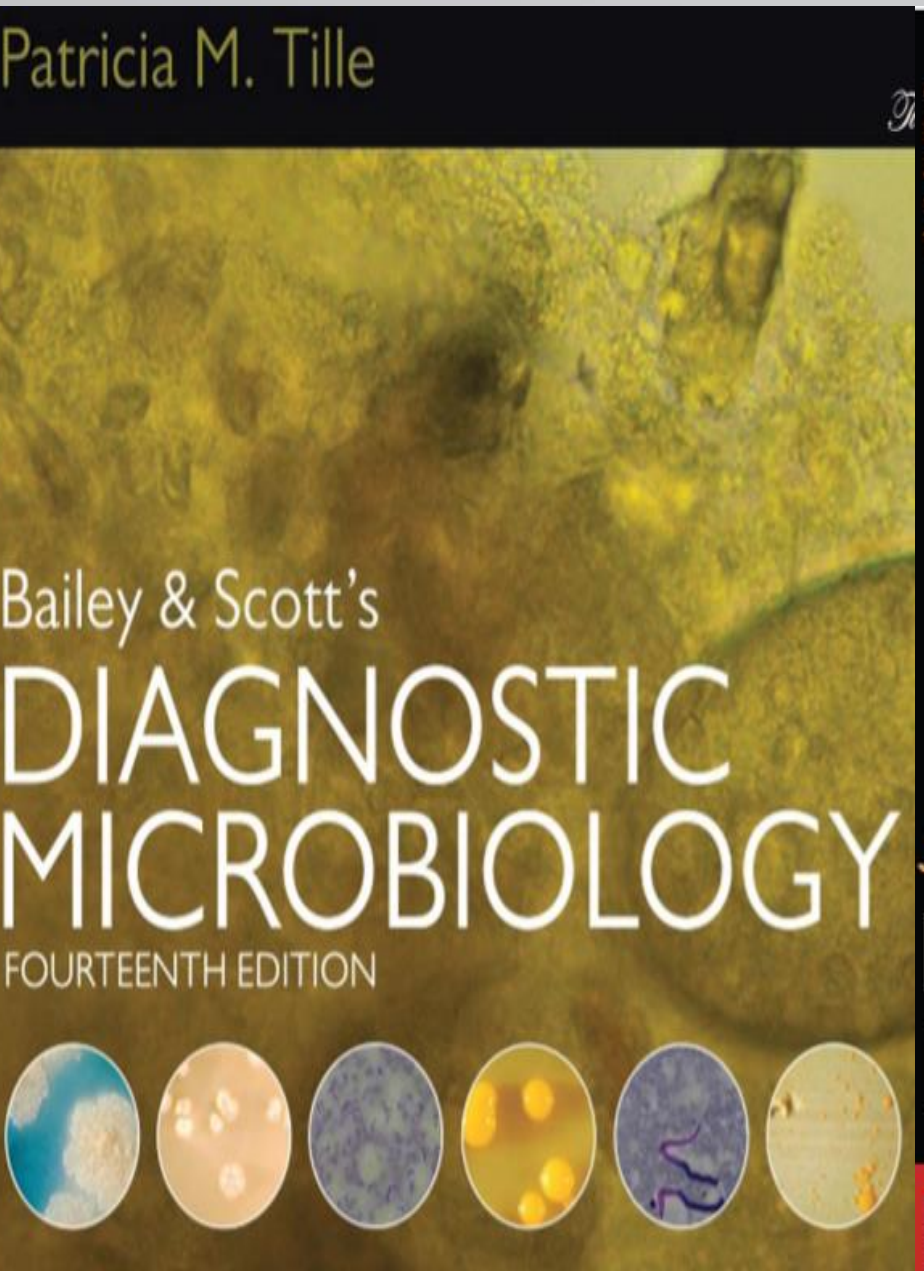
PROKARYOTIC CELL STRUCTURE

Lecture I

Ass.Pro. Asra'a Adnan Abdul-Jalil

References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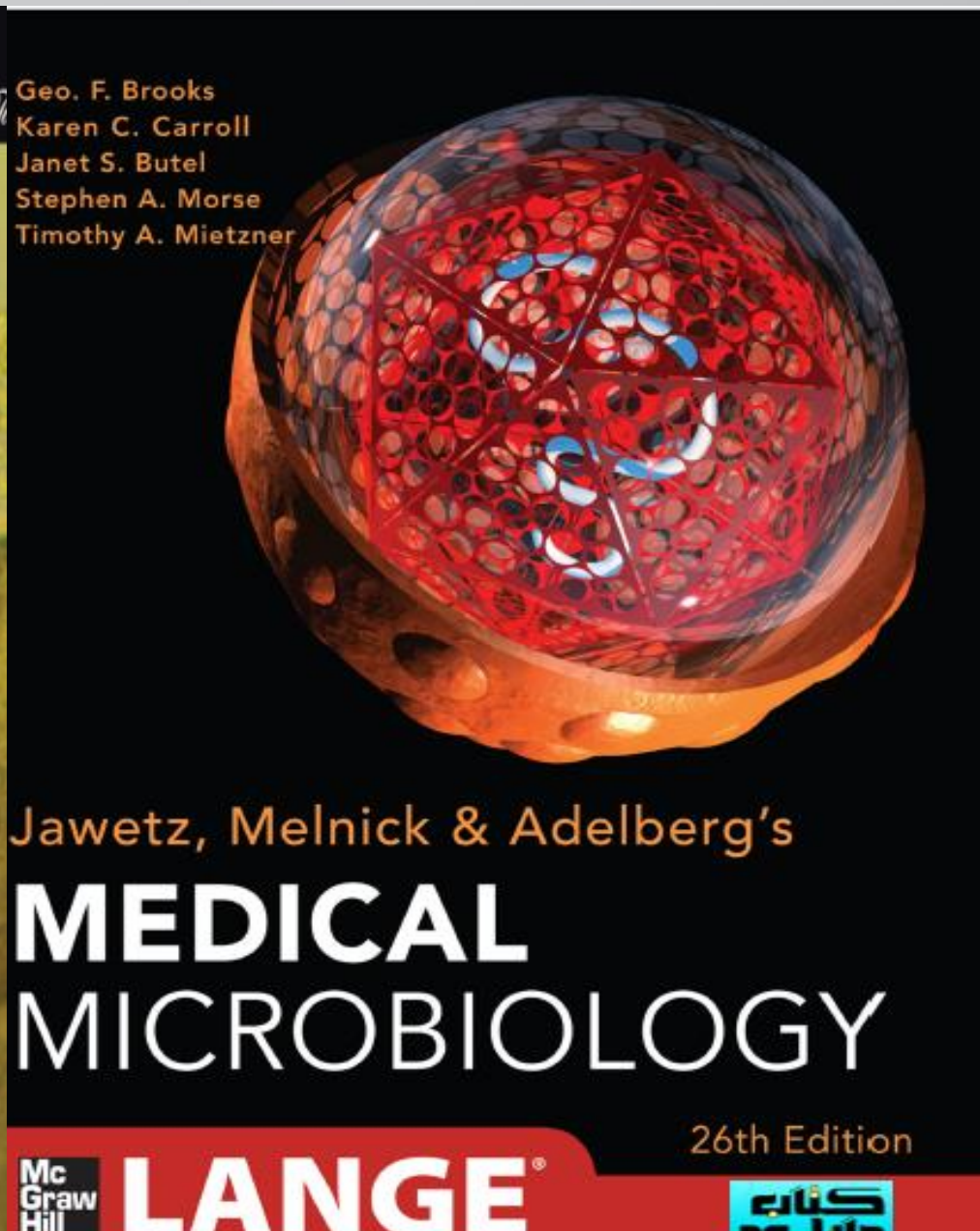
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Syllibus

- ✓ Anatomy of bacteria
- ✓ Genetics
- ✓ Recombinant DNA biotechnology.

Introduction

- Based on key characteristics, all cells are classified into two basic types: prokaryotic and eukaryotic

- The prokaryotic cell is simpler than the eukaryotic cell at every level, with one exception: The cell envelope is more complex.

Eukaryotic and Prokaryotic Cells

- bacteria are single-cell prokaryotic microorganisms.

- Fungi and parasites are single-cell or multicellular eukaryotic organisms, as are plants and all higher animals.
- Viruses are dependent on host cells for survival and therefore are not considered cellular organisms but rather infectious agents.
- Prions which are abnormal infectious proteins, are also not considered living cells

Eukaryotic cell vs. Prokaryotic cell

- The presence of membrane-enclosed organelles that have specific cellular functions like ER, Golgi apparatus, Mitochondria, Lysosomes & Nucleus.

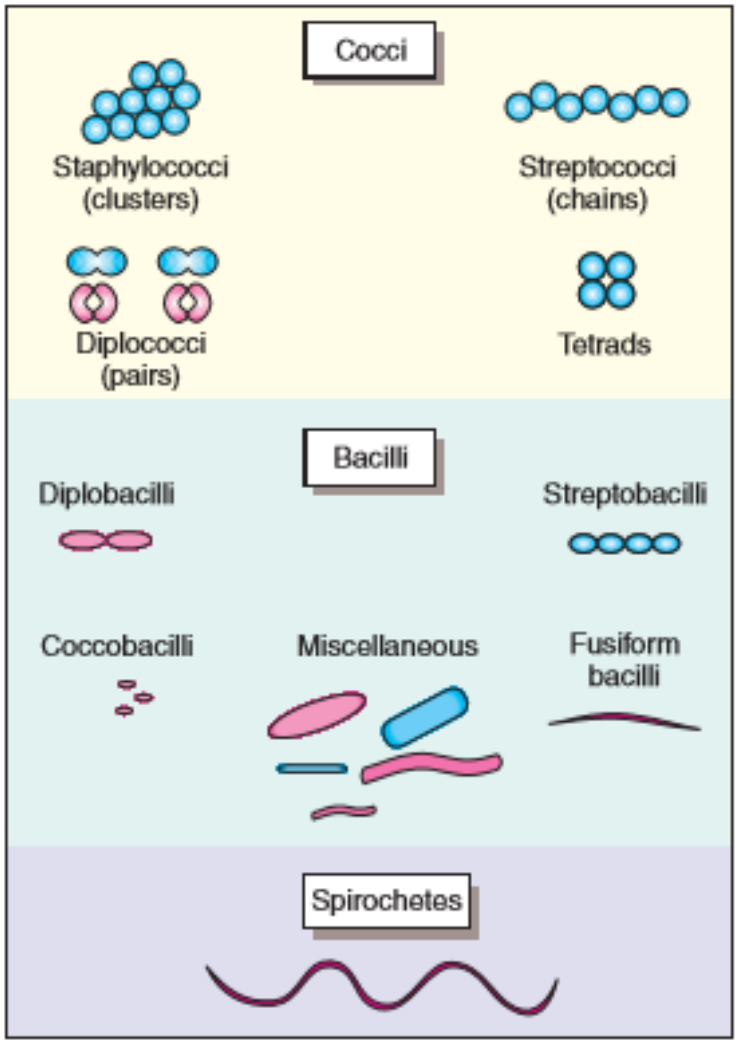
- eukaryotic cells have an infrastructure, or cytoskeleton, that provides support for cellular structure, organization, and movement.

- Prokaryotic cells, such as bacteria, do not contain organelles. All functions take place in the cytoplasm or cytoplasmic membrane of the cell.

- One notable structure present only in prokaryotic bacterial cells is a cell wall composed of peptidoglycan

Bacterial Morphology

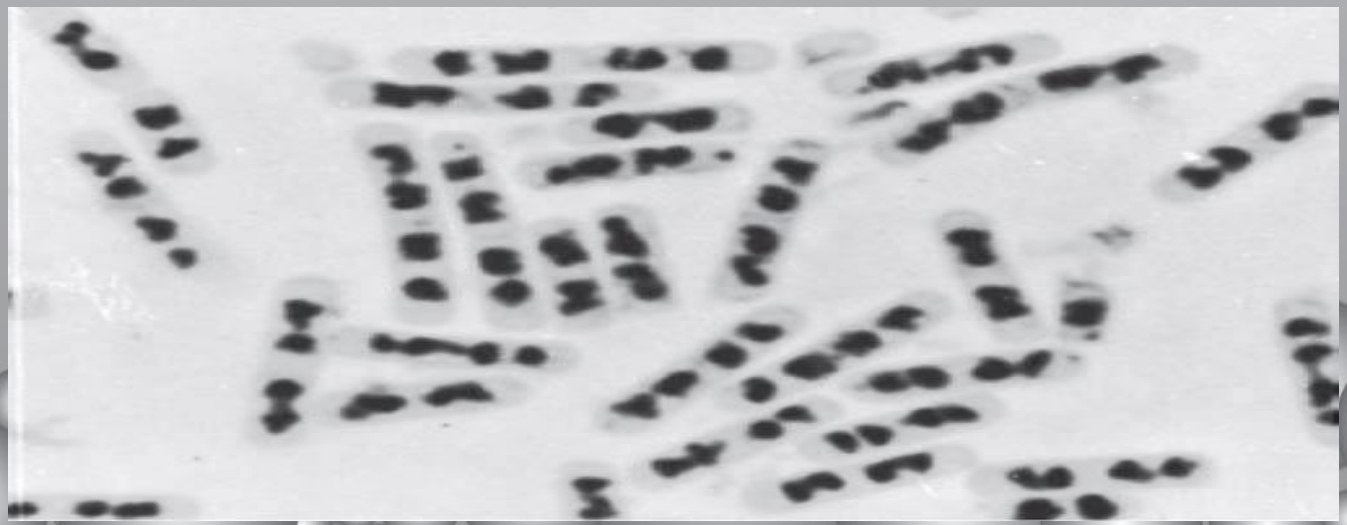
- Most clinically relevant bacterial species range in size from **0.25** to **1** μm in width and 1 to 3 μm in length, thus requiring microscopy for visualization.
- Gram stain is a fundamental staining technique used in bacterial identification schemes. This staining procedure separates almost all medically relevant bacteria into two general types: gram-positive bacteria, which stain a deep blue or purple, and gram-negative bacteria, which stain a pink to red.
- Common bacterial cellular morphologies include cocci (circular), coccobacilli (ovoid), and bacilli (rod shaped), as well as fusiform (pointed end), curved, or spiral shapes.
- Cells may characteristically occur singly, in pairs, or grouped as tetrads, clusters, or in chains.



• **Figure 6-4** Examples of common bacterial cellular morphologies, Gram staining reactions, and cellular arrangements.

Bacterial cell components

- Bacterial cell components can be divided into those that make up the outer cell structure and its appendages (cell envelope) and those associated with the cell's interior
- **The Nucleoid:**
- Prokaryotes have no true nuclei; instead they package their DNA in a structure known as the **nucleoid**. The nucleoid can be seen with the light microscope in stained material.



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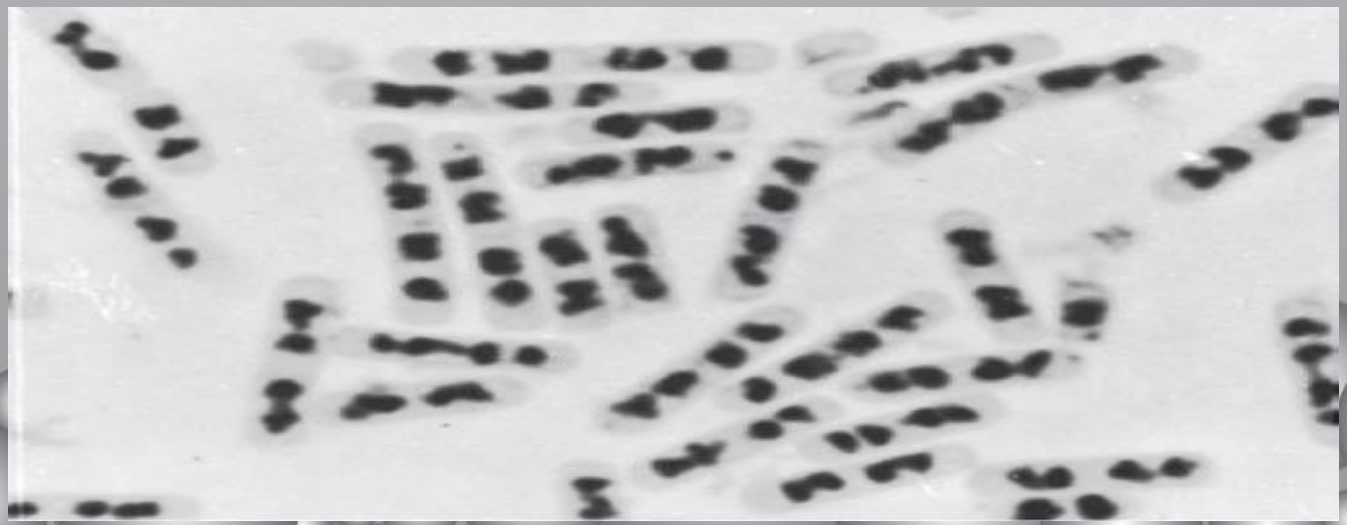
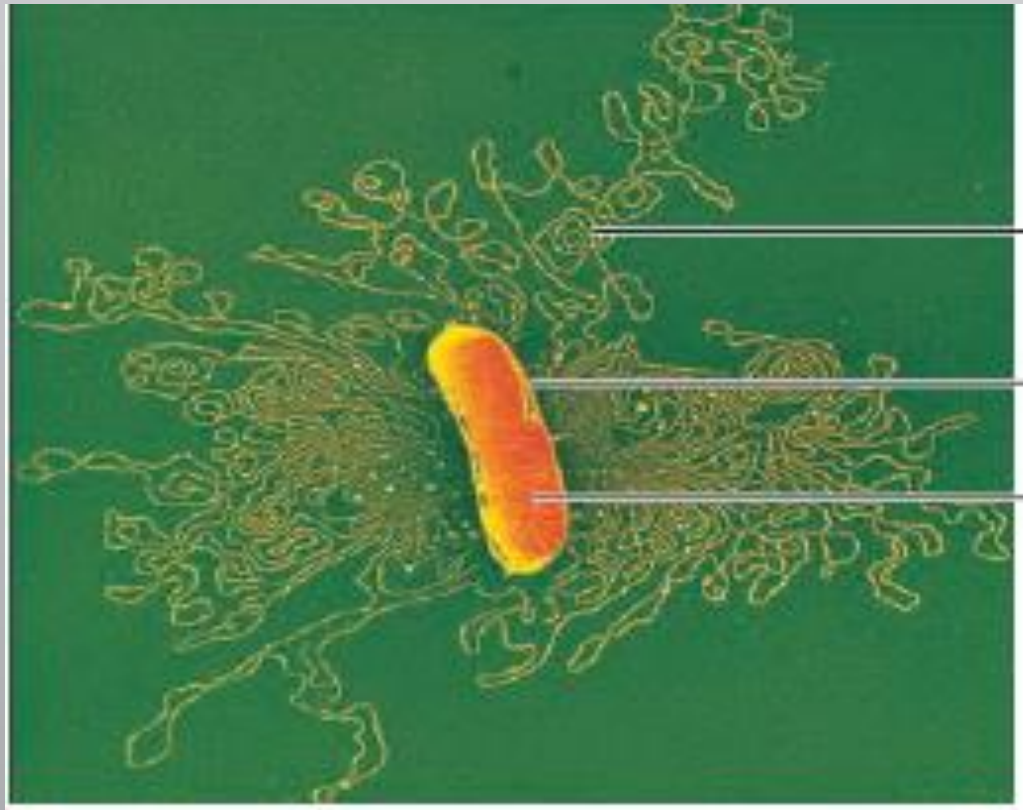


- It is Feulgen positive, indicating the presence of DNA. The negatively charged DNA is at least partially neutralized by small polyamines and magnesium ions, but histone-like proteins exist in bacteria and presumably play a role similar to that of histones in eukaryotic chromatin.
- Nucleoids are stainable with the Feulgen stain, which is specific for DNA

- Electron micrographs of a typical prokaryotic cell reveal the absence of a nuclear membrane and a mitotic apparatus. The exception to this rule is the planctomycetes, a divergent group of aquatic bacteria, which have a nucleoid surrounded by a nuclear envelope consisting of two membranes.

