BIOL 230
MICROBIOLOGY
Hard Copy of E-Text SoftChalk Lessons for Classroom Use

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Unit 1:

Introduction to Microbiology and Prokaryotic Cell Anatomy

I. Introduction to Microbiology: Basic Groups of Microbes; Prokaryotic and Eukaryotic Cells; TheThree Domain System.

II. The Prokaryotic Cell: Shapes and Arrangements of Bacteria; Prokaryotic Cell Anatomy.

An introduction to microorganisms
BASIC GROUPS OF MICROBES AND AN INTRODUCTION TO MICROORGANISMS

Basic Groups of Microbes and an Introduction to Microorganisms

Fundamental Statements for this Lesson:

1. Microorganisms are typically too small to be seen with the naked eye.
2. Bacteria, fungi, viruses, protozoa, and algae are the major groups of microorganisms.
3. The vast majority of microorganisms are not harmful but rather beneficial.
4. Microbiota refers to all of the microorganisms that live in a particular environment.
5. A microbiome is the entire collection of genes found in all of the microbes associated with a particular host.
6. The microbiome of the human body - especially in the intestinal tract - aid in the digestion of many foods, the regulation of multiple host metabolic pathways, and the regulation the body's immune defenses.

Common Course Objective

1. Define microbiology and be able to distinguish what types of organisms it encompasses
2. Identify the types of microorganisms that are studied in microbiology and give examples of each.

Detailed Learning Objectives for this Lesson

1. State three harmful effects and four beneficial effects associated with the activities of microorganisms.

2*. Define microbiota and microbiome.

3*. Briefly describe two different beneficial things the human microbiome does for the normal function of our body.

4*. State several diseases associated with a change in our "normal" microbiota.

4. List and recognize a description of each of the 5 basic groups of microbes.

(*) = Common theme throughout the course

Think-Pair-Share Questions

This tube contains 7 milliliters of a culture of *Escherichia coli*. The total number of bacteria in this tube is equal to:

A. The number of people in Baltimore city.

B. The number of people in Maryland.

C. The number of people in North America.

D. The number of people in the world.

Basic Groups of Microbes

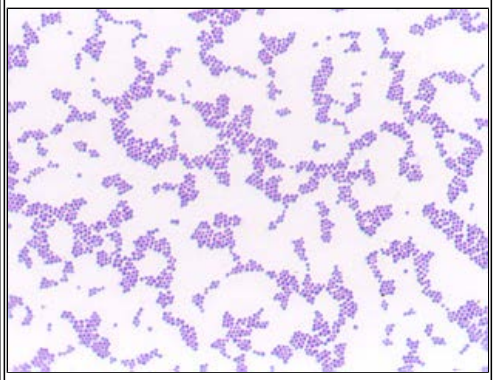
Microorganisms are the dominant life forms on earth, are found in almost every conceivable environment, and are essential to sustaining life on this planet.

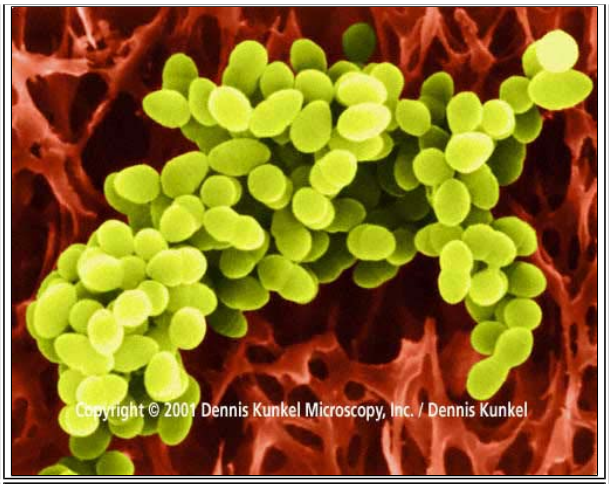
There are **5 basic groups of microorganisms**:

a. **Bacteria**

Bacteria are typically unicellular, microscopic, prokaryotic organisms that reproduce by binary fission (see *Staphylococcus aureus* shown in **Fig. 1** and **Fig. 2**).

Fig. 1: Photomicrograph of *Staphylococcus aureus*	**Fig. 2: Scanning Electron Micrograph of *Staphylococcus aureus***





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b. **Fungi: yeasts and molds**

Yeasts are typically unicellular, microscopic, eukaryotic fungi that reproduce asexually by budding (see *Saccharomyces cerevisiae* shown in **Fig. 3** and **Fig. 4**)

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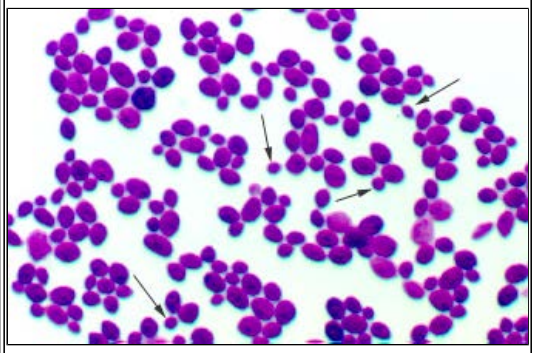
Fig. 3: Photomicrograph of *Saccharomyces cerevisiae*	**Fig. 4: Scanning Electron Micrograph of *Saccharomyces cerevisiae***
 Note budding (arrows)	
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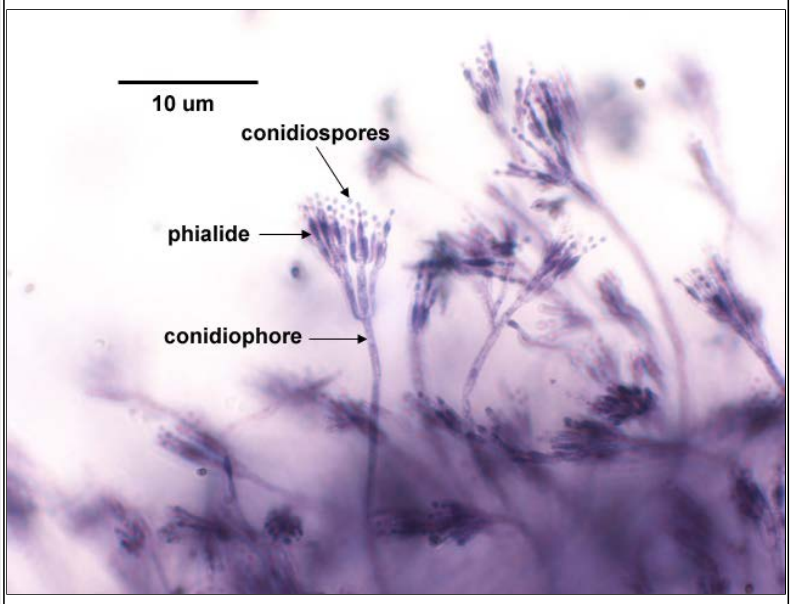
Molds are typically filamentous, eukaryotic fungi that reproduce by producing asexual reproductive spores (see *Penicillium roqueforti* in **Fig. 5** and in **Fig. 6**)

Fig. 5: Photomicrograph of Conidiospores of the Mold *Penicillium*	**Fig. 6: Electron Micrograph of the mold *Penicillium* showing asexual reproductive spores called Conidiospores**



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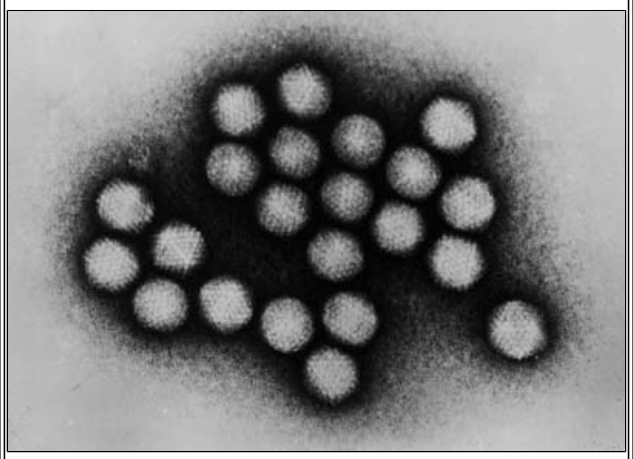
c. **Viruses**

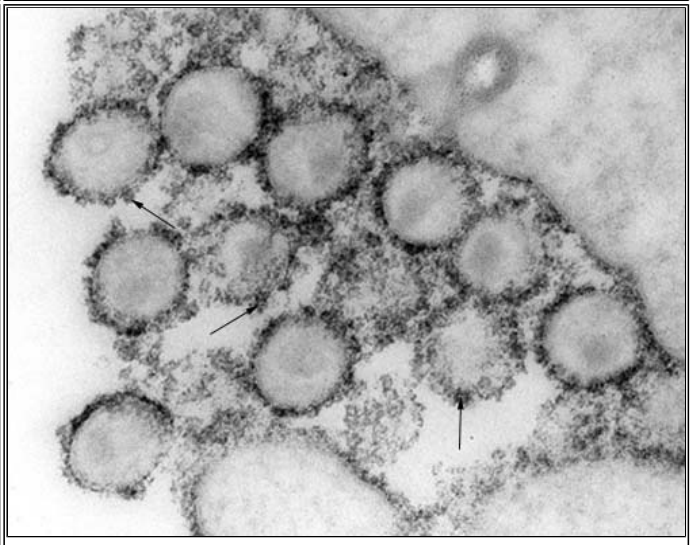
Viruses are typically submicroscopic, acellular infectious particles that can only replicate inside a living host cell. The vast majority of viruses possess either DNA or RNA but not both (see adenoviruses in **Fig. 7** and HIV-1 in **Fig. 8**).

Fig. 7:Transmission Electron Micrograph of Adenovirus



Courtesy of the Centers for Disease Control and Prevention.

Fig. 8:Transmission Electron Micrograph of HIV-1 Stained to Show Surface Glycoproteins



Glycoproteins (arrows) are seen on the viral envelope.

Courtesy of the Centers for Disease Control and Prevention.

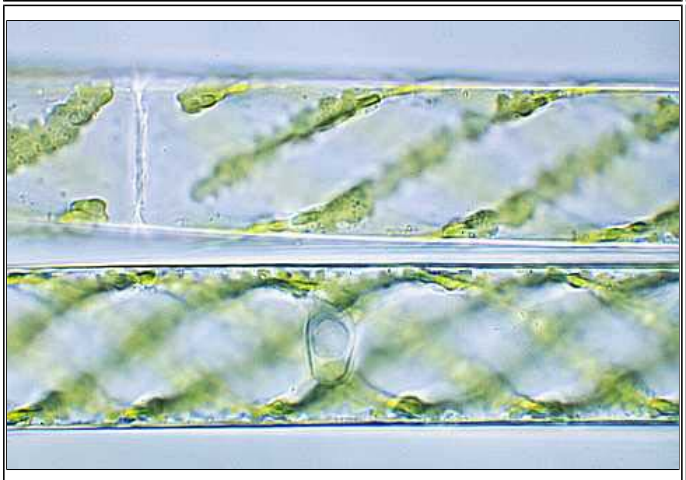
d. **Protozoa**

Protozoa are typically unicellular, microscopic, eukaryotic organisms that lack a cell wall (see *Amoeba proteus* in **Fig. 9** and *Paramecium multimicronucleatum* in **Fig. 10**).

Fig. 9: Photomicrograph of *Amoeba proteus*	Fig. 10: Electron Micrograph of *Paramecium*
	
Image: Microbial Inhabitants of Freshwater--Amoeba proteus. © Stephen Durr, author. Licensed for use, ASM MicrobeLibrary.	Courtesy of Dennis Kunkel Microscopy, Inc. Copyright © 2001 Dennis Kunkel Microscopy, Inc. Used with Permission

e. **algae**

Algae are typically eukaryotic microorganisms that carry out photosynthesis (see *Spirogyra* species in **Fig 11** and *Euglena* in **Fig. 12**).

Fig. 11: Photomicrograph of *Spirogyra* species.	Fig. 12: Photomicrograph of *Euglena*
	
Courtesy of Micrographia.com	Courtesy of Micrographia.com

For more information: Preview of prokaryotic and eukaryotic cells.

To gain an appreciation of the small size of microbes, view this excellent interactive illustration of cell size and scale.

Courtesy of Learn.Gene tics, University of Utah Health Sciences

Think-Pair-Share Questions

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B. The number of people in Maryland.

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Crossword Activity

In this course we will be looking at various fundamental concepts of microbiology, with particular emphasis on their relationships to human health. The **overall goal** is to **better understand the total picture of infectious diseases** in terms of **host-infectious agent interaction**. We will look at various groups of microbes and learn what they might do to establish infection and harm the body, we will look at the body to see the ways in which it defends itself against these microbes, and we will learn what can be done to help the body in its defense efforts.

Most people tend to think of microorganisms as **harmful** because of their roles in causing infectious diseases in humans and other animals, and agricultural loss as a result of infectious diseases of plants and the spoilage of food. The fact is, however, **the vast majority of microorganisms are not harmful but rather beneficial**. Without them there would be no life on earth. Therefore, we will start this course by looking at a few of the many **benefits** from microbial activity on this planet.

1. **Food production**

   Many food products employ microorganisms in their production. These include the microbial fermentation processes used to produce yogurt, buttermilk, cheeses, alcoholic beverages, leavened breads, sauerkraut, pickles, and Kim chi.

2. **Energy production and cleaning up the environment**

   Methane, or natural gas, is a product of methanogenic microorganisms. Many aquatic microbes capture light energy and store it in molecules used as food then used by other organisms. Animal wastes, domestic refuse, biomass, and grain can be converted to biofuels such as ethanol and methane by microorganisms. In addition, through a process called bioremediation

3. **Sustaining agriculture**

   Through their roles in recycling nitrogen, carbon, and sulfur, microorganism are able to convert these essential elements into forms that can be used by plants in their growth. They are also essential in enabling ruminant animals such as cows and sheep to digest cellulose from the grasses they eat**.**

4. **Production of useful natural gene products or products from bioengineering.** Examples include specific enzymes. antibiotics, vaccines, and medications such as human insulin, interferons, and growth hormones.

5. **The human microbiota and microbiome: Where we be without microorganisms?**

While the typical human body contains an estimated 37 trillion human cells, it also contains over 100 trillion bacteria and other microbes . The human body has 3 times as many bacterial cells as it does human cells! (It should be noted that in January 2016, a **revised estimate** was published that proposes that

the number of **human cells to be around 30 trillion** - the vast majority of these being red blood cells - while the number of **bacterial cells is approximately 39 trillion**. This of course varies with age, height, and weight of the individual as well as other factors.)

It is estimated the mass of the human microbiota is 2.5 pounds. **The complex mutually beneficial symbiotic relationship** . It is now recognized that **the millions of genes associated with the microbiota**. These collective microbial genes are referred to as the human microbiome . There are c**urrently an estimated 5, 000,000 - 10,000,000 genes from over 1000 species that constitute the human microbiome** compared to the approximately 20,000 - 23,000 genes that make up the human genome. There are approximately 300 non-human genes in the human body for every human gene.

a. **Regulation of Host Metabolism**

The mutually beneficial interaction between the human host and its resident microbiota is essential to human health. Microbial genes produce metabolites essential to the host while human genes contribute to development of the microbiota. The microbiome aids in the following:

1. **The digestion of many foods**, especially plant polysaccharides that would normally be indigestible by humans.

2. **The regulation of many host metabolic pathways**. The metabolism of many substrates in the human body is carried out by a **combination of genes from both the microbiome and the human genome**. Within the intestinal tract there is constant chemical communication not only between microbial species but also between microbial cells and human cells. Multiple factors, including **diet, antibiotic use, disease, life style, and a person's environment can alter the composition of the microbiota within the gastrointestinal tract and, as a result, influence host biochemistry and the body's susceptibility to disease**.

3. **Metabolic disorders** such as diabetes, nonalcoholic fatty liver disease, hypertension, obesity, gastric ulcers, colon cancer, and possibly some mood and behavior changes through hormone signaling have been linked to alterations in the microbiota.

b. **Regulation of Immunity**

There is ever growing evidence that **commensal bacteria of the gastrointestinal tract, as well as parasitic gastrointestinal helminths, may have coevolved with the human body over the past 200,000 year in such a way that genes from the human microbiota may play a significant role in regulating the human immune responses** by providing a series of checks and balances that prevent the immune system from being too aggressive and causing an autoimmune attack upon the body's own cells, while still remaining aggressive enough to recognize and remove harmful pathogens. The microbiota affects the development of the immune system while the immune system influences the composition of the microbiota.

As exposure to and colonization with these once common human organisms has drastically changed over time as a result of less exposure to mud, animal and human feces,and helminth ova, coupled with ever increasing antibiotic use that destroys normal flora, improved sanitation, changes in the human diet, increased rate of cesarean sections,decreased rate of breastfeeding, and improved methods of processing and preserving of food, **the rates of allergies, allergic asthma, and autoimmune diseases (inflammatory bowel disease, Crone's disease, irritable bowel syndrome, type-1 and type-2 diabetes, and multiple sclerosis for example) have dramatically increased in developed countries while remaining relatively low in undeveloped and more agrarian parts of the world**.

Self Quiz for Basic Groups of Microbes and an Introduction to Microorganisms

Quiz Group

Back to Unit 1 Table of Contents

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Assignment for the Next Lecture Period in My Traditional Class Sections: Flipped-Class Assignment

Read and study the following section under I. Introduction:

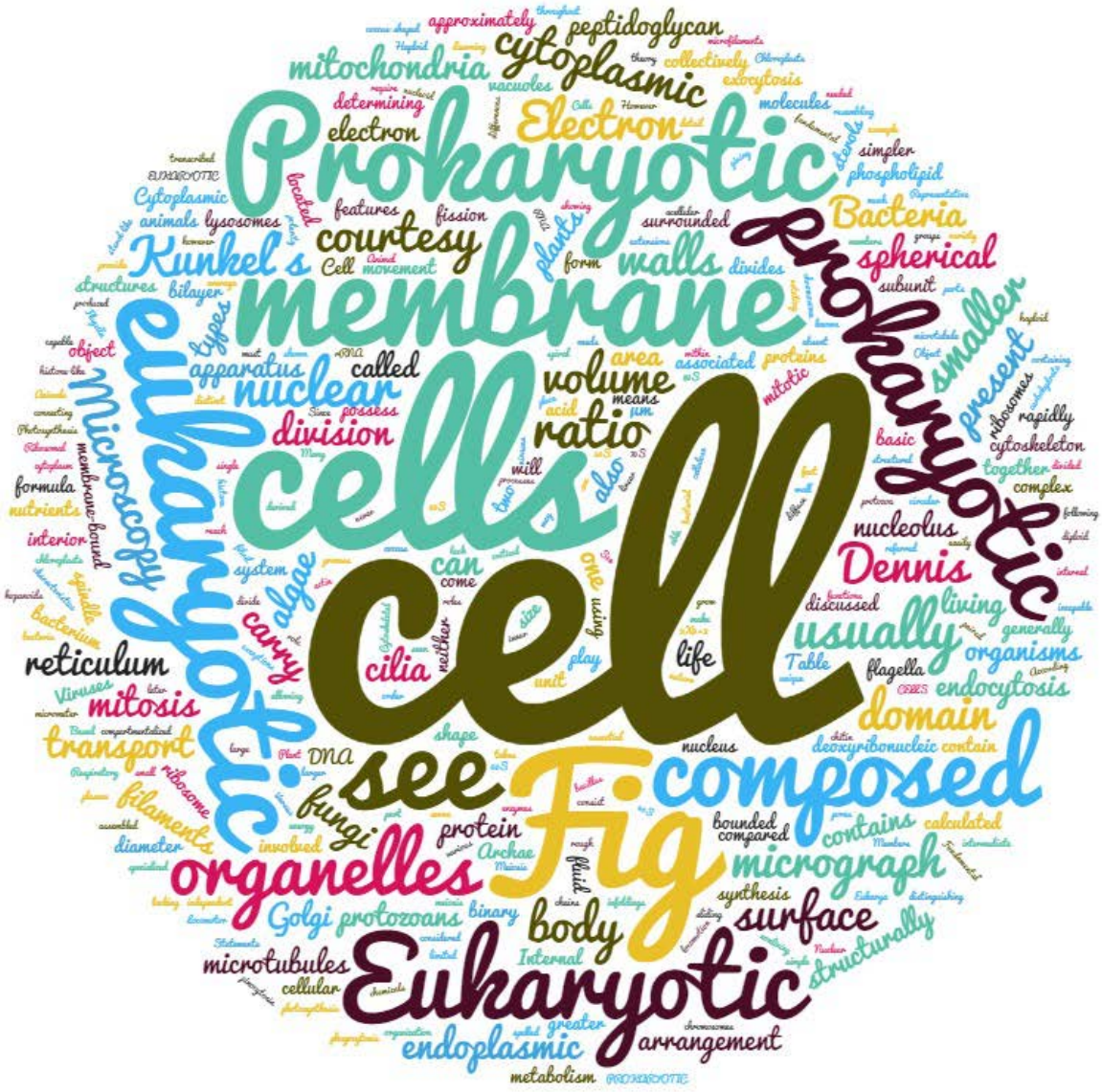
B. Cellular Organization: Prokaryotic and Eukaryotic Cells in your E-text and answer the 3 learning objectives for this section.

We will be doing a classroom group activity on this section so it is critical that you come prepared. I will be assuming that you have done this

preparatory assignment.

A comparison of prokaryotic and eukaryotic cells
PROKARYOTIC AND EUKARYOTIC CELLS

Prokaryotic and Eukaryotic Cells



Fundamental Statements for this Lesson:

1. ***There are two basic types of cells in nature: prokaryotic and eukaryotic.***
2. ***Prokaryotic cells are structurally simpler than eukaryotic cells.***
3. ***The smaller a cell, the greater its surface to volume ratio.***
4. ***The smaller the surface to volume ratio, the more structurally complex (compartmentalized) a cell needs to be in order to carry out life functions.***
5. ***There are fundamental differences between prokaryotic and eukaryotic cells.***
6. ***Bacteria are prokaryotic cells; fungi, protozoa, algae, plants, and animals are composed of eukaryotic cells.***
7. ***Viruses are not cells so they are neither prokaryotic nor eukaryotic. They can replicate only inside a living cell.***

Common Course Objective

Distinguish between prokaryotic and eukaryotic cells and state which microbes fit into each group.

Detailed Learning Objectives for this Lesson

1. Briefly describe why, in terms of differences in cell size, a eukaryotic cell is structurally more complex and compartmentalized than a cell that is prokaryotic.

2.* When given a description, determine whether a cell is prokaryotic or eukaryotic and explain why.

3. Briefly state why viruses are not considered as prokaryotic nor eukaryotic.

(*) = Common theme throughout the course

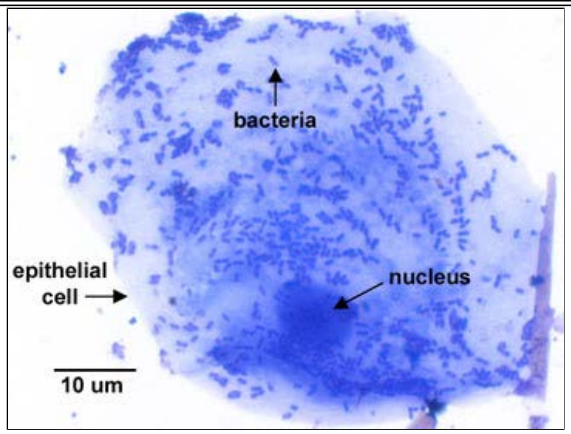
TPS Questions (These TPS questions will be done as an in-class activity in my traditional BIOL 230 classes.)

A. Overview

According to the cell theory, the cell is the basic unit of life. All living organisms are composed of one or more cells. Based on the organization of their cellular structures, all living cells can be divided into two groups: prokaryotic and eukaryotic (also spelled procaryotic and eucaryotic). Animals, plants, fungi, protozoans, and algae all possess **eukaryotic cell types**. Only bacteria have **prokaryotic cell types**.

Prokaryotic cells are generally much smaller and more simple than eukaryotic **(see Fig. 1)**. Prokaryotic cells are, in fact, able to be structurally more simple because of their small size. **The smaller a cell, the greater is its surface-to-volume ratio** (the surface area of a cell compared to its volume).

Fig. 1: Photomicrograph of a eukaryotic human cell from the mouth on which normal prokaryotic bacteria are growing.



The surface area of a spherical object can be calculated using the following formula:

$$S = 4 \pi r^2$$

The volume of a spherical object can be calculated using the formula:

$$V = 4/3 \pi r^3$$

For example, a spherical cell 1 micrometer (µm) in diameter - the average size of a coccus-shaped prokaryotic bacterium - has a surface-to-volume ratio of approximately 6:1, while a spherical eukaryotic cell having a diameter of 20 µm has a surface-to-volume ratio of approximately 0.3:1.

A large surface-to-volume ratio, as seen in smaller prokaryotic cells, means that nutrients can easily and rapidly reach any part of the cells interior. However, in the larger eukaryotic cell, the limited surface area when compared to its volume means nutrients cannot rapidly diffuse to all interior parts of the cell. That is why **eukaryotic cells require a variety of specialized internal organelles to carry out metabolism, provide energy, and transport chemicals throughout the cell**. Both, however, must carry out the same life processes. Features distinguishing prokaryotic and eukaryotic cells are discussed on the following pages. **All of these features will be discussed in detail later in Unit 1.**

Self Check

B. Comparison of Eukaryotic and Prokaryotic Cells

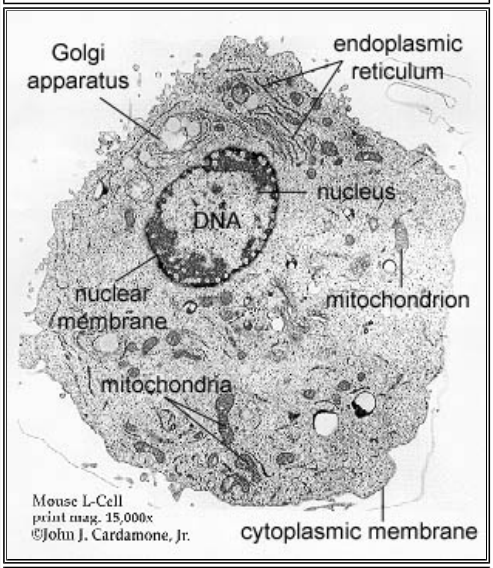
1. Nuclear Body

Eukaryotic cell

a. The nuclear body is bounded by a nuclear membrane having pores connecting it with the endoplasmic reticulum (**see Fig. 2** and **Fig. 3**).
b. It contains one or more paired, linear chromosomes composed of deoxyribonucleic acid (DNA) associated with histone proteins.
c. A nucleolus is present. Ribosomal RNA (rRNA) is transcribed and assembled in the nucleolus.

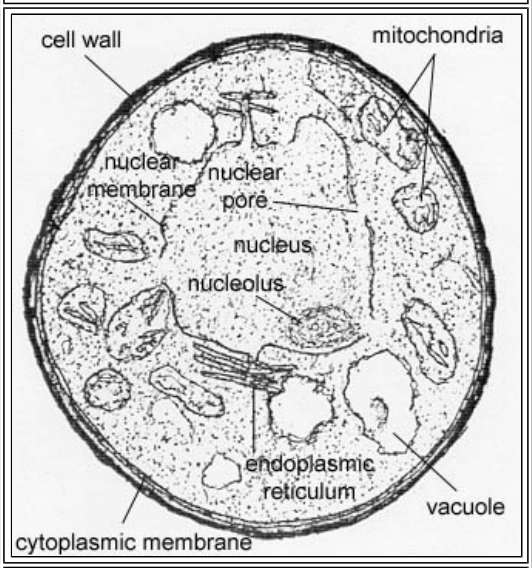
d. The nuclear body is called a nucleus.

Fig. 2: Transmission electron micrograph of a eukaryotic animal cell.



Hot Spot Explore

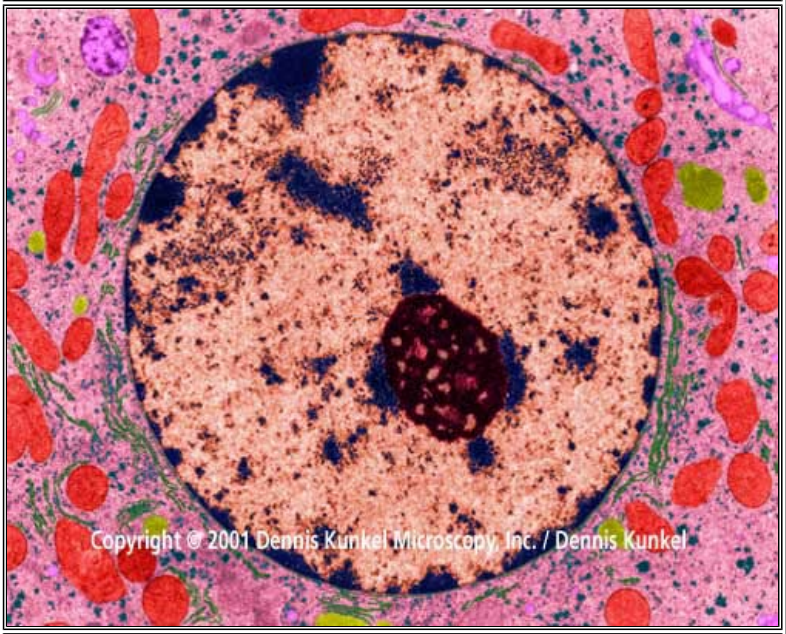
Fig. 3: Transmission electron micrograph of the yeast Candida albicans, a eukaryotic cell.



Hot Spot Explore

Transmission electron micrograph of a nucleus

Courtesy of Dennis Kunkel's Microscopy

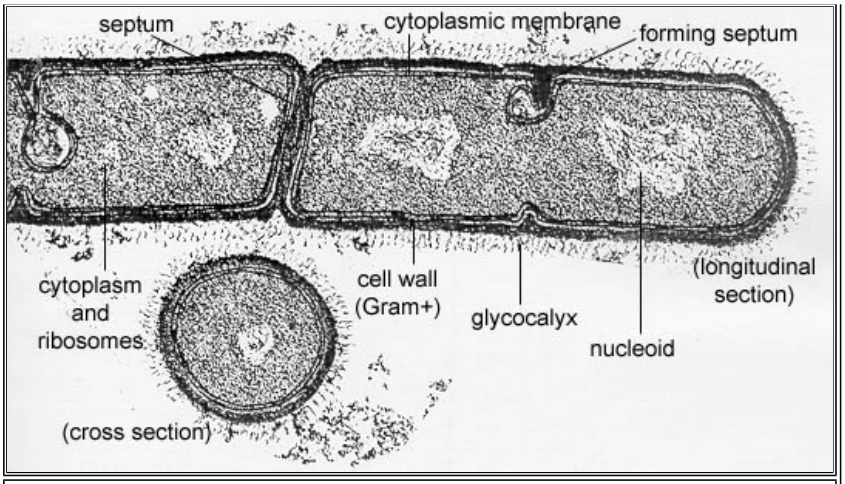


Hot Spot Explore

Prokaryotic cell

a. The nuclear body is typically not bounded by a nuclear membrane (**see Fig. 4**).
b. It usually contains one circular chromosome composed of deoxyribonucleic acid (DNA) associated with histone-like proteins.
c. There is no nucleolus.
d. The nuclear body is called a nucleoid.

Fig. 4: Transmission electron micrograph of a prokaryotic bacterium.



Hot Spot Explore

Sorting Activity

B. Comparison of Eukaryotic and Prokaryotic Cells

2. Cell Division

Eukaryotic cell

a. The nucleus divides by mitosis.
b. Haploid (1N) sex cells in diploid or 2N organisms are produced through meiosis.

For more information: Review of mitosis

Prokaryotic cell

a. The cell usually divides by binary fission. There is no mitosis.
b. Prokaryotic cells are haploid Meiosis is not needed.

Sorting Activity

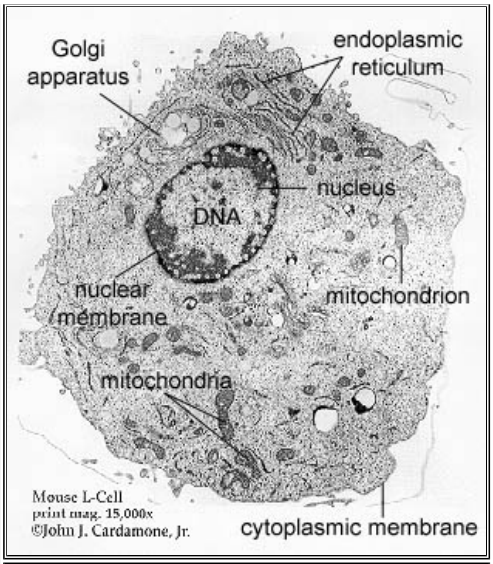
B. Comparison of Eukaryotic and Prokaryotic Cells

3. Cytoplasmic Membrane - Also Known as a Cell Membrane or Plasma Membrane

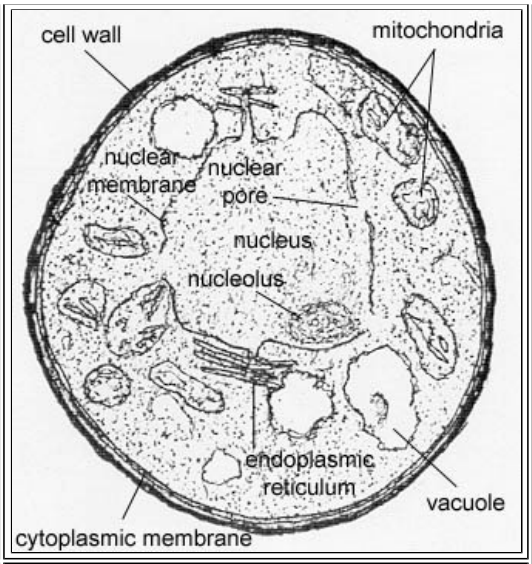
Eukaryotic cell

a. The cytoplasmic membrane (**see Fig. 2 and Fig. 3**) is a fluid phospholipid bilayer (**see Fig. 5**) containing sterols (**see Fig. 6**).
b. The membrane is capable of endocytosis (phagocytosis and pinocytosis) and exocytosis.

Fig. 2: Transmission electron micrograph of a eukaryotic animal cell.	**Fig. 3: Transmission electron micrograph of the yeast Candida albicans, a eukaryotic cell.**



Hot Spot Explore



Hot Spot Explore

Fig. 5: Diagram of a cytoplasmic membrane.

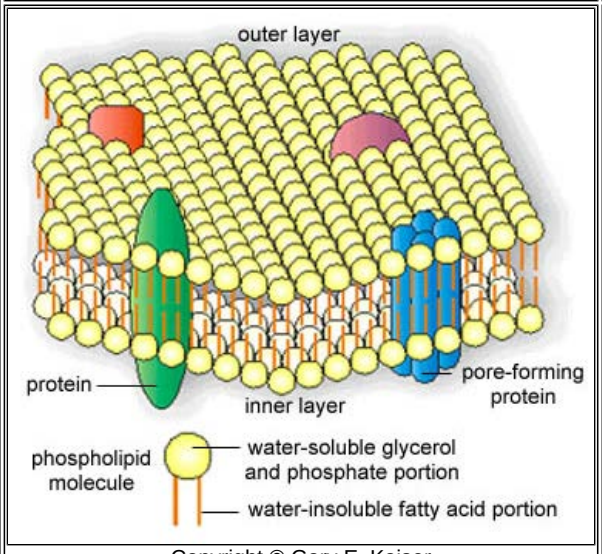
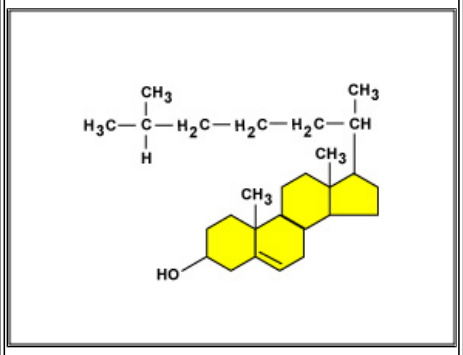


Fig. 6: A cholesterol molecule.

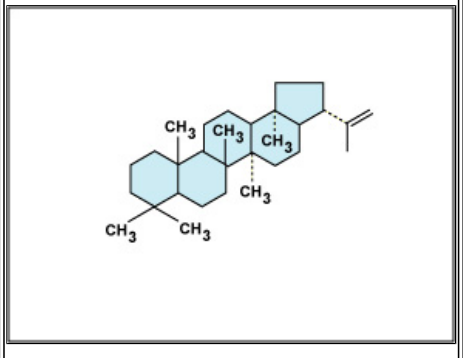


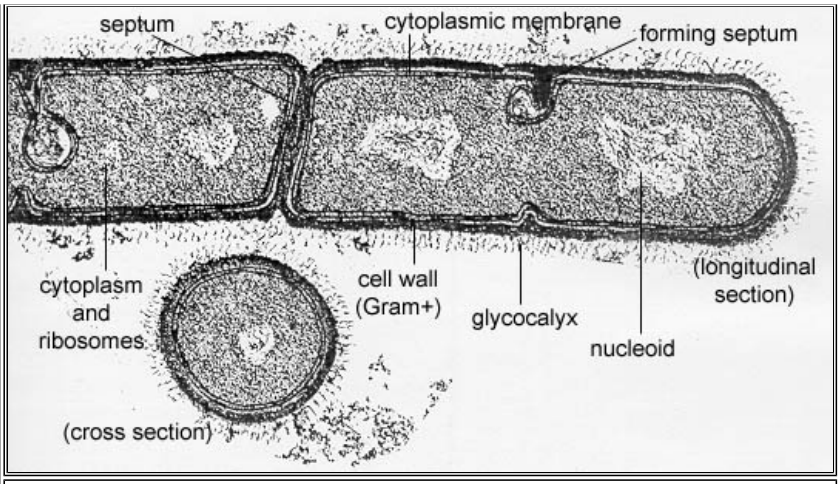
Prokaryotic cell

a. The cytoplasmic membrane (**see Fig. 4**); is a fluid phospholipid bilayer (**see Fig. 5**) usually lacking sterols . Bacteria generally contain sterol-like molecules called hopanoids (**see Fig. 7**).
b.The membrane is incapable of endocytosis and exocytosis.

Fig. 4: Transmission electron micrograph of a prokaryotic bacterium.

Fig. 7: Molecule of diplotene, a bacterial hopanoid.





Hot Spot Explore

Self Check

B. Comparison of Eukaryotic and Prokaryotic Cells

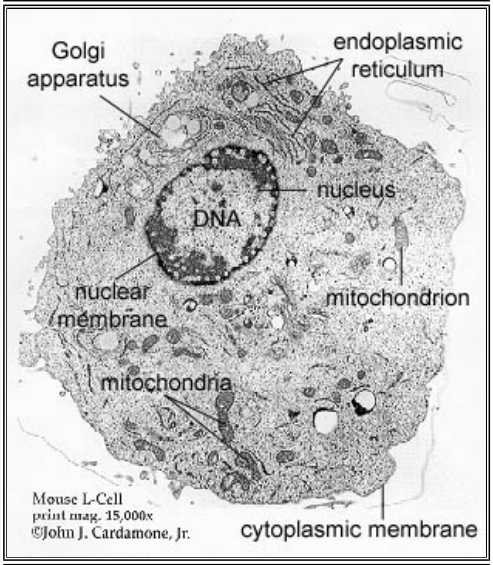
4. Cytoplasmic Structures

Eukaryotic cell

a. The ribosomes are composed of a 60S and a 40S subunit that come together during protein synthesis to form an 80S ribosome.

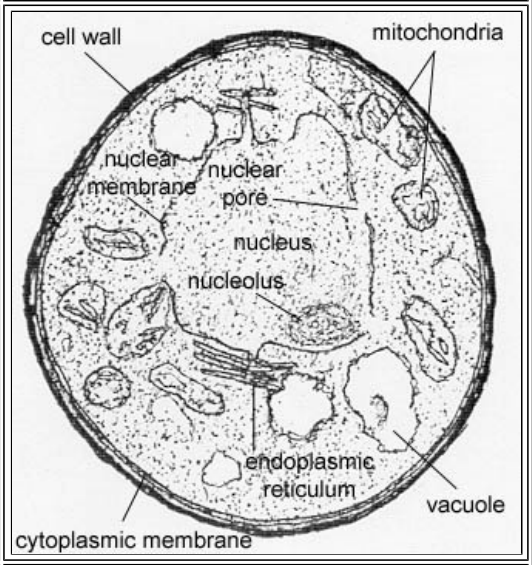
b. Internal membrane-bound organelles such as mitochondria, endoplasmic reticulum, Golgi apparatus, vacuoles, and lysosomes are typically present (**see Fig. 2** and **Fig. 3**).

Fig. 2: Transmission electron micrograph of a eukaryotic animal cell.



Hot Spot Explore

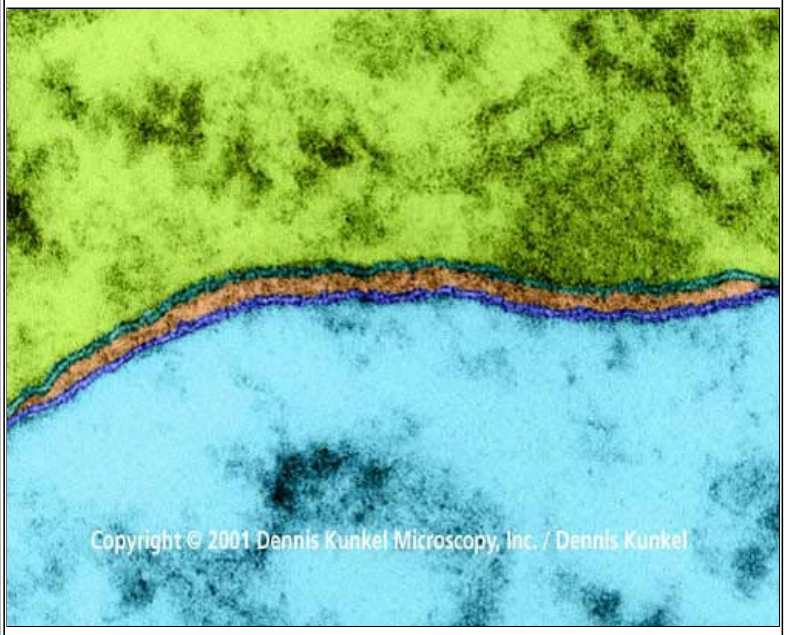
Fig. 3: Transmission electron micrograph of the yeast Candida albicans, a eukaryotic cell.

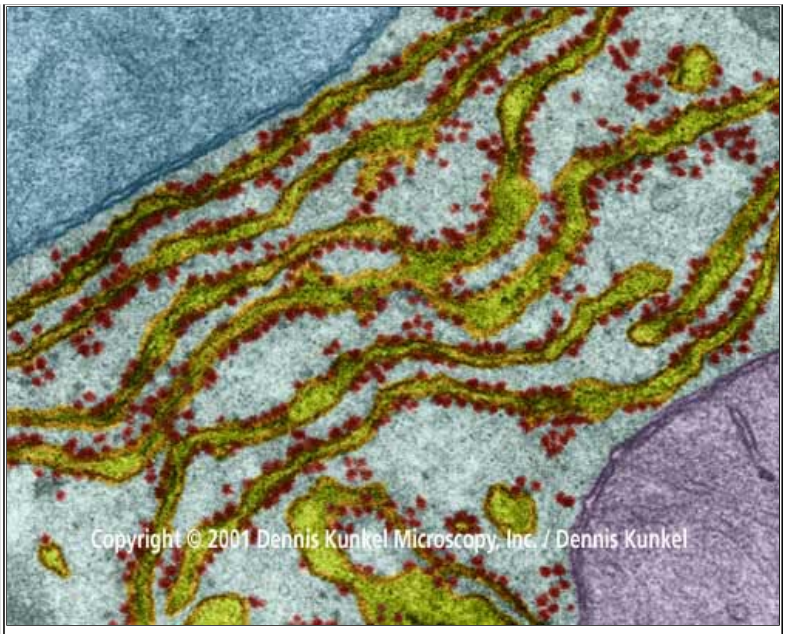


Hot Spot Explore

c. Chloroplasts serve as organelles for photosynthesis.
d. A mitotic spindle involved in mitosis is present during cell division.
e. A cytoskeleton is present. It contains microtubules, actin micofilaments, and intermediate filaments. These collectively play a role in giving shape to cells, allowing for cell movement, movement of organelles within the cell and endocytosis, and cell division.

Transmission electron micrograph of a cytoplasmic membrane Courtesy of Dennis Kunkel's Microscopy	**Transmission electron micrograph of rough endoplasmic reticula** Courtesy of Dennis Kunkel's Microscopy





Hot Spot Explore

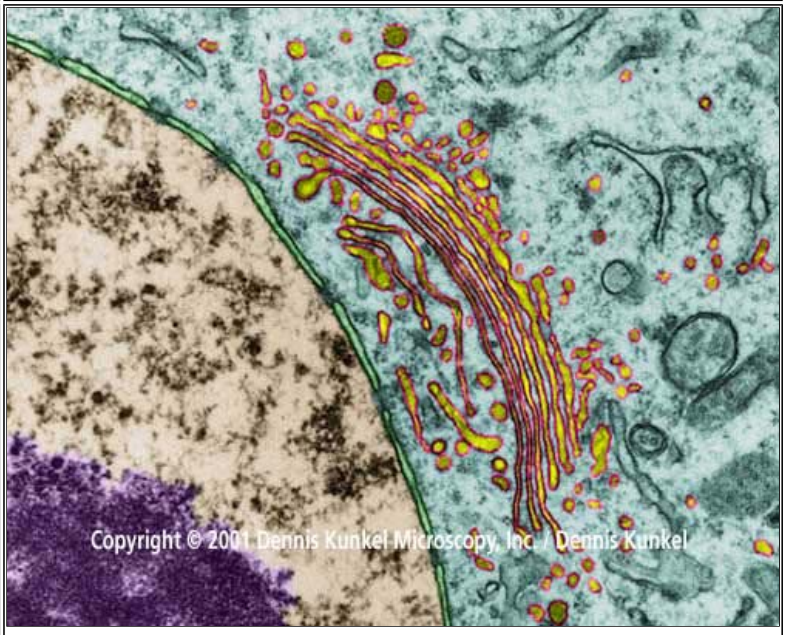
Transmission electron micrograph of mitochondria and rough endoplasmic reticula

Courtesy of Dennis Kunkel's Microscopy

Hot Spot Explore

Transmission electron micrograph of a nucleus and Golgi apparatus

Courtesy of Dennis Kunkel's Microscopy



Hot Spot Explore

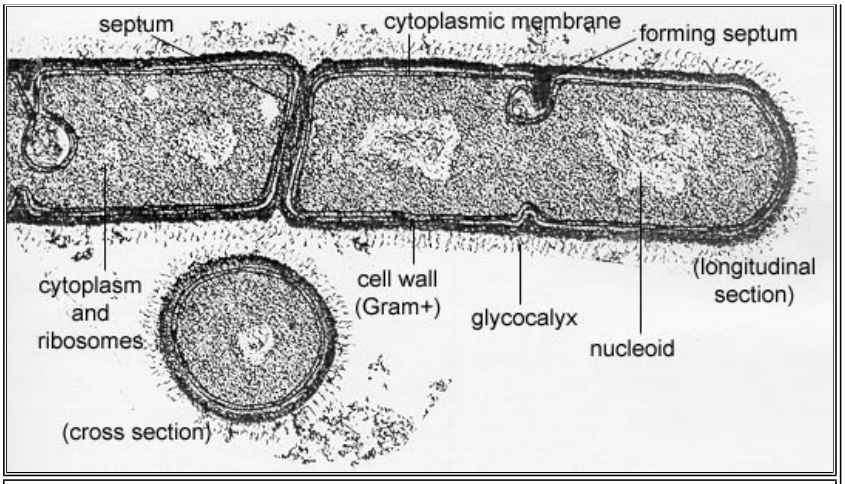
Learn more: The density of ribosomal subunits: 60S and 40S.

Prokaryotic cell

a. Internal membrane-bound organelles such as mitochondria, endoplasmic reticulum, Golgi apparatus, vacuoles, and lysosomes are absent (**see Fig. 4**)

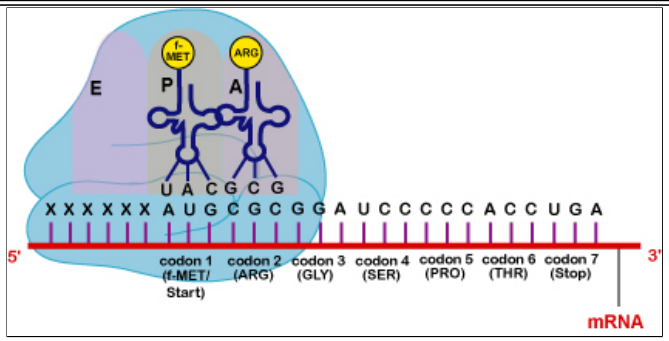
b. The ribosomes are composed of a 50S and a 30S subunit that come together during protein synthesis to form a 70S ribosome **(see Fig. 8)**.
c. There are no chloroplasts. Photosynthesis usually takes place in infoldings or extensions derived from the cytoplasmic membrane.
d. There is no mitosis and no mitotic spindle.
e. The various structural filaments in the cytoplasm collectively make up the prokaryotic cytoskeleton. Cytoskeletal filaments play essential roles in determining the shape of a bacterium (coccus, bacillus, or spiral) and are also critical in the process of cell division by binary fission and in determining bacterial polarity.

Fig. 4: Transmission electron micrograph of a prokaryotic bacterium.



Hot Spot Explore

Fig. 8: Illustration of a prokaryotic ribosome during translation.



Hot Spot Explore

Learn more: The density of ribosomal subunits: 50S and 30S.

Learn more: prokaryotic cells with internal membrane-bound compartments?

Sorting Activity

B. Comparison of Eukaryotic and Prokaryotic Cells

5. Respiratory Enzymes and Electron Transport Chains

Eukaryotic cell

The **electron transport system** is located in the inner membrane of the mitochondria. It contributes to the production of ATP molecules via **chemiosmosis**.

Transmission electron micrograph of a mitochondrion

Courtesy of jonlieffmd.com

Flash animation illustrating the development of proton motive force as a result of chemiosmosis and ATP production by ATP synthase.
Copyright © Gary E. Kaiser
html5 version of animation for iPad illustrating the development of proton motive force as a result of chemiosmosis and ATP production by ATP synthase
In an electron transport system, energy from electron transfer during oxidation-reduction reactions enables certain carriers to pump protons (H^+) across a membrane. As the (H^+) concentration increases on one side of the membrane, an electrochemical gradient called proton motive force develops. As the accumulating protons follow the electrochemical gradient back across the membrane through an ATP synthase complex, the movement of the protons provides energy for synthesizing ATP from ADP and phosphate.

Prokaryotic cell

The **electron transport system** is located in the cytoplasmic membrane. It contributes to the production of ATP molecules via **chemiosmosis**.

Flash animation illustrating ATP production by chemiosmosis during aerobic respiration in a prokaryotic bacterium.
Copyright © Gary E. Kaiser
html5 version of animation for iPad illustrating ATP production by chemiosmosis during aerobic respiration in a prokaryotic bacterium.
NADH and $FADH_2$ carry protons (H^+) and electrons (e^-) to the electron transport chain located in the membrane. The energy from the transfer of electrons along the chain transports protons across the membrane and creates an electrochemical gradient or proton motive force. At the end of the electron transport system, protons, electrons, and oxygen molecules combine to form water. As the accumulating protons follow the electrochemical gradient back across the membrane through an ATP synthase complex, the movement of the protons (proton motive force) provides energy for synthesizing ATP from ADP and phosphate.

For more information: Review of the electron transport system and chemiosmosis.

Self Check

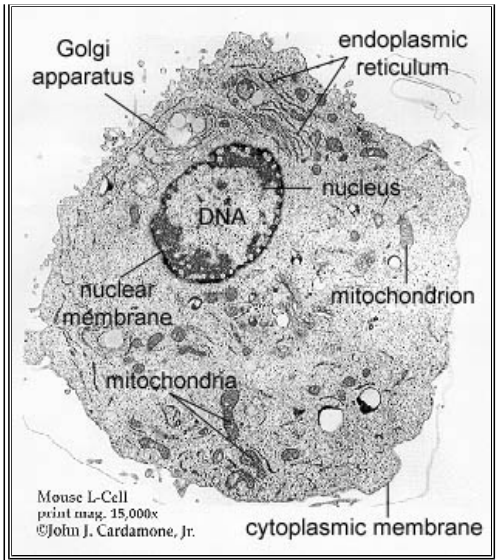
B. Comparison of Eukaryotic and Prokaryotic Cells

6. Cell Wall

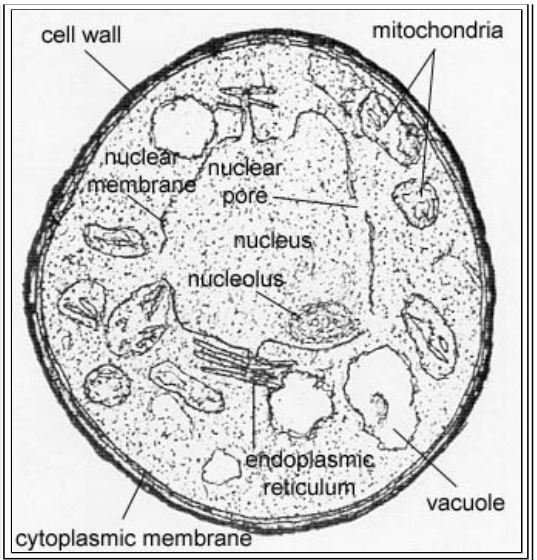
Eukaryotic cell

a. Plant cells, algae, and fungi have cell walls, usually composed of cellulose or chitin. Eukaryotic cell walls are never composed of peptidoglycan (**see Fig. 3**).
b. Animal cells and protozoans lack cell walls (**see Fig. 2**).

Fig. 2: Transmission electron micrograph of a eukaryotic animal cell.	**Fig. 3: Transmission electron micrograph of the yeast Candida albicans, a eukaryotic cell.**



Hot Spot Explore

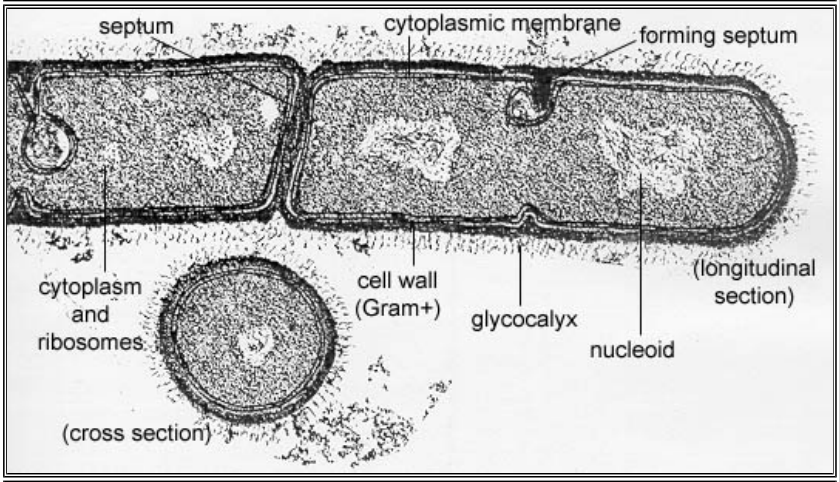


Hot Spot Explore

Prokaryotic cell

a. With few exceptions, members of the domain *Bacteria* have cell walls composed of peptidoglycan (**see Fig. 4**).
b. Members of the domain *Archae* have cell walls composed of protein, a complex carbohydrate, or unique molecules resembling but not the same as peptidoglycan.

