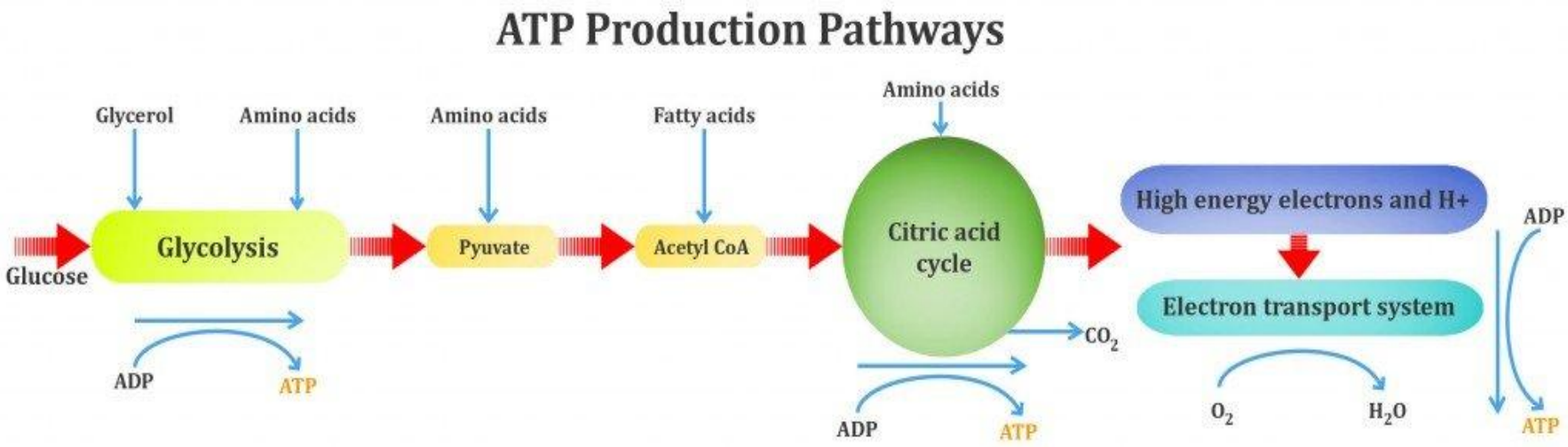
ELECTRON TRANSPORT SYSTEM

Electron Flow in Organotrophy, Lithotrophy, and Phototrophy (Chapter 14)

Lecture 19

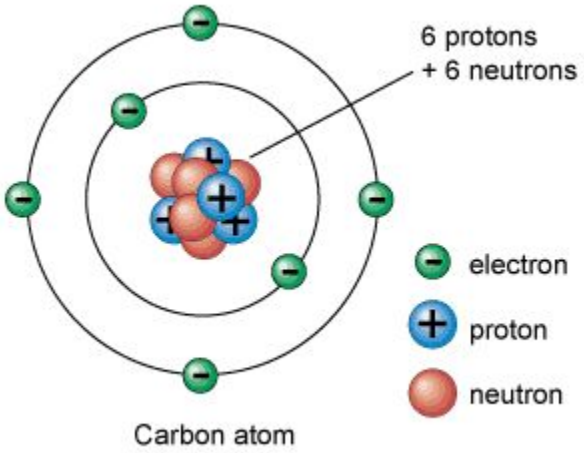


Energy, redox Reactions, and Enzymes

https://ecampusontario.pressbooks.pub/microbio/chapter/energy-matter-and-enzymes/

Lecture Overview

- Electron transport systems (ETS) or Electron Transport Chain: ETC)
- The proton motive force
- The respiratory ETS
- ATP synthase
- Anaerobic respiration
- Lithotrophy
- Phototrophy



Introduction

- We have learned previously how microorganisms can catabolize nutrients and obtain energy in the form of energy carriers
- ATP and GTP can produce energy by hydrolysis and cleavage of the phosphate bond(s).
- However, NADH and FABH2 need to be transformed into ATP.
- Mot of the energy yield comes from successive redox (coupled reduction and oxidation) steps within an electron transport system (ETS).
- The types of metabolisms that use an ETS include organotrophy (organic electron donors), lithotrophy (inorganic electron donors), and phototrophy (electrons are excited by light absorption).
- Our focus will mainly be on ETS organotrophy.

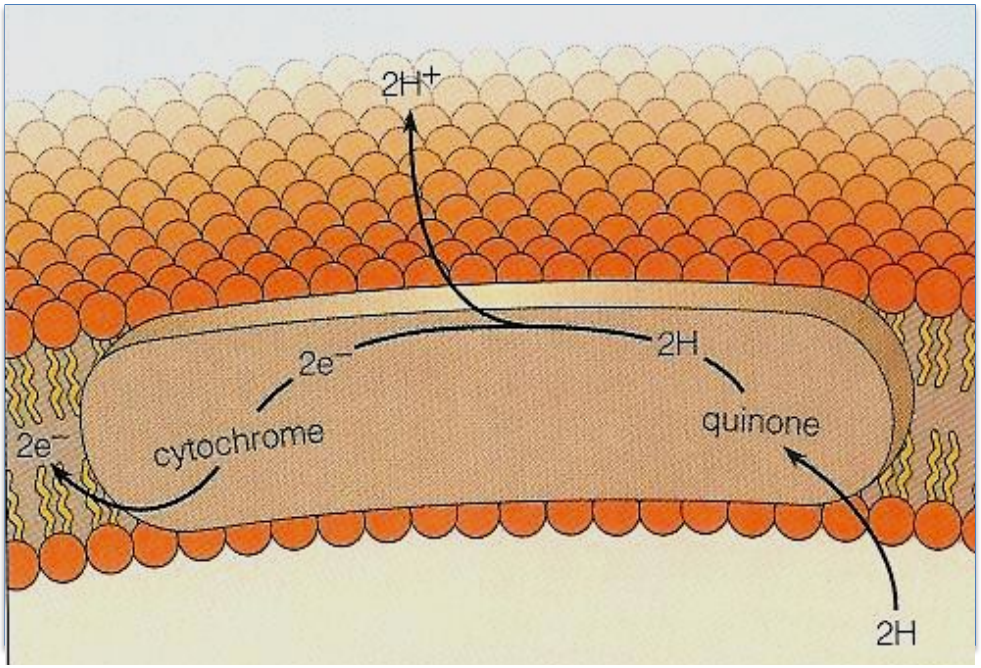
Flow of electrons

- **Microbes transfer energy by moving electrons**.
  - Electrons move from **reduced food molecules** onto **energy carriers**, then onto **membrane protein carriers**, and then onto **oxygen or oxidized minerals.**
- **The electron transport system generates a "proton motive force"** that drives protons across the membrane.
  - The Proton motive force stores energy to make ATP

Energy transfer pathway

Flow of electron from

Donor to acceptor



Reduced food molecules

e-

Energy carriers

e-

Cytochrom e

e-

Oxygen or Oxydized minerals

Proton potential

- In each step of the ETS, a molecule becomes reduced (gains an electron), while the molecule donating the electron becomes oxidized (loses an electron).
- Some of the energy from the electron transferred is stored across the membrane in the form of an electrochemical potential (voltage).
- The potential is composed of the chemical concentration gradient of protons (H+ ions).
- The proton potential drives ATP synthesis

Electron transport system

- The **electron transport system** (ETS) or electron transport chain (ETC) generates a **proton motive force that drives protons across the membrane**
- The **proton motive force stores energy to make ATP**
- A s*imilar process occurs in mitochondria of animals and photosynthetic membrane of plant chloroplasts.*

The ETS is embedded in the membrane

- The **ETS can convert its energy into an ion potential or electrochemical potential between two compartments separated by a membrane**
- The ion potential is most commonly a **proton (H+) potential or proton motive force (PMF)**
- PMF drives essential cell processes such as synthesis of ATP

Complete redox reaction

Basic principle: Combination of 2 redox couple

e- Acceptor	**:**	**Reduction**
e-Donor	**:**	**Oxidation**

- **Aerobic oxidation of NADH pairs a strong electron donor (NADH) with a strong electron acceptor O2.**
- NADH oxidation via the ETS provide the cell with a huge amount of potential energy.
- To understand the energy gained in this system, please practice using the **"electron tower"**

Table 14.1 "Electron tower" of standard reduction potentials.[a]

Electron acceptor	→	electron donor	$E^{\circ\prime}$ (mV)[b]
$CO_2 + 4H^+ + 4e^-$	→	$[CH_2O]$ glucose + H_2O	−430
$2H^+ + 2e^-$	→	H_2	−420
$NAD^+ + 2H^+ + 2e^-$	→	$NADH + H^+$	−320
$S^0 + H^+ + e^-$	→	HS^-	−280
$CO_2 + 2H^+ + 3H_2 + 2e^-$	→	$CH_4 + 2H_2O$	−240
$SO_4^{2-} + 10H^+ + 8e^-$	→	$H_2S + 4H_2O$	−220
$FAD + 2H^+ + 2e^-$	→	$FADH_2$	−220[c]
$FMN + 2H^+ + 2e^-$	→	$FMNH_2$	−190
$Menaquinone + 2H^+ + 2e^-$	→	Menaquinol	−74
$Fumarate + 2H^+ + 2e^-$	→	Succinate	33
$Ubiquinone + 2H^+ + 2e^-$	→	Ubiquinol	+110
$NO_3^- + 2H^+ + 2e^-$	→	$NO_2^- + H_2O$	+420
$NO_2^- + 8H^+ + 6e^-$	→	$NH_4^+ + 2H_2O$	+440
$MnO_2 + 4H^+ + 2e^-$	→	$Mn^{2+} + 2H_2O$	+460
$NO_3^- + 6H^+ + 5e^-$	→	$\frac{1}{2}N_2 + 3H_2O$	+740
$Fe^{3+} + e^-$	→	Fe^{2+} (at pH 2)	+770
$\frac{1}{2}O_2 + 2H^+ + 2e^-$	→	H_2O	+820

[a]Values taken from Rudolf K. Thauer, Kurt Jungermann, and Karl Decker. 1977. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriological Reviews* 41:100–180.

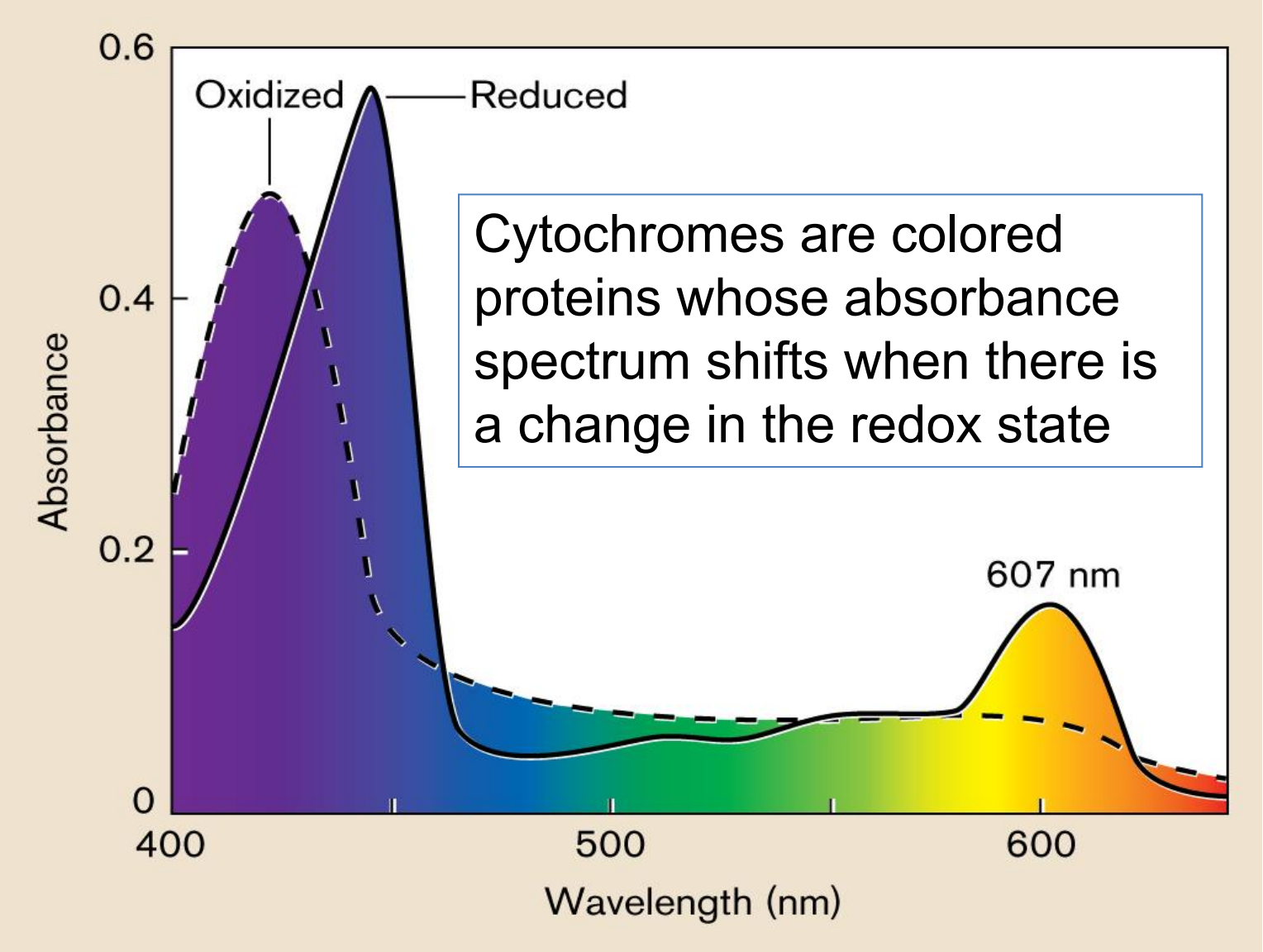
[b]Voltage potentials for redox couples when all concentrations are 1 M at pH 7.