The nucleoid of most bacterial cells consists of a single continuous circular molecule ranging in size from 0.58 to almost 10 million base Pairs (???). However, a few bacteria have been shown to have two, three, or even four dissimilar chromosomes. For example, *Vibrio cholerae* and *Brucella melitensis* have two dissimilar chromosomes. There are exceptions to this rule of circularity because some prokaryotes (eg, *Borrelia burgdorferi* and *Streptomyces coelicolor*) have been shown to have a linear chromosome.

- In bacteria, the number of chromosomes, depend on the growth conditions Rapidly growing bacteria have more nucleoids per cell than slowly growing ones; however, when multiple copies are present, they are all the same (prokaryotic cells are haploid).



B

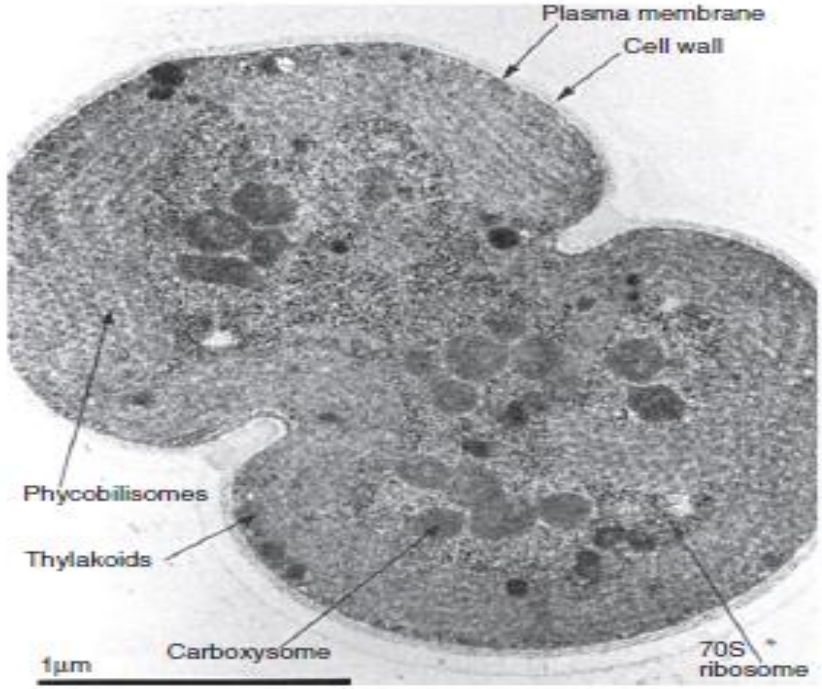
FIGURE 2-6 The nucleoid. **A:** Color-enhanced transmission electron micrograph of *Escherichia coli* with the DNA shown in *red*. (© CNRI/SPL/Photo Researchers, Inc.) **B:** Chromosome released from a gently lysed cell of *E coli*. Note how tightly packaged the DNA must be inside the bacterium. (© Dr. Gopal Murti/SPL/Photo Researchers.)

- **Cytoplasmic Structures**

- Prokaryotic cells lack autonomous plastids, such as mitochondria and chloroplasts; the electron transport enzymes are localized instead in the cytoplasmic membrane. The photosynthetic pigments (carotenoids, bacteriochlorophyll) of photosynthetic bacteria are contained in intracytoplasmic membrane systems of various morphologies

- Some photosynthetic bacteria have specialized nonunit membrane-enclosed structures called **chlorosomes.**
- In some Cyanobacteria (formerly known as blue-green algae), the photosynthetic membranes often form multilayered structures known as thylakoids. The major accessory pigments used for light harvesting are the phycobilins found on the outer surface of the thylakoid membranes.

A



B

FIGURE 2-7 **A:** Thin section of *Synechococcus lividus* showing an extensive thylakoid system. The phycobillisomes lining these thylakoids are clearly visible as granules at location t (85,000×). (Reproduced with permission from Elizabeth Gentt/Visuals Unlimited.) **B:** Thin section of *Synechocystis* during division. Many structures are visible. (Reproduced with permission from Carlsberg Research Communications 42:77-98, 1977, With kind permission of Springer Science+Business Media.)

Bacteria often store reserve materials in the form of insoluble granules, which appear as refractile bodies in the cytoplasm when viewed by phase contrast microscopy. These so-called inclusion bodies almost always function in the storage of energy or as a reservoir of structural building blocks. Most cellular inclusions are bounded by a thin nonunit membrane consisting of lipid, which serves to separate the inclusion from the cytoplasm proper. One of the most common inclusion bodies consists of poly-β-hydroxybutyric acid (PHB), a lipid-like compound consisting of chains of β-hydroxybutyric acid units connected through ester linkages. PHB is produced when the source of nitrogen, sulfur, or phosphorous is limited and there is excess carbon in the medium

Another storage product formed by prokaryotes when carbon is in excess is glycogen, which is a polymer of glucose. PHB and glycogen are used as carbon sources when protein and nucleic acid synthesis are resumed.

PROKARYOTIC CELL STRUCTURE -CELL ENVELOPE-

Lecture II

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The Cell Envelope

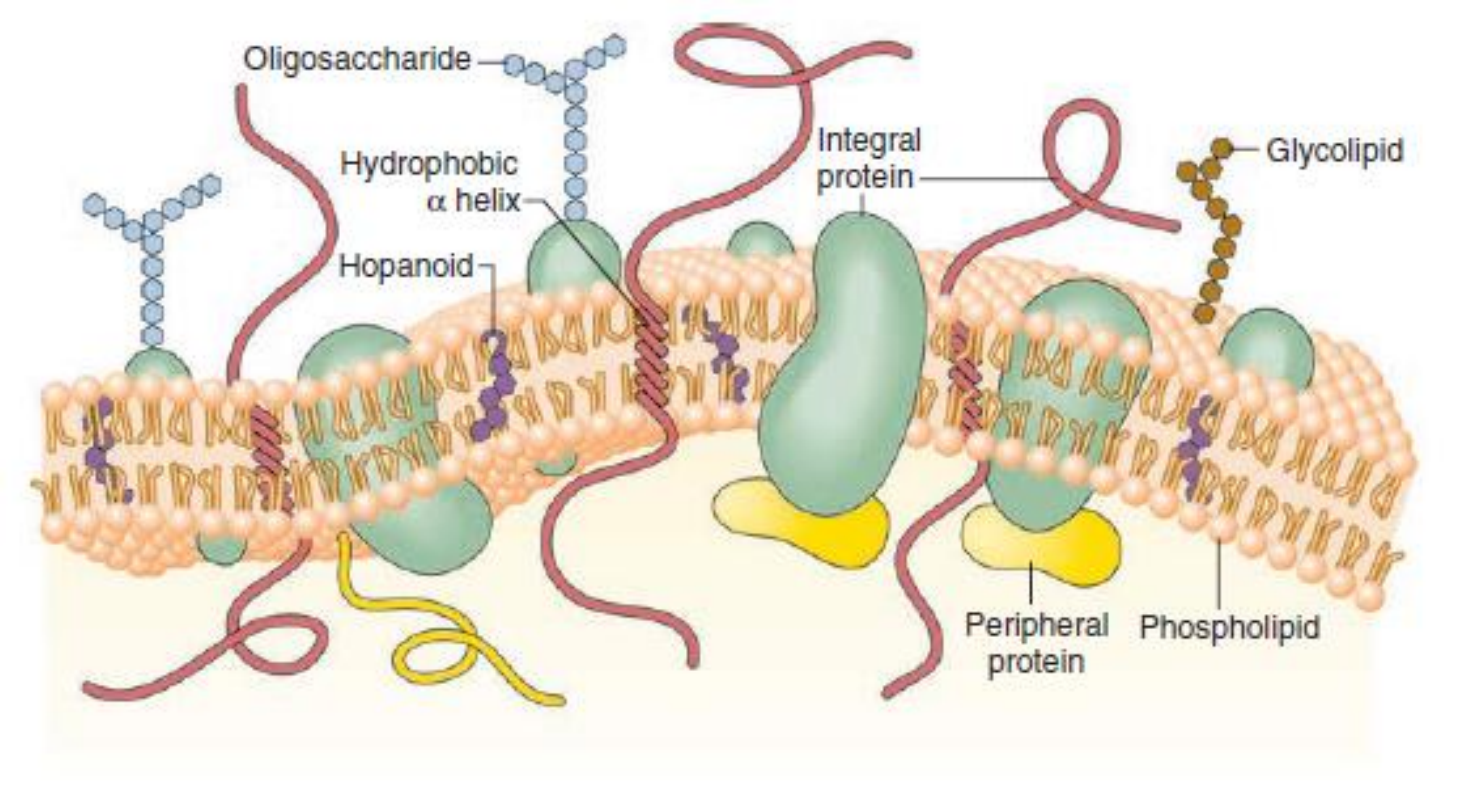
Prokaryotic cells are surrounded by complex envelope layers that differ in composition among the major groups. These structures protect the organisms from hostile environments,

such as extreme osmolarity, harsh chemicals, and even antibiotics.

The Cell Membrane

- **A. Structure**

The bacterial cell membrane, also called the cytoplasmic membrane, is visible in electron micrographs of thin sections. It is a typical “unit membrane” composed of phospholipids and upward of 200 different kinds of proteins. Proteins account for approximately 70% of the mass of the membrane, which is a considerably higher proportion than that of mammalian cell membranes.



Bacterial Plasma Membrane Structure. This diagram of the fluid mosaic model of bacterial membrane structure shown the integral proteins (green and red) floating in a lipid bilayer. Peripheral proteins (yellow) are associated loosely with the inner membrane surface. Small spheres represent the hydrophilic ends of membrane phospholipids and wiggly tails, the hydrophobic fatty acid chains. Other membrane lipids such as hopanoids (purple) may be present. For the sake of clarity, phospholipids are shown proportionately much larger size than in real membranes

The membranes of prokaryotes are distinguished from those of eukaryotic cells by the absence of sterols, the only exception being mycoplasmas that incorporate sterols, such as cholesterol, into their membranes when growing in sterol-containing media.

• B. Function

The major functions of the cytoplasmic membrane are

(1) selective permeability and transport of solutes;

(2) electron transport and oxidative phosphorylation in aerobic species.

(3) excretion of hydrolytic exoenzymes.

(4) bearing the enzymes and carrier molecules that function in the biosynthesis of DNA, cell wall polymers, and membrane lipids; and

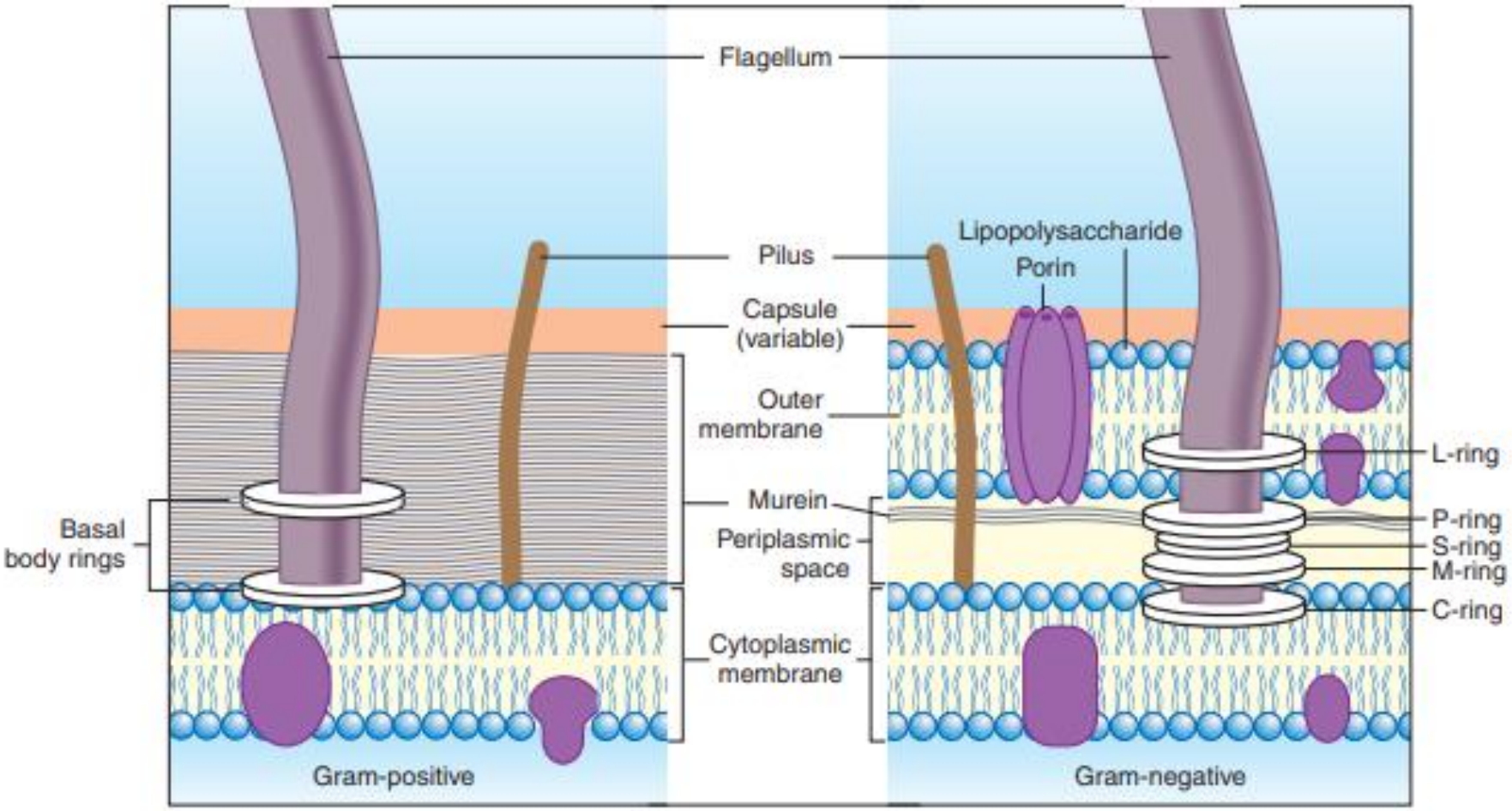
(5) bearing the receptors and other proteins of the chemotactic and other sensory transduction systems.

At least 50% of the cytoplasmic membrane must be in the semifluid state for cell growth to occur. At low temperatures, this is achieved by greatly increased synthesis and incorporation of unsaturated fatty acids into the phospholipids of the cell membrane.

The Cell Wall(murein layer)

The internal osmotic pressure of most bacteria ranges from 5 to 20 atm as a result of solute concentration via active transport. In most environments, this pressure would be sufficient to burst the cell were it not for the presence of a high-tensile strength cell wall. The bacterial cell wall owes its strength to a layer composed of a substance variously referred to as murein, mucopeptide, or peptidoglycan (all are synonyms). Most bacteria are classified as gram-positive or gram negative according to their response to the Gram-staining procedure. This procedure was named for the histologist Hans Christian Gram, who developed this differential staining procedure in an attempt to stain bacteria in infected tissues. The Gram stain depends on the ability of certain bacteria (the gram-positive bacteria) to retain a complex of crystal violet (a purple dye) and iodine after a brief wash with alcohol or acetone. Gram-negative bacteria do not retain the dye–iodine complex and become translucent, but they can then be counterstained with safranin (a red dye)

- **The Gram stain can be used to divide most bacterial species into two large groups: those that take up the basic dye, crystal violet (i.e., gram-positive bacteria), and those that allow the crystal violet dye to wash out easily with the decolorizer alcohol or acetone (i.e., gram-negative bacteria). Thus, gram-positive bacteria look purple under the microscope, and gram-negative bacteria look red. The distinction between these two groups turns out to reflect fundamental differences in their cell envelopes.**
- **The most common stain in bacteriology is the Gram stain, which helps the clinician to visualize rods, cocci, white blood cells, red blood cells, or squamous epithelial cells present in the sample.**



• **Figure 2-13** General structures of the gram-positive and gram-negative bacterial cell envelopes. The outer membrane and periplasmic space are present only in the envelope of gram-negative bacteria. In addition to porins, bacterial membranes contain additional proteins involved in stabilizing the layers of the cellular structure, adherence, or sorting and reacting to chemical signals. The murein layer is substantially more prominent in gram-positive envelopes. (Modified from Niedhardt FC, Ingraham JL, Schaechter M, editors: *Physiology of the bacterial cell: a molecular approach*, Sunderland, MA, 1990, Sinauer Associates.)

