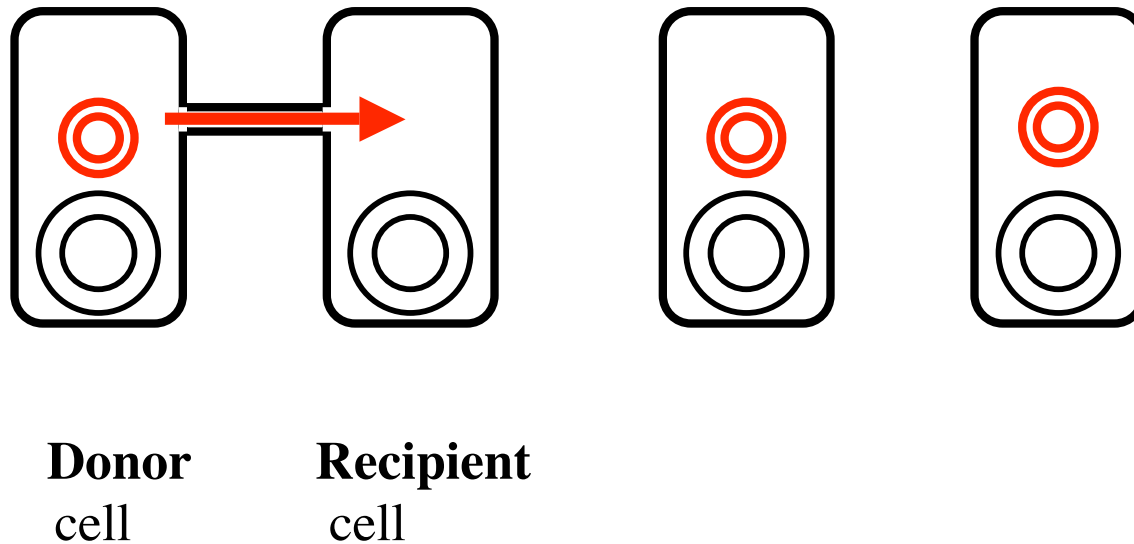
Figure 9.15

Gene Transfer: Uptake and Exchange of DNA

- Allows a cell to acquire new genes and the traits they confer
- Also known as **horizontal gene transfer**
 - Ex. *bla* Gene encodes **beta lactamase** (penicillinase)
 - Enzyme that **destroys penicillin**
 - Acquiring *bla* makes a microorganism **resistant** to penicillin and it **survives exposure** to the antibiotic
 - Development of **antibiotic resistant pathogens** is a big problem for treatment of bacterial infections
- **3 Mechanisms**
 1. **Conjugation**
 2. **Transformation**
 3. **Transduction**

1. Conjugation

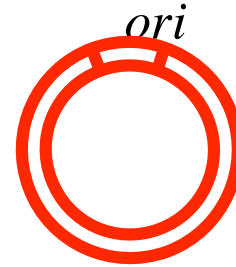
- Transmission of a **plasmid** from 1 cell to another through a pilus



- The plasmid is replicated during transfer and the donor retains a copy of the plasmid

Plasmids

- Circular DNA molecules that are **separate** from the chromosome
- **Smaller** than the chromosome with **fewer genes**
Approx. 1/10 to 1/100 size of chromosome
- Contain an *ori* and are **replicated** in the cell
 - May be 1 or many (> 100) copies in one cell
- Plasmids usually aren't needed by cell
- Under certain circumstances a gene on a plasmids may be advantageous to cell
Ex. *bla* on a plasmid allows cell to produce penicillinase and survive exposure to the antibiotic
- Plasmids with antibiotic resistance genes are called **R plasmids**
 - Spread of R plasmids to pathogens can make an antibiotic useless for treating infectionsEx. Vancomycin-resistant *Enterococcus* and *Staph. aureus*
 - Have the *vanA* gene on an R plasmid



- **Virulence genes**

- Help pathogens infect a host and cause disease
- Some encode toxins or enzymes that digest host tissue

Ex. *Clostridium tetani* causes tetanus (lockjaw)