[c]This value is for free FAD; FAD bound to a specific flavoprotein has a different $E^{\circ\prime}$ that depends on its protein environment.

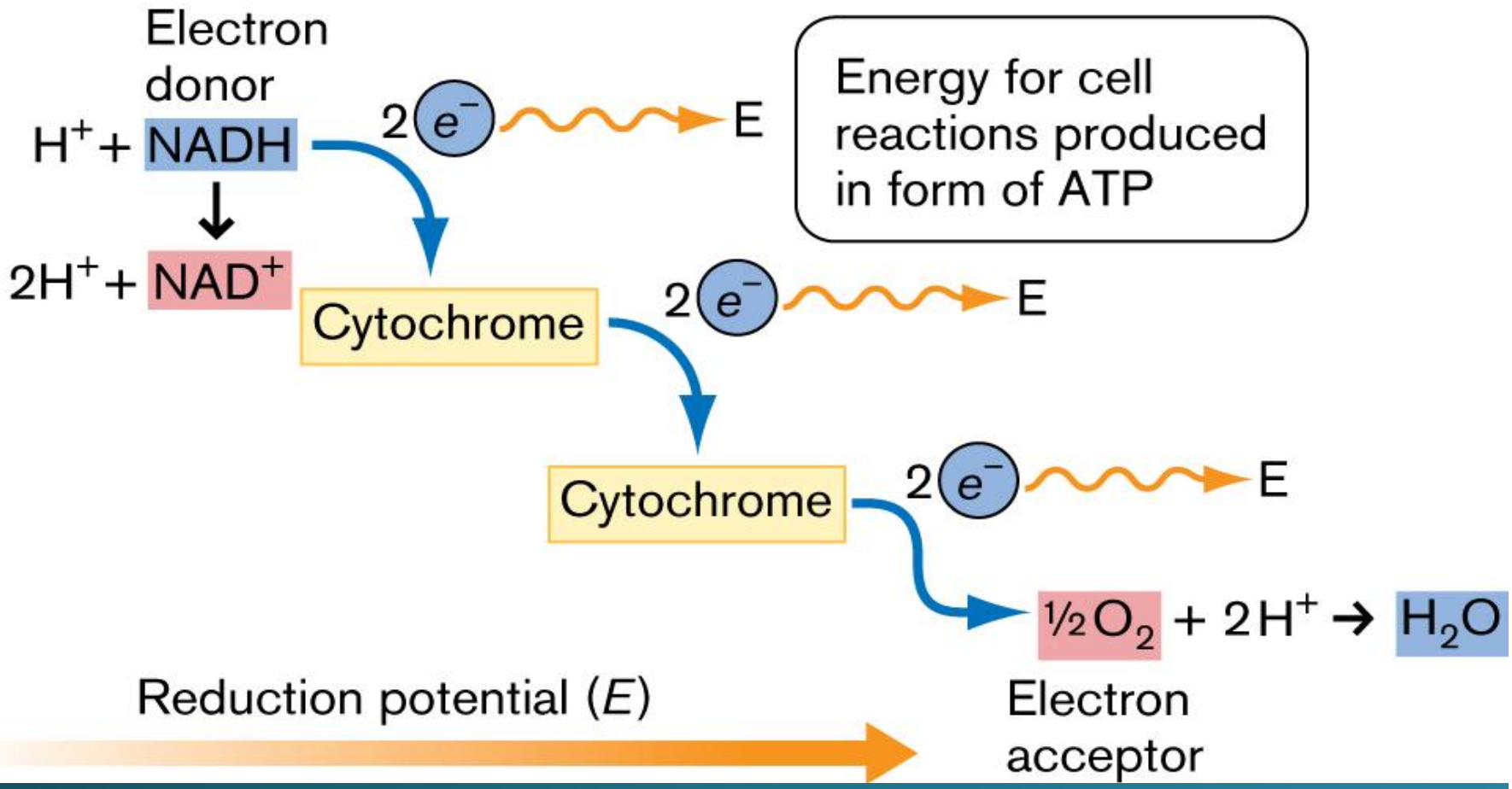
Cytochrome is a component of the ETS

- **The ETS is composed of electron carriers (proteins and molecules that can accept and then donate electrons)**
- **Cytochromes** are important components of the ETS
- **Cytochromes** are located in the inner membranes
- **Cytochromes** can receive electrons (reduced state), then donate electrons (oxidized state)
- Reduction and oxidation of cytochomes are associated with a shift in light absorption (Fig.14.3)
- The ETS is illustrated in Fig.14.4

Light absorbance spectrum of a cytochrome.



Electron transport system



Each cytochrome molecule receives electrons from a stronger donor and transfer them to a stronger electron acceptor.
This principle applies to all electron transport chain

Summary of ETS

- The **reduction potential E** for a complex redox reaction must be positive to yield energy for metabolism. The standard reduction potential **$E^{o'}$** assumes all reactant concentration equal 1 M, at pH7
- **Concentrations of e- donors and e- acceptors** in the environment influence the actual reduction potential E experienced by the cell
- **The ETS is embedded in a membrane that separate two compartments in order to maintain an ion gradient generated by ETS**
- The ETS is composed of protein complexes and cofactors. Protein complexes called **oxidoreductases** include **cytochromes** and **noncytochrome proteins**.

Electron Transport Chain: Oxidative phosphorylation

https://www.youtube.com/watch?v=LsRQ5_EmxJA

- Excellent video on Electron Transport Chain: Chemiosmotic Theory.

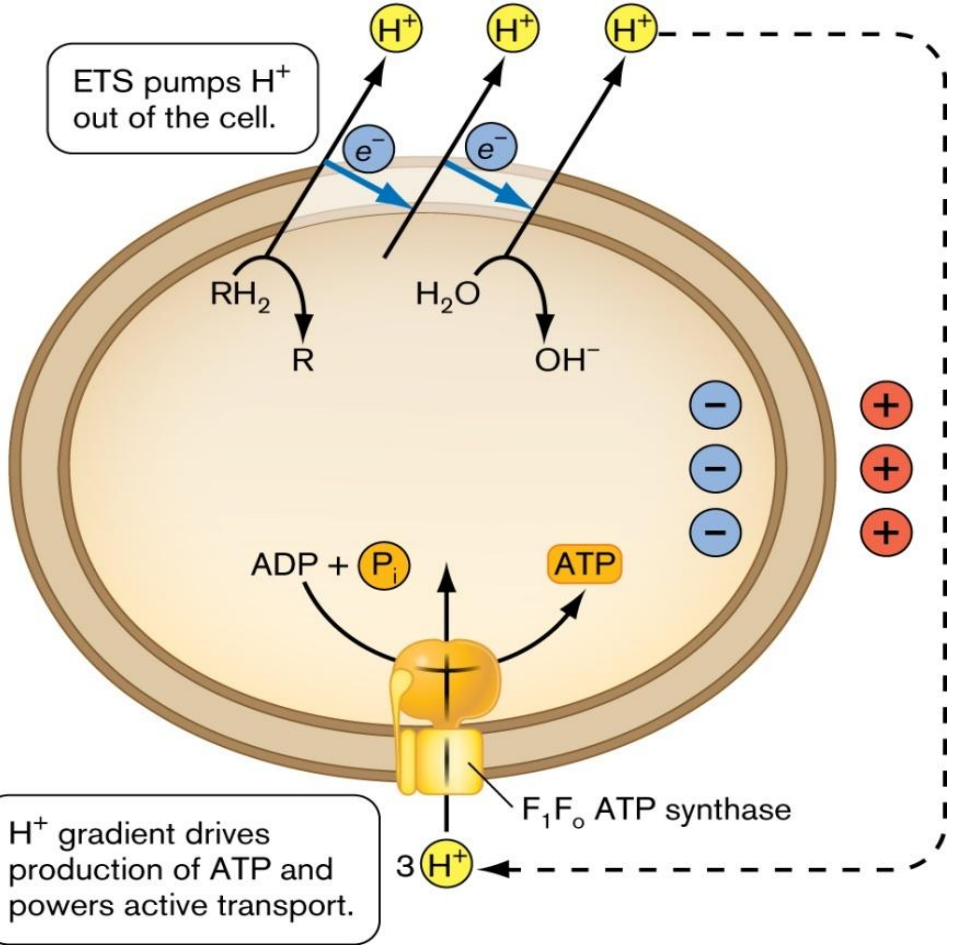
- *Note that the author described the ETC in eukaryotes, but the basic principles are similar in organotrophs.*