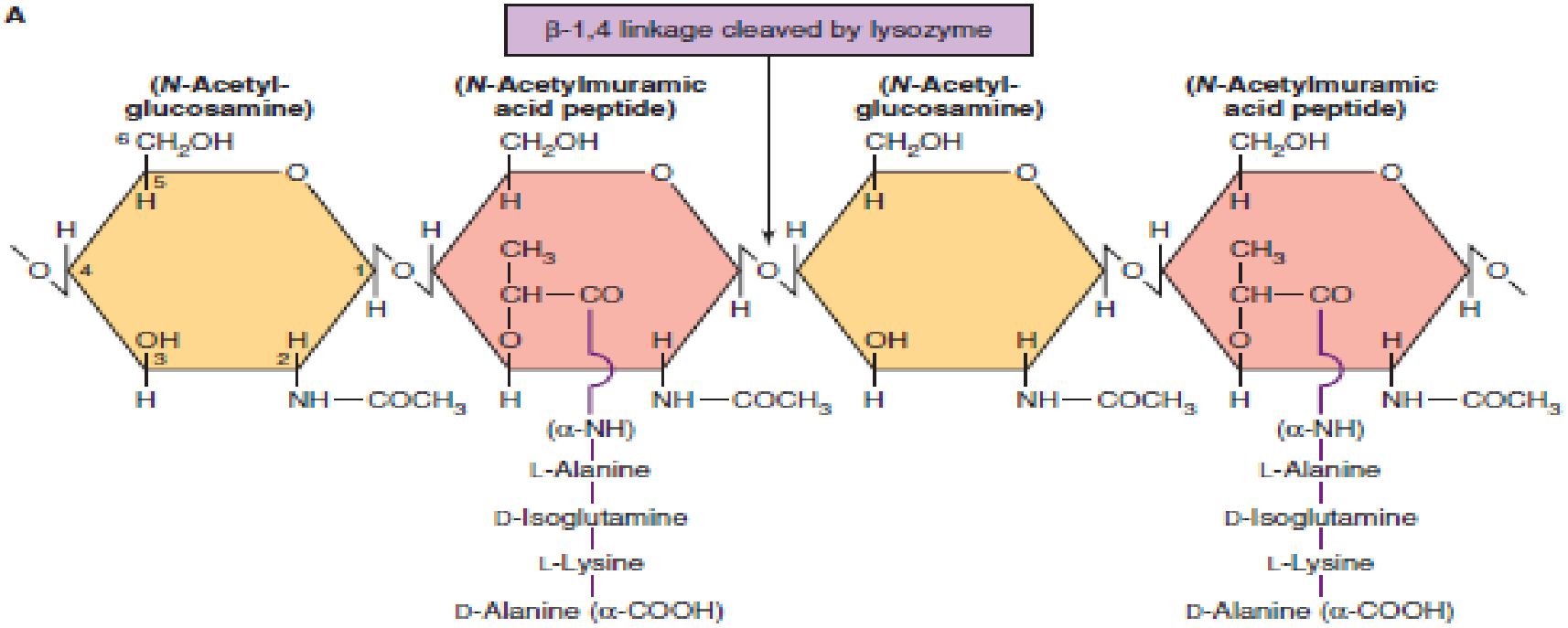
In addition to giving osmotic protection, the cell wall plays an essential role in cell division as well as serving as a primer for its own biosynthesis. Various layers of the wall are the sites of major antigenic determinants of the cell surface, and one component—the lipopolysaccharide of gram-negative cell walls—is responsible for the nonspecific endotoxin activity of gram-negative bacteria. The cell wall is, in general, non-selectively permeable; one layer of the gram-negative wall, however—the outer membrane—hinders the passage of relatively large molecules.

A. The Peptidoglycan Layer(murein) structure

Peptidoglycan is a complex polymer consisting, for the purposes of description, of three parts: a backbone, composed of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid connected by β1→4 linkages; a set of identical tetrapeptide side chains attached to *N*-acetylmuramic acid; and a set of identical peptide cross-bridges . The backbone is the same in all bacterial species; the tetrapeptide side chains and the peptide cross-bridges vary from species to species. In many gram-negative cell walls, the cross-bridge consists of a direct peptide linkage between the diaminopimelic acid (DAP) amino group of one side chain and the carboxyl group of the terminal d-alanine of a second side chain.

Diaminopimelic acid is a unique element of bacterial cell walls. It is never found in the cell walls of *Archaea* or eukaryotes.

The fact that all peptidoglycan chains are cross-linked means that each peptidoglycan layer is a single giant molecule. In gram-positive bacteria, there are as many as 40 sheets of peptidoglycan, comprising up to 50% of the cell wall material; in gram-negative bacteria, there appears to be only one or two sheets, comprising 5–10% of the wall material. Bacteria owe their shapes, which are characteristic of particular species, to their cell wall structure.

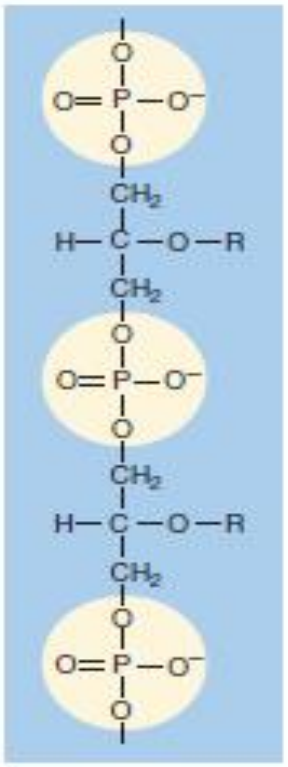


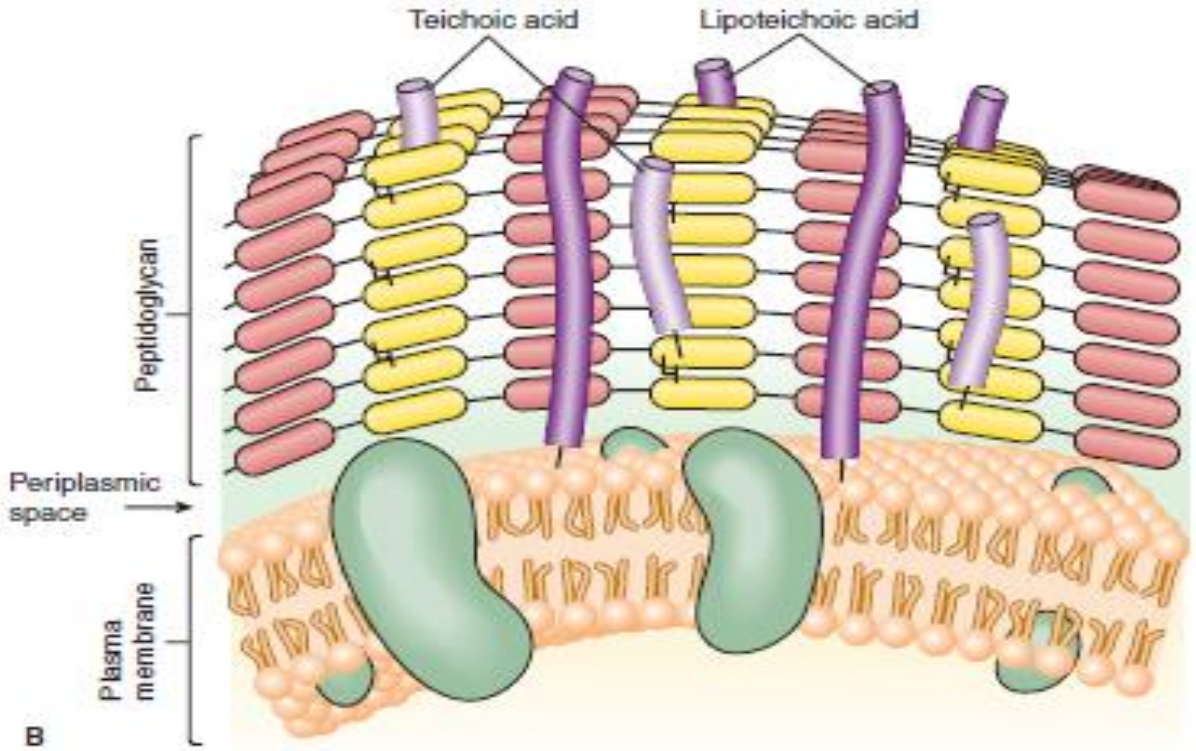
segment of the peptidoglycan of ***Staphylococcus aureus.*** **The backbone of the polymer consists of alternating subunits of *N*-acetylglucosamine and *N*-acetylmuramic acid connected by β1→4 linkages. The muramic acid residues are linked to short peptides, the composition of which varies from one bacterial species to another. In some species, the l-lysine residues are replaced by diaminopimelic acid, an amino acid that is found in nature only in prokaryotic cell walls. Note the d-amino acids, which are also characteristic constituents of prokaryotic cell walls**

- **B. Special Components of Gram-Positive Cell Walls**

Most gram-positive cell walls contain considerable amounts of **teichoic** and **teichuronic acids**, which may account for up to 50% of the dry weight of the wall and 10% of the dry weight of the total cell. In addition, some gram-positive walls may contain polysaccharide molecules.

1. Teichoic and teichuronic acids—The term *teichoic acids* encompasses all wall, membrane, or capsular polymers containing glycerophosphate or ribitol phosphate residues. These polyalcohols are connected by phosphodiester linkages and usually have other sugars and d-alanine attached. Because they are negatively charged, teichoic acids are partially responsible for the negative charge of the cell surface as a whole. There are two types of teichoic acids: **wall teichoic acid (WTA)**, covalently linked to peptidoglycan, and **membrane teichoic acid**, covalently linked to membrane glycolipid. Because the latter are intimately associated with lipids, they have been called **lipoteichoic acids (LTA**





A: Teichoic acid structure. The segment of a teichoic acid made of phosphate, glycerol, and a side chain, R. R may represent
D-alanine, glucose, or other molecules. **B:** Teichoic and lipoteichoic acids of the gram-positive envelope.

The **teichuronic acids** are similar polymers, but the repeat units include sugar acids (eg, *N*-acetylmannosuronic or d-glucosuronic acid) instead of phosphoric acids. They are synthesized in place of teichoic acids when phosphate is limiting.

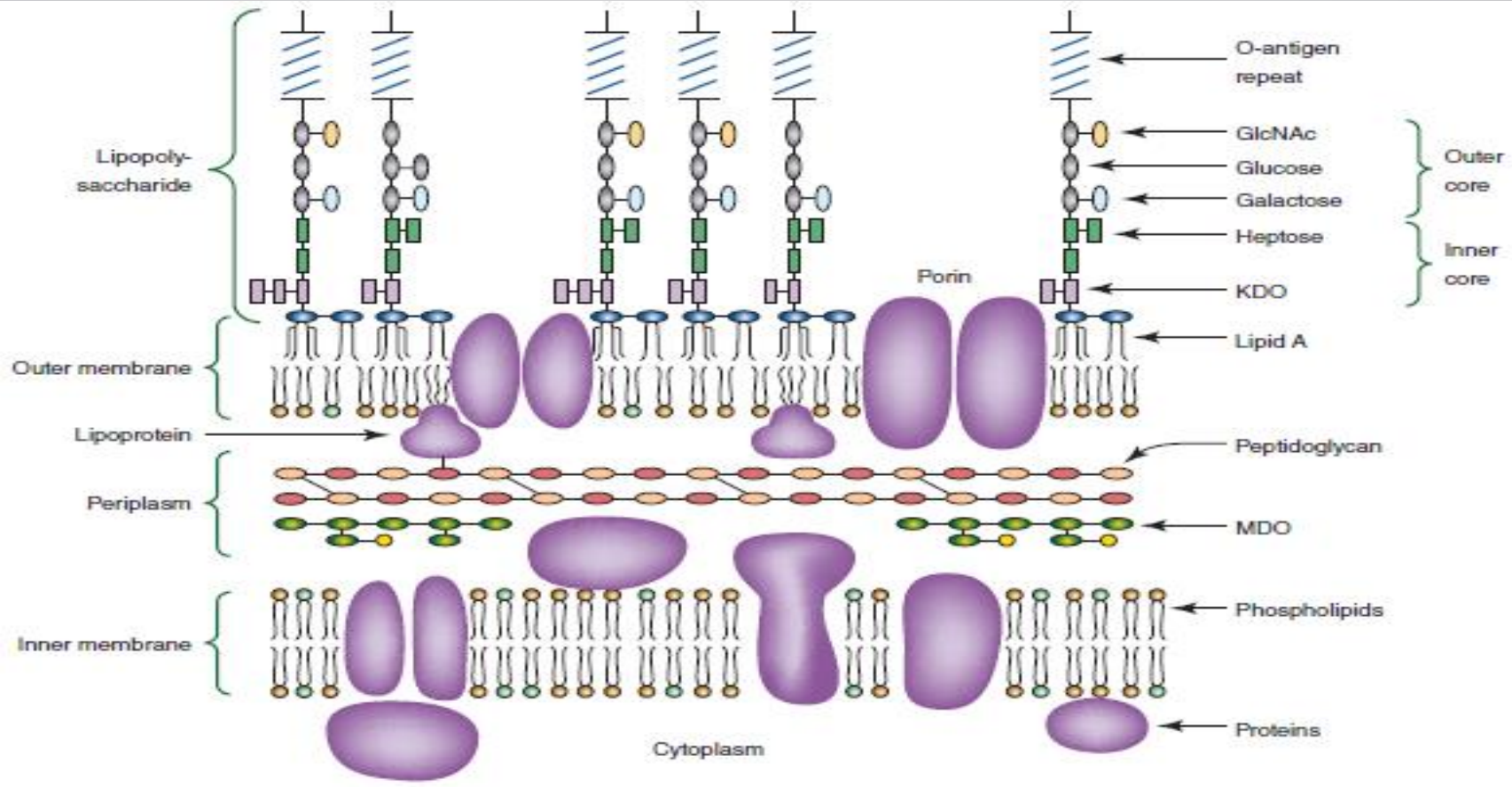
WTA and LTA make up a matrix that provides functions relating to the elasticity, porosity, and electrostatic properties of the envelope.

Special Components of Gram-Negative Cell Walls

Gram-negative cell walls contain three components that lie outside of the peptidoglycan layer: lipoprotein, outer membrane,and lipopolysaccharide.

1. Outer membrane—The outer membrane is chemically distinct from all other biological membranes. It is a bilayered structure; its inner leaflet resembles in composition that of the cell membrane, and its outer leaflet contains a distinctive component, a **lipopolysaccharide (LPS)** . As a result, the leaflets of this membrane are asymmetrical, and the properties of this bilayer differ considerably from those of a symmetrical biologic membrane such as the cell membrane. the outer membrane has special channels, consisting of protein molecules called **porins** that permit the passive diffusion of low-molecular-weight hydrophilic compounds such as sugars, amino acids, and certain ions.

Large antibiotic molecules penetrate the outer membrane relatively slowly, which accounts for the relatively **high antibiotic resistance** of gram negative bacteria. The permeability of the outer membrane.



Molecular representation of the envelope of a gram-negative bacterium. *Ovals* and *rectangles* represent sugar residues, and *circles* depict the polar head groups of the glycerophospholipids (phosphatidylethanolamine and phosphatidylglycerol).

2. **Lipopolysaccharide (LPS)—**The LPS of gram-negative cell walls consists of a complex glycolipid, called lipid A, to which is attached a polysaccharide made up of a core and a terminal series of repeat units. The lipid A component is embedded in the outer leaflet of the membrane anchoring the LPS. LPS is synthesized on the cytoplasmic membrane and transported to its final exterior position. The presence of LPS is required for the function of many outer membrane proteins. Lipid A consists of phosphorylated glucosamine disaccharide units to which are attached a number of long-chain fatty acids. and B is similar in all gram-negative species that have LPS and includes two characteristic sugars, ketodeoxyoctanoic acid (KDO) and a heptose.

Each species, however, contains a unique repeat unit, The repeat units are usually linear trisaccharides or branched tetra- or pentasaccharides. The repeat unit is referred to as the O antigen. The hydrophilic carbohydrate chains of the O-antigen cover the bacterial surface and exclude hydrophobic compounds.

- Lipopolysaccharide, which is extremely toxic to animals, has been called the **endotoxin** of gram-negative bacteria because it is firmly bound to the cell surface and is released only when the cells are lysed. When LPS is split into lipid A and polysaccharide, all of the toxicity is associated with the former.

The periplasmic space—The space between the inner and outer membranes, called the **periplasmic space**, contains the peptidoglycan layer and a gel-like solution of proteins. The periplasmic space is approximately 20–40% of the cell volume, which is far from insignificant. The periplasmic proteins include binding proteins for specific substrates (eg, amino acids, sugars, vitamins, and ions), hydrolytic enzymes. alkaline phosphatase and 52-nucleotidase) that break down nontransportable substrates into transportable ones, and detoxifying enzymes (eg, β-lactamase and aminoglycoside phosphorylase) that inactivate certain antibiotics.

Surface appendages

Capsule & Glycocalyx

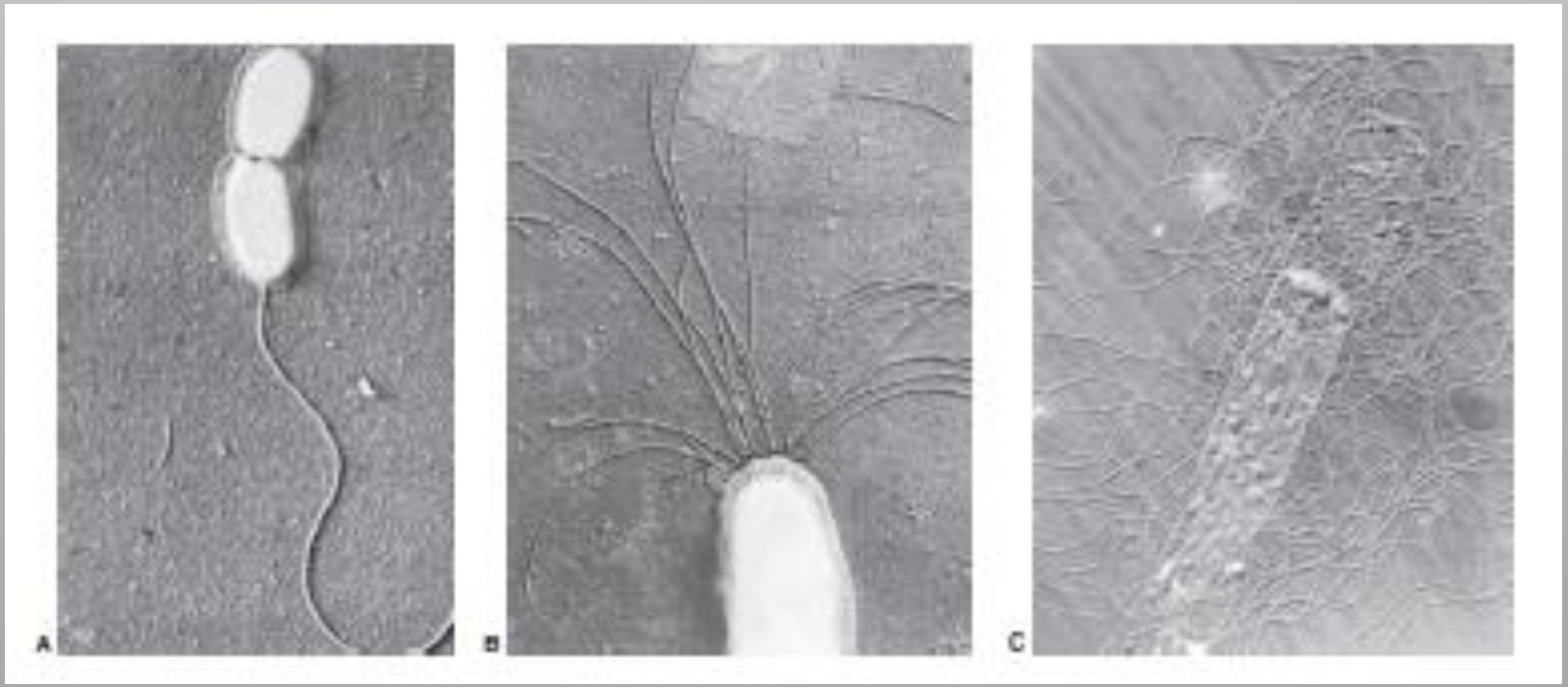
Many bacteria synthesize large amounts of extracellular polymer when growing in their natural environments. With one known exception (the poly-D-glutamic acid capsules of *Bacillus anthracis* and *Bacillus licheniformis*), the extracellular material is polysaccharide .The terms **capsule** and **slime layer** are frequently used to describe polysaccharide layers; the more inclusive term **glycocalyx** is also used. Glycocalyx is defined as the polysaccharide-containing material lying outside the cell.