Fig. 4: Transmission electron micrograph of a prokaryotic bacterium.



Hot Spot Explore

Self Check

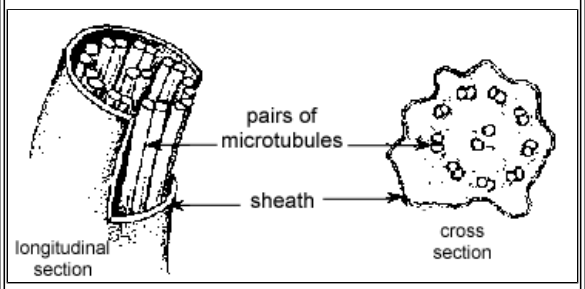
B. Comparison of Eukaryotic and Prokaryotic Cells

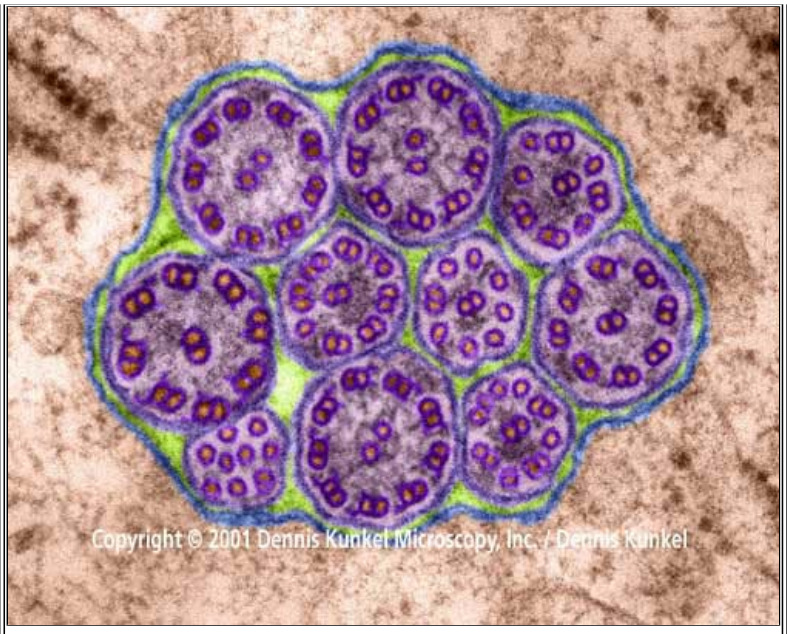
7. Locomotor Organelles

Eukaryotic cell

Eukaryotic cells may have flagella or cilia. Flagella and cilia are organelles involved in locomotion and in eukaryotic cells consist of a distinct arrangement of sliding microtubules surrounded by a membrane. The microtubule arrangement is referred to as a 2X9+2 arrangement **(see Fig. 9A and 9B)**.

Fig. 9A: Illustration of a eukaryotic flagellum.	**Fig. 9B: Transmission electron micrograph of epithelial cilia showing microtubules.** Courtesy of Dennis Kunkel's Microscopy





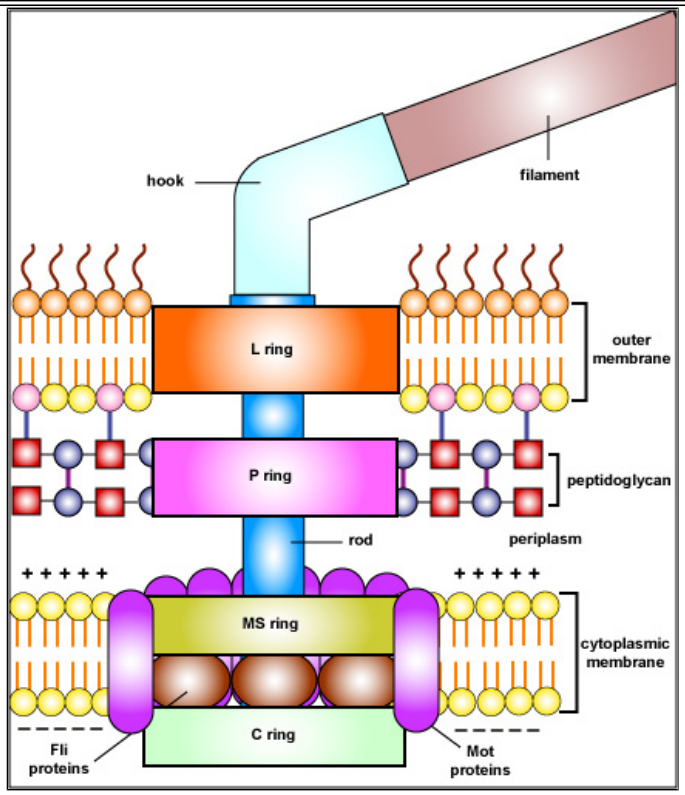
Hot Spot Explore

YouTube movie of motile sperm.

Prokaryotic cell

Many prokaryotes have flagella, each composed of a single, rotating fibril and usually not surrounded by a membrane **(see Fig. 10)**. There are no cilia.

Fig. 10: Illustration of a prokaryotic flagellum.



A movie of motile Rhodobacter spheroides with fluorescent labelled-flagella

Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.

B. Comparison of Eukaryotic and Prokaryotic Cells

8. Representative Organisms

Eukaryotic cell

The domain *Eukarya*: animals, plants, algae, protozoans, and fungi (yeasts, molds, mushrooms).

Prokaryotic cell

The domain *Bacteria* and the domain *Archae*.

Since viruses are **acellular**- they contain no cellular organelles, cannot grow and divide, and carry out no independent metabolism - they are considered neither prokaryotic nor eukaryotic. **Because viruses are not cells and have no cellular organelles, they can only replicate and assemble inside a living host cell.** They turn the host cell into a factory for manufacturing viral parts and viral enzymes and assembling the viral components.

Viruses, which possess both **living and nonliving characteristics**, will be discussed in Unit 4. **Recently, viruses have been declared as living entities** based on the large number of protein folds encoded by viral genomes that are shared with the genomes of cells. This indicates that viruses likely arose from multiple ancient cells.

Sorting Activity

Concept map for Prokaryotic and Eukaryotic Cells

TPS Questions (will be done as an in-class activity in my traditional BIOL 230 classes)

Self Quiz for Prokaryotic and Eukaryotic Cells

Sorting Activity

Quiz Group

Back to Unit 1 Table of Contents

Back to Softchalk Lesson Table of Contents

Assignment for Unit-1, Lecture 2 (Traditional Class Sections)

ASSIGNMENT FOR UNIT-1, LECTURE -2 (Traditional Class Sections)

Flipped-class assignment

Read II. The Prokaryotic Cell: Bacteria: B. Prokaryotic Cell Structure, 1. The Cytoplasmic Membrane in your E-text and answer the 3 learning objectives for this section.

We will be doing a classroom group activity on this section so it is critical that you come prepared. I will be assuming that you have done this preparatory assignment.

A overview of the three domain system of classification
THE THREE DOMAIN SYSTEM

Classification: The Three Domain System

Fundamental Statements for this Lesson:

1. ***Phylogeny refers to the evolutionary relationships between organisms.***
2. ***Organisms can be classified into one of three domains based on differences in the sequences of nucleotides in the cell's ribosomal RNAs (rRNA), the cell's membrane lipid structure, and its sensitivity to antibiotics.***
3. ***The three domains are the Archaea, the Bacteria, and the Eukarya.***
4. ***Prokaryotic organisms belong either to the domain Archaea or the domain Bacteria; organisms with eukaryotic cells belong to the domain Eukarya.***
5. ***Microorganism transfer genes to other microorganisms through horizontal gene transfer - the transfer of DNA to an organism that is not its offspring.***

Common Course Objective

Distinguish between prokaryotic and eukaryotic cells and state which microbes fit into each group.

Explain how phylogenic and phenotypic classification schemes are different.

Detailed Learning Objectives for this Lesson

1. Define phylogeny.

2. Name the 3 Domains of the 3 Domain system of classification and recognize a description of each.

3. Name the four kingdoms of the Domain Eukarya and recognize a description of each.

4. Define horizontal gene transfer.

The Earth is 4.6 billion years old and microbial life is thought to have first appeared between 3.8 and 3.9 billion years ago; in fact, 80% of Earth's history was exclusively microbial life. Microbial life is still the dominant life form on Earth. It has been estimated that the total number of microbial cells on Earth on the order of 2.5×10^{30} cells, making it the major fraction of biomass on the planet.

Phylogeny refers to the evolutionary relationships between organisms. The Three Domain System, proposed by Woese and others, is an evolutionary model of phylogeny based on **differences in the sequences of nucleotides in the cell's ribosomal RNAs (rRNA)**, as well as **the cell's membrane lipid structure** and **its sensitivity to antibiotics**.

Comparing rRNA structure is especially useful. Because rRNA molecules throughout nature carry out the same function, their structure changes very little over time. Therefore similarities and dissimilarities in rRNA nucleotide sequences are a good indication of how related or unrelated different cells and organisms are.

There are various hypotheses as to the origin of prokaryotic and eukaryotic cells. Because all cells are similar in nature, it is generally thought that all cells came from a common ancestor cell termed the last universal common ancestor (LUCA). These LUCAs eventually evolved into three different cell types, each representing a domain. The three domains are the *Archaea*, the *Bacteria*, and the *Eukarya*.

More recently various fusion hypotheses have begun to dominate the literature. One proposes that the diploid or 2N nature of the eukaryotic genome occurred after the fusion of two haploid or 1N prokaryotic cells. Others propose that the domains *Archaea* and *Eukarya* emerged from a common archaeal-eukaryotic ancestor that itself emerged from a member of the domain *Bacteria*. Some of the evidence behind this hypothesis is based on a "superphylum" of bacteria called **PVC**, members of which share some characteristics with both archaea and eukaryotes. There is growing evidence that eukaryotes may have originated within a subset of archaea.

In any event, it is accepted today that there are three distinct domains of organisms in nature: *Bacteria, Archaea*, and *Eukarya*. A description of the three domains follows:

1. The *Archaea* (archaebacteria)

The *Archaea* possess the following characteristics:

a. *Archaea* are **prokaryotic cells**.

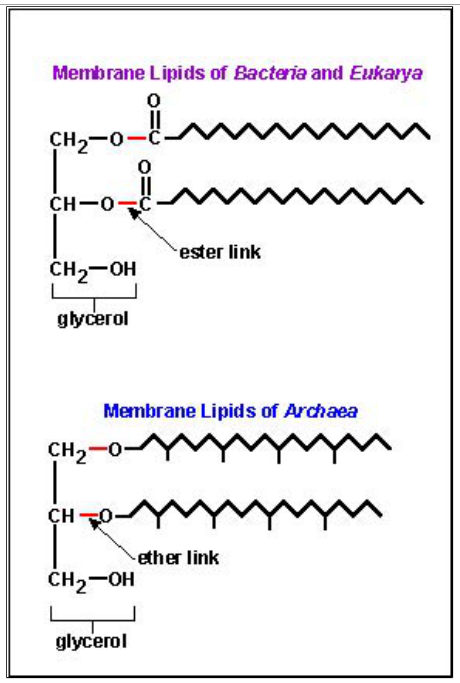
b. Unlike the *Bacteria* and the *Eukarya*, the *Archaea* have membranes composed of **branched hydrocarbon chains** (many also containing rings within the hydrocarbon chains) **attached to glycerol by ether linkages (see Fig. 1).**

c. The cell walls of *Archaea* **contain no peptidoglycan**.

d. *Archaea* are not sensitive to some antibiotics that affect the *Bacteria*, but are sensitive to some antibiotics that affect the *Eukarya*.

e. *Archaea* contain **rRNA that is unique to the *Archaea*** as indicated by the presence molecular regions distinctly different from the rRNA of *Bacteria* and *Eukarya*.

Fig. 1: Membrane phospholipids in the domains *Archae, Bacteria*, and *Eukarya*



Archaea often live in extreme environments and include methanogens, extreme halophiles, and hyperthermophiles. One reason for this is that the ether-containing linkages in the *Archaea* membranes is more stabile than the ester-containing linkages in the *Bacteria* and *Eukarya* and are better able to withstand higher temperatures and stronger acid concentrations.

2. The *Bacteria* (eubacteria)

The *Bacteria* possess the following characteristics:

a. *Bacteria* are **prokaryotic cells**.

b. Like the *Eukarya*, they have membranes composed of **unbranched fatty acid chains attached to glycerol by ester linkages (see Fig. 1)**.

c. The cell walls of *Bacteria*, unlike the *Archaea* and the Eukarya, **contain peptidoglycan**.

d. *Bacteria* are sensitive to traditional antibacterial antibiotics but are resistant to most antibiotics that affect *Eukarya*.

e. *Bacteria* contain **rRNA that is unique to the *Bacteria*** as indicated by the presence molecular regions distinctly different from the rRNA of *Archaea* and *Eukarya*.

Bacteria include mycoplasmas, cyanobacteria, Gram-positive bacteria, and Gram-negative bacteria.

3. The *Eukarya* (eukaryotes)

The *Eukarya* (also spelled *Eucarya*) possess the following characteristics:

a. *Eukarya* have **eukaryotic cells**.

b. Like the *Bacteria*, they have membranes composed of **unbranched fatty acid chains attached to glycerol by ester linkages** (see **Fig. 1**).

c. Not all *Eukarya* possess cells with a cell wall, but for those *Eukarya* having a cell wall, that wall **contains no peptidoglycan**.

d. *Eukarya* are resistant to traditional antibacterial antibiotics but are sensitive to most antibiotics that affect eukaryotic cells.

e. *Eukarya* contain **rRNA that is unique to the *Eukarya*** as indicated by the presence molecular regions distinctly different from the rRNA of *Archaea* and *Bacteria*.

The *Eukarya* are subdivided into the following kingdoms:

a. Protista Kingdom

Protista are simple, predominately unicellular eukaryotic organisms. Examples includes slime molds, euglenoids, algae, and protozoans.

b. Fungi Kingdom

Fungi are unicellular or multicellular organisms with eukaryotic cell types. The cells have cell walls but are not organized into tissues. They do not carry out photosynthesis and obtain nutrients through absorption. Examples include sac fungi, club fungi, yeasts, and molds.

c. Plantae Kingdom

Plants are multicellular organisms composed of eukaryotic cells. The cells are organized into tissues and have cell walls. They obtain nutrients by photosynthesis and absorption. Examples include mosses, ferns, conifers, and flowering plants.

d. Animalia Kingdom

Animals are multicellular organisms composed of eukaryotic cells. The cells are organized into tissues and lack cell walls. They do not carry out photosynthesis and obtain nutrients primarily by ingestion. Examples include sponges, worms, insects, and vertebrates.

It used to be thought that the changes that allow microorganisms to adapt to new environments or alter their virulence capabilities was a relatively slow process occurring within an organism primarily through mutations, chromosomal rearrangements, gene deletions and gene duplications. Those changes would then be passed on to that microbes progeny and natural selection would occur. This gene transfer from a parent organism to its offspring is called vertical gene transmission.

It is now known that microbial genes are transferred not only vertically from a parent organism to its progeny, but also horizontally to relatives that are only distantly related, eg, other species and other genera. This latter process is known as horizontal gene transfer. Through mechanisms such as transformation, transduction, and conjugation, genetic elements such as plasmids, transposons, integrons, and even chromosomal DNA can readily be spread from one microorganism to another. (These mechanisms will be discussed in detail under Bacterial Genetics.) As a result, the old three-branched "tree of life" in regard to microorganisms now appears to be more of a **"net of life"** in the journal *Science*.

Microbes are known to live in remarkably diverse environments, many of which are extremely harsh. This amazing and rapid adaptability is a result of their ability to quickly modify their repertoire of protein functions by modifying, gaining, or losing their genes. This gene expansion predominantly takes place by horizontal gene transfer.

Self Quiz for Classification: The Three Domain System

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Sizes, shapes, arrangements. and forms of bacteria
SIZES, SHAPES, ARRANGEMENTS OF BACTERIA

Size, Shapes, Arrangements, and Forms of Bacteria

Fundamental Statements for this Lesson:

1. There are three basic shapes of bacteria: coccus, bacillus, and spiral.
2. Based on planes of division, the coccus shape can appear in several distinct arrangements: diplococcus, streptococcus, tetrad, sarcina, and staphylococcus.
3. The bacillus shape can appear as a single bacillus, a streptobacillus, or a coccobacillus.
4. The spiral shape can appear in several forms: vibrio, spirillum, and spirochete.
5. The metric unit micrometer (1/1,000,000 or 10^{-6} of a meter) is used to measure bacterial size.

Common Course Objective

Describe how the different shape, arrangements, and forms of bacteria can be used in identification.

Detailed Learning Objectives for this Lesson

1*. List the three basic shapes of bacteria.

2*. List and describe 5 different arrangements of cocci.

3. Define and give the abbreviation for the metric unit of length termed micrometer and state the average size of a coccus-shaped bacterium and a rod-shaped bacterium.

4. List and describe 2 different arrangements of bacilli.

5*. List and describe 3 different spiral forms of bacteria.

(*) = Common theme throughout the course

Introduction

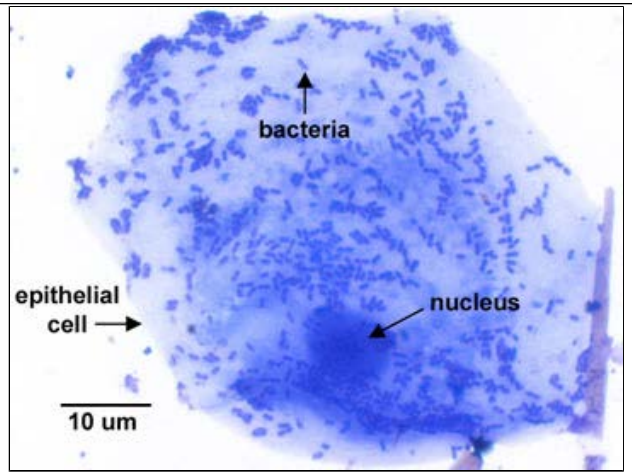
Bacteria are:

a. prokaryotic.

b. single-celled, microscopic organisms (Exceptions have been discovered that can reach sizes just visible to the naked eye. They include *Epulopiscium fishelsoni*, a bacillus-shaped bacterium that is typically 80 micrometers (µm) in diameter and 200-600 µm long, and *Thiomargarita namibiensis*, a spherical bacterium between 100 and 750 µm in diameter.)

c. generally much **smaller** than eukaryotic cells.

Bacteria on a Human Epithelial Cell from the Mouth



The bacteria are the small dark purple dots and dashes on the light blue cell. The oval purple mass in the center is the nucleus of the epithelial cell.

d. very complex despite their small size. Even though bacteria are single-celled organisms, they are able to communicate with one another through a process called quorum sensing. In this way they can function as a multicellular population rather than as individual bacteria. This will be discussed in greater detail in Unit 2.

For more information: Bacterial communication through quorum sensing

To gain an appreciation of the small size of microbes, view this excellent interactive illustration of cell size and scale.

Courtesy of Learn.Genetics, University of Utah Health Sciences

Bacterial Shapes

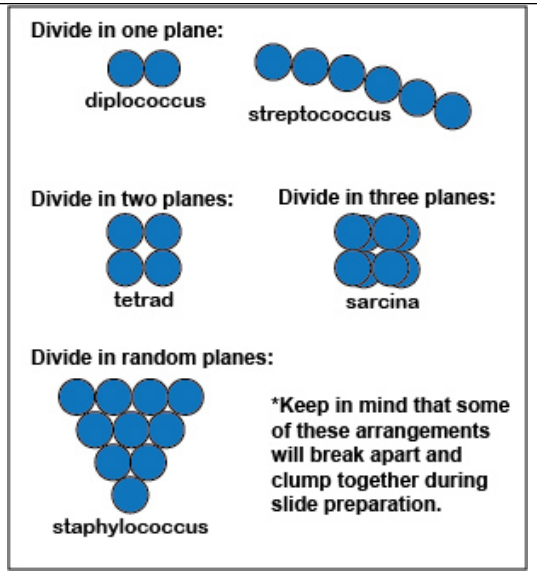
Bacterial cell shape is determined primarily by a protein called MreB. MreB forms a spiral band – a simple cytoskeleton – around the interior of the cell just under the cytoplasmic membrane. It is thought to define shape by recruiting additional proteins that then direct the specific pattern of bacterial cell growth. For example, bacillus-shaped bacteria that have an inactivated MreB gene become coccoid shaped, and coccus-shaped bacteria naturally lack the MreB gene.

Most bacteria come in one of **three basic shapes**: coccus, rod or bacillus, and spiral.

Coccus

The cocci are spherical or oval bacteria having one of **several distinct arrangements** (**see Fig. 1**) based on their planes of division.

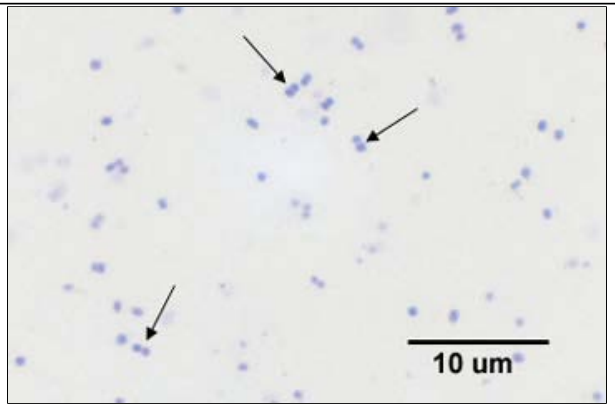
Fig. 1: Arrangements of cocci.



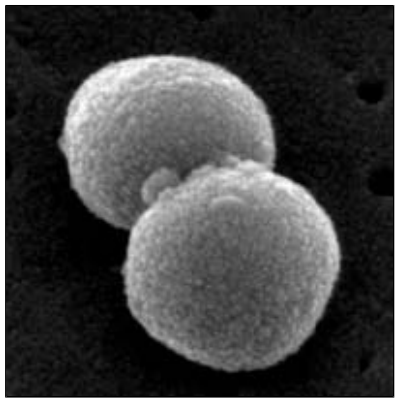
a. Division in **one plane** produces either a diplococcus or streptococcus arrangement.

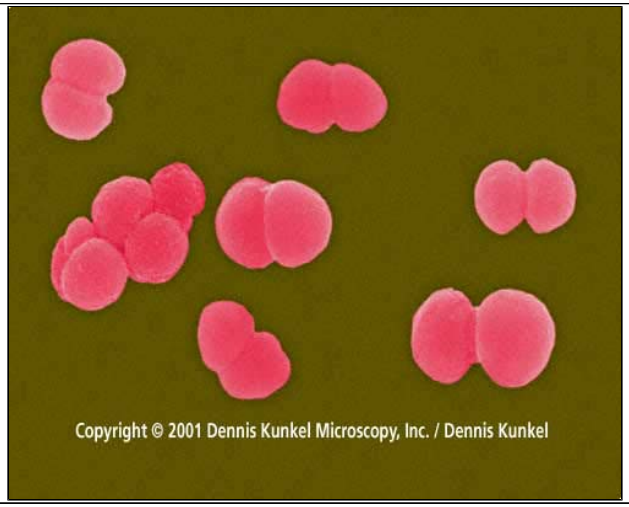
1. **diplococcus arrangement**: cocci arranged in pairs (**see Fig. 2**)

Fig. 2: Photomicrograph showing diplococcus arrangements of cocci.



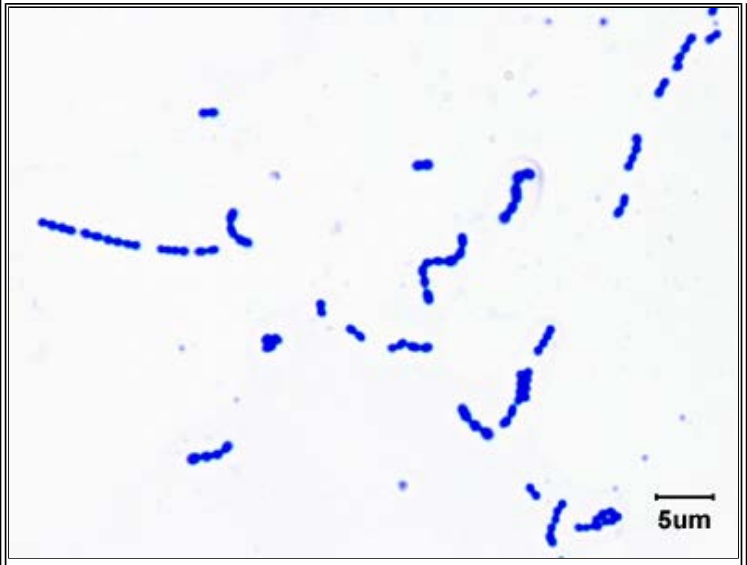
Scanning electron micrograph of *Streptococcus pneumoniae*, a diplococcus. **Courtesy of the Centers for Disease Control and Prevention.**	**Scanning electron micrograph of a *Neisseria*,** a diplococcus. Courtesy of Dennis Kunkel's Microscopy





2. **streptococcus arrangement**: cocci arranged in chains (**See Fig. 3**)

Fig. 3: Photomicrograph showing streptococcus arrangements of cocci.



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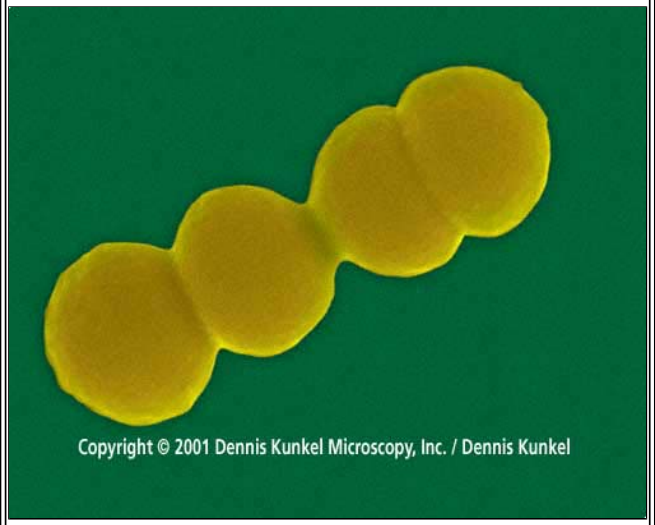
Scanning electron micrograph of a *Streptococcus pyogenes*, a streptococcus.

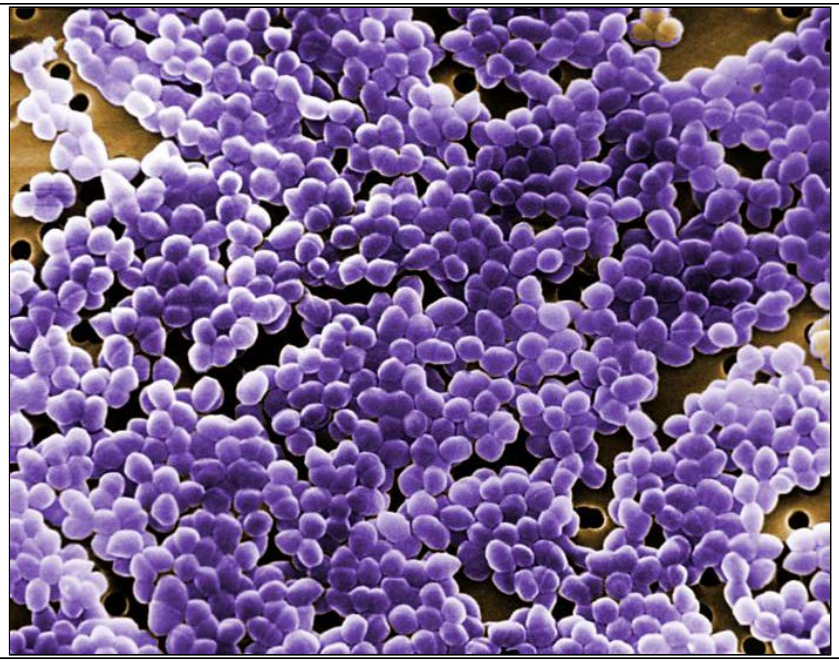
Courtesy of Dennis Kunkel's Microscopy



Transmission electron micrograph of an *Enterococcus* species, a streptococcus.

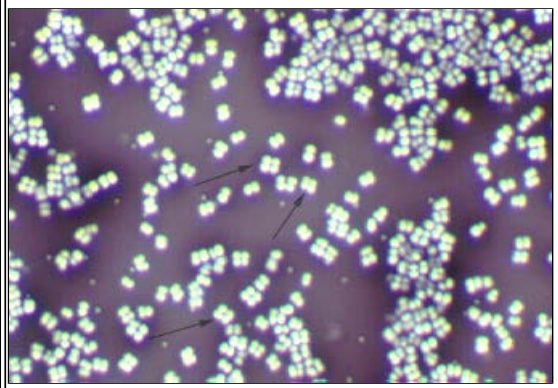
Image provided by Janice Haney Carr

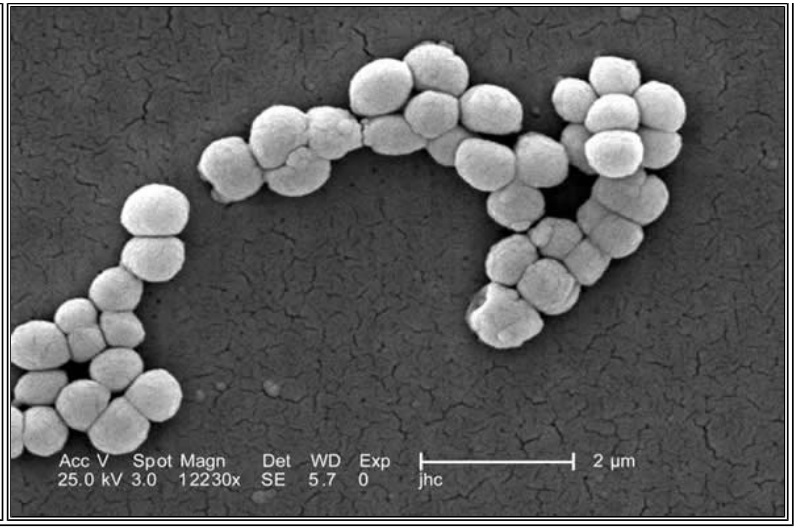
Courtesy of the Centers for Disease Control and Prevention.



b. Division in **two planes** produces a tetrad arrangement.

tetrad arrangement: cocci arranged in squares of 4 (**See Fig. 4**)

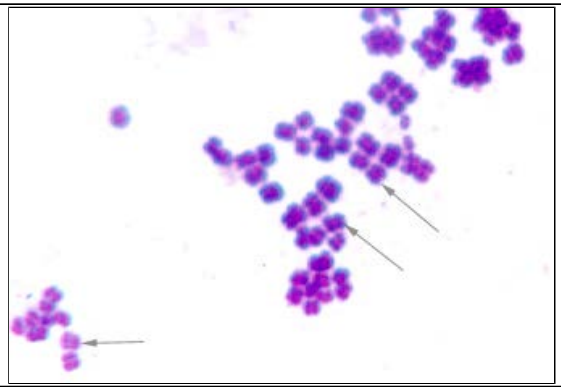
Fig. 4: Photomicrograph showing tetrad arrangements of cocci.	**Scanning electron micrograph of *Micrococcus luteus*** showing several tetrads **Image provided by Janice Haney Carr** **Courtesy of the Centers for Disease Control and Prevention.**
 Copyright © Gary E. Kaiser	



c. Division in **three planes** produces a **sarcina** arrangement.

sarcina arrangement: cocci in arranged cubes of 8 (**See Fig. 5**)

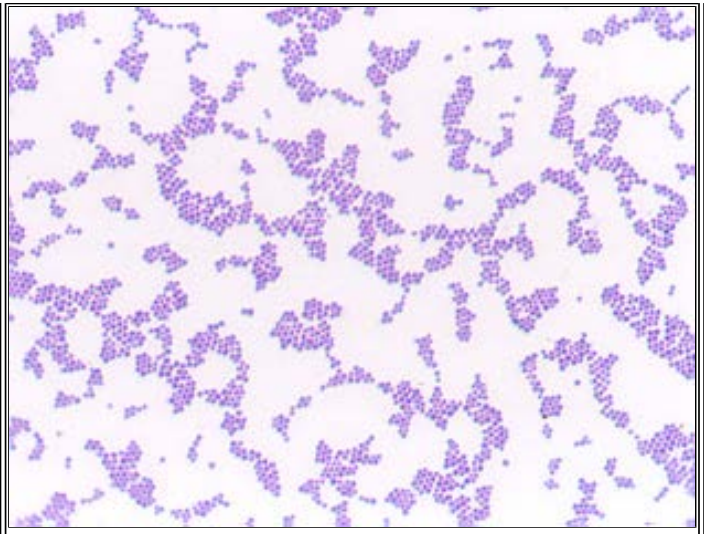
Fig. 5: Photomicrograph showing sarcina arrangements of cocci.

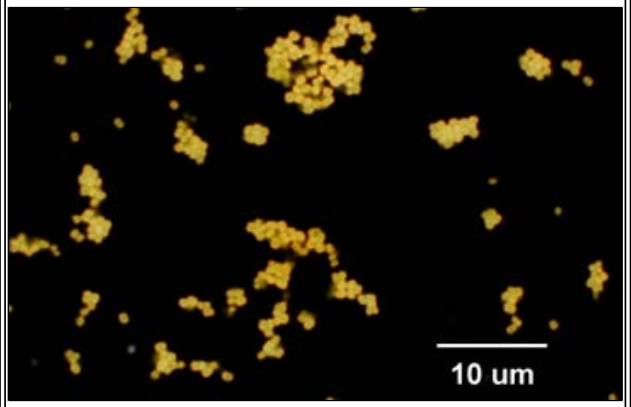


d. Division in **random planes** produces a staphylococcus arrangement.

staphylococcus arrangement: cocci arranged in irregular, often grape-like clusters. (**See Fig. 6**)

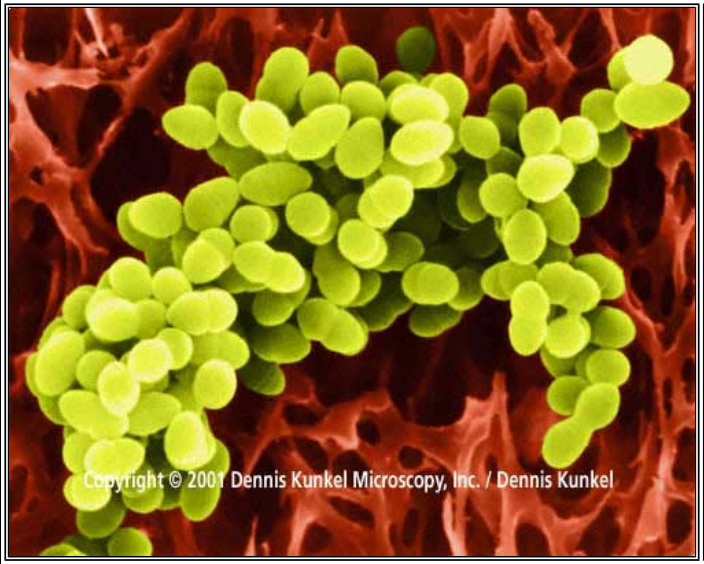
Fig. 6A: Photomicrograph showing staphylococcus arrangements of cocci.	**Fig. 6B: Photomicrograph showing a negative image of staphylococcus arrangements of cocci.**





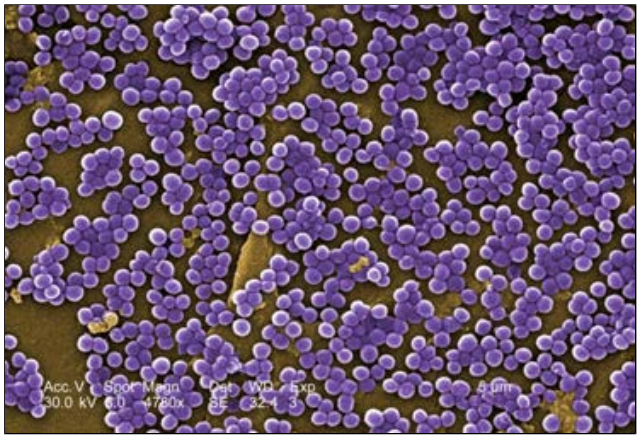
Scanning electron micrograph of Staphylococcus aureus, a staphylococcus.

Courtesy of Dennis Kunkel's Microscopy.



Scanning electron micrograph of methicillin-resistant Staphylococcus aureus (MRSA).

Image provided by Joyce Ayers.
Courtesy of the Centers for Disease Control and Prevention.



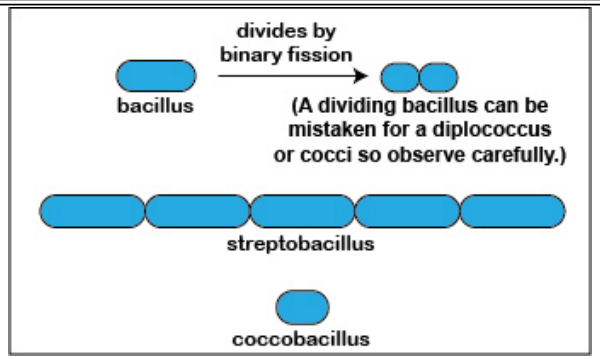
An average coccus is about **0.5-1.0 micrometer (µm) in diameter**.

DragNDrop Activity

Rod or Bacillus

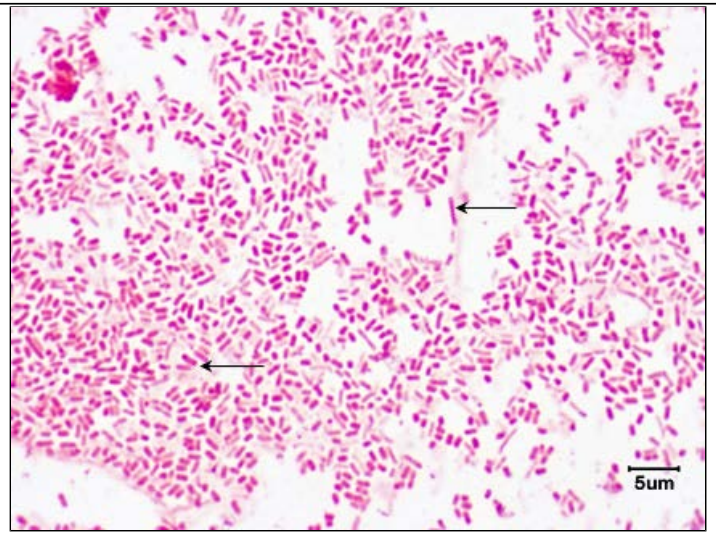
Bacilli are cylindrical, rod-shaped bacteria. Bacilli all divide in one plane producing a bacillus, streptobacillus, or coccobacillus **arrangement (see Fig. 7)**

Fig. 7: Arrangements of bacilli.

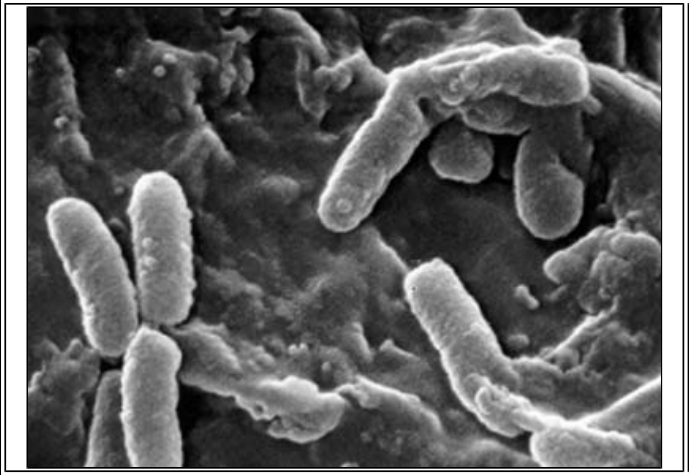
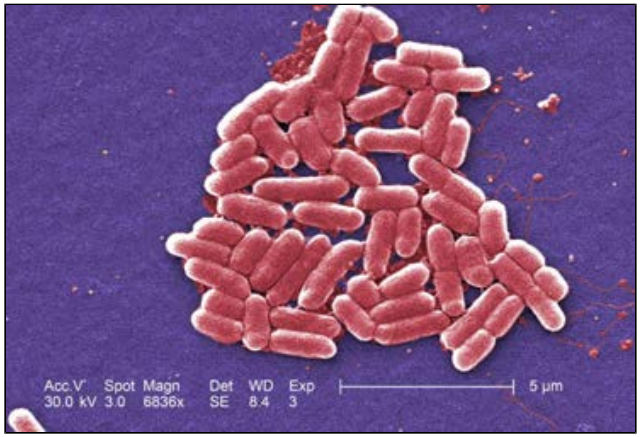


a. **bacillus**: single bacilli (**See Fig. 8**)

Fig. 8: Photomicrograph of single bacilli.

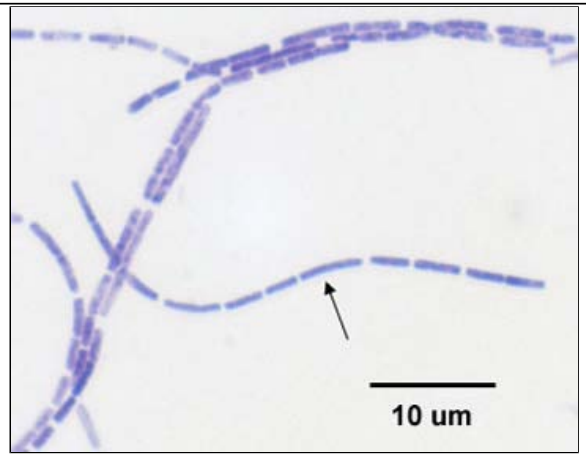


Scanning electron micrograph of *Pseudomonas aeruginosa*, a bacillus. **Image provided by Janice Carr.** **Courtesy of the Centers for Disease Control and**	**Scanning electron micrograph of *Escherichia coli* O157H7, a bacillus.** **Image provided by Janice Carr.** **Courtesy of the Centers for Disease Control and**

Prevention.	Prevention.
	

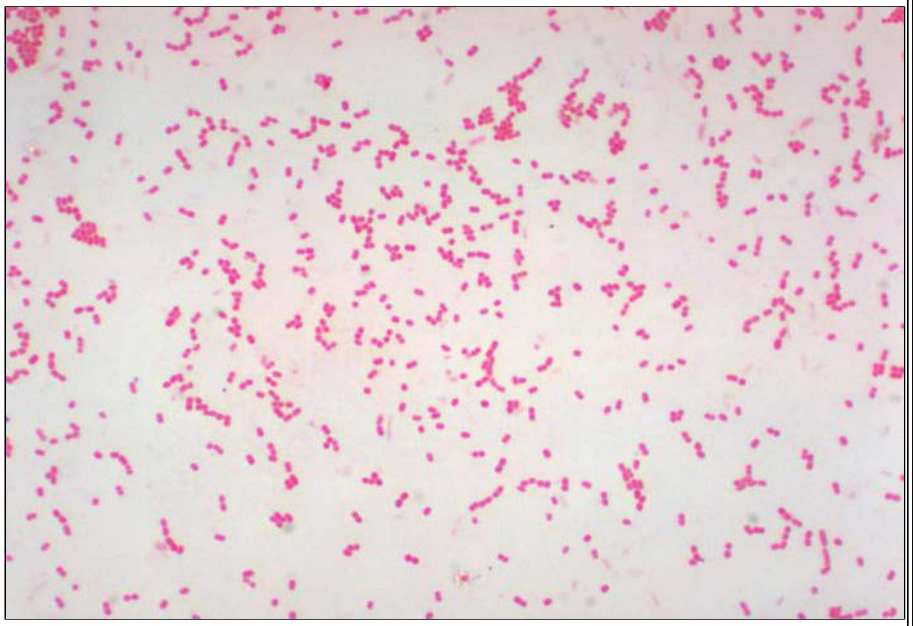
b. **streptobacillus**: bacilli arranged in chains (**See Fig. 9**)

Fig. 9: Photomicrograph of a streptobacillus.

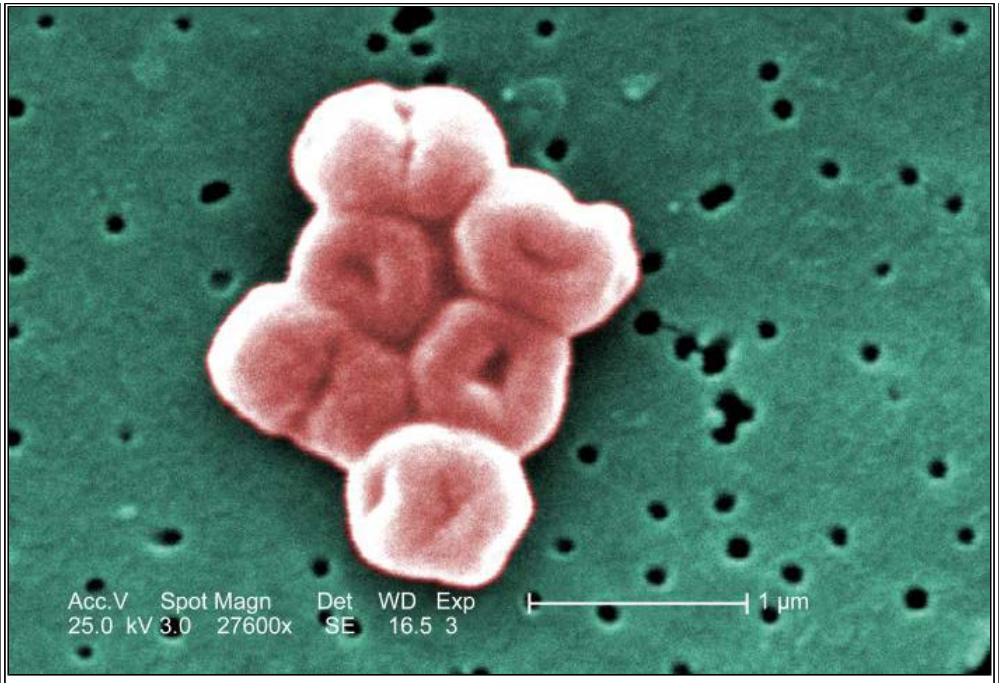


c. a **coccobacillus**: oval and similar to a coccus

Photomicrograph of the coccobacillus *Acinetobacter*.	Scanning electron micrograph of the coccobacillus *Acinetobacter*.



Courtesy of Centers for Disease Control and Prevention.



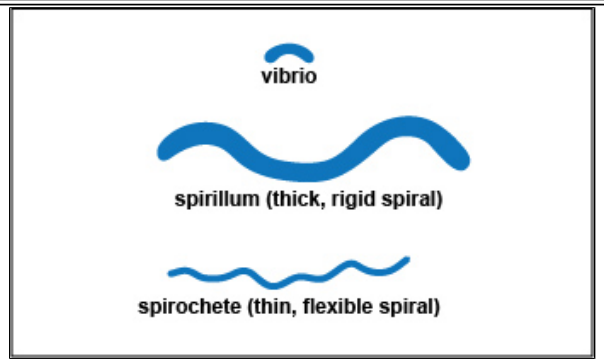
Courtesy of Janice Haney Carr, Centers for Disease Control and Prevention.

An average bacillus is **0.5-1.0 µm wide by 1.0-4.0 µm long**.

Spiral

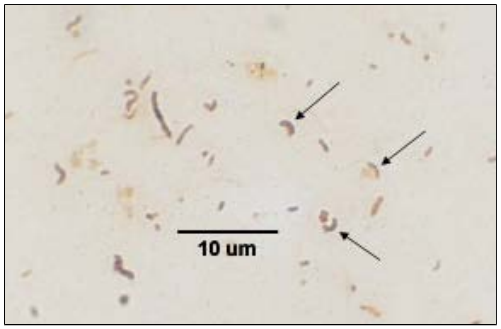
Spirals come in one of three **forms**, a vibrio, a spirillum, or a spirochete (**see Fig. 10**)

Fig. 10: Forms of spirals.



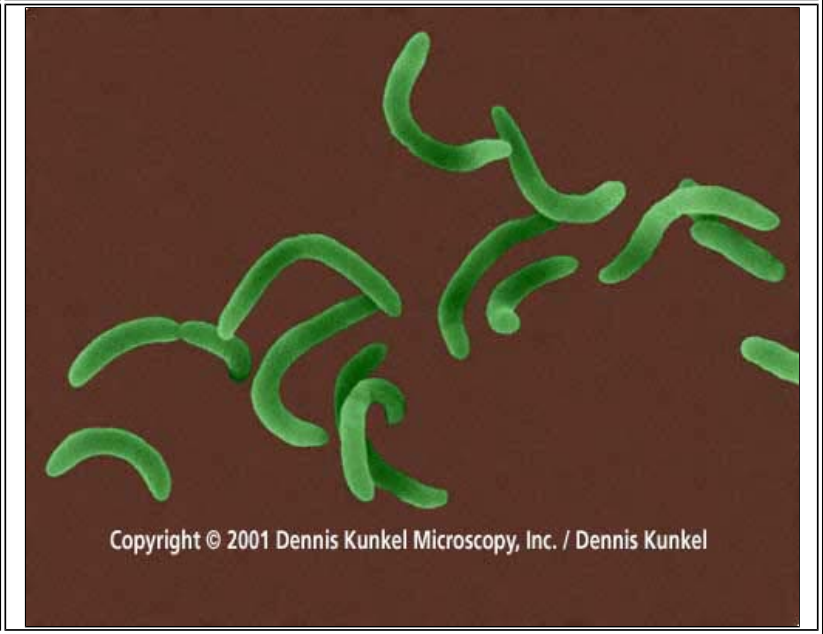
a. **vibrio**: a curved or comma-shaped rod (**see Fig. 11**)

Fig. 11: Photomicrograph of a vibrio.



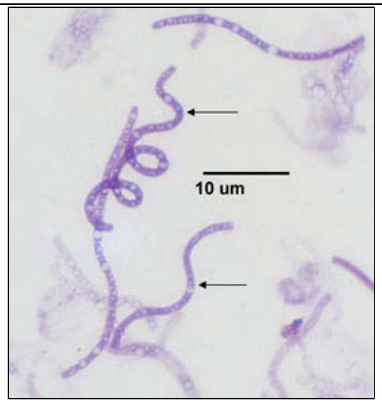
Scanning electron micrograph of a Vibrio cholerae, a vibrio.

Courtesy of Dennis Kunkel's Microscopy



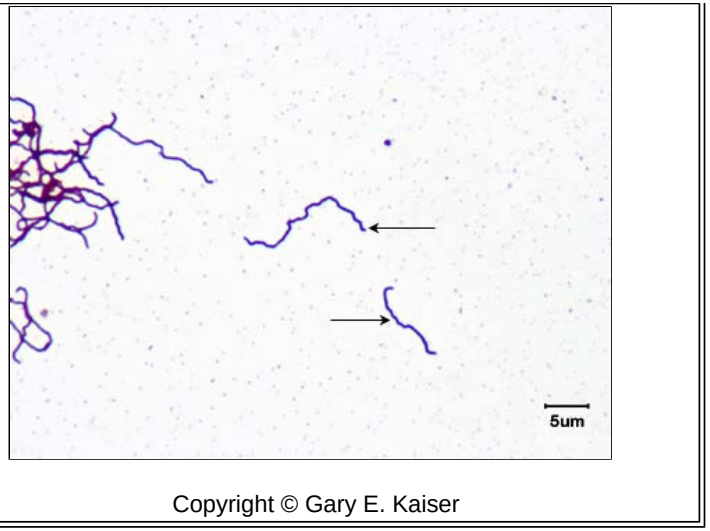
b. **spirillum**: a thick, rigid spiral (**see Fig. 12**)

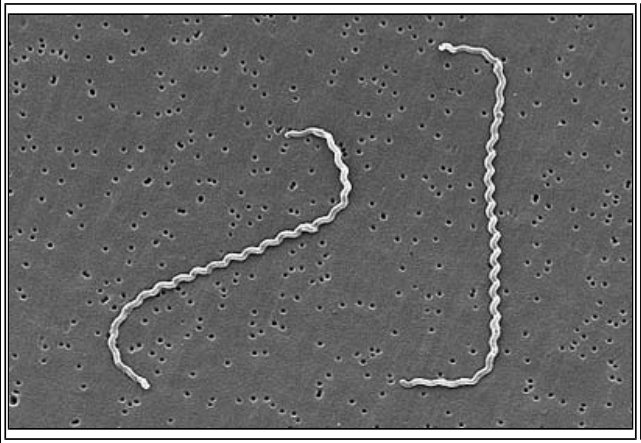
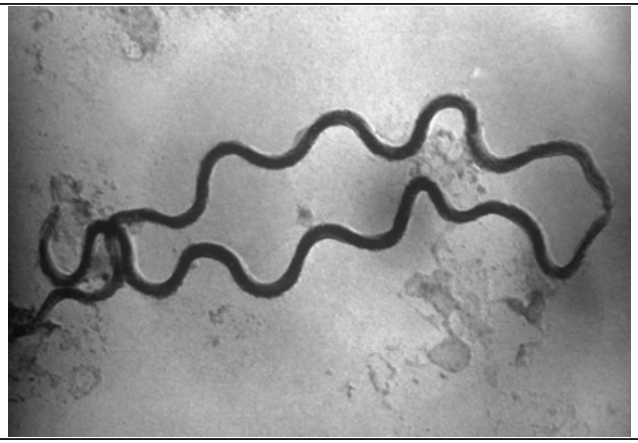
Fig. 12: Photomicrograph of a spirillum.



c. **spirochete**: a thin, flexible spiral (**see Fig. 13**)

Fig. 13: Photomicrograph of a spirochete.



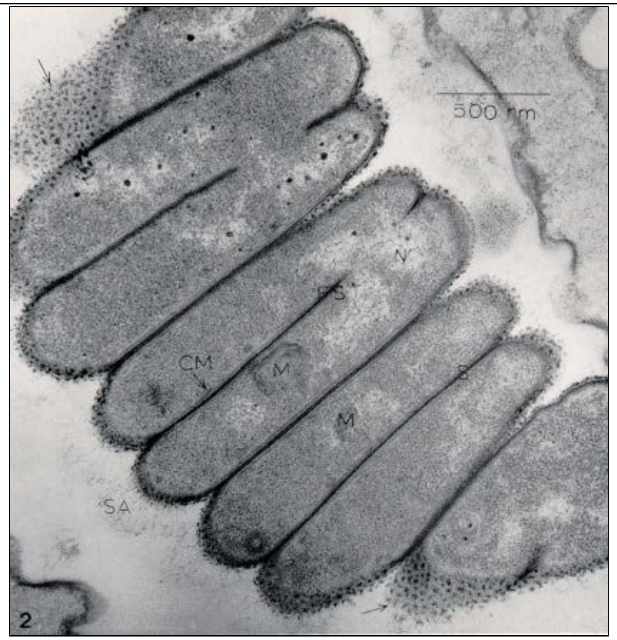
Scanning electron micrograph of the spirochete *Leptospira*. **Image provided by Rob Weyant.** **Courtesy of the Centers for Disease Control and Prevention.**	**Scanning electron micrograph of the spirochete *Treponema pallidum*.** **Image provided by Joyce Ayers.** **Courtesy of the Centers for Disease Control and Prevention.**
	

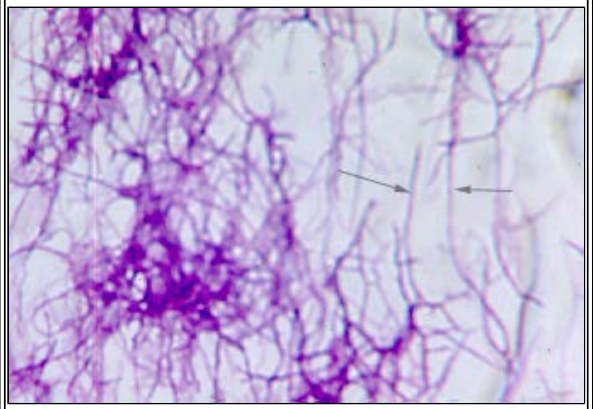
Spirals range in size from **1 µm to over 100 µm in length**.