The proton motive force

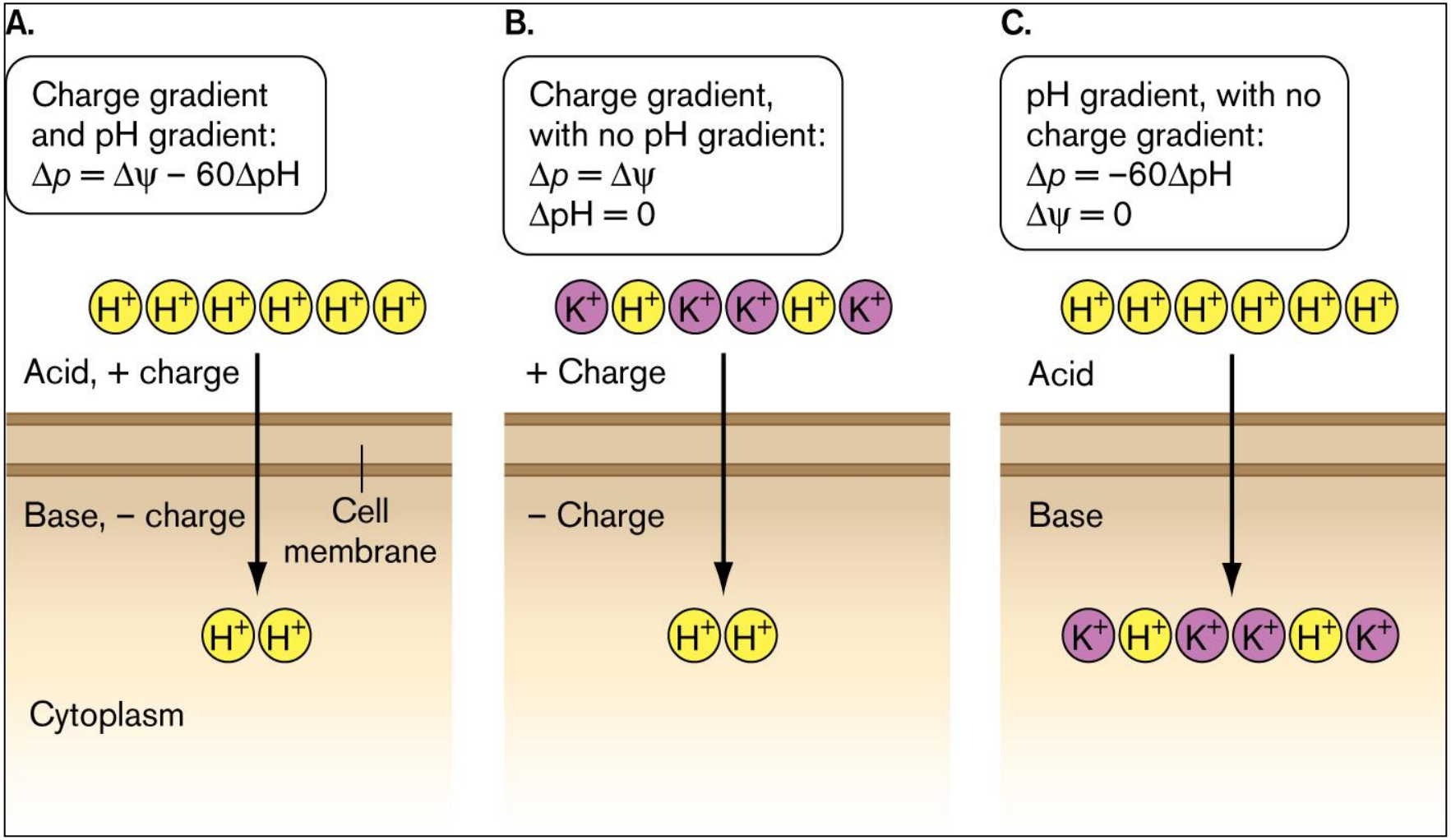
- The sequential transfer of e- from one ETS protein to the next yield energy to pump ions (in most cases H+) across the membrane: **Proton pump**
- Proton pumping generates a proton motive force , also called proton potential, which is composed of the **H+ concentration difference** as well as the **charge difference across the membrane**
- The proton motive force (PMF) drives many different cell processes:
  - **ATP synthesis** as discussed previously under nutrient transports
  - **Flagellar rotation** (Bacteria swim using rotary motions powered by a proton current

The transfer of H^+ through a proton pump generates an electrochemical gradient of protons, called a proton motive force.

- PMF drives the conversion of ADP to ATP through **ATP synthase**
- This process is known as the **chemiosmotic theory**.



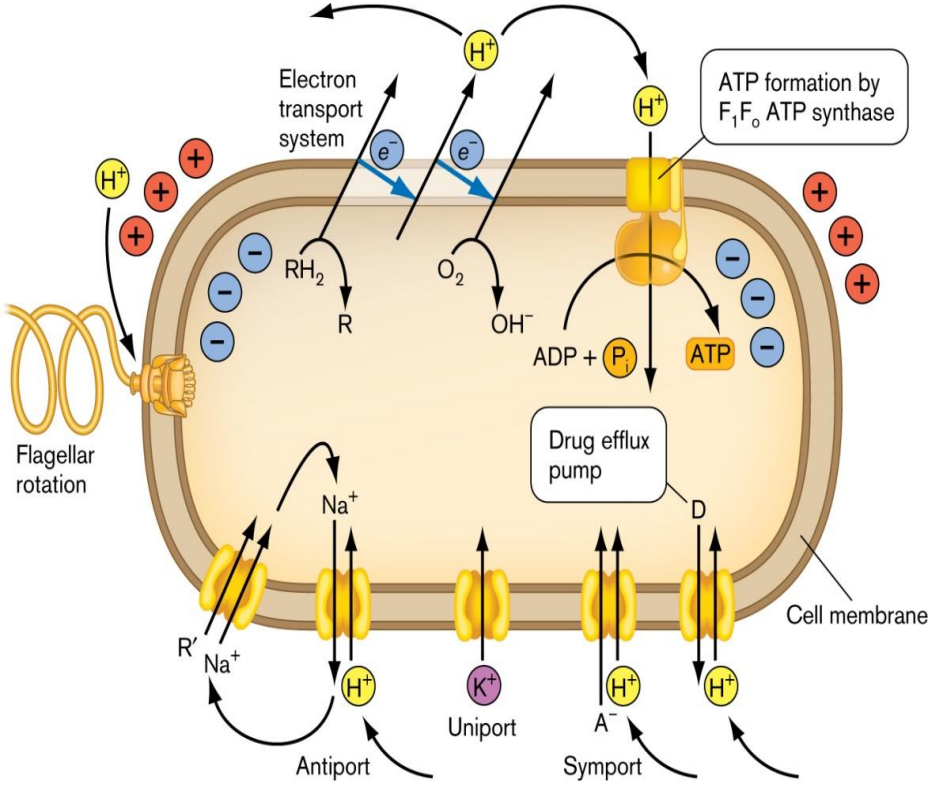
Electrical Potential and pH Difference



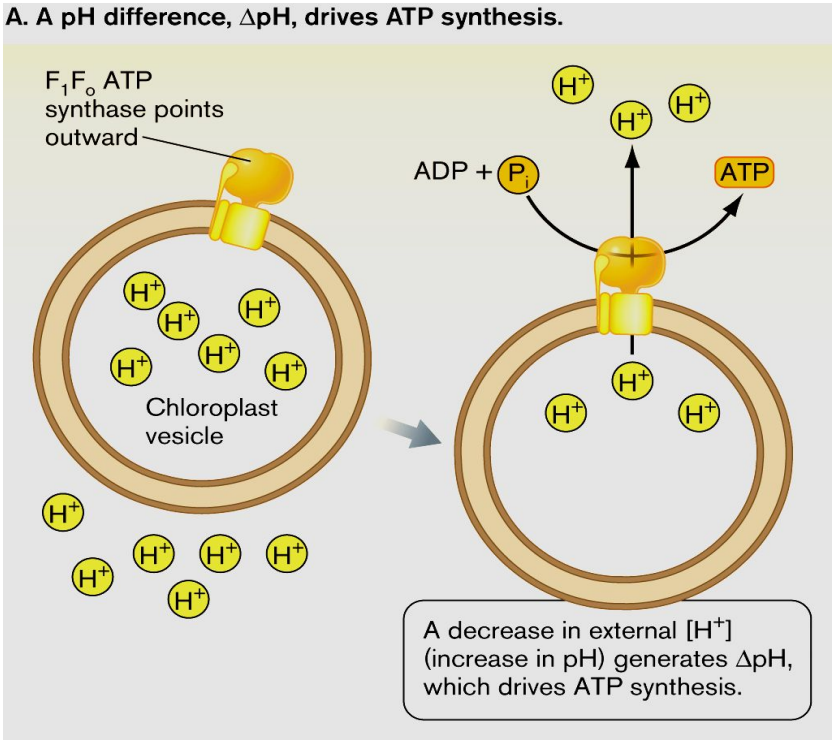
Δp drives many cell Functions

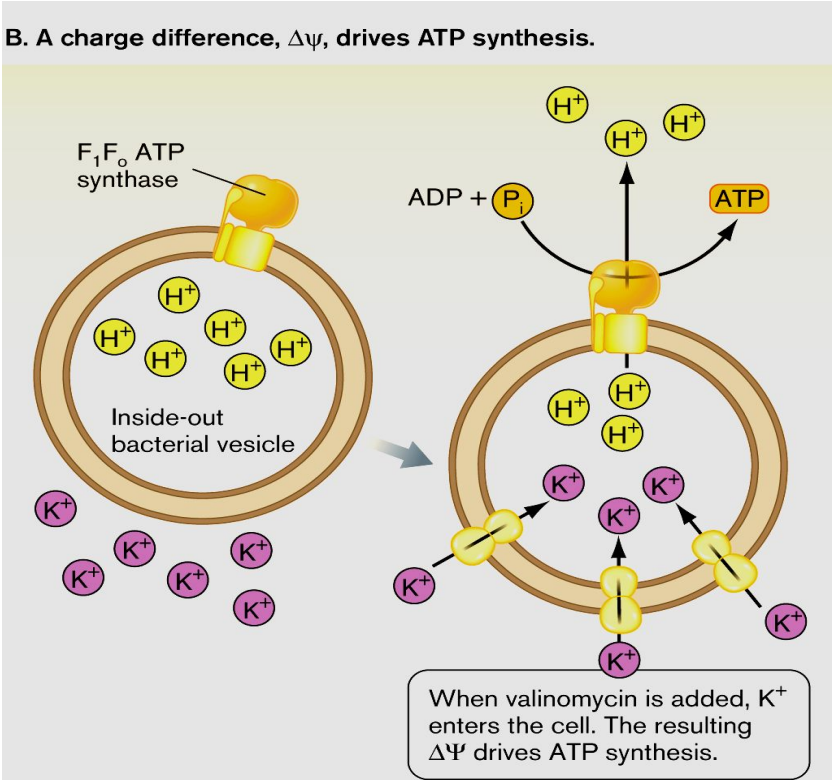
Processes driven by the proton motive force (Δp)

- **Rotation of flagella**
- **Uptake of nutrients**
- **Efflux of toxic drugs.**



Either pH difference or charge difference drives ATP synthesis.





The ETS components include enzymatic reactions

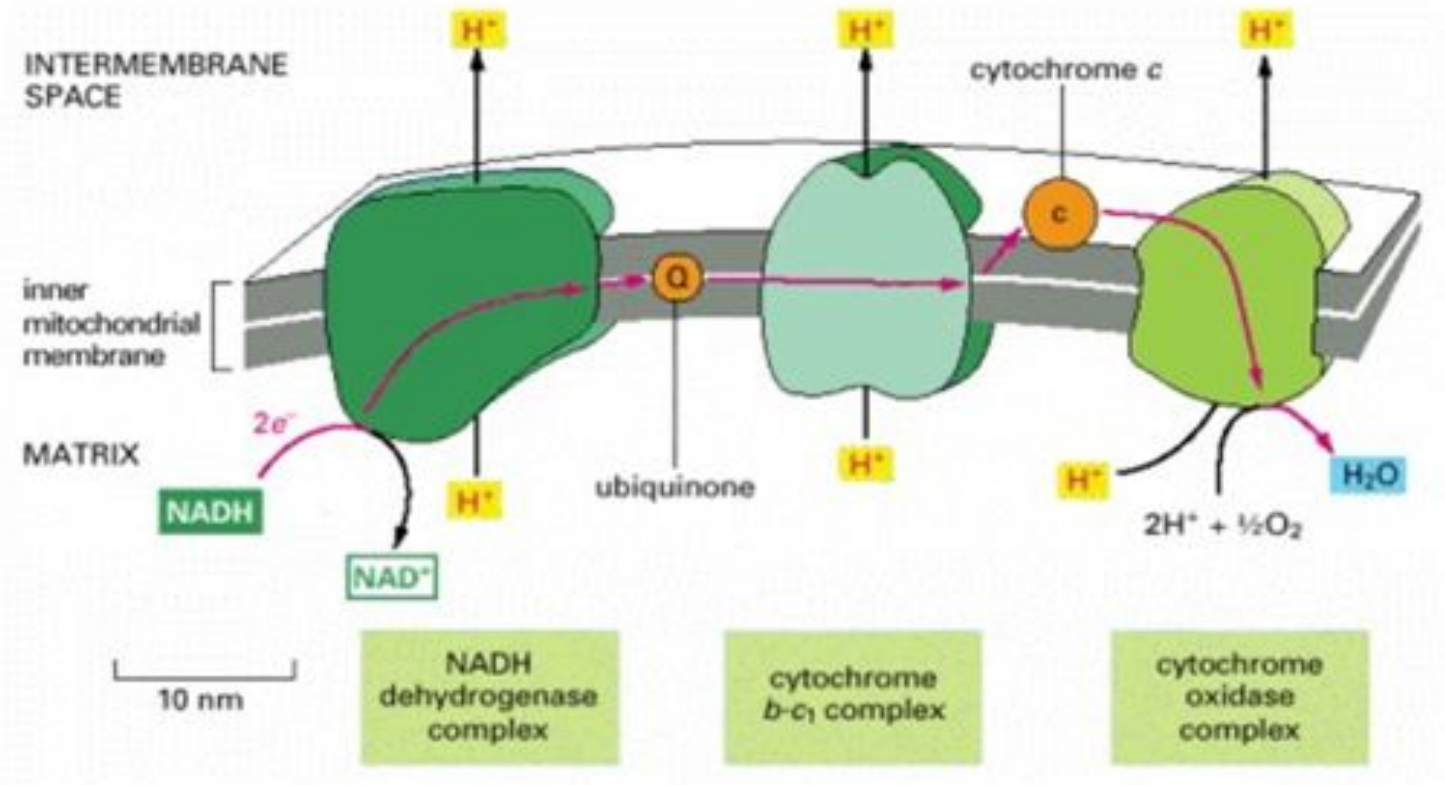
- **Oxidoreductases** catalyzes the removal of remove e- (oxidation of one substrate), and the donation of e- (reduction of another substrate)
- **Dehydrogenases**: Oxidoreductases that accept e- from NADH or FADH2 are also called dehydrogenases because their reaction releases H+
- **Oxidases** catalyzes the removal of remove e-