The capsule contributes to the invasiveness of pathogenic bacteria—encapsulated cells are protected from phagocytosis unless they are coated with anticapsular antibody. The glycocalyx plays a role in the adherence of bacteria to surfaces in their environment, including the cells of plant and animal hosts.

Flagella

Bacterial flagella are thread-like appendages composed entirely of protein, 12–30 nm in diameter. They are the organs of locomotion for the forms that possess them. Three types of arrangement are known: **monotrichous** (single polar flagellum), **lophotrichous** (multiple polar flagella), and **peritrichous** (flagella distributed over the entire cell).

Bacterial flagellation



monotrichous **Polytrichous** **peritrichous**

A bacterial flagellum is made up of several thousand molecules of a protein subunit called **flagellin**. In a few organisms (eg, Caulobacter), flagella are composed of two types of flagellin, but in most, only a single type is found. The flagellum is formed by the aggregation of subunits to form a helical structure.

Pili (Fimbriae)

Many gram-negative bacteria possess rigid surface appendages called **pili** (L "hairs") or **fimbriae** (L "fringes"). They are shorter and finer than flagella; similar to flagella, they are composed of structural protein subunits termed **pilins**.

Some pili contain a single type of pilin, others more than one. Minor proteins termed **adhesins** are located at the tips of pili and are responsible for the attachment properties. Two classes can be distinguished: ordinary pili, which play a role in the adherence of symbiotic and pathogenic bacteria to host cells, and sex pili, which are responsible for the attachment of donor and recipient cells in bacterial conjugation. Pili are illustrated in Figure 2-27, in which the sex pili have been coated with phage particles for which they serve as specific receptors.

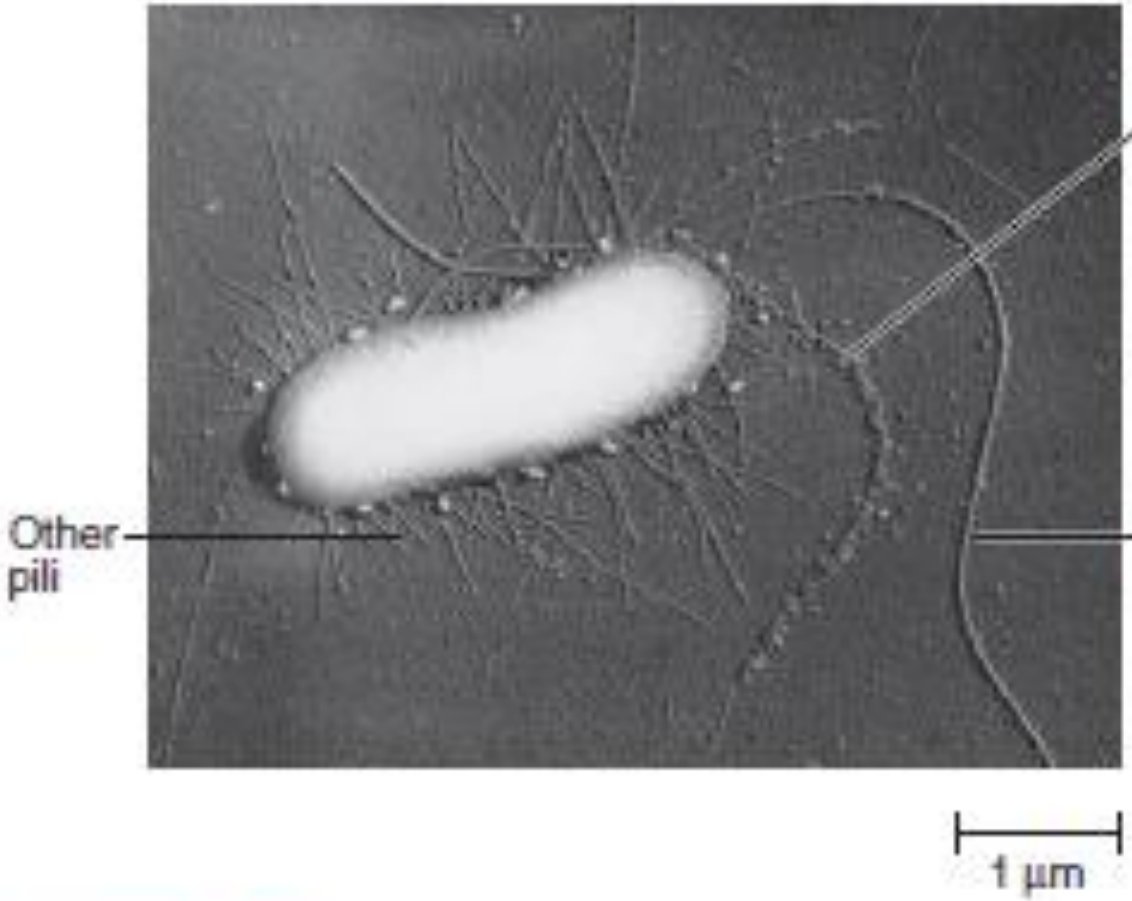
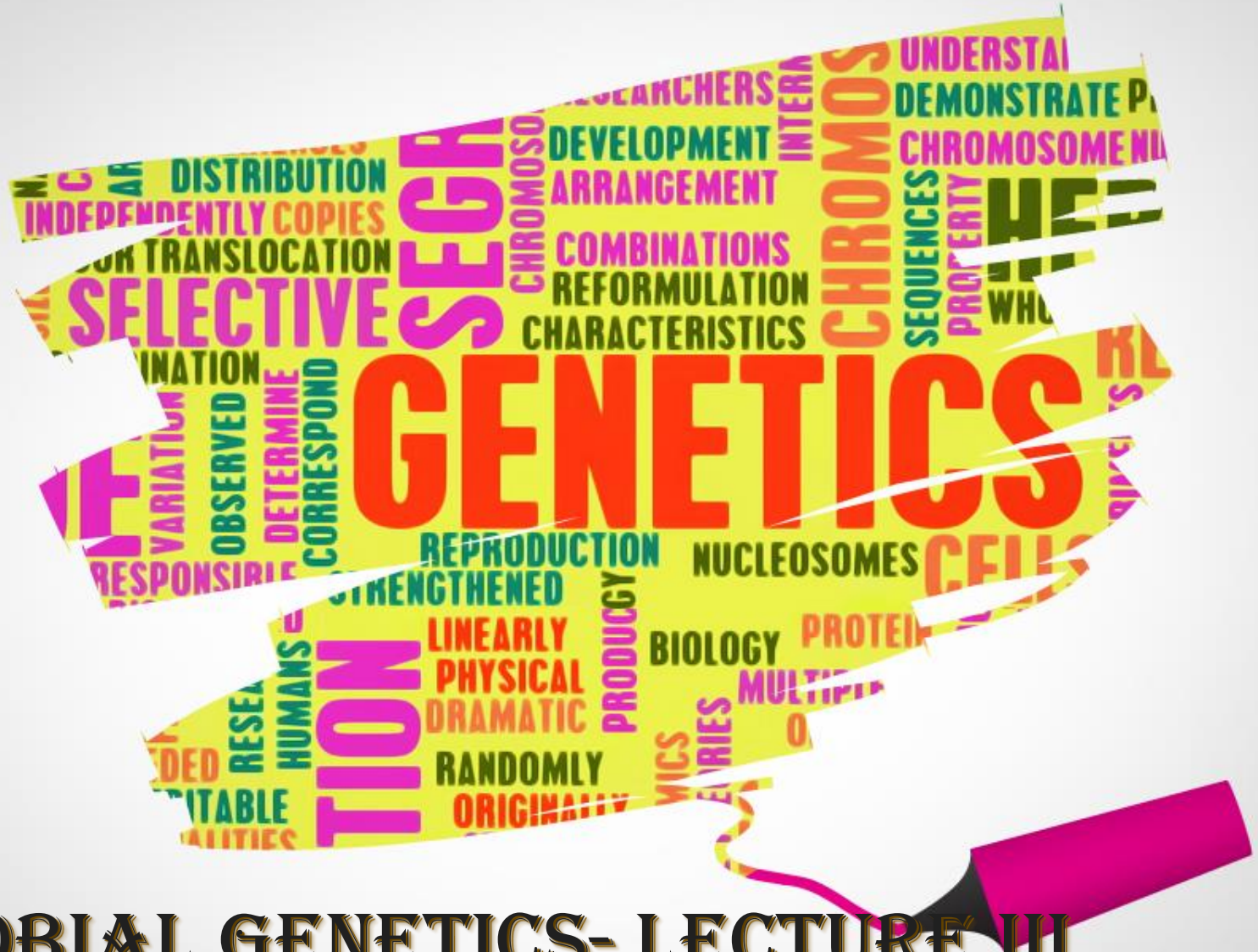


FIGURE 2-27 Pili. Pili on an *Escherichia coli cell*. The short pili (fimbriae) mediate adherence; the sex pilus is involved in DNA transfer. (Courtesy of Dr. Charles Brinton, Jr.)



MICROBIAL GENETICS- LECTURE III

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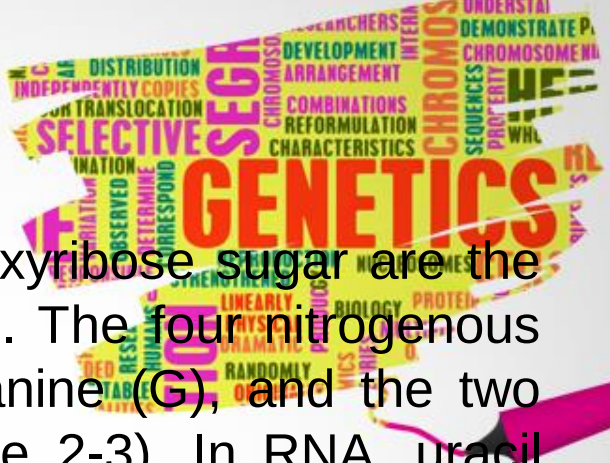
Nucleic Acid Structure and Organization

- Genetic information in bacteria is stored as a sequence of **DNA bases.**

- In bacteriophages and viruses, genetic information can be stored as a sequences of ribonucleic acid (RNA). RNA rarely exists as a double-stranded molecule. There are four major types of RNA (messenger RNA [mRNA], transfer RNA [tRNA], and ribosomal RNA [rRNA]) along with a variety of noncoding RNA (ncRNA), molecules that play key roles in gene expression

- Prokaryotic, or prenuclear, organisms do not have membrane-bound organelles, and the cells' genetic material is therefore not enclosed in a nucleus. Eukaryotic, or "true nucleus," organisms have the genetic material enclosed in a nuclear envelope.

- Most DNA molecules are double stranded, with complementary bases (A-T; G-C) paired by hydrogen bonding in the center of the molecule. The orientation of the two DNA strands is antiparallel: One strand is chemically oriented in a 5′→3′direction, and its complementary strand runs 3′→5′.

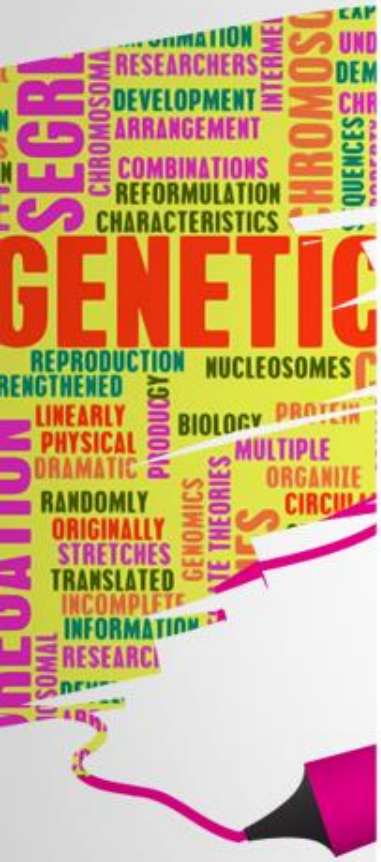


- The bases that are covalently linked to each deoxyribose sugar are the key to the genetic code within the DNA molecule. The four nitrogenous bases include two purines, adenine (A) and guanine (G), and the two pyrimidines, cytosine (C) and thymine (T) (Figure 2-3). In RNA, uracil replaces thymine. The combined sugar, phosphate, and a base form a single unit referred to as a nucleotide (adenosine triphosphate [ATP], guanidine triphosphate [GTP], cytosine triphosphate [CTP], and thymidine triphosphate [TTP] or uridine triphosphate [UTP]).

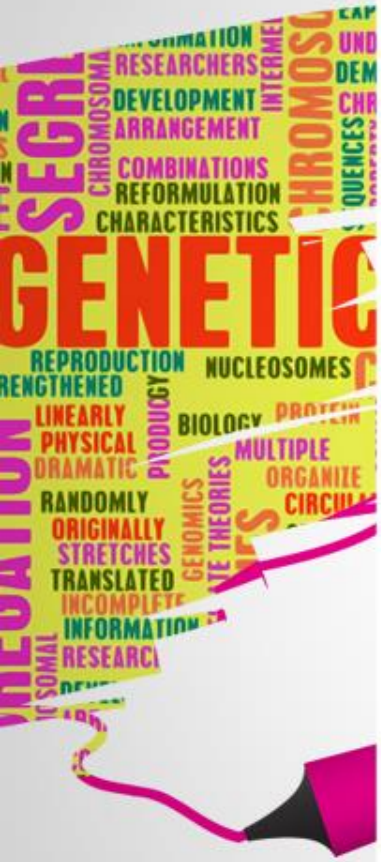
- **The complementarity of the bases enables one strand (template strand) to provide the information for copying or expression of information in the other strand (coding strand)**

- **The base pairs are stacked within the center of the DNA double helix , and they determine its genetic information. Each turn of the helix has one major groove and one minor groove.**

Nucleic acid structure



- **The length of a DNA molecule is usually expressed in thousands of base pairs, or kilobase pairs (kbp) . Whereas a small virus may contain a single DNA molecule of less than 0.5 kbp, the single DNA genome that encodes *Escherichia coli* is greater than 4000 Kbp. each base pair is separated from the next by about 0.34 nm, or 3.4×10^{-7}mm**



