An Introduction to Medical Microbiology

Microbiology is a subject which deals with living organisms that are individually too small to be seen with the naked eye. It considers the microscopic forms of life and deals about their reproduction, physiology, and participation in the process of nature, helpful and harmful relationship with other living things, and significance in science and industry.

All ecosystems contain microorganisms. Billions of them populate the healthy human body and some of them may be recorded as participants in bodily functions. One of the classical example that the bacteria play a role in the degradation of intestinal contents.

Microbes in Our Lives

1. Living things too small to be seen with the unaided eye are called microorganisms.

2. Microorganisms are important in maintaining Earth's ecological balance.

3. Some microorganisms live in humans and other animals and are needed to maintain good health.

4. Some microorganisms are used to produce foods and chemicals.

5. Some microorganisms cause disease.

Comparing Prokaryotic and Eukaryotic Cells: An Overview

The world of living things is classified in the three **domains** bacteria, archaea, and eucarya. In this system, each domain is subdivided into **kingdoms**. Pathogenic microorganisms are found in the domains **bacteria** and **eucarya**.

Prokaryotes and eukaryotes both contain nucleic acids, proteins, lipids, and carbohydrates. They use the same kinds of chemical reactions to metabolize food, build proteins, and store energy. It is primarily the structure of cell walls and membranes, and the absence of organelles (specialized cellular structures that have specific functions), that distinguish prokaryotes from eukaryotes.

The chief distinguishing characteristics of **prokaryotes** (from the Greek words meaning prenucleus) are as follows:

1. Typically their DNA is not enclosed within a membrane and is usually a singular, circularly arranged chromosome. Gemma obscuriglobus has a double membrane around its nucleus. (Some bacteria, such as Vibrio cholerae, have two chromosomes, and some bacteria have a linearly arranged chromosome.)

2. Their DNA is not associated with histones (special chromosomal proteins found in eukaryotes); other proteins are associated with the DNA.

3. They generally lack organelles. Advances in microscopy reveal a few membrane-enclosed organelles (for example, some inclusions). However, prokaryotes lack other membraneenclosed organelles such as nuclei, mitochondria, and chloroplasts.

4. Their cell walls almost always contain the complex polysaccharide peptidoglycan.

5. They usually divide by binary fission, where DNA is copied, and the cell splits into two cells. This involves fewer structures and processes than eukaryotic cell division.

Eukaryotes (from the Greek words meaning true nucleus) have the following distinguishing characteristics:

1. Their DNA is found in the cell's nucleus, which is separated from the cytoplasm by a nuclear membrane, and the DNA is found in multiple chromosomes.

Table 1.2 Characteristics of Prokaryotic (Eubacteria) and Eukaryotic (Fungi, Protozoans) Microorganisms

Characteristic	**Prokaryotes** (bacteria)	**Eukaryotes** (fungi, protozoans)
Nuclear structure	Circular DNA molecule not covered with proteins	Complex of DNA and basic proteins
Localization of nuclear structure	Dense tangle of DNA in cytoplasm; no nuclear membrane; nucleoid or nuclear equivalent	In nucleus surrounded by nuclear membrane
DNA	Nucleoid and plasmids	In nucleus and in mitochondria
Cytoplasm	No mitochondria and no endoplasmic reticulum, 70S ribosomes	Mitochondria and endoplasmic reticulum, 80S ribosomes
Cell wall	Usually rigid wall with murein layer; exception: mycoplasmas	Present only in fungi: glucans, mannans, chitin, chitosan, cellulose
Reproduction	Asexual, by binary transverse fission	In most cases sexual, possibly asexual

Some Branches of Microbiology

Bacteriology: Small single-celled prokaryotic organisms.

Mycology: The fungi, a group of eukaryotes that includes both microscopic eukaryotes (molds and yeasts) and larger organisms (mushrooms, puffballs)

Protozoology: The protozoa—animal-like and mostly single-celled eukaryotes**.**

Virology: Viruses—minute, noncellular particles that parasitize cells.

Parasitology: Parasitism and parasitic organisms—traditionally including pathogenic protozoa, helminth worms, and certain insects.

Microbial Taxonomy

'Systematics' is the term used to define the study of the diversity of life and their relationships.

'taxonomy' tends to be restricted to the theory and practice of classifying organisms.

Classification attempts to group organisms according to their similarity.

TAXON:- A group or category of related organisms.

There are two key characteristics of taxa are:

1-Members of **lower level** taxa (e.g. Species) are **more similar** to each other than are members of **higher level** taxa (eg. Kingdom or domain).

2-Member of specific taxa are more similar to each other than any are to members of different specific taxa found at the same **hierarchical** متسلسل level (eg. Humans are more similar to apes, i.e., comparison between species, than either is similar to, for example, *Escherichia coli*). Thus once you know that two individuals are member of the same **taxon**, you can inter certain similarities between the two organisms.

BINOMIAL NOMENCLATURE

- Organisms are named using binomial nomenclature (viruses are exceptions)

- Binomial nomenclature employs the names of the two level taxa, genus and species, to name a specie. Binomial nomenclature includes:

i. Genus comes before species (e.g., *Escherichia coli*)

ii. Genus name is always capitalized (e.g., *Escherichia*)

iii. Species name is never capitalized (e.g., *coli*)

iv. Both names are always either ***italicized*** or **underlined** (e.g *Escherichia coli*)

v. The genus name may be used alone, but not the species name (i.e saying or writing "*Escherichia* " alone is legitimate while saying or writing " coli" is not)

Domain (Bacteria, Archaea, Eukarya): **Bacteria**

Kingdom: **Bacteria**

Phylum/Division: **Proteobacteria**

Class: **Zymobacteria**

Order: **Enterobacterales**

Family: **Enterobacteriaceae**

Genus: ***Escherichia***

Species: ***coli***

Scientific name: ***Escherichia coli***

Bacteria

Bacteria are single-celled prokaryotic microorganisms, and their DNA is not contained within a **separate nucleus** as in **eukaryotic** cells. They are approximately 0.1–10.0 µm in size and exist in various shapes, including **spheres** (cocci), **curves**, **spirals** and **rods** (bacilli).

Bacterial Classification

Bacterial classification depends on the following characteristics.