Exceptions to the above shapes

There are exceptions to the three basic shapes of coccus, bacillus, and spiral. They include sheathed, stalked, filamentous, square, star-shaped, spindle-shaped, lobed, trichome-forming, and pleomorphic bacteria.

Transmission electron micrograph of *Alysiella filiformis*, a filamentous trichome-forming bacterium	**Light microscope photograph of an *Actinomyces*, a filamentous bacterium.**





Ultrasmall Bacteria: 150 could fit in a single *Escherichia coli*

Ultrasmall bacteria have been discovered in groundwater that was passed through a filter with a pore size of 0.2 micrometers µm). They showed an **average length of only 323 nanometers (nm) and an average width of 242 nm**. They contain DNA, an average of 42 ribosomes per bacterium, and possessed pili. It is thought that they use these pili to attach to other bacteria from which they scavenge nutrients. Because the surface to volume ratio is even greater than in more traditional sized bacteria, they might be better designed to take up scarce nutrients from more nutrient-poor environments.

Electron micrographs of ultrasmall bacteria from Science Alert

Concept map for Shapes and Arrangements of Bacteria

DragNDrop Activity

ASSIGNMENT FOR UNIT-1, LECTURE -2

Flipped-class assignment
Read II. The Prokaryotic Cell: Bacteria: B. Prokaryotic Cell Structure, 1. The Cytoplasmic Membrane in your E-text and answer the 3 learning objectives for this section.

We will be doing a classroom group activity on this section so it is critical that you come prepared. I will be assuming that you have done this preparatory assignment.

Self Quiz for Size, Shapes, Arrangements, and Forms of Bacteria

Quiz Group

Quiz Group

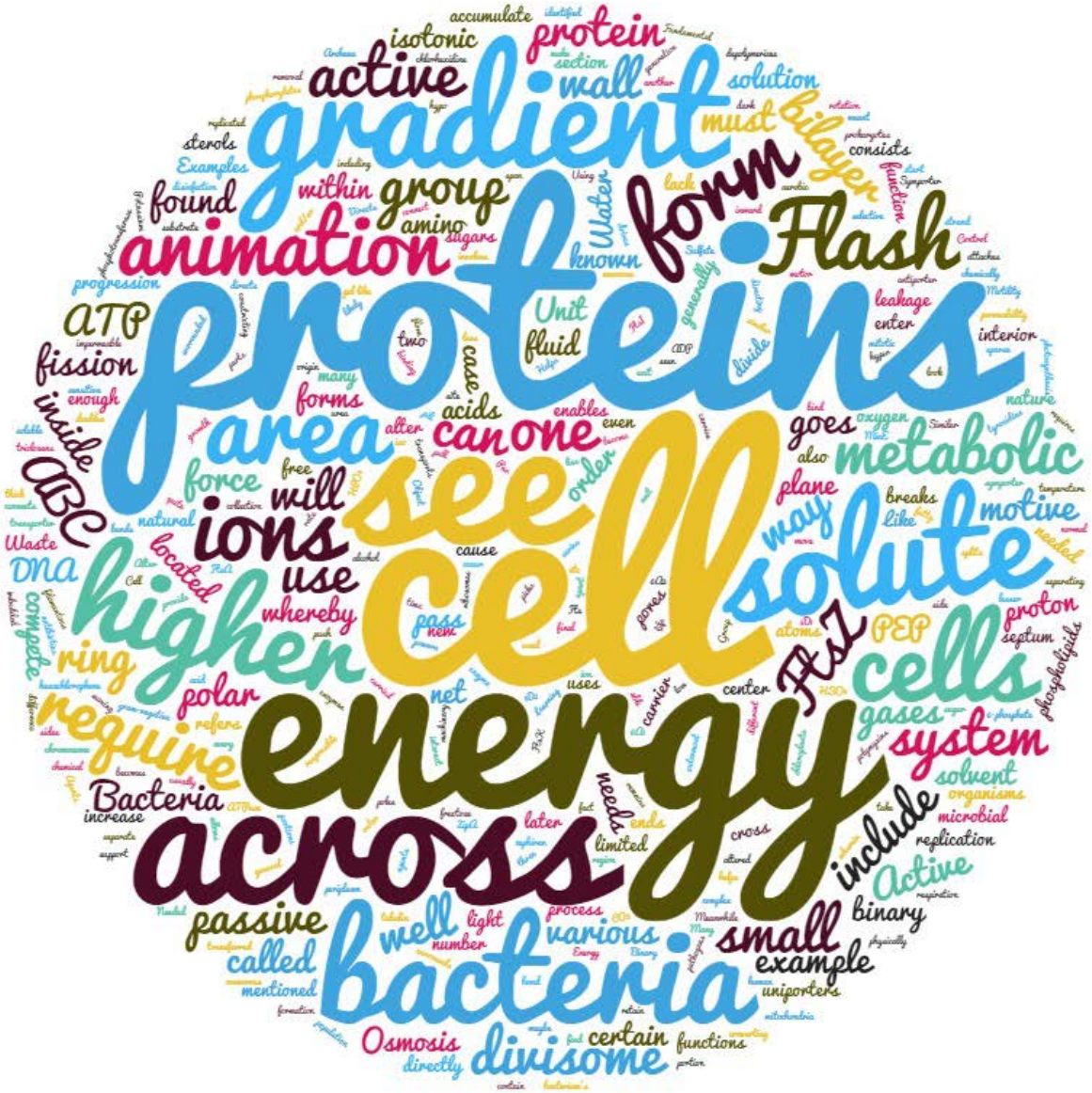
Identify Activity

Back to Unit 1 Table of Contents

Back to Softchalk Lessons Table of Contents

The cytoplasmic membrane and cellular transport in bacteria
THE BACTERIAL CYTOPLASMIC MEMBRANE

The Cytoplasmic Membrane



Fundamental Statements for this Lesson:
1. The bacterial cytoplasmic membrane is a fluid phospholipid bilayer that encloses the bacterial cytoplasm.
2. The cytoplasmic membrane is semipermeable and determines what molecules enter and leave the bacterial cell.
4. Passive diffusion is the net movement of gases or small uncharged polar molecules such as water across a membrane from an area of higher concentration to an area of lower concentration.
5. Passive diffusion is powered by the potential energy of a concentration gradient and does not require the expenditure of metabolic energy or the use of transport proteins.
6. Facilitated diffusion is powered by the potential energy of a concentration gradient and does not require the expenditure of metabolic energy, but it does require the use of transport proteins.
7. A solution refers to solute dissolved in a solvent.
8. Osmosis is the movement of water across a membrane from an area of higher water (lower solute) concentration to an area of lower water (higher solute) concentration by both passive diffusion and facilitated diffusion.
9. Active transport is a process whereby the cell uses both transport proteins and metabolic energy to transport substances across the membrane against the concentration gradient.
10. Most molecules and ions that a cell needs to concentrate within the cytoplasm in order to support life require active transport for entry into the cell.
11. In order to colonize any environment, a bacterium must be able to effectively use its transport systems to compete with other bacteria, as well as the cells of other organisms – such as human cells - for limited nutrients.
12. Bacteria divide by binary fission and increase their numbers by geometric progression.

13. Some antimicrobial agents alter the microbial cytoplasmic membranes and cause leakage of cellular needs.

Common Course Objectives

1. Identify the parts of a bacterium and their physiological purpose.
2. Explain how the cell membrane is selectively permeable.
3. Compare and contrast passive diffusion, facilitated diffusion, and active transport and give examples for each.
4. Predict what would happen if a cell was put into solutions of different tonicities.
5. Explain how certain parts of the bacteria can be targeted in order to kill the bacteria.

Detailed Learning Objectives for this Lesson

1*. State the chemical composition and major function of the cytoplasmic membrane in bacteria.

2. Briefly describe the fluid phospholipid bilayer arrangement of biological membranes.

3. State the net flow of water when a cell is placed in an isotonic, hypertonic, or hypotonic environment and relate this to the solute concentration.

4. Define the following means of transport:

a. passive diffusion
b. osmosis
c. facilitated diffusion
d. transport through channel proteins
e. transport through uniporter
f. active transport
g. transport through antiporter
h. transport through symporter
i. the ABC transport system
j. group translocation

5*. State how the antibiotic polymyxin and disinfectants such as orthophenylphenol, chlorhexidine, hexachlorophene, zephiran, and alcohol affect bacteria.

6*. Define binary fission and geometric progression and relate this to bacteria being able to astronomically increase their numbers in a relatively short period of time.

7. Briefly describe the process of binary fission in bacteria, stating the functions of Par proteins, the divisome, and FtsZ proteins.

(*) = Common theme throughout the course

In this and several following sections we are going to look at the various anatomical parts that make up a bacterium. We will not deal with the *Archaea* in this course but rather will concentrate on the *Bacteria*. A typical bacterium usually consists of:

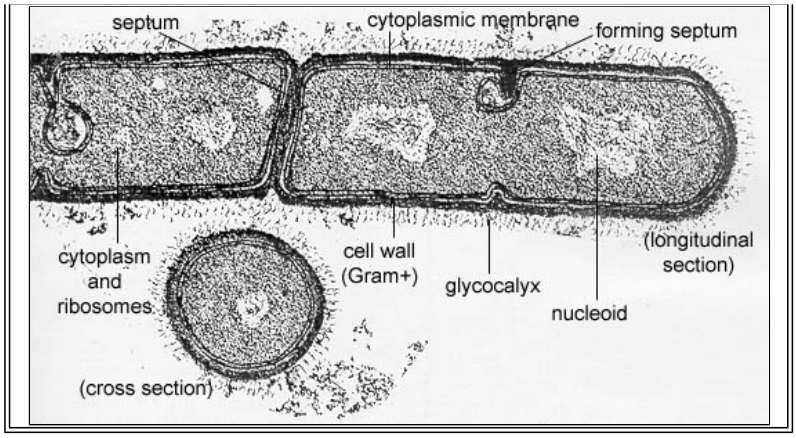
- a cytoplasmic membrane surrounded by a peptidoglycan cell wall and maybe an outer membrane;
- a fluid cytoplasm containing a nuclear region (nucleoid) and numerous ribosomes; and
- often various external structures such as a glycocalyx, flagella, and pili.

Because a cytoplasmic membrane surrounds all cells in nature, we will start with this structure.

The Cytoplasmic Membrane

The cytoplasmic membrane, also called a cell membrane or plasma membrane, is about 7 nanometers (nm; 1/1,000,000,000 m) thick. It lies internal to the cell wall and encloses the cytoplasm of the bacterium (**see Fig. 1**).

Fig. 1: Transmission electron micrograph of a *Bacillus* species.

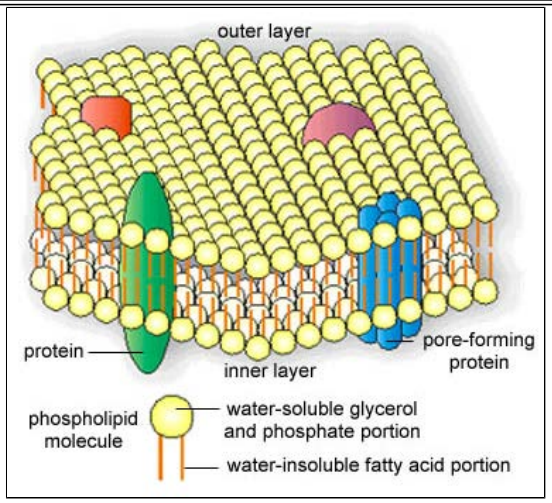


A. Structure and Composition

Like all biological membranes in nature, the bacterial cytoplasmic membrane is composed of **phospholipid and protein molecules**. In electron micrographs, it appears as 2 dark bands separated by a light band and is actually a **fluid phospholipid bilayer** imbedded with proteins (**see Fig. 2**). With the exception of the mycoplasmas, the only bacteria that lack a cell wall, prokaryotic membranes lack sterols. Many bacteria, however, do contain sterol-like molecules called hopanoids. Like the sterols found in eukaryotic cell membranes, the hopanoids most likely **stabilize the bacterial cytoplasmic membrane and regulate membrane fluidity**.

The phospholipid bilayer is arranged so that the **polar ends** of the molecules (the phosphate and glycerol portion of the phospholipid that is soluble in water) form the **outermost and innermost surface** of the membrane while the **non-polar ends** (the fatty acid portions of the phospholipids that are insoluble in water) form the **center** of the membrane (**see Fig. 2**).

Fig. 2. Illustration of a cytoplasmic membrane.



Self Check

Learn More: Fluid Mosaic Model of the Cell Membrane

Concept Map for the Bacterial Cytoplasmic Membrane

B. Functions

The cytoplasmic membrane is a **selectively permeable membrane that determines what goes in and out of the organism**. All cells must take in and retain all the various chemicals needed for metabolism. Water, dissolved gases such as carbon dioxide and oxygen, and lipid-soluble molecules simply diffuse across the phospholipid bilayer. Water-soluble ions generally pass through small pores - less than 0.8 nm in diameter - in the membrane . All other molecules require carrier molecules to transport them through the membrane.

Materials move across the bacterial cytoplasmic membrane by passive diffusion, facilitated diffusion, and active transport.

1. Passive Diffusion

Passive diffusion is the net movement of gases or small uncharged polar molecules across a phospholipid bilayer membrane from an area of **higher concentration to an area of lower concentration** (**see Fig. 3A and 3B in the Slideshow Activity below**) . Examples of gases that cross membranes by passive diffusion include N_2, O_2, and CO_2; examples of small polar molecules include ethanol, H_2O, and urea.

Figs. 3A and 3B: Passive diffusion
Slideshow Activity
Copyright © Gary E. Kaiser

All molecules and atoms possess kinetic energy (energy of motion). If the molecules or atoms are not evenly distributed on both sides of a membrane, the difference in their concentration forms a **concentration gradient** that represents a form of potential energy (stored energy). The net movement of these particles will therefore be down their concentration gradient - from the area of higher concentration to the area of lower concentration. **Diffusion is powered by the potential energy of a concentration gradient and does not require the expenditure of metabolic energy**.

Flash animation illustrating passive diffusion of oxygen.
Copyright © Gary E. Kaiser
html5 version of animation for iPad illustrating passive diffusion of oxygen.
Passive diffusion is the net movement of gases or small uncharged polar molecules across a phospholipid bilayer membrane from an area of higher concentration to an area of lower concentration . Examples of gases that cross membranes by passive diffusion include N_2, O_2, and CO_2; examples of small polar molecules include ethanol, H_2O, and urea.

a. Osmosis is the diffusion of **water** across a membrane from an area of **higher water concentration** (lower solute concentration) **to lower water concentration** (higher solute concentration). **Osmosis is powered by the potential energy of a concentration gradient and does not require the expenditure of metabolic energy**. While water molecules are small enough to pass between the phospholipids in the cytoplasmic membrane, their transport can be enhanced by water transporting transport proteins known as aquaporins. The aquaporins form channels that span the cytoplasmic membrane and transport water in and out of the cytoplasm (see channel proteins below).

To understand osmosis, one must understand what is meant by a **solution**. A solution consists of a solute dissolved in a solvent. In terms of osmosis, **solute** refers to all the molecules or ions dissolved in the water (the solvent). When a solute such as sugar dissolves in water, it forms weak hydrogen bonds with water molecules. While free, unbound water molecules are small enough to pass through membrane pores, water molecules bound to solute are not (**see Fig. 4A and 4B in the Slideshow Activity below**).Therefore, **the higher the solute concentration, the lower the concentration of free water molecules capable of passing through the membrane**.

Figs 4A and 4B: Sugar and salt forming hydrogen bonds with water
Slideshow Activity
Copyright © Gary E. Kaiser

A cell can find itself in one of three environments: isotonic, hypertonic, or hypotonic. (The prefixes iso-, hyper-, and hypo- refer to the **solute concentration**).

In an **isotonic** environment (**see Fig. 5A and 5B in the Slideshow Activity below**), both the water and solute concentration are the same inside and outside the cell and water goes into and out of the cell at an equal rate.

Figs. 5A and 5B: A cell in an isotonic environment
Slideshow Activity
Copyright © Gary E. Kaiser

Flash animation illustrating osmosis in an isotonic environment.
Copyright © Gary E. Kaiser
html5 version of animation for iPad illustrating osmosis in an isotonic environment.
Osmosis is the diffusion of water across a membrane from an area of higher water concentration (lower solute concentration) to lower water concentration (higher solute concentration). In an isotonic environment, both the water and solute concentration are the same inside and outside the cell and water flows into and out of the cell at an equivalent rate.

If the **environment is hypertonic** (**see Fig. 6A and 6B in the Slideshow Activity below**) the water concentration is greater inside the cell while the solute concentration is higher outside (the interior of the cell is hypotonic to the surrounding hypertonic environment). Water goes out of the cell.

Figs. 6A and 6B: A cell in a hypertonic environment
Slideshow Activity Copyright © Gary E. Kaiser

Flash animation illustrating osmosis in a hypertonic environment.
Copyright © Gary E. Kaiser
html5 version of animation for iPad illustrating osmosis in a hypertonic environment.
Osmosis is the diffusion of water across a membrane from an area of higher water concentration (lower solute concentration) to lower water concentration (higher solute concentration). If the environment is hypertonic, the water concentration is greater inside the cell while the solute concentration is higher outside; the interior of the cell is hypotonic to the surrounding hypertonic environment. The net flow of water is out of the cell.

In an **environment that is hypotonic** (**see Fig. 7A and 7B in the Slideshow Activity below**) the water concentration is greater outside the cell and the solute concentration is higher inside (the interior of the cell is hypertonic to the hypotonic surroundings). Water goes into the cell.

Figs. 7A and 7B: A cell in a hypotonic environment
Slideshow Activity Copyright © Gary E. Kaiser

Flash animation showing osmosis in a hypotonic environment.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing osmosis in a hypotonic environment.
Osmosis is the diffusion of water across a membrane from an area of higher water concentration (lower solute concentration) to lower water concentration (higher solute concentration). In an environment that is hypotonic, the water concentration is greater outside the cell and the solute concentration is higher inside; the interior of the cell is hypertonic to the hypotonic surroundings. The net flow of water is into the cell.

Sorting Activity

Concept Map for the Bacterial Cytoplasmic Membrane

2. Facilitated Diffusion

Facilitated diffusion is the transport of substances across a membrane by transport proteins, such as **uniporters and channel proteins**, along a concentration gradient from an area of **higher concentration to lower concentration**. Facilitated diffusion is powered by the potential energy of a concentration gradient and does not require the expenditure of metabolic energy.

a. **Uniporter**: Uniporters are transport proteins that **transport a substance from one side of the membrane to the other** (**see Fig. 8A and 8B in the Slideshow Activity below**). Potassium ions (K^+) can enter bacteria through uniporters.

Figs. 8A and 8B: A uniporter
Slideshow Activity Copyright © Gary E. Kaiser

Flash animation showing transport by way of a uniporter.

Copyright © Gary E. Kaiser
html5 version of animation showing transport by way of a uniporter.
Uniporters are transport proteins that transport a substance across a membrane down a concentration gradient from an area of greater concentration to lesser concentration. The transport is powered by the potential energy of a concentration gradient and does not require metabolic energy. Interaction between the substrate and the uniporter causes a conformational change in the uniporter enabling the substrate to cross the membrane. Amino acids, nucleosides, sugars, and other small molecules are often transported across membranes by uniporters.

b. Channel Proteins: Channel proteins transport water or certain ions down either a concentration gradient, in the case of water, or an electric potential gradient in the case of certain ions, from an area of higher concentration to lower concentration. While water molecules can directly cross the membrane by passive diffusion, as mentioned above, channel proteins called aquaporins can enhance their transport.

Flash animation showing transport of water across a membrane by channel proteins.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing transport of water across a membrane by channel proteins.
Channel proteins transport water and certain ions down either a concentration gradient, in the case of water, or an electric potential gradient in the case of certain ions, from an area of higher concentration to lower concentration. While water molecules can directly cross the membrane by passive diffusion, as mentioned above, their transport can be enhanced by channel proteins called aquaporins.

Learn More: Facilitated Diffusion by way of a Uniporter

Learn More: Facilitated Diffusion by way of Channel Proteins

Quiz Group

Concept Map for the Bacterial Cytoplasmic Membrane

3. Active Transport

Active transport is a process whereby the cell uses **both transport proteins and metabolic energy** to transport substances across the membrane **against the concentration gradient**. In this way, active transport allows cells to accumulate needed substances even when the concentration is lower outside.

Active transport enables bacteria to successfully compete with other organisms for limited nutrients in their natural habitat, and as will be seen in Unit 2, enables pathogens to compete with the body's own cells and normal flora bacteria for the same nutrients.

The energy is provided by **proton motive force**, **the hydrolysis of ATP**, or the breakdown of some other high-energy compound such as phosphoenolpyruvate (PEP).

Proton motive force is an energy gradient resulting from hydrogen ions (protons) moving across the membrane from greater to lesser hydrogen ion concentration. ATP is the form of energy cells most commonly use to do cellular work. PEP is one of the intermediate high-energy phosphate compounds produced at the end of glycolysis.

For more information: Review of proton motive force
For more information: a review of ATP
For more information: a review of glycolysis

Specific **transport proteins** (carrier proteins) are required in order to transport the majority of molecules a cell requires across its cytoplasmic membrane. This is because the concentration of nutrients in most natural environments is typically quite low. Transport proteins allow cells to accumulate nutrients from even a sparse environment.

Transport proteins involved in active transport include antiporters, symporters, the proteins of the ATP-binding cassette (ABC) system, and the proteins involved in group translocation.

a. **Antiporter** : Antiporters are transport proteins that **transport one substance across the membrane in one direction while simultaneously transporting a second substance across the membrane in the opposite direction**. Antiporters in bacteria generally use the potential energy of electrochemical gradients from protons (H^+), that is, proton motive force to co-transport ions, glucose, and amino acids against their concentration gradient (**see Fig. 9A and 9B in the Slideshow Activity below**)

. Sodium ions (Na^+) and protons (H^+), for example, are co-transported across bacterial membranes by antiporters.

Figs. 9A and 9B: Antiporters
Slideshow Activity

Flash animation showing transport by way of an antiporter.
html5 version of animation for iPad showing transport by way of an antiporter.
Antiporters are transport proteins that simultaneously transport two substances across the membrane in opposite directions; one against the concentration gradient and one with the concentration gradient. Energy for transport of the substance moving against it's concentration gradient is provided by the potential energy stored in the electrochemical gradient of sodium ions (Na^+) or protons (H^+). The sodium-proton antiporter is an example of an antiporter found in bacteria.

b. **Symporter** : Symporters are transport proteins that **simultaneously transport two substances across the membrane in the same direction.** Symporters use the potential energy of electrochemical gradients from protons (H^+), that is, proton motive force to co-transport ions, glucose, and amino acids against their concentration gradient (**see Fig. 10A and 10B in the Slideshow Activity below**). Sulfate (HSO_4^-) and protons (H^+) as well as phosphate (HPO_4^-) and protons (H^+) are co-transported across bacterial membranes by symporters.

Figs. 10A and 10B: Symporters
Slideshow Activity

Flash animation showing transport by way of a symporter.
html5 version of animation for iPad showing transport by way of a symporter.
Symporters are transport proteins that simultaneously transport two substances across the membrane in the same direction; one against the concentration gradient and one with the concentration gradient. Energy for transport of the substance moving against it's concentration gradient is provided by the potential energy stored in the electrochemical gradient of sodium ions (Na^+) or protons (H^+). Sugars, phosphate ions, and sulfate ions are examples of molecules or ions that can be transported across bacterial membranes by symporters.

c. **ATP-binding cassette (ABC) system:** An example of an ATP-dependent active transport found in various gram-negative bacteria is the **ATP-binding cassette (ABC) system**. This involves substrate-specific binding proteins located in the bacterial periplasm, the gel-like substance between the bacterial cell wall and cytoplasmic membrane. The periplasmic-binding protein picks up the substance to be transported and carries it to a membrane-spanning transport protein. Meanwhile, an ATP-hydrolyzing protein breaks ATP down into ADP, phosphate, and energy. It is this energy that powers the transport of the substrate, by way of the membrane-binding transporter, across the membrane and into the cytoplasm (**see Fig. 11A through11D in the Slideshow Activity below**). Examples of active transport include the transport of certain sugars and amino acids. Over 200 different ABC transport systems have been found in bacteria.

Figs. 11A through 11D: The ATP-binding cassette (ABC) system
Slideshow Activity

Flash animation showing an "ABC" transport system.
html5 version of animation for iPad showing an "ABC" transport system.
In the ATP-binding cassette (ABC) system of transport, a high affinity binding protein located in the periplasm between the cytoplasmic membrane and the cell wall picks up the substance to be transported and carries it to the membrane-spanning transporter. The actual transport across the membrane is powered by the energy provided by the breakdown of ATP by an ATP-hydrolyzing protein.

Learn More: ABC Transporter Animation

d. **Group translocation** is another form of active transport that can occur in prokaryotes. In this case, a substance is chemically altered during its transport across a membrane so that once inside, the cytoplasmic membrane becomes impermeable to that substance and it remains within the cell.

An example of group translocation in bacteria is the phosphotransferase system. A high-energy phosphate group from phosphoenolpyruvate (PEP) is

transferred by a series of enzymes to glucose. The final enzyme both phosphorylates the glucose and transports it across the membrane as glucose 6-phosphate (**see Fig. 12A through12D in the Slideshow Activity below**). (This is actually the first step in glycolysis.) Other sugars that are transported by group translocation are mannose and fructose.

Figs. 12A through 12D: Group translocation
Slideshow Activity
Copyright © Gary E. Kaiser

Flash animation showing group translocation.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing group translocation.
An example of group translocation in bacteria is the phosphotransferase system. A high-energy phosphate group from phosphoenolpyruvate (PEP) is transferred by a series of enzymes to glucose. The final enzyme both phosphorylates the glucose and transports it across the membrane as glucose 6-phosphate.

Learn More: Group Translocation

Quiz Group

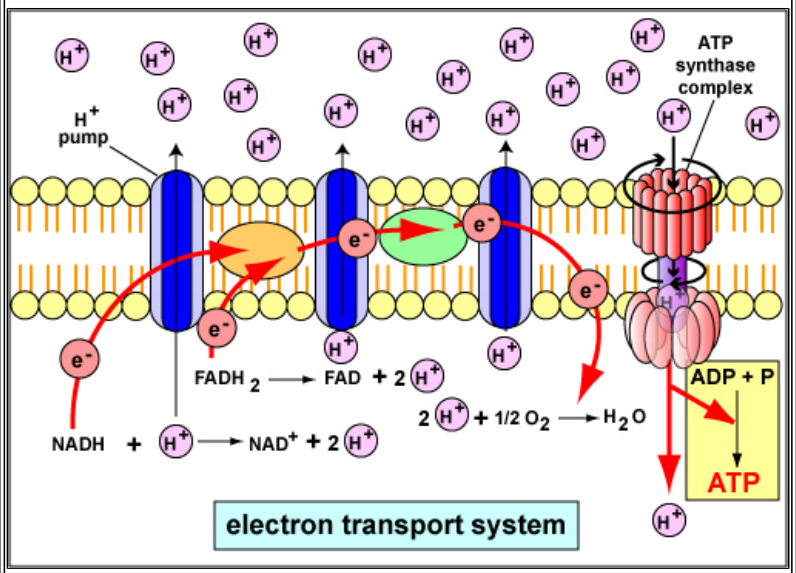
Concept Map for the Bacterial Cytoplasmic Membrane

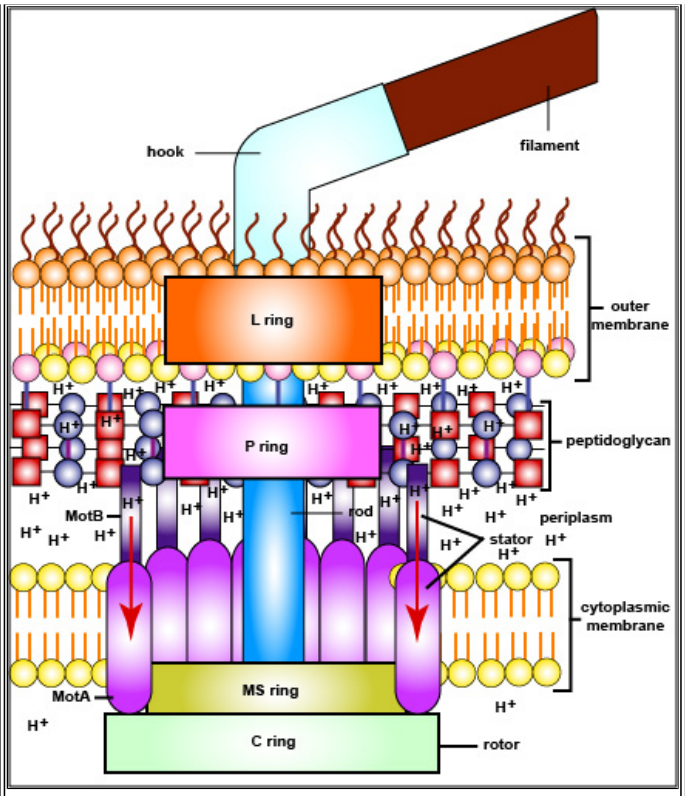
C. Functions of the cytoplasmic membrane other than selective permeability

A number of other functions are associated with the bacterial cytoplasmic membrane and associated proteins of a collection of cell division machinery known as the divisome. In fact, many of the functions associated with specialized internal membrane-bound organelles in eukaryotic cells are carried out generically in bacteria by the cytoplasmic membrane. Functions associated with the bacterial cytoplasmic membrane and/or the divisome include:

1. energy production. The electron transport system (**see Fig. 13**) for bacteria with aerobic and anaerobic respiration, as well as photosynthesis for bacteria converting light energy into chemical energy is located in the cytoplasmic membrane.

2. motility. The motor that drives rotation of bacterial flagella (**see Fig. 14**) is located in the cytoplasmic membrane.

Fig. 13: ATP Production during Aerobic Respiration by Oxidative Phosphorylation involving an Electron Transport System and Chemiosmosis	**Fig. 14: Illustration of the Structure of a Bacterial Flagellum**
 Copyright © Gary E. Kaiser	



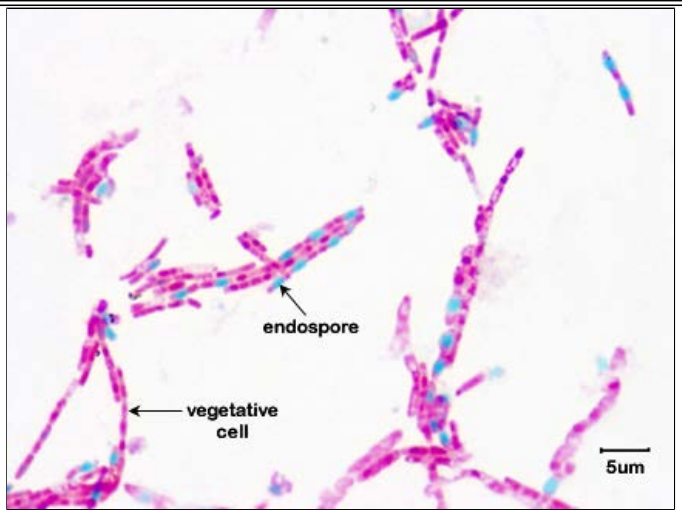
Movie of motile Rhodobacter spheroides with fluorescent labelled-flagella.

Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.

3. waste removal. Waste byproducts of metabolism within the bacterium must exit through the cytoplasmic membrane.

4. formation of endospores (discussed later in this unit).

Fig. 15: Photomicrograph of endospores in *Bacillus megaterium*.



Concept map for the cytoplasmic membrane, domain *Bacteria*.

D. Binary fission

Bacteria divide by binary fission wherein one bacterium splits into two. Therefore, bacteria increase their numbers by **geometric progression** whereby their population doubles every generation time.

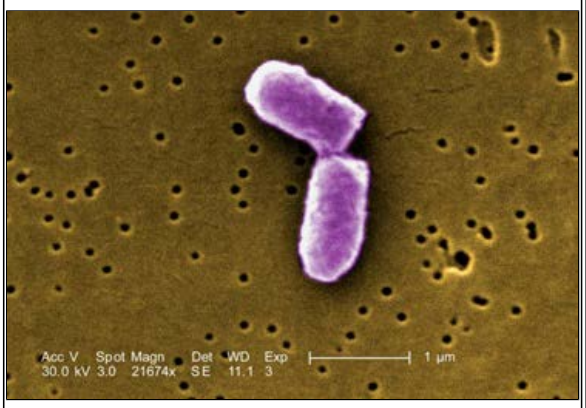
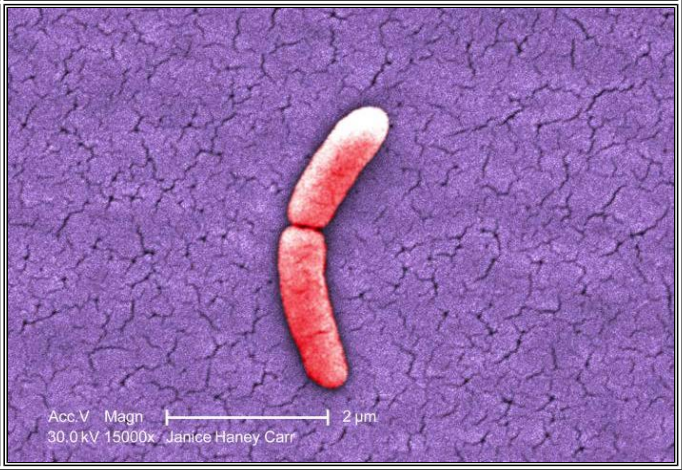
In general it is thought that during DNA replication (discussed in Unit 6), each strand of the replicating bacterial DNA attaches to proteins at what will become the cell division plane. For example, **Par proteins** function to separate bacterial chromosomes to opposite poles of the cell during cell division. They bind to the origin of replication of the DNA and physically pull or push the chromosomes apart, similar to the mitotic apparatus of eukaryotic cells.

In the center of the bacterium, a group of proteins called **Fts** proteins(filamentous temperature sensitive proteins) interact to form a ring at the cell division plane. These proteins form the **cell division apparatus known as the divisome** and are directly involved in bacterial cell division by binary fission (**see Fig. 1 and Fig. 13 in the Slideshow Activity below**).

Figs. 1 and 13: Binary fission in bacteria
Slideshow Activity

The divisome is responsible for directing the synthesis of new cytoplasmic membrane and new peptidoglycan to form the division septum. The function of a number of divisome proteins have been identified, including:

- MinE: Directs formation of the FtsZ ring and divisome complex at the bacterium's division plane.
- FtsZ: Similar to tubulin in eukaryotic cells, FtsZ forms a constricting ring at the division site. As FtsZ depolymerizes, it directs an inward growth of the cell wall to form the division septum. It is found in both *Bacteria* and *Archaea*, as well as in mitochondria and chloroplasts.
- ZipA: A protein that connects the FtsZ ring to the bacterial cytoplasmic membrane.
- FtsA: An ATPase that breaks down ATP to provide energy for cell division and also helps connect the FtsZ ring to the bacterial cytoplasmic membrane.
- FtsK: Helps in separating the replicated bacterial chromosome.
- FtsI: Needed for peptidoglycan synthesis.

Scanning electron micrograph of dividing *Escherichia coli* Image provided by Evangeline Sowers and Janice Haney Carr. Courtesy of the Centers for Disease Control and Prevention.	**Scanning electron micrograph of dividing *Salmonella typhimurium.*** Provided by Janice Haney Carr. Courtesy of the Centers for Disease Control and Prevention.
	

Self Check

Concept Map for the Bacterial Cytoplasmic Membrane

E. Using Antimicrobial Agents that Alter the Cytoplasmic Membrane to Control Bacteria

As will be discussed later in Unit 2, a very few antibiotics, such as polymyxins and tyrocidins as well as many disinfectants and antiseptics, such as orthophenylphenol, chlorhexidine, hexachlorophene, zephiran, alcohol, triclosans, etc., used during disinfection and decontamination **alter the microbial cytoplasmic membranes** and cause **leakage of cellular needs**.

For more information: Preview of chemotherapeutic control of bacteria

Concept Map for the Bacterial Cytoplasmic Membrane

Self Quiz for the Cytoplasmic Membrane

Quiz Group

Quiz Group

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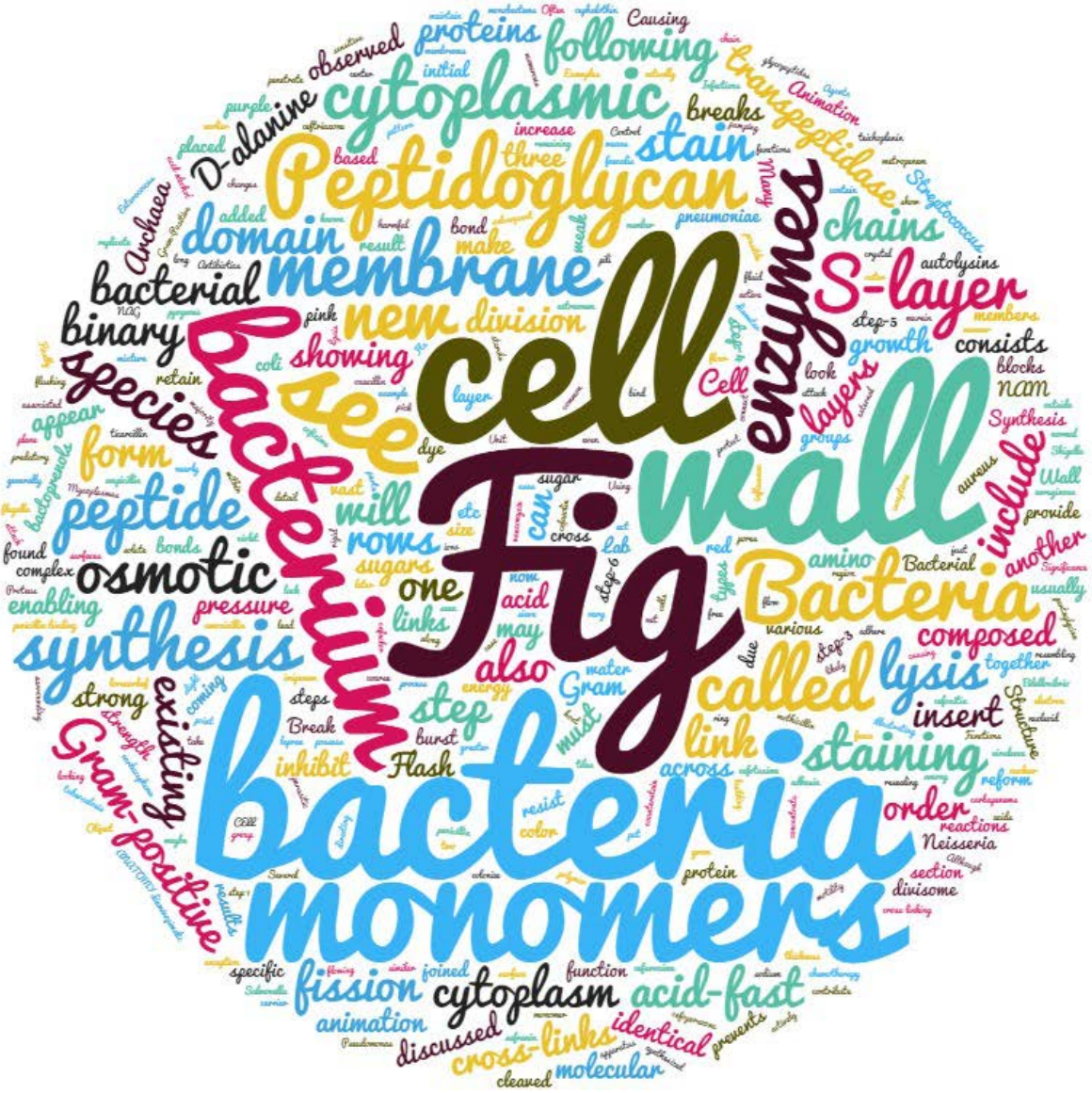
The peptidoglycan cell wall in bacteria
THE PEPTIDOGLYCAN CELL WALL

The Peptidoglycan Cell Wall



Fundamental Statements for this Lesson:

1. The vast majority of the domain Bacteria have a rigid cell wall composed of peptidoglycan.
2. The peptidoglycan cell wall surrounds the cytoplasmic membrane and prevents osmotic lysis.
3. Peptidoglycan is composed of interlocking chains of building blocks called peptidoglycan monomers.
4. In order to grow following binary fission, bacteria have to synthesize new peptidoglycan monomers in the cytoplasm, transport those monomers across the cytoplasmic membrane, put breaks in the existing cell wall so the monomers can be inserted, connect the monomers to the existing peptidoglycan, and cross-link the rows and layers of peptidoglycan.
5. Many antibiotics inhibit peptidoglycan synthesis in bacteria and lead to osmotic lysis of the bacteria.
6. Most bacteria can be placed into one of three groups based on their color after specific staining procedures are performed: Gram-positive, Gram-negative, or acid-fast. These staining reactions are due to fundamental differences in the bacterial cell wall.
7. Gram-positive bacteria stain purple after Gram staining while Gram-negative bacteria stain pink.
8. Acid-fast bacteria stain red after acid-fast staining.

Common Course Objectives

1. Identify the parts of a bacterium and their physiological purpose.
2. Describe how peptidoglycan cell walls form.
3. Explain the difference between a Gram-positive cell wall, a Gram-negative cell wall, and an acid-fast cell wall.

4. Explain how certain parts of the bacteria can be targeted in order to kill the bacteria.

Detailed Learning Objectives for this Lesson

1. State the 3 parts of a peptidoglycan monomer and state the function of peptidoglycan in bacteria.

2**. Briefly describe how bacteria synthesize peptidoglycan, indicating the roles of autolysins, bactoprenols, transglycosylases, and transpeptidases.

3*. Briefly describe how antibiotics such as penicillins, cephalosporins, and vancomycin affect bacteria and relate this to their cell wall synthesis.

4*. State what color Gram-positive bacteria stain after Gram staining.

5*. State what color Gram-negative bacteria stain after Gram staining.

6. State what color acid-fast bacteria stain after acid-fast staining.

(*) = Common theme throughout the course

(**) = More depth and common theme

TPS Question

In this and several following sections we are going to look at the various anatomical parts that make up a bacterium. We will not deal with the *Archaea* in this course but rather will concentrate on the *Bacteria*. A typical bacterium usually consists of:

- a cytoplasmic membrane surrounded by a peptidoglycan cell wall and maybe an outer membrane;
- a fluid cytoplasm containing a nuclear region (nucleoid) and numerous ribosomes; and
- often various external structures such as a glycocalyx, flagella, and pili.

We will now look at the peptidoglycan cell wall found in members of the domain *Bacteria*.

The Peptidoglycan Cell Wall

The mycoplasmas are the only bacteria that naturally lack a cell wall. Mycoplasmas maintain a nearly even pressure between the outside environment and the cytoplasm by actively pumping out sodium ions. Their cytoplasmic membranes also contain sterols that most likely provide added strength. All other bacteria have a cell wall.

The remaining bacteria in the domain *Bacteria*, with the exception of a few bacteria, have a semirigid cell wall containing peptidoglycan. (While bacteria belonging to the domain *Archaea* also have a semirigid cell wall, it is composed of chemicals distinct from peptidoglycan such as protein or pseudomurein. We will not take up the *Archaea* here.)

A. Function of Peptidoglycan

Peptidoglycan **prevents osmotic lysis**. As seen earlier under the cytoplasmic membrane, bacteria concentrate dissolved nutrients (solute) through active transport. As a result, the bacterium's cytoplasm is usually hypertonic to its surrounding environment and the net flow of free water is into the bacterium. Without a strong cell wall, the bacterium would burst from the osmotic pressure of the water flowing into the cell.

For more information: Review of the cytoplasmic membrane

B. Structure and Composition of Peptidoglycan

With the exceptions above, members of the domain *Bacteria* have a cell wall containing a semirigid, tight knit molecular complex called **peptidoglycan**.

Peptidoglycan, also called murein, is a vast polymer consisting of **interlocking chains of identical peptidoglycan monomers** (**see Fig. 2A and Fig. 2B in the first Slideshow Activity below**). A **peptidoglycan monomer** consists of two joined amino sugars, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), with a pentapeptide coming off of the NAM (**see Fig. 1A and Fig. 1B in the second Slideshow Activity below**). The types and the order of amino acids in the pentapeptide, while almost identical in Gram-positive and Gram-negative bacteria, show some slight variation among the domain *Bacteria*.

Figs. 1A and 1B: Peptidoglycan monomers
Slideshow Activity

Figs. 2A and 2B: Structure of peptidoglycan
Slideshow Activity
Copyright © Gary E. Kaiser

The peptidoglycan monomers are synthesized in the cytosol of the bacterium where they attach to a membrane carrier molecule called bactoprenol. As discussed below, the bactoprenols transport the peptidoglycan monomers across the cytoplasmic membrane and work with the enzymes discussed below to insert the monomers into existing peptidoglycan enabling bacterial growth following binary fission.

Once the new peptidoglycan monomers are inserted, glycosidic bonds then link these **monomers into the growing chains** of peptidoglycan. These **long sugar chains are then joined to one another by means of peptide cross-links between the peptides coming off of the NAMs**. By linking the rows and layers of sugars together in this manner, the peptide cross-links provide tremendous strength to the cell wall, enabling it to function similar to a molecular chain link fence around the bacterium.

Animation of Peptidoglycan

Quiz Group

C. Synthesis of Peptidoglycan

In order for bacteria to increase their size following binary fission, links in the peptidoglycan must be broken, new peptidoglycan monomers must be inserted, and the peptide cross links must be resealed.

The following sequence of events occur:

1. Bacterial enzymes called **autolysins:**

   a) Break the glycosidic bonds between the peptidoglycan monomers at the point of growth along the existing peptidoglycan; and

   b) Break the peptide cross-bridges that link the rows of sugars together (**see Fig. 3, steps 1-3 in the Slideshow Activity below**).

Fig 3, Steps 1-3: Autolysins breaking glycosidic bonds and peptide cross-bridges
Slideshow Activity
Copyright © Gary E. Kaiser

Flash animation showing the role of autolysins in the synthesis of peptidoglycan.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing the role of autolysins in the synthesis of peptidoglycan.

2. The **peptidoglycan monomers are synthesized in the cytosol** and bind to bactoprenol.The bactoprenols transport the peptidoglycan monomers across the cytoplasmic membrane and interacts with transglycosidases to insert the monomers into existing peptidoglycan (**see Fig. 4, step-1 through Fig. 4, step-6 in the Slideshow Activity below).**

Fig. 4, Steps 1-6:Synthesis of peptidoglycan monomers and the role of bactoprenol
Slideshow Activity
Copyright © Gary E. Kaiser

Flash animation showing the synthesis of peptidoglycan monomers and the role of bactoprenol.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing the synthesis of peptidoglycan monomers and the role of bactoprenols.

3. Transglycosylase (transglycosidase) enzymes insert and link new peptidoglycan monomers into the breaks in the peptidoglycan (**see Fig. 5, step 1 and Fig. 5, step 2 in the Slideshow Activity below**)

Fig. 5, Steps 1-2: Transglycosylase enzymes inserting and linking new peptidoglycan monomers into the breaks in the peptidoglycan
Slideshow Activity Copyright © Gary E. Kaiser

.

Flash animation showing the role of transglycosylase in the synthesis of peptidoglycan.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing the role of transglycosylase in the synthesis of peptidoglycan.

4. Finally, **transpeptidase** enzymes reform the peptide cross-links between the rows and layers of peptidoglycan to make the wall strong (**see Fig. 7, step 1 and Fig. 7, step 2 in the Slideshow Activity below**).

Fig. 7, Steps 1-2: Transpeptidase enzymes forming peptide cross-links between the rows and layers of peptidoglycan
Slideshow Activity Copyright © Gary E. Kaiser

In *Escherichia coli*, the terminal D-alanine is cleaved from the pentapeptides to form a tetrapeptides. This provides the energy to bond the D-alanine of one tetrapeptide to the diaminopimelic acid of another tetrapeptide **(see Fig. 1B)**. In the case of *Staphylococcus aureus*, the terminal D-alanine is cleaved from the pentapeptides to form a tetrapeptides. This provides the energy to bond a pentaglycine bridge (5 molecules of the amino acid glycine) from the D-alanine of one tetrapeptide to the L-lysine of another **(see Fig. 1A)**.

Flash animation showing the role of transpeptidase in the synthesis of peptidoglycan.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing the role of transpeptidase in the synthesis of peptidoglycan.

Flash animation summarizing the synthesis of peptidoglycan
Copyright © Gary E. Kaiser
html5 version of animation for iPad summarizing the synthesis of peptidoglycan

Concept Map for the peptidoglycan and peptidoglycan synthesis.

TPS Question

In the center of the bacterium, a group of proteins called Fts (filamentous temperature sensitive) proteins interact to form a ring at the cell division plane. These proteins form the cell division apparatus known as the divisome and are directly involved in bacterial cell division by binary fission (**see Fig. 7A and Fig. 7B in the Slideshow Activity below**). The divisome is responsible for directing the synthesis of new cytoplasmic membrane and new peptidoglycan to form the division septum.

Figs. 7A and 7B: The role of the divisome in bacterial binary fission

Slideshow Activity

Self Check

D. Antimicrobial Agents that Inhibit Peptidoglycan Synthesis Causing Bacterial Lysis

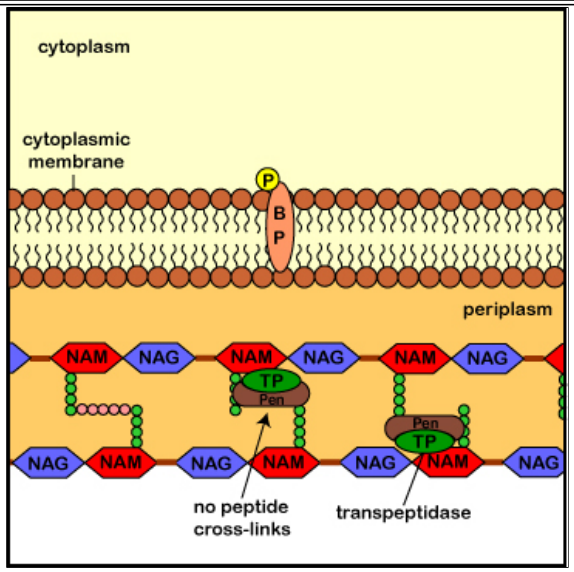
Many antibiotics work by **inhibiting normal synthesis of peptidoglycan** in bacteria causing them to burst as a result of **osmotic lysis**.

As just mentioned, in order for bacteria to increase their size following binary fission, enzymes called **autolysins** break the peptide cross links in the peptidoglycan, transglycosylase enzymes then insert and link new peptidoglycan monomers into the breaks in the peptidoglycan, and **transpeptidase** enzymes reform the peptide cross-links between the rows and layers of peptidoglycan to make the wall strong.

Interference with this process results in a weak cell wall and lysis of the bacterium from osmotic pressure. Examples include the **penicillins** (penicillin G, methicillin, oxacillin, ampicillin, amoxicillin, ticarcillin, etc.), the **cephalosporins** (cephalothin, cefazolin, cefoxitin, cefotaxime, cefaclor, cefoperazone, cefixime, ceftriaxone, cefuroxime, etc.), the **carbapenems** (imipenem, metropenem), the **monobactems** (aztreonem), the carbacephems (loracarbef), and the **glycopeptides** (vancomycin, teichoplanin).

- For example, **penicillins and cephalosporins bind to the transpeptidase enzymes** (also called penicillin-binding proteins) responsible for resealing the cell wall as new peptidoglycan monomers are added during bacterial cell growth. This blocks the transpeptidase enzymes from cross-linking the sugar chains and results in a weak cell wall and subsequent osmotic lysis of the bacterium **(see Fig. 8)**.
-

Fig. 8: Penicillin inhibiting peptidoglycan synthesis.



During normal bacterial growth, bacterial enzymes called autolysins put breaks in the peptidoglycan in order to allow for insertion of peptidoglycan building blocks (monomers of NAG-NAM-pentapeptide). These monomers are then attached to the growing end of the bacterial cell wall with transglycosidase enzymes. Finally, transpeptidase enzymes join the peptide of one monomer with that of another in order to provide strength to the cell wall. Penicillins and cephalosporins bind to the transpeptidase enzyme and block the formation of the peptide cross-links. This results in a weak cell wall and osmotic lysis of the bacterium.

Flash animation illustrating how penicillins inhibit peptidoglycan synthesis.
Copyright © Gary E. Kaiser
html5 version of animation for ipad showing how penicillins inhibit the synthesis of peptidoglycan.

Antimicrobial chemotherapy will be discussed in greater detail later in Unit 2 under **Control of Bacteria by Using Antibiotics and Disinfectants**.

For more information: Preview of chemotherapeutic control of bacteria

Concept map for peptidoglycan and peptidoglycan synthesis.

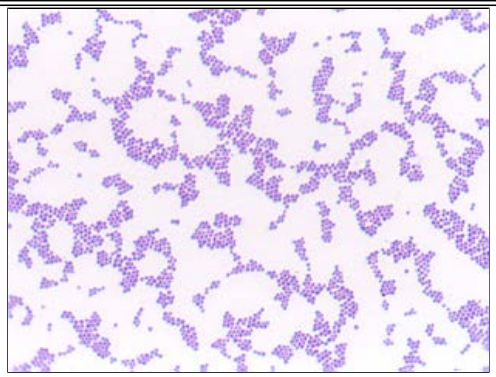
Self Check

E. Gram-Positive, Gram-Negative, and Acid-Fast Bacteria

Most bacteria can be placed into one of three groups based on their color after specific staining procedures are performed: Gram-positive, Gram-negative, or acid-fast.

1. Gram-positive: retain the initial dye crystal violet during the Gram stain procedure and appear **purple** when observed through the microscope (**see Fig. 9**). Common Gram-positive bacteria of medical importance include *Streptococcus pyogenes, Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus faecalis,* and *Clostridium* species.

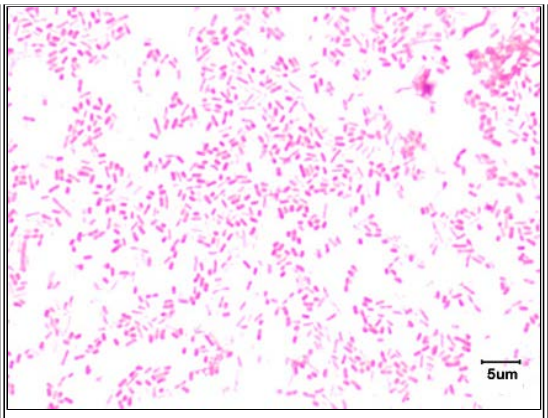
Fig. 9: Photomicrograph of a Gram stain of *Staphylococcus aureus*, a Gram-positive staphylococcus.



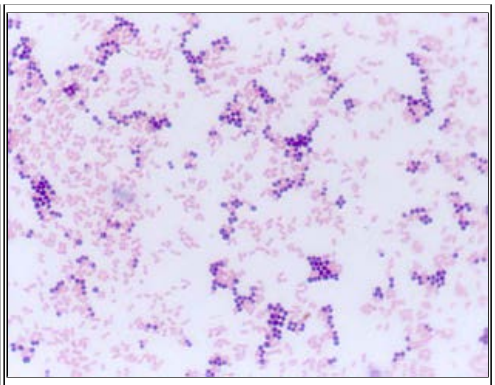
Note gram-positive (purple) cocci in clusters.