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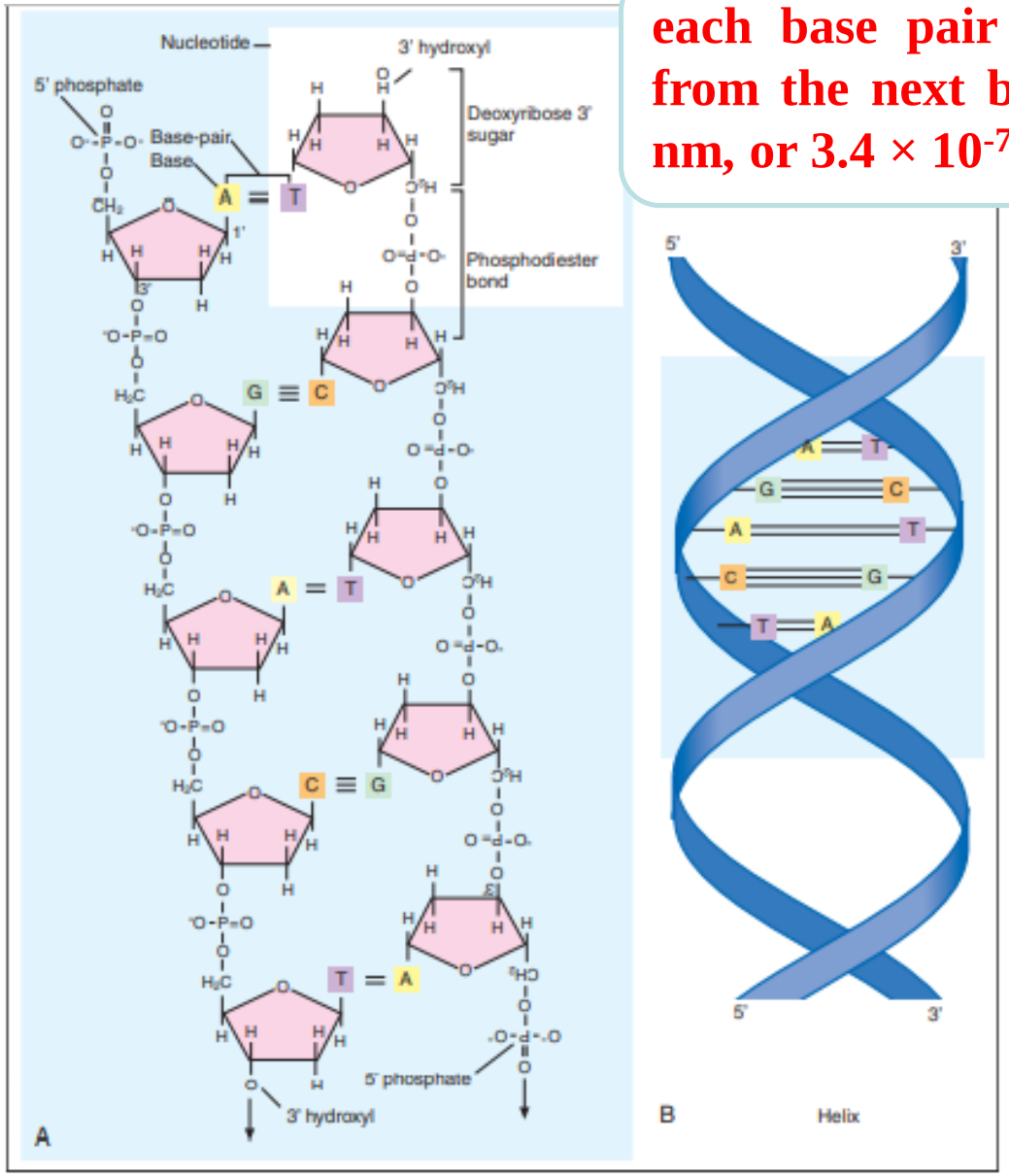
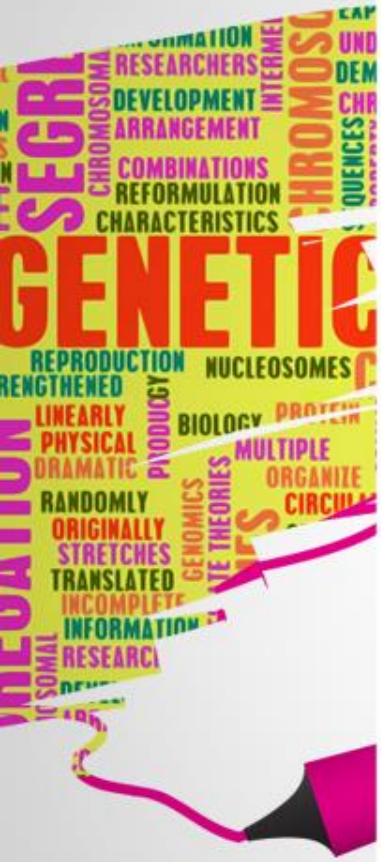
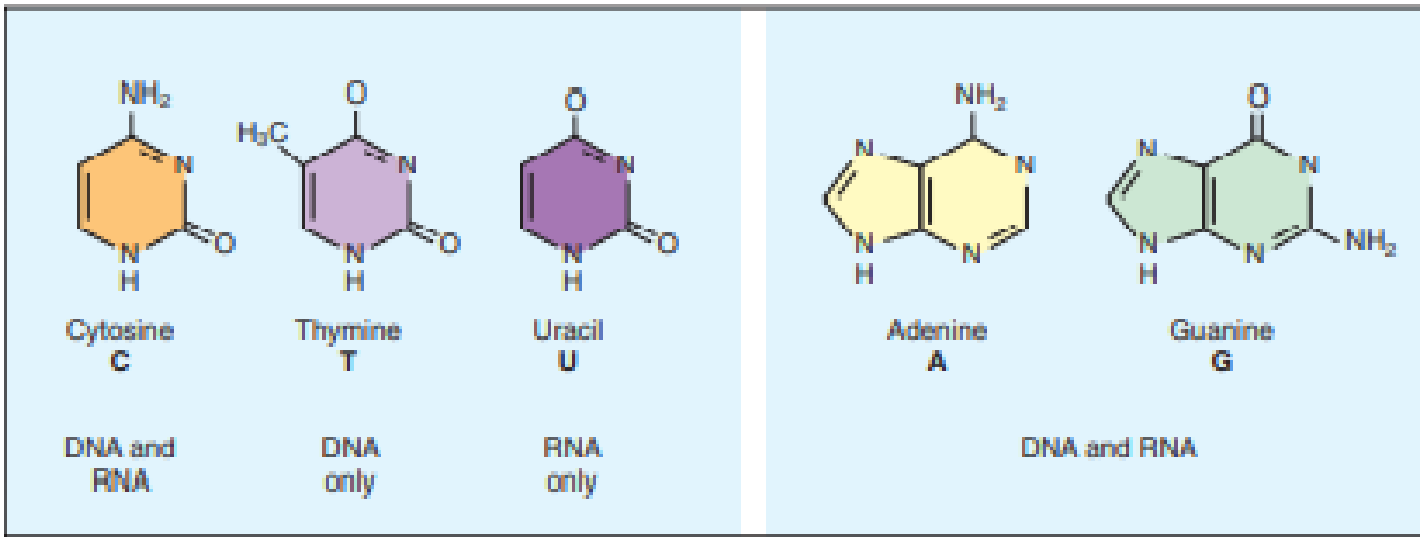


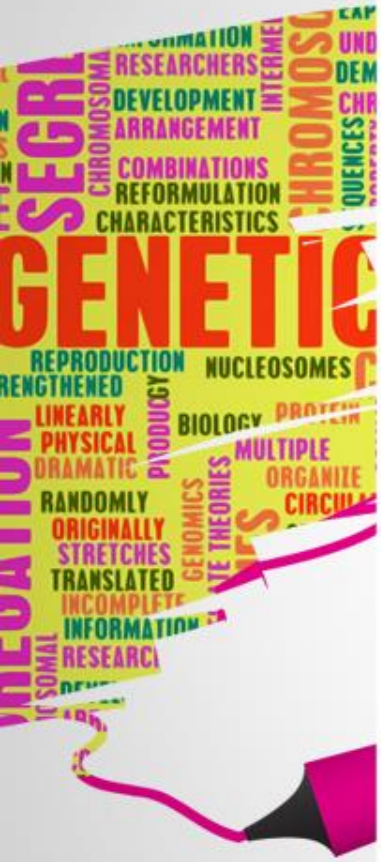
• **Figure 2-2** **A,** Molecular structure of deoxyribonucleic acid (DNA) depicting nucleotide structure, phosphodiester bonds connecting nucleotides, and complementary base pairing (*A*, adenine; *T*, thymine; *G*, guanine; *C*, cytosine) between antiparallel nucleic acid strands. **B,** 5′ and 3′ antiparallel polarity and double-helix configuration of DNA.



Nitrogen Bases



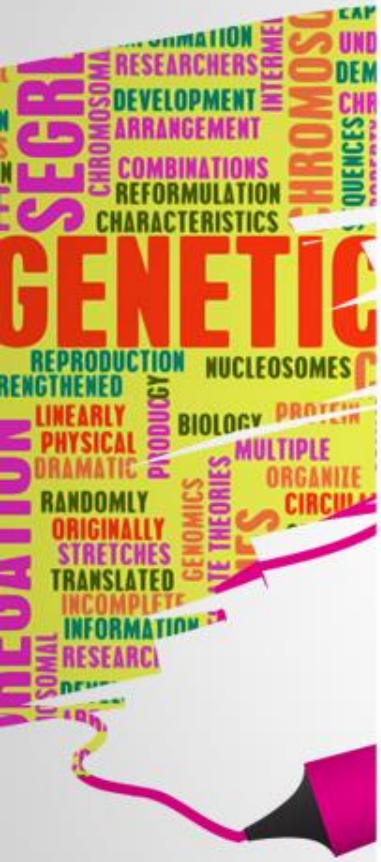
• **Figure 2-3** Molecular structure of nucleic acid bases. Pyrimidines: cytosine, thymine, and uracil. Purines: adenine and guanine.



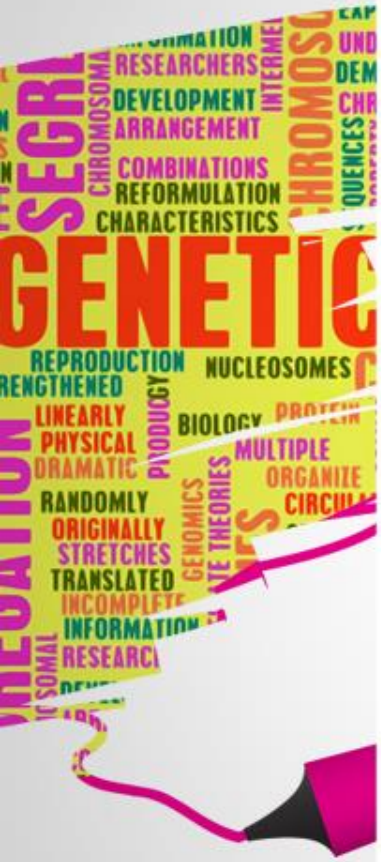
Notes

- Gene: A DNA sequence that encodes for a specific product (RNA or protein). All the genes in an organism comprise the organism's genome. The size of a gene and an entire genome is usually expressed in the number of base pairs (bp) present (e.g., kilobases [10^3 bases], megabases [10^6 bases]).

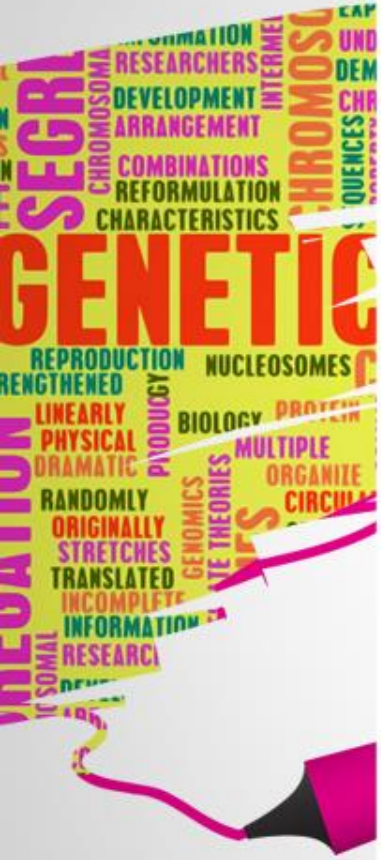
- The genome is organized into discrete elements known as chromosomes. The set of genes within a given chromosome are arranged in a linear fashion, but the number of genes per chromosome is variable. Similarly, although the number of chromosomes per cell is consistent for a given species.



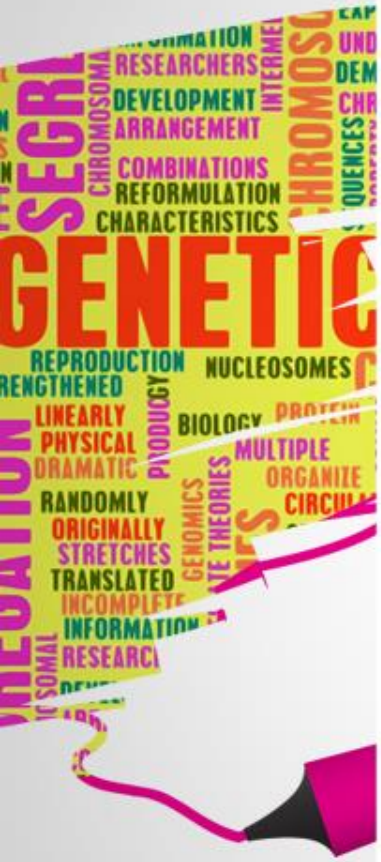
- **RNA most frequently occurs in single-stranded form. The base uracil (U) replaces thymine (T) in DNA, so the complementary bases that determine the structure of RNA are A-U and C-G. The overall structure of single-stranded RNA molecules is determined by pairing between bases within the strand-forming loops, with the result that single-stranded RNA molecules assume a compact structure capable of expressing genetic information contained in DNA.**

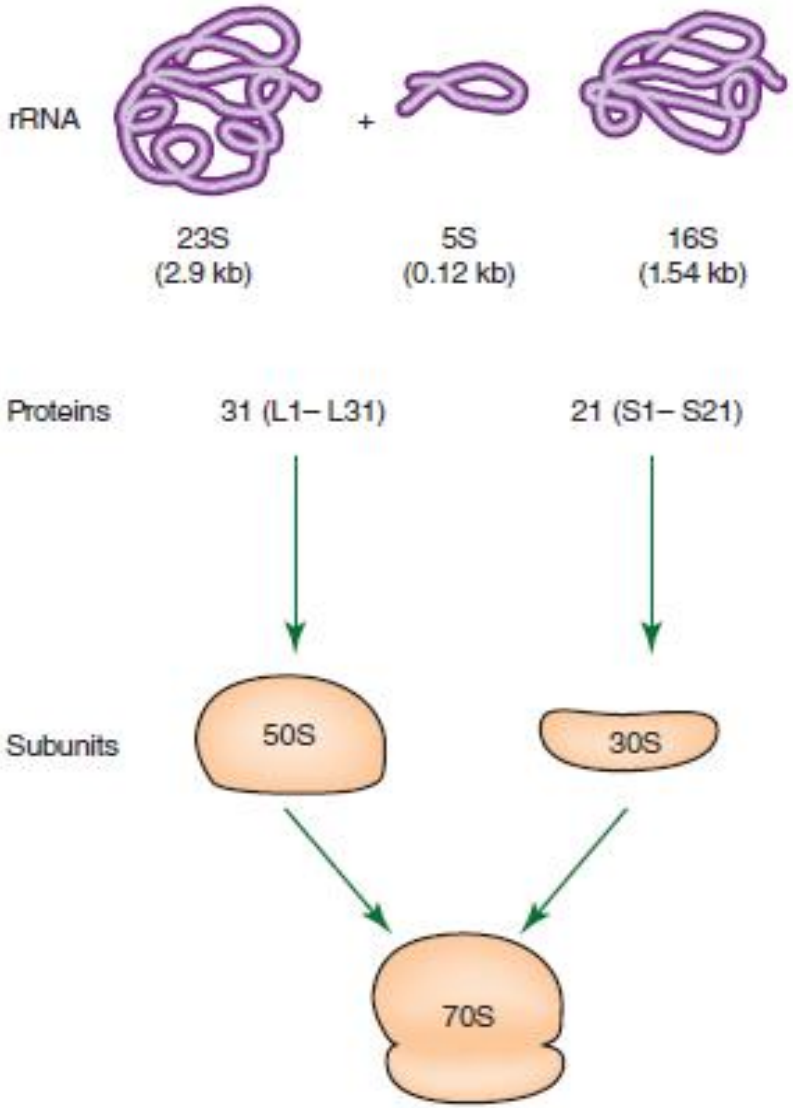


- **The most general function of RNA is communication of DNA gene sequences in the form of messenger RNA (mRNA) to ribosomes. These processes are referred to as transcription and translation. mRNA is transcribed as the RNA complement to the coding DNA strand. This mRNA is then translated by ribosomes. The ribosomes, which contain both ribosomal RNA (rRNA) and proteins, translate this message into the primary structure of proteins via aminoacyl-transfer RNAs (tRNAs).**

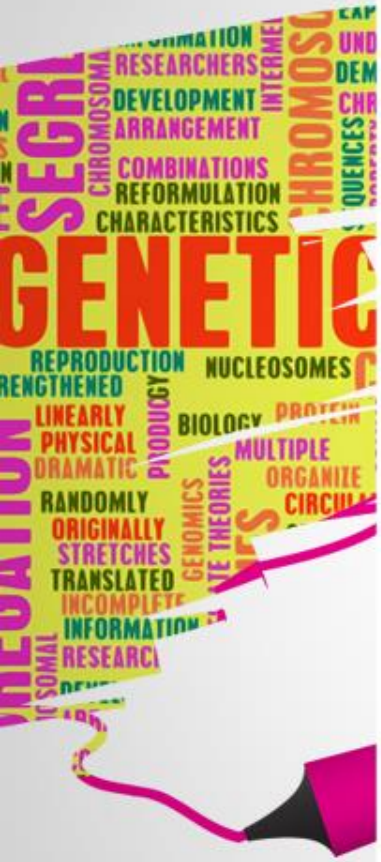


- **RNA molecules range in size from the small tRNAs, which contain fewer than 100 bases, to mRNAs, which may carry genetic messages extending to several thousand bases. Bacterial ribosomes contain three kinds of rRNA, with respective sizes of 120, 1540, and 2900 bases and a number of proteins.**

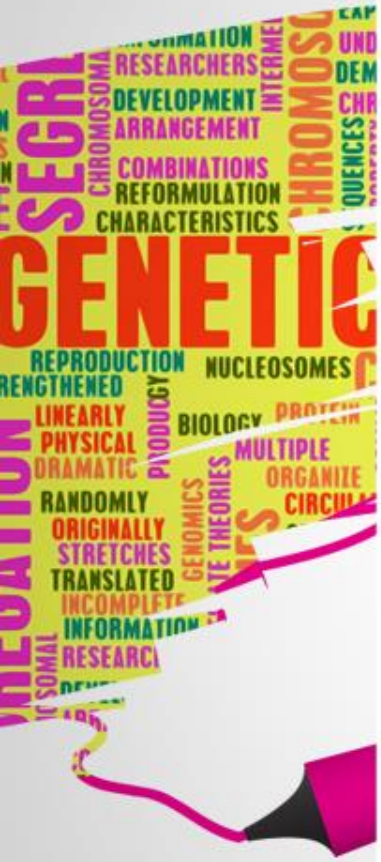


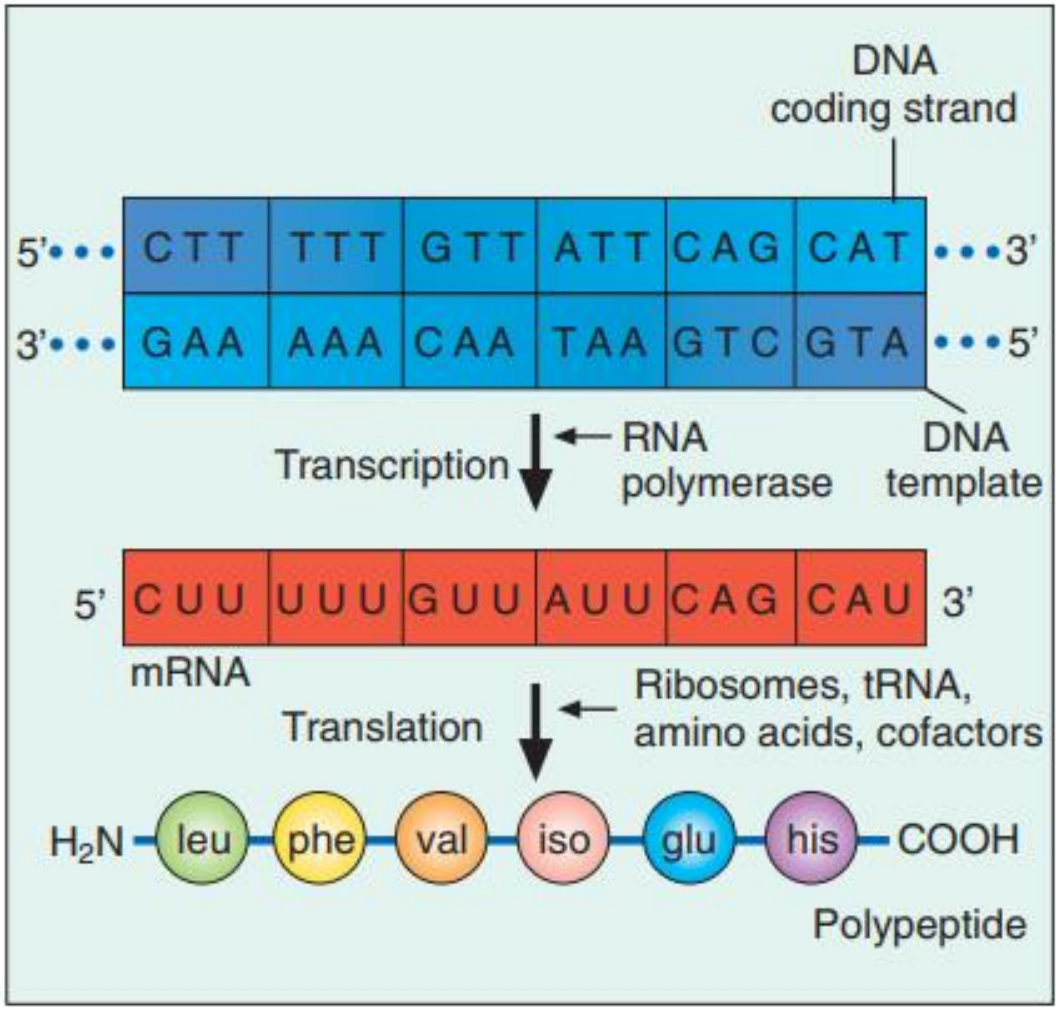


The composition of a ribosome containing one copy each of the 16S, 23S, and 5S RNAs as well as many proteins. The proteins of the large 50S subunit are designated L1–L31. The proteins of the small 30S subunit are designated S1–S21.