1. Morphology and arrangement
2. Staining
3. Cultural characteristics
4. Biochemical reactions
5. Antigenic structure
6. Base composition of bacterial DNA.

Morphology and staining of bacteria are the commonly used characteristics to classify bacteria.

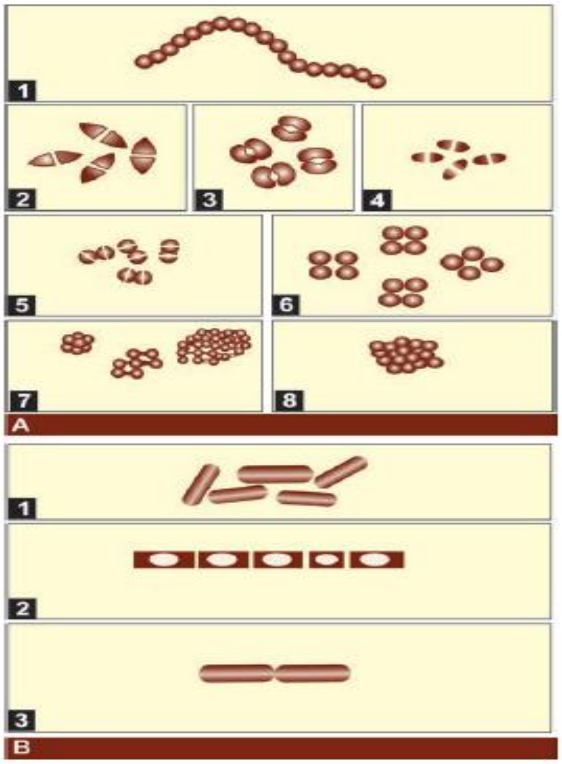
Morphology of bacteria

When bacteria are visualized under light microscope, the following morphology are seen.

1. Cocci (singular coccus): Round or oval bacteria measuring about 0.5-1.0µmb in diameter.They are found insingle, pairs, chains or clusters.

2. Bacilli (singular bacillus): Stick-like bacteria with rounded, tapered مستدق الطرف, square or swollen ends; with a size measuring 1-10µm in length by 0.3-1.0µm in width.

3. Coccobacilli (singular coccobacillus): Short rods.



4. Spiral: Spiral shaped bacteria with regular or irregular distance between twisting. Eg. Spirilla and spirochaetes.

Depends to the arrangement the bacteria can be classified into:

- Single cell (coccus كروي, bacillus عصوي and curved or spiral shape منحني او حلزوني).
- Diplococci كرويات ثنائية (2,3,4,5)
- Streptococci كرويات بشكل المسبحة(1)
- Staphylococci(8) كرويات بشكل عناقيد العنب
- Streptobacilli (2, 3 in bacilli shape) عصويات بشكل المسبحة
- Tetrads (6) كرويات تترتب بهية الرباعيات(اربع خلايا)
- Cubic (7) كرويات تترتب بهيئة مكعبات

2. Staining of bacteria

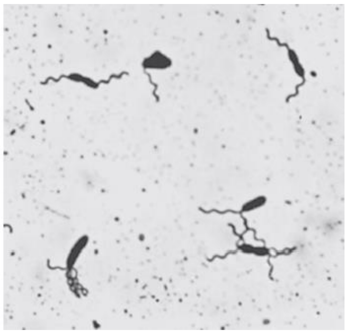
Bacterial staining is the process of coloring of colorless bacterial structural components using stains (dyes).

Staining reactions are made possible because of the physical phenomena of capillary osmosis, solubility, adsorption, and absorption of stains or dyes by cells of microorganisms.

Types of microbiological stains

- Basic stains
- Acidic stains
- Neutral stains.

Bacterial staining methods include:



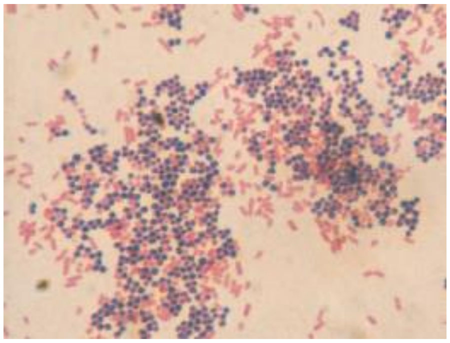
Flagellar stain Jawetz, P.39

- Simple stain.. (ex. Methylene blue stain)
- Differential stain. (ex. Gram stain, Acid fast stain)

- Special stain. (Spore stain, Flagellar stain)