Oxidoreductase Protein Complexes

A respiratory electron transport system includes at least 3 functional components

1. An initial substrate **oxidoreductase** (or **dehydrogenase**)
2. A mobile electron carrier
3. A terminal **oxidase**

The path of electrons through the three respiratory enzyme complexes

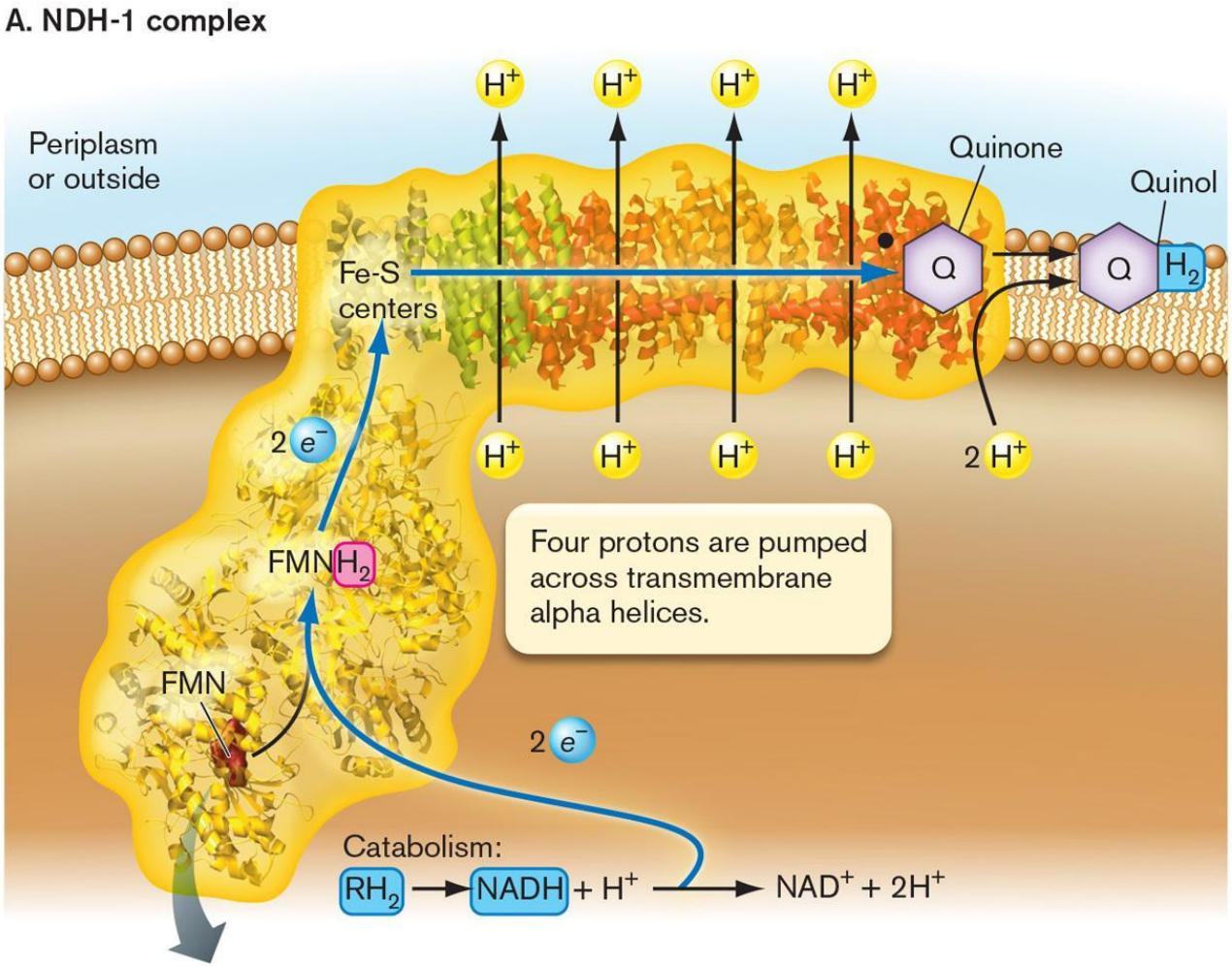


1. The substrate dehydrogenase receives a pair of electrons from an organic substrate, such as NADH, or an inorganic substrate, such as H_2.
2. It donates the electrons *ultimately* to a mobile electron carrier, such as quinone.

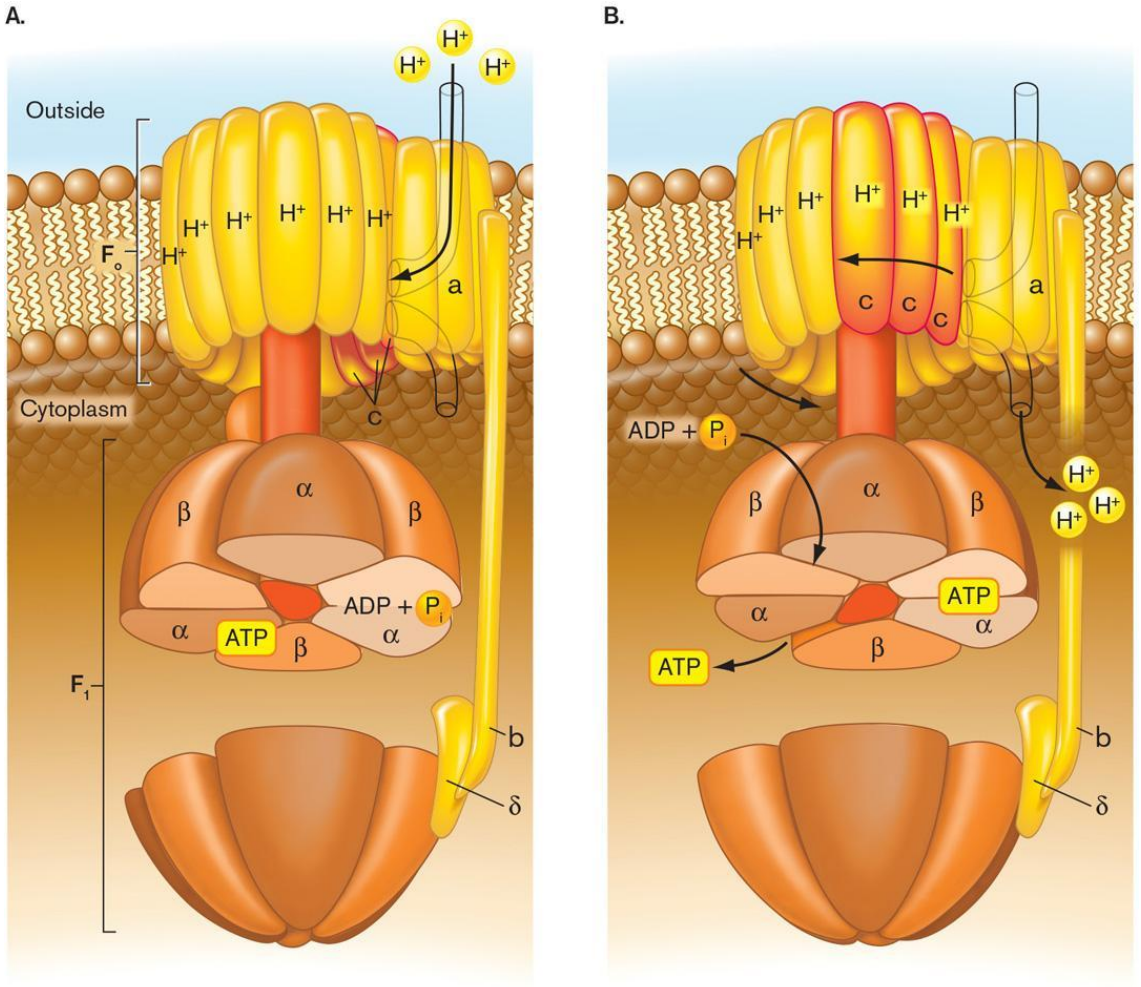
   Quinone picks up $2H^+$ from the solution and is thus reduced to quinol. There are many quinones, each with a different side chain; so for simplicity they are collectively referred to as Q and QH_2.
3. **The oxidation of NADH and reduction of Q is coupled to pumping $4H^+$ across the membrane**

Oxidoreductase Protein Complexes – 3

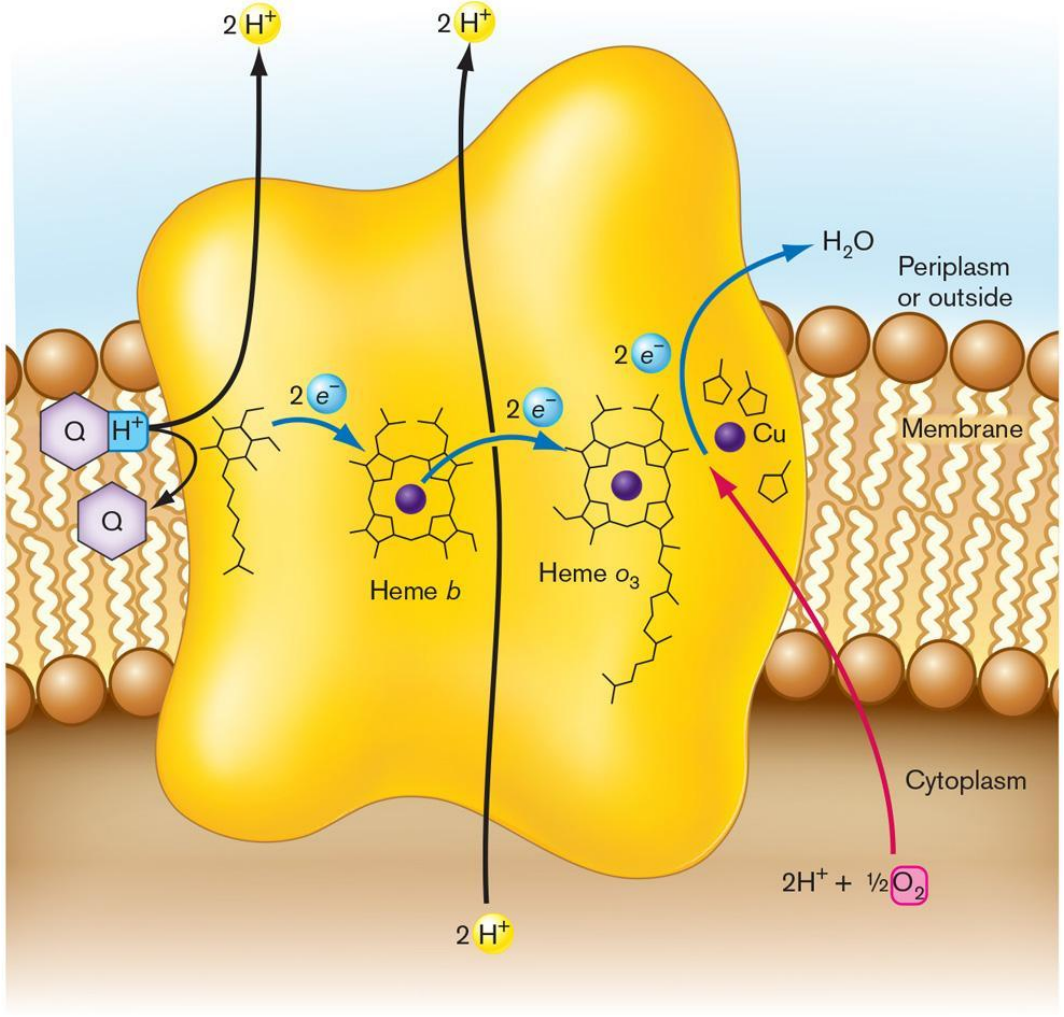
The oxidation of NADH and reduction of Q is coupled to pumping $4H^+$ across the membrane