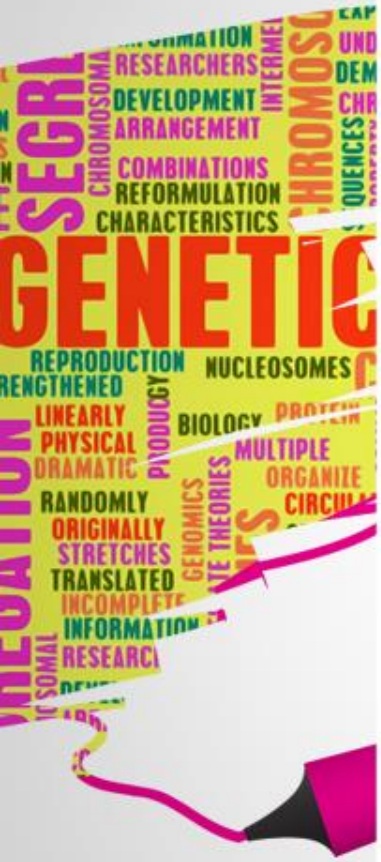


- **few RNA molecules have been shown to function as enzymes (ribozymes). For example, the 23S RNA in the 50S ribosomal subunit catalyzes the formation of the peptide bond during protein synthesis. Recently, a new class of RNA molecules called small interfering RNA (siRNA) was described in plants. siRNAs are double-stranded RNA molecules, 20–25 nucleotides in length, that play a variety of roles in biology. Some have been shown to function as regulators by either binding near the 5′end of an mRNA, preventing ribosomes from translating that message, or base pairing directly with a strand of DNA near the promoter, preventing transcription.**



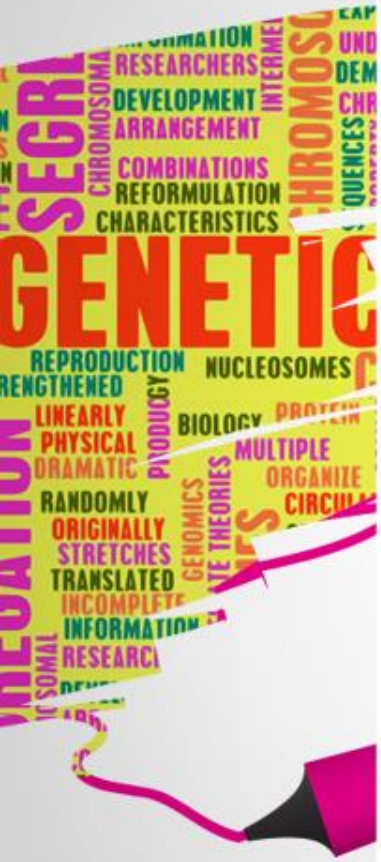


• **Figure 2-5** Overview of gene expression components: transcription for production of mRNA and translation for production of a polypeptide (protein).



Nonchromosomal Elements

- Although the bacterial chromosome represents the majority of a cell's genome, not all genes are confined to the chromosome. Many genes may also be located on **plasmids** and **transposable elements**. Both of these extrachromosomal elements are able to replicate and encode information for the production of various cellular products. Many of these elements replicate by integration into the host chromosome, whereas others, referred to as **episomes(???)**, are capable of replication independently of the host chromosome.



Plasmids

- Plasmids exist as double-stranded, closed, circular, autonomously replicating extrachromosomal genetic elements ranging in size from 1 to 2 kilobases up to 1 mega base or more. The number of plasmids per bacterial cell varies extensively, and each plasmid is composed of several genes. Some genes encode products that mediate plasmid replication and transfer between bacterial cells, whereas others encode products that provide a specialized function, such as a determinant of antimicrobial resistance or a unique metabolic process. Unlike most chromosomal genes, plasmid genes do not usually encode for products essential for viability.

Recombinant DNA technology

Lecture IV

Ass.Pro. Asra'a Adnan Abdul-Jalil

Prokaryotic Genome

- Most prokaryotic genes are carried on the bacterial chromosome
- Covalently closed DNA circles (bacterial chromosomes and plasmids), which contain genetic information necessary for their own replication, are called replicons
- Some bacterial species are efficient at causing disease in higher organisms because they possess specific genes for pathogenic determinants. These genes are often clustered together in the DNA and are referred to as pathogenicity islands. These gene segments can be quite large (up to 200 kbp) and encode a collection of virulence genes.
- Pathogenicity islands (1) have a different G + C content from the rest of the genome; (2) are closely linked on the chromosome to tRNA genes; (3) are flanked by direct repeats; and (4) contain diverse genes important for pathogenesis, including, antibiotic resistance, adhesins, invasins, and exotoxins.
- Genes essential for bacterial growth (often referred to as “housekeeping genes”) are carried on the chromosome

- **REPLICATION**

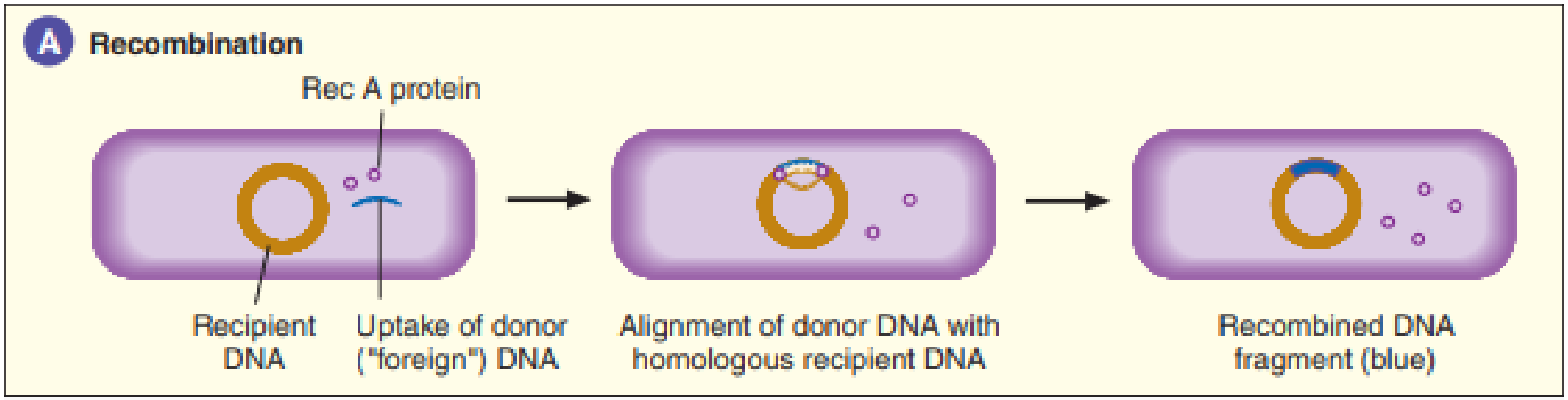
Double-stranded DNA is synthesized by **semiconservative replication**. As the parental duplex unwinds, each strand serves as a template (ie, the source of sequence information) for DNA replication. New strands are synthesized with their bases in an order complementary to that in the preexisting strands. When synthesis is complete, each daughter molecule contains one parental strand and one newly synthesized strand.

Recombinant DNA technology

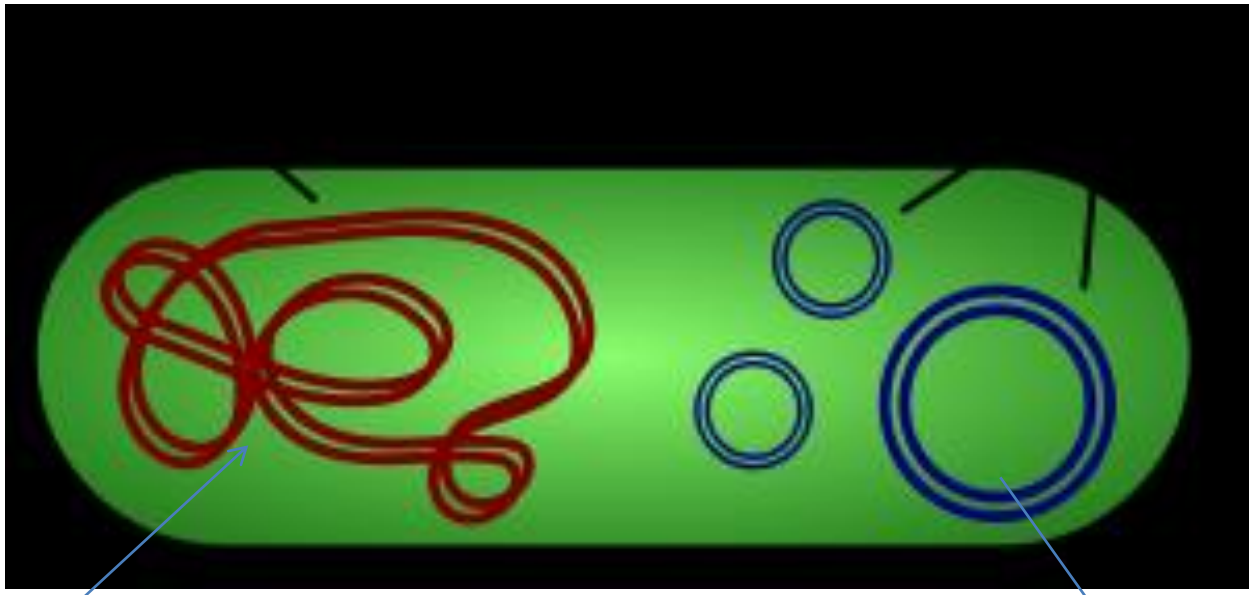
Other names

(Genetic modification) OR (DNA cloning) OR (genetic engineering). Adding of foreign DNA(desired gene) to microbial cells, plant cells or animal cells to form genetically Modified Organism(GMO)

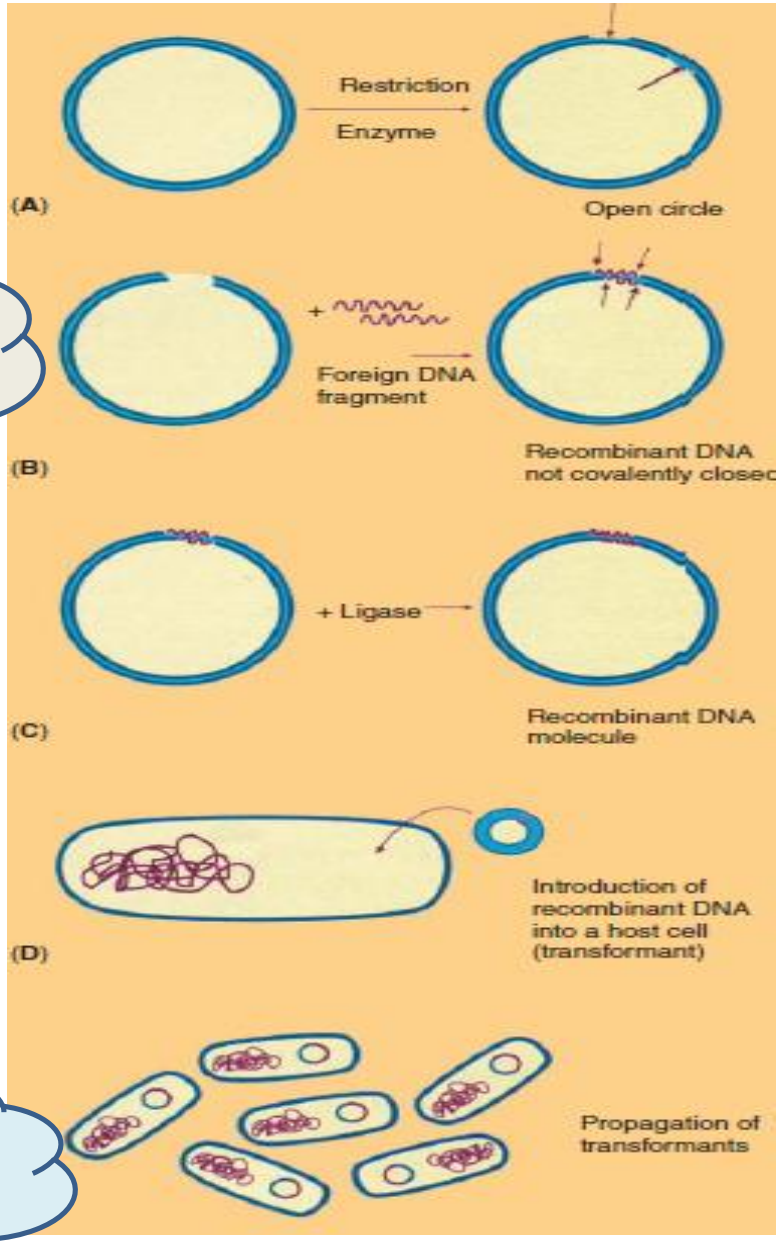
Plasmids usually used as a vector for a foreign DNA after treating them with Restriction enzymes such as ECORI. The cloning technique is very suitable to obtain large amounts of a specific DNA fragment, by fusing such a fragment to an appropriate vector and transferring the construct to a host that can easily be cultivated to high cell densities. The recombinant DNA molecules, which can then be isolated from the cell mass, form an abundant source for the specific DNA fragment and it is important for pharmaceutical biotechnology.



Plasmids



Bovine albumin



Bacillus subtilis

Principle of cloning a foreign DNA fragment

Genetic Exchange

- An organism's ability to undergo recombination depends on the acquisition of "foreign" DNA from a donor cell. The three mechanisms by which bacteria physically exchange DNA are transformation, transduction, and conjugation.

- **Restriction enzymes** (restriction endonucleases) provide bacteria with a mechanism to distinguish between their own DNA and DNA from other biologic sources. These enzymes hydrolyze (cleave) DNA at restriction sites determined by specific DNA sequences ranging from 4 to 13 bases

Enzyme	Source	Cutting sequence*
EcoR1	*Escherichia coli*	G↓AATT C
		C TTAA↑G
Pst1	*Providencia stuartii*	C TGCA↓G
		G↑ACGT C
Taq1	*Thermus aquaticus*	T↓CG A
		A GC↑T
Hinf1	*Hemophilus influenzae*	G↓ANT C
		C TNA↑G
Msp1	*Moraxella species*	C↓CG G
		G GC↑C
HaeIII	*Hemophilus aegyptus*	GG↓CC
		CC↑GG

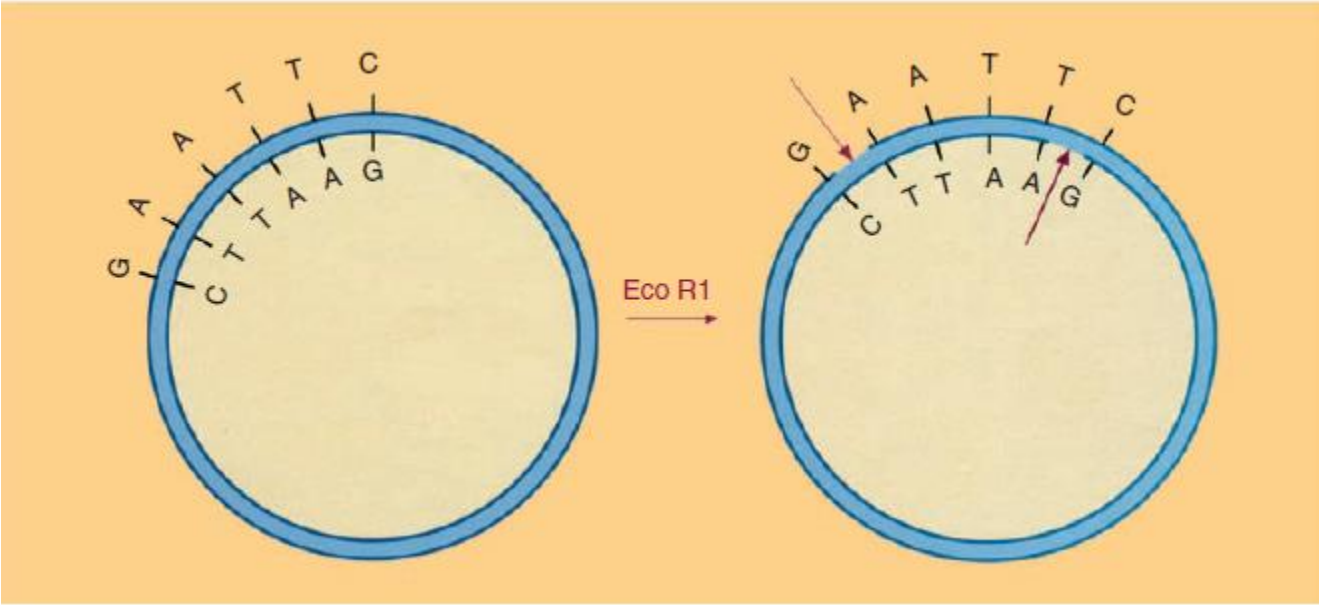
*N, no base preference.
Note: Open space in the recognition site indicates the endonucleolytic cut by the enzyme.

Some restriction enzymes, their origin, and their recognition site.

Notes

- Each bacterial strain that possesses a restriction system is able to distinguish these recognition sites in its own DNA by modifying them through methylation of adenine or cytosine residues within the site.

- The DNA composition of microorganisms is remarkably fluid. DNA can be transferred from one organism to another, and that DNA can be stably incorporated in the recipient, permanently changing its genetic composition. This process is called horizontal gene transfer to differentiate it from the inheritance of parental genes, a process called vertical inheritance.



Treatment of a plasmid with an unique EcoR1 site. This restriction enzyme will open the plasmid and make it amenable for manipulation.