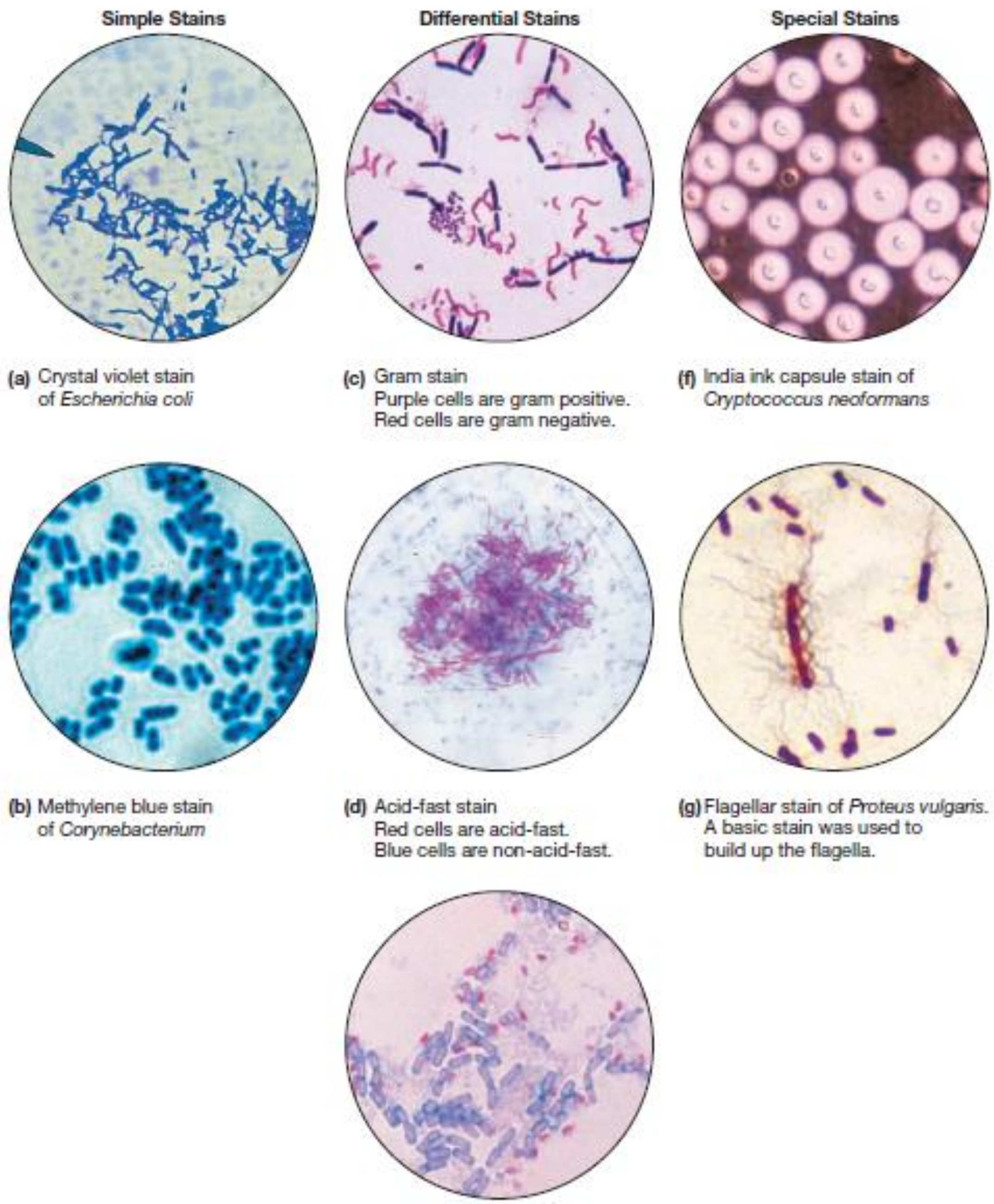
According to the Gram-stain technique the bacterial cells classified into:

Gram-positive bacteria (purpule color), and Gram-negative bacteria (Pink- color).



Gram-stain Prescott, P. 28

Some bacterial cell cannot be stained with Gram- sain according to the difference of structure in the bacterial cell wall, thus it must be stained with other techniques such as Acid- Fast stain.



Bacterial staining Methods

"Prescott, microbiology, p27

Bacterial Cell Structure.... (part one)

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

The general property of the bacterial cell includes....

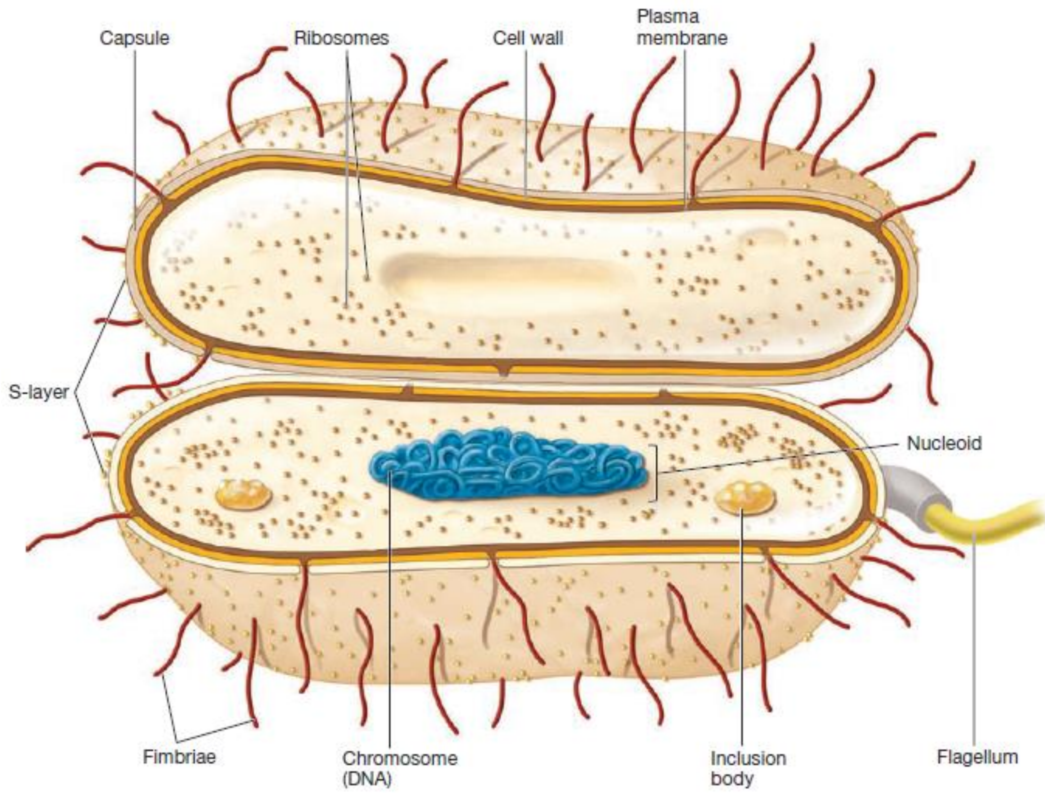
- Typical prokaryotic cell
- Contain both DNA and RNA
- Most grow in artificial media
- Replicate by binary fission
- Almost all contain rigid cell wall
- Sensitive to antimicrobial agent

Bacterial structure is considered at three levels.

1. Cell envelope proper: Cell wall and cell membrane.

2. Cellular element enclosed with in the cell envelope: Mesosomes, ribosomes, nuclear apparatus, polyamines and cytoplasmic granules.