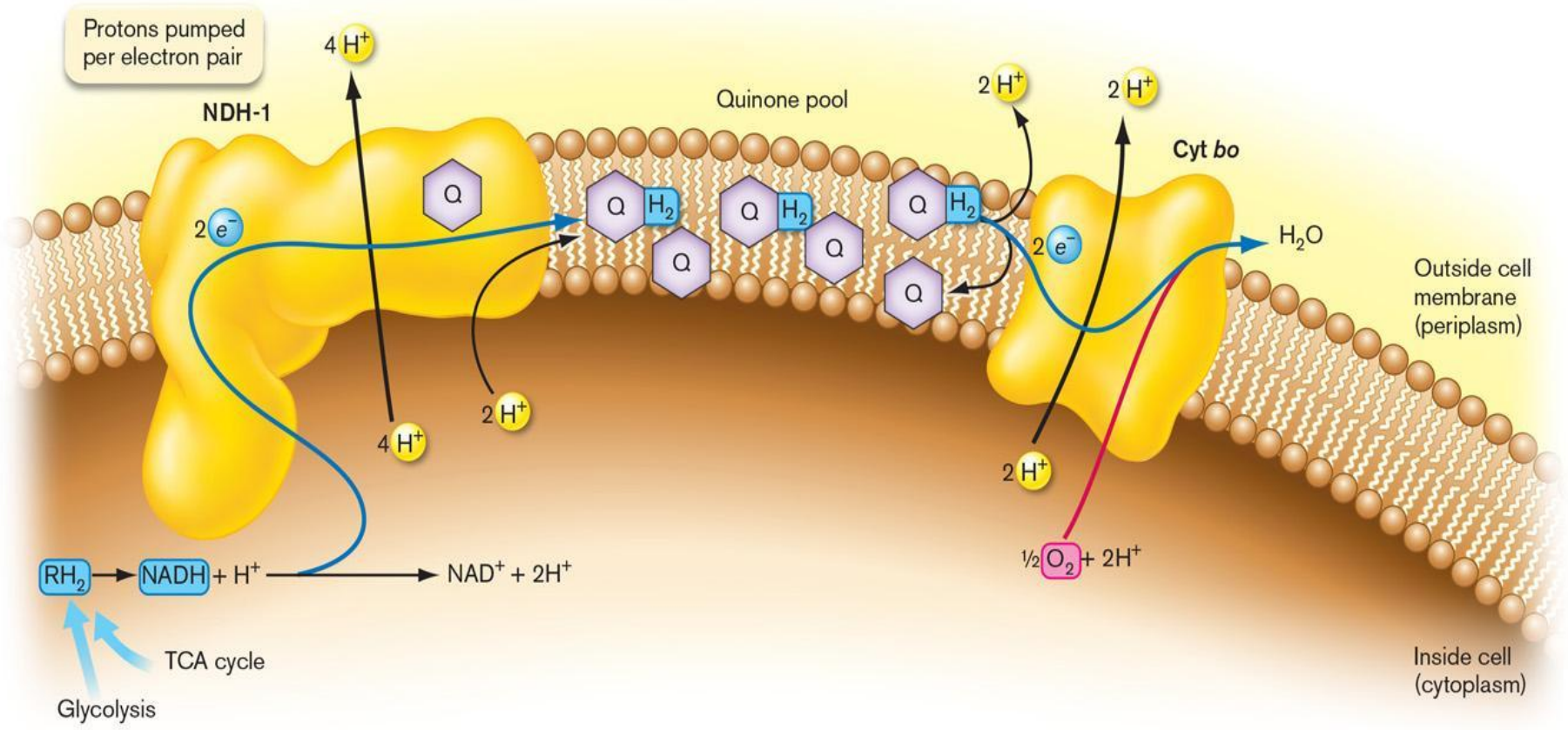
The Proton Potential Drives ATP Synthesis – 2