2. Gram-negative: decolorize during the Gram stain procedure, pick up the counterstain safranin, and appear **pink** when observed through the microscope (**see Fig. 10**). Common Gram-negative bacteria of medical importance include *Salmonella* species, *Shigella* species, *Neisseria gonorrhoeae, Neisseria meningitidis, Haemophilus influenzae, Escherichia coli, Klebsiella pneumoniae, Proteus* species, and *Pseudomonas aeruginosa*. Also see **Fig. 11**: Gram stain of a mixture of Gram-positive and Gram-negative bacteria.

Fig. 10: Photomicrograph of a Gram stain of *Escherichia coli*, a Gram-negative bacillus.	**Fig. 11: Gram stain of a mixture of Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*.**



Note Gram-negative (pink) bacilli.

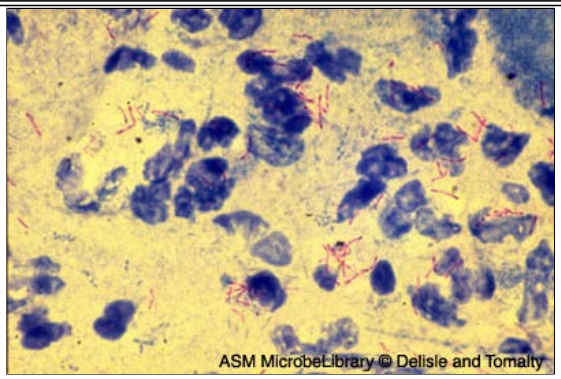


Note Gram-negative (pink)bacilli and Gram-positive (purple) cocci.

For more information: Preview of the Gram stain

3. Acid-fast: resist decolorization with an acid-alcohol mixture during the acid-fast stain procedure, retain the initial dye carbolfuchsin and appear **red** when observed through the microscope (**see Fig. 12**). Common acid-fast bacteria of medical importance include *Mycobacterium tuberculosis, Mycobacterium leprae,* and *Mycobacterium avium-intracellulare* complex.

Fig. 12: Acid-fast stain of the sputum from a person with tuberculosis.



Note acid-fast (red) bacilli.

For more information: Preview of the acid-fast stain

These staining reactions are due to fundamental differences in their cell wall as will be discussed in Lab 6 and Lab 16. We will now look at each of these three bacterial cell wall types.

Concept Map for the peptidoglycan and peptidoglycan synthesis.

Self Check

Self Quiz for Peptidoglycan and Peptidoglycan Synthesis

Quiz Group

Identify Activity

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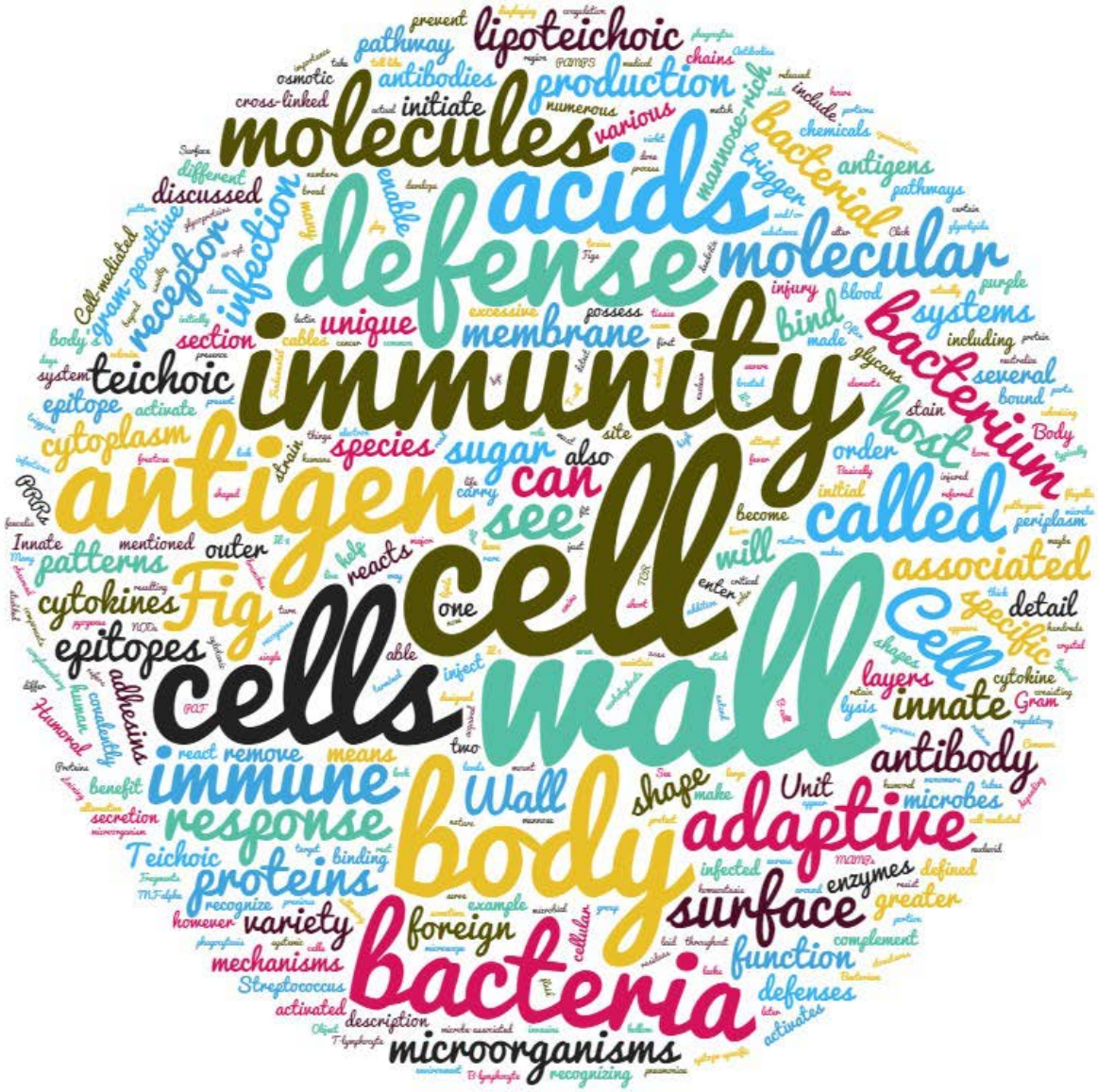
The Gram-positive cell envelope in bacteria
THE GRAM-POSITIVE CELL WALL

The Gram-Positive Cell Wall (Cell Envelope)



Fundamental Statements for this Lesson:

1. Because of the nature of their cell wall, Gram-positive bacteria stain purple after Gram staining.
2. The Gram-positive cell wall consists of many interconnected layers of peptidoglycan and lacks an outer membrane.
3. Peptidoglycan prevents osmotic lysis in the hypotonic environment in which most bacteria live.
4. Teichoic acids and lipoteichoic acids are interwoven through the peptidoglycan layers.
5. Surface proteins embedded in the cell wall can function as adhesins, secretion systems, and enzymes.
6. The Gram-positive cell wall activates both the body's innate immune defenses and its adaptive immune defenses.
7. The body activates innate immunity by recognizing molecules unique to microorganisms that are not associated with human cells called pathogen-associated molecular patterns or PAMPs. PAMPs bind to Pattern-recognition receptors (PRRs) on defense cells to trigger the production of inflammatory cytokines.
8. Inflammation is the means by which the body delivers defense cells and defense molecules to an infection site ,however, excessive inflammation can be harmful and even deadly to the body.
9. PAMPs associated with the Gram-positive cell wall include peptidoglycan monomers, teichoic acids, lipoteichoic acids, and mannose-rich sugar chains.
10. An antigen is a molecular shape that reacts with antigen receptors on lymphocytes to initiate an adaptive immune response.
11. Cell wall molecules can also trigger adaptive immunity such as the production of antibody molecules against bacterial cell wall antigens.Opsonizing antibodies made against cell wall antigens can bind bacteria to phagocytes for more efficient phagocytosis. Antibodies against cell wall adhesins can block attachment of bacteria to host cell receptors.

Common Course Objectives

1. Identify the parts of a bacterium and their physiological purpose.
2. Explain how various bacterial structures can contribute to bacterial colonization of a host.
3. Explain how various bacterial structures can contribute to the initiation of immune defenses
4. Explain the difference between a Gram-positive cell wall, a Gram-negative cell wall, and an acid-fast cell wall.
5. Provide a diagnosis for a "patient" when given a list of symptoms and a description of the bacterium.

Detailed Learning Objectives for this Lesson

1*. State what color Gram-positive bacteria stain after the Gram stain procedure.

2**. Describe the composition of a Gram-positive cell wall and indicate the possible beneficial functions to the bacterium of peptidoglycan, teichoic acids, and surface proteins.

3*. Briefly describe how PAMPs of the Gram-positive cell wall can promote inflammation.

4*. State the function of bacterial adhesins, secretion systems, and invasins.

5*. Define antigen and epitope.

6*. Briefly describe how opsonizing antibodies can promote phagocytosis and how antibodies made against cell wall adhesins can block colonization.

(*) = Common theme throughout the course

(**) = More depth and common theme

Highlighted Bacterium

1. Read the description of *Enterococcus*, and match the bacterium with the description of the organism and the infection it causes.

In this section on Prokaryotic Cell Anatomy we are looking at the various anatomical parts that make up a bacterium. As mentioned in the introduction to this section, a typical bacterium usually consists of:

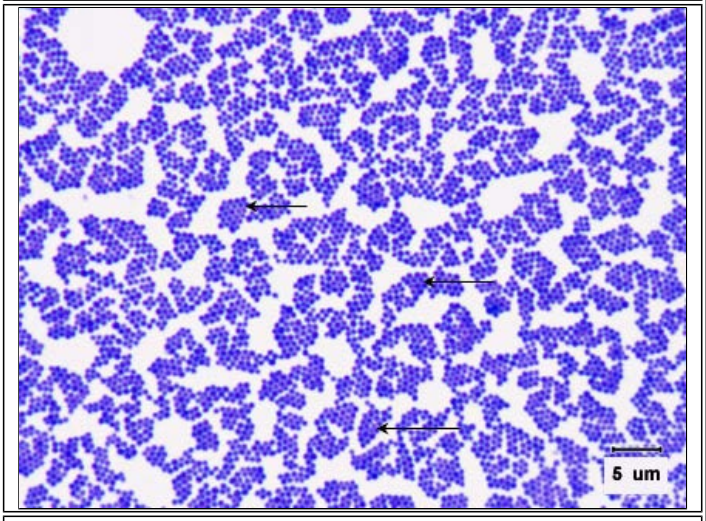
- a cytoplasmic membrane surrounded by a peptidoglycan cell wall and maybe an outer membrane;
- a fluid cytoplasm containing a nuclear region (nucleoid) and numerous ribosomes; and
- often various external structures like a glycocalyx, flagella, and pili.

We will now look at the Gram-positive bacterial cell wall.

The Gram-Positive Cell Wall (Cell Envelope)

As mentioned in the previous section on peptidoglycan, Gram-positive bacteria are those that retain the initial dye crystal violet during the Gram stain procedure and appear **purple (see Fig. 1)** when observed through the microscope. As we will learn in lab, this is a result of the structure and function of the Gram-positive cell wall.

Fig. 1: Photomicrograph of a Gram stain of *Staphylococcus aureus*, a Gram-positive staphylococcus.



Note gram-positive (purple) cocci in clusters.

For more information: Preview of the Gram stain

Flash animation illustrating the interactions of the Gram stain reagents at the molecular level.

Common Gram-positive bacteria of medical importance include *Streptococcus pyogenes, Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus faecalis,* and *Clostridium* species.

Highlighted Bacterium: *Enterococcus* species

Click on this link, read the description of *Enterococcus*, and be able to match the bacterium with its description on an exam.

Self Check

A. Structure and Composition of the Gram-Positive Cell Wall

The term cell envelope is often used to indicate all the various layers that envelope a bacterial cell. In terms of Gram-positive bacteria, the cell envelope would include the cytoplasmic membrane, the peptidoglycan cell wall with its teichoic acids, and if present, an S-layer and capsule or glycocalyx. In this section we will be concerned with just the cell wall of Gram-positive bacteria.

1. In electron micrographs, the **Gram-positive cell wall** appears as a broad, dense wall 20-80 nm thick and consisting of numerous interconnecting layers of **peptidoglycan** (**See Figs. 2A and 2B**). Chemically, 60 to 90% of the Gram-positive cell wall is peptidoglycan. In Gram-positive bacteria it is thought that the peptidoglycan is laid down in cables of several cross-linked glycan strands approximately 50 nm wide. These cables then themselves become cross-linked for further cell wall strength.

For more information: Peptidoglycan

Fig. 2A: Electron Micrograph of a Gram-Positive Cell Wall.

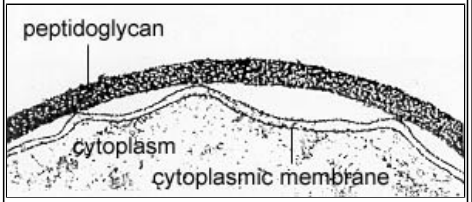
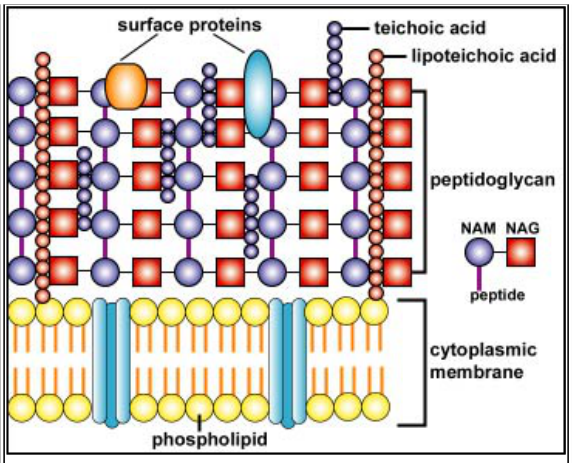


Fig. 2B: Illustration of the Structure of a Gram-Positive Cell Wall.



The Gram-positive cell wall appears as dense layer typically composed of numerous rows of peptidoglycan, and molecules of lipoteichoic acid, wall teichoic acid and surface proteins.

2. Interwoven in the cell wall of Gram-positive are **teichoic acids and lipoteichoic acids.** Teichoic acids extend through and beyond the rest of the cell wall and are polyalcohols composed of polymers of glycerol, phosphates, and the sugar alcohol ribitol and are covalently bound to the peptidoglycan. Teichoic acids covalently bound to cytoplasmic membrane lipids are called lipoteichoic acids **(see Fig. 2B)**.

3. The outer surface of the peptidoglycan is studded with **surface proteins** that differ with the strain and species of the bacterium **(see Fig. 2B)**.

4. The **periplasm** is the gelatinous material between the peptidoglycan and the cytoplasmic membrane.

Concept map for the Gram-positive cell wall.

B. Functions of the Gram-Positive Cell Wall Components

1. The **peptidoglycan** in the Gram-positive cell wall **prevents osmotic lysis.**

2. The **teichoic acids** probably help make the cell wall stronger **(see Fig. 2B)**.

3. The **surface proteins (see Fig. 2B)** in the bacterial peptidoglycan, depending on the strain and species, carry out a variety of activities.

    a. Some surface proteins function as **enzymes**.

    b. Other proteins serve as **adhesins**. Adhesins enable the bacterium to **adhere intimately to host calls** and other surfaces in order to colonize those cells and resist flushing **(See Fig. 3**).

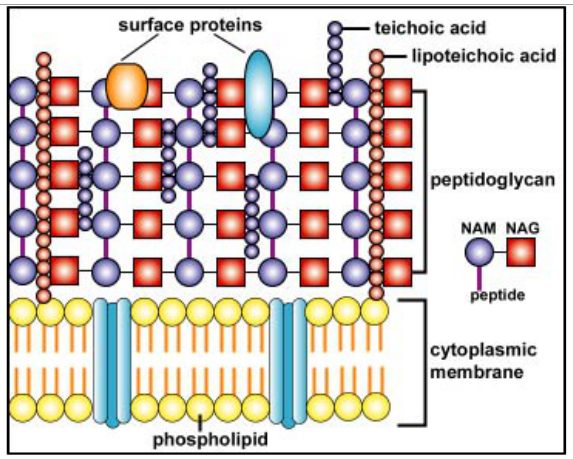
    c. Many bacteria involved in infection have the ability to **co-opt the functions of host cells for the bacterium's own benefit**. This is done by way of bacterial secretions systems that enable the bacterium to directly inject bacterial effector molecules into the cytoplasm of the host cell in order to alter its cellular machinery or cellular communication to the benefit of the bacteria. They do this by producing secretion systems such as the type 3 secretion system that produces hollow, needle-like tubes called injectisomes. Certain bacteria, for example, inject invasins into the cytoplasm of the host cell that enable the bacterium to **enter that cell.**

The role of these cell wall surface proteins will be discussed in greater detail later in Unit 3 under Bacterial Pathogenicity.

4. The periplasm contains enzymes for nutrient breakdown.

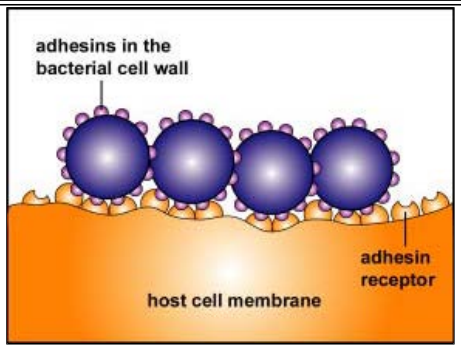
Fig. 2B: Illustration of the Structure of a

Gram-Positive Cell Wall.



The Gram-positive cell wall appears as dense layer typically composed of numerous rows of peptidoglycan, and molecules of lipoteichoic acid, wall teichoic acid and surface proteins.

Fig. 3: Bacterial Adhesins



Surface proteins called adhesins in the bacterial cell wall bind to receptor molecules on the surface of a susceptible host cell enabling the bacterium to make intimate contact with the host cell, adhere, colonize, and resist flushing.

Flash animation showing a bacterium using adhesins to adhere to a host cell.

html5 version of animation for iPad showing a bacterium using adhesins to adhere to a host cell.

Surface proteins called adhesins in the bacterial cell wall bind to receptor molecules on the surface of a susceptible host cell enabling the bacterium to make intimate contact with the host cell, adhere, colonize, and resist flushing

Flash animation showing bacteria injecting invasins into a non-immune host cells in order to enter that cell by phagocytosis.

html5 version of animation for iPad showing bacteria secreting invasions into a non-immune host cell in order to enter that cell by phagocytosis.

Some bacteria produce molecules called invasins that activate the host cell's cytoskeletal machinery enabling bacterial entry into the cell by phagocytosis. By entering the cytoplasm of the host cell, it has a ready supply of nutrients and is able to protect the bacteria from complement, antibodies, and certain other body defenses. Many bacteria use injectosomes, such as a type 3 secretion system, to inject effector molecules into the host cell's cytoplasm.

For more information: Preview of the ability to invade host cells

For more information: Preview of the ability to adhere to host cells

Concept map for the Gram-positive cell wall.

Sorting Activity

C. Significance of Gram-Positive Cell Wall Components to the Initiation of Body Defenses

The body has two immune systems: **the innate immune system** and the **adaptive immune syste**m.

1. **Innate immunity** is an **antigen-nonspecific** defense mechanisms that a host uses **immediately or within several hours after exposure to almost any microb**e. This is the immunity one is born with and is the initial response by the body to eliminate microbes and prevent infection.

2. **Adaptive (acquired) immunity** refers to **antigen-specific** defense mechanisms that **take several days to become protective** and are designed to react with and remove a specific antigen. This is the immunity one develops throughout life.

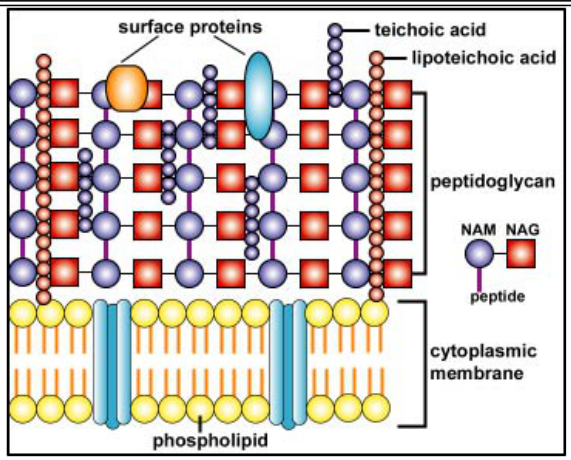
Initiation of Innate Immunity

In order to protect against infection, one of the things the body must initially do is detect the presence of microorganisms. The body does this by **recognizing molecules unique to microorganisms that are not associated with human cells**. These unique molecules are called **pathogen-associated molecular patterns** or PAMPs. (Because all microbes, not just pathogenic microbes, possess PAMPs, pathogen-associated molecular patterns are sometime referred to as microbe-associated molecular patterns or MAMPs.)

Fragments of peptidoglycan and teichoic acids are PAMPS associated with the cell wall of Gram-positive bacteria **(See Fig. 2B)**. In addition, bacteria and other microorganisms also possess **mannose-rich glycans** (short carbohydrate chains with the sugar mannose or fructose as the terminal sugar) that function as PAMPs. These mannose-rich glycans are common in microbial glycoproteins and glycolipids but rare in those of humans **(see Fig. 4)**.

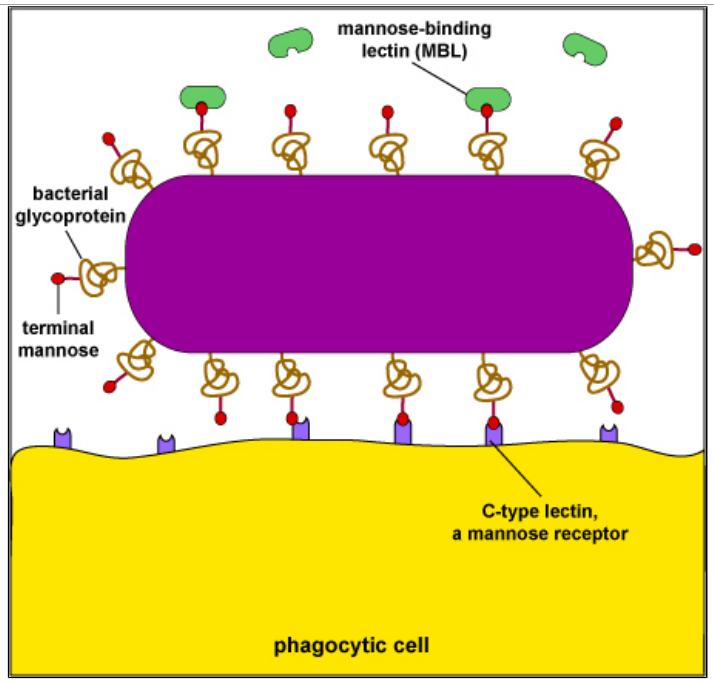
Fig. 2B: Illustration of the Structure of a

Gram-Positive Cell Wall.



The Gram-positive cell wall appears as dense layer typically composed of numerous rows of peptidoglycan, and molecules of lipoteichoic acid, wall teichoic acid and surface proteins.

Fig. 4: Recognition of Bacteria Mannose by Mannose Receptors.



Mannose-rich glycans are short carbohydrate chains with the sugar mannose or fructose as the terminal sugar. They are commonly found in microbial glycoproteins and glycolipids but are rare in those of humans. (Human glycoproteins and glycolipids typically have terminal N-acetylglucosamine and sialic acid groups.) C-type lectins, found on the surface of phagocytes, are endocytic pattern recognition receptors that bind to mannose-rich glycans in order to attach microbes to phagocytes. Mannose-binding lectin (MBL), also known as mannan-binding protein, is a soluble pattern recognition receptor in plasma and tissue fluid that binds to mannose-rich glycans on microbes in order to activate the lectin complement pathway.

These PAMPS bind to **pattern-recognition receptors or PRRs** on a variety of defense cells of the body and **trigger such innate immune defenses as inflammation, fever, and phagocytosis**.

For more information: Preview of pathogen-associated molecular patterns (PAMPs)

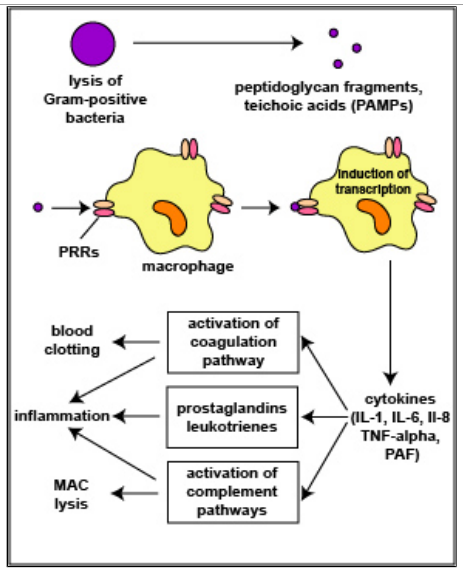
For more information: Preview of pattern-recognition receptors (PRRs)

Inflammation is the first response to infection and injury and is critical to body defense. Basically, the inflammatory response is an attempt by the body to restore and maintain homeostasis after injury. **Most of the body defense elements are located in the blood, and inflammation is the means by which body defense cells and body defense chemicals leave the blood and enter the tissue around an injured or infected site**.

Body defense cells such as macrophages, and dendritic cells have pattern recognition receptors such as toll-like receptors on their surface that are specific for the peptidoglycan fragments and lipoteichoic acids in the Gram-positive cell wall and/or to NODs in their cytoplasm that are specific for peptidoglycan fragments.

The binding of these cell wall components to their corresponding pattern recognition receptors triggers the macrophages to release various defense regulatory chemicals called cytokines, including IL-1, IL-6, IL-8, TNF-alpha, and PAF. The cytokines then bind to cytokine receptors on target cells and initiate inflammation and activate both the complement pathways and the coagulation pathway **(see Fig. 5)**.

Fig. 5: The Effects of Peptidoglycan Fragments and Lipoteichoic Acid Released During Gram-Positive Infections.



The lysis of Gram-positive bacteria causes them to release peptidoglycan fragments, teichoic acids, lipoteichoic acids, and mannose-rich glycans from their cell wall. These cell wall components, in turn, bind to pattern recognition receptors (PRRs) that are specific for these cell wall components and are found on the surface of body defense cells called macrophages. This triggers the macrophages to release various defense regulatory chemicals called cytokines, including IL-1, IL-6, IL-8, TNF-alpha, and PAF. The cytokines then bind to cytokine receptors on target cells and initiate inflammation and activate both the complement pathways and the coagulation pathway. These Gram-positive cell wall components can also bind first to binding proteins circulating in the blood that subsequently carry them to CD14 molecules on the macrophages.

For more information: Preview of cytokines

For more information: Preview of Inflammation

The peptidoglycan and teichoic acids also **activate the alternative complement pathway and the lectin pathway**, defense pathways that play a variety of roles in body defense.

For more information: Preview of the complement pathways

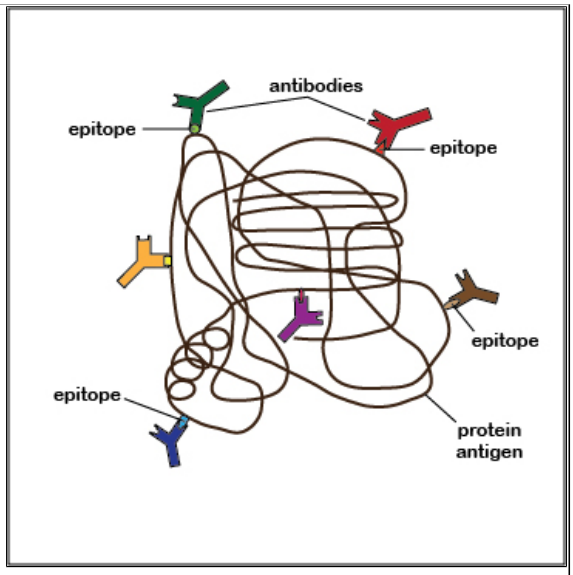
Innate immunity will be discussed in greater detail in Unit 5.

Initiation of Adaptive Immunity

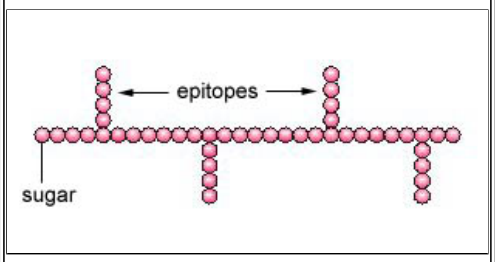
Proteins and polysaccharides associated with the Gram-positive cell wall function as antigens and initiate adaptive immunity. An **antigen** is defined as **a molecular shape that reacts with antibody molecules and with antigen receptors on lymphocytes**. We recognize those molecular shapes as foreign or different from our body's molecular shapes because they fit specific antigen receptors on our B-lymphocytes and T-lymphocytes, **the cells that carry out adaptive immunity**.

The actual portions or fragments of an antigen that react with antibodies and with receptors on B-lymphocytes and T-lymphocytes are called epitopes. An epitope is typically a group of 5-15 amino acids with a unique shape that makes up a portion of a protein antigen (**see Fig. 6A**) , or 3-4 sugar residues branching off of a polysaccharide antigen (**see Fig. 6B**). **A single microorganism has many hundreds of different shaped epitopes that our lymphocytes can recognize as foreign and mount an adaptive immune response against**.

Fig. 6A: Illustration of Epitopes of a Protein Antigen	Fig. 6B: Illustration of Epitopes of a Polysaccharide Antigen



Proteins have many epitopes of different specificities. During humoral immunity, antibodies are made to fit each epitope of each antigen.



The body recognizes an antigen as foreign when epitopes of that antigen bind to B-lymphocytes and T-lymphocytes by means of epitope-specific receptor molecules having a shape complementary to that of the epitope. The epitope receptor on the surface of a B-lymphocyte is called a B-cell receptor and is actually an antibody molecule. The receptor on a T-lymphocyte is called a T-cell receptor (TCR).

There are two major branches of the adaptive immune responses: humoral immunity and cell-mediated immunity.

1. **Humoral immunity**: Humoral immunity involves the production of **antibody molecules** in response to an antigen and is mediated by B-lymphocytes. Through a variety of mechanisms, these antibodies are able to remove or neutralize microorganisms and their toxins after binding to their epitopes. For example, antibodies made against cell wall antigens can **stick bacteria to phagocytes**, a process called opsonization. Antibodies made against cell wall adhesins can **prevent bacteria from adhering to and colonizing host cells**.

Flash Animation of Phagocytosis by Opsonizing Antibodies
The Fab portion of IgG binds to epitopes of a microbe. The Fc portion can now attach the microbe to Fc receptors on phagocytes for enhanced attachment, also known as opsonization. Once attached to the phagocyte by way of IgG, the microbe can be engulfed more efficiently and placed in a phagosome, and destroyed by lysosomes.
html5 version of animation for iPad showing opsonization by way of IgG.

Flash Animation of Antibodies Blocking Bacterial Adherance
The Fab portion of the antibodies made against epitopes of adherence structures such as cell wall adhesins bind and block the bacteria from adhering to receptors on the host cell membrane. As a result, the bacteria are unable to colonize and may be flushed away.
html5 version of animation for iPad showing antibodies blocking bacterial adherence to host cell

2. **Cell-mediated immunity**: Cell-mediated immunity involves the **production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines** in response to an antigen and is mediated by T-lymphocytes. These defense cells help to **remove infected cells** and **cancer cells** displaying foreign epitopes.

Adaptive immunity will be discussed in greater detail in Unit 6.

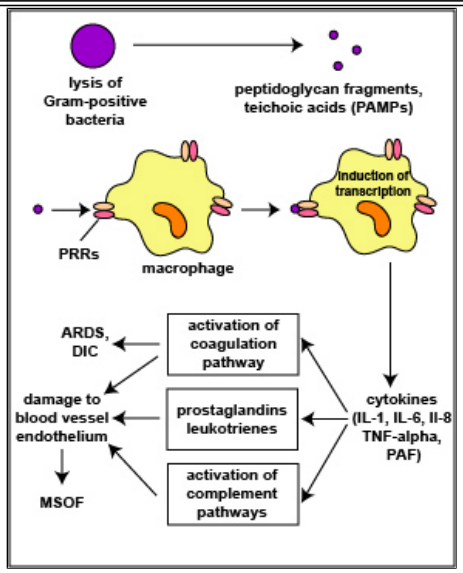
Concept map for the Gram-positive cell wall.

DragNDrop Activity

D. Significance of Gram-Positive Cell Wall Components to Bacterial Pathogenicity

During **severe** systemic infections with large numbers of bacteria present, however, **high levels of Gram-positive PAMPs are released** resulting in **excessive cytokine production** by the macrophages and other cells and this, in turn, can **harm the body (see Fig. 7)**.

Fig. 7: The Harmful Effects of Peptidoglycan Fragments and Lipoteichoic Acid Released During Gram-Positive Infections.



The lysis of gram-positive bacteria causes them to release peptidoglycan fragments, teichoic acids, lipoteichoic acids, and mannose-rich glycans from their cell wall. These cell wall components, in turn, bind to pattern recognition receptors (PRRs) that are specific for these cell wall components and are found on the surface of body defense cells called macrophages.This triggers the macrophages to release various defense regulatory chemicals called cytokines, including IL-1, IL-6, IL-8, TNF-alpha, and PAF. The cytokines then bind to cytokine receptors on target cells stimulating the production of inflammatory mediators such as prostaglandins and leukotrienes as well as activating both the complement pathways and the coagulation pathway. Excessive production of clotting factors may lead to ARDS and DIC while an overproduction of prostaglandins, leukotrienes, and complement proteins can damage the vascular endothelium resulting in shock and MSOF.

(LPS, lipopolysaccharide; IL-1, interleukin-1; IL-6, interleukin-6; IL-8, interleukin-8, TNF-alpha, tumor necrosis factor-alpha; PAF, platelet-activating factor; ARDS, acute respiratory distress syndrome; DIC, disseminated intravascular coagulation; MSOF, multiple system organ failure.)

Flash animation illustrating signaling toll-like receptors on defense cells: LTA and TLR-2/TLR-6

html5 version of animation for iPad illustrating signaling toll-like receptors on defense cells: LTA and TLR-2/TLR-6.

1) Gram-positive bacteria release lipoteichoic acid (LTA) from their cell wall.
2) The LTA binds to a TLR-2/TLR-6 pair on defense cells such as macrophages and dendritic cells.
3) The binding of LTA to TLR-2/TLR-6 enables regulatory molecules within the cell - Mal, MyD88, Tram, and Trif - to trigger reactions that activate a master regulator of inflammation called NF-kappa B. Activated NF-kappa B enters the cell's nucleus and switches on genes coding for cytokines such as:

a. Interleukin-1 (IL-1) and Tumor necrosis factor-alpha (TNF-alpha): enhance inflammatory responses;
b. Interleukin-8 (IL-8): aids in the ability of white blood cells to leave the blood vessels and enter the tissue; a chemoattractant for phagocytes;
c. Interleukin-6 (IL-6) promotes B-lymphocyte activity; and
d. Interleukin-12 (IL-12): promotes T-lymphocyte activity. (5)

4) Cytokine genes are transcribed into mRNA molecules that goes to the cytoplasm to be translated into inflammatory cytokines that are subsequently secreted from the cell.

Concept map for the Gram-positive cell wall.

For more information: Preview of inflammatory Gram-positive cell wall components
For more information: Preview of cytokines
For more information: Preview of inflammation

Self Check

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Streptococcus pyogenes*
- *Streptococcus pneumoniae*
- *Staphylococcus aureus*
- *Enterococcus* species

Self Quiz for the Gram-Positive Cell Wall

Quiz Group

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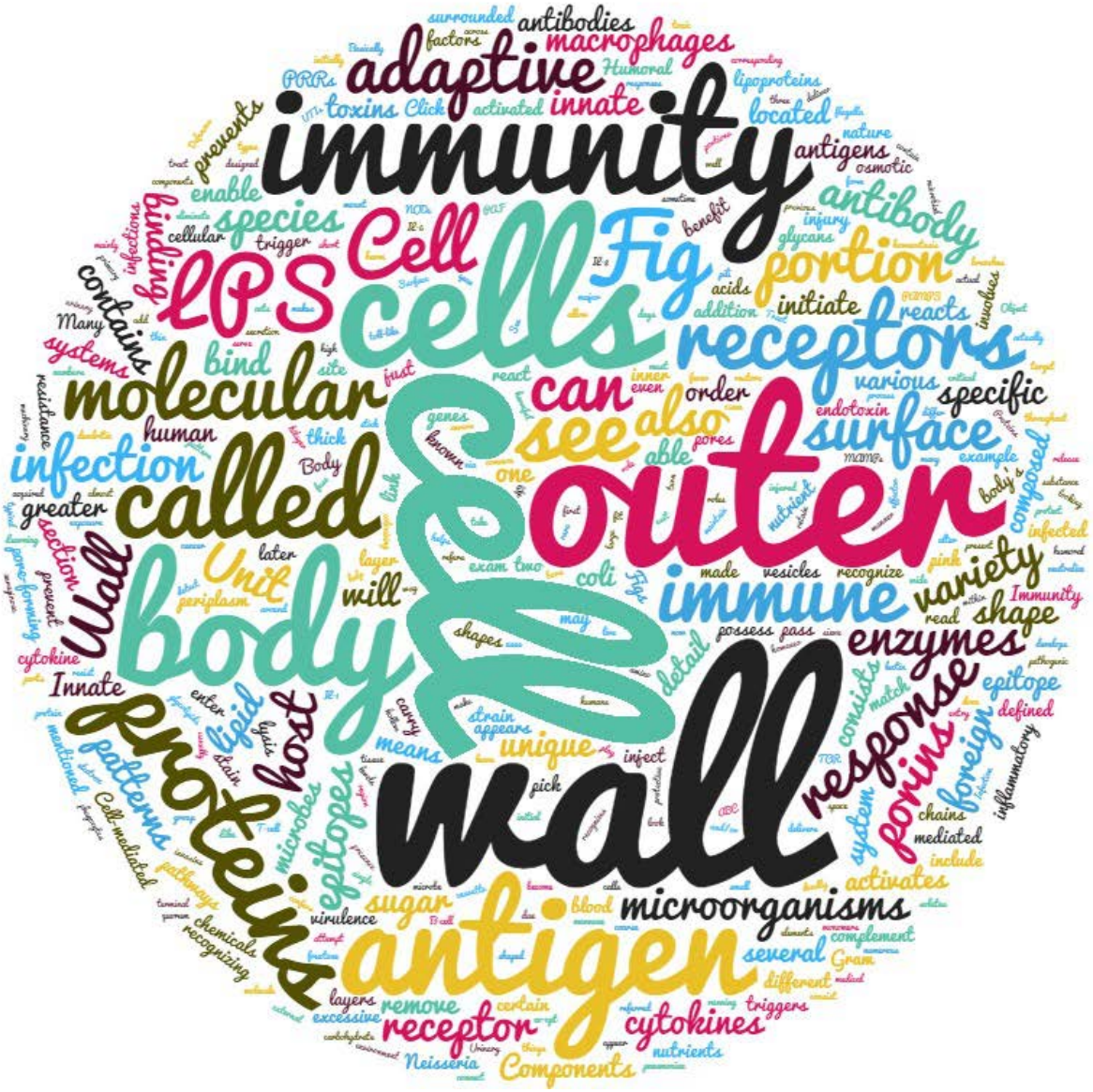
Back to Softchalk Lessons Table of Contents

The Gram-negative cell envelope in bacteria
THE GRAM-NEGATIVE CELL WALL

The Gram-Negative Cell Wall (Cell Envelope)



Fundamental Statements for this Lesson:

1. Because of the nature of their cell wall, Gram-negative bacteria stain pink after Gram staining.
2. The Gram-negative cell wall consists of 2-3 interconnected layers of peptidoglycan surrounded by an outer membrane.
3. Peptidoglycan prevents osmotic lysis in the hypotonic environment in which most bacteria live.
4. The outer membrane is a semipermeable structure that contains pore-forming proteins called porins that allow nutrients to pass through the outer membrane.
5. Surface proteins embedded in the cell wall can function as adhesins, secretion systems, and enzymes.
6. The Gram-negative cell wall activates both the body's innate immune defenses and its adaptive immune defenses.
7. The body activates innate immunity by recognizing molecules unique to microorganisms that are not associated with human cells called pathogen-associated molecular patterns or PAMPs. PAMPs bind to Pattern-recognition receptors (PRRs) on defense cells to trigger the production of inflammatory cytokines.
8. Inflammation is the means by which the body delivers defense cells and defense molecules to an infection site, however, excessive inflammation, can be harmful and even deadly to the body.
9. PAMPs associated with the Gram-negative cell wall include peptidoglycan monomers, lipopolysaccharide (LPS), porins, and mannose-rich sugar chains.
10. An antigen is a molecular shape that reacts with antigen receptors on lymphocytes to initiate an adaptive immune response.
11. Cell wall molecules can also trigger adaptive immunity such as the production of antibody molecules against bacterial cell wall antigens. Opsonizing antibodies made against cell wall antigens can bind bacteria to phagocytes for more efficient phagocytosis.Antibodies against cell wall adhesins can block attachment of bacteria to host cell receptors.

Common Course Objectives

1. Identify the parts of a bacterium and their physiological purpose.
2. Explain how various bacterial structures can contribute to bacterial colonization of a host.
3. Explain how various bacterial structures can contribute to the initiation of immune defenses
4. Explain the difference between a Gram-positive cell wall, a Gram-negative cell wall, and an acid-fast cell wall.
5. Provide a diagnosis for a "patient" when given a list of symptoms and a description of the bacterium.

Detailed Learning Objectives for this Lesson

1. State what color Gram-negative bacteria stain after the Gram stain procedure.

2**. Describe the composition of a Gram-negative cell wall and indicate the possible beneficial functions to the bacterium of peptidoglycan, the outer membrane, lipopolysaccharides, porins, and surface proteins.

3*. Briefly describe how LPS and other PAMPs of the Gram-negative cell wall can promote inflammation.

4*. State the function of bacterial adhesins, secretion systems, and invasins.

5. Define periplasm.

6*. Define antigen and epitope.

7*. Briefly describe how opsonizing antibodies can promote phagocytosis and how antibodies against cell wall adhesins can block colonization.

(*) = Common theme throughout the course

(**) = More depth and common theme

Highlighted Bacterium

1. Read the description of *Escherichia coli*, and match the bacterium with the description of the organism and the infection it causes.

Highlighted Disease: Urinary Tract Infections (UTIs)

1. Define the following:

a. urethritis
b. cystitis
c. pyelonephritis

2. Name at least 4 risk factors for UTIs.

3. Name the most common bacterium to cause UTIs; name at least 3 other bacteria that commonly cause UTIs.

4. Name at least 3 common symptoms of UTIs.

TPS Questions

In this section on Prokaryotic Cell Anatomy we are looking at the various anatomical parts that make up a bacterium. As mentioned in the introduction to this section, a typical bacterium usually consists of:

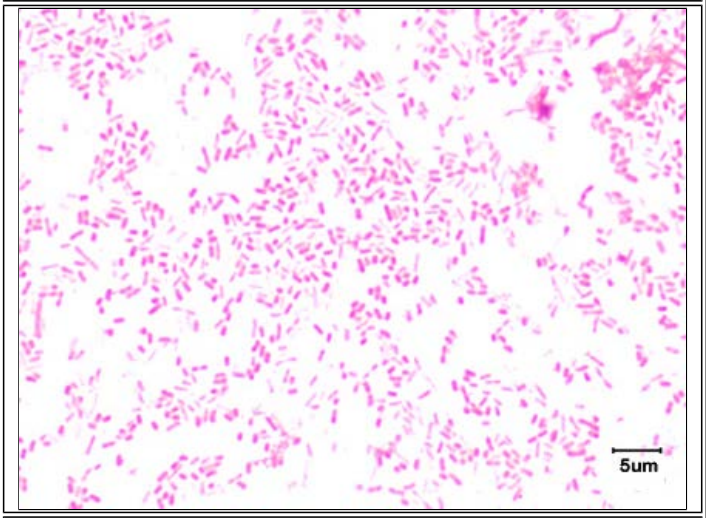
- a cytoplasmic membrane surrounded by a peptidoglycan cell wall and maybe an outer membrane;
- a fluid cytoplasm containing a nuclear region (nucleoid) and numerous ribosomes; and
- often various external structures like a glycocalyx, flagella, and pili.

We will now look at the Gram-negative bacterial cell wall.

The Gram-Negative Cell Wall (Cell Envelope)

As mentioned in the previous section on peptidoglycan, Gram-negative bacteria are those that decolorize during the Gram stain procedure, pick up the counterstain safranin, and appear **pink (see Fig. 1)**.

Fig. 1: Gram Stain of *Escherichia coli*



Note Gram-negative (pink) bacilli.

As we will learn in lab, this is a result of the structure and function of the Gram-negative cell wall.

For more information: Preview of the Gram stain

Flash animation illustrating the interactions between the various Gram stain reagents at the molecular level.

Common Gram-negative bacteria of medical importance include *Salmonella* species, *Shigella* species, *Neisseria gonorrhoeae, Neisseria meningitidis, Haemophilus influenzae, Escherichia coli, Klebsiella pneumoniae, Proteus* species, and *Pseudomonas aeruginosa*.

Highlighted Bacterium: *Escherichia coli*

Click on this link, read the description of *Escherichia coli*, and be able to match the bacterium with its description on an exam.

Highlighted Infection: Urinary Tract Infections (UTIs)

Click on this link, read the description of urinary tract infections (UTIs), and be able to match the infection with its description on an exam.

Self Check

A. Structure and Composition of the Gram-Negative Cell Wall

The term cell envelope is often used to indicate all the various layers that envelope a bacterial cell. In terms of Gram-negative bacteria, the cell envelope would include the cytoplasmic membrane, the peptidoglycan cell wall, the outer membrane, and if present, a capsule or glycocalyx. In this section we will be concerned with just the cell wall and outer membrane of Gram-negative bacteria.

In electron micrographs, the **Gram-negative cell wall (see Figs. 2A and 2B)** appears **multilayered**. It consists of:

1. **A thin, inner wall composed of peptidoglycan**

The peptidoglycan portion of the Gram-negative cell wall is generally 2-3 nanometers (nm) thick and contains just 2-3 layers of **peptidoglycan** (**see Fig. 2B**). Chemically, only 10 to 20% of the Gram-negative cell wall is peptidoglycan.

For More Information: Review of Peptidoglycan

Fig. 2A: Electron Micrograph of a Gram-Negative Cell Wall.

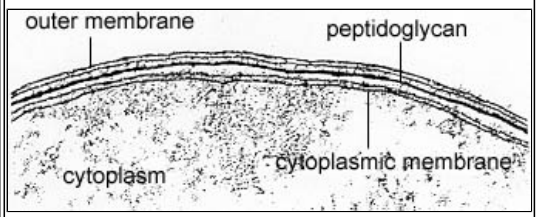
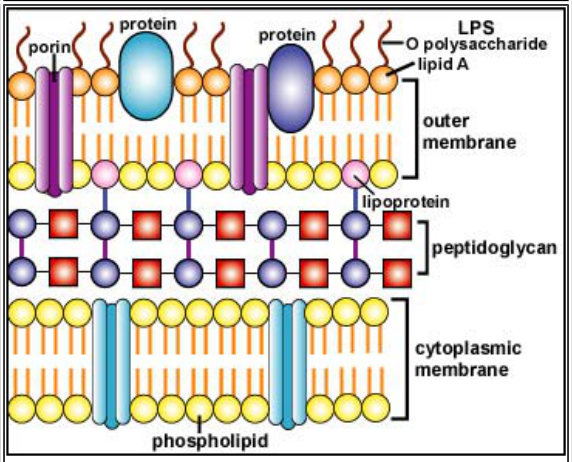


Fig. 2B: Illustration of the Structure of a Gram-Negative Cell Wall.



The Gram-negative cell wall is composed of a thin, inner layer of peptidoglycan and an outer membrane consisting of molecules of phospholipids, lipopolysaccharides (LPS), lipoproteins and surface proteins. The lipopolysaccharide consists of lipid A and O polysaccharide.

2. **An outer membrane**

The outer membrane of the Gram-negative cell wall appears as a lipid bilayer about 7 nm thick. It is composed of **phospholipids, lipoproteins, lipopolysaccharides (LPS), and proteins**. **Phospholipids** are located mainly in the inner layer of the outer membrane, as are the **lipoproteins** that connect the outer membrane to the peptidoglycan **(See Figs. 2A and 2B)**. The **lipopolysaccharides**, located in the outer layer of the outer membrane, consist of a lipid portion called **lipid A** embedded in the membrane and a polysaccharide portion extending outward from the bacterial surface. The LPS portion of the outer membrane is also known as endotoxin.

In addition, **pore-forming proteins called porins (see Fig. 2B)** span the outer membrane. The porins function as channels for the entry and exit of solutes through the outer membrane of the Gram-negative cell wall.

3. The outer membrane of the Gram-negative cell wall is studded with **surface proteins** that differ with the strain and species of the bacterium **(see Fig. 2B)**.

4. The periplasm is the gelatinous material between the outer membrane, the peptidoglycan, and the cytoplasmic membrane. This periplasmic space is about 15nm wide and contains a variety of hydrolytic enzymes for nutrient breakdown, periplasmic binding proteins for transport via the ATP-binding cassette (ABC) system, and chemoreceptors for chemotaxis (discussed under Bacterial Flagella later in this Unit).

Concept map for the Gram-negative cell wall.

B. Functions of the Gram-Negative Cell Wall Components

1. The **peptidoglycan** in the Gram-negative cell wall **prevents osmotic lysis**.

2. The outer membrane of the Gram-negative cell wall confers several functions:

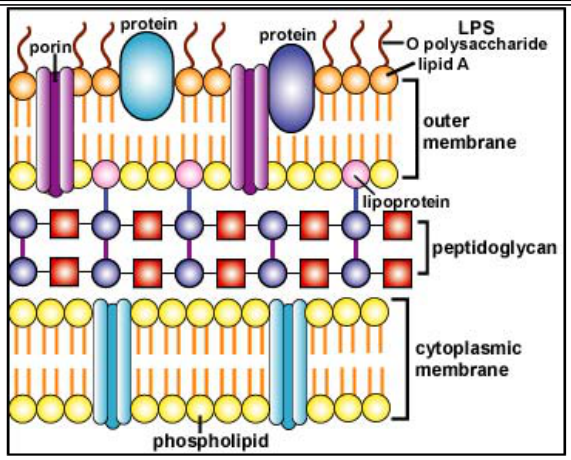
a. Like the cytoplasmic membrane discussed previously in Unit 1, is semipermeable and acts as a coarse molecular sieve. Many small molecules may pass through due to pores running through the membrane. These pores are composed of proteins called **porins (see Fig. 2B)**.

b. Because of its semipermeable nature, the outer membrane helps retain certain enzymes and **prevents some toxic substances, such as penicillin G and lysozyme, from entering.**

c. The LPS from the outer membrane of the Gram-negative cell wall **(see Fig. 2B)** is thought to add strength to the outer membrane, in a manner similar to the glycopeptides and teichoic acids of the gram-positive cell wall.

d. The outer membrane may also form vesicles that contain quorum signaling molecules, enzymes, toxins, virulence factors, and even antibiotic resistance genes. These vesicles can then fuse with the outer membrane of other Gram-negative bacteria enabling them to communicate, obtain virulence factors, pick up resistance genes, or deliver toxins to human cells.

Fig. 2B: Illustration of the Structure of a Gram-Negative Cell Wall.



The Gram-negative cell wall is composed of a thin, inner layer of peptidoglycan and an outer membrane consisting of molecules of phospholipids, lipopolysaccharides (LPS), lipoproteins and surface proteins. The lipopolysaccharide consists of lipid A and O polysaccharide.

3. The **surface proteins (see Fig. 2B)** in the bacterial peptidoglycan, depending on the strain and species, carry out a variety of activities.

a. Some surface proteins function as **enzymes**.

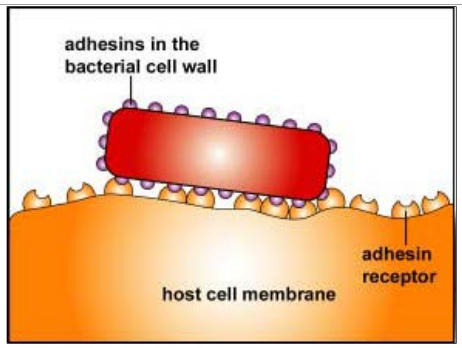
b. Other proteins serve as **adhesins**. Adhesins enable the bacterium to **adhere intimately to host calls** and other surfaces in order to colonize those cells and resist flushing **(See Fig. 3**).

c. Many bacteria involved in infection have the ability to **co-opt the functions of host cells for the bacterium's own benefit**. This is done by way of bacterial secretions systems that enable the bacterium to directly inject bacterial effector molecules into the cytoplasm of the host cell in order to alter its cellular machinery or cellular communication to the benefit of the bacteria. They do this by producing secretion systems such as the type 3 secretion system that produces hollow, needle-like tubes called injectisomes. Certain bacteria, for example, inject invasins into the cytoplasm of the host cell that enable the bacterium to **enter that cell.**

The role of these cell wall surface proteins will be discussed in greater detail later in Unit 3 under Bacterial Pathogenicity.

4. The periplasm contains **enzymes for nutrient breakdown as well as periplasmic binding protein**s to facilitate the transfer of nutrients across the cytoplasmic membrane.

Fig. 3: Bacterial Adhesins



Surface proteins called adhesins in the bacterial cell wall bind to receptor molecules on the surface of a susceptible host cell enabling the bacterium to make intimate contact with the host cell, adhere, colonize, and resist flushing.

Flash animation showing a bacterium using adhesins to adhere to a host cell.

html5 version of animation for iPad showing a bacterium using adhesins to adhere to a host cell.

Surface proteins called adhesins in the bacterial cell wall bind to receptor molecules on the surface of a susceptible host cell enabling the bacterium to make intimate contact with the host cell, adhere, colonize, and resist flushing.

Flash Animation showing a bacterium using adhesins to resist being flushed out of the urethra.

html5 version of animation for iPad showing a bacterium using adhesins to resist being flushed out of the urethra.

Surface proteins called adhesins in the bacterial cell wall bind to receptor molecules on the surface of a susceptible host cell enabling the bacterium to make intimate contact with the host cell, adhere, colonize, and resist flushing.

Flash Animation showing a bacterium without adhesins being flushed out of the urethra

html5 version of animation for iPad showing a bacterium without adhesins being flushed out of the urethra.

Without the necessary cell wall adhesins, the bacteria are flushed from the body.

Flash animation showing bacteria injecting invasins into a non-immune host cells in order to enter that cell by phagocytosis.

html5 version of animation for iPad showing bacteria secreting invasions into a non-immune host cell in order to enter that cell by phagocytosis.

Some bacteria produce molecules called invasins that activate the host cell's cytoskeletal machinery enabling bacterial entry into the cell by phagocytosis. By entering the cytoplasm of the host cell, it has a ready supply of nutrients and is able to protect the bacteria from complement, antibodies, and certain other body defenses. Many bacteria use injectosomes, such as a type 3 secretion system, to inject effector molecules into the host cell's cytoplasm.

For more information: Preview of the ability to adhere to host cells

For more information: Preview of the ability to invade host cells

Concept map for the Gram-negative cell wall.