3. Cellular element external to the cell envelope: Flagellum, Pilus and Glycocalyx.



Bacterial Cell Structure

"Prescott, microbiology, p27"

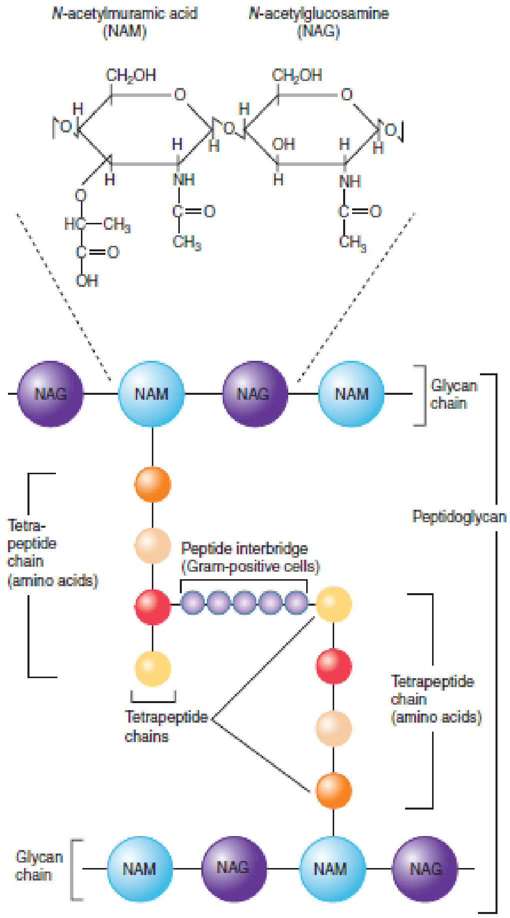
- Willey, J. M., Sherwood, L., Woolverton, C. J., **Prescott**, L. M., & Willey, J. M. (2011). **Prescott's microbiology**. New York: McGraw-Hill.

Cell wall structure

The bacterial cell wall composed of a substance variously referred to as **murein**, **mucopeptide**, or **peptidoglycan** (all, including “cell wall,” are synonyms).

Most bacteria are classified as Gram-positive or Gram-negative according to their response to the Gram-staining procedure.

Peptidoglycan is a complex polymer consisting, for the purposes of description, of **three parts**: a **backbone**, composed of alternating *N*-acetylglucosamine and *N*-acetylmuramic acidconnected by β1→4 linkages; a set of identical tetrapeptide side chains attached to *N*-acetylmuramic acid; and a set of identical peptide cross-bridges.



Components and structure of peptidoglycan.

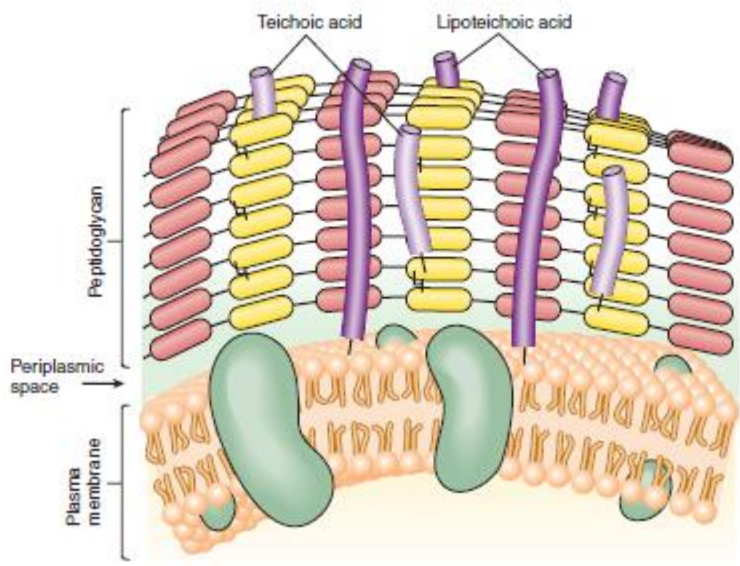
Chemical structure of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM); the ring structures of the two molecules are glucose. Glycan chains are composed of alternating subunits of NAG and NAM joined by covalent bonds. Adjacent glycan chains are cross-linked via their tetrapeptide chains to create peptidoglycan.(Jawetz, p. 25)

- **Brooks**, G. F., Jawetz, E., Melnick, J. L., & Adelberg, E. A. (2013). Jawetz, Melnick & Adelberg's medical microbiology (26th edition.). New York : London: McGraw-Hill Medical.

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

Special Components of Gram-Positive Cell Walls

- **Teichoic and teichuronic acids.**
- **Polysaccharides**



Gram-positive cell wall (Envelope), "Jawetz, p.26"

Special Components of Gram-Negative Cell Walls

- **Outer membrane:** The outer membrane is chemically distinct from all other biological membranes. It is a bilayer structure; its inner leaflet resembles in composition that of the cytoplasmic membrane, and its outer leaflet contains a distinctive component, a lipopolysaccharide (LPS).

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.

- **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 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 Acid-Fast Cell Wall**

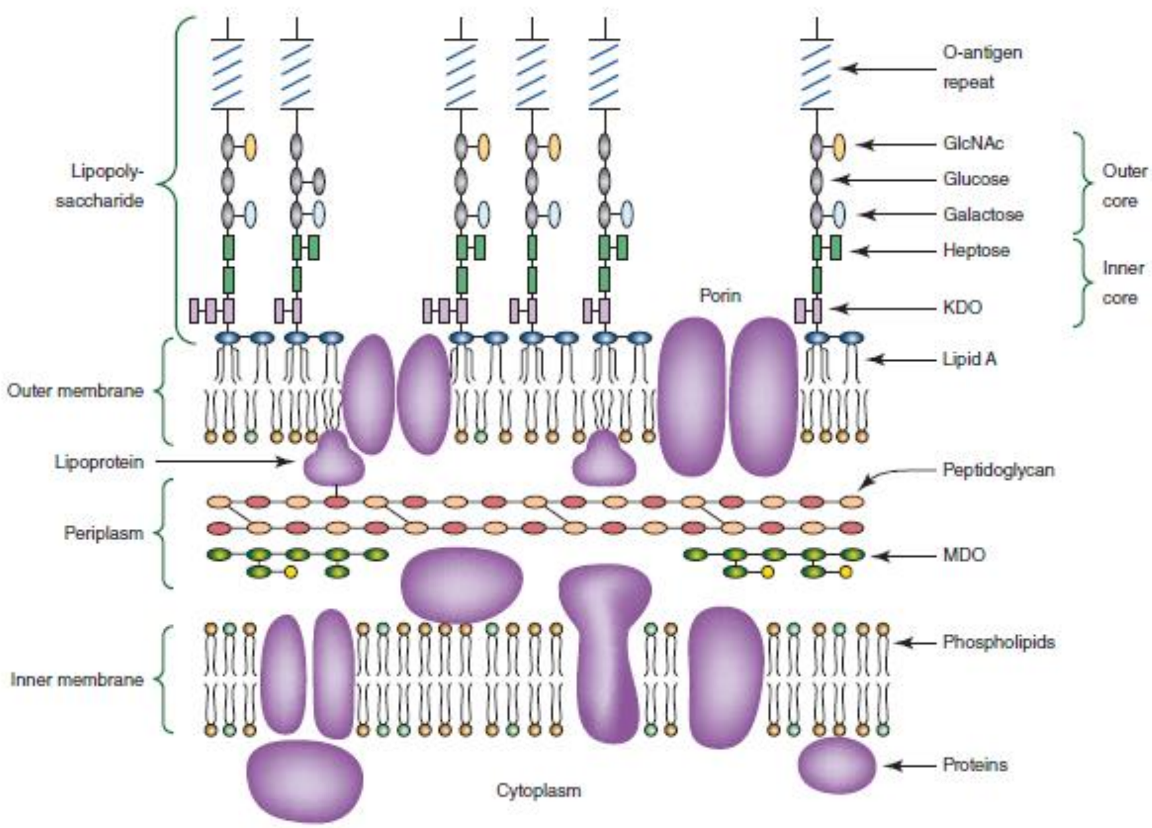
Some bacteria, notably the tubercle bacillus (*Mycobacterium tuberculosis*) and its relatives, have cell walls that contain substantial amounts of waxes, complex branched hydrocarbons (70–90 carbons long) known as **mycolic acids.**