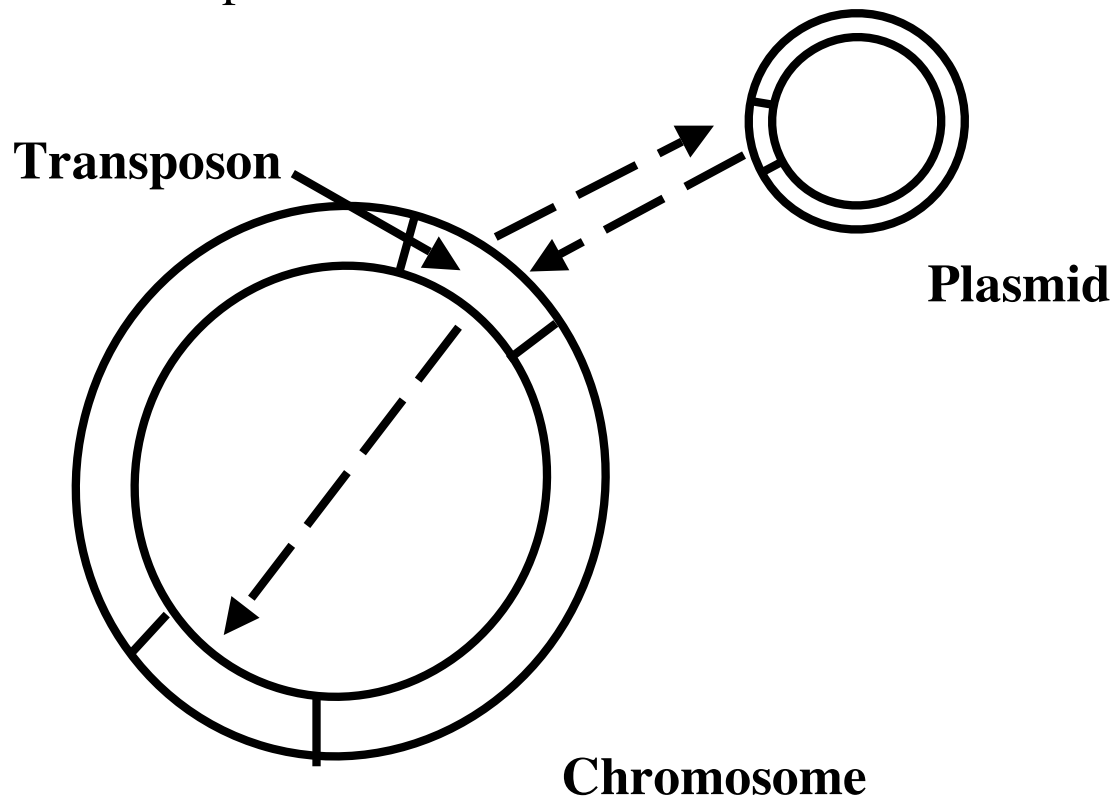
tetX Plasmid gene that encodes a neurotoxin

Recombination

- Joining together of separate DNA molecules
- **In cells**, integrates DNA into chromosome and plasmids
 - 1.) Plasmid or viral DNA may integrate into a chromosome
 - 2.) Transposons may move to another location or integrate into a plasmid or chromosome