TPS Questions

Sorting Activity

C. Significance of Gram-Negative Cell Wall Components to the Initiation of Body Defenses

The body has two immune systems: **the innate immune system** and the **adaptive immune syste**m.

1. **Innate immunity** is an **antigen-nonspecific** defense mechanisms that a host uses **immediately or within several hours after exposure to almost any microb**e. This is the immunity one is born with and is the initial response by the body to eliminate microbes and prevent infection.

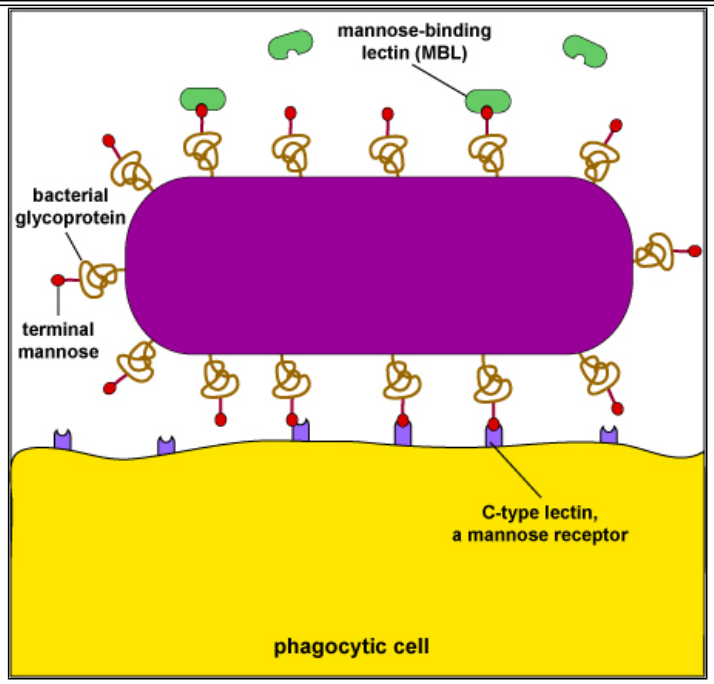
2. **Adaptive (acquired) immunity** refers to **antigen-specific** defense mechanisms that **take several days to become protective** and are designed to react with and remove a specific antigen. This is the immunity one develops throughout life.

Initiation of Innate Immunity

In order to protect against infection, one of the things the body must initially do is detect the presence of microorganisms. The body does this by **recognizing molecules unique to microorganisms that are not associated with human cells**. These unique molecules are called **pathogen-associated molecular patterns** or PAMPs. (Because all microbes, not just pathogenic microbes, possess PAMPs, pathogen-associated molecular patterns are sometime referred to as microbe-associated molecular patterns or MAMPs.)

LPS, porins, and fragments of peptidoglycan are PAMPs associated with the cell wall of Gram-negative bacteria. In addition, bacteria and other microorganisms also possess **mannose-rich glycans** (short carbohydrate chains with the sugar mannose or fructose as the terminal sugar) that function as PAMPs. These mannose-rich glycans are common in microbial glycoproteins and glycolipids but rare in those of humans **(see Fig. 4)**.

Fig. 4: Recognition of Bacteria Mannose by Mannose Receptors.



Mannose-rich glycans are short carbohydrate chains with the sugar mannose or fructose as the terminal sugar. They are commonly found in microbial glycoproteins and glycolipids but are rare in those of humans. (Human glycoproteins and glycolipids typically have terminal N-acetylglucosamine and sialic acid groups.) C-type lectins, found on the surface of phagocytes, are endocytic pattern recognition receptors that bind to mannose-rich glycans in order to attach microbes to phagocytes. Mannose-binding lectin (MBL), also known as mannan-binding protein, is a soluble pattern recognition receptor in plasma and tissue fluid that binds to mannose-rich glycans on microbes in order to activate the lectin complement pathway.

These PAMPS bind to **pattern-recognition receptors or PRRs** on a variety of defense cells of the body and **trigger such innate immune defenses as inflammation, fever, and phagocytosis**.

For more information: Preview of pathogen-associated molecular patterns (PAMPs)

For more information: Preview of pattern-recognition receptors (PRRs)

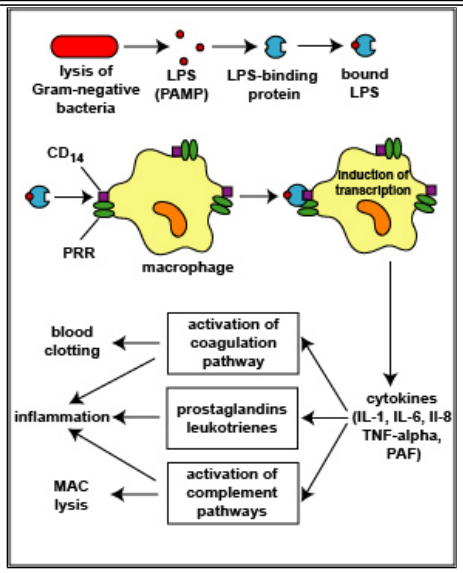
Inflammation is the first response to infection and injury and is critical to body defense. Basically, the inflammatory response is an attempt by the body to restore and maintain homeostasis after injury. **Most of the body defense elements are located in the blood, and inflammation is the means by which body defense cells and body defense chemicals leave the blood and enter the tissue around an injured or infected site**.

Body defense cells such as macrophages, and dendritic cells have pattern recognition receptors such as toll-like receptors on their surface that are specific for the peptidoglycan fragments and LPS in the Gram-negative cell wall and/or to NODs in their cytoplasm that are specific for peptidoglycan fragments.

The binding of these cell wall components to their corresponding pattern recognition receptors triggers the macrophages to release various defense regulatory chemicals called cytokines, including IL-1, IL-6, IL-8, TNF-alpha, and PAF. The cytokines then bind to cytokine receptors on target cells and initiate inflammation and activate both the complement pathways and the coagulation pathway **(see Fig. 54)**.

The LPS binds to a LPS-binding protein circulating in the blood and this complex, in turn, binds to a receptor molecule (CD14) found on the surface of body defense cells called macrophages. This is thought to promote the ability of the toll-like receptor pair TLR-4/TLR4 to respond to the LPS. The binding of these cell wall components to their corresponding pattern recognition receptors triggers macrophages to release various defense regulatory chemicals called cytokines, including IL-1, IL-6, IL-8, TNF-alpha, and PAF. The cytokines then bind to cytokine receptors on target cells and initiate inflammation and activate both the complement pathways and the coagulation pathway **(see Fig. 5)**.

Fig. 5: Physiologic Action of Lipopolysaccharide (LPS) from the Gram-Negative Cell Wall



The lysis of Gram-negative bacteria causes them to release PAMPs such as lipopolysaccharide (LPS; endotoxin) from the outer membrane of their cell wall. The LPS binds to a LPS-binding protein circulating in the blood and this complex, in turn, binds to a receptor molecule (CD14) found on the surface of body defense cells called macrophages. This is thought to promote the ability of the pattern recognition receptor (PRR) called TLR-4 to respond to the LPS, triggering the macrophage to release various defense regulatory chemicals called cytokines, including IL-1, IL-6, IL-8, TNF-alpha, and PAF. The cytokines then bind to cytokine receptors on target cells and initiate inflammation and activate both the complement pathways and the coagulation pathway. LPS can also bind directly to TLR-4 molecules.

(LPS, lipopolysaccharide;TLR, toll-like receptor; IL-1, interleukin-1; IL-6, interleukin-6; IL-8, interleukin-8, TNF-alpha, tumor necrosis factor-alpha; PAF, platelet-activating factor.) This will be discussed in greater detail under Bacterial Pathogenicity.

Flash animation showing the release of LPS from the cell wall of a Gram negative bacterium and its subsequent binding to pattern-recognition receptors on a macrophage.

html5 version of animation for iPad showing the release of LPS from the cell wall of a Gram negative bacterium and its subsequent binding to pattern-recognition receptors on a macrophage.

1) Gram-negative bacteria release lipopolysaccharide (LPS; endotoxin) from the outer membrane of their cell wall.
2) The LPS binds to a pair of TLR-4s on defense cells such as macrophages and dendritic cells. LPS also binds to LPS-binding protein in the plasma and tissue fluid. The LPS-binding protein promotes the binding of LPS to the CD14 receptors. At that point the LPS-binding protein comes off and the LPS-CD14 bind to TLR-4.
3) The binding of LPS to TLR-4 enables regulatory molecules within the cell - Mal, MyD88, Tram, and Trif - to trigger reactions that activate a master regulator of inflammation called NF-kappa B. Activated NF-kappa B enters the cell's nucleus and switches on genes coding for cytokines such as:

a. Interleukin-1 (IL-1) and Tumor necrosis factor-alpha (TNF-alpha): enhance inflammatory responses;
b. Interleukin-8 (IL-8): aids in the ability of white blood cells to leave the blood vessels and enter the tissue; a chemoattractant for phagocytes;
c. Interleukin-6 (IL-6) promotes B-lymphocyte activity; and
d. Interleukin-12 (IL-12): promotes T-lymphocyte activity. (5)

4) Cytokine genes are transcribed into mRNA molecules that goes to the cytoplasm to be translated into inflammatory cytokines that are subsequently secreted from the cell.

For more information: Preview of cytokines

For More Information: Preview of Inflammation

Concept map for the Gram-negative cell wall.

The LPS aso **activates the alternative complement pathway and the lectin pathway**, defense pathways that play a variety of roles in body defense.

For more information: Preview of the complement pathways

Innate immunity will be discussed in greater detail in Unit 5.

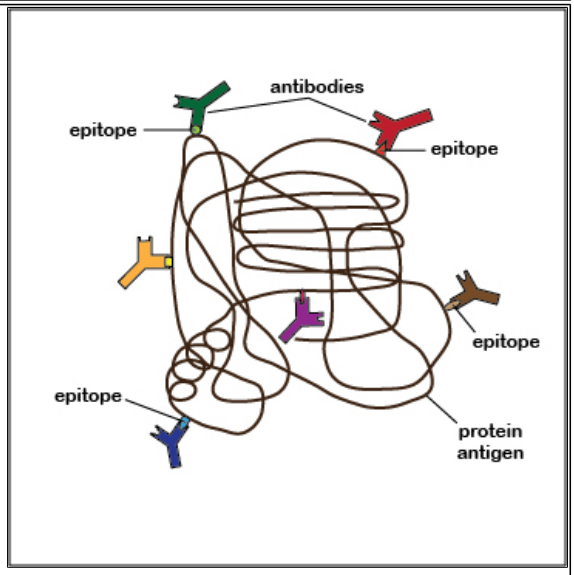
Concept map for the Gram-positive cell wall.

Initiation of Adaptive Immunity

Proteins and polysaccharides associated with the Gram-negative cell wall function as antigens and initiate adaptive immunity. An **antigen** is defined as a **molecular shape that reacts with antibody molecules and with antigen receptors on lymphocytes**. We recognize those molecular shapes as foreign or different from our body's molecular shapes because they fit specific antigen receptors on our B-lymphocytes and T-lymphocytes, **the cells that carry out adaptive immunity**.

The actual portions or fragments of an antigen that react with antibodies and with receptors on B-lymphocytes and T-lymphocytes are called epitopes. An epitope is typically a group of 5-15 amino acids with a unique shape that makes up a portion of a protein antigen (**see Fig. 6A**) , or 3-4 sugar residues branching off of a polysaccharide antigen (**see Fig. 6B**). **A single microorganism has many hundreds of different shaped epitopes that our lymphocytes can recognize as foreign and mount an adaptive immune response against**.

Fig. 6A: Illustration of Epitopes of a Protein Antigen



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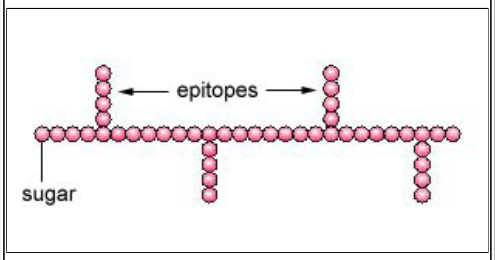
Proteins have many epitopes of different specificities. During humoral immunity, antibodies are made to fit each epitope of each antigen.

Fig. 6B: Illustration of Epitopes of a Polysaccharide Antigen



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The body recognizes an antigen as foreign when epitopes of that antigen bind to B-lymphocytes and T-lymphocytes by means of epitope-specific receptor molecules having a shape complementary to that of the epitope. The epitope receptor on the surface of a B-lymphocyte is called a B-cell receptor and is actually an antibody molecule. The receptor on a T-lymphocyte is called a T-cell receptor (TCR).

There are two major branches of the adaptive immune responses: humoral immunity and cell-mediated immunity.

1. **Humoral immunity**: Humoral immunity involves the production of **antibody molecules** in response to an antigen and is mediated by B-lymphocytes. Through a variety of mechanisms, these antibodies are able to remove or neutralize microorganisms and their toxins after binding to their epitopes. For example, antibodies made against cell wall antigens can **stick bacteria to phagocytes**, a process called opsonization. Antibodies made against cell wall adhesins can **prevent bacteria from adhering to and colonizing host cells**.

Flash Animation of Phagocytosis by Opsonizing Antibodies

The Fab portion of IgG binds to epitopes of a microbe. The Fc portion can now attach the microbe to Fc receptors on phagocytes for enhanced attachment, also known as opsonization. Once attached to the phagocyte by way of IgG, the

microbe can be engulfed more efficiently and placed in a phagosome, and destroyed by lysosomes.

html5 version of animation for iPad showing opsonization by way of IgG.

Flash Animation of Antibodies Blocking Bacterial Adherance

The Fab portion of the antibodies made against epitopes of adherence structures such as cell wall adhesins bind and block the bacteria from adhering to receptors on the host cell membrane. As a result, the bacteria are unable to colonize and may be flushed away.

html5 version of animation for iPad showing antibodies blocking bacterial adherence to host cell

2. **Cell-mediated immunity**: Cell-mediated immunity involves the **production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines** in response to an antigen and is mediated by T-lymphocytes. These defense cells help to **remove infected cells** and **cancer cells** displaying foreign epitopes.

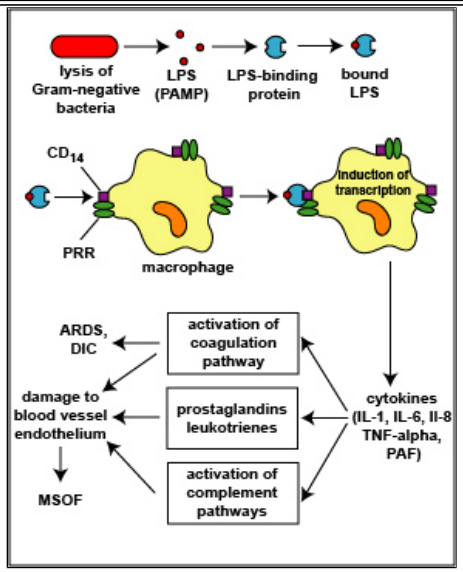
Adaptive immunity will be discussed in greater detail in Unit 6.

DragNDrop Activity

D. Significance of Gram-Negative Cell Wall Components to Bacterial Pathogenicity

The lipid A portion of the LPS portion in the outer membrane is also known as **endotoxin**. During severe systemic infections with large numbers of bacteria present, high levels of LPS are released resulting in excessive cytokine production by the macrophages and other cells and this, in turn, can harm the body **(see Fig. 7)**.

Fig. 7: Harmful Effects of Lipopolysaccharide (LPS; Endotoxin) Released from the Gram-Negative Cell Wall



The lysis of Gram-negative bacteria causes them to release PAMPs such as lipopolysaccharide (LPS; endotoxin) from the outer membrane of their cell wall. The LPS binds to a LPS-binding protein circulating in the blood and this complex, in turn, binds to a receptor molecule (CD14) found on the surface of body defense cells called macrophages. This is thought to promote the ability of the pattern recognition receptor (PRR) called TLR-4 to respond to the LPS, triggering the macrophage to release various defense regulatory chemicals called cytokines, including IL-1, IL-6, IL-8, TNF-alpha, and PAF. The cytokines then bind to cytokine receptors on target cells stimulating the production of inflammatory mediators such as prostaglandins and leukotrienes as well as activating both the complement pathways and the coagulation pathway. Excessive production of clotting factors may lead to ARDS and DIC while an overproduction of prostaglandins, leukotrienes, and complement proteins can damage the vascular endothelium resulting in shock and MSOF.

(LPS, lipopolysaccharide; IL-1, interleukin-1; IL-6, interleukin-6; IL-8, interleukin-8, TNF-alpha, tumor necrosis factor-alpha; PAF, platelet-activating factor; ARDS, acute respiratory distress syndrome; DIC, disseminated intravascular coagulation;

MSOF, multiple system organ failure.)

Concept map for the Gram-negative cell wall.

For More Information: Preview of Inflammatory Gram-Negative Cell Wall Components

For More Information: Preview of Cytokines

For More Information: Preview of Inflammation

Self Check

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Salmonella* species
- *Shigella* species
- *Neisseria gonorrhoeae*
- *Neisseria meningitidis*
- *Haemophilus influenzae*
- *Escherichia coli*
- *Klebsiella* species
- *Proteus* species
- *Pseudomonas aeruginosa*

Self Quiz for the Gram-Negative Cell Wall

Quiz Group

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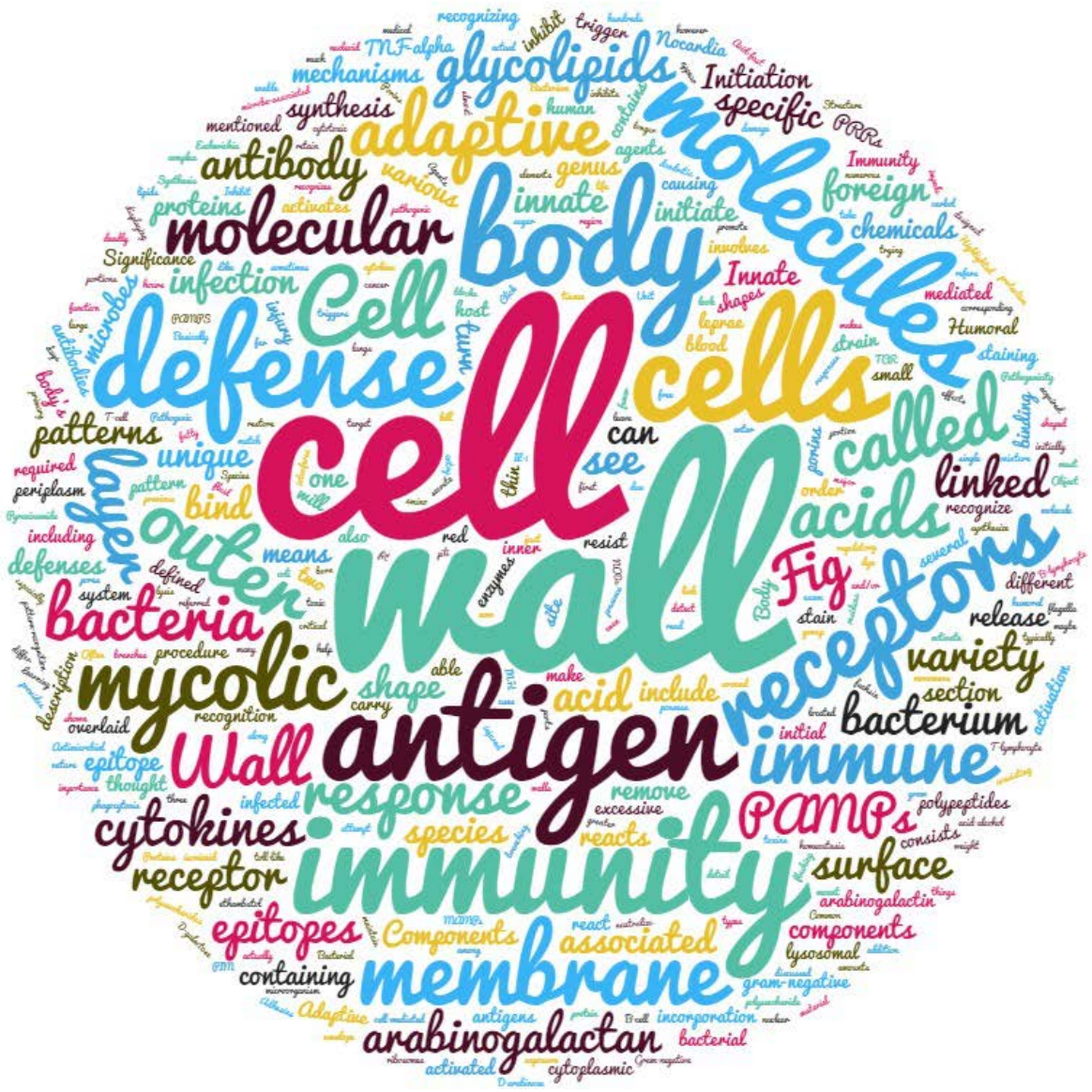
Back to Softchalk Lessons Table of Contents

The acid-fast cell envelope in bacteria
THE ACID-FAST CELL WALL

The Acid-Fast Cell Wall (Cell Envelope)



Fundamental Statements for this Lesson:

1. Because of the nature of their cell wall, acid-fast bacteria stain red after acid-fast staining.
2. The genus Mycobacterium and the genus Nocardia are among the few bacteria possessing an acid-fast cell wall.
3. The acid-fast cell wall consists of a thin, inner layer of peptidoglycan linked to a layer of arabinogalactin, which in turn is linked to an outer membrane containing mycolic acids and overlaid with a variety of polypeptides and glycolipids.
4. Porins are required to transport small hydrophilic molecules through the outer membrane of the acid-fast cell wall.
5. The acid-fast cell wall activates both the body's innate immune defenses and its adaptive immune defenses.
6. The body activates innate immunity by recognizing molecules unique to microorganisms that are not associated with human cells called pathogen-associated molecular patterns or PAMPs. PAMPs bind to Pattern-recognition receptors (PRRs) on defense cells to trigger the production of inflammatory cytokines.
7. Inflammation is the means by which the body delivers defense cells and defense molecules to an infection site, however, excessive inflammation, can be harmful and even deadly to the body.
8. PAMPs associated with the acid-fast cell wall include peptidoglycan monomers, arabinogalactin, and mycolic acids.
9. Cell wall molecules can also trigger adaptive immunity such as the production of antibody molecules against the bacterium.
10. A few antimicrobial chemotherapeutic agents inhibit acid-fast cell wall synthesis

Common Course Objectives

1. Identify the parts of a bacterium and their physiological purpose.
2. Explain how various bacterial structures can contribute to bacterial colonization of a host.
3. Explain how various bacterial structures can contribute to the initiation of immune defenses
4. Explain the difference between a Gram-positive cell wall, a Gram-negative cell wall, and an acid-fast cell wall.
5. Provide a diagnosis for a "patient" when given a list of symptoms and a description of the bacterium.

Detailed Learning Objectives for this Lesson

1. State what color acid-fast bacteria stain after the acid-fast stain procedure and briefly describe why.

2**. Describe the composition of an acid-fast cell wall and indicate the beneficial function to the bacterium of peptidoglycan, arabinogalactin, mycolic acid, and porins.

(**) = More depth and common theme

Highlighted Bacterium

1. Read the description of *Mycobacterium tuberculosis* and match the bacterium with the description of the organism and the infection it causes.

TPS Question 1

TPS Question 2

In this section on Prokaryotic Cell Anatomy we are looking at the various anatomical parts that make up a bacterium. As mentioned in the introduction to this section, a typical bacterium usually consists of:

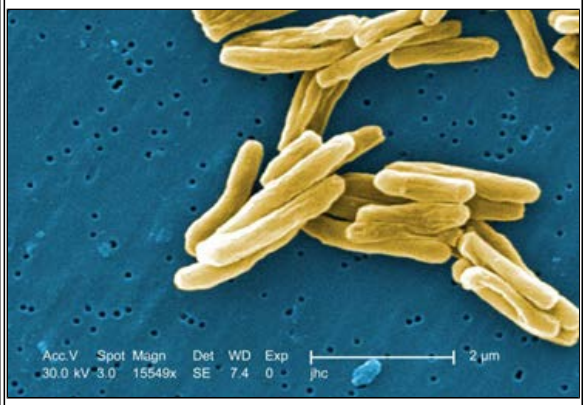
- a cytoplasmic membrane surrounded by a peptidoglycan cell wall and maybe an outer membrane;
- a fluid cytoplasm containing a nuclear region (nucleoid) and numerous ribosomes; and
- often various external structures like a glycocalyx, flagella, and pili.

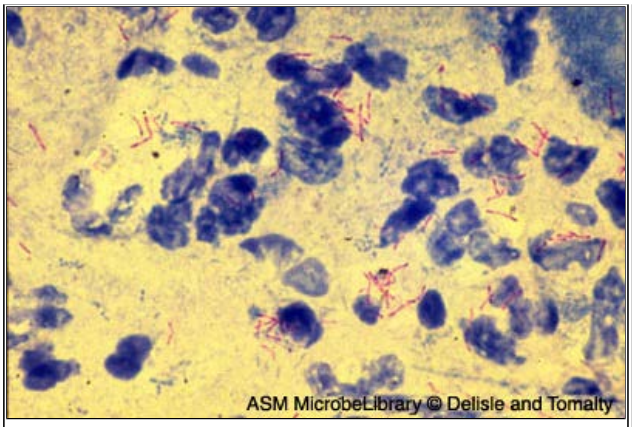
We will now look at the acid-fast bacterial cell wall.

The Acid-Fast Cell Wall (Cell Envelope)

Acid-fast bacteria stain poorly with the Gram stain procedure, **appearing weakly Gram-positive or Gram-variable**. They are usually characterized using the acid-fast staining procedure. As mentioned in the previous section on peptidoglycan, bacteria with an acid-fast cell wall **resist decolorization with an acid-alcohol mixture during the acid-fast staining procedure** , **retain the initial dye carbol fuchsin and appear red**. Common acid-fast bacteria of medical importance include *Mycobacterium tuberculosis* **(See Fig. 1A and 1B)**, *Mycobacterium leprae, Mycobacterium avium-intracellulare* complex, and *Nocardia* species.

Fig. 1A: Scanning electron micrograph of Mycobacterium tuberculosis. Image provided by Dr. Ray Butler and Janice Carr. Courtesy of the Centers for Disease Control and Prevention.	**Fig. 1B: Photomicrograph showing a positive acid-fast stain of Mycobacterium tuberculosis in sputum.**





Note the reddish acid-fast bacilli among the blue normal flora and white blood cells in the sputum that are not acid-fast.

Preview of the Acid-Fast Stain from Lab 16.

Self Check

A. Structure and Composition of the Acid-Fast Cell Wall

The term cell envelope is often used to indicate all the various layers that envelope a bacterial cell. In terms of acid-fast bacteria, the cell envelope would include the cytoplasmic membrane, the peptidoglycan cell wall, the arabinogalactan layer, the mycolic acid-containing outer membrane, and if present, a capsule or glycocalyx. In this section we will be concerned with just the cell wall and outer membrane of acid-fast bacteria.

Acid-fast bacteria are Gram-positive, but in addition to **peptidoglycan**, the outer membrane or envelope of the acid-fast cell wall of contains **large amounts of glycolipids, especially mycolic acids** that in the genus *Mycobacterium*, make up approximately 60% of the acid-fast cell wall.

1. The **acid-fast cell wall** of Mycobacterium has a **thin, inner layer of peptidoglycan (see Fig. 2)**.

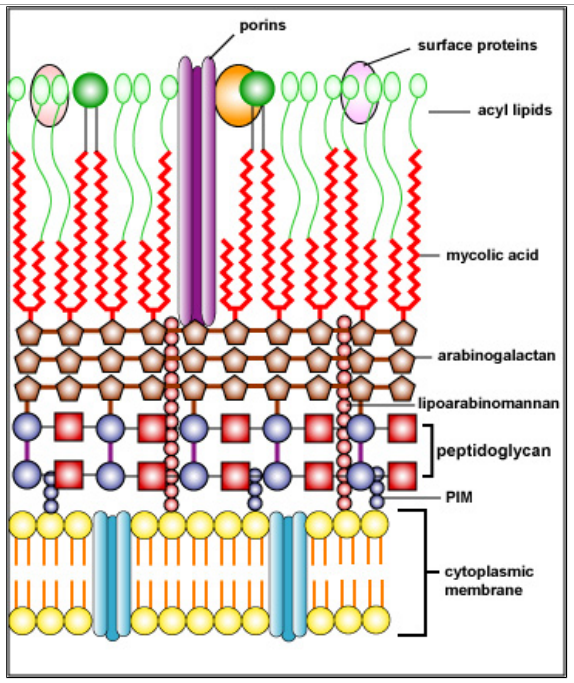
2. The peptidoglycan layer is, in turn, linked to **arabinogalactan** (D-arabinose and D-galactose) as shown in **Fig. 2**.

3. The arabinogalactan is then linked to an outer membrane containing high-molecular weight mycolic acids. The **arabinogalactan/mycolic acid layer** is overlaid with a **layer of polypeptides and mycolic acids** consisting of free lipids, glycolipids, and peptidoglycolipids. Other glycolipids include lipoarabinomannan and phosphatidyinositol mannosides (PIM). Like the outer membrane of the Gram-negative cell wall, **porins are required to transport small hydrophilic molecules through the outer membrane of the acid-fast cell wall (see Fig. 2)**.

4. The **outer surface of the acid-fast cell wall is studded with surface proteins** that differ with the strain and species of the bacterium.

5. The **periplasm** is the gelatinous material between the peptidoglycan and the cytoplasmic membrane.

Fig.2: Structure of an Acid-Fast Cell Wall



In addition to peptidoglycan, the acid-fast cell wall of *Mycobacterium* contains a large amount of glycolipids, especially mycolic acids. The peptidoglycan layer is linked to arabinogalactan (D-arabinose and D-galactose) which is then linked to high-molecular weight mycolic acids. The arabinogalactan/mycolic acid layer is overlaid with a layer of polypeptides and mycolic acids consisting of free lipids, glycolipids, and peptidoglycolipids. Other glycolipids include lipoarabinomannan and phosphatidyinositol mannosides (PIM). Like the outer membrane of the gram-negative cell wall, porins are required to transport small hydrophilic molecules through the outer membrane of the acid-fast cell wall. Because of its unique cell wall, when it is stained by the acid-fast procedure, it will resist decolorization with acid-alcohol and stain red, the color of the initial stain, carbol fuchsin. With the exception of a very few other acid-fast bacteria such as *Nocardia*, all other bacteria will be decolorized and stain blue, the color of the methylene blue counterstain.

Concept map for the acid-fast cell wall.

B. Functions of the Acid-Fast Cell Wall Components

1. The **peptidoglycan** prevents osmotic lysis.

For more information: Review of peptidoglycan

2. The **arabinogalactan layer** is **linked to both the peptidoglycan and to the mycolic acid outer membrane** and probably **provides additional strengt**h to the cell wall.

3. The **mycolic acids and other glycolipids** also **impede the entry of chemicals** causing the organisms to **grow slowly** and be **more resistant to chemical agents and lysosomal components of phagocytes than most bacteria** (see Fig. 2). There are **far fewer porins** in the acid-fast cell wall compared to the gram-negative cell wall and the **pores are much longer**. This is thought to **contribute significantly to the lower permeability of acid-fast bacteria**.

4. The **surface proteins** in the acid-fast cell wall, depending on the strain and species, carry out a variety of activities.

   a. Functioning as **enzymes**.

   b. Serving as adhesins. Adhesins enable the bacterium to **adhere intimately to host cells** and other surfaces in order to colonize and resist flushing.

5. The periplasm contains **enzymes for nutrient breakdown**.

Concept map for the acid-fast cell wall.

TPS Question 1

Sorting Activity

C. Significance of Acid-Fast Cell Wall Components to the Initiation of Body Defenses

The body has two immune systems: **the innate immune system** and the **adaptive immune syste**m.

1. **Innate immunity** is an **antigen-nonspecific** defense mechanisms that a host uses **immediately or within several hours after exposure to almost any microb**e. This is the immunity one is born with and is the initial response by the body to eliminate microbes and prevent infection.

2. **Adaptive (acquired) immunity** refers to **antigen-specific** defense mechanisms that **take several days to become protective** and are designed to react with and remove a specific antigen. This is the immunity one develops throughout life.

Initiation of Innate Immunity

In order to protect against infection, one of the things the body must initially do is detect the presence of microorganisms. The body does this by **recognizing molecules unique to microorganisms that are not associated with human cells**. These unique molecules are called **pathogen-associated molecular patterns or** PAMPs. Pathogenic *Mycobacterium* species such as *Mycobacterium tuberculosis* and *Mycobacterium leprae* release **mycolic acid, arabinogalactan, and peptidoglycan fragments** from their acid-fast cell wall. (Because all microbes, not just pathogenic microbes, possess PAMPs, pathogen-associated molecular patterns are sometime referred to as microbe-associated molecular patterns or MAMPs.)

These PAMPS bind to pattern-recognition receptors or PRRs on a variety of defense cells of the body causing them to synthesize and secrete a variety of proteins called cytokines. These cytokines can, in turn promote innate immune defenses such as inflammation, phagocytosis, activation of the complement pathways, and activation of the coagulation pathway.

For more information: Preview of pathogen-associated molecular patterns (PAMPs)

For more information: Preview of pattern-recognition receptors (PRRs)

Inflammation is the first response to infection and injury and is critical to body defense. Basically, the inflammatory response is an attempt by the body to restore and maintain homeostasis after injury. **Most of the body defense elements are located in the blood, and inflammation is the means by which body defense cells and body defense chemicals leave the blood and enter the tissue around an injured or infected site**.

Body defense cells called macrophages, and dendritic cells **have pattern recognition receptors such as toll-like receptors on their surface that are specific for the peptidoglycan fragments and mycolic acids in the acid-fast cell wall and/or to NODs in their cytoplasm that are specific for peptidoglycan fragments**. The binding of these cell wall components to their corresponding pattern recognition receptors triggers the macrophages to **release various defense regulatory chemicals called** cytokines, including IL-1 and TNF-alpha. The cytokines then bind to cytokine receptors on target cells and **initiate inflammation and activate both the complement pathways and the coagulation pathway**.

Innate immunity will be discussed in greater detail in Unit 5.

For more information: Preview of cytokines

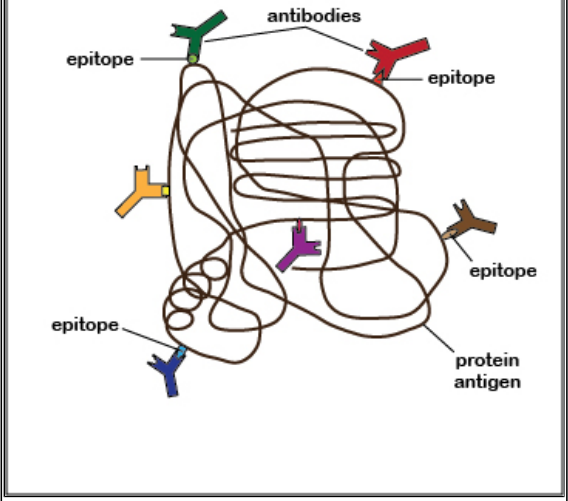
For more information: Preview of inflammation

Initiation of Adaptive Immunity

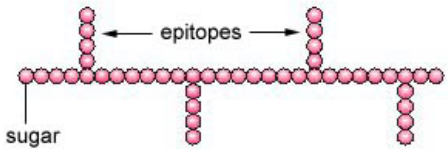
Proteins and polysaccharides associated with the acid-fast cell wall function as antigens and initiate adaptive immunity. An **antigen** is defined as **a molecular shape that reacts with antibody molecules and with antigen receptors on lymphocytes**. We recognize those molecular shapes as foreign or different from our body's molecular shapes because they fit specific antigen receptors on our B-lymphocytes and T-lymphocytes, **the cells that carry out adaptive immunity**.

The actual portions or fragments of an antigen that react with antibodies and with receptors on B-lymphocytes and T-lymphocytes are called epitopes. An **epitope** is typically a group of 5-15 amino acids with a unique shape that makes up a portion of a protein antigen (**see Fig. 3A**), or 3-4 sugar residues branching off of a polysaccharide antigen (**see Fig. 3B**). **A single microorganism has many hundreds of different shaped epitopes that our lymphocytes can recognize as foreign and mount an adaptive immune response against**.

Fig. 3A: Illustration of Epitopes of a Protein Antigen	Fig. 3A: Illustration of Epitopes of a Protein Antigen



Proteins have many epitopes of different specificities. During humoral immunity, antibodies are made to fit each epitope of each antigen.



The body recognizes an antigen as foreign when epitopes of that antigen bind to B-lymphocytes and T-lymphocytes by means of epitope-specific receptor molecules having a shape complementary to that of the epitope. The epitope receptor on the surface of a B-lymphocyte is called a B-cell receptor and is actually an antibody molecule. The receptor on a T-lymphocyte is called a T-cell receptor (TCR).

There are two major branches of the adaptive immune responses: humoral immunity and cell-mediated immunity.

1. **Humoral immunity**: Humoral immunity involves the production of **antibody molecules** in response to an antigen and is mediated by B-lymphocytes. Through a variety of mechanisms, these antibodies are able to remove or neutralize microorganisms and their toxins after binding to their epitopes.

2. **Cell-mediated immunity**: Cell-mediated immunity involves the **production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines** in response to an antigen and is mediated by T-lymphocytes. These defense cells help to **remove infected cells and cancer cells** displaying foreign epitopes.

Adaptive immunity will be discussed in greater detail in Unit 6.

Concept map for the acid-fast cell wall.

DragNDrop Activity

D. Significance of Acid-Fast Cell Wall Components to Bacterial Pathogenicity

Most of the damage in the lungs during tuberculosis is thought to be due to the **inflammatory effects from excessive TNF-alpha production**, along with the **release of toxic lysosomal components of the macrophages** trying to kill the *Mycobacterium tuberculosis*.

Highlighted Bacterium: *Mycobacterium tuberculosis*

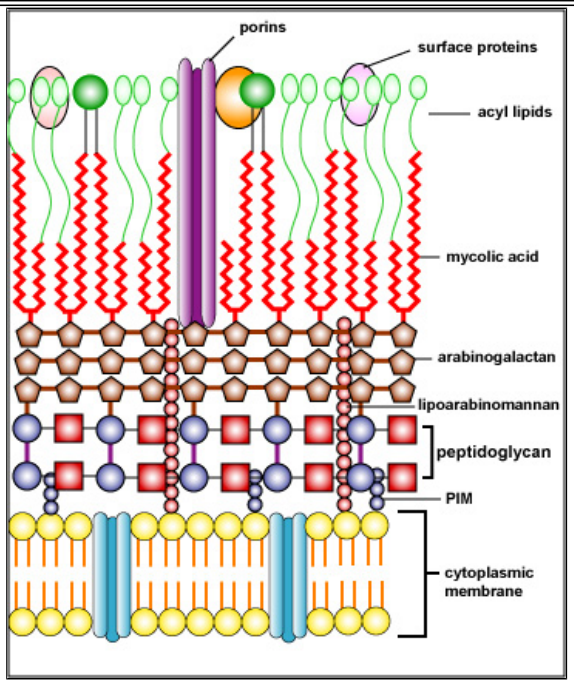
Click on this link, read the description of *Mycobacterium tuberculosis*, and be able to match the bacterium with its description on an exam.

Self Check

E. Antimicrobial Agents that Inhibit Acid-Fast Cell Wall Synthesis to Control *Mycobacterium* Species

INH (isoniazid) blocks the incorporation of mycolic acid into acid-fast cell walls while ethambutol interferes with the incorporation of arabinoglactan (**see Fig. 2**) . Both inhibit synthesis of the acid-fast cell wall. Pyrazinamide inhibits fatty acid synthesis in *Mycobacterium tuberculosis.*

Fig.2: Structure of an Acid-Fast Cell Wall



In addition to peptidoglycan, the acid-fast cell wall of *Mycobacterium* contains a large amount of glycolipids, especially mycolic acids. The peptidoglycan layer is linked to arabinogalactan (D-arabinose and D-galactose) which is then linked to high-molecular weight mycolic acids. The arabinogalactan/mycolic acid layer is overlaid with a layer of polypeptides and mycolic acids consisting of free lipids, glycolipids, and peptidoglycolipids. Other glycolipids include lipoarabinomannan and phosphatidyinositol mannosides (PIM). Like the outer membrane of the gram-negative cell wall, porins are required to transport small hydrophilic molecules through the outer membrane of the acid-fast cell wall. Because of its unique cell wall, when it is stained by the acid-fast procedure, it will resist decolorization with acid-alcohol and stain red, the color of the initial stain, carbol fuchsin. With the exception of a very few other acid-fast bacteria such as *Nocardia*, all other bacteria will be decolorized and stain blue, the color of the methylene blue counterstain.

Concept map for the acid-fast cell wall.

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Mycobacterium tuberculosis*
- *Mycobacterium leprae*
- *Mycobacterium avium-intracellulare* complex

TPS Question 2

Self Quiz for the Acid-Fast Cell Wall

Quiz Group

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Bacterial cytoplasm
THE CYTOPLASM

In this section on Prokaryotic Cell Anatomy we are looking at the various anatomical parts that make up a bacterium. A typical bacterium usually consists of:

- a cytoplasmic membrane surrounded by a peptidoglycan cell wall and maybe an outer membrane;
- a fluid cytoplasm containing a nuclear region (nucleoid) and numerous ribosomes; and
- often various external structures such as a glycocalyx, flagella, and pili.

We will now look at the cytoplasm.

The Cytoplasm

Fundamental Statements for this Lesson:

1. In bacteria, the cytoplasm refers to anything enclosed by the cytoplasmic membrane.
2. The liquid portion of the cytoplasm is called the cytosol.
3. The cytoplasm is the site of most bacterial metabolism.
4. During catabolic reactions larger molecules are broken down to obtain cellular building block molecules and energy; during anabolic reactions cellular molecules and macromolecules are synthesized.

Common Course Objectives

1. Identify the parts of a bacterium and their physiological purpose.

Detailed Learning Objectives

1. Define the following:
   a. exoenzymes
   b. endoenzymes.
   c. cytosol
2. State the primary function of the bacterial cytoplasm.
3. Define the following:
   a. metabolism
   b. catabolic reactions
   c. anabolic reactions.

The Cytoplasm

A. Structure and Composition

In bacteria, the cytoplasm refers to everything enclosed by the cytoplasmic membrane. About 80% of the cytoplasm of bacteria is composed of water. Within the cytoplasm can be found nucleic acids (DNA and RNA), enzymes and amino acids, carbohydrates, lipids, inorganic ions, and many low molecular weight compounds. The liquid component of the cytoplasm is called the **cytosol**. Some groups of bacteria produce cytoplasmic inclusion bodies that carry out specialized cellular functions.

B. Functions

While bacteria secrete exoenzymes to hydrolyze macromolecules into smaller molecules capable of being transported across the cytoplasmic membrane, the cytoplasm is the **site of most bacterial metabolism**. This includes **catabolic reactions** in which molecules are broken down in order to obtain building block molecules for more complex cellular molecules and macromolecules, and **anabolic reactions** used to synthesize cellular molecules and macromolecules. The chemical reactions occurring within the bacterium are under the control of endoenzymes.

The various structural filaments in the cytoplasm collectively make up the prokaryotic **cytoskeleton**. Prokaryotic cells possess analogs for all of the cytoskeletal proteins found in eukaryotic cells, as well as cytoskeletal proteins with no eukaryotic homologues. Cytoskeletal filaments play essential roles in **determining the shape of a bacterium** (coccus, bacillus, or spiral) and are also **critical in the process of cell division** by binary fission and in determining bacterial polarity.

Self Quiz for the Cytoplasm

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The bacterial chromosome and the nucleoid
THE BACTERIAL NUCLEOID AND CHROMOSOME

The Nucleoid and Chromosome

Fundamental Statements for this Lesson:

1. The genome is the sum of an organism's genetic material.
2. Bacteria contain a single chromosome of double-stranded deoxyribonucleic acid (DNA).
3. The region of the bacterial cytoplasm where the chromosome is located and visible when viewed with an electron microscope called the nucleoid.
4. The bacterial chromosome is typically a physical and genetic circle, becomes supercoiled,and is not surrounded by a nuclear membrane.
5. Bacteria do not carry out mitosis or meiosis.
6. DNA topoisomerase enzymes are used to supercoil and relax the bacterial chromosome during DNA replication and transcription.
7. Like eukaryotic DNA, prokaryotic DNA replicates by sequential unwinding of the two DNA parent strands and the subsequent complementary base pairing of DNA nucleotides with each parent strand.
8. During DNA replication the nitrogenous base adenine forms hydrogen bonds with thymine and guanine forms hydrogen bonds with cytosine.
9. Genes located along the DNA are transcribed into RNA molecules, primarily messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA). Messenger RNA is then translated into protein at the ribosomes. (Transcription and translation are coupled in bacteria.)
10. During transcription, ribonucleic acid (RNA) is synthesized by complementary base pairing of ribonucleotides with deoxyribonucleotides to match a portion of one strand of DNA called a gene.
11. During translation, specific tRNA molecules pick up specific amino acids, transfer those amino acids to the ribosomes, and insert them in their proper place according to the mRNA "message."
12. Bacterial and viral genomes act as PAMPs to stimulate innate immunity.
13. Some antibacterial chemotherapeutic agents inhibiting normal nucleic acid replication in bacteria.

Common Course Objectives

1. Identify the parts of a bacterium and their physiological purpose.
2. Explain how various bacterial structures can contribute to bacterial colonization of a host.
3. Explain how various bacterial structures can contribute to the initiation of immune defenses.
4. Explain how certain parts of the bacteria can be targeted in order to kill the bacteria.
5. Summarize where and how transcription and translation occurs in prokaryotic cells.

Detailed Learning Objectives for this Lesson

1. Define genome.

2. Describe the composition of the bacterial chromosome.

3*. Name the enzymes that enables bacterial DNA to become circular, supercoiled, and unwind during DNA replication.

4*. Briefly describe the process of DNA replication.

5. State the function of the following enzymes in bacterial DNA replication:

   a. DNA polymerase III
   b. DNA polymerase II
   c. DNA helicase
   d. primase
   e. DNA ligase

6*. State the function of DNA.

7*. In terms of protein synthesis, briefly describe the process of transcription and translation.

8*. Briefly state how the following antibacterial chemotherapeutic agents affect bacteria:

   a. fluoroquinolones (norfloxacin, lomefloxacin, fleroxacin, ciprofloxacin, enoxacin, trovafloxacin, etc.)

   b. trimethoprim and sulfamethoxazole

   (*) = Common theme throughout the course

TPS Questions

In this section on Prokaryotic Cell Anatomy we are looking at the various anatomical parts that make up a bacterium. As mentioned in the introduction to this section, a typical bacterium usually consists of:

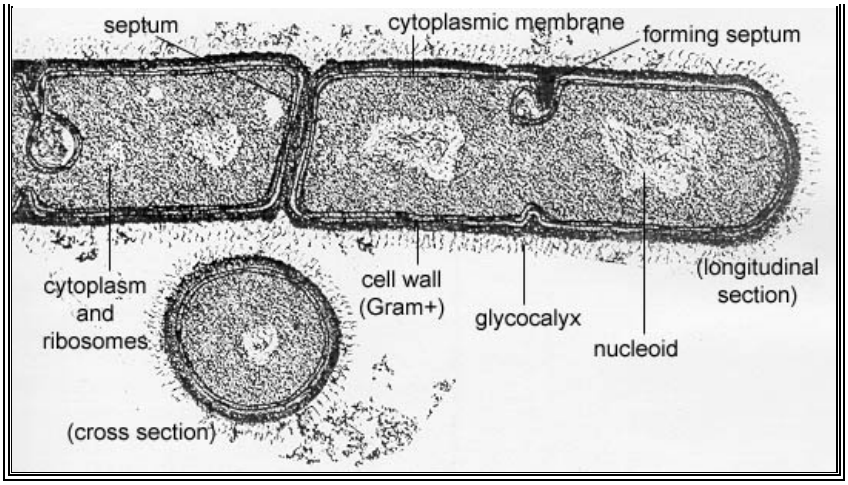
- a cytoplasmic membrane surrounded by a peptidoglycan cell wall and maybe an outer membrane;
- a fluid cytoplasm containing a nuclear region (nucleoid) and numerous ribosomes; and
- often various external structures like a glycocalyx, flagella, and pili.

We will now look at the bacterial chromosome located in the nuclear region called the nucleoid.

A. Structure and Composition of the Bacterial Chromosome

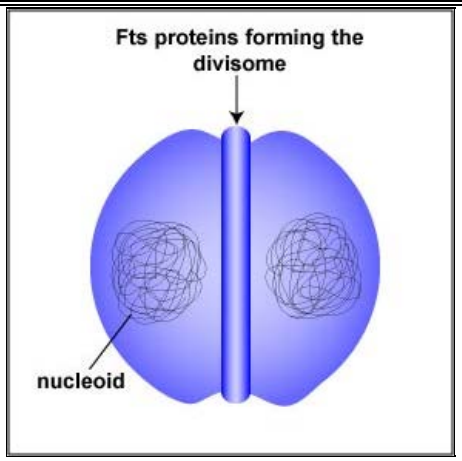
The term genome refers to the sum of an organism's genetic material. The bacterial genome is composed of a single molecule of **chromosomal deoxyribonucleic acid or DNA** and is located in a region of the bacterial cytoplasm visible when viewed with an electron microscope called the nucleoid. Unlike the eukaryotic nucleus, the bacterial nucleoid **has no** nuclear membrane **or** nucleoli **(see Fig. 1)**.

Fig .1: Transmission Electron Micrograph of a Prokaryotic Cell



In general it is thought that during DNA replication (discussed in Unit 6), each strand of the replicating bacterial DNA attaches to proteins at what will become the cell division plane. For example, Par proteins function to separate bacterial chromosomes to opposite poles of the cell during cell division. They bind to the origin of replication of the DNA and physically pull or push the chromosomes apart, similar to the mitotic apparatus of eukaryotic cells. **(See Fig. 2**).

Fig. 2: Bacterial Division



It is thought that during DNA replication, each strand of the replicating bacterial DNA attaches to proteins at what will become the cell division plane. For example, Par proteins function to separate bacterial chromosomes to opposite poles of the cell during cell division. They bind to the origin of replication of the DNA and physically pull or push the chromosomes apart, similar to the mitotic apparatus of eukaryotic cells.

In the center of the bacterium, a group of proteins called Fts (filamentous temperature sensitive) proteins interact to form a ring at the cell division plane. These proteins form the cell division apparatus known as the divisome and are directly involved in bacterial cell division by binary fission. The divisome is responsible for directing the synthesis of new cytoplasmic membrane and new peptidoglycan to form the division septum.

In the center of the bacterium, a group of proteins called Fts (filamentous temperature sensitive) proteins interact to form a ring at the cell division plane. These proteins form the cell division apparatus known as the **divisome** and are directly involved in bacterial cell division by binary fission. The divisome is responsible for directing the synthesis of new cytoplasmic membrane and new peptidoglycan to form the division septum.

Since bacteria are haploid, that is they have only one chromosome and only reproduce asexually, there is also **no** meiosis in bacteria.

For more information: Review of prokaryotic DNA replication

The bacterial chromosome is one long, single molecule of double stranded, helical, supercoiled DNA. In most bacteria, the two ends of the double-stranded DNA

covalently bond together to form both a physical and genetic circle. The chromosome is generally around 1000 µm long and frequently contains as many as 3500 genes **(see Fig. 2)**. *E. coli*, a bacterium that is 2-3 µm in length, has a chromosome approximately 1400 µm long.

To enable a macromolecule this large to fit within the bacterium, histone-like proteins bind to the DNA, segregating the DNA molecule into around 50 chromosomal domains and making it more compact. A group of enzymes called **DNA topoisomerases** then **supercoil each domain around itself, forming a compacted mass of DNA** approximately 0.2 µm in diameter. In actively growing bacteria, projections of the nucleoid extend into the cytoplasm. Presumably, these projections contain DNA that is being transcribed into mRNA. **Supercoils are both inserted and removed by topoisomerases**.

DNA topoisomerases are, therefore, essential in the unwinding, replication, and rewinding of the circular, supercoiled bacterial DNA. **In order for the long molecule of DNA to fit within the bacterium, the DNA must be supercoiled**. However, **this supercoiled DNA must be uncoiled and relaxed in order for DNA polymerase to bind for DNA replication and RNA polymerase to bind for transcription of the DNA**. For example, a topoisomerase called **DNA gyrase catalyzes the negative supercoiling of the circular DNA found in bacteria**. **Topoisomerase IV**, on the other hand, is **involved in the relaxation of the supercoiled circular DNA**, enabling the separation of the interlinked daughter chromosomes at the end of bacterial DNA replication.

Quiz Group

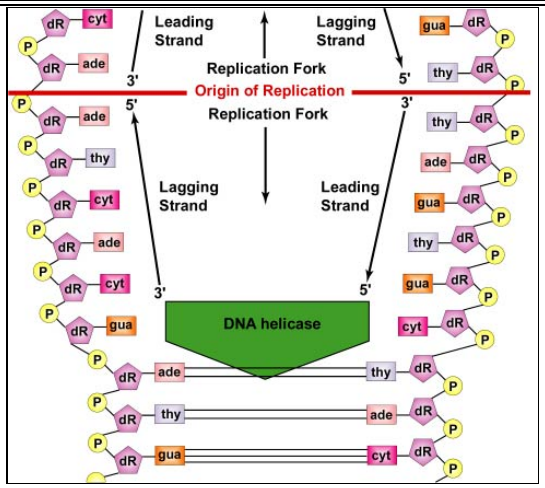
B. DNA Replication in Bacteria

DNA Replication in Bacteria

In general, DNA is replicated by **uncoiling of the helix, strand separation by breaking of the hydrogen bonds between the complementary strands, and synthesis of two new strands by complementary base pairing**. Replication begins at a specific site in the DNA called the **origin of replication** (*ori*C).

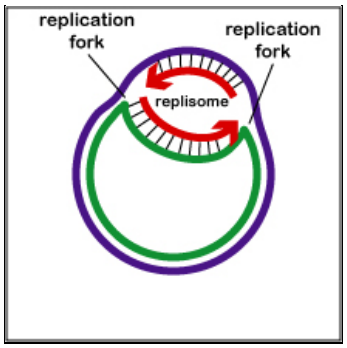
DNA replication is **bidirectional** from the origin of replication. To begin DNA replication, unwinding enzymes called **DNA helicases** cause short segments of the two parent DNA strands to unwind and separate from one another at the origin of replication to form two "Y"-shaped **replication forks**. These replication forks are the actual site of DNA copying **(see Fig. 4)**. All the proteins involved in DNA replication aggregate at the replication forks to form a replication complex called a replisome **(see Fig. 5)**.

Fig. 4: DNA Replication by Complementary Base Pairing: Unwinding by DNA Helicase



Replication begins at a specific site in the DNA called the origin of replication. Unwinding enzymes called DNA helicases cause the two parent DNA strands to unwind and separate from one another in both directions at this site to form two "Y"-shaped replication forks. These replication forks are the actual site of DNA copying. During replication within the fork, helix destabilizing proteins (not shown here) bind to the single-stranded regions preventing the strands from rejoining.