Protoplasts, Spheroplasts, and L Forms

Removal of the bacterial wall may be accomplished by **hydrolysis** with **lysozyme** (as described above) or by **blocking peptidoglycan synthesis** with an **antibiotic** such as penicillin.

In osmotically protective media, such treatments liberate **protoplasts** from Gram-positive cells and **spheroplasts** from Gram-negative cells.

If such cells are able to grow and divide, they are called **L forms**. L forms are difficult to cultivate and usually require a medium that is solidified with agar as well as having the right osmotic strength.



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). The core region shown is that of *E. coli* K-12, a strain that does not normally contain an O-antigen repeat unless transformed with an appropriate plasmid. MDO, membrane-derived oligosaccharides. (Jawetz, p. 27)**

Capsule and Glycocalyx

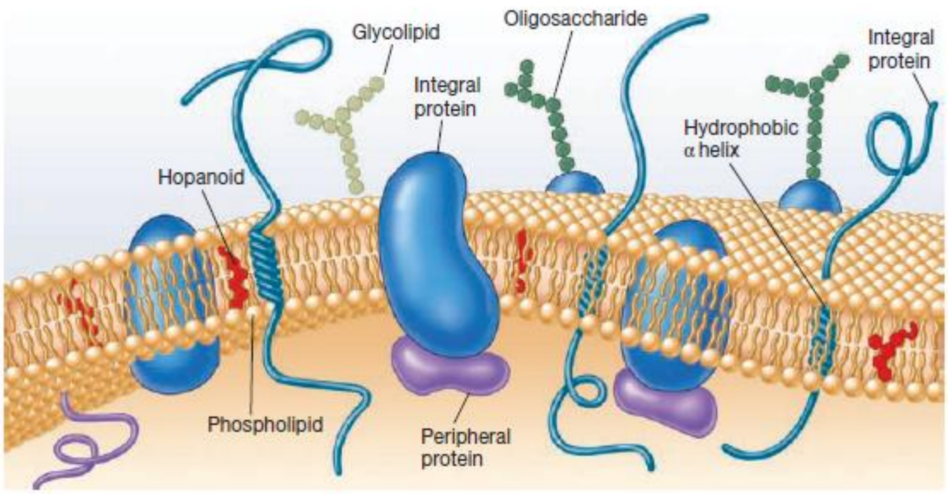
Many bacteria synthesize copious amounts of **extracellular polymers** when growing in their **natural** environments. With few exceptions (the **poly-*d* glutamic acid** capsules of ***Bacillus anthracis***), and the **mixed amino acid** capsule of ***Yersinia pestis***, **the extracellular** material is **polysaccharide**. The terms capsule and slime layer are frequently used to describe polysaccharide layers; the more inclusive شمولي term **glycocalyx** is also used. Glycocalyx is defined as the polysaccharide-containing material lying outside the cell. A **condensed**, **well-defined** layer **closely surrounding** the cell that **excludes particles**, such as India ink, is referred to as a **capsule**. If the glycocalyx is **loosely associated** with the cell and **does not exclude particles**, it is referred to as a **slime layer**.

Cell membrane (also named as cytoplasmic membrane)

- It is a delicate tri-laminar unit membrane.
- It accounts for 30% of the dry weight of bacterial cell.
- It is composed of 60% protein, 20-30% lipids and 10-20%carbohydrate.

The Fluid Mosaic Model of Membrane Structure

The most widely accepted model for membrane structure is the fluid mosaic model of Singer and Nicholson, which proposes that membranes are lipid bilayers within which proteins float. Most membrane-associated lipids are structurally asymmetric, with polar and nonpolar ends, and are called amphipathic. The polar ends interact with water and are hydrophilic; the nonpolar hydrophobic ends are insoluble in water and tend to associate with one another.



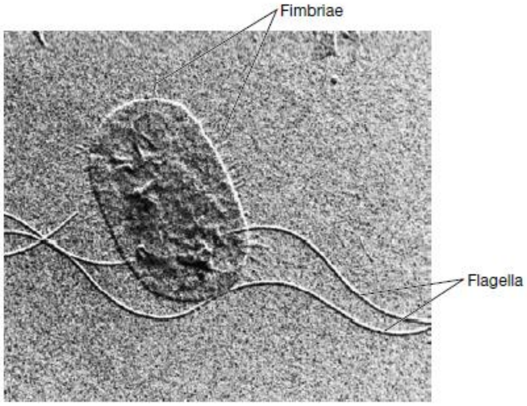
Bacterial Plasma Membrane Structure. This diagram of the fluid mosaic model of bacteria membrane structure shows the integral proteins (blue) floating in a lipid bilayer. Peripheral proteins (purple) are associated loosely with the inner membrane surface. Small spheres represent the hydrophilic ends of membrane phospholipids and wiggly tails, the hydrophobic fatty acid chains.Other membrane lipids such as hopanoids (red) may be present. (Prescott, p. 45)

Flagella

Bacterial flagella are thread-like appendages composed entirely of protein. They are threadlike locomotor appendages extending outward from the plasma membrane and cell wall.