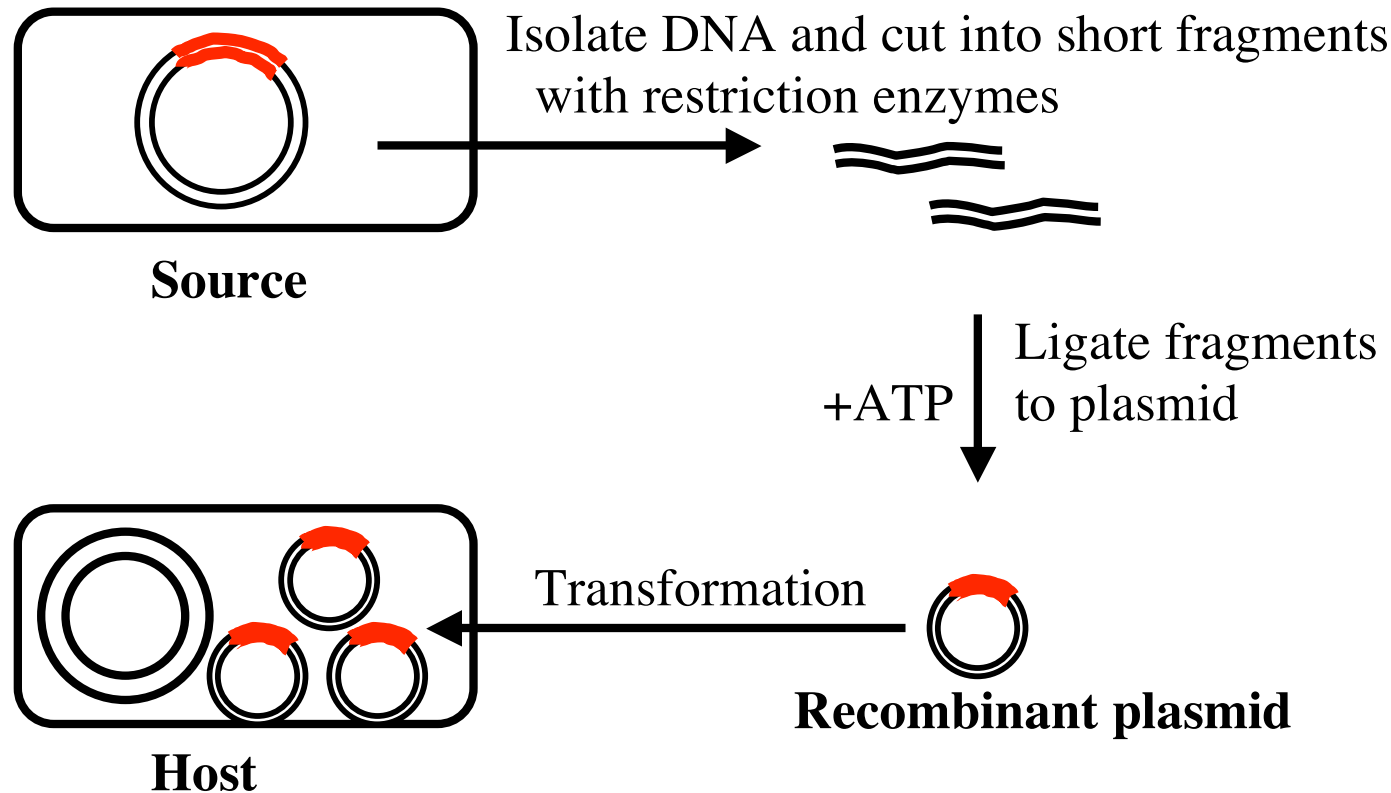
Transposons

- Segments of DNA that carry **mobile genes** (“jumping genes”)
- Recombination allows them to move from 1 chromosomal location to another or between a plasmid and the chromosome



- Antibiotic resistance genes on transposons can hitch rides on plasmids and spread to other cells

- **Recombinant DNA** can be also be artificially created in a test tube by mixing DNA fragments, enzymes and ATP
- **Gene cloning** involves isolating a gene and splicing it into a plasmid

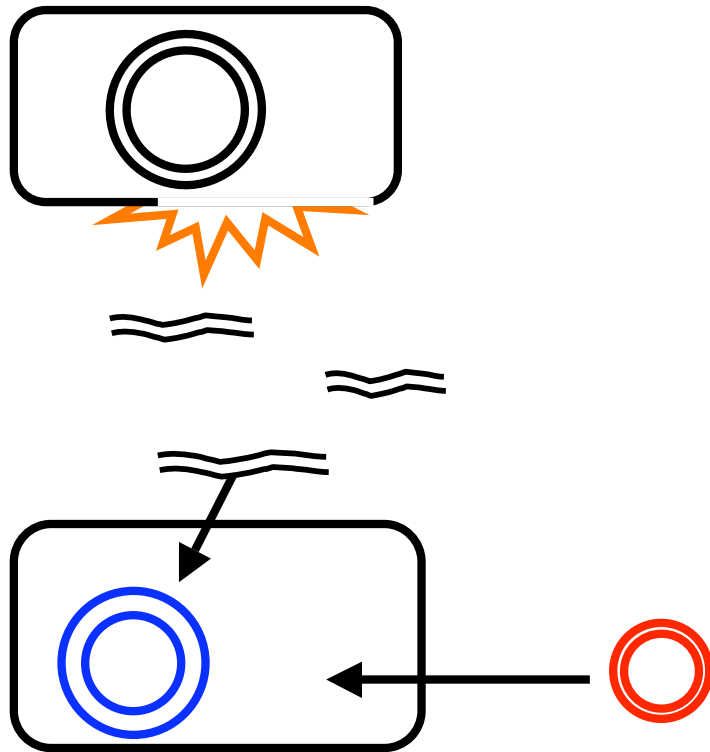


- Transforming the recombinant plasmid into a host cell allows the gene to be replicated and expressed
- Used in biotechnology to produce valuable proteins on large industrial scales
Ex. Human insulin gene expressed in *E. coli* to manufacture insulin for use by diabetics