Fig. 5: Bidirectional Circular DNA Replication in Bacteria

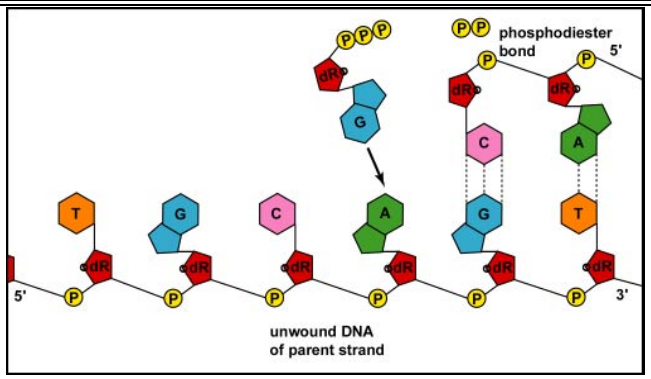


DNA replication (arrows) occurs in both directions from the origin of replication in the circular DNA found in most bacteria. All the proteins involved in DNA replication aggregate at the replication forks to form a replication complex called a replisome. The lagging DNA strand loops out from the leading strand and this enables the replisome to move along both strands pulling the DNA through as replication occurs. It is the actual DNA, not the DNA polymerase that moves during bacterial DNA replication.

Single-strand binding proteins bind to the single-stranded regions so the two strands do not rejoin. Unwinding of the double-stranded helix generates positive supercoils ahead of the replication fork. Enzymes called **topoisomerases** counteract this by producing breaks in the DNA and then rejoin them to form negative supercoils in order to relieve this stress in the helical molecule during replication.

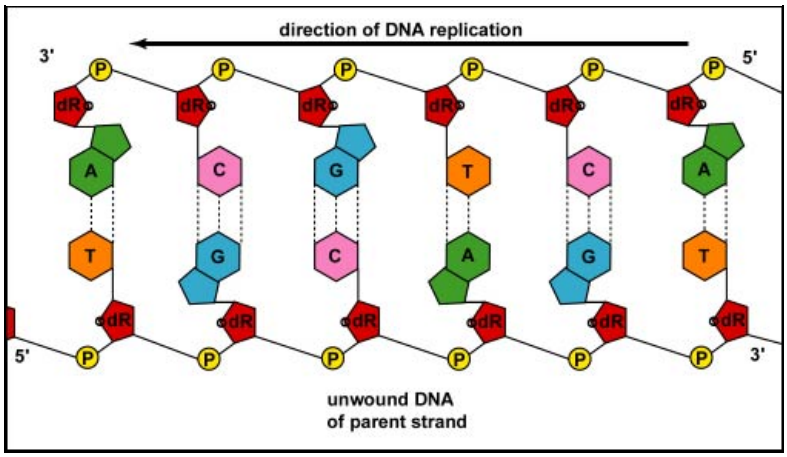
As the strands continue to unwind and separate in both directions around the entire DNA molecule, **new complementary strands are produced by the hydrogen bonding of free DNA nucleotides with those on each parent strand**. As the new nucleotides line up opposite each parent strand by hydrogen bonding, enzymes called **DNA polymerases join the nucleotides by way of phosphodiester bonds**. Actually, the nucleotides lining up by complementary base pairing are deoxynucleotide triphosphates, composed of a nitrogenous base, deoxyribose, and three phosphates. As the phosphodiester bond forms between the 5' phosphate group of the new nucleotide and the 3' OH of the last nucleotide in the DNA strand, two of the phosphates are removed providing energy for bonding **(see Fig. 6**). In the end, each parent strand serves as a template to synthesize a complementary copy of itself, resulting in the formation of two identical DNA molecules **(see Fig. 7)**. In bacteria, Par proteins function to separate bacterial chromosomes to opposite poles of the cell during cell division. They bind to the origin of replication of the DNA and physically pull or push the chromosomes apart, similar to the mitotic apparatus of eukaryotic cells. Fts proteins, such as FtsK in the divisome, also help in separating the replicated bacterial chromosome.

Fig. 6: DNA Replication by Complementary Base Pairing



As the two strands of DNA unwind and separate in both directions, the hydrogen bonding of free DNA nucleotides with those on each parent strand produces new complementary strands. As the new nucleotides line up opposite each parent strand by hydrogen bonding, enzymes called DNA polymerases join the nucleotides by way of phosphodiester bonds. Actually, the nucleotides lining up by complementary base pairing are deoxynucleoside triphosphates, composed of a nitrogenous base, deoxyribose, and three phosphates. As the phosphodiester bond forms between the 5' phosphate group of the new nucleotide and the 3' OH of the last nucleotide in the DNA strand, two of the phosphates are removed providing energy for bonding.

Fig. 7: DNA Replication by Complementary Base Pairing



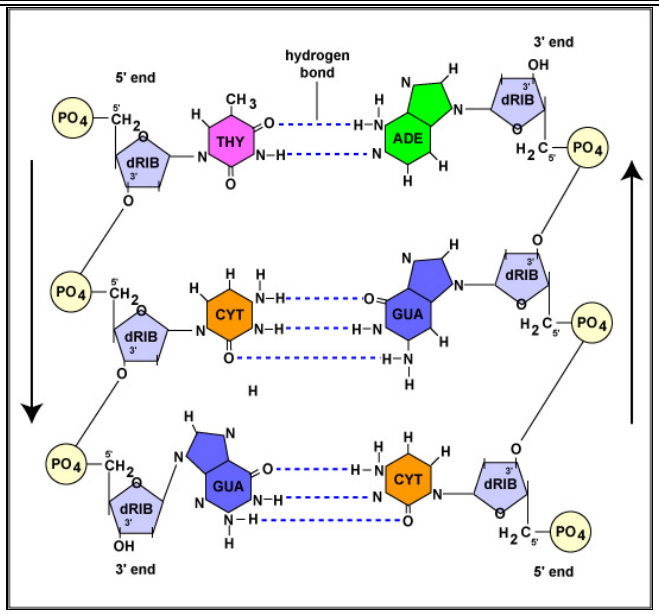
DNA replication in a 5' to 3' direction.

GIF animation illustrating DNA replication by complementary base pairing

In reality, DNA replication is more complicated than this because of the nature of the DNA polmerases. **DNA polymerase** enzymes are **only able to join the phosphate group at the 5' carbon of a new nucleotide to the hydroxyl (OH) group of the 3' carbon of a nucleotide already in the chain**. As a result, DNA can only be synthesized in a **5' to 3' direction** while copying a parent strand running in a 3' to 5' direction.

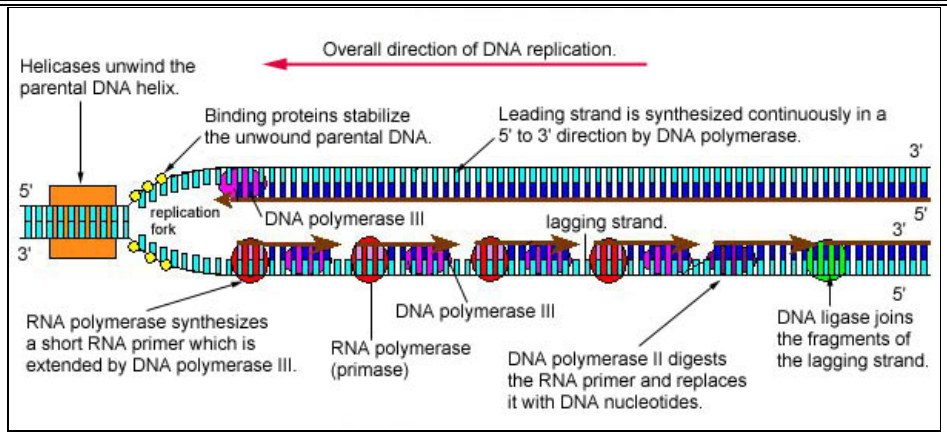
Remember, as mentioned above, each DNA strand has two ends. The **5' end** of the DNA is the one with the terminal **phosphate group** on the 5' carbon of the deoxyribose; the **3' end** is the one with a terminal **hydroxyl (OH) group** on the deoxyribose of the 3' carbon of the deoxyribose **(see Fig. 8)**. The two strands are antiparallel, that is they run in opposite directions. Therefore, one parent strand - the one running 3' to 5' and called the **leading strand** - can be copied **directly** down its entire length **(see Fig. 9)**. However, the other parent strand - the one running 5' to 3' and called the **lagging strand** - must be copied discontinuously in **short fragments** (Okazaki fragments) of around 100-1000 nucleotides each as the DNA unwinds. This occurs, as mentioned above, at the replisome. The lagging DNA strand loops out from the leading strand and this enables the replisome to move along both strands pulling the DNA through as replication occurs. It is the actual DNA, not the DNA polymerase that moves during bacterial DNA replication **(see Fig. 5)**.

Fig. 8: Chemical Structure of DNA



The phosphate of one deoxyribonucleotide binding to the 3' carbon of the deoxyribose of another forms the sugar-phosphate backbone of the DNA (the sides of the "ladder"). The hydrogen bonds between the complementary nucleotide bases (adenine-thymine; guanine-cytosine) form the rungs. Note the antiparallel nature of the DNA. One strand ends in a 5' phosphate and the other ends in a 3' hydroxyl.

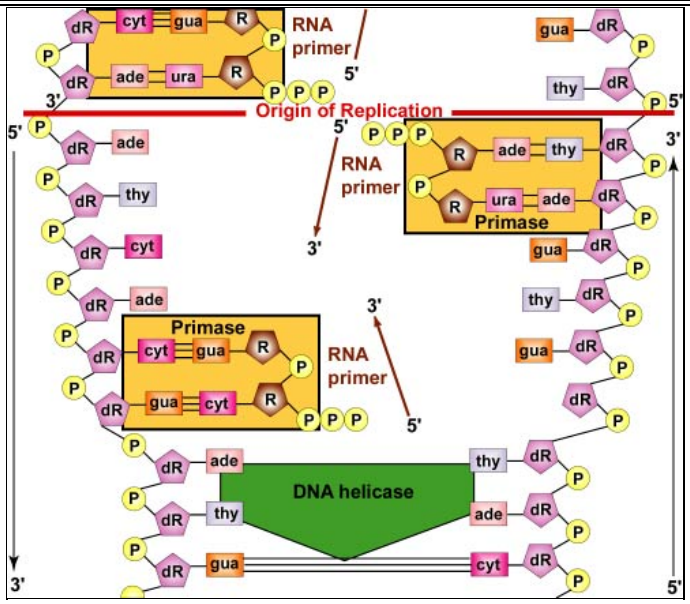
Fig. 9: Replication of Leading and Lagging DNA Strands



The leading strand is made continuously in a 5' to 3' direction by DNA polymerase III as the DNA helicase unwinds the parental DNA helix. However, because the parental DNA strands are antiparallel, the lagging strand must be made in short fragments. RNA polymerase (primase) synthesizes a short RNA primer which is extended by DNA polymerase III. DNA polymerase II then digests the RNA primer and replaces it with DNA. Finally, DNA ligase joins the fragments of the lagging strand together.

In addition, **DNA polymerase enzymes cannot begin a new DNA chain from scratch**. They can only attach new nucleotides onto 3' OH group of a nucleotide in a preexisting strand. Therefore, to start the synthesis of the leading strand and each DNA fragment of the lagging strand, an **RNA polymerase complex called a primase** is required. The primase, which is capable of joining RNA nucleotides without requiring a preexisting strand of nucleic acid, first adds several comlementary RNA nucleotides opposite the DNA nucleotides on the parent strand. This forms what is called an **RNA primer (see Fig. 10)**.

Fig. 10: DNA Replication by Complementary Base Pairing: Producing an RNA Primer

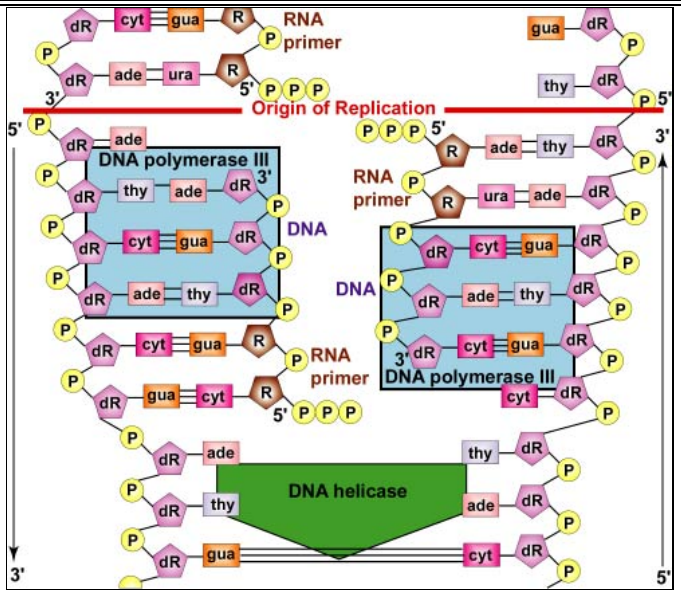


DNA polymerases cannot begin a new DNA chain from scratch. They can only attach new nucleotides onto 3' OH group of a nucleotide in a preexisting strand. Therefore, to start the synthesis of the leading strand and each DNA fragment of the lagging strand, an RNA polymerase complex called a primosome or primase (not shown here) is required. The primase, which is capable of joining RNA nucleotides without requiring a preexisting strand of nucleic acid, first adds several comlementary RNA nucleotides opposite the DNA nucleotides on the parent strand. This forms what is called an RNA primer. The free ribonucleoside triphosphates line up by complementary base pairing with the nucleotides on each parent strand of the unwound DNA in the replication fork and are then joined together by the primase.

DNA polymerase III then replaces the primase and is able to add DNA nucleotides to the RNA primer **(see Fig. 11)**. Later, DNA polymerase II digests away the RNA primer and replaces the RNA nucleotides of the primer with the proper DNA nucleotides to fill the gap **(see Fig. 12)**. Finally, the DNA fragments themselves

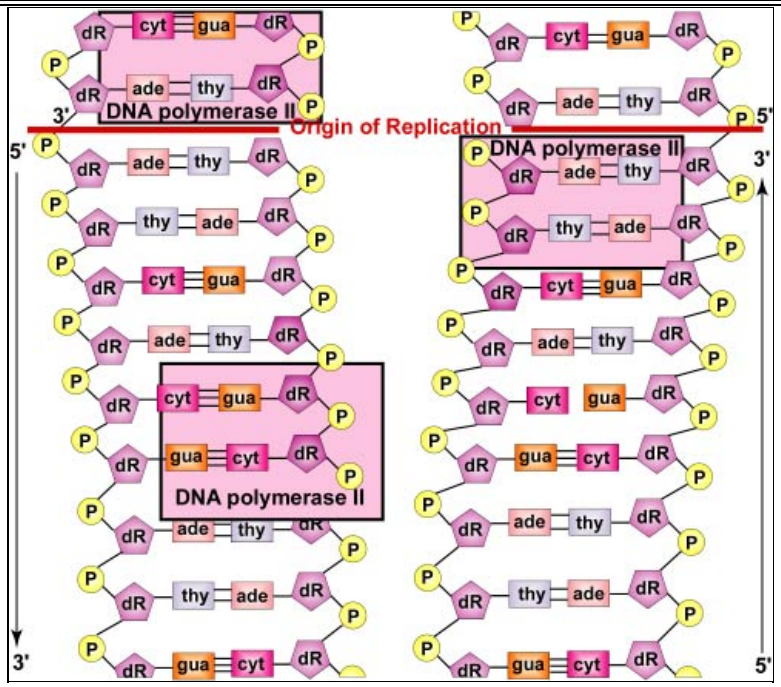
are hooked together by the enzyme **DNA ligase (see Fig. 9)**. Yet even with this complicated procedure, a 1000 micrometer-long macromolecule of tightly-packed, supercoiled DNA can make an exact copy of itself in only about 10 minutes time under optimum conditions, inserting nucleotides at a rate of about 1000 nucleotides per second!

Fig. 11: DNA Replication by Complementary Base Pairing: Adding DNA Nucleotides to the RNA Primer



DNA polymerase III replaces the primase and is able to add DNA nucleotides to the RNA primer. As the free deoxyribonucleoside triphosphates line up by complementary base pairing with the nucleotides on each parent strand of the unwound DNA in the replication fork, the phosphate on the 5 carbon of the newest building block lining up then forms a phosphodiester bond with the 3 carbon of the last nucleotide in the growing strand. During the process, two phosphates are lost . Because the parent strands are antiparallel and DNA can only be replicated in a 5 to 3 direction, the the two new strands must be synthesized in opposite directions.

Fig. 12: DNA Replication by Complementary Base Pairing: Replacing RNA Primer with DNA



Eventually, DNA polymerase II digests away the RNA primer and replaces the RNA nucleotides of the primer with the proper DNA nucleotides to fill the gap. Finally, the DNA fragments on the lagging strand are hooked together by the enzyme DNA ligase.

GIF animation illustrating the replication of leading and lagging strands of DNA

Animation of DNA Replication

Courtesy of HHMI's Biointeractive.

Quiz Group

C. Functions of the Bacterial Chromosome

The chromosome is the genetic material of the bacterium. Genes located along the DNA are transcribed into RNA molecules, primarily messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA). Messenger RNA is then translated into protein at the ribosomes.

Transcription

Ribonucleic acid (RNA) is synthesized by complementary base pairing of ribonucleotides with deoxyribonucleotides to match a portion of one strand of DNA called a gene. Although genes are present on both strands of DNA, only one strand is transcribed for any given gene. Following transcription of genes into mRNA, 30S and 50S ribosomal subunits attach to the mRNA and tRNA inserts the correct amino acids which are subsequently joined to form a polypeptide or a protein through a process called translation.

Animation of Transcription of DNA

Courtesy of HHMI's Biointeractive.

Translation

During translation, specific tRNA molecules pick up specific amino acids, transfer those amino acids to the ribosomes, and insert them in their proper place according to the mRNA "message." This is done by the anticodon portion of the tRNA molecules complementary base pairing with the codons along the mRNA.

In bacteria, transcription and translation are coupled. RNA polymerase binds to the 30S ribosomal subunit of prokaryotic ribosomes to form a transcription and translation machine called an **expressome**. As the DNA is being unwound and transcribed into complementary mRNA by RNA polymerase, the mRNA is being fed into the translational center of the ribosome where it is being translated into a polypeptide.

Coupled transcription and translation in bacteria via the bacterial expressome.

Science News.

In general then, **DNA determines what proteins and enzymes an organism can synthesize and, therefore, what chemical reactions it is able to carry out**.

For more information: Review of RNA

For more information: Review of transcription

For more information: Review of translation

Animation of Translation

Courtesy of HHMI's Biointeractive.

Concept map for the bacterial chromosome and nucleoid

Quiz Group

D. The Bacterial Epigenome

The epigenome refers to a variety of chemical compounds that modify the genome typically by adding a methyl (CH_3) group to the nucleotide base adenine at specific locations along the DNA molecule. This methylation can, in turn, either repress or activate transcription of specific genes.

By basically turning genes on or off, the epigenome enables the bacterial genome to interact with and respond to the bacterium's environment. The epigenome can be inherited just like the genome.

All cells, including human cells, possess an epigenome. **Just as the bacterial epigenome can affect the bacterial genome, bacteria, can affect our epigenome and subsequently modify the function of our genome** by causing either DNA methylation of nucleotides or by modifying our histone proteins. **The resulting modification can either help activate various genes involved in immune defenses, or, in the case of some pathogens, suppress immune response genes**.

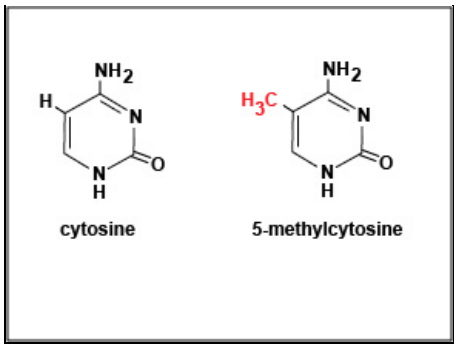
For more information: Article on the human epigenome

E. Significance of the Chromosome to the Initiation of Body Defense

In order to protect against infection, one of the things the body must initially do is detect the presence of microorganisms. The body does this by **recognizing molecules unique to microorganisms that are not associated with human cells**. These unique molecules are called **pathogen-associated molecular patterns** or **PAMPS**. (Because all microbes, not just pathogenic microbes, possess PAMPs, pathogen-associated molecular patterns are sometimes referred to as microbe-associated molecular patterns or MAMPs.)

Bacterial and viral genomes contain a high frequency of **unmethylated cytosine-guanine (CpG) dinucleotide sequences** (a cytosine lacking a methyl or CH3 group and located adjacent to a guanine; **see Fig. 13**). Mammalian DNA has a low frequency of cytosine-guanine dinucleotides and most are methylated. These unmethylated cytosine-guanine dinucleotide sequences in bacterial DNA are PAMPS that bind to **pattern-recognition receptors** on a variety of defense cells of the body and **triggers innate immune defenses such as inflammation, fever, and phagocytosis**.

Fig. 13: Methylated and Non-methylated Cytosine



For more information: Preview of pathogen-associated molecular patterns (PAMPs)

For more information: Preview of pattern-recognition receptors (PRRs)

Concept map for the bacterial chromosome and nucleoid

Self Check

F. Antimicrobial Agents that Inhibiting Normal Nucleic Acid Replication in Bacteria

Some antibacterial chemotherapeutic affect bacteria by inhibiting normal nucleic acid replication.

- The **fluoroquinolones** (norfloxacin, lomefloxacin, fleroxacin, ciprofloxacin, enoxacin, trovafloxacin, etc.) work by **inhibiting one or more of the** topoisomerases, the enzymes needed for bacterial nucleic acid synthesis.

- Co-trimoxazole, a combination of **sulfamethoxazole and trimethoprim**, **block enzymes in the bacteria pathway required for the synthesis of tetrahydrofolic acid, a cofactor needed for bacteria to make the nucleotide bases thymine, guanine, uracil, and adenine**. Without the tetrahydrofolic acid, the bacteria cannot synthesize DNA or RNA.

Flash animation illustrating a normal enzyme reaction
Copyright © Gary E. Kaiser
html5 version of animation for iPad illustrating a normal enzyme reaction
Without the antibiotic binding to a bacterial enzyme, the activate site of the enzyme is able to react with its substrate.

Flash animation illustrating antimicrobial agents may inactivate a bacterial enzyme
Copyright © Gary E. Kaiser
html5 version of animation for iPad illustrating antimicrobial agents may inactivate a bacterial enzyme
When an antibiotic binds to a bacterial enzyme, it may alter the activate site of the enzyme and prevent it from reacting with its substrate.

Antimicrobial chemotherapy will be discussed in greater detail later in Unit 2 under **Control of Bacteria by Using Antibiotics and Disinfectants**.

For more information: Preview of chemotherapeutic control of bacteria

Concept map for the bacterial chromosome and nucleoid

TPS Questions

Self Quiz for the Bacterial Chromosome and Nucleoid

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Plasmids and transposons
PLASMIDS AND TRANSPOSONS

Plasmids and Transposons

Fundamental Statements for this Lesson:

1. Many bacteria often contain small nonchromosomal DNA molecules called plasmids.
2. While plasmids are not essential for normal bacterial growth and bacteria may lose or gain them without harm, they can provide an advantage under certain environmental conditions.
3. Plasmids code for synthesis of a few proteins not coded for by the bacterial chromosome.
4. Transposons (jumping genes) are small pieces of DNA that encode enzymes that enable the transposon to, move from one DNA location to another.
5. Transposons may be found as part of a bacterium's chromosome or in plasmids
6. Integrons are transposons that can carry multiple gene clusters called gene cassettes that move as a unit from one piece of DNA to another
7. Horizontal gene transfer is a process in which an organism transfers genetic material to another cell that is not its offspring.
8. Horizontal gene transfer is able to cause rather large-scale changes in a bacterial genome.
9. The ability of Bacteria and Archaea to adapt to new environments as a part of bacterial evolution, most frequently results from the acquisition of new genes through horizontal gene transfer rather than by the alteration of gene functions through mutations.

Common Course Objectives

1. Identify the parts of a bacterium and their physiological purpose.

2. Explain how various bacterial structures can contribute to bacterial colonization of a host.
3. Compare and contrast mutation and horizontal gene transfer as methods of enabling bacteria to respond to selective pressures and adapt to new environments.
4. Describe pathogenicity islands and how they are primarily transferred from one bacterium to another.

Detailed Learning Objectives for this Lesson

1*. Describe plasmids and indicate their possible benefit to bacteria. 2. State the function of the following:

a*. transposons

b. integrons

c. episome

d*. conjugative plasmid

3*. State the most common way plasmids are transmitted from one bacterium to another.

4*. Define horizontal gene transfer.

(*) = Common theme throughout the course

TPS Question

In this section on Prokaryotic Cell Anatomy we are looking at the various anatomical parts that make up a bacterium. As mentioned in the introduction to this section, a typical bacterium usually consists of:

- a cytoplasmic membrane surrounded by a peptidoglycan cell wall and maybe an outer membrane;
- a fluid cytoplasm containing a nuclear region (nucleoid) and numerous ribosomes; and
- often various external structures like a glycocalyx, flagella, and pili.

We will now look at plasmids and transposons.

Plasmids and Transposons

In addition to the nucleoid, many bacteria often contain small nonchromosomal DNA molecules called plasmids. Plasmids usually contain between 5 and 100 genes. Plasmids are not essential for normal bacterial growth and bacteria may lose or gain them without harm. They can, however, **provide an advantage under certain environmental conditions**. For example, under normal environmental growth conditions, bacteria are not usually exposed to antibiotics and having a plasmid coding for an enzyme capable of denaturing a particular antibiotic is of no value. However, if that bacterium finds itself in the body when the particular antibiotic that the plasmid-coded enzyme is able to degrade is being given to treat an infection, the bacterium containing the plasmid is able to survive and grow.

A. Structure and Composition

Plasmids are small molecules of double stranded, helical, **non-chromosomal DNA**. In most plasmids the two ends of the double-stranded DNA molecule that make up plasmids covalently bond together forming a physical circle. Some plasmids, however, have linear DNA.

Plasmids replicate independently of the host chromosome, but some plasmids, called episomes, are able to insert or integrate into the host cell's chromosome where their replication is then regulated by the chromosome.

Although some plasmids can be transmitted from one bacterium to another by transformation and by generalized transduction, the most common mechanism of plasmid transfer is conjugation. Plasmids that can be transmitted by cell-to-cell contact are called conjugative plasmids. They contain genes coding for proteins involved in both DNA transfer and and the formation of mating pairs.

Flash animation illustrating transfer of conjugative plasmids and mobilizable plasmids.
Copyright © Gary E. Kaiser
html5 version of animation for iPad illustrating transfer of conjugative plasmids and mobilizable plasmids.

Conjugation involves a donor bacterium that contains a conjugative plasmid and a recipient cell that does not. A conjugative plasmid is self-transmissible, in that it possesses all the necessary genes for that plasmid to transmit itself to another bacterium by conjugation. Conjugation genes known as *tra* genes enable the bacterium to form a mating pair with another organism, while *oriT* (origin of transfer) genes determine where on the plasmid DNA transfer is initiated. In addition, mobilizable plasmids that lack the *tra* genes for self-transmissibility but possess the *oriT* genes for initiation of DNA transfer may also be transferred by conjugation if the bacterium containing them also possesses a conjugative plasmid. The *tra* genes of the conjugative plasmid enable a mating pair to form and the *oriT* genes of the mobilizable plasmid enable the DNA to moves through the conjugative bridge.

B. Functions

Plasmids **code for synthesis of a few proteins not coded for by the nucleoid**. For example, **R-plasmids**, found in some Gram-negative bacteria, often have genes coding for both production of a conjugation pilus (discussed later in this unit) and multiple antibiotic resistance. Through a process called conjugation, the conjugation pilus enables the bacterium to transfer a copy of the R-plasmids to other bacteria, making them also multiple antibiotic resistant and able to produce a conjugation pilus. In addition, some exotoxins, such as the tetanus exotoxin, *Escherichia coli* enterotoxin, and *E.coli* shiga toxin discussed later in Unit 2 under Bacterial Pathogenicity, are also coded for by plasmids. Thousands of different plasmids are known to exist.

Flash animation illustrating the transfer of R-plasmids coding for mating pair formation and multiple antibiotic resistance.
Copyright © Gary E. Kaiser
html5 version of animation for iPad illustrating the transfer of R plasmids coding for mating pair formation and multiple antibiotic resistance.
R plasmids are conjugative plasmids coding for mating pair formation and also multiple antibiotic resistance. A conjugative plasmid is self-transmissible, in that it possesses all the necessary genes for that plasmid to transmit itself to another bacterium by conjugation. Conjugation genes known as *tra* genes enable the bacterium to form a mating pair with another organism, while *oriT* (origin of transfer) genes determine where on the plasmid DNA transfer is initiated. The plasmid also possess genes coding for resistance to a number of different antibiotics.

C. Transposons

Transposons (transposable elements or "jumping genes") are small pieces of DNA that encode enzymes that transpose the transposon, that is, **move it from one DNA location to another**, either on the same molecule of DNA or on a different molecule. Transposons may be found as part of a bacterium's nucleoid (**conjugative transposons**) or in plasmids and are usually between one and twelve genes long. A transposon contains a number of genes, coding for antibiotic resistance or other traits, flanked at both ends by insertion sequences coding for an enzyme called **transpoase**. Transpoase is the enzyme that catalyzes the cutting and resealing of the DNA during transposition. Thus, such transposons are able to cut themselves out of a bacterial nucleoid or a plasmid and insert themselves into another nucleoid or plasmid and contribute in the transmission of antibiotic resistance among a population of bacteria.

Flash animation illustrating transposons in a bacterium.
Copyright © Gary E. Kaiser
html5 version of animation for iPad illustrating transposons in a bacterium.
Transposons (transposable elements or "jumping genes" are small pieces of DNA that encode enzymes that transpose the transposon, that is, move it from one DNA location to another, either on the same molecule of DNA or on a different molecule. A transposon contains a number of genes, coding for antibiotic resistance or other traits, flanked at both ends by insertion sequences coding for an enzyme called transpoase. Transpoase is the enzyme that catalyzes the cutting and resealing of the DNA during transposition. Thus, such transposons are able to cut themselves out of a bacterial chromosome or a plasmid and insert themselves into another chromosome or plasmid and contribute in the transmission of antibiotic resistance among a population of bacteria.

Plasmids can also acquire a number of different antibiotic resistance genes by means of integrons. Integrons are transposons that can carry **multiple gene clusters called gene cassettes that move as a unit from one piece of DNA to another**. An enzyme called integrase enables these gene cassettes to integrate and accumulate within the integron. In this way, a number of different antibiotic resistance genes can be transferred as a unit from one bacterium to another.

Plasmids and conjugative transposons are very important in horizontal gene transfer in bacteria. Horizontal gene transfer, also known as lateral gene transfer, is a process in which an organism transfers genetic material to another organism that is not its offspring. The ability of *Bacteria* and *Archaea* to adapt to new environments as a part of bacterial evolution most frequently results from the acquisition of new genes through horizontal gene transfer rather than by the alteration of gene functions through mutations. (It is estimated that as much as 20% of the genome of *Escherichia coli* originated from horizontal gene transfer.)

Horizontal gene transfer is able to cause rather large-scale changes in a bacterial genome. For example, certain bacteria contain multiple virulence genes called pathogenicity islands that are located on large, unstable regions of the bacterial genome. These pathogenicity islands can be transmitted to other bacteria by

horizontal gene transfer. However, if these transferred genes provide no selective advantage to the bacteria that acquire them, they are usually lost by deletion. In this way the size of the bacterium's genome can remain approximately the same size over time.

For more information: Preview of horizontal gene transfer in bacteria.

Because bacteria are always taking in new DNA from horizontal gene transfer or being infected by bacteriophages, **bacteria have developed a system for removing viral nucleic acid or DNA from self-serving or harmful plasmids**. This system represents a type of adaptive immunity in bacteria, and is carried out by clustered, regularly interspaced, short palindromic repeat (CRISPR) sequences and CRISPR-associated (Cas) proteins that possess nuclease activity. **The CRISPR/Cas system targets specific foreign DNA sequences in bacteria for destruction**.

Applications of CRISPR technology has now become a common tool used in molecular biology for CRISPR/nuclease mediated genome editing (genetic engineering) in a wide variety of different cell types. CRISPER is an RNA-guided gene-editing platform that makes use of a bacterially derived protein (Cas9) and a synthetic guide RNA to introduce a double strand break at a specific location within the genome. Molecular biologists are now beginning to use this to carry out highly efficient, targeted alterations of genome sequence and gene expression and hope to eventually use it to repair damaged or dysfunctional genes. **(See Movie below.)**

Concept map for Plasmids and Transposons

TPS Question

Self Quiz for Plasmids and Transposons

Quiz Group

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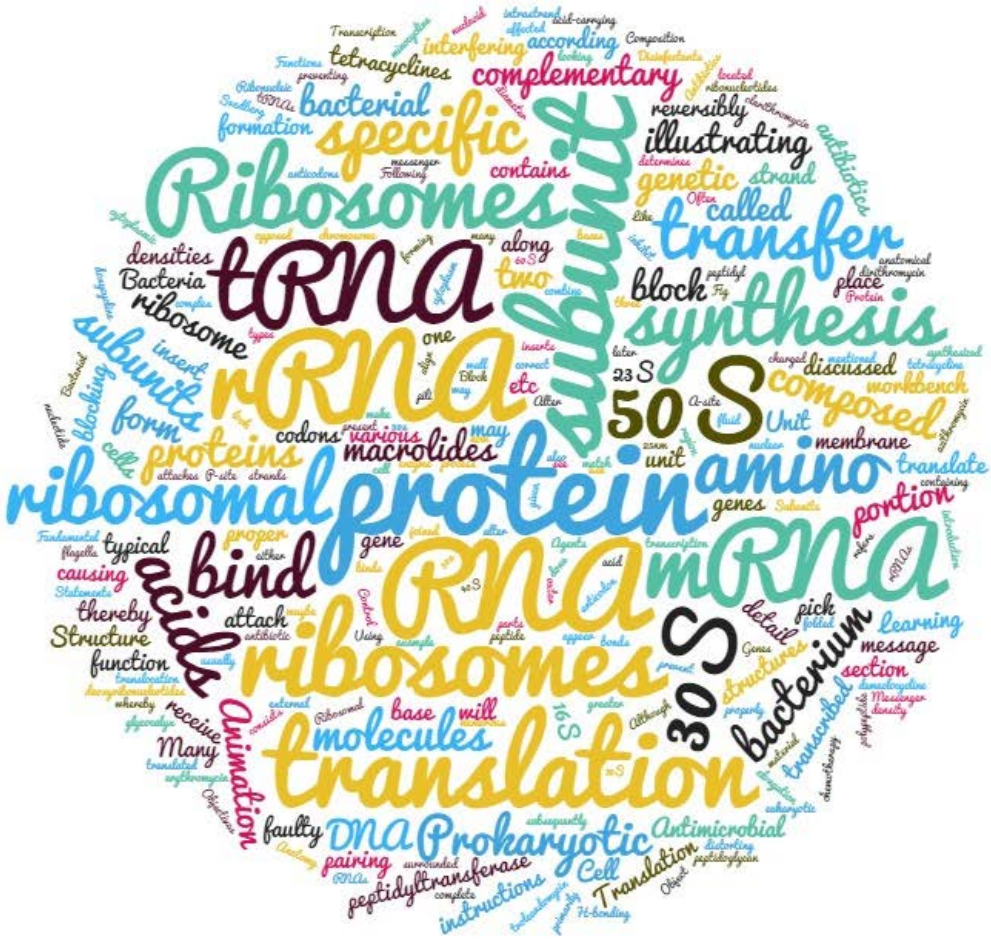
THE PROKARYOTIC CELL:
RIBOSOMES

Ribosomes



Fundamental Statements for this Learning Object:

1. Ribosomes are composed of ribosomal RNA (rRNA) and protein.
2. Bacterial ribosomes are composed of two subunits with densities of 50S and 30S, as opposed to 60S and 40S in eukaryotic cells.
3. Ribosomes function as a workbench for protein synthesis whereby they receive and translate genetic instructions for the formation of specific proteins.
4. During translation, specific tRNA molecules pick up specific amino acids, transfer those amino acids to the ribosomes, and insert them in their proper place according to the mRNA "message."
5. In bacteria, transcription and translation are coupled.
6. Many antibiotics bind to either the 30S or the 50S subunit of bacterial ribosomes, interfering with translation and thereby causing faulty protein synthesis.

Common Course Objectives

1. Identify the parts of a bacterium and their physiological purpose.
2. Explain how certain parts of the bacteria can be targeted in order to kill the bacteria.
3. Summarize where and how transcription and translation occurs in prokaryotic cells.

Detailed Learning Objectives for this Lesson

1. Describe the structure and chemical composition of bacterial ribosomes and state their function.

2. In terms of protein synthesis, briefly describe the process of transcription and translation.

3. State, in a general sense, how antibiotics like neomycin, tetracycline, doxycycline, erythromycin, and azithromycin affect bacterial growth.

TPS Questions

In this section on Prokaryotic Cell Structure we are looking at the various organelles or structures that make up a bacterium. As mentioned in the introduction to this section, a typical bacterium usually consists of:

- a cytoplasmic membrane surrounded by a peptidoglycan cell wall and maybe an outer membrane;
- a fluid cytoplasm containing a nuclear region (nucleoid) and numerous ribosomes; and
- often various external structures like a glycocalyx, flagella, and pili.

We will now look at ribosomes.

Bacterial Ribosomes

A. Structure and Composition

Ribosomes are composed of **ribosomal RNA (rRNA) and protein**. Prokaryotic cells have three types of rRNA: 16S rRNA, 23S rRNA, and 5S rRNA. Like transfer RNA (tRNA), rRNAs use intrastrand H-bonding between comlementary nucleotide bases to form complex folded structures.

Ribosomes are composed of two subunits with densities of 50S and 30S. ("S" refers to a unit of density called the Svedberg unit.) The 30S subunit contains 16S rRNA and 21 proteins; the 50S subunit contains 5S and 23S rRNA and 31 proteins.

For more information: Review of ribosomal subunit densities: 50S and 30S

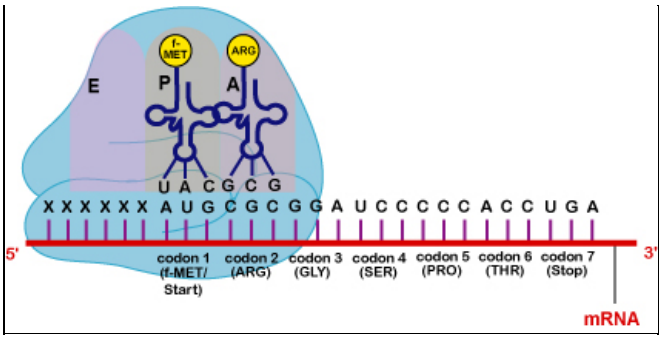
The two subunits combine during protein synthesis to form a complete 70S ribosome about 25nm in diameter. A typical bacterium may have as many as 15,000 ribosomes.

To view **electron micrograph showing the nucleoid and ribosomes in a *Streptococcus***, see the Rockefeller University home page.

B. Functions

Ribosomes function as a **workbench for protein synthesis**, that is, they receive and translate genetic instructions for the formation of specific proteins. During protein synthesis, mRNA attaches to the 30S subunit and amino acid-carrying transfer RNAs (tRNA) attach to the 50S subunit **(see Fig. 1)**. Protein synthesis is discussed in detail in Unit 7.

Fig. 1: 70S Ribosomal Subunit during Translation



The 70S prokaryotic ribosome consists of a 50S and a 30S subunit. "S" refers to a unit of density called the Svedberg unit.

The chromosome is the genetic material of the bacterium. Genes located along the DNA are transcribed into RNA molecules, primarily messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA). Messenger RNA is then translated into protein at the ribosomes.

In bacteria, transcription and translation are coupled. RNA polymerase binds to the 30S ribosomal subunit of prokaryotic ribosomes to form a transcription and translation machine called an **expressome**. As the DNA is being unwound and transcribed into complementary mRNA by RNA polymerase, the mRNA is being fed into the translational center of the ribosome where it is being translated into a polypeptide.

Coupled transcription and translation in bacteria via the bacterial expressome.

Science News.

Transcription

Ribonucleic acid (RNA) is synthesized by complementary base pairing of ribonucleotides with deoxyribonucleotides to match a portion of one strand of DNA called a gene. Although genes are present on both strands of DNA, only one strand is transcribed for any given gene. Following transcription of genes into mRNA, 30S and 50S ribosomal subunits attach to the mRNA and tRNA inserts the correct amino acids which are subsequently joined to form a polypeptide or a protein through a process called translation.

Translation

During translation, specific tRNA molecules pick up specific amino acids, transfer those amino acids to the ribosomes, and insert them in their proper place according to the mRNA "message." This is done by the anticodon portion of the tRNA molecules complementary base pairing with the codons along the mRNA.

Flash animation summarizing translation in bacteria

html5 version of animation illustrating translation in bacteria

For more information: Review of RNA

For more information: Review of transcription

For more information: Review of translation

TPS Questions

DragNDrop Activity

C. Antimicrobial Agents that Alter Prokaryotic Ribosomal Subunits and Block Translation in Bacteria

Many antibiotics **alter bacterial ribosomes**, interfering with translation and thereby causing **faulty protein synthesis**. The portion of the ribosome to which the antibiotic binds determines how translation is effected. For example:

- The **tetracyclines** (tetracycline, doxycycline, demeclocycline, minocycline, etc.) **bind reversibly to the 30S subunit**, distorting it in such a way that the **anticodons of charged tRNAs cannot align properly with the codons of the mRNA**.

Flash animation illustrating how tetracyclines bind to the 30S ribosomal subunit and block translation
Copyright © Gary E. Kaiser
Flash animation illustrating how tetracyclines bind to the 30S ribosomal subunit and block translation
The tetracyclines (tetracycline, doxycycline, demeclocycline, minocycline, etc.) block bacterial translation by binding reversibly to the 30S subunit and distorting it in such a way that the anticodons of the charged tRNAs cannot align properly with the codons of the mRNA.

- The **macrolides** (erythromycin, azithromycin, clarithromycin, dirithromycin, troleandomycin, etc.) **bind reversibly to the 50S subunit**. They appear to inhibit elongation of the protein by preventing the enzyme peptidyltransferase from forming peptide bonds between the amino acids. They may also prevent the transfer of the peptidyl tRNA from the A-site to the P-site.

Flash animation illustrating how macrolides bind to the 50S ribosomal subunit and block translation by blocking peptidytransferase.
Copyright © Gary E. Kaiser
Flash animation illustrating how macrolides bind to the 50S ribosomal subunit and block translation by blocking peptidytransferase.
The macrolides (erythromycin, azithromycin, clarithromycin, dirithromycin, troleandomycin, etc.) bind reversibly to the 50S subunit.They can inhibit elongation of the protein by the peptidyltransferase, the enzyme that forms peptide bonds between the amino acids.

Flash animation illustrating how macrolides bind to the 50S ribosomal subunit and block translation by preventing the transfer of the peptidyl tRNA from the A-site to the P-site.
Copyright © Gary E. Kaiser
Flash animation illustrating how macrolides bind to the 50S ribosomal subunit and block translation by preventing the transfer of the peptidyl tRNA from the A-site to the P-site.
The macrolides (erythromycin, azithromycin, clarithromycin, dirithromycin, troleandomycin, etc.) bind reversibly to the 50S subunit. They appear to inhibit elongation of the protein by preventing the enzyme peptidyltransferase from forming peptide bonds between the amino acids. They may also prevent the transfer of the peptidyl tRNA from the A-site to the P-site as shown here.

For More Information: Preview of Chemotherapeutic Control of Bacteria

Concept map for Ribosomes

Self Quiz for Bacterial Ribosomes

Quiz Group

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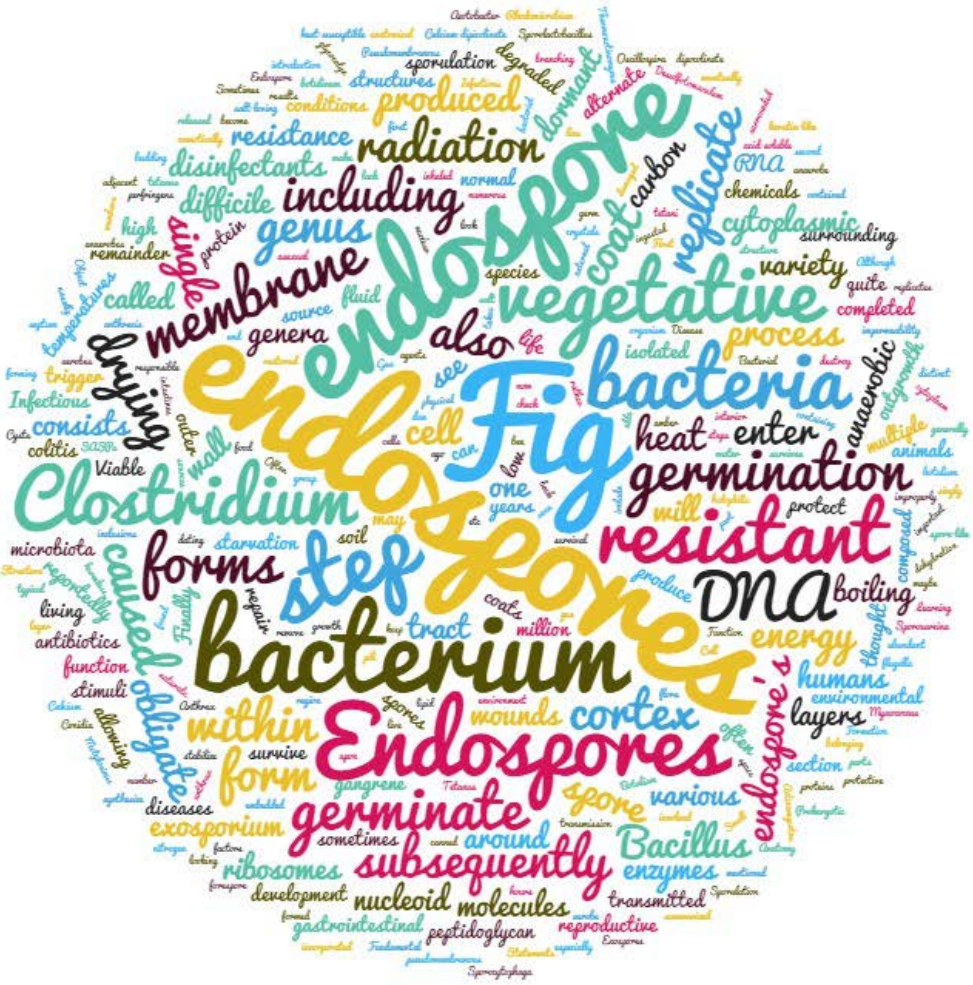
Bacterial endospores
BACTERIAL ENDOSPORES

Bacterial Endospores



Fundamental Statements for this Lesson:

1. Endospores are dormant alternate life forms produced by a few genera of bacteria.
2. The genus Bacillus (an obligate aerobe often living in the soil) and the genus Clostridium (an obligate anaerobe living in the gastrointestinal tract of animals) produce endospores.
3. Under conditions of starvation, a single endospore forms within a bacterium through a process called sporulation, after which the remainder of the bacterium is degraded.
4. The completed endospore consists of multiple layers of resistant coats (including a cortex, a spore coat, and sometimes an exosporium) surrounding a nucleoid, some ribosomes, RNA molecules, and enzymes.
5. Endospores are quite resistant to high temperatures (including boiling), most disinfectants, low energy radiation, and drying.
6. The endospore survives until a variety of environmental stimuli trigger germination, allowing outgrowth of a single vegetative bacterium.
7. Infectious diseases such as anthrax, tetanus, gas gangrene, botulism, and pseudomembranous colitis are transmitted to humans by endospores.

Common Course Objectives

1. Identify the parts of a bacterium and their physiological purpose.
2. Articulate why endospore formation enhances bacterial survival and recall the medically important endospore formers and the diseases they cause.
3. Provide a diagnosis for a "patient" when given a list of symptoms and a description of the bacterium.

Detailed Learning Objectives for this Lesson

1. Name 2 common genera of bacteria capable of producing endospores and state which is an obligate anaerobe.

2*. Briefly discuss the function of a bacterial endospore.

3. Describe the structure of a bacterial endospore.

4*. Define sporulation and germination.

5. Name three infections that may be transmitted to humans by endospores.

(*) = Common theme throughout the course

(**) = More depth and common theme

Highlighted Bacterium

1. Read the description of *Clostridium tetani* and match the bacterium with the description of the organism and the infection it causes.

TPS Question

In this section on Prokaryotic Cell Anatomy we are looking at the various anatomical parts that make up a bacterium. As mentioned in the introduction to this section, a typical bacterium usually consists of:

- a cytoplasmic membrane surrounded by a peptidoglycan cell wall and maybe an outer membrane;
- a fluid cytoplasm containing a nuclear region (nucleoid) and numerous ribosomes; and
- often various external structures like a glycocalyx, flagella, and pili.

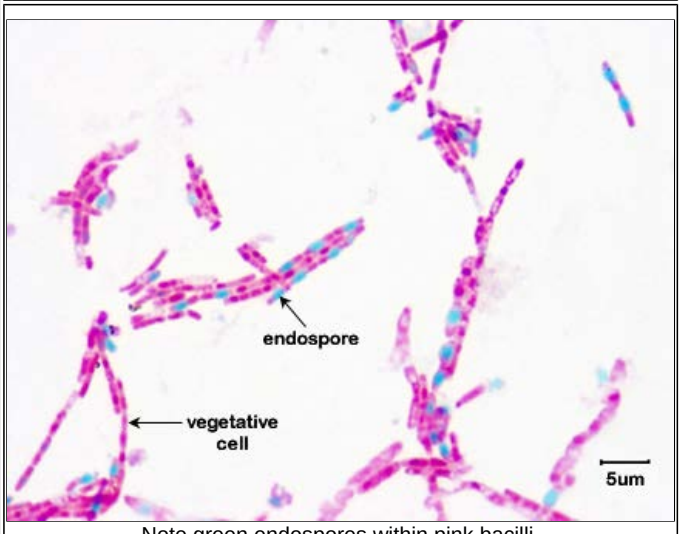
We will now look at bacterial endospores.

Bacterial Endospores

Endospores are **dormant alternate life forms** produced by the genus *Bacillus*, the genus *Clostridium*, and a number other genera of bacteria, including *Desulfotomaculum, Sporosarcina, Sporolactobacillus, Oscillospira,* and *Thermoactinomyces*.

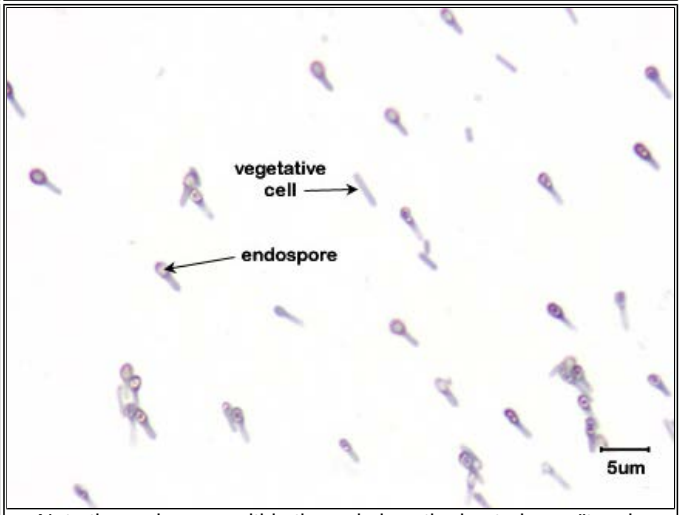
Bacillus species **(see Fig. 1)** are obligate aerobes that live in soil while *Clostridium* species **(see Fig. 2**) are obligate anaerobes often found as normal flora of the gastrointestinal tract in animals.

Fig. 1: Endospore stain of *Bacillus megaterium*



Note green endospores within pink bacilli.

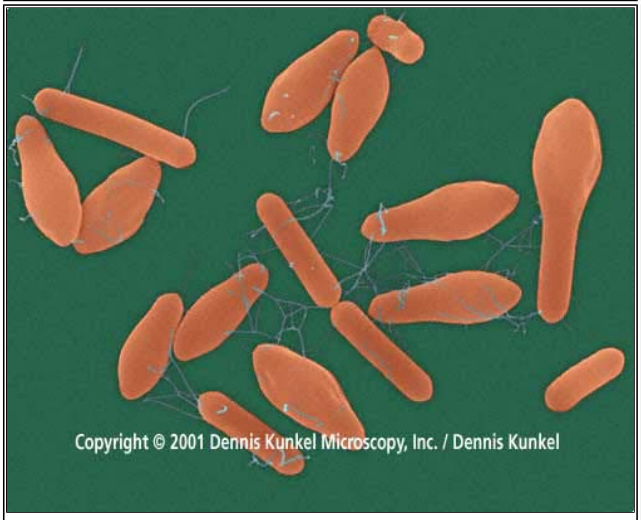
Fig. 2: Endospore stain of *Clostridium tetani*



Note the endospore within the rod gives the bacterium a "tennis racquet" shape (arrows).

Scanning electron micrograph of *Clostridium botulinum* with endospore.

Courtesy of Dennis Kunkel's Microscopy.

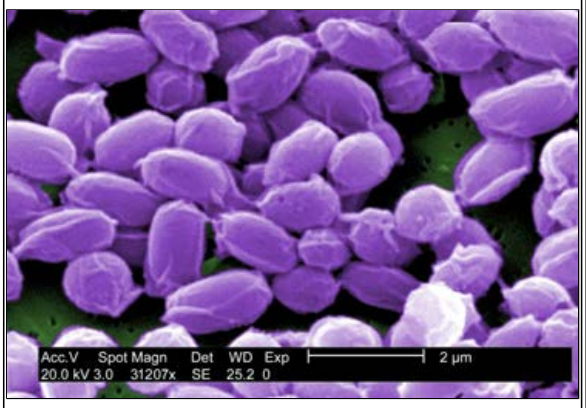


Scanning electron micrograph of Clostridium botulinum with endospores. The spore-containing end appears swollen or club-shaped. Flagella are also visible.

Scanning electron micrograph of *Bacillus anthracis* endospores.

Image provided by Janice Haney Carr.

Courtesy of the Centers for Disease Control and Prevention.



Quiz Group

A. Formation of Endospores

Under conditions of starvation, especially the lack of carbon and nitrogen sources, a single endospores form within some of the bacteria. The process is called sporulation.

First the DNA replicates **(Slideshow Fig. 3, step 1)** and a cytoplasmic membrane septum forms at one end of the cell **(Slideshow Fig. 3, step 3)**. A second layer of cytoplasmic membrane then forms around one of the DNA molecules **(Slideshow Fig. 3, step 4)** - the one that will become part of the endospore - to form a forespore **(Slideshow Fig. 3, step 5)**. Both of these membrane layers then synthesize peptidoglycan in the space between them to form the first protective coat, the cortex **(Slideshow Fig. 3, step 6)** that lies next to the germ cell wall that will eventually form the cell wall of the bacterium upon germination. Calcium dipocolinate is also incorporated into the forming endospore. A spore coat composed of a keratin-like protein then forms around the cortex **(Slideshow Fig. 3, step 7)**. Sometimes an outer membrane composed of lipid and protein and called an exosporium is also seen **(Slideshow Fig. 3, step 8)**.