Bacterial species often differ distinctively in their patterns of flagella distribution and these patterns are useful in identifying bacteria. Four types of arrangement are known: **monotrichous** (trichous means hair) (single polar flagellum), it is said to be a **polar** flagellum., **lophotrichous** (lopho means tuft) (multiple polar flagella), **amphitrichous** (amphi means on both sides)(single flagellum found at each of two opposite poles), and **peritrichous (**Flagella are spread fairly evenly over the whole surface) (peri means around) (multiple flagella distributed over the entire cell).

A bacterial flagellum is made up of several protofilaments, each made up of thousands of molecules of a protein subunit called **flagellin**..



Flagellar Distribution. Examples of various patterns of flagellation as seen in the light microscope. (a) Monotrichous polar (*Pseudomonas*). (b) Lophotrichous (*Spirillum*). (c) Peritrichous (*Proteus vulgaris*, X600). (Prescott, p.68)

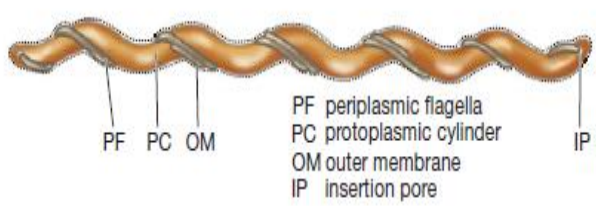
Swarming: An increasing number of bacterial species has been found to exhibit an interesting type of motility called swarming. This motility occurs on moist surfaces and is a type of group behavior in which cells move in unison across the surface.

Swarmming phenomena

Twitching الحركة الوخزية: motility is characterized by short, intermittent, jerky motions of up to several micrometers in length and is normally seen on very moist surfaces.

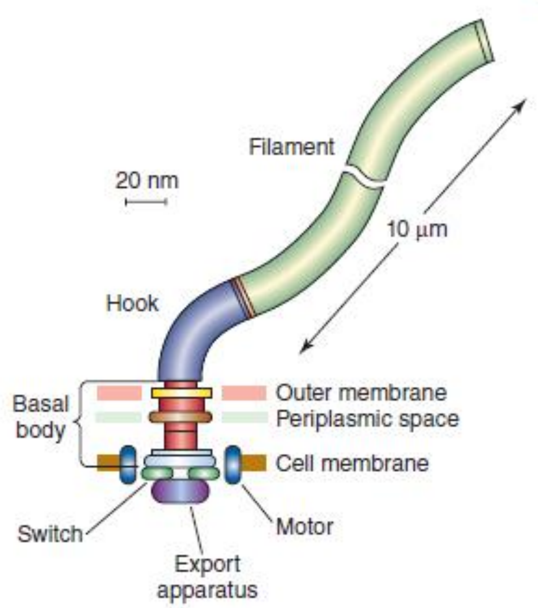
In contrast to the jerky movement of twitching motility, **gliding motility** is smooth.

Spirochete Motility: Spirochetes have flagella that work in a distinctive manner. In many spirochetes, multiple flagella arise from each end of the cell and wind around the cell (. The flagella do not extend outside the cell wall but rather remain in the periplasmic space and are covered by the outer membrane.



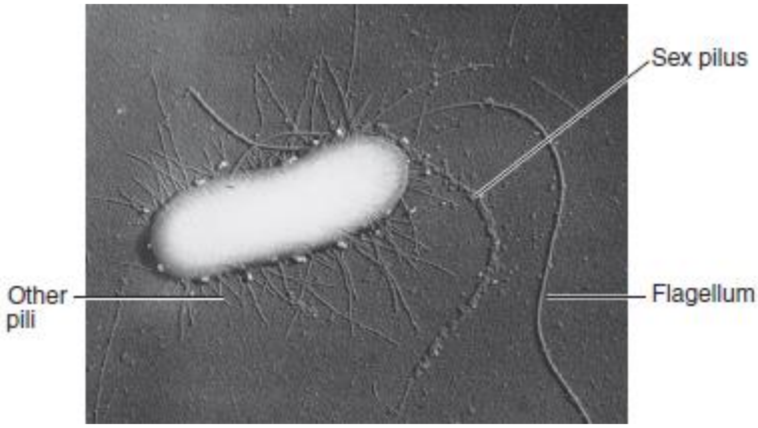
They are highly **antigenic** (H antigens), and some of the immune responses to infection are directed against these proteins.

The flagellum is attached to the bacterial cell body by a complex structure consisting of a **hook** and a **basal body**. Flagella are made stepwise. **First**, the **basal body** is assembled and inserted into the cell envelope. Then **the hook** is added, and **finally**, the **filament** is assembled progressively by the addition of flagellin subunits to its growing tip.



Pili and Fimbriae

Many procaryotes have short, fine, hairlike appendages that are thinner than flagella. These are usually called fimbriae (s., fimbria). Another type of pillus called sex pillus. Fimbriae are responsible for more than attachment. Type IV fimbriae are present at one or both poles of bacterial cells. They can aid in attachment to objects, and also are required for the twitching motility that occurs in some bacteria such as *P. aeruginosa*, *Neisseria gonorrhoeae*, and some strains of *E. coli*.



Pili of different bacteria are antigenically distinct and elicit the formation of antibodies by the host.

Endospores

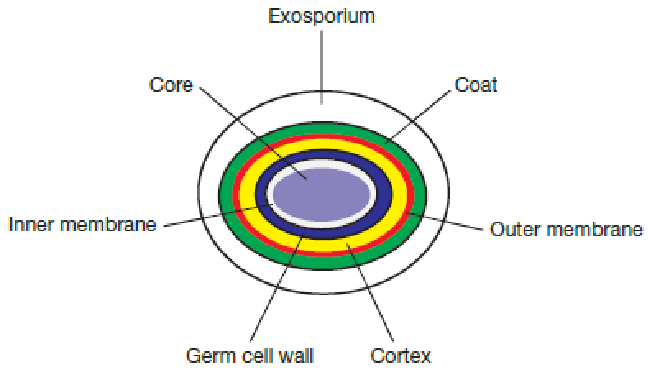
Members of several bacterial genera can form endospores. The two most common are Gram-positive rods: the obligately aerobic genus ***Bacillus*** and the obligately anaerobic genus ***Clostridium***.