2. Transformation

- Uptake of naked DNA from the environment by a cell and integration into chromosome

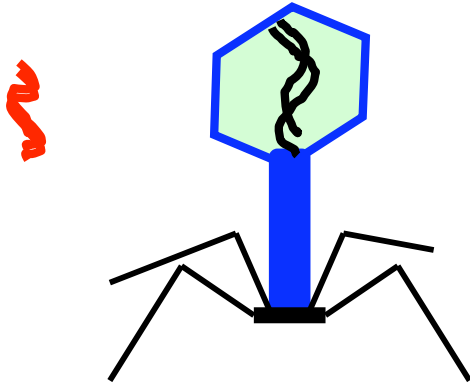
Ex. Uptake of DNA released when cells lyse



- In biotechnology transformation is used to introduce entire plasmids into bacterial cells

3. Transduction

- Transfer of bacterial DNA by bacteriophage



- Bacteriophages infect cells by injecting their DNA into the cell
- When new viral particles are produced, some of the bacterial DNA may be packaged into the virus
- Infection of another cell transfers the DNA to the new cell

Figure 9.25
Generalized
transduction

Mutation

- A permanent **change in the sequence** of bases of DNA
- **Spontaneous mutations** occur when mistakes are made during replication of DNA that are not corrected

1.) Nucleotide with wrong base may be incorporated

```
---T-A-C-G-A-G-G-C---  
---A-T-G-A-T-C-C-G---
```

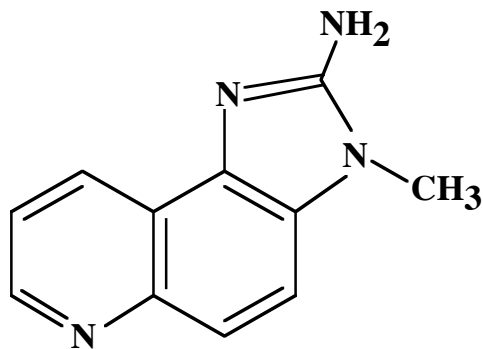
2.) Extra nucleotides may be added

3.) Nucleotides may be deleted

- **Mutation rate** is low because cell corrects most mistakes
- Most mutations lower fitness of microorganisms and are eventually eliminated from population

Induced mutations

- Mutation rate is increased by **mutagens** that **damage DNA**
 - **Physical mutagens**
Ex. X rays, ultraviolet light
 - **Chemical mutagens**
Ex. nitrous acid, mustard gas
- Mutagens may be **carcinogenic** in higher animal cells
Ex. Heterocyclic amines



IQ (2-Amino-3-methylimidazo[4,5-f]quinoline)

- Formed during cooking of meat (protein)
DNA damage may cause a cell to become cancerous
- Damage by uv light can cause skin cancer

Potential effects of mutations

1. Point mutation

- 1 base change in the sequence

Met Thr Cys Leu

ATG-ACG-TGC-CTG---- Wild type sequence

ATG-**CCG**-TGC-CTG---- Alters amino acid sequence

Lys

- May or may not affect structure and function of a protein

2. Frameshift mutation

- Results from insertion or deletion of nucleotide(s)
- Shifts the reading frame of codons during translation
- Drastically alters amino acid sequence --protein will be nonfunctional

Insertion



ATG-ACG-TGC-CTT-GAC---

Met Thr Cys Leu Asp

ATG-ACG-**ATG-CCT-TGA**-C---

Met Thr **Met Pro Stop**

3. Insertion or deletion

Effects of mutation on microorganisms

- **No effect** (silent mutation or one that doesn't disrupt function of crucial cellular components)
- **Death** –most mutations are deleterious to cell
 - Billions of years of evolution have selected for the most efficient phenotypic traits that allow a microorganism to survive and compete with others in their environment
- **Change in cell morphology**
 - Ex. Loss of ability to synthesize flagella
- **Loss or gain of virulence** (ability to cause disease)
 - Ex. Capsules help pathogens evade immune system of host
- Increased **resistance to antibiotics**
 - Ex. Antibiotic's target in cell may be changed so that the antibiotic can't affect it
- **Requirement** for a specific **nutrient** for growth
 - Ex. Loss of ability to synthesize an amino acid
- Increase or decrease in level of **gene expression**
 - Mutations in promoter region or in regulatory genes may affect binding of RNA polymerase