Finally, the remainder of the bacterium is degraded and the endospore is released **(Slideshow Fig. 3, step 9)**. Sporulation generally takes around 15 hours. The process is summarized in **(Slideshow Fig. 3).**

Fig. 3, steps 1-9: Formation of endospores

Slideshow Activity

GIF animation showing endospore formation
GIF animation showing endospore germination

Ordering Activity

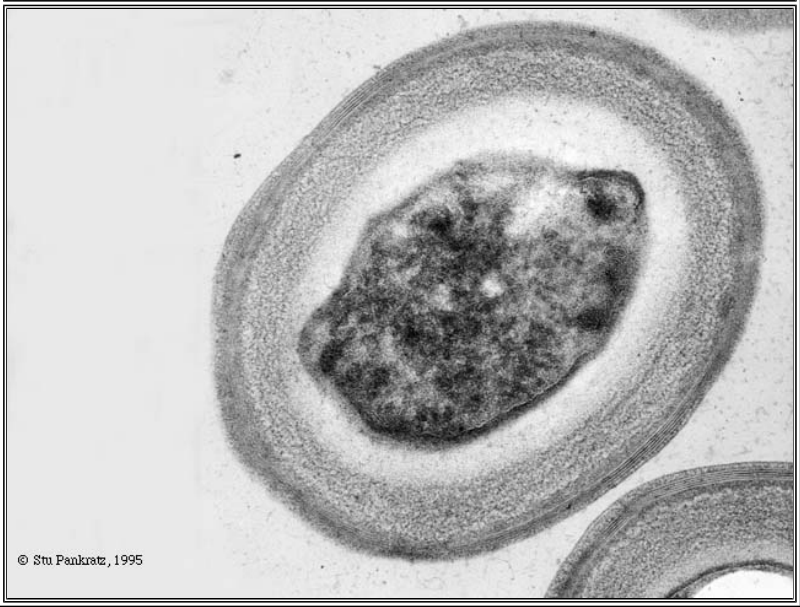
B. Endospore Structure

The completed endospore consists of multiple layers of resistant coats (including a **cortex,** a **spore coat**, and sometimes an **exosporium**) surrounding a nucleoid, some ribosomes, RNA molecules, and enzymes **(Slideshow Fig. 3, step 10)**.

Transmission electron micrograph of an endospore of *Bacillus stearothermophilus*.

Image by Stu Pankratz.

Courtesy of the Microbe Zoo web page of Michigan State University.



Slideshow Activity

(Some bacteria produce spore-like structures distinct from endospores. **Exospores** are heat resistant spores produced by a budding process in members of the genus *Metylosinus* and *Rhodomicrobium*. **Cysts** are resistant to drying and are formed singly within vegetative cells by *Azotobacter, Myxococcus*, and *Sporocytophaga*. **Conidia** are heat-susceptible asexual reproductive spores produced by various genera of branching bacteria belonging to the group Actinomycetes.)

C. Function of Endospores

An endospore is not a reproductive structure but rather a **resistant, dormant survival form** of the organism. Endospores are quite resistant to high temperatures (including boiling), most disinfectants, low energy radiation, drying, etc. The endospore can then survive until a variety of environmental stimuli trigger germination, allowing outgrowth of a single vegetative bacterium as shown in **(Slideshow Fig. 3, steps 11 and 12)**. Viable endospores have reportedly been isolated from the GI tract of a bee embedded in amber between 25 and 40 million years ago. Viable endospores of a halophilic (salt-loving) bacteria have also reportedly been isolated from fluid inclusions in salt crystals dating back over 250 million years!

Fig. 4: Scanning Electron Micrograph of the Germination of an Endospores of *Clostridium sporogenes.*

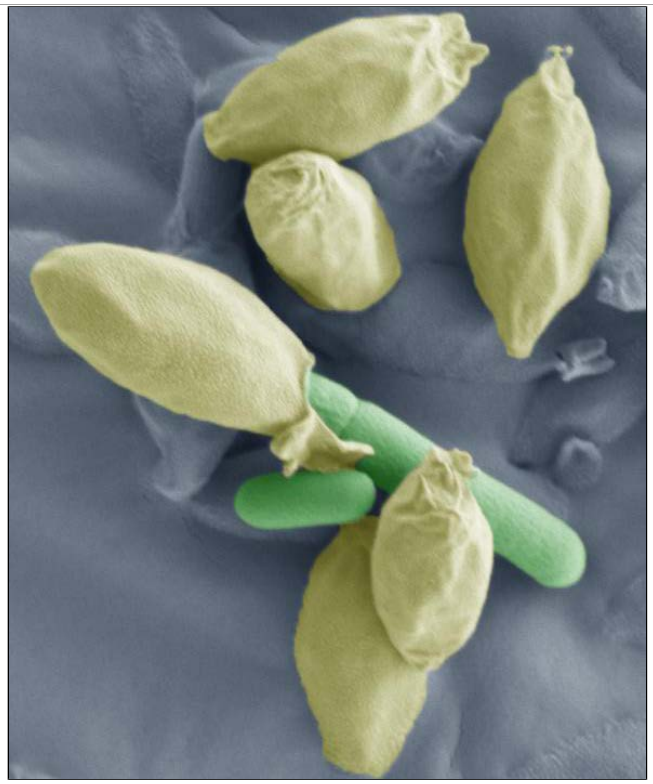


Image provided by Kathryn Cross, Institute of Food Research.

Fig. 3, steps 11-12:Germination of endospores

Slideshow Activity

Bacterial **endospores** are resistant to antibiotics, most disinfectants, and physical agents such as radiation, boiling, and drying. The impermeability of the spore coat is thought to be responsible for the endospore's resistance to chemicals. The heat resistance of endospores is due to a variety of factors:

- Calcium-dipicolinate, abundant within the endospore, may stabilize and protect the endospore's DNA.
- Small acid-soluble proteins (SASPs) saturate the endospore's DNA and protect it from heat, drying, chemicals, and radiation. They also function as a carbon and energy source for the development of a vegetative bacterium during germination.
- The cortex may osmotically remove water from the interior of the endospore and the dehydration that results is thought to be very important in the endospore's resistance to heat and radiation.
- Finally, DNA repair enzymes contained within the endospore are able to repair damaged DNA during germination.

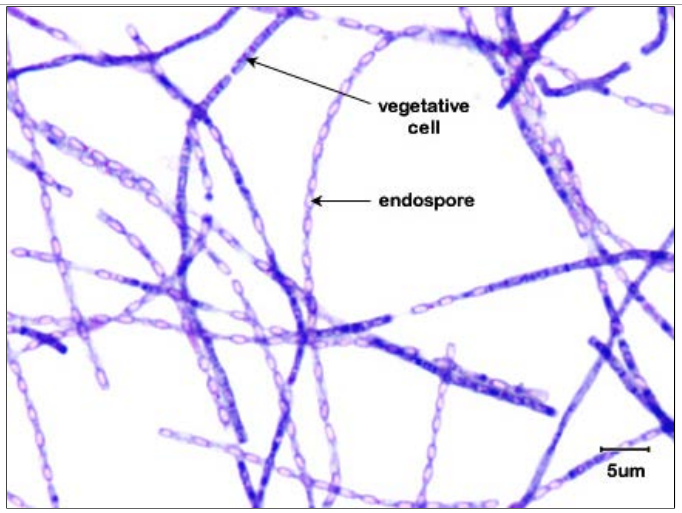
TPS Question

D. Endospores and Infectious Disease

Although harmless themselves until they germinate, they are **involved in the transmission of some diseases to humans**. Infections transmitted to humans by endospores include:

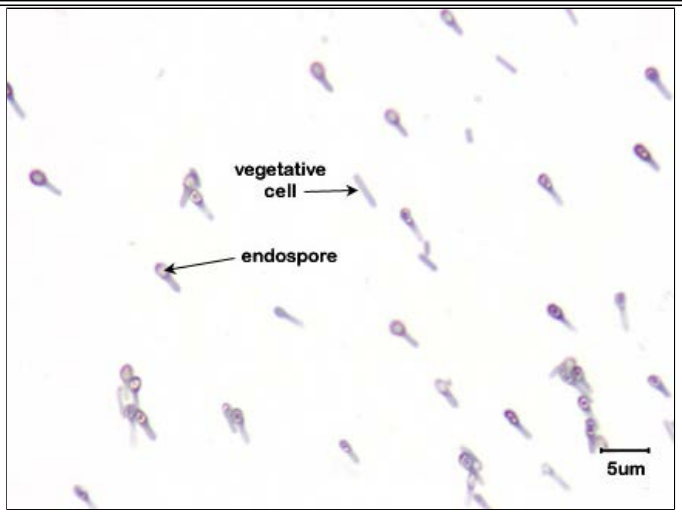
1. Anthrax, caused by *Bacillus anthracis*; endospores can be inhaled, ingested, or enter wounds where they germinate and the vegetative bacteria subsequently replicate.

Endospore stain of *Bacillus anthracis*



2. Tetanus, caused by *Clostridium tetani*; endospores enter anaerobic wounds where they germinate and the vegetative bacteria subsequently replicate.

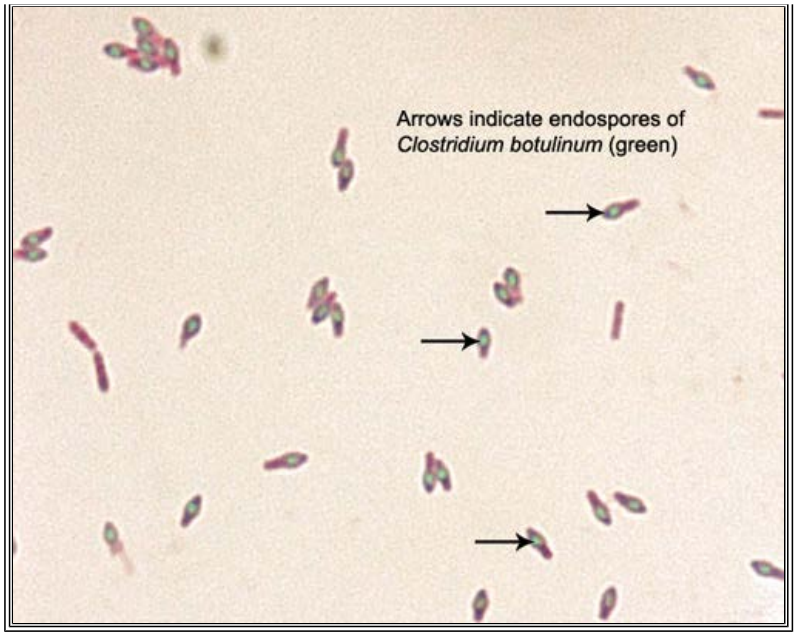
Endospore stain of *Clostridium tetani*



3. Botulism, caused by *Clostridium botulinum*; endospores enter the anaerobic environment of improperly canned food where they germinate and subsequently replicate.

Endospore stain of *Clostridium botulinum*

Courtesy of the Public Health Image Library (Images), Centers for Disease Control and Prevention. Licensed for use, ASM MicrobeLibrary.

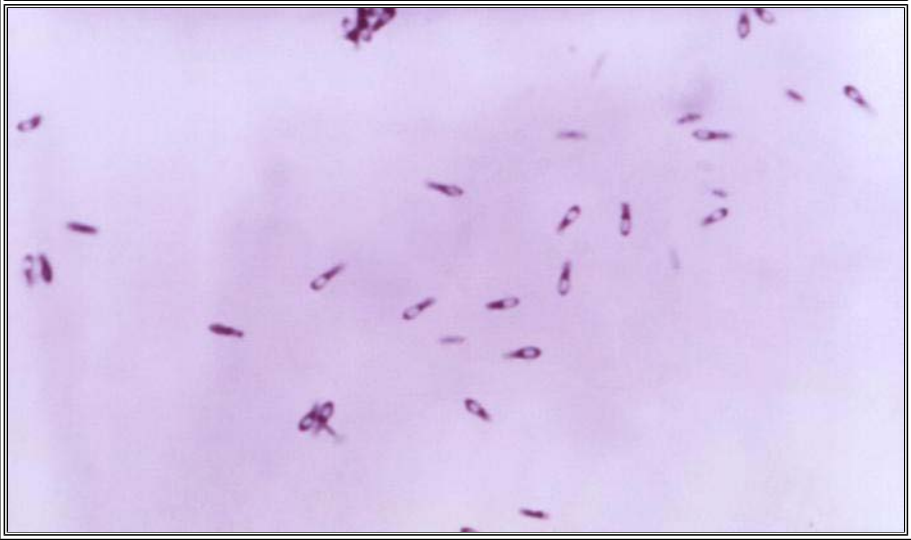


4. Gas gangrene, caused by *Clostridium perfringens;*•endospores enter anaerobic wounds where they germinate and the vegetative bacteria subsequently replicate.

Endospore stain of *Clostridium perfringens*

Image provided by Dr. Holderman.

Courtesy of the Centers for Disease Control and Prevention.

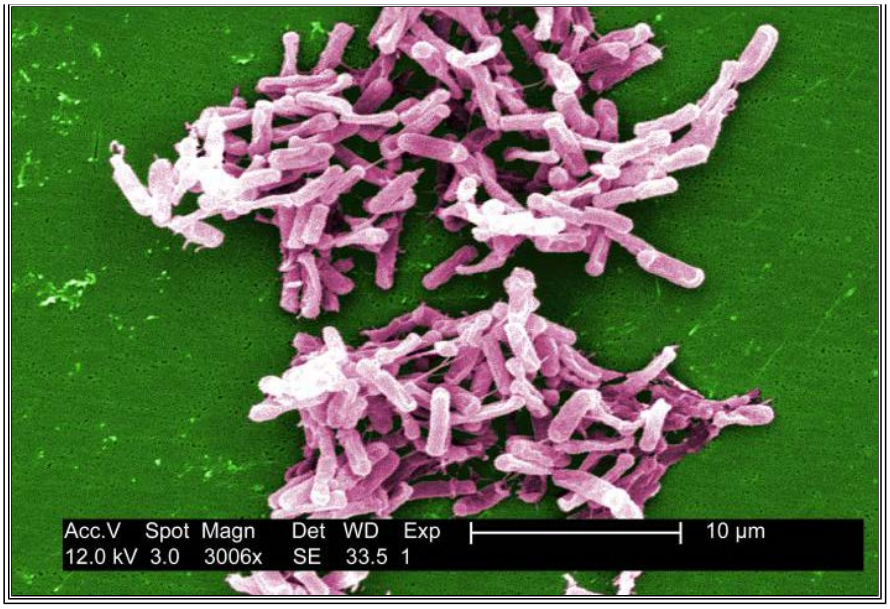


5. Pseudomembranous colitis caused by *Clostridium difficile;* antibiotics destroy the normal microbiota of the intestines that keep the growth of *C. difficile* in check while the endospores of *C. difficile* survive and subsequently germinate and replicate before the microbiota is restored.

Endospore stain of *Clostridium perfringens*

Image provided by Lois S. Wiggs.

Courtesy of the Centers for Disease Control and Prevention.



Highlighted Bacterium: *Clostridium tetani*

Click on this link, read the description of *Clostridium tetani*, and be able to match the bacterium with its description on an exam.

Concept map for Bacterial Endospores

E-Medicine article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Bacillus anthracis*
- *Clostridium tetani*
- *Clostridium perfringens*
- *Clostridium botulinum*

Self Quiz for Bacterial Endospores

Quiz Group

Back to Unit 1 Table of Contents

Back to Softchalk Lessons Table of Contents

The bacterial glycocalyx and biofilms
THE BACTERIAL GLYCOCALYX AND BIOFILMS

The Bacterial Glycocalyx and Biofilms

Fundamental Statements for this Lesson:

1. All bacteria secrete some sort of glycocalyx, an outer viscous covering of fibers extending from the bacterium.
2. An extensive, tightly bound glycocalyx adhering to the cell wall is called a capsule.
3. Phagocytosis involves several distinct steps including attachment of the microbe to the phagocyte through unenhanced or enhanced attachment, ingestion of the microbe and its placement into a phagosome, and the destruction of the microbe after fusion of lysosomes with the phagosome.
4. Capsules enable bacteria to resist unenhanced attachment by covering up bacterial PAMPs so they are unable to bind to endocytic pattern-recognition receptors.
5. The glycocalyx also enables some bacteria to adhere to environmental surfaces, colonize, and resist flushing.
6. The body's adaptive immune defenses can eventually overcome bacterial capsules by producing opsonizing antibodies (IgG) against the capsule that are able to stick the capsule to the phagocyte.
7. Biofilms are groups of bacteria attached to a surface and enclosed in a common secreted adhesive matrix and are functional, interacting, and growing bacterial communities.
8. Most bacteria in nature exist as biofilm populations.
9. Many chronic and difficult-to-treat infections are caused by bacteria in biofilms.
10. An antigen is a molecular shape that reacts with antigen receptors on lymphocytes to initiate an adaptive immune response.
11. The actual portions or fragments of an antigen that react with antibodies and with receptors on B-lymphocytes and T-lymphocytes are called epitopes.
12. Adaptive (acquired) immunity refers to antigen-specific defense mechanisms that take several days to become protective and are designed to react with and remove a specific antigen. This is the immunity one develops throughout life.
13. Humoral immunity involves the production of antibody molecules in response to an antigen.
14. Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigens. These defense cells help to remove infected cells and cancer cells displaying foreign epitopes.

15. The bacterial glycocalyx (capsule) functions as an antigen and can trigger the production of opsonizing antibody molecules that bind to epitopes of the glycocalyx. These opsonizing antibodies can bind bacteria to phagocytes for more efficient phagocytosis.

Common Course Objectives

1. Identify the parts of a bacterium and their physiological purpose.
2. Explain how various bacterial structures can contribute to bacterial colonization of a host.
3. Explain how various bacterial structures can contribute to the initiation of immune defenses.
4. Describe the different ways in which antibodies play a role in removing and/or neutralizing microbes and toxins.
5. Provide a diagnosis for a "patient" when given a list of symptoms and a description of the bacterium.

Detailed Learning Objectives for this Lesson

1*. State the chemical composition and 2 common functions of a bacterial glycocalyx.

2*. Briefly describe the following steps in phagocytosis:

a. unenhanced attachment

b. enhanced attachment

c. engulfment

d. destruction

3*. Briefly describe how a capsule might initially enable some bacteria to resist being phagocytosed by white blood cells.

4*. Briefly describe how opsonizing antibodies made against bacterial capsules help protect the body.

5*. Define biofilm and state at least 3 advantages of biofilm formation to bacteria.

6*. Compare and contrast innate immunity and adaptive immunity.

7*. Define antigen and epitope.

8*. Define humoral immunity and cell-mediated immunity.

9*. Briefly describe how opsonizing antibodies can promote phagocytosis.

(*) = Common theme throughout the course

Highlighted Bacterium

1. Read the description of *Strepococcus pneumoniae* and match the bacterium with the description of the organism and the infection it causes.

TPS Questions

In this section on Prokaryotic Cell Anatomy we are looking at the various anatomical parts that make up a bacterium. As mentioned in the introduction to this section, a typical bacterium usually consists of:

- a cytoplasmic membrane surrounded by a peptidoglycan cell wall and maybe an outer membrane;
- a fluid cytoplasm containing a nuclear region (nucleoid) and numerous ribosomes; and
- often various external structures like a glycocalyx, flagella, and pili.

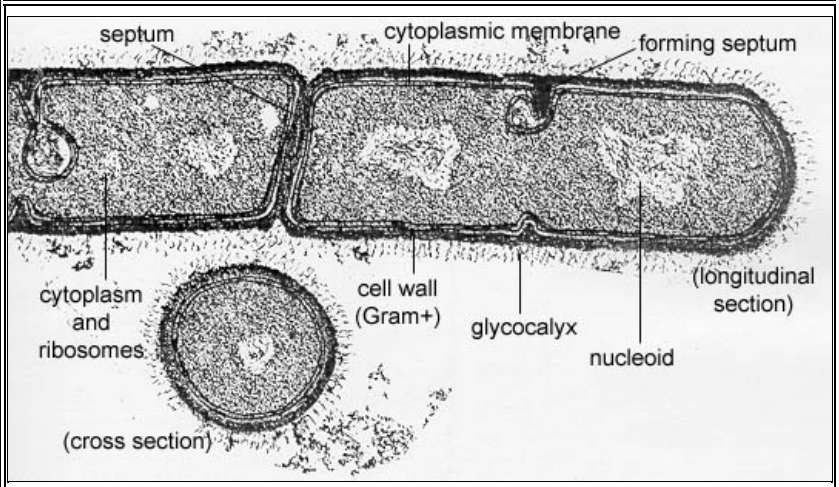
We will now look at the bacterial glycocalyx and biofilms.

The Bacterial Glycocalyx and Biofilms

1. The Glycocalyx (Capsules and Slime Layers)

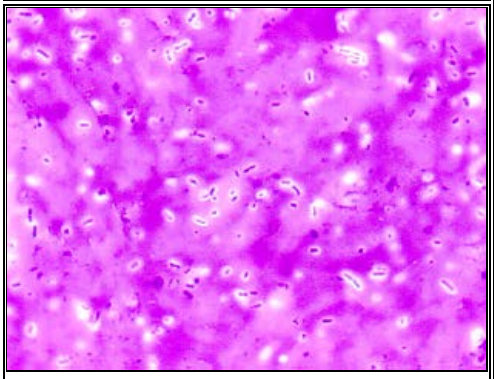
All bacteria secrete some sort of glycocalyx, an outer viscous covering of fibers extending from the bacterium **(see Fig. 1, Fig. 2, and Fig. 3)**. If it appears as an extensive, tightly bound accumulation of gelatinous material adhering to the cell wall, it is called a capsule as shown in the photomicrograph in **Fig. 2**. If the glycocalyx appears unorganized and more loosely attached, it is referred to as a slime layer.

Fig. 1: Transmission Electron Micrograph of a Prokaryotic Cell (*Bacillus megaterium*)



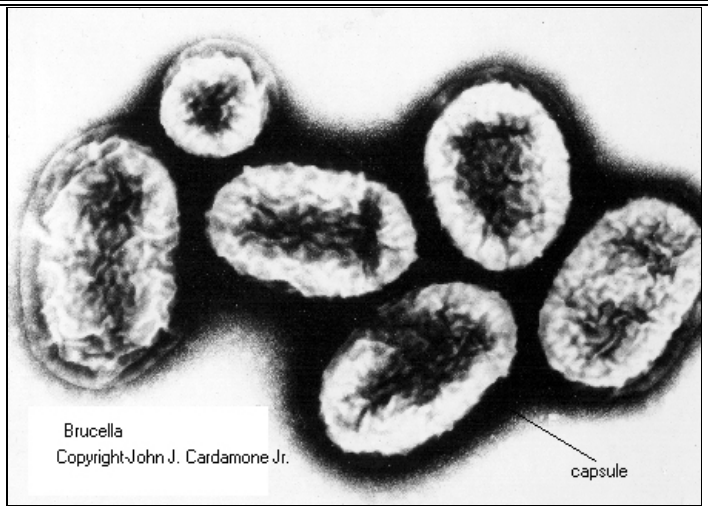
Note glycocalyx surrounding the cell wall.

Fig. 2: Capsule stain of *Enterobacter aerogenes*



Note colorless capsules surrounding purple bacilli.

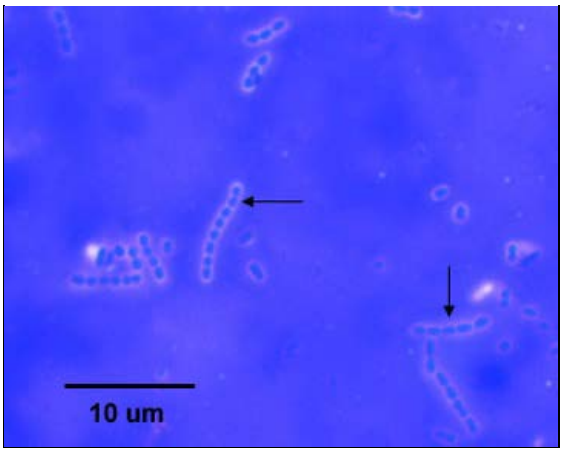
Fig. 3.Encapsulated *Brucella* species



A. Structure and Composition

The glycocalyx is usually a viscous **polysaccharide or polypeptide slime**. Actual production of a glycocalyx often depends on environmental conditions.

A capsule stain of *Streptococcus lactis*.



Note the capsule (arrow) surrounding the streptococcus.

Quiz Group

B. Functions and Significance to Bacterial Pathogenicity

Although a number of functions have been associated with the glycocalyx, such as protecting bacteria against drying, trap nutrients, etc., for our purposes there are two very important functions. The glycocalyx enables certain bacteria to **resist phagocytic engulfment** by white blood cells in the body or protozoans in soil and water. The glycocalyx also enables some bacteria to **adhere** to environmental surfaces (rocks, root hairs, teeth, etc.), **colonize**, and **resist flushing**.

1. Preview of the Steps in Phagocytosis

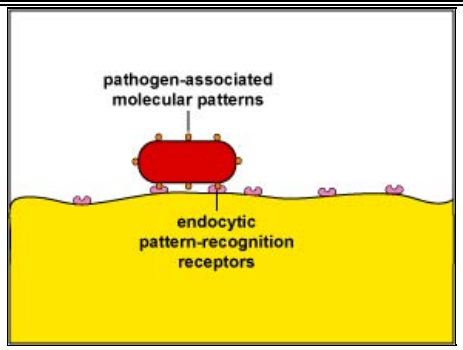
As will be seen in Unit 5, there are several steps involved in phagocytosis.

a. Attachment

First the **surface of the microbe must be attached to the cytoplasmic membrane of the phagocyte**. Attachment of microorganisms is necessary for ingestion and may be **unenhanced or enhanced**.

1. **Unenhanced attachment** is a general recognition of what are called **pathogen-associated molecular patterns** or PAMPs - components of common molecules such as peptidoglycan, teichoic acids, lipopolysaccharide, mannans, and glucans common in microbial cell walls but not found on human cells - by means of glycoprotein known as endocytic pattern-recognition receptors on the surface of the phagocytes **(see Fig. 4)**.

Fig. 4: Unenhanced Attachment of Bacteria to Phagocytes



Glycoprotein molecules known as pattern-recognition receptors are found on the surface of phagocytes. They are so named because they recognize and bind to pathogen-associated molecular patterns - components of common molecules such as peptidoglycan, teichoic acids, lipopolysaccharide, mannans, and glucans - found in many microorganisms.

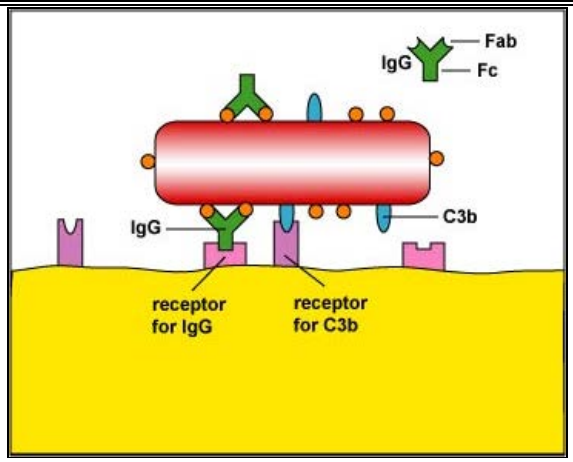
Flash animation illustrating the function of endocytic pattern-recognition receptors on phagocytes.
Copyright © Gary E. Kaiser
html5 version of animation for iPad illustrating the function of endocytic pattern-recognition receptors on phagocytes.
Glycoprotein molecules known as endocytic pattern-recognition receptors are found on the surface of phagocytes. They are so named because they recognize and bind to pathogen-associated molecular patterns - molecular components associated with microorganisms but not found as a part of eukaryotic cells. These include bacterial molecules such as peptidoglycan, lipoteichoic acids, mannans, and lipopolysaccharide (LPS). These receptors enable the phagocyte to attach to the cell wall of the microorganism so it can be engulfed and destroyed by lysosomes.

For more information: Preview of pathogen-associated molecular patterns (PAMPs)

For more information: Preview of pattern-recognition receptors (PRRs)

2. **Enhanced attachment** is the attachment of microbes to phagocytes by way of an antibody molecule called IgG or proteins produced during the complement pathways called C3b and C4b **(see Fig. 5)**. Molecules such as IgG and C3b that promote enhanced attachment are called opsonins and the process is called opsonization. Enhanced attachment is much more specific and efficient than unenhanced.

Fig. 5: Enhanced Attachment of Bacteria to Phagocytes



One of the functions of certain antibody molecules known as IgG is to stick antigens such as bacterial proteins and polysaccharides to phagocytes. The tips of the antibody, the Fab portion, have a shape that fits epitopes, portions of an antigen with a complementary shape. The stalk of the antibody is called the Fc portion and is able to bind to Fc receptors on phagocytes. Also, when body defense pathways known as the complement pathways are activated, one of the beneficial defense proteins made is called C3b. C3b binds by one end to bacterial surface proteins and by the other end to C3b receptors on phagocytes. The IgG and C3b are also known as opsonins and the process of enhanced attachment is also called opsonization.

Flash animation illustrating the function of enhanced attachment (opsonization) by IgG.
Copyright © Gary E. Kaiser
html 5 version of animation for iPad illustrating the function of enhanced attachment (opsonization) by IgG.
The Fab portion of IgG binds to epitopes of a microbe. The Fc portion can now attach the microbe to Fc receptors on phagocytes for enhanced attachment, also known as opsonization. Once attached to the phagocyte by way of IgG, the microbe can be engulfed more efficiently and placed in a phagosome, and destroyed by lysosomes.

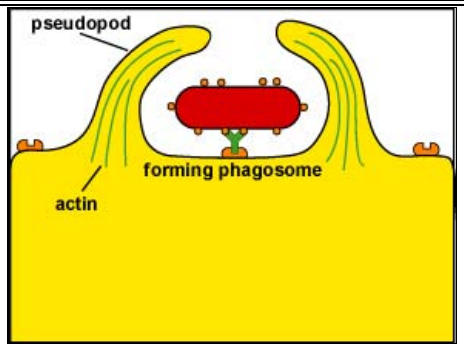
For more information: Preview of antibodies

For more information: Preview of the benefits of the complement pathways

b. Engulfment

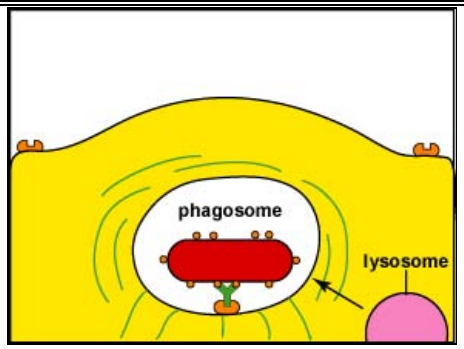
Following attachment, polymerization and then depolymerization of actin filaments **send pseudopods out to engulf the microbe (see Fig. 6)** and **place it in a vesicle called a** phagosome **(see Fig. 7)**.

Fig. 6: Formation of Pseudopods by Rearrangement of Actin Molecules



Following attachment, polymerization and depolymerization of actin molecules send pseudopods out to engulf the bacterium and place it in a vesicle called a phagosome.

Fig. 7: Placing the Bacterium in a Phagosome



Following engulfment, the bacterium is placed in a vesicle called a phagosome.

Flash animation summarizing phagocytosis through unenhanced attachment.

html5 version of animation for iPad summarizing phagocytosis through unenhanced attachment.

Unenhanced attachment is a general recognition of what are called pathogen-associated molecular patterns or PAMPs- components of common molecules such as peptidoglycan, teichoic acids, lipopolysaccharide, mannans, and glucans common in microbial cell walls but not found on human cells - by means of glycoproteins known as endocytic pattern-recognition receptors on the surface of the phagocytes. Following attachment, polymerization and then depolymerization of actin filaments send pseudopods out to engulf the microbe and place it in a vesicle called a phagosome. Finally, lysosomes, containing digestive enzymes and microbicidal chemicals, fuse with the phagosome containing the ingested microbe and the microbe is destroyed.

Flash animation summarizing phagocytosis through enhanced attachment (opsonization).

html5 version of animation for iPad summarizing phagocytosis through enhanced attachment (opsonization).

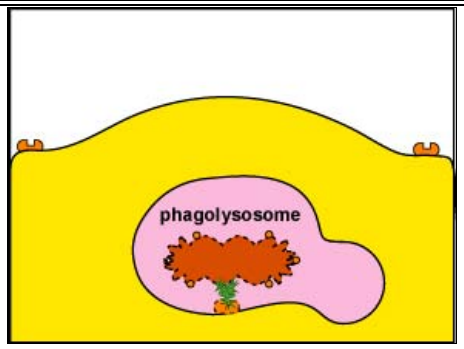
Enhanced attachment is the attachment of microbes to phagocytes by way of molecules such as the antibody molecule IgG or proteins produced during the complement pathways called C3b and C4b. Following attachment, polymerization and then depolymerization of actin filaments send pseudopods out to engulf the microbe and

place it in a vesicle called a phagosome. Finally, lysosomes, containing digestive enzymes and microbicidal chemicals, fuse with the phagosome containing the ingested microbe and the microbe is destroyed.

c. Destruction

Finally, lysosomes, containing digestive enzymes and microbicidal chemicals, fuse with the phagosome containing the ingested microbe and the microbe is destroyed **(see Fig. 8)**.

Fig. 8: Fusion of Phagosome and Lysosome



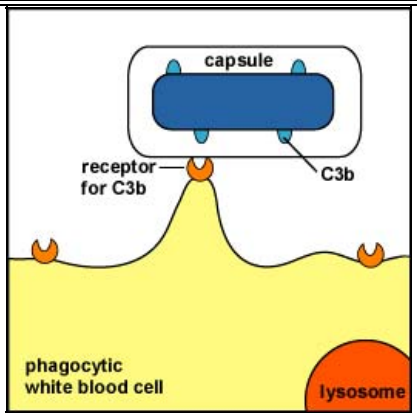
The lysosome its digestive enzymes and microbicidal chemicals fuses with the phagosome containing the ingested bacteria to form a phagolysosome and the bacterium is killed.

Quiz Group

2. Role of glycocalyx in resisting phagocytosis

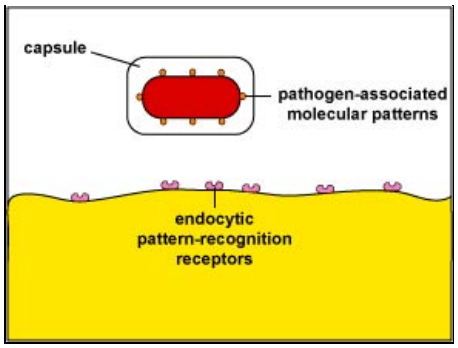
Capsules enable bacteria to resist phagocytosis by evading the complement and antibody body defense responses. For example, **capsules can resist unenhanced attachment** by preventing the glycoprotein receptors on phagocytes from recognizing the bacterial cell wall components and mannose-containing carbohydrates **(see Fig. 10)**. Also, **some capsules simply cover the C3b** that does bind to the bacterial surface and **prevent the C3b receptor on phagocytes from making contact with the C3b (see Fig. 9)**. This will be discussed in greater detail later in Unit 3 under Bacterial Pathogenesis.

Fig. 9: Bacterial Capsule Preventing C3b Receptors on Phagocytes from Binding to C3b Attached to a Bacterial Cell Wall



In some bacteria, the capsule covers the opsonin C3b bound to the bacterial cell wall so that it cannot bind to C3b receptors (called CR1) on the surface of phagocytes.

Fig. 10: Capsules Blocking the Unenhanced Attachment of Bacteria to Phagocytes



Glycoprotein molecules known as endocytic pattern-recognition receptors are found on the surface of phagocytes. They are so named because they recognize and bind to pathogen-associated molecular patterns - components of common molecules such as peptidoglycan, teichoic acids, lipopolysaccharide, mannans, and glucans - found in many microorganisms. Capsules can cover up these surface molecules preventing their attachment to the endocytic pattern-recognition sites on the phagocyte.

Flash animation illustrating how capsules can block unenhanced attachment of pathogen-associated molecular patterns to endocytic pattern-recognition receptors on phagocytes.

html5 version of animation for iPad illustrating how capsules can block unenhanced attachment of pathogen-associated molecular patterns to endocytic pattern-recognition receptors on phagocytes.

Glycoprotein molecules known as endocytic pattern-recognition receptors are found on the surface of phagocytes. They are so named because they recognize and bind to pathogen-associated molecular patterns - molecular components associated with microorganisms but not found as a part of eukaryotic cells. These include bacterial molecules such as peptidoglycan, lipoteichoic acids, and lipopolysaccharide (LPS). These receptors enable the phagocyte to attach to the cell wall of the microorganism so it can be engulfed and destroyed by lysosomes. Capsules can cover the pathogen-associated molecular patterns blocking their binding to endocytic pattern-recognition receptors.

Examples of bacteria that use their capsule to resist phagocytic engulfment include *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, *Neisseria meningitidis*, *Bacillus anthracis*, and *Bordetella pertussis*.

For more information: Preview of the ability to resist phagocytic engulfment

The body's immune defenses, however, can eventually get around the capsule by producing opsonizing antibodies (IgG) against the capsule. The antibody then sticks the capsule to the phagocyte. In vaccines against pneumococccal pneumonia and *Haemophilus influenzae* type b, it is capsular polysaccharide that is given as the antigen in order to stimulate the body to make opsonizing antibodies against the encapsulated bacterium.

Flash animation showing phagocytosis of an encapsulated bacterium through opsonization.

html5 version of animation for iPad showing phagocytosis of an encapsulated bacterium through opsonization.

The Fab portion of IgG binds to epitopes of a capsule. The Fc portion can now attach the capsule to Fc receptors on phagocytes for enhanced attachment. Once attached to the phagocyte by way of IgG, the encapsulated bacterium can be engulfed more efficiently and placed in a phagosome.

Highlighted Bacterium: *Streptococcus pneumoniae*

Click on this link, read the description of *Streptococcus pneumoniae*, and be able to match the bacterium with its description on an exam.

Movie of an encapsulated bacterium resisting engulfment by a neutrophil.

Phagocytosis. © James Sullivan, author. Licensed for use, ASM MicrobeLibrary.

TPS Questions

3. Role of glycocalyx in adhering to and colonizing environmental surfaces

The glycocalyx also enables some bacteria to **adhere** to environmental surfaces (rocks, root hairs, teeth, etc.), **colonize**, and **resist flushing**. For example, many normal flora bacteria produce a capsular polysaccharide matrix or glycocalyx to form a biofilm on host tissue as discussed below.

Concept map for Glycocalyx and Biofilms

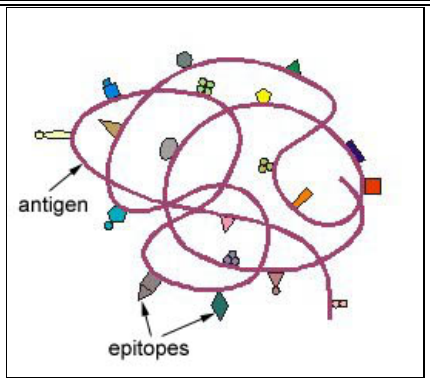
C. Significance of the glycocalyx in the Initiation of Body Defense

Initiation of Adaptive Immunity

Polysaccharides or proteins associated with bacterial capsules function as antigens and initiate adaptive immunity. An **antigen** is defined as **a molecular shape that reacts with antibody molecules and with antigen receptors on lymphocytes**. We recognize those molecular shapes as foreign or different from our body's molecular shapes because they **fit specific antigen receptors on our B-lymphocytes and T-lymphocytes**, the cells that carry out adaptive immunity.

The actual portions or fragments of an antigen that react with antibodies and with receptors on B-lymphocytes and T-lymphocytes are called **epitopes**. An epitope is typically a group of 5-15 amino acids with a unique shape that makes up a portion of a protein antigen (**see Fig. 11A**), or 3-4 sugar residues branching off of a polysaccharide antigen (**see Fig. 11B**). **A single microorganism has many hundreds of different shaped epitopes that our lymphocytes can recognize as foreign and mount an adaptive immune response against**.

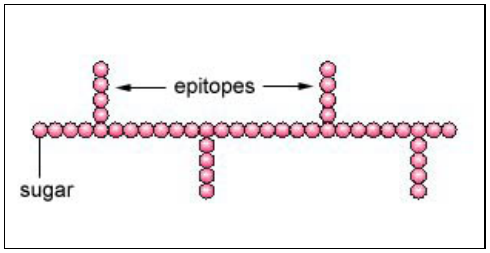
Fig. 11A: Illustration of Epitopes of a Protein Antigen



Note many epitopes of different specificities.

Copyright © Gary E. Kaiser

Fig. 11B: Illustration of Epitopes of a Polysaccharide Antigen



Note many epitopes of the same specificity.

Copyright © Gary E. Kaiser

The body recognizes an antigen as foreign when **epitopes of that antigen bind to B-lymphocytes and T-lymphocytes by means of epitope-specific receptor molecules having a shape complementary to that of the epitope**. The epitope receptor on the surface of a B-lymphocyte is called a B-cell receptor and is actually an antibody molecule. The receptor on a T-lymphocyte is called a T-cell receptor (TCR).

There are two major branches of the adaptive immune responses: humoral immunity and cell-mediated immunity.

1. Humoral immunity: Humoral immunity involves the production of antibody molecules in response to an antigen and is mediated by B-lymphocytes. Through a variety of mechanisms, these antibodies are able to remove or neutralize microorganisms and their toxins after binding to their epitopes. For example, antibodies made against capsular antigens can stick bacteria to phagocytes, a process called opsonization.

Flash animation showing phagocytosis of an encapsulated bacterium by way of opsonizing antibodies

html5 version of animation for iPad showing phagocytosis of an encapsulated bacterium by way of opsonizing antibodies.

The Fab portion of IgG binds to epitopes of a capsule. The Fc portion can now attach the capsule to Fc receptors on phagocytes for enhanced attachment. Once attached to the phagocyte by way of IgG, the encapsulated bacterium can be engulfed more efficiently and placed in a phagosome.

2. Cell-mediated immunity: Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigen and is mediated by T-lymphocytes. These defense cells help to remove infected cells and cancer cells displaying foreign epitopes.

Adaptive immunity will be discussed in greater detail later in the course.

For More Information: Review of antigens and epitopes

Self Check

Quiz Group

2. Biofilms

Many pathogenic bacteria, as well as normal flora and many environmental bacteria, form complex bacterial communities as biofilms. Biofilms are groups of bacteria attached to a surface and enclosed in a common secreted adhesive matrix, typically polysaccharide in nature.

Bacteria in biofilms are often able to communicate with one another by a process called quorum sensing (discussed later in Unit 2) and are able to interact with and adapt to their environment as a population of bacteria rather than as individual bacteria. By living as a community of bacteria as a biofilm, these bacteria are better able to:

- resist attack by antibiotics;
- trap nutrients for bacterial growth and remain in a favorable niche;
- adhere to environmental surfaces and resist flushing;
- live in close association and communicate with other bacteria in the biofilm; and
- resist phagocytosis and attack by the body's complement pathways.

For More Information: Preview of Quorum Sensing

Biofilms are, therefore, functional, interacting, and growing bacterial communities. Biofilms even contain their own water channels for delivering water and nutrients throughout the biofilm community.

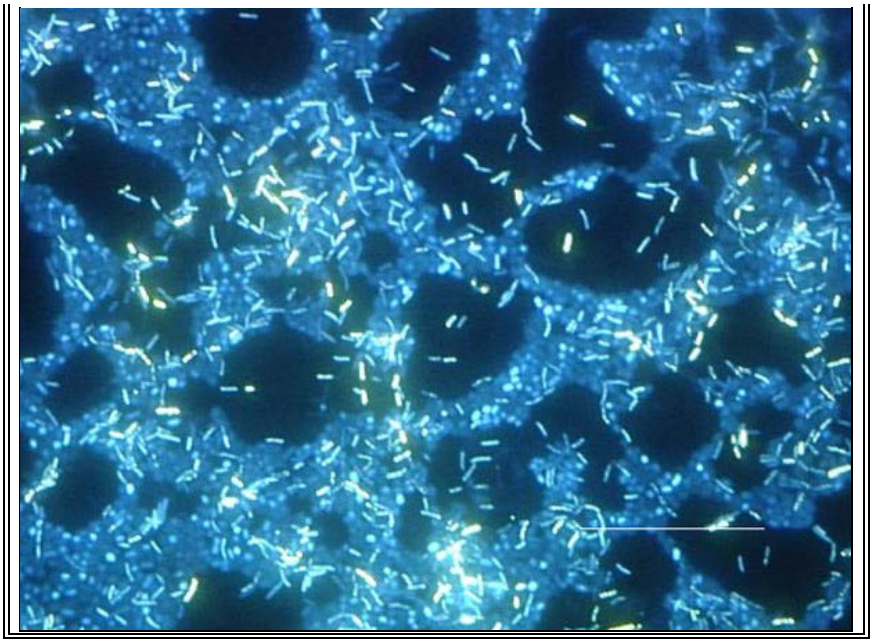
Link to an electron micrograph of a biofilm of *Haemophilus influenzae*

from Biomedcentral.com

Photomicrograph of a biofilm with water channels

Photograph by Rodney M. Donlan: "Biofilms: Microbial Life on Surfaces"

Courtesy of Centers for Disease Control and Prevention



To initiate biofilm formation, planktonic bacteria (free individual bacteria not in a biofilm) contact an environmental surface through their motility or by random collision. These planktonic bacteria then attach to that surface using pili or cell wall adhesins. This attachment then signals the expression of genes involved in quorum sensing and, ultimately, biofilm formation. As the biofilm matrix is secreted, motile bacteria lose their flagella and become nonmotile.

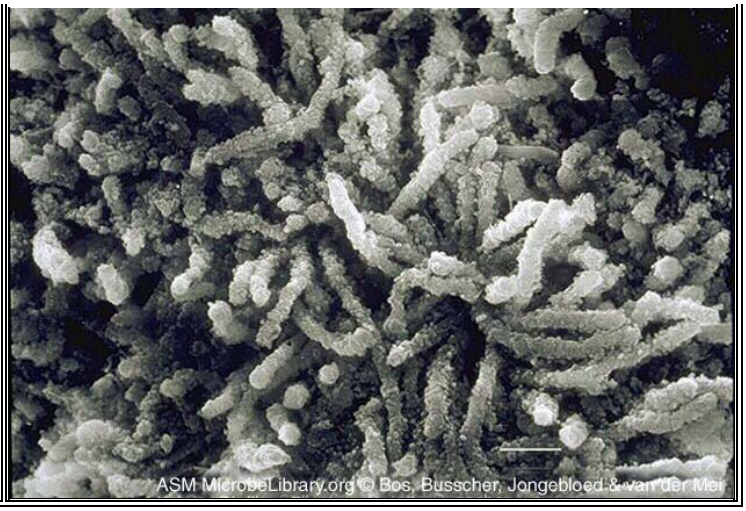
Planktonic *Pseudomonas aeruginosa*, for example, uses its polar flagellum to move through water or mucus and make contact with a solid surface such as the body's mucous membranes. It then can use pili and cell wall adhesins to attach to the epithelial cells of the mucous membrane. Attachment activates signaling and quorum sensing genes to eventually enable the population of *P. aeruginosa* to start synthesizing a polysaccharide biofilm composed of alginate. As the biofilm grows, the bacteria lose their flagella to become nonmotile and secrete a variety of enzymes that enable the population to obtain nutrients from the host cells. Eventually the biofilm mushrooms up and develops water channels to deliver water and nutrients to all the bacteria within the biofilm. As the biofilm begins to get too crowded with bacteria, quorum sensing enables some of the *Pseudomonas* to again produce flagella, escape the biofilm, and colonize a new location **(See Slideshow Figs. 11A-11G below)**.

Figs.11A - 11G: Biofilm formation by *Pseudomonas aeruginosa*

Slideshow Activity

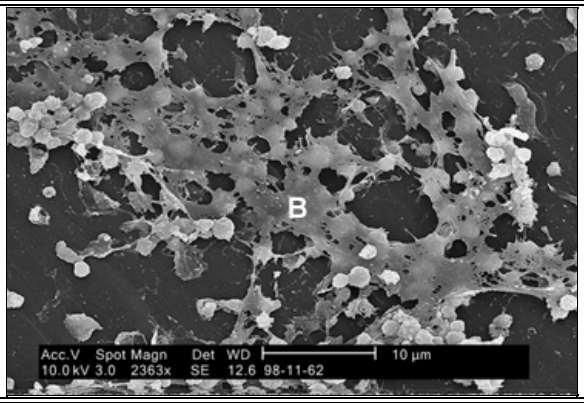
Streptococcus mutans, and *Streptococcus sobrinus* , two bacteria implicated in initiating dental caries, break down sucrose into glucose and fructose. *Streptococcus mutans* can uses an enzyme called dextransucrase to convert sucrose into a sticky polysaccharide called dextran that forms a biofilm enabling the bacteria to adhere to the enamel of the tooth and form plaque. This will be discussed in greater detail later in Unit 2 under Bacterial Pathogenicity. *S. mutans* and *S. sobrinus* also ferment glucose in order to produce energy. The fermentation of glucose results in the production of **lactic acid** that is released onto the surface of the tooth and initiates decay.

Scanning electron micrograph of *Streptococcus* growing in the enamel of a tooth. **© Lloyd Simonson, author. Licensed for use, ASM MicrobeLibrary.**	**Scanning electron micrograph of dental plaque**. **© H. Busscher, H. van der Mei, W. Jongebloed, R Bos, authors. Licensed for use, ASM MicrobeLibrary.**



Scanning electron micrograph of *Staphylococcus aureus* forming a biofilm in an indwelling catheter

Image provided by Janice Carr.
Courtesy of the Centers for Disease Control and Prevention.



A number of biofilm-forming bacteria, such as uropathogenic *Escherichia coli* (UPEC), enterohemorrhagic *E. coli* (EHEC), *Citrobacter* species, *Salmonella* species, and *Mycobacterium tuberculosis* are able to **produce amyloid fibers that can play a role in such processes as attachment to host cells, invasion of host cells, and biofilm formation**. **Curli** is an example of such an amyloid fiber produced by UPEC and *Salmonella*.

Many chronic and difficult-to-treat infections are caused by bacteria in biofilms. Within biofilms, bacteria grow more slowly, exhibit different gene expression than free planktonic bacteria, and are more resistant to antimicrobial agents such as antibiotics because of the reduced ability of these chemicals to penetrate the dense biofilms matrix. Biofilms have been implicated in tuberculosis, kidney stones, *Staphylococcus* infections, Legionnaires' disease, and periodontal disease. It is further estimated that as many as 10 million people a year in the US may develop biofilm-associated infections as a result of invasive medical procedures and surgical implants.

Concept map for Glycocalyx and Biofilms

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Streptococcus pneumoniae*
- *Haemophilus influenzae*
- *Neisseria meningitidis*
- *Bacillus anthracis*
- *Bordetella pertussis*

Self Check

Self Quiz for the Bacterial Glycocalyx and Biofilms

Quiz Group

Back to Unit 1 Table of Contents

Back to Softchalk Lessons Table of Contents

Bacterial flagella and motility
BACTERIAL FLAGELLA

Bacterial Flagella

Fundamental Statements for this Lesson:

1. Many bacteria are motile and use flagella to swim through liquid environments.
2. The basal body of a bacterial flagellum functions as a rotary molecular motor, enabling the flagellum to rotate and propel the bacterium through the surrounding fluid.
3. Bacterial flagella appear in several arrangements, each unique to a particular organism.
4. Motility serves to keep bacteria in an optimum environment via taxis.
5. Taxis refers to a motile response to an environmental stimulus enabling the net movement of bacteria towards some beneficial attractant or away from some harmful repellent.
6. Most bacterial flagella can rotate both clockwise and counterclockwise enabling to stop and change direction.
7. The protein flagellin that forms the filament of bacterial flagella functions as a pathogen-associated molecular pattern or PAMP that binds to pattern-recognition receptors or PRRs on a variety of defense cells of the body to trigger innate immune defenses.
8. Motility and chemotaxis probably help some intestinal pathogens to move through the mucous layer so they can attach to the epithelial cells of the mucous membranes and colonize the intestines.
9. Innate immunity is an antigen-nonspecific defense mechanisms that a host uses immediately or within several hours after exposure to almost any microbe. This is the immunity one is born with and is the initial response by the body to eliminate microbes and prevent infection.
10. An antigen is a molecular shape that reacts with antigen receptors on lymphocytes to initiate an adaptive immune response.
11. The actual portions or fragments of an antigen that react with antibodies and with receptors on B-lymphocytes and T-lymphocytes are called epitopes.
12. Adaptive (acquired) immunity refers to antigen-specific defense mechanisms that take several days to become protective and are designed to react with and remove a specific antigen. This is the immunity one develops throughout life.

13. Humoral immunity involves the production of antibody molecules in response to an antigen.
14. Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigens. These defense cells help to remove infected cells and cancer cells displaying foreign epitopes.
15. Antibodies made against bacterial flagella can immobilize bacteria and/or promote phagocytosis.
16. Motility enables some spirochetes to penetrate deeper in tissue and enter the lymphatics and bloodstream and disseminate to other body sites.

Common Course Objectives

1. Identify the parts of a bacterium and their physiological purpose.
2. Explain how various bacterial structures can contribute to bacterial colonization of a host.
3. Explain how various bacterial structures can contribute to the initiation of immune defenses.
4. Describe the different ways in which antibodies play a role in removing and/or neutralizing microbes and toxins.
5. Provide a diagnosis for a "patient" when given a list of symptoms and a description of the bacterium.

Detailed Learning Objectives for this Lesson

1. Describe the basic structure of a bacterial flagellum and state its function.

2. State what provides the energy for bacterial flagellar rotation.

3. Define the following flagellar arrangements:

    a. monotrichous
    b. lophotrichous
    c. amphitrichous
    d. peritrichous
    e. axial filaments

4. Define taxis.

5. Compare and contrast how bacteria with peritrichous flagella and bacteria with polar flagella carry out taxis.

6*. Compare and contrast innate and adaptive immunity.

7*. State how bacterial flagella may play a role in the initiation of innate immune defenses.

8*. Briefly describe how bacterial flagella and chemotaxis may play a role in the Pathogenicity of some bacteria.

9*. Briefly describe how antibodies made against bacterial flagella can immobilize bacteria and/or promote phagocytosis.

(*) = Common theme throughout the course

Highlighted Bacterium

1. Read the description of *Treponema pallidum* and match the bacterium with the description of the organism and the infection it causes.

TPS Questions

In this section on Prokaryotic Cell Anatomy we are looking at the various anatomical parts that make up a bacterium. As mentioned in the introduction to this section, a typical bacterium usually consists of:

- a cytoplasmic membrane surrounded by a peptidoglycan cell wall and maybe an outer membrane;
- a fluid cytoplasm containing a nuclear region (nucleoid) and numerous ribosomes; and
- often various external structures like a glycocalyx, flagella, and pili.

We will now look at the bacterial flagella.

Bacterial Flagella

A. Structure and Composition

A bacterial flagellum has 3 basic parts: a filament, a hook, and a basal body.

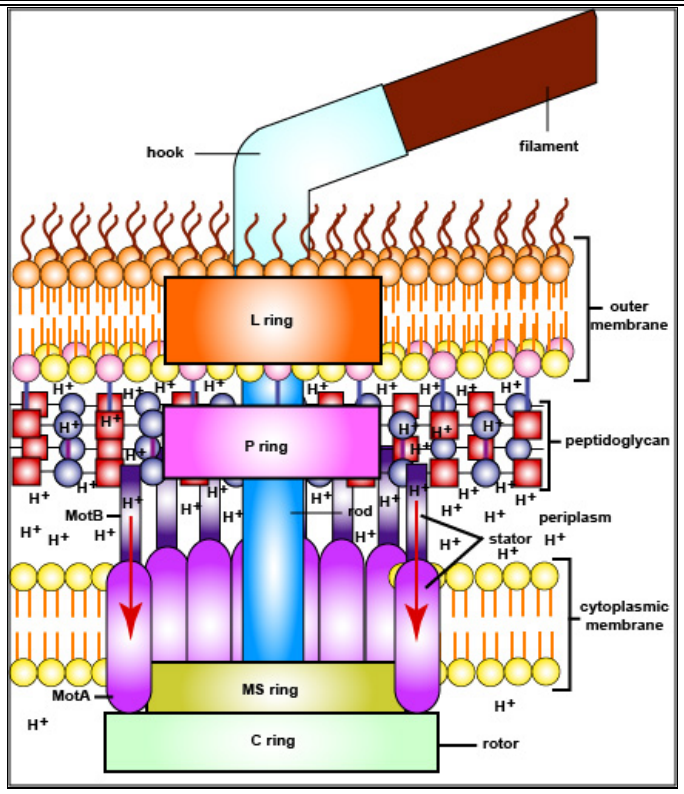
1) The **filament** is the rigid, helical structure that extends from the cell surface. It is composed of the protein **flagellin** arranged in helical chains so as to form a hollow core. During synthesis of the flagellar filament, flagellin molecules coming off of the ribosomes are transported through the hollow core of the filament where they attach to the growing tip of the filament causing it to lengthen. With the exception of a few bacteria, such as *Bdellovibrio* and *Vibrio cholerae*, the flagellar filament is not surrounded by a sheath **(see Fig. 1)**.