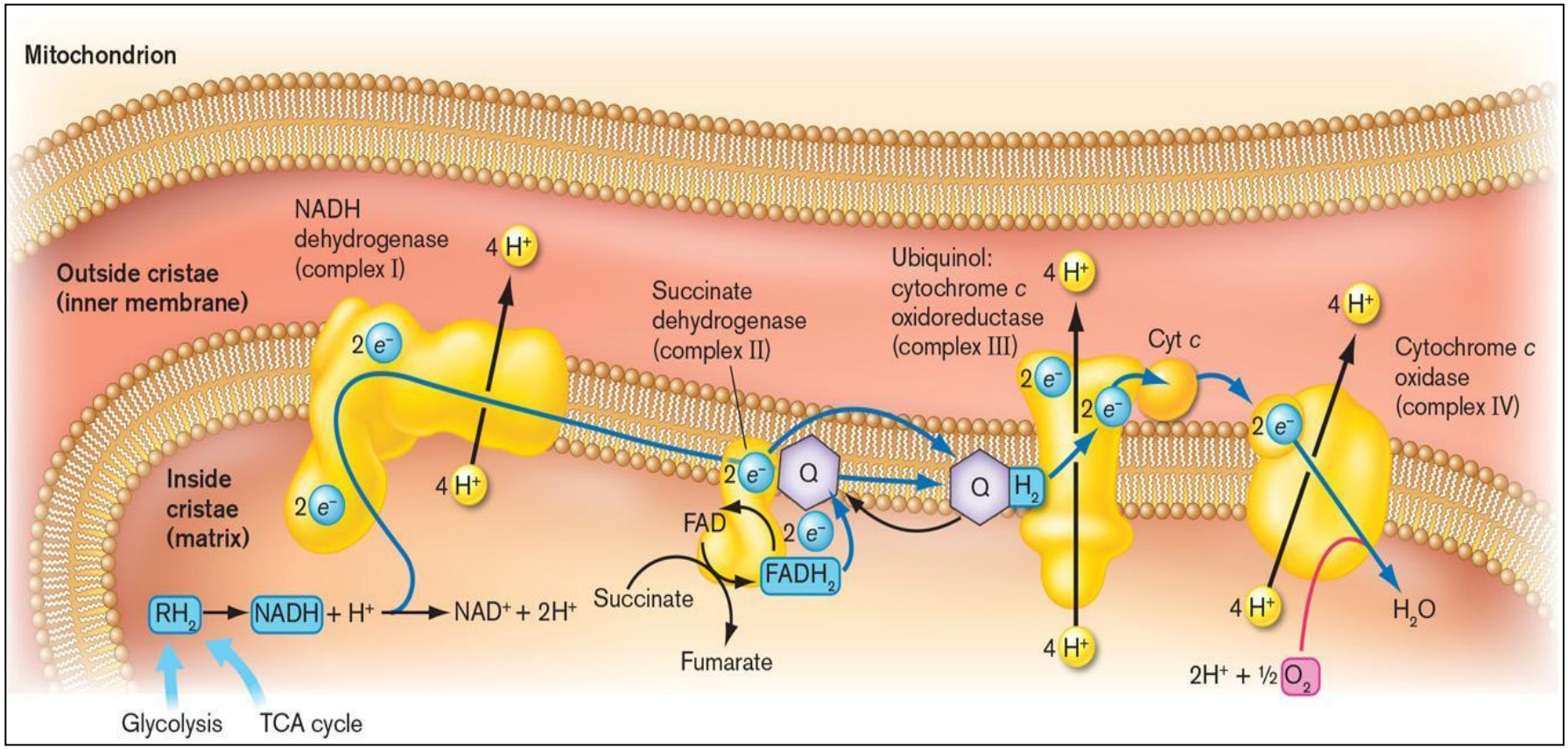
Oxidoreductase Protein Complexes – 6



Oxidoreductase Protein Complexes – 7

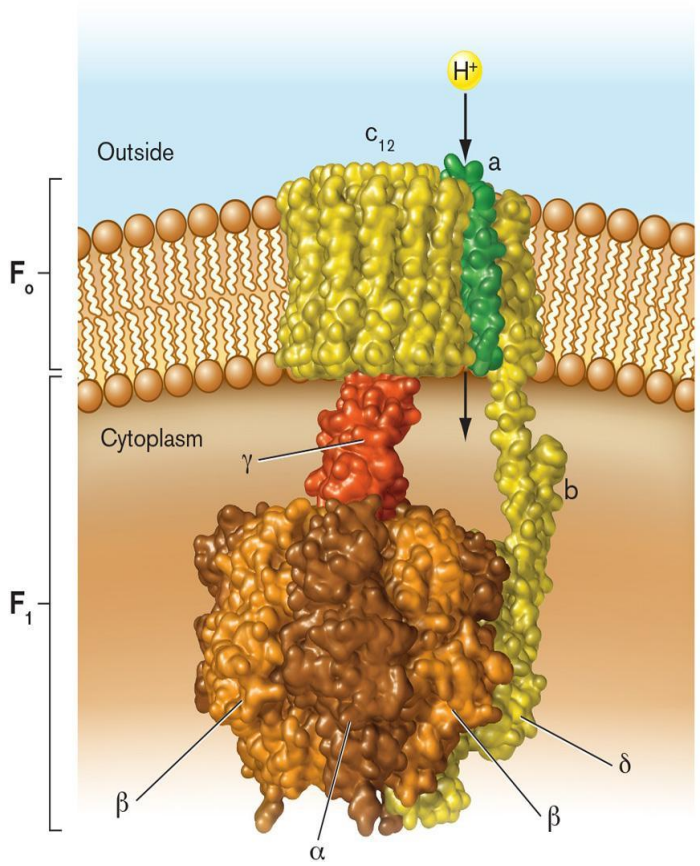


Mitochondrial Electron Transport

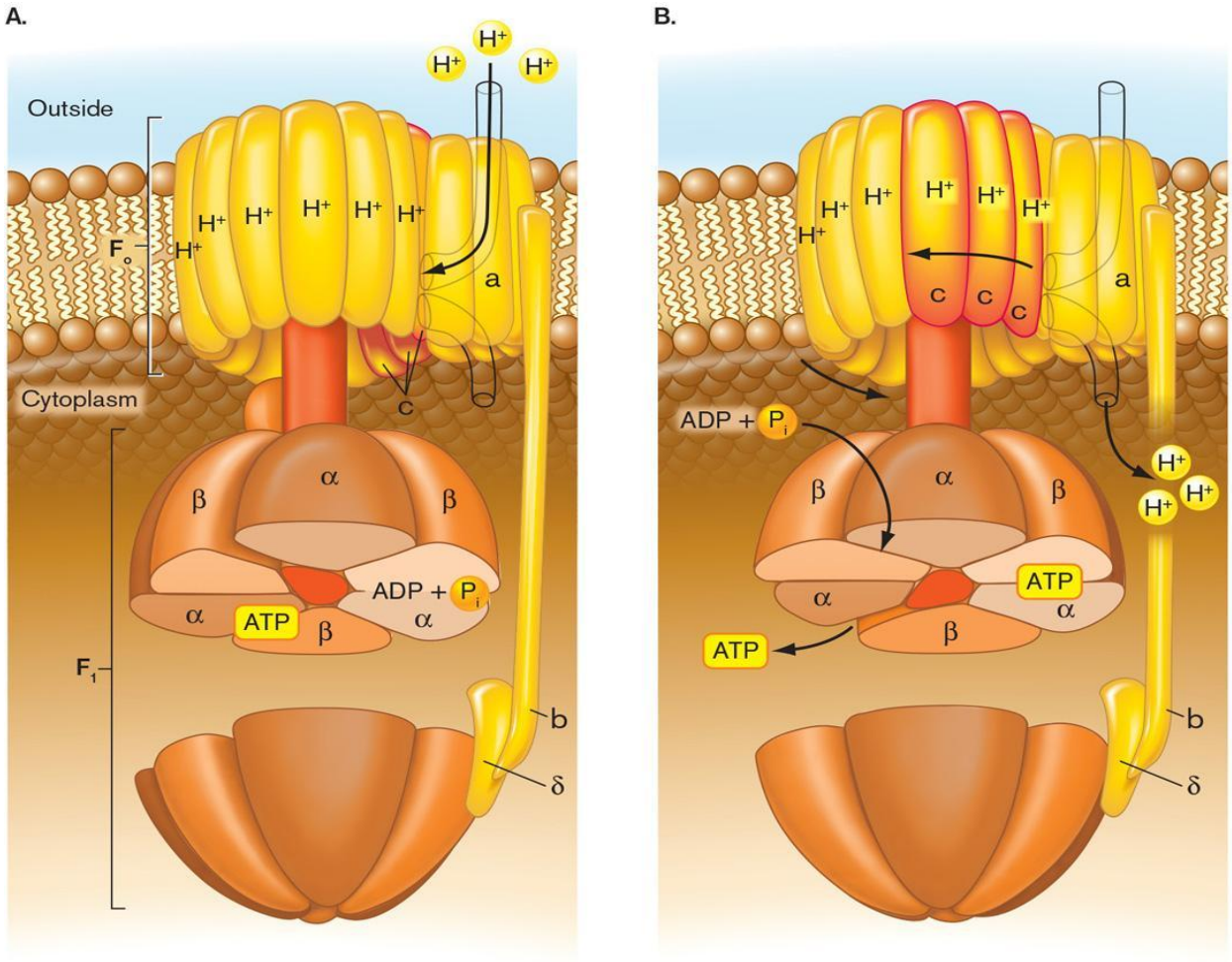


The Proton Potential Drives ATP Synthesis – 1

- The F_1F_o ATP synthase is a highly conserved protein complex, made of two parts:
  - F_o: embedded in the membrane
    - Proton flow through the subunit causes its rotation in the membrane.
  - F_1: protrudes in the cytoplasm
    - Rotation of the F_o subunit causes the F_1 γ subunit to turn, changing the conformation of the α and β subunits.
    - This conformational change catalyzes ATP synthesis.



The Proton Potential Drives ATP Synthesis – 2



Electron Transport Chain

- hhttp://www.youtube.com/watch?v=xbJ0nbzt5Kw&feature=related
- NDSU Virtual Cell Animations Project animation 'Cellular Respiration (Electron Transport Chain)'. For more information please see http://vcell.ndsu.edu/animations

- https://www.youtube.com/watch?v=3y1dO4nNaKY Gradients (ATP synthase)
- NDSU Virtual Cell Animations Project animation 'Gradients (ATP Synthase)'. For more information please see http://vcell.ndsu.edu/animations

Terminal electron acceptors

- In organotrophy or chemoorganotrophy: It is a form of metabolism in which organic molecules donate electrons, and the terminal electron acceptor is O2.
- Alternative electron acceptor is Nitrate (NO3)
- Some electron acceptors are organic molecules, such as fumarate (note that fumaric acid is a key chemical intermediate in the Krebs cycle)

- **Oxidized forms of nitrogen**
  - Nitrate is successively reduced as follows:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow 1/2\ N_2O \rightarrow 1/2\ N_2$$

nitrate nitrite nitric oxide nitrous oxide nitrogen gas

  - In general, any given species can carry out only one or two transformations in the series.

- **Oxidized forms of sulfur**
  - Sulfate is successively reduced by many bacteria as follows:

$$SO_4^{2-} \rightarrow SO_3^{2-} \rightarrow 1/2\ S_2O_3^{2-} \rightarrow S^0 \rightarrow H_2S$$

sulfate sulfite thiosulfate sulfur hydrogen sulfide

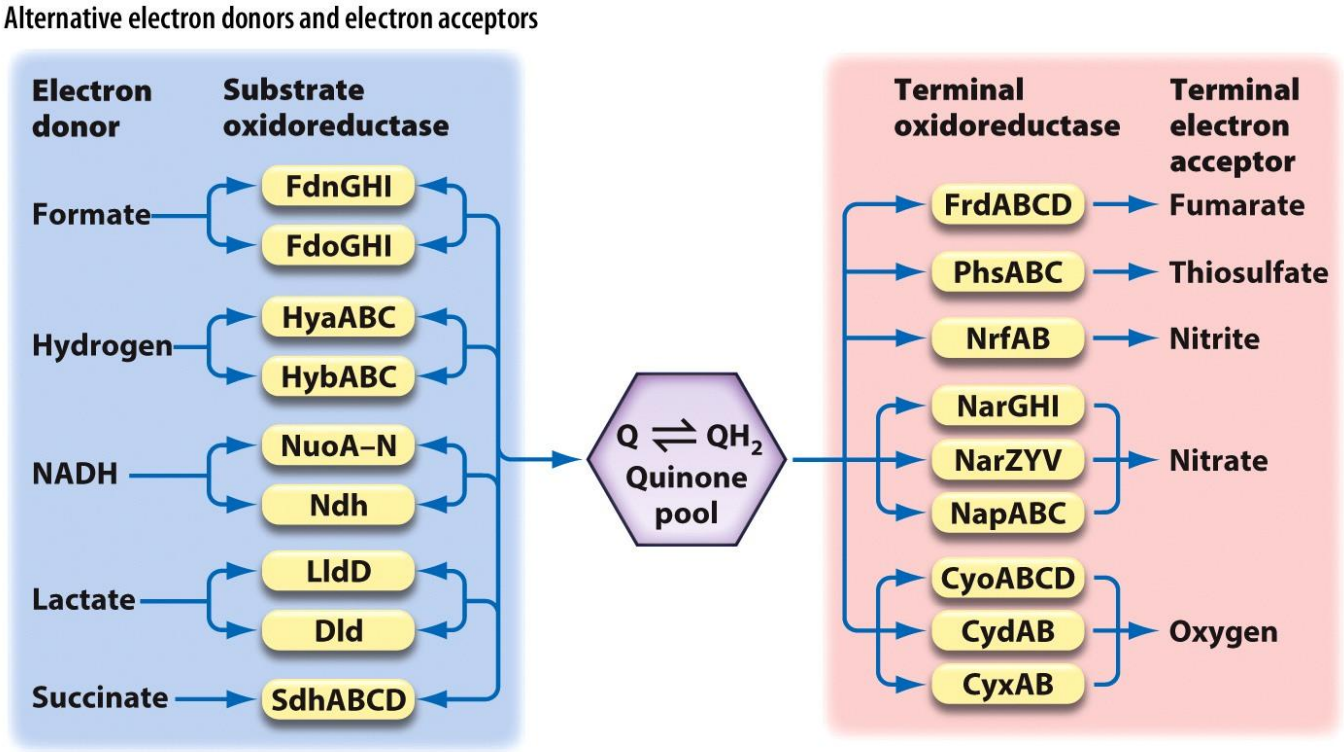
Anaerobic Respiration in Organotrophs

- **Obligate aerobes** are organisms that grow only using O_2 as a terminal electron acceptor.
  - Include animals, plants, and many bacteria
- **Anaerobic respiration**. Other prokaryotes use a wide range of terminal electron acceptors, including metals, oxidized ions of nitrogen, and sulfur.
  - This **anaerobic respiration** generally occurs in environments where oxygen is scarce
    - Wetland soil and the human digestive tract

Electron Acceptors and Donors

- **Anaerobic respiration is unique to prokaryotes**.
  - They usually possess alternative electron donors and electron acceptors.

FYI



Microbiology: An Evolving Science, Third Edition Figure 14.18

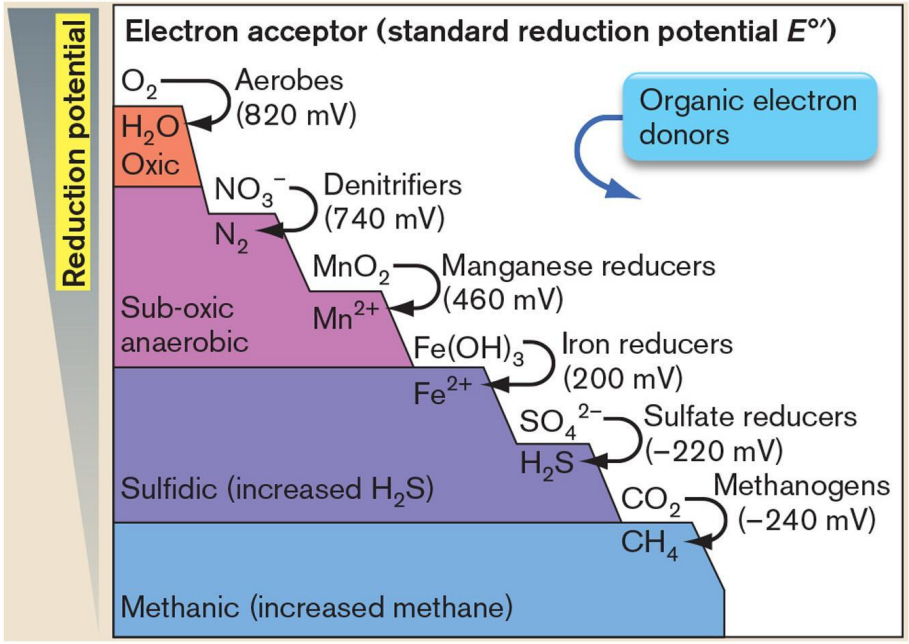
Figure 14.18

Dissimilatory Metal Reduction

- An important class of anaerobic respiration involves the reduction of metal cations, or **dissimilatory metal reduction**.
  - In contrast to minerals reduced for the purpose of incorporation into cell components (assimilatory metal reduction)
- The **metals most commonly reduced through anaerobic respiration are:**
  - Iron ($Fe^{3+} \rightarrow Fe^{2+}$)
  - Manganese ($Mn^{4+} \rightarrow Mn^{2+}$)

Dissimilatory Metal Reduction – 2

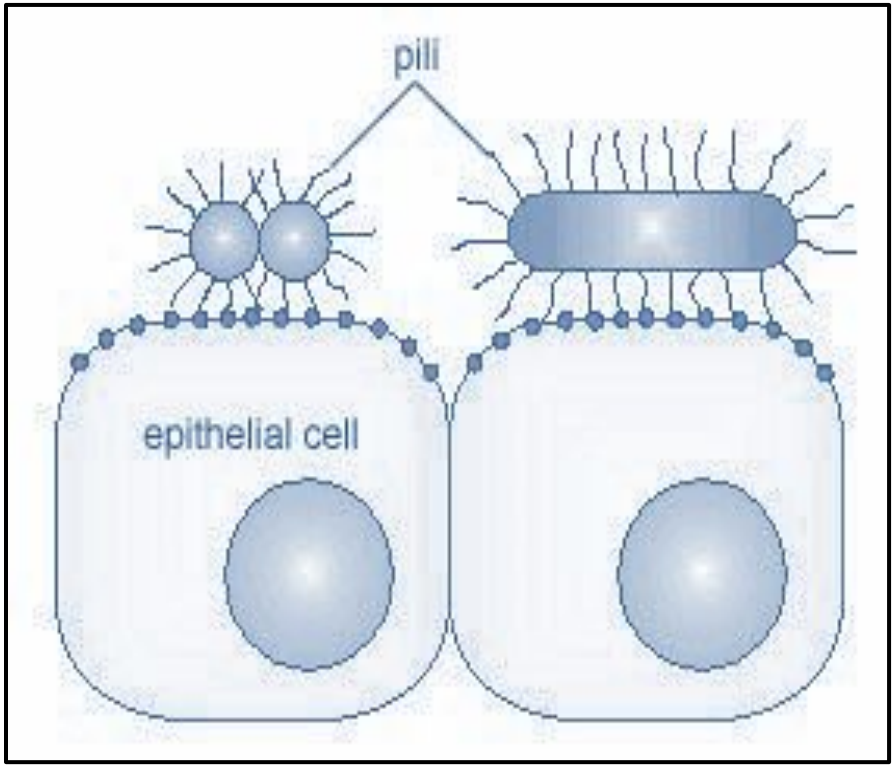
- Anaerobic environments, such as the bottom of a lake, offer a series of different electron acceptors.
- As each successive terminal electron acceptor is used up, its reduced form appears; the next-best electron acceptor is then used, generally by a different microbe species.