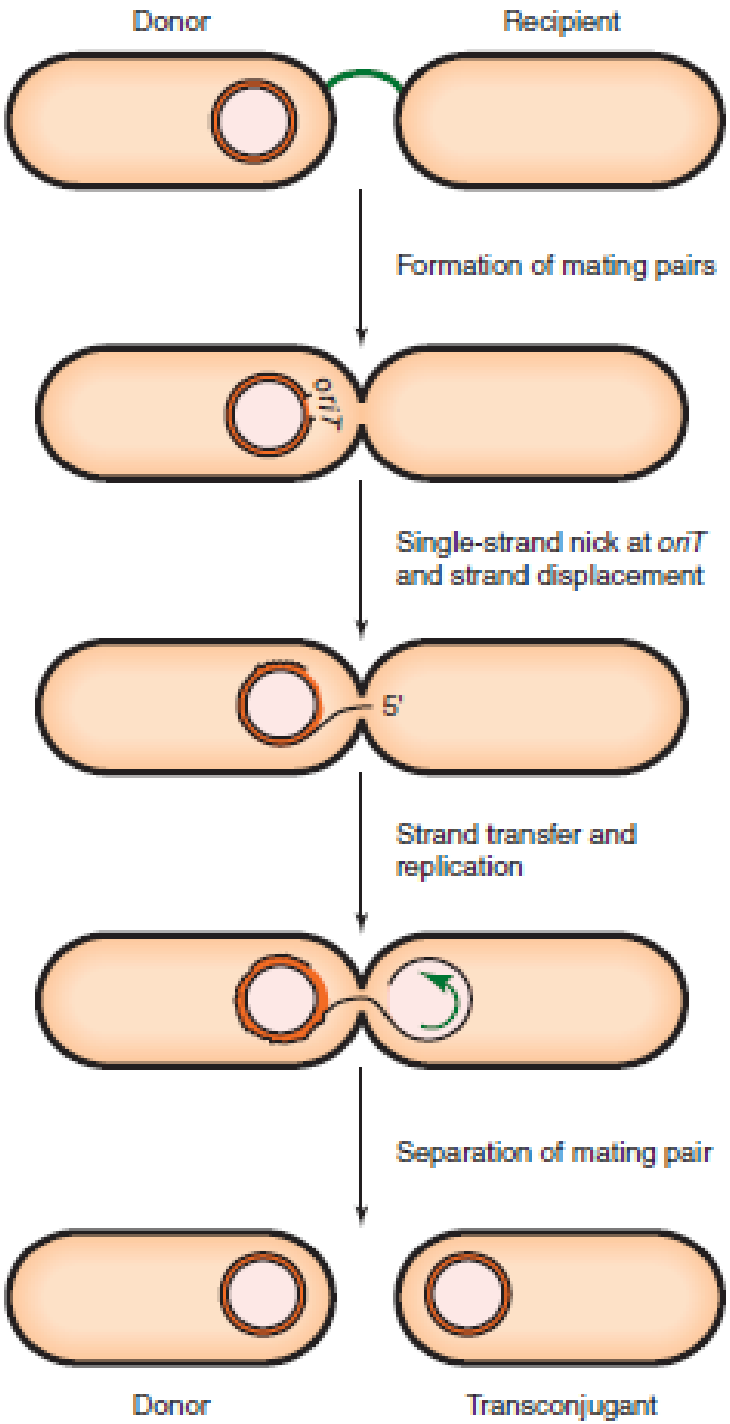
Mechanisms of Gene Transfer

I. **Conjugation** requires donor cell-to-recipient cell contact to transfer only one strand of DNA. The recipient completes the structure of double-stranded DNA by synthesizing the strand that complements the strand acquired from the donor.

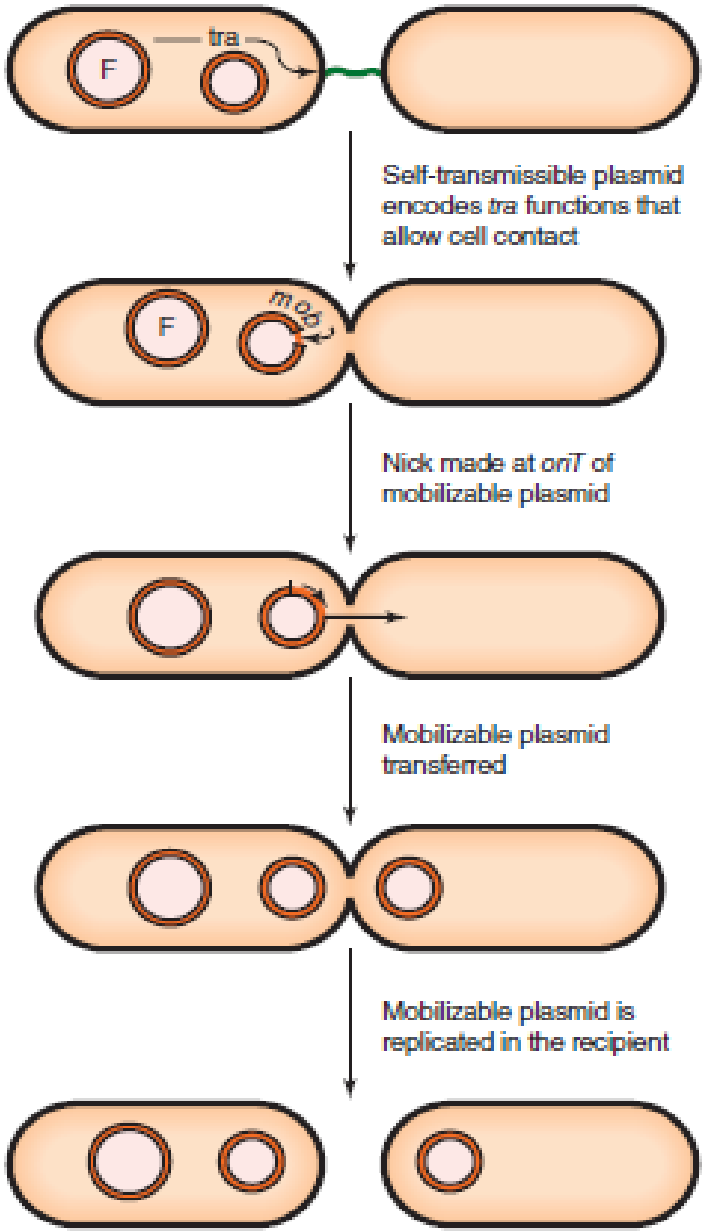
- Plasmids are most frequently transferred by conjugation. Genetic functions required for transfer are encoded by the *tra* genes, which are carried by self-transmissible **plasmids**. Some self-transmissible plasmids can mobilize other plasmids or portions of the chromosome for transfer.

The donor cell produces a pilus, which is encoded by the plasmid and contacts a potential recipient cell that does not contain the plasmid. Retraction of the pilus brings the cells into close contact, and a pore forms in the adjoining cell membranes. Formation of the mating pair signals the plasmid to begin transfer from a single-stranded nick at *oriT.* The nick is made by plasmid encoded *tra* functions.

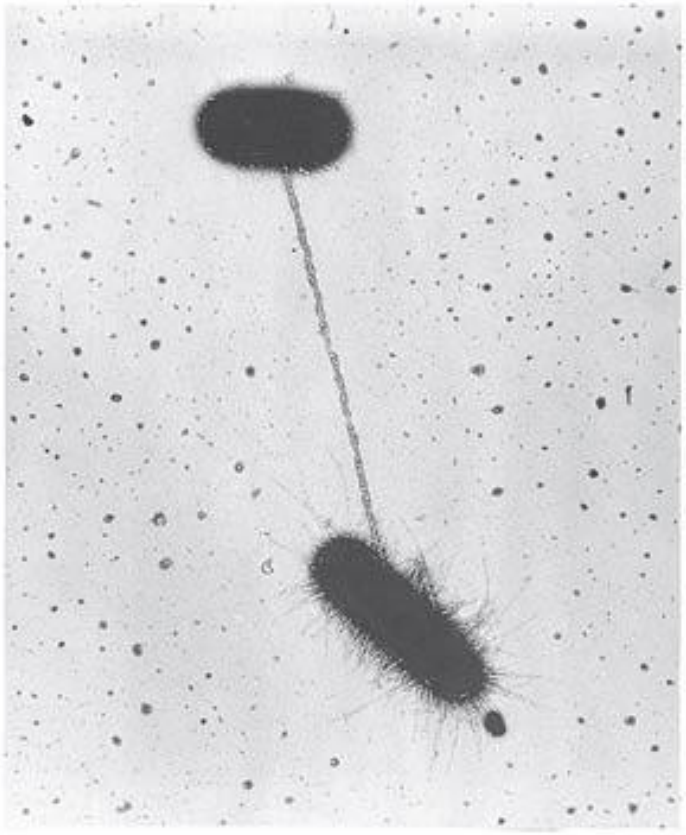
The 5′ end of a single strand of the plasmid is transferred to the recipient through the pore. During transfer, the plasmid in the donor is replicated. Replication of the single strand in the recipient proceeds by a different mechanism with RNA primers. Both cells now contain double-stranded plasmids, and the mating pair separates.



The donor cell carries two plasmids, a self-transmissible plasmid, F, which encodes the *tra* functions that promote cell contact and plasmid transfer, and a mobilizable plasmid. The *mob* functions encoded by the mobilizable plasmid make a single-stranded nick at *oriT* in the *mob* region. Transfer and replication of the mobilizable plasmid then occur.



Mechanism of plasmid mobilization.



male and a female cell joined by an F pilus (sex pilus).

II. Transformation, the direct uptake of "naked" donor DNA by the recipient cell, may be natural or forced. Forced transformation is induced in the laboratory, where, after treatment with high salt and temperature shock, many bacteria are rendered competent for the uptake of extracellular plasmids The capacity to force bacteria to incorporate extracellular plasmids by transformation is fundamental to genetic engineering.

Direct uptake of donor DNA by recipient bacteria depends on their competence for transformation. Natural competence is unusual among bacteria, and some of these strains are transformable only in the presence of competence factors, produced only at a specific point in the growth cycle. Other strains readily undergo natural transformation, and these organisms offer promise for genetic engineering because of the ease with which they incorporate modified DNA into their chromosomes. Naturally competent transformable bacteria are found in several genera and include *Bacillus subtilis, Haemophilus influenzae, Neisseria gonorrhoeae, Neisseria meningitidis,* and *Streptococcus pneumoniae.*

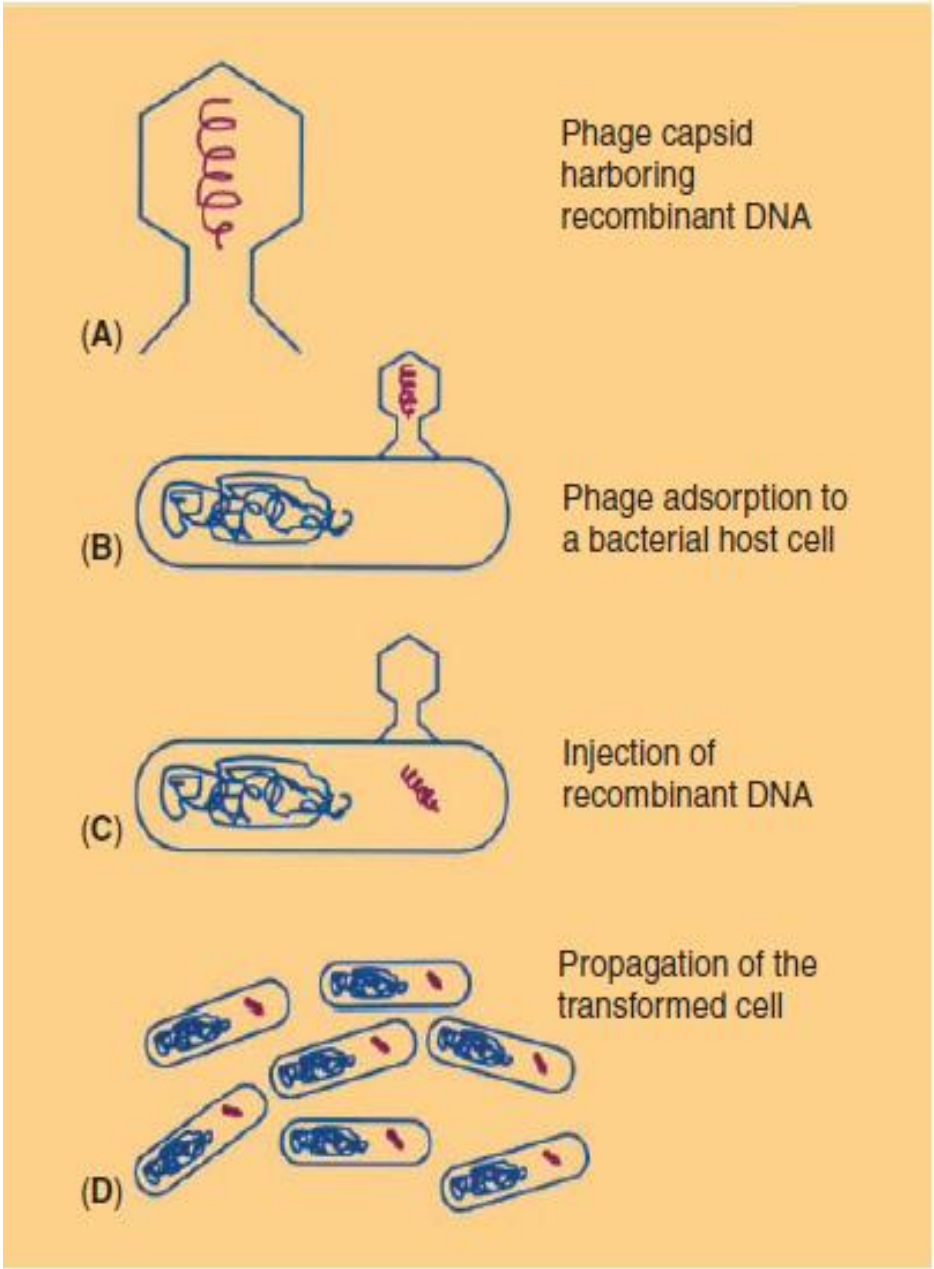
Most bacteria are unable to undergo natural transformation. In these cases, transformation can be forced by treatment with calcium chloride and temperature shock.

Transformation with engineered recombinant plasmids by this procedure is a cornerstone of modern molecular biology because it enables DNA from diverse biologic sources to be established as part of well-characterized bacterial replicons.

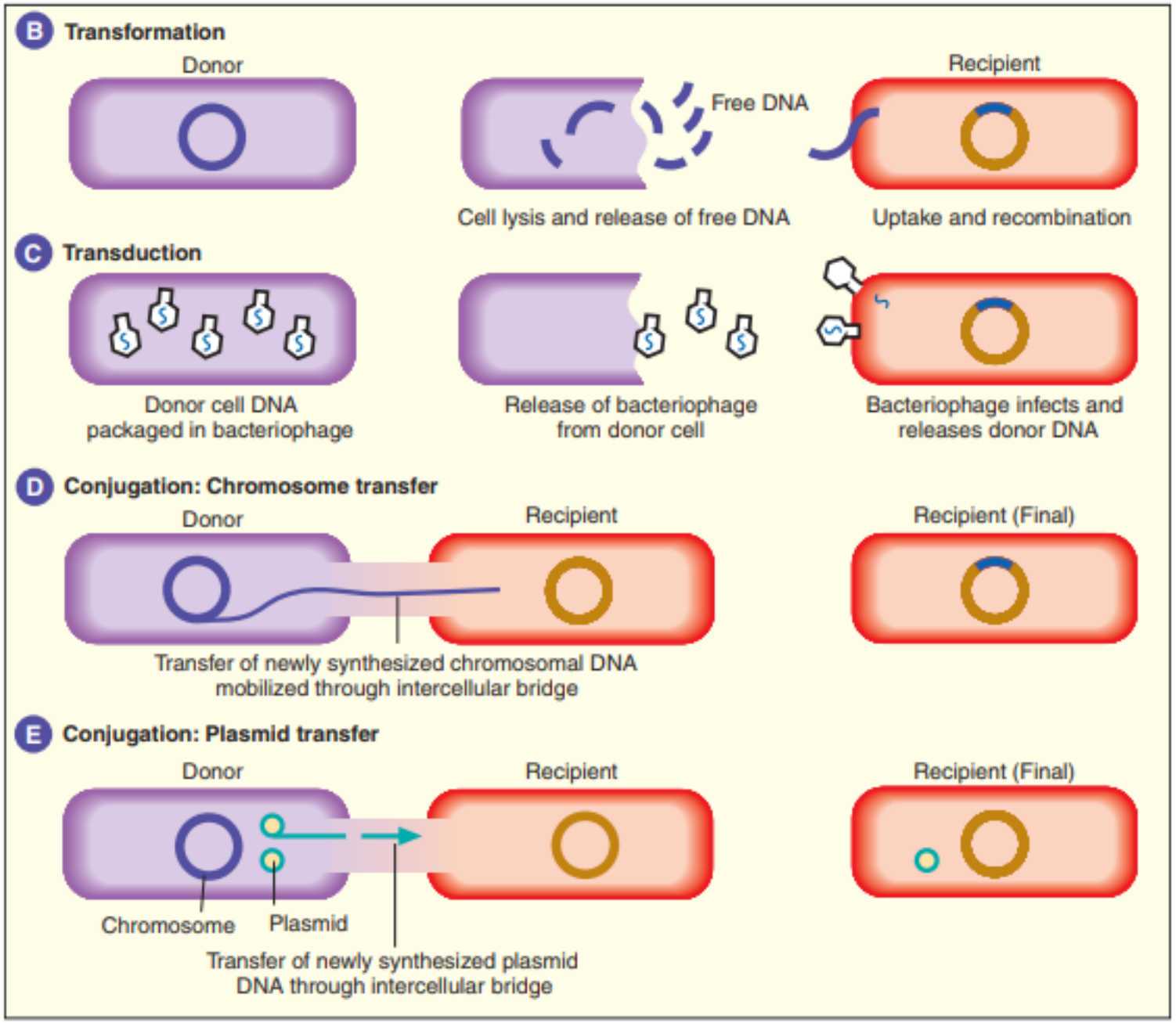
III. Transduction

Transduction is phage-mediated genetic recombination in bacteria. In simplest terms, a transducing particle might be regarded as bacterial nucleic acid in a phage coat **Pathogenicity islands** are often transported by phages. For example, two phages transport pathogenicity islands responsible for converting a benign form of *Vibrio cholerae* into the pathogenic form responsible for epidemic cholera . These phage encode genes for cholera toxin (responsible for symptoms) and bundle-forming pili (responsible for attachment).

In **transduction**, donor DNA is carried in a phage coat and is transferred into the recipient by the mechanism used for phage infection.