2) The **hook** is a **flexible coupling between the filament and the basal body (see Fig. 1)**.

3) The **basal body** consists of a rod and a series of rings that anchor the flagellum to the cell wall and the cytoplasmic membrane **(see Fig. 1)**. Unlike eukaryotic flagella, the bacterial flagellum has no internal fibrils and does not flex. Instead, the basal body **acts as a rotary molecular motor, enabling the flagellum to rotate** and propel the bacterium through the surrounding fluid. In fact, the flagellar motor rotates very rapidly. (Some flagella can rotate up to 300 revolutions per second!)

The MotA and MotB proteins form the stator of the flagellar motor and function to generate torque for rotation of the flagellum. The MS and C rings function as the rotor **(see Fig. 1)**. Energy for rotation comes from the proton motive force provided by protons moving through the Mot proteins along a concentration gradient from the peptidoglycan and periplasm towards the cytoplasm.

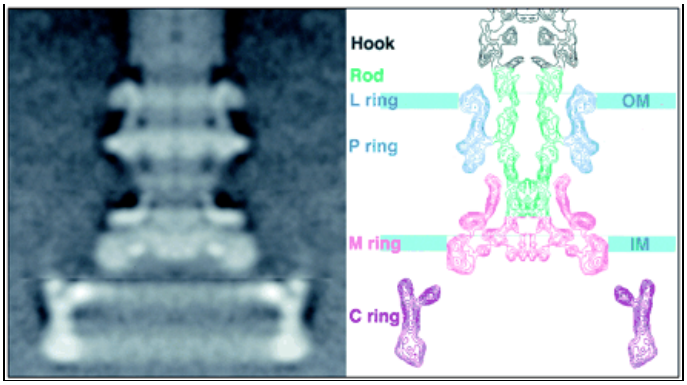
Fig. 1: Structure of a Bacterial Flagellum



The filament of the bacterial flagellum is connected to a hook which, in turn, is attached to a rod. The basal body of the flagellum consists of a rod and a series of rings that anchor the flagellum to the cell wall and the cytoplasmic membrane. In gram-negative bacteria, the L ring anchors the flagellum to the lipopolysaccharide layer of the outer membrane while the P ring anchors the flagellum to the peptidoglycan portion of the cell wall. The MS rings are located in the cytoplasmic membrane and the C ring (the rotor) in the cytoplasm. The stator, composed of MotA and MotB proteins surround the MS and C rings of the motor and function to generate torque for rotation of the flagellum. Energy for rotation comes from the proton motive force provided by protons moving through the Mot proteins.

For more information: A review of proton motive force

Fig. 2: The structure of the extracted bacterial flagellar motor



The structure of the extracted bacterial flagellar motor was described in a seminal paper by Dennis Thomas, David Morgan, and David DeRosier (1999, Rotational Symmetry of the C-ring and a Mechanism for the Flagellar Rotary Motor. Proc. Natl. Acad. Sci. USA *96*, 10134-10139). The left panel is an image of the flagellar basal body obtained by averaging electron micrographs of many individual flagellar basal structures. At the bottom is the cytoplasmic C ring, which extends from the M ring, the portion of the structure that resides within the cytoplasmic membrane (IM). Above them are the P and L rings, which are located in the periplasm and outer membrane (OM), respectively. The L and P rings are threaded on an axel-like rod, which traverses the surface layers of Gram-negative bacteria. The right panel is a contour plot of the density of the averaged image. Three of the five proteins essential for the torque generation are located in the C and M rings, and a mechanism for torque generation was proposed that is based on the symmetry mismatch between these two rings.

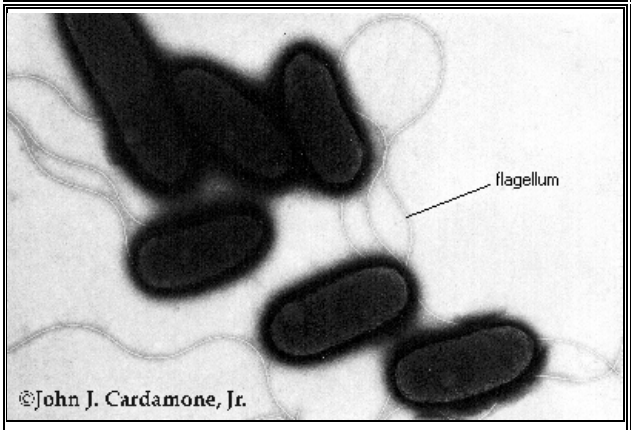
Courtesy of Molecular Biology of the Cell. May 1, 2000; 11 (5).

Image by Professor Lucy Shapiro.

The structure of the extracted bacterial flagellar motor was described in a seminal paper by Dennis Thomas, David Morgan, and David DeRosier (1999, Rotational Symmetry of the C-ring and a Mechanism for the Flagellar Rotary Motor. Proc. Natl. Acad. Sci. USA *96*, 10134-10139). The left panel is an image of the flagellar basal body obtained by averaging electron micrographs of many individual flagellar basal structures. At the bottom is the cytoplasmic C ring, which extends from the M ring, the portion of the structure that resides within the cytoplasmic membrane (IM). Above them are the P and L rings, which are located in the periplasm and outer membrane (OM), respectively. The L and P rings are threaded on an axel-like rod, which traverses the surface layers of Gram-negative bacteria. The right panel is a contour plot of the density of the averaged image. Three of the five proteins essential for the torque generation are located in the C and M rings, and a mechanism for torque generation was proposed that is based on the symmetry mismatch between these two rings. *Professor Lucy Shapiro.*

Bacteria flagella (see **Fig. 3** and **Fig. 4**) are 10-20 µm long and between 0.01 and 0.02 µm in diameter.

Fig.3: Electron Micrograph of Bacteria with Flagella



© John J. Cardamone Jr. Used with permission.

Fig. 4: Transmission Electron Micrograph of *Escherichia coli* O157:H7

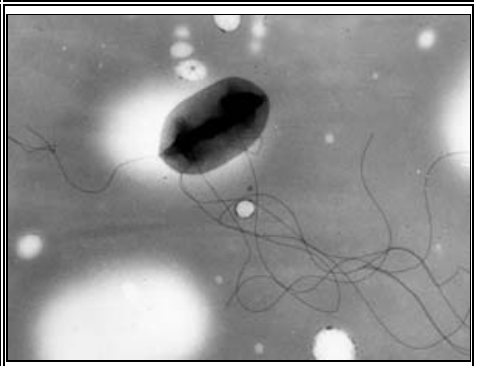


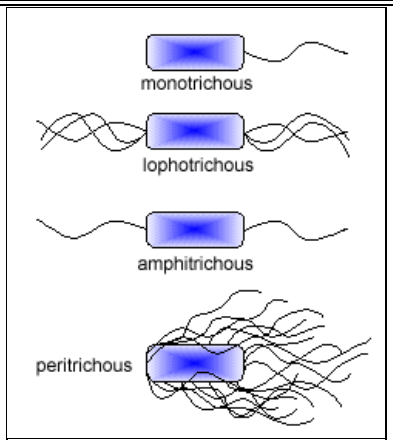
Image provided by Elizabeth White.
Courtesy of the Centers for Disease Control and Prevention.

Self Check

B. Flagellar Arrangements

Bacterial flagella come in a number of distinct arrangements as shown in **Fig. 5**.

Fig. 5: Bacterial Flagellar Arrangements



1. **monotrichous**: a single flagellum, usually at one pole.

Fig. 5A: Monotrichous Flagellum of *Vibrio cholerae*

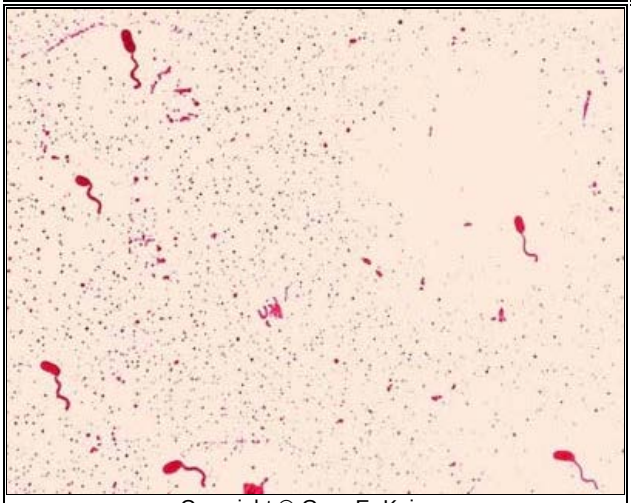


Fig. 5B: Scanning electron micrograph showing monotrichous flagellum of *Vibrio*

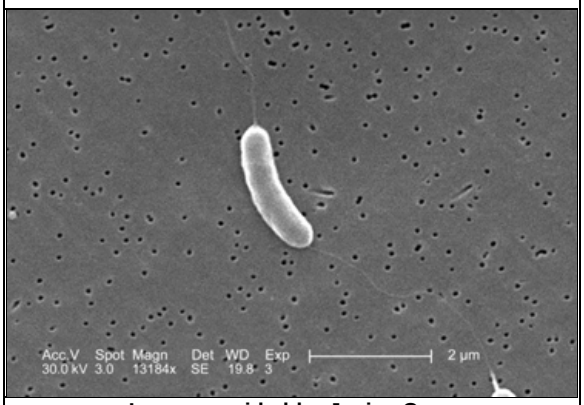
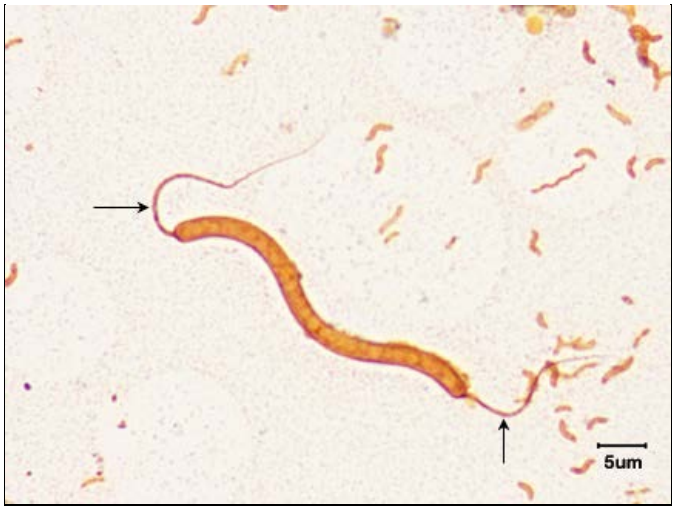


Image provided by Janice Carr.
Courtesy of the Centers for Disease Control and Prevention.

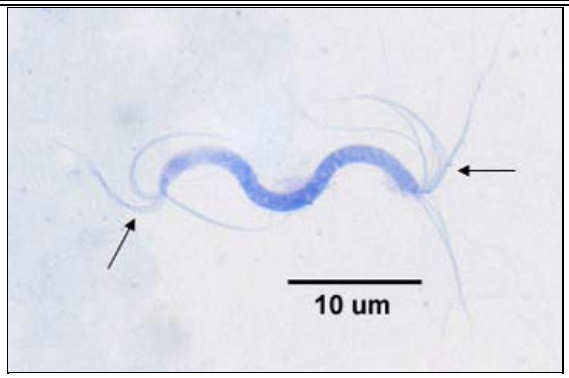
2. **amphitrichous**: a single flagellum at both ends of the organism.

Fig. 5C: *Spirillum* with Amphitrichous Arrangement of Flagella



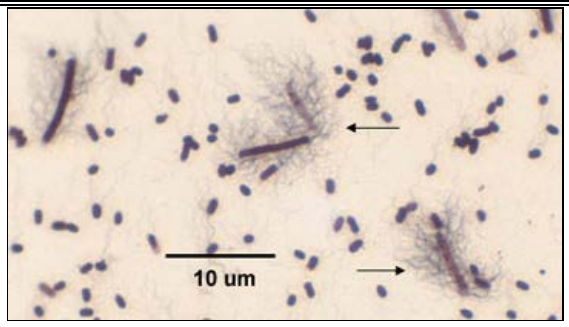
3. **lophotrichous**: two or more flagella at one or both poles.

Fig. 5D: *Spirillum* with Lophotrichous Arrangement of Flagella



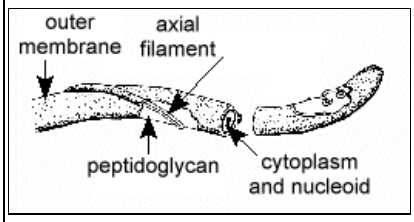
4. **peritrichous**: flagella over the entire surface.

Fig. 5E: Flagella Stain of *Proteus* Showing Peritrichous Arrangement of Flagella



5. **axial filaments**: internal flagella found only in the spirochetes. Axial filaments are composed of from two to over a hundred axial fibrils (or endoflagella) that extend from both ends of the bacterium between the outer membrane and the cell wall, often overlapping in the center of the cell. (see Fig. 6 and Fig. 7). A popular theory as to the mechanism behind spirochete motility presumes that as the endoflagella rotate in the periplasmic space between the outer membrane and the cell wall, this could cause the corkscrew-shaped outer membrane of the spirochete to rotate and propel the bacterium through the surrounding fluid.

Fig. 6: Spirochete Axial Filaments	Fig. 7: Scanning Electron Micrograph of *Leptospira interrogans*



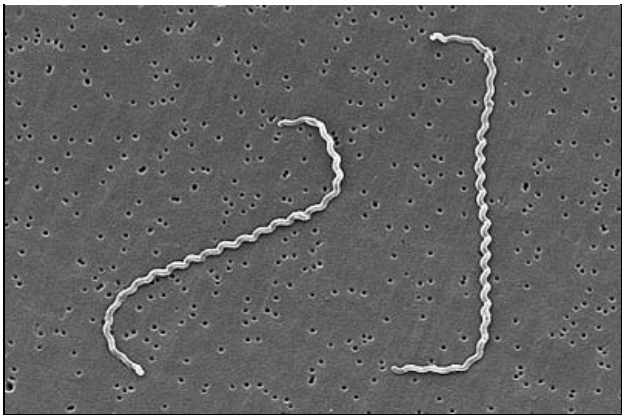


Image provided by Rob Weyant.
Courtesy of the Centers for Disease Control and Prevention.

Axial filaments of the spirochete Leptospira; Midlands Technical College, Bio 255 course site

Concept map for Bacterial Flagella

DragNDrop Activity

C. Functions

Flagella are the organelles of **locomotion** for most of the bacteria that are capable of motility. Two proteins in the flagellar motor, called MotA and MotB, form a proton channel through the cytoplasmic membrane and **rotation of the flagellum is driven by a proton gradient**. This driving proton motive force occurs as protons accumulating in the space between the cytoplasmic membrane and the cell wall as a result of the electron transport system travel through the channel back into the bacterium's cytoplasm. Most bacterial flagella can rotate both counterclockwise and clockwise and this rotation contributes to the bacterium's ability to change direction as it swims. A protein switch in the molecular motor of the basal body controls the direction of rotation.

1. A bacterium with peritrichous flagella

If a bacterium has a peritrichous arrangement of flagella, counterclockwise rotation of the flagella causes them to form a single bundle that propels the bacterium in **long, straight or curved runs without a change in direction**.Counterclockwise rotation causes the flagellum to exhibit a left-handed helix. During a run, that lasts about one second, the bacterium moves 10 - 20 times its length before it stops. This occurs when some of the the flagella rotate clockwise, disengage from the bundle, and trigger a tumbling motion. Clockwise rotation causes the flagellum to assume a right-handed helix. A tumble only lasts about one-tenth of a second and no real forward progress is made. After a "tumble", the direction of the next bacterial run is random because every time the bacterium stops swimming, Brownian motion and fluid currents cause the bacterium to reorient in a new direction.

Phase contrast microscopy of motile *Escherichia coli.* Flagella are not visible with under phase contrast microscopy. Note runs and tumbles. Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.	Movie #1 of motile *Escherichia coli* with fluorescent- labelled flagella. This technique allows the the flagella to be seen as the bacteria swim. Note some flagella leaving the flagellar bundle to initiate tumbling. Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.	Movie #2 of motile *Escherichia coli* with fluorescent- labelled flagella. This technique allows the the flagella to be seen as the bacteria swim. Note some flagella leaving the flagellar bundle to initiate tumbling. Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.	Movie of tethered *Escherichia coli* showing clockwise and counterclockwise rotation. Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.

When bacteria with a peritrichous arrangement grow on a nutrient-rich solid surface, they can exhibit a swarming motility wherein the bacteria elongate, synthesize additional flagella, secrete wetting agents, and move across the surface in coordinated manner.

Swarming motility in *Escherichia coli*. Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.

2. A bacterium with polar flagella

Most bacteria with polar flagella, like the peritrichous above, can rotate their flagella both clockwise and counterclockwise. If the flagellum is rotating counterclockwise, it pushes the bacterium forward. When it rotates clockwise, it pulls the bacterium backward. These bacteria change direction by changing the

rotation of their flagella.

Phase contrast movie of motile *Pseudomonas*. *Pseudomonas* has a single polar flagellum that can rotate both counterclockwise and clockwise but is not visible under phase contrast microscopy. From YouTube.	Movie showing motility of *Spirillum volutans,* a spiral-shaped bacterium with a bundle of flagella at either end.. Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.

Some bacteria with polar flagella can only rotate their flagellum clockwise. In this case, clockwise rotation pushes the bacterium forward. Every time the bacterium stops, Brownian motion and fluid currents cause the bacterium to reorient in a new direction.

Movie of *Rhodobacter spheroides* with fluorescent-labelled flagella. The flagellum can only rotate clockwise.
Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.

Concept map for Bacterial Flagella

Self Check

D. Taxis

Around half of all known bacteria are motile. Motility serves to keep bacteria in an optimum environment via taxis. **Taxis is a motile response to an environmental stimulus**. Bacteria can respond to chemicals (chemotaxis), light (phototaxis), osmotic pressure (osmotaxis), oxygen (aerotaxis), and temperature (thermotaxis).

Chemotaxis

is a **response to a chemical gradient of attractant or repellent molecules** in the bacterium's environment.

- **In an environment that lacks a gradient of attractant or repellent, the bacterium moves randomly.** In this way the bacterium keeps searching for a gradient.
- **In an environment that has a gradient of attractant or repellent, the net movement of the bacterium is towards the attractant or away from the repellent.**

If a bacterium has a **peritrichous arrangement of flagella**, such as *Escherichia coli, Salmonella, Proteus*, and *Enterobacter*, **counterclockwise rotation of the flagella causes them to form a single bundle that propels the bacterium in long, straight or curved runs without a change in direction**. **Clockwise rotation of some of the flagella in the bundle causes those flagella to be pushed apart from the bundle triggering a tumbling motion**. Every time the bacterium tumbles it reorients itself in a new direction. In the presence of a chemical gradient, these movements become biased. When the bacterium is moving away from higher concentrations of repellents or towards higher concentrations of attractants the runs become longer and the tumbles less frequent.

Movie of tethered *Escherichia coli* Switching from clockwise rotation to counterclockwise rotation as attractant is added.
Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.

Most bacteria with **polar flagella**, such as *Pseudomonas aeruginosa*, can rotate their flagella both clockwise and counterclockwise. **If the flagellum is rotating counterclockwise, it pushes the bacterium forward**. **When it rotates clockwise, it pulls the bacterium backward**. These bacteria change direction by changing the rotation of their flagella. Some bacteria with polar flagella, such as *Rhodobacter sphaeroides*, can only rotate their flagellum clockwise. In this case, clockwise rotation pushes the bacterium forward. Every time the bacterium stops, it reorients itself in a new direction.

A more detailed look at chemotaxis in *Escherichia coli.*

Chemotaxis is regulated by **chemoreceptors** located in the cytoplasmic membrane or periplasm of the bacterium bind chemical attractants or repellents. In most cases, this leads to either the methylation or demethylation of methyl-accepting chemotaxis proteins (MCPs) that in turn, eventually trigger either a counterclockwise or clockwise rotation of the flagellum. An increasing concentration of attractant or decreasing concentration of repellent (both conditions beneficial) causes less tumbling and longer runs; a decreasing concentration of attractant or increasing concentration of repellent (both conditions harmful) causes normal tumbling and a greater chance of reorienting in a "better" direction. As a result, the organism's net movement is toward the optimum environment.

E. Significance of Flagella in the Initiation of Body Defense

The body has two immune systems: **the innate immune system** and the **adaptive immune system**.

1. **Innate immunity** is an **antigen-nonspecific defense mechanisms** that a host uses **immediately or within several hours after exposure to almost any microbe**. This is the immunity one is born with and is the initial response by the body to eliminate microbes and prevent infection.

2. **Adaptive (acquired) immunity** refers to **antigen-specific** defense mechanisms that **take several days to become protective** and are designed to react with and remove a specific antigen. This is the immunity one develops throughout life.

Initiation of Innate Immunity

In order to protect against infection, one of the things the body must initially do is detect the presence of microorganisms. The body does this by **recognizing molecules unique to microorganisms that are not associated with human cells**. These unique molecules are called **pathogen-associated molecular patterns** or PAMPs. (Because all microbes, not just pathogenic microbes, possess PAMPs, pathogen-associated molecular patterns are sometimes referred to as microbe-associated molecular patterns or MAMPs.)

The protein flagellin in bacterial flagella is a PAMP that binds to **pattern-recognition receptors** or PRRs on a variety of defense cells of the body and **triggers innate immune defenses** such as inflammation**,** fever, and phagocytosis.

For More Information: Preview of Pathogen-Associated Molecular Patterns (PAMPs)
For More Information: Preview of Pattern-Recognition Receptors

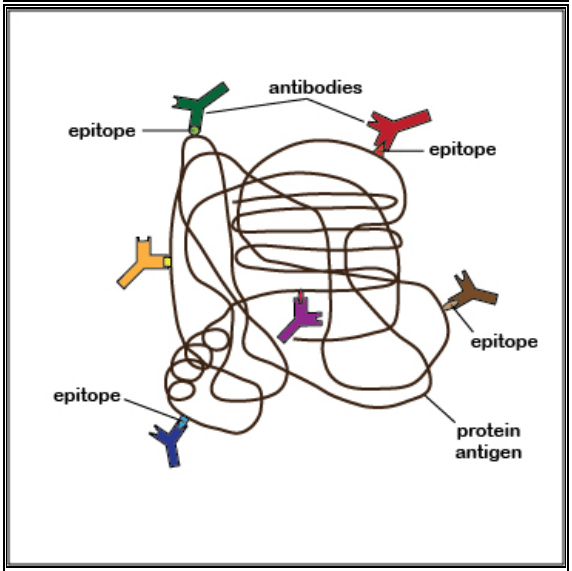
Initiation of Adaptive Immunity

Proteins associated with bacterial flagella function as antigens and initiate adaptive immunity. An antigen is defined as **a molecular shape that reacts with antibody molecules and with antigen receptors on lymphocytes**. We recognize those molecular shapes as foreign or different from our body's molecular shapes because they **fit specific antigen receptors on our B-lymphocytes and T-lymphocytes**, the cells that carry out adaptive immunity.

The actual portions or fragments of an antigen that react with antibodies and with receptors on B-lymphocytes and T-lymphocytes are called **epitopes**. An epitope is typically a **group of 5-15 amino acids with a unique shape that makes up a portion of a protein antigen (see fig. 8A**), or **3-4 sugar residues branching off of a polysaccharide antigen (see Fig. 8B)**. **A single microorganism has many hundreds of different shaped epitopes that our lymphocytes can recognize as foreign and mount an adaptive immune response against**.

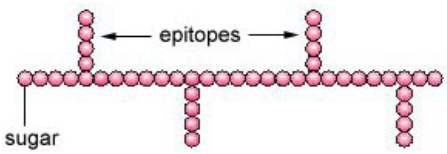
The body recognizes an antigen as foreign when **epitopes of that antigen bind to B-lymphocytes and T-lymphocytes by means of epitope-specific receptor molecules having a shape complementary to that of the epitope**. The epitope receptor on the surface of a B-lymphocyte is called a B-cell receptor and is actually an antibody molecule. The receptor on a T-lymphocyte is called a T-cell receptor (TCR).

Fig. 8A: Illustration of epitopes of a protein antigen.



Proteins have many epitopes of different specificities. During humoral immunity, antibodies are made to fit each epitope of each antigen.

Fig. 8B: Illustration of epitopes of a polysaccharide antigen.



Polysaccharides have many epitopes but of similar specificities.

There are two major branches of the adaptive immune responses: humoral immunity and cell-mediated immunity.

1. Humoral immunity: Humoral immunity involves the production of antibody molecules in response to an antigen and is mediated by B-lymphocytes. Through a variety of mechanisms, these antibodies are able to remove or neutralize microorganisms and their toxins after binding to their epitopes. For example, antibodies made against flagellar antigens can **stick bacteria to phagocytes**, a process called opsonization.They can also **interfere with bacterial motility**. Antibodies made against the flagella of motile bacteria can bind to these locomotor organelles and **arrest the organism's movement blocking its ability to spread**.

Flash animation illustrating phagocytosis by way of opsonizing antibodies
html5 version of animation for iPad showing phagocytosis by opsonizing antibodies.
The Fab portion of IgG binds to epitopes of a microbe. The Fc portion can now attach the microbe to Fc receptors on phagocytes for enhanced attachment, also known as opsonization. Once attached to the phagocyte by way of IgG, the microbe can be engulfed more efficiently and placed in a phagosome, and destroyed by lysosomes. In this case, opsonizing antibodies would be mage against the bacterial flagella.

2. Cell-mediated immunity: Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigen and is mediated by T-lymphocytes. These defense cells help to remove infected cells and cancer cells displaying foreign epitopes.

Adaptive immunity will be discussed in greater detail later in the course.

For More Information: Review of antigens and epitopes

F. Significance of Motility to Bacterial Pathogenicity

Motility and chemotaxis probably help some intestinal pathogens to **move through the mucous layer so they can attach to the epithelial cells of the mucous membranes**. In fact, many bacteria that can colonize the mucous membranes of the bladder and the intestines are motile. Motility probably helps these bacteria move through the mucus in places where it is less viscous.

Flash animation showing a motile bacterium contacting a host cell by swimming through the mucus.
Copyright © Gary E. Kaiser
html5 version of animation showing a motile bacterium contacting a host cell by swimming through the mucus.
The mucosal surfaces of the bladder and the intestines constantly flush bacteria away in order to prevent colonization. Motile bacteria that can swim chemotactically toward mucosal surfaces may have a better chance to make contact with the mucous membranes, attach, and colonize. Many bacteria that can colonize the mucous membranes of the bladder and the intestines are motile. Motility probably helps these bacteria move through the mucus in places where it is less viscous.

Motility and chemotaxis also enable spirochetes to move through viscous environments and penetrate cell membranes. Examples include *Treponema pallidum*, *Leptospira*, and *Borrelia burgdorferi*. Because of their thinness, their internal flagella (axial filaments), and their motility, spirochetes are more readily able to penetrate host mucous membranes, skin abrasions, etc., and enter the body. Motility and invasins may also enable the spirochetes to **penetrate deeper in tissue and enter the lymphatics and bloodstream** and disseminate to other body sites.

Flash animation showing spirochetes using motility to enter a blood vessel.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing spirochetes using motility to enter a blood vessel.
A combination of motility and invasins appears to helps *Borrelia bergdorferi* and *Treponema pallidum* to invade

and exit blood vessels by passing between and through endothelial cells. This enables these spirochetes to disseminate to other locations in the body. One tip of the spirochete attaches to the host cell and some form of invasin apparently causes the host cell to release digestive enzymes that enable the spirochete with its corkscrewing motility to penetrate the host cell membrane.

Movie of motile Borrelia bergdorferi, the spirochete that causes Lyme disease.
Note corkscrewing motility.
From You Tube, courtesy of CytoVivo.

Electron micrograph of *Treponema pallidum* invading a host cell.

This will be discussed in more detail under **Bacterial Pathogenesis** in Unit 3.

TPS Questions

For More Information: Preview of the ability to contact host cells

For More Information: Preview of the ability to invade host cells

Highlighted Bacterium: *Treponema pallidum*

Click on this link, read the description of *Treponema pallidum*, and be able to match the bacterium with its description on an exam.

Concept map for Bacterial Flagella

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Treponema pallidum*
- *Leptospira*
- *Borrelia burgdorferi*
- *Helicobacter pylori*

Self Check

Self Quiz for Bacterial Flagella

Quiz Group

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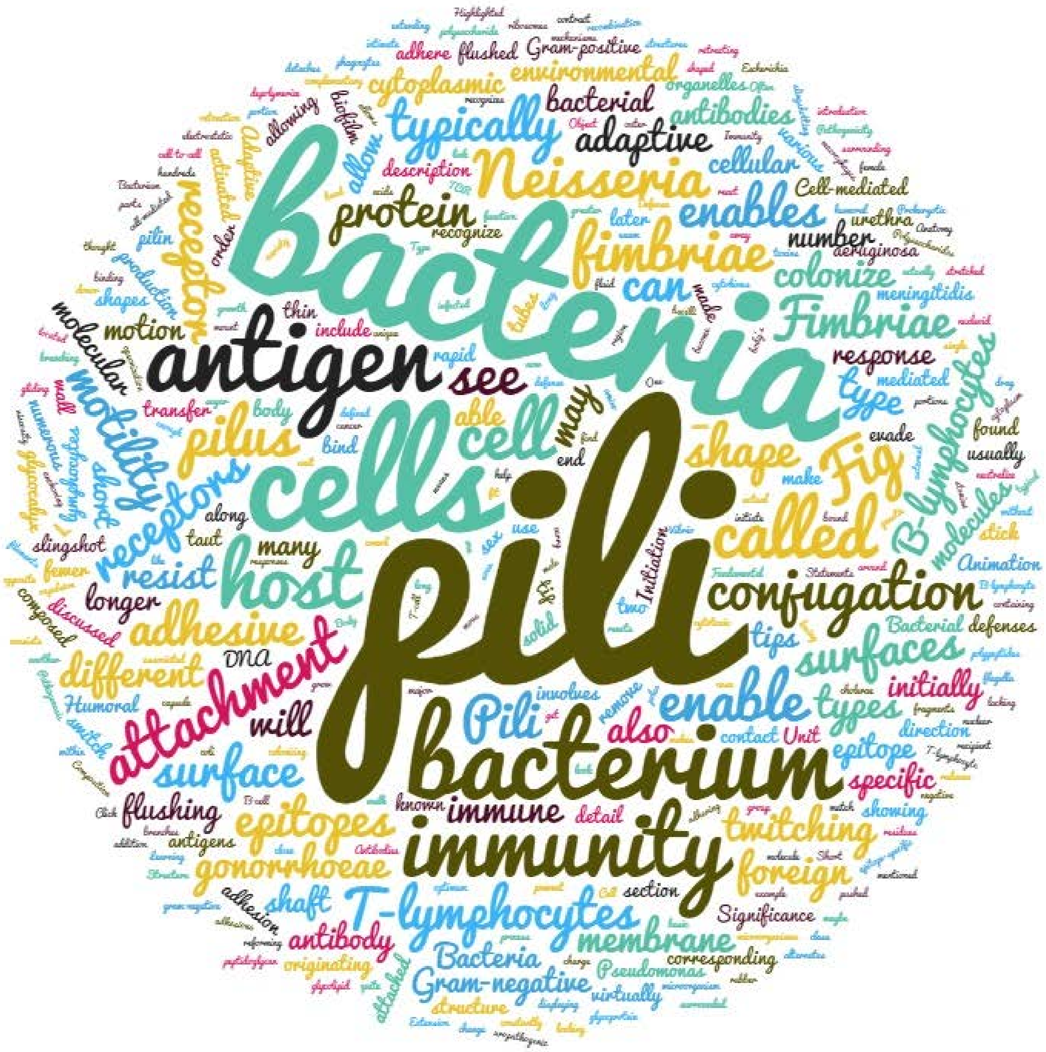
Bacterial pili and fimbriae
BACTERIAL FIMBRIAE AND PILI

Bacterial Fimbriae and Pili



Fundamental Statements for this Lesson:

1. Fimbriae and pili are thin, protein tubes originating from the cytoplasmic membrane found in virtually all Gram-negative bacteria but not in many Gram-positive bacteria. Pili are typically longer and fewer in number than fimbriae.
2. The short attachment pili or fimbriae are organelles of adhesion allowing bacteria to colonize environmental surfaces or cells and resist flushing.
3. Antibodies made against bacterial pili may block colonization and/or promote opsonization.
4. The long conjugation pilus enables conjugation in Gram-negative bacteria.
5. The pilus has a shaft composed of a protein called pilin with an adhesive tip structure at the end having a shape corresponding to that of specific receptors on a host cell.
6. The same bacterium may switch the adhesive tips of the pili in order to adhere to different types of cells and evade immune defenses.
7. Type IV pili not only allow for attachment but also enable a twitching motility that enables bacteria to "crawl" or "walk" over the surfaces to which they have attached by extending and retracting their type IV pili.

Common Course Objectives

1. Identify the parts of a bacterium and their physiological purpose.
2. Explain how various bacterial structures can contribute to bacterial colonization of a host.
3. Provide a diagnosis for a "patient" when given a list of symptoms and a description of the bacterium.

Detailed Learning Objectives for this Lesson

1*. State the chemical composition, structure, and function of the short adhesion pili of bacteria.

2*. Briefly describe how antibodies made against the adhesive tip of bacterial pili may block colonization and/or promote phagocytosis.

3. State the function of a bacterial conjugation (sex) pilus.

4*. Define bacterial conjugation.

5*. State how the ability to change the shape of the adhesive tip of its pili could be an advantage to a bacterium.

6*. Briefly describe twitching motility induced by type IV pili.

(*) = Common theme throughout the course

Highlighted Bacterium

1. Read the description of *Neisseria gonorrhoeae* and match the bacterium with the description of the organism and the infection it causes.

TPS Questions

In this section on Prokaryotic Cell Anatomy we are looking at the various anatomical parts that make up a bacterium. As mentioned in the introduction to this section, a typical bacterium usually consists of:

- a cytoplasmic membrane surrounded by a peptidoglycan cell wall and maybe an outer membrane;
- a fluid cytoplasm containing a nuclear region (nucleoid) and numerous ribosomes; and
- often various external structures like a glycocalyx, flagella, and pili.

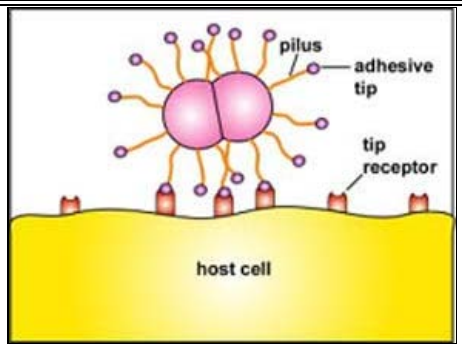
We will now look at the bacterial fimbriae and pili.

Bacterial Fimbriae and Pili

A. Structure and Composition

Pili and fimbriae are thin, protein tubes originating from the cytoplasmic membrane of many bacteria. Both are able to stick bacteria to surfaces but pili are typically longer and fewer in number than fimbriae. They are found in virtually all Gram-negative bacteria but not in many Gram-positive bacteria. The fimbriae and pili have a shaft composed of a protein called pilin. At the end of the shaft is the **adhesive tip structure having a shape corresponding to that of specific glycoprotein or glycolipid receptors on a host cell (see Fig. 1)**.

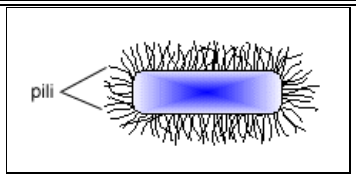
Fig. 1: Illustration of Adhesive Tips of Bacterial Pili Binding to Host Cell Receptors



There are two basic types of pili:

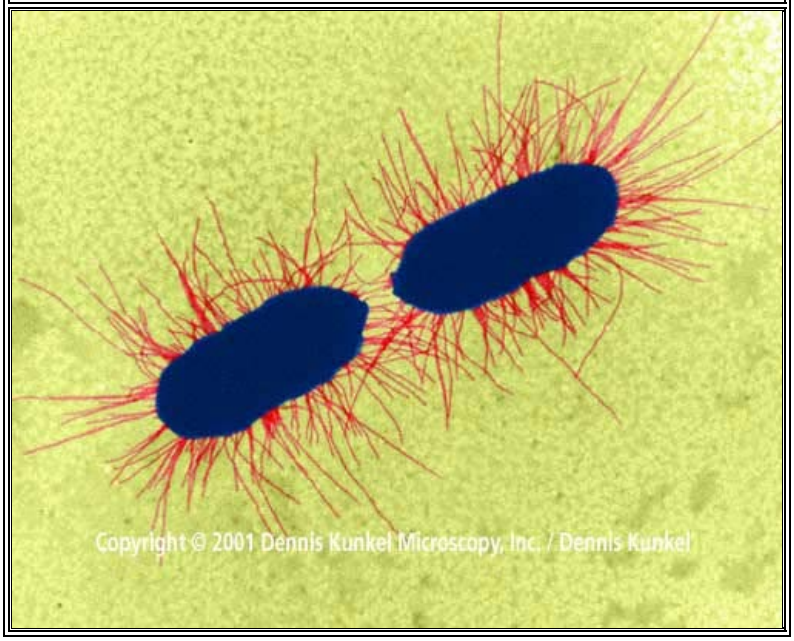
1. **Short attachment pili**, also known as fimbriae, are usually short and quite numerous **(see Fig. 2)** and enable bacteria to **colonize environmental surfaces or cells and resist flushing**.

Fig. 2: Illustration of Bacterial Pili



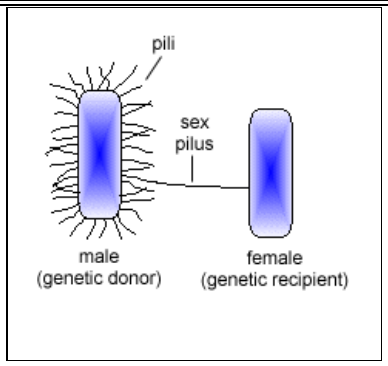
Scanning electron micrograph *Escherichia coli* with pili

Courtesy of Dennis Kunkel's Microscopy.



2. **Long conjugation pili**, also called "F" or sex pili **(see Fig. 3)**, that are longer and very few in number. The **conjugation pilus** enables conjugation. As will be seen later in this unit, conjugation is the transfer of DNA from one bacterium to another by cell-to-cell contact. In Gram-negative bacteria it is typically the transfer of DNA from a donor or male bacterium with a sex pilus to a recipient or female bacterium to enable genetic recombination.

Fig. 3: Illustration of a Conjugation (Sex) Pilus



Scanning electron micrograph *Escherichia coli* with a conjugation pilus

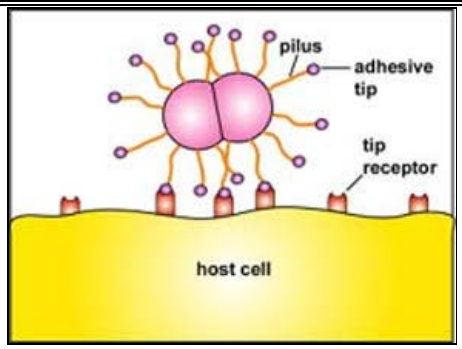
Courtesy of Dennis Kunkel's Microscopy.

Quiz Group

B. Significance of Pili to Bacterial Pathogenicity

The short **attachment pili** or **fimbriae** are organelles of **adhesion** allowing bacteria to **colonize environmental surfaces or cells and resist flushing**. The pilus has a shaft composed of a protein called pilin. At the end of the shaft is the **adhesive tip structure having a shape corresponding to that of specific glycoprotein or glycolipid receptors on a host cell (see Fig. 1)**.

Fig. 1: Illustration of Adhesive Tips of Bacterial Pili Binding to Host Cell Receptors



Because both the bacteria and the host cells have a negative charge, pili may enable the bacteria to bind to host cells without initially having to get close enough to be pushed away by electrostatic repulsion. Once attached to the host cell, the pili can depolymerize and enable adhesions in the bacterial cell wall to make more intimate contact.

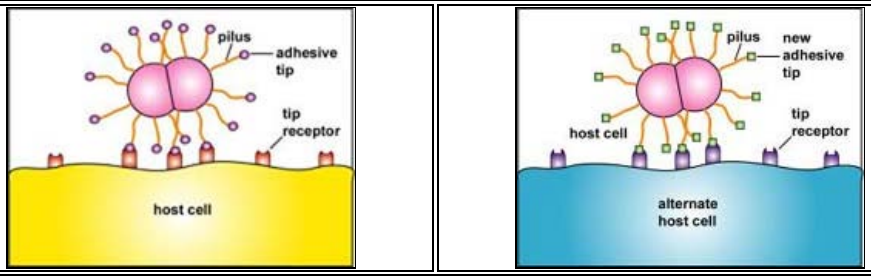
Flash animation showing a bacterium using pili and cell wall adhesins to adhere to a host cell.

html5 version of animation for iPad showing a bacterium using pili and cell wall adhesins to adhere to a host cell.

Pili enable some organisms to adhere to receptors on target host cells. The pilus has a shaft composed of a protein called pilin. At the end of the shaft is the adhesive tip structure having a shape corresponding to that of specific glycoprotein or glycolipid receptors on a host cell. Because both the bacteria and the host cells have a negative charge, pili may enable the bacteria to bind to host cells without initially having to get close enough to be pushed away by electrostatic repulsion. Once attached to the host cell, the pili can depolymerize and this enables bacterial cell wall adhesins to bind to adhesin receptors on the host cell. This allows the bacterial cell wall to make more intimate contact with the host cell and enables the bacterium to colonize the host cell and resist flushing.

Bacteria are constantly losing and reforming pili as they grow in the body and the **same bacterium may switch the adhesive tips of the pili** in order to adhere to different types of cells and evade immune defenses **(see Fig. 4)**. This will be discussed in detail later in Unit 3 under **Bacterial Pathogenesis**.

Fig. 4: Bacteria Altering the Adhesive Tips of Their Pili



By genetically altering the adhesive tips of their pili, certain bacteria are able to: 1) adhere to and colonize different cell types with different receptors, and 2) evade antibodies made against the previous pili.

For more information: Preview of the ability to adhere to host cells

Examples of bacteria that use pili to initially colonize host cells include *Neisseria gonorrhoeae*, *Neisseria meningitidis*, uropathogenic strains of *Escherichia coli*, and *Pseudomonas aeruginosa*.

Highlighted Bacterium: *Neisseria gonorrhoeae*

Click on this link, read the description of *Neisseria gonorrhoeae*, and be able to match the bacterium with its description on an exam.

Flash animation showing bacteria lacking pili being flushed out of the urethra.

html5 version of animation for iPad showing bacteria lacking pili being flushed out of the urethra.

Without the pili the bacteria are flushed from the body.

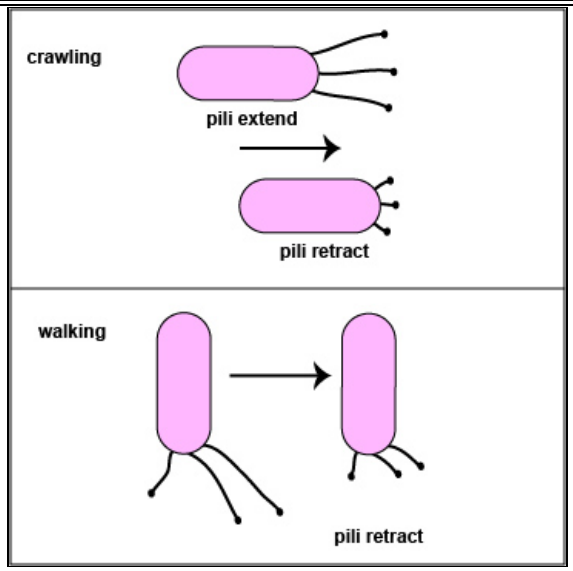
Flash animation showing how bacteria with pili may resist being flushed out of the urethra.

html5 version of animation for iPad showing how bacteria with pili may resist being flushed out of the urethra.

Bacterial pili bind to receptor molecules on the surface of a susceptible host cell enabling the bacterium to make contact with the host cell, adhere, colonize, and resist flushing.

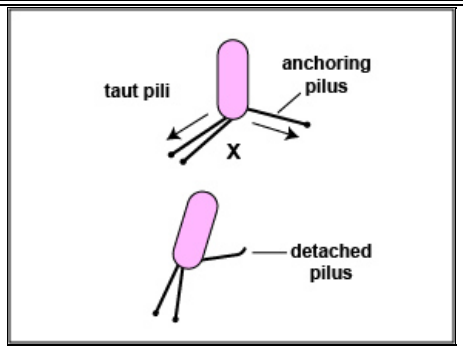
One class of pili, known as **type IV pili,** not only allow for attachment but also enable a twitching motility. They are located at the poles of bacilli and allow for a gliding motility along a solid surface such as a host cell. Extension and retraction of these pili allows the bacterium to drag itself along the solid surface **(see Fig. 5)**. In addition, bacteria can use their type IV pili to "slingshot" the bacterium over a cellular surface. In this case, as the pili contract they are thought to become taut like a stretched rubber band. When an anchoring pilus detaches, the taut pili "slingshot" the bacterium in the opposite direction **(see Fig. 6)**. This motion typically alternates with the twitching motility and enables a more rapid motion and direction change than with the twitching motility because the rapid slingshotting motion reduces the viscosity of the surrounding biofilm.

Fig. 5: Type IV Pili-Induced Dragging of a Bacterium



Some bacteria use type IV pili to move along surfaces within a biofilm. (Biofilms are to viscous for bacteria to swim through with flagella.) The bacterium extends and anchors its pili. As the pili retract the bacterium is pulled forward. This enables the bacterium to drag themselves through biofilms on environmental surfaces and alternates with a type-IV pili-induced slingshotting motion (see Fig. 6). The top illustration shows a bacterium dragging itself or "crawling" along a surface. Bacteria with polar pili are also able to pull themselves upright and "walk" along the surface as shown in the bottom illustration.

Fig. 6: Type IV Pili-Induced Slingshotting of a Bacterium



Some bacteria use type IV pili to move along surfaces within a biofilm. (Biofilms are to viscous for bacteria to swim through with flagella.) A bacterium sitting at location "X" extends and anchors its pili. As the pili contract, the pili become stretched or taut. As the anchoring pilus on the right detaches, the taut pili "slingshot" the bacterium to the left. This motion alternates with the twitching motility (see Fig. 5) also caused by type IV pili and enables a more rapid motion and direction change than with the twitching motility because the rapid slingshotting motion reduces the viscosity of the surrounding biofilm.

This enables bacteria with these types of pili within a biofilm to move around a cellular surface and find an optimum area on that cell for attachment and growth

once they have initially bound. Bacteria with type IV pili include *Pseudomonas aeruginosa, Neisseria gonorrhoeae, Neisseria meningitidis*, and *Vibrio cholerae*.

Flash animation showing a bacterium using type IV pili to drag itself (twitching motility) along a surface.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing a bacterium using type IV pili to drag itself (twitching motility) along a surface.
Some bacteria use type IV pili to move along surfaces within a biofilm. (Biofilms are to viscous for bacteria to swim through with flagella.) The bacterium extends and anchors its pili. As the pili retract the bacterium is pulled forward. This enables the bacteria to drag themselves through biofilms on environmental surfaces with a twitching motility. This twitching motility alternates with a type-IV pili-induced slingshotting motion.

Flash animation showing a bacterium using type IV pili to slingshot itself along a surface.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing a bacterium using type IV pili to slingshot itself along a surface
Some bacteria use type IV pili to move along surfaces within a biofilm. (Biofilms are to viscous for bacteria to swim through with flagella.) It starts with a bacterium extending and anchoring its pili. As the pili contract, the pili become stretched or taut. As the anchoring pilus detaches, the taut pili 'slingshot' the bacterium in the opposite direction. This motion alternates with the twitching motility also caused by type IV pili and enables a more rapid motion and direction change than with the twitching motility because the rapid slingshotting motion reduces the viscosity of the surrounding biofilm.

Movie showing *Pseudomonas* using type IV pili to "walk" on end following binary fission.
Courtesy of Gerard Wong, UCLA Bioengineering, CNSI

Movie of twitching motility of *Pseudomonas* due to type IV pili.
Courtesy of Dr. Howard Berg, Roland Institute, Harvard University

Retraction of pili of *Pseudomonas* used in twitching motility.
Courtesy of Dr. Howard Berg, Roland Institute, Harvard University

TPS Questions

Concept map for Bacterial Pili and Fimbriae

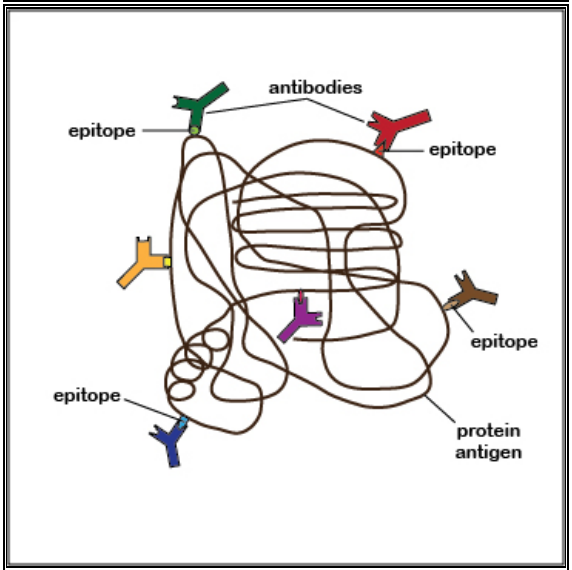
C. Significance of Fimbriae and Pili in the Initiation of Body Defense

Initiation of Adaptive Immunity

Proteins associated with bacterial flagella function as antigens and initiate adaptive immunity. An antigen is defined as **a molecular shape that reacts with antibody molecules and with antigen receptors on lymphocytes**. We recognize those molecular shapes as foreign or different from our body's molecular shapes because they **fit specific antigen receptors on our B-lymphocytes and T-lymphocytes**, the cells that carry out adaptive immunity.

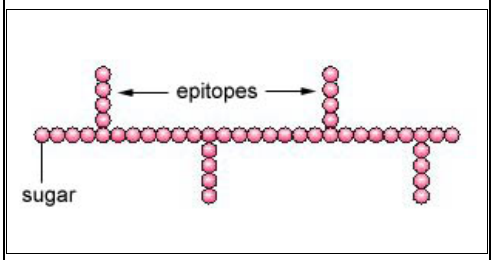
The actual portions or fragments of an antigen that react with antibodies and with receptors on B-lymphocytes and T-lymphocytes are called **epitopes**. An epitope is typically a group of 5-15 amino acids with a unique shape that makes up a portion of a protein antigen **(see Fig. 7A)**, or **3-4 sugar residues branching off of a polysaccharide antigen (see Fig. 7B)**. **A single microorganism has many hundreds of different shaped epitopes that our lymphocytes can recognize as foreign and mount an adaptive immune response against**.

Fig. 7A: Illustration of epitopes of a protein antigen.



Proteins have many epitopes of different specificities.

Fig. 7B: Illustration of epitopes of a polysaccharide antigen.



Polysaccharides have many epitopes but of similar specificities.

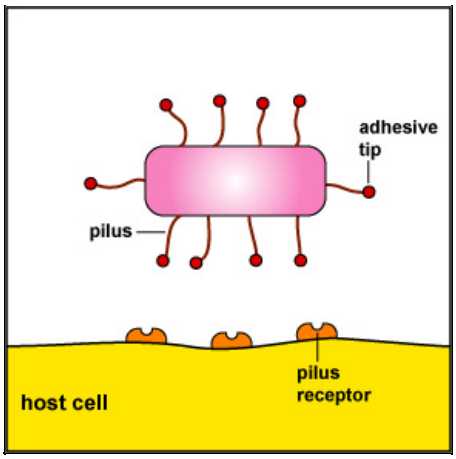
The body recognizes an antigen as foreign when **epitopes of that antigen bind to B-lymphocytes and T-lymphocytes by means of epitope-specific receptor molecules having a shape complementary to that of the epitope**. The epitope receptor on the surface of a B-lymphocyte is called a B-cell receptor and is actually an antibody molecule. The receptor on a T-lymphocyte is called a T-cell receptor (TCR).

There are two major branches of the adaptive immune responses: humoral immunity and cell-mediated immunity.

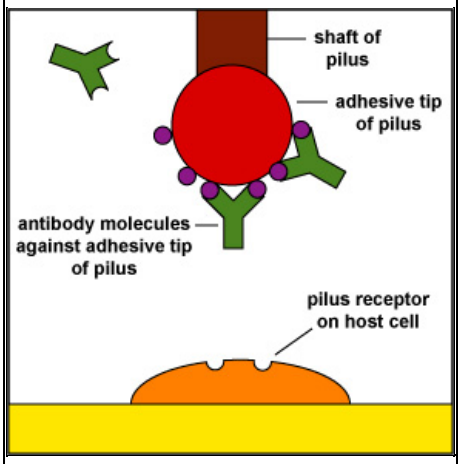
1. Humoral immunity: Humoral immunity involves the production of antibody molecules in response to an antigen and is mediated by B-lymphocytes. Through a variety of mechanisms, these antibodies are able to remove or neutralize microorganisms and their toxins after binding to their epitopes. For example, antibodies made against the adhesive tips of bacterial pili **can prevent bacteria from adhering to and colonizing host cells** (see **Fig. 8A** and **Fig. 8B**). Antibodies made against pili antigens can also **stick bacteria to phagocytes**, a process called opsonization.

Fig. 8A: Illustration of Bacterial Adherence via Pili.

Fig. 8B: Illustration of Antibodies Blocking Bacterial Adherence.



The Fab portion of the antibodies made against epitopes of adherence structures such as pili bind and block the bacteria from adhering to the host cell membrane. As a result, the bacteria may be flushed away. Antibody molecules made against the adhesive tip of pili block the adhesive tip from binding to receptors on host cell membranes.



Antibody molecules made against the adhesive tip of pili block the adhesive tip from binding to receptors on host cell membranes. This prevents the bacterium from colonizing the host cells.

Flash animation illustrating phagocytosis by opsonizing antibodies

html5 version of animation for iPad showing phagocytosis by opsonizing antibodies.

The Fab portion of IgG binds to epitopes of a microbe. The Fc portion can now attach the microbe to Fc receptors on phagocytes for enhanced attachment, also known as opsonization. Once attached to the phagocyte by way of IgG, the microbe can be engulfed more efficiently and placed in a phagosome, and destroyed by lysosomes. In this case, antibodies would be made against the bacterial pili.

For example, antibodies made against pili antigens can **stick bacteria to phagocytes**, a process called opsonization.

2. Cell-mediated immunity: Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigen and is mediated by T-lymphocytes. These defense cells help to remove infected cells and cancer cells displaying foreign epitopes.

Adaptive immunity will be discussed in greater detail later in the course.

For more information: Review of antigens and epitopes

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Neisseria gonorrhoeae*
- *Neisseria meningitidis*
- *Escherichia coli*
- *Pseudomonas aeruginosa*
- *Vibrio cholerae*

Self Quiz for Bacterial Fimbriae and Pili

Sorting Activity

Self Check

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