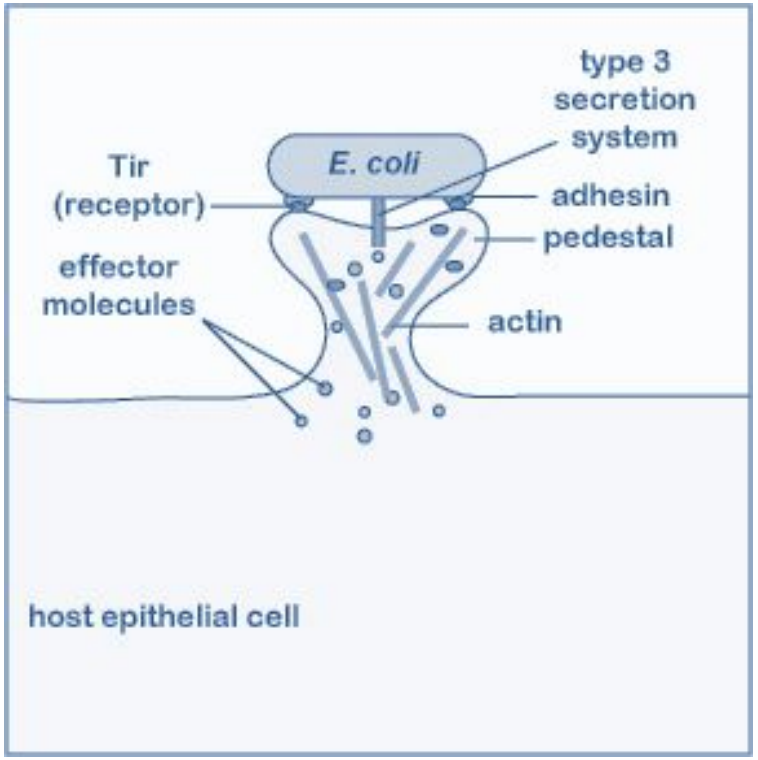


Chapter Summary

- Electron transport systems (ETS) consist of membrane-embedded proteins that transfer electrons from an initial electron donor to a TEA that leaves the cell.
- The ETS complexes generate a proton motive force that can drive ATP synthesis and other cell functions.
- Electron carriers contain metal ions and/or conjugated, double-bonded ring structures.
- An ETS includes at least three functional components:
  - Substrate dehydrogenase, mobile electron carrier, and terminal oxidase
- The F_1F_o ATP synthase is a membrane-embedded protein complex.
  - Three protons drive each F_1F_o cycle, synthesizing one molecule of ATP.

BACTERIAL PATHOGENESIS
Lecture 20 (Ch.25)





Chapter Overview

- Host-pathogen interactions
- How microbes attach to host cells
- How toxins subvert host functions
- How toxins and effectors are deployed
- How pathogens survive within their hosts
- Tools used to probe pathogenesis

Introduction

- Mammals have elaborated physical, chemical, and immunological defenses that protect against disease-causing microbes.
  - However, every fortress has its weakness.
    - Pathogenic microbes exploit those weaknesses, and the result is disease.
- The fundamental question of microbial pathogenesis is this:
  - How an organism too small to be seen with the naked eye can kill a human a million times larger?

The Language of Pathogenesis

The Language of Pathogenesis – 1

- By definition, a **parasite** is an organism that receives benefits at the expense of a host.
  - In practice, the term "**parasite**" refers to disease-causing protozoa and worms. Bacterial, viral, and fungal parasitic agents of disease are called **pathogens**.
  - **Ectoparasites** live on the surface of the host;
  - **Endoparasites** live inside the host's body.
- An **infection** occurs when a pathogen or parasite enters or begins to grow on a host. However, the term "infection" does not necessarily imply overt disease.

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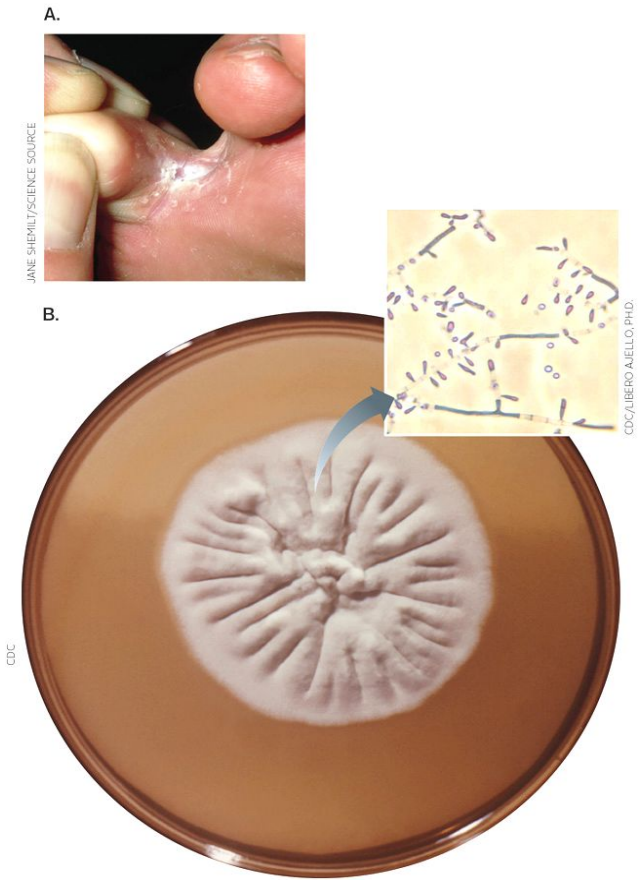
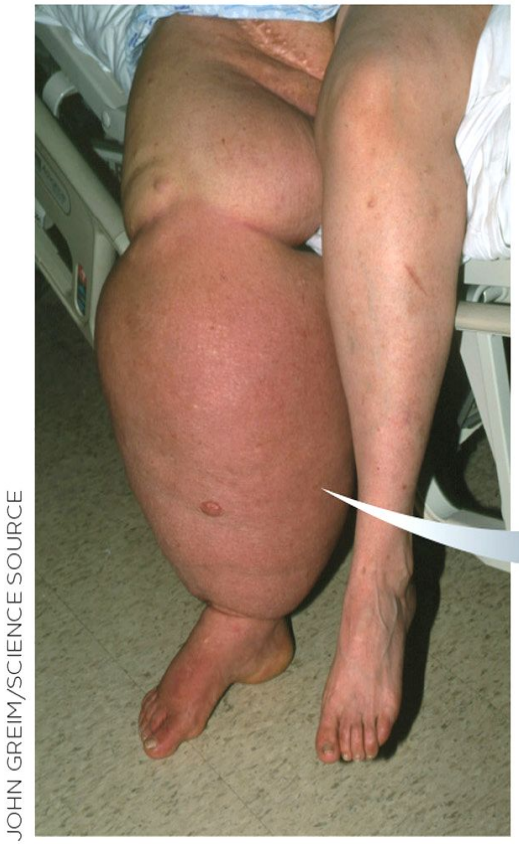
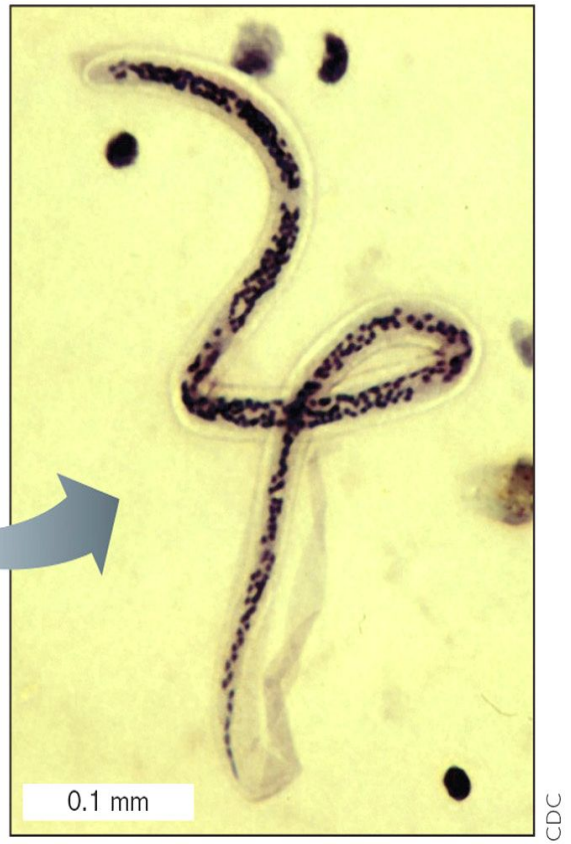
The Language of Pathogenesis – 3

- **Primary pathogens**: cause disease in healthy hosts
  - For example: ***Shigella flexneri***, the cause of bacillary dysentery
- **Opportunistic pathogens**: cause disease only in compromised hosts or following entry into unprotected sites
  - For example: ***Pneumocystis jirovecii***, the cause of life-threatening infections in AIDS patients
- Some microbes even enter a **latent state** during infection, in which the infectious organism cannot be found by culture.
  - For example: herpesvirus, the cause of **cold sores**

The Language of Pathogenesis – 2







The Language of Pathogenesis – 4

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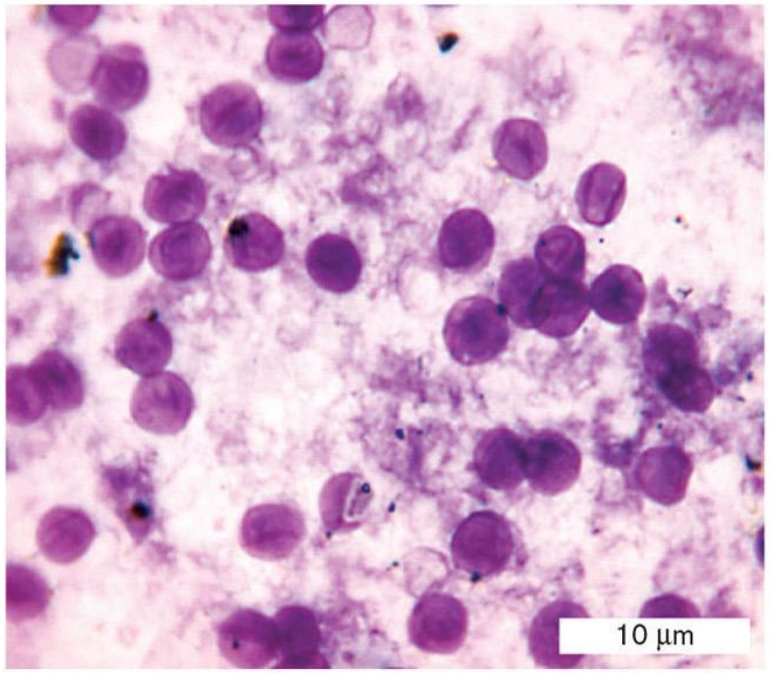
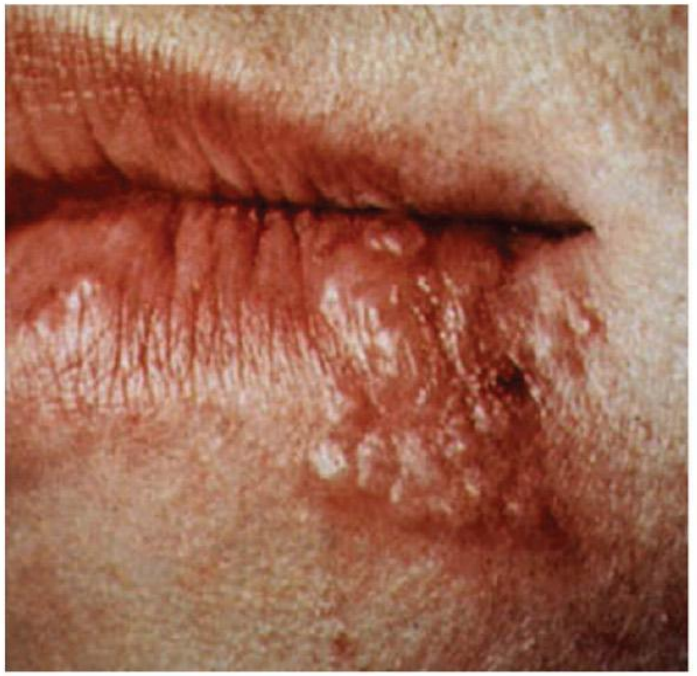


CDC/LOIS NORMAN

B.