The process, **sporulation**, is triggered by near depletion of any of several nutrients (carbon, nitrogen, or phosphorous). Each cell forms a single internal spore that is liberated when the mother cell undergoes autolysis.

The spore is a **resting cell**, **highly resistant to desiccation**, **heat**, and **chemical agents**; when returned to favorable nutritional conditions and activated, the spore **germinates** to produce a **single vegetative cell**. The **location** of an endospore within a cell is species-specific and can be used to determine the identity of a bacterium.

Properties of Endospores

1. **Core**—The core is the spore protoplast. It contains a complete chromosome, all the components of the proteinsynthesizing apparatus, and an energy-generating system based on glycolysis.

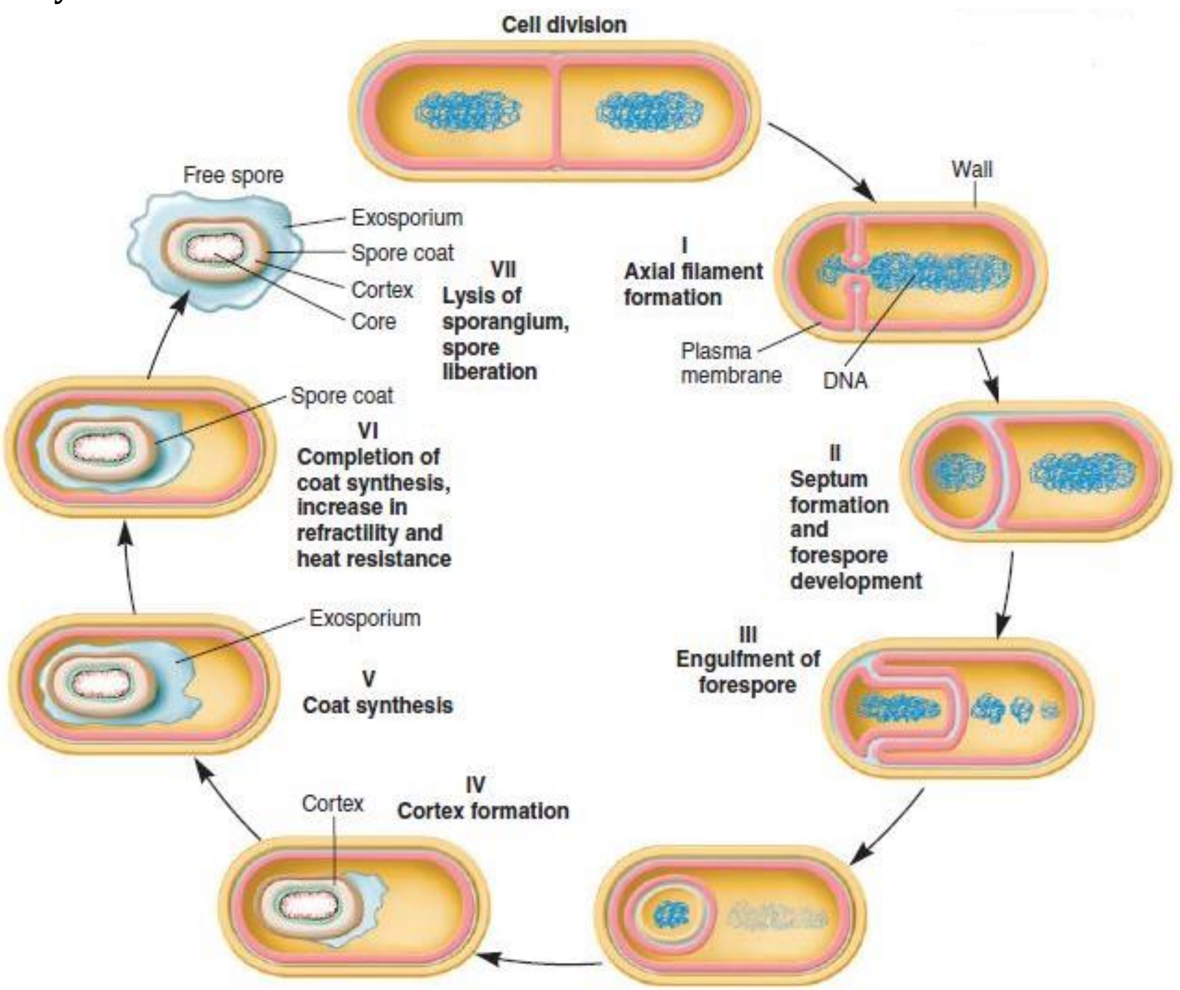


2. **Spore wall**—The innermost layer surrounding the inner spore membrane is called the spore wall.

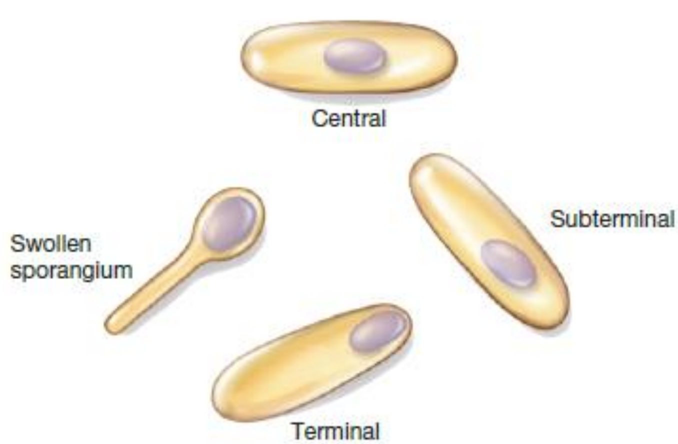
3. **Cortex**—The cortex is the thickest layer of the spore envelope. It contains an unusual type of peptidoglycan, with many fewer cross-links than are found in cell wall peptidoglycan. Cortex peptidoglycan is extremely sensitive to lysozyme, and its autolysis plays a role in spore germination.