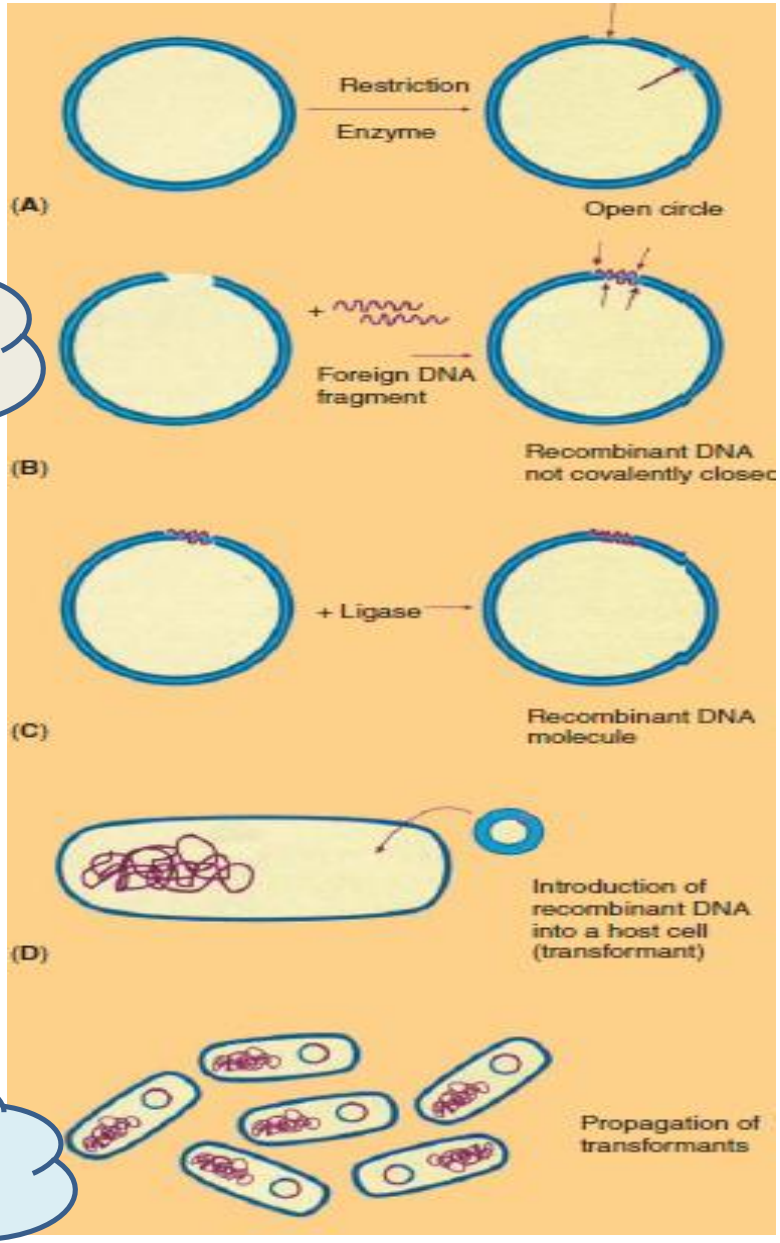


Phage as a mediator for transfer of recombinant DNA



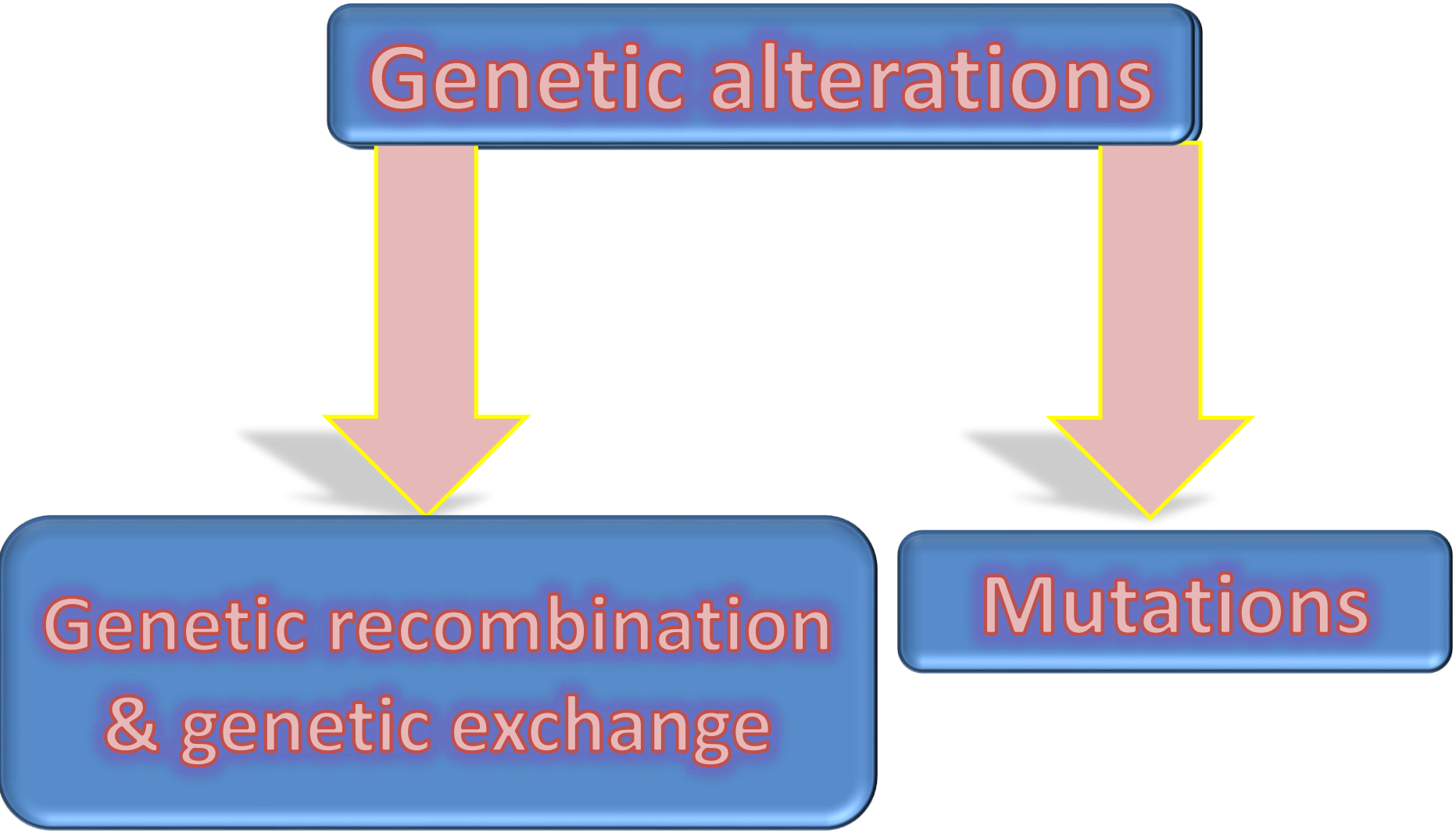
• **Figure 2-8 A,** Genetic recombination. The mechanisms of genetic exchange between bacteria are transformation **(B),** transduction **(C),** and conjugational transfer of chromosomal **(D)** and plasmid **(E)** DNA.

Bovine albumin



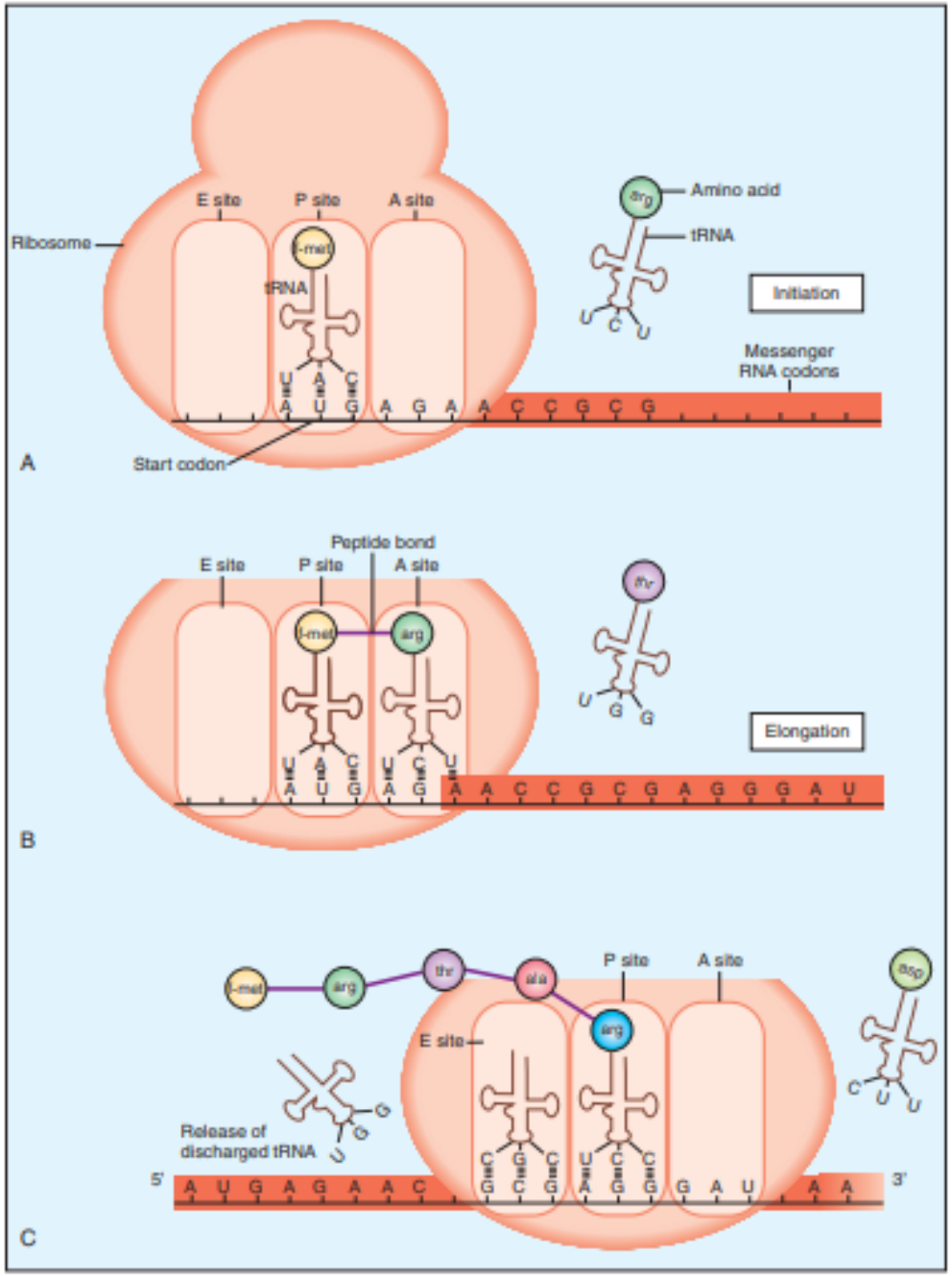
Bacillus subtilis

Principle of cloning a foreign DNA fragment


Genetic alterations
Genetic recombination & genetic exchange
Mutations

Mutation

- ✓ Mutation is defined as an alteration in the original nucleotide sequence of a gene or genes within an organism's genome; that is, a change in the organism's genotype.
- ✓ This alteration may involve a single DNA base in a gene, an entire gene, or several genes.
- ✓ Mutational changes in the sequence may arise spontaneously, perhaps by an error made during DNA replication.
- ✓ Alternatively, mutations may be induced by mutagens (i.e., chemical or physical factors) in the environment or by biologic factors, such as the introduction of foreign DNA into the cell.
- ✓ Alterations in the DNA base sequence can result in changes in the base sequence of mRNA during transcription. This, in turn, can affect the types and sequences of amino acids that will be incorporated into the protein during translation



• **Figure 2-6** Overview of translation in which mRNA serves as the template for the assembly of amino acids into polypeptides. The three steps include initiation **(A)**, elongation (**B** and **C**), and termination (not shown).

MUTATION AND GENE REARRANGEMENT

- The mutations include **base substitutions**, **deletions**, **insertions**, and **rearrangements**.

Occurrence of a mispaired base is minimized by enzymes associated with **mismatch repair**, a mechanism that essentially proofreads a newly synthesized strand to ensure that it perfectly complements its template.

Rearrangements are the result of deletions that remove large portions of genes or even sets of genes

Mutagens

Physical Mutagens

damage DNA by linking neighboring thymine bases to form dimers.

Chemical Mutagens

may act by altering either the chemical or the physical structure of DNA. Reactive chemicals alter the structure of bases in DNA. For example, nitrous acid (HNO_2) substitutes hydroxyl groups for amino groups.

- https://meet.google.com/nsk-ahpr-erb?hs=122&authuser=0
- https://meet.google.com/fun-tmig-qif?hs=122&authuser=0

Antimicrobial Chemotherapy

Ass.Pro. Asra'a Adnan Abdul-Jalil

HISTORY

- ✓The current era of antimicrobial chemotherapy began in 1935 with the discovery of the sulfonamides.
- ✓In 1940, it was demonstrated that penicillin, discovered in 1929, could be an effective therapeutic substance.
- ✓During the next 25 years, research on chemotherapeutic agents centered largely on substances of microbial origin called antibiotics.
- ✓The isolation, concentration, purification, and mass production of penicillin were followed by the development of streptomycin, tetracyclines, chloramphenicol, and many other agents

MECHANISMS OF ACTION OF ANTIMICROBIAL DRUGS

- The mechanisms of action of antimicrobial drugs can be discussed under four headings:

1. Inhibition of cell wall synthesis
2. Inhibition of cell membrane function
3. Inhibition of protein synthesis (ie, inhibition of translation and transcription of genetic material)
4. Inhibition of nucleic acid synthesis

PEPTIDOGLYCAN

- The cell wall contains a chemically distinct complex polymer “mucopeptide” (“peptidoglycan”) consisting of polysaccharides and a highly cross-linked polypeptide. The polysaccharides regularly contain the amino sugars N-acetylglucosamine and acetylmuramic acid. The latter is found only in bacteria. To the amino sugars are attached short peptide chains. The final rigidity of the cell wall is imparted by cross-linking of the peptide chains (eg, through pentaglycine bonds) as a result of transpeptidation reactions carried out by several enzymes. The peptidoglycan layer is much thicker in the cell wall of gram-positive than of gram-negative bacteria.

PEPTIDOGLYCAN

- ✓All β-lactam drugs are selective inhibitors of bacterial cell wall synthesis and therefore active against growing bacteria.

- ✓The initial step in drug action consists of binding of the drug to cell receptors (penicillin-binding proteins [PBPs]). There are at least six different PBPs (molecular weight [MW], 40–120 kilodaltons [kD]),

ß-LACTAM RESISTANCE

- There are two types of resistance mechanisms:
- ✓ One is caused by the absence of some PBPs and occurs as a result of chromosomal mutation;
- ✓ failure of the β-lactam drug to activate the autolytic enzymes in the cell wall. As a result, the organism is inhibited but not killed. Such tolerance has been observed especially with staphylococci and certain streptococci.
- Examples of agents acting by inhibition of cell wall synthesis are penicillins, the cephalosporins, vancomycin, and cycloserine. Several other drugs, including bacitracin, teicoplanin, vancomycin, ristocetin, and novobiocin, inhibit early steps in the biosynthesis of the peptidoglycan.

INHIBITION OF CELL MEMBRANE FUNCTION

- The cytoplasm of all living cells are bounded by the cytoplasmic membrane,
- which serves as a selective permeability barrier and carries out active transport functions and thus controls the internal composition of the cell.
- If the functional integrity of the cytoplasmic membrane is disrupted, macromolecules and ions escape from the cell, and cell damage or death ensues.
- The cytoplasmic membrane of bacteria and fungi has a structure different from that of animal cells and can be more readily disrupted by certain agents.

INHIBITION OF CELL MEMBRANE FUNCTION

- Detergents, which contain lipophilic and hydrophilic groups, disrupt cytoplasmic membranes and kill the cell .
- One class of antibiotics, the polymyxins, consists of detergent-like cyclic peptides that selectively damage membranes containing phosphatidylethanolamine, a major component of bacterial membranes. A number of antibiotics specifically interfere with biosynthetic functions of the cytoplasmic membranes (eg, nalidixic acid and novobiocin inhibit DNA synthesis, and novobiocin also inhibits teichoic acid synthesis). A third class of membrane-active agents is the ionophores, compounds that permit rapid diffusion of specific cations through the membrane. Valinomycin, for example, specifically mediates the passage of potassium ions

INHIBITION OF PROTEIN SYNTHESIS

- It is established that macrolides, lincosamides, tetracyclines, glycylcyclines, aminoglycosides, and chloramphenicol can inhibit protein synthesis in bacteria. The precise mechanisms of action differ among these classes of drugs.
- Whereas bacteria have 70S ribosomes, mammalian cells have 80S ribosomes. The subunits of each type of ribosome, their chemical composition, and their functional specificities are sufficiently different to explain why antimicrobial drugs can inhibit protein synthesis in bacterial ribosomes without having a major effect on mammalian ribosomes. In normal microbial protein synthesis, the mRNA message is simultaneously “read” by several ribosomes that are strung out along the mRNA strand. These are called polysomes

INHIBITION OF PROTEIN SYNTHESIS

- Macrolides, Azalides, and Ketolides These drugs (erythromycins, azithromycin, clarithromycin, and roxithromycin and the ketolide telithromycin) bind to the 50S subunit of the ribosome, and the binding site is a 23S rRNA.

- Aminoglycosides bind to a specific receptor protein the 30S subunit of the microbial ribosome. Second, the aminoglycoside blocks the normal activity of the “initiation complex” of peptide formation (mRNA + formyl methionine + tRNA).

INHIBITION OF NUCLEIC ACID SYNTHESIS

- Examples of drugs acting by inhibition of nucleic acid synthesis are the quinolones, pyrimethamine, rifampin, sulfonamides, trimethoprim, and trimetrexate. Rifampin inhibits bacterial growth by binding strongly to the DNA-dependent RNA polymerase of bacteria. Thus, it inhibits bacterial RNA synthesis. Rifampin resistance results from a change in RNA polymerase because of a chromosomal mutation that occurs with high frequency. The mechanism of rifampin action on viruses is different. It blocks a late stage in the assembly of poxviruses. All quinolones and fluoroquinolones inhibit microbial DNA synthesis by blocking DNA gyrases, topoisomerase enzymes that play key roles in DNA replication and repair

RESISTANCE TO ANTIMICROBIAL DRUGS

1. Microorganisms produce enzymes that destroy the active drug. Examples: Staphylococci resistant to penicillin G produce a β-lactamase that destroys the drug.

2. Microorganisms change their permeability to the drug. Examples: Tetracyclines accumulate in susceptible bacteria but not in resistant bacteria. Streptococci have a natural permeability barrier to aminoglycosides

3. Microorganisms develop an altered structural target for the drug. Examples: Erythromycin-resistant. organisms have an altered receptor on the 50S subunit of the ribosome, resulting from methylation of a 23S ribosomal RNA.