4. **Coat.** The coat is composed of a keratin-like protein containing many intramolecular disulfide bonds.

5. **Exosporium**—The exosporium is composed of proteins, lipids, and carbohydrates.



The mature endospore occupies a characteristic location in the mother cell (referred to as the sporangium), depending on the species of bacteria. Endospores may be centrally located, close to one end (subterminal), or terminal. Sometimes an endospore is so large that it swells the sporangium.

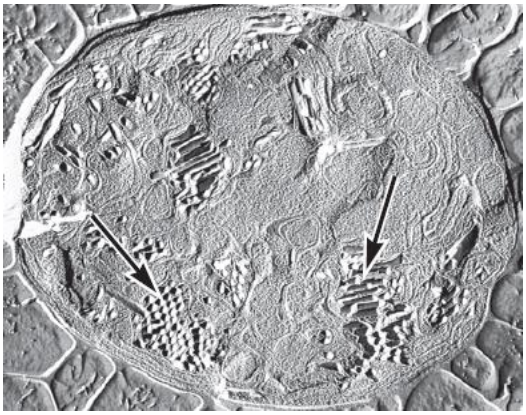


Inclusions

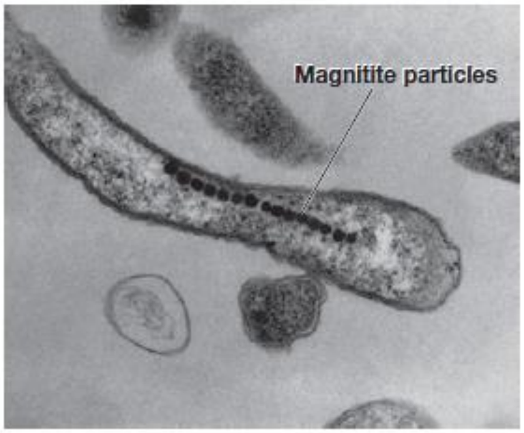
Inclusions are common in all cells. They are formed by the aggregation of substances that may be either organic or inorganic. Inclusions can take the form of granules حبات صغيرة, crystals بلورات, or globulesقطرات; some are amorphous.

Several important inclusions are found:

1. **Carbon granules**.
2. **Polyphosphate granules**.
3. **Carboxysomes**.
4. The **gas vacuole** provides buoyancy طفو to some aquatic bacteria.
5. Aquatic magnetotactic bacteria use **magnetosomes** to orient themselves in Earth's magnetic field.
6. **Metachromatic granules**.
7. **Polysaccharide Granules**.
8. **Lipid Inclusions.**
9. **Sulfur Granules.**



Gas Vacuoles are Clusters of Gas Vesicles. A freezefracture preparation of Anabaena flosaquae (389,000) showing gas vesicles and gas vacuoles. Both longitudinal and cross-sectional views of gas vesicles are indicated by arrows.



Magnetosomes. Transmission electron micrograph of the magnetotactic bacterium *Magnetospirillum magnetotacticum*.

Nucleoid

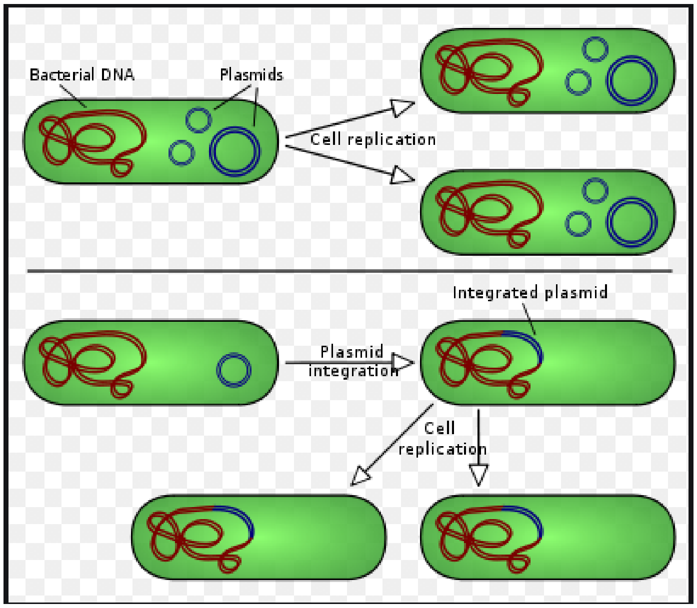
The nucleoid is an irregularly shaped region that contains the cell's chromosome and numerous proteins. The chromosomes of most bacteria are a single circle of doublestranded deoxyribonucleic acid (DNA), but some bacteria have a linear chromosome, and some bacteria, such as *Vibrio cholerae* and *Borrelia burgdorferi* (causative agents of cholera and Lyme disease, respectively), have more than one chromosome. A few bacteria (e.g., the very large bacteria *Thiomargarita* are polyploid.

Bacterial chromosomes are longer than the length of the cell. An important and still unanswered question is: how these microbes manage to fit their chromosomes into the relatively small space occupied by the nucleoid?

Answer: **Supercoiling** is thought to be important. It produces a dense, central core of DNA (*DNA is a polymer of deoxyribonucleotides*)with loops of DNA extending out from the core. Several nucleoid-associated proteins (NAPs) help compact the chromosome by causing the chromosome to bend and fold.

Plasmids

In addition to the genetic material present in the nucleoid, many bacteria contain **extrachromosomal DNA** molecules called plasmids. Plasmids use the cell's DNA-synthesizing machinery to replicate, but their replication is not linked to any particular stage of the cell cycle. Thus regulation of plasmid and chromosomal replication are **independent**. However, some plasmids are able to **integrate into the chromosome**. Such plasmids are called **episomes** and when integrated are replicated as part of the chromosome. Plasmids are inherited stably during cell division, but they are not always equally apportioned into daughter cells and sometimes are lost. The loss of a plasmid is called **curing**.



Ribosomes

All eukaryotic and prokaryotic cells contain ribosomes, where protein synthesis takes place. Prokaryotic ribosomes differ from eukaryotic ribosomes in the number of proteins and rRNA molecules they contain; they are also somewhat smaller and less dense than ribosomes of eukaryotic cells.