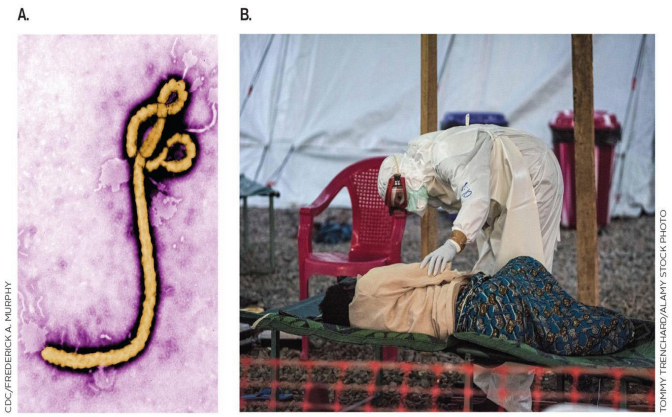


CDC/HERMANN

A. ***Pneumocytis jirovechi*** cysts in bronchoalveolar materials
B. Cold sore produced by a reactivated **herpesvirus** hiding latent in nerve cells

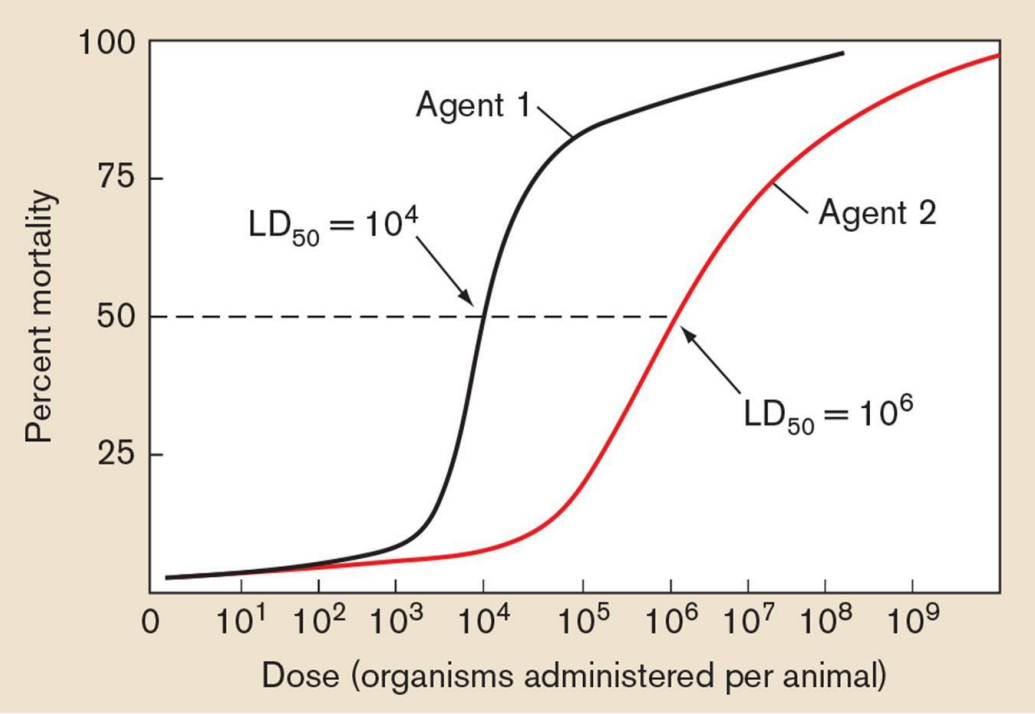
The Language of Pathogenesis – 5

- **Pathogenicity** refers to an organism's ability to cause disease. It is defined in terms of . . .
  - how easily an organism causes disease (infectivity)
  - how severe that disease is (virulence)
  - the specific genetic makeup of the pathogen
- **Virulence** is a measure of the degree, or severity, of disease.
  - For example, Ebola is highly virulent, whereas rhinoviruses are not.



The Language of Pathogenesis – 6

- Virulence is measured by determining the **infectious dose (ID_{50}, # required to cause infection, but not death, in 50% of hosts) and/or the lethal dose (LD_{50}, # required to cause death in 50% of hosts).**



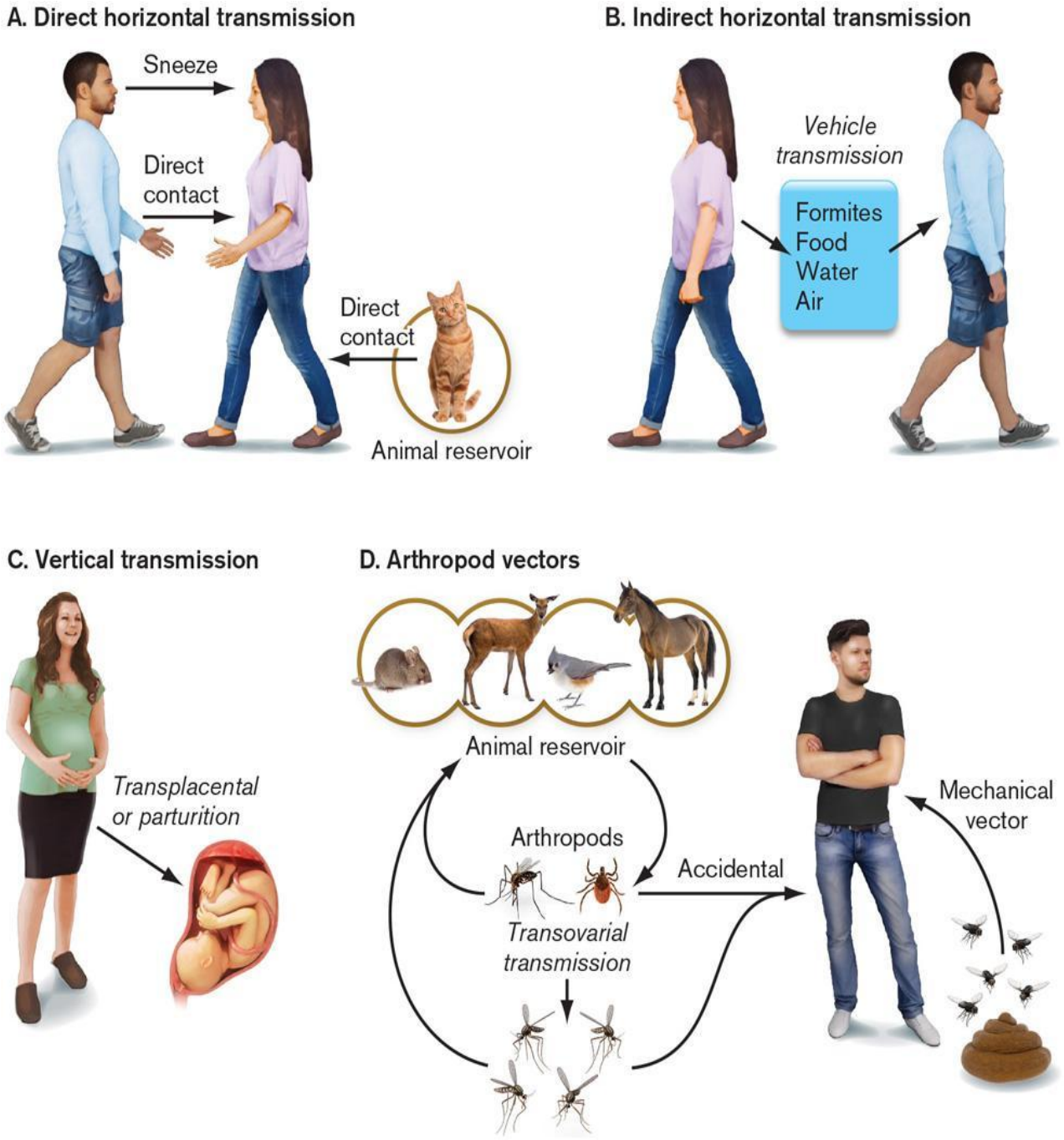
Immunopathogenesis

- It is often “friendly fire” by our immune system reacting to a pathogen that causes major tissue and organ damage.
- The term **immunopathogenesis** applies when the immune response to a pathogen is a contributing cause of pathology and disease.
- To fully understand any infectious disease, researchers must study both the pathogenic mechanisms of the pathogen and the disease symptoms caused by immunopathogenesis.
- Ex. Lyme disease caused by the spirochete *Borrelia burdorferi* is associated with stimulates the production of strong inflammatory cytokines, including TNF alpha, IL-1
- beta and IL-6.

Infection Cycles

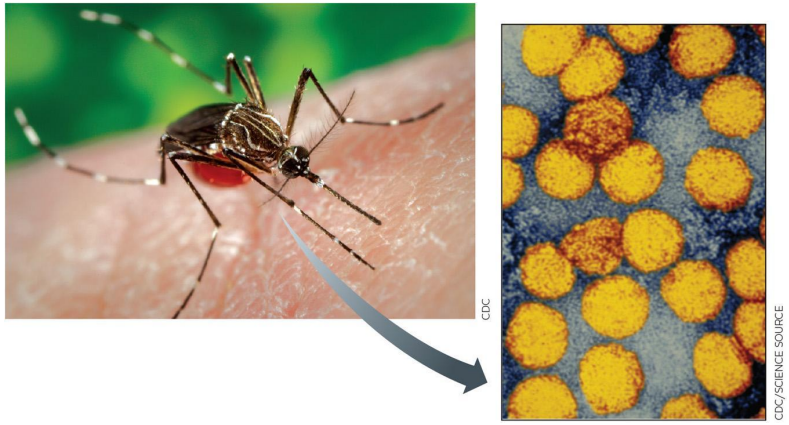
Infection Cycles – 1

- The **infection cycle** describes the route of transmission of an infectious organism.
  - **Horizontal transmission**: passage from one person or animal to another within the same generation
    - Can be **direct** (e.g., handshaking) or **indirect** (e.g., sharing contaminated objects)
    - **Fomites**: inanimate objects (e.g., doorknobs, hand towels, utensils)
    - **Vehicles**: ingested or inhaled materials (e.g., food, water, air)
  - **Vertical transmission**: passage from a mother to her fetus during pregnancy (transplacental) or birth (parturition)


A. Direct horizontal transmission
Sneeze
Direct contact
Direct contact
Animal reservoir
B. Indirect horizontal transmission
Vehicle transmission
Formites
Food
Water
Air
C. Vertical transmission
Transplacental or parturition
D. Arthropod vectors
Animal reservoir
Arthropods
Transovarial transmission
Accidental
Mechanical vector

Infection Cycles – 3

- Complex infection cycles often involve **vectors** as intermediaries (usually arthropods like mosquitoes, ticks, mites, or flies).
  - For example, a mosquito vector transfers the virus that causes yellow fever from infected to uninfected individuals.



Infection Cycles – 4

- A **reservoir** is an animal, bird, or arthropod that normally harbors the pathogen, often without exhibiting disease.
- In the case of yellow fever, the mosquito is not only the **vector** but the **reservoir** as well, because the insect can pass the virus to future generations of mosquitoes through vertical transmission.
- The virus causing eastern equine encephalitis (EEE), however, uses **birds as a reservoir**.
- Reservoirs are critically important for the survival of a pathogen and as a source of infection.

Portals of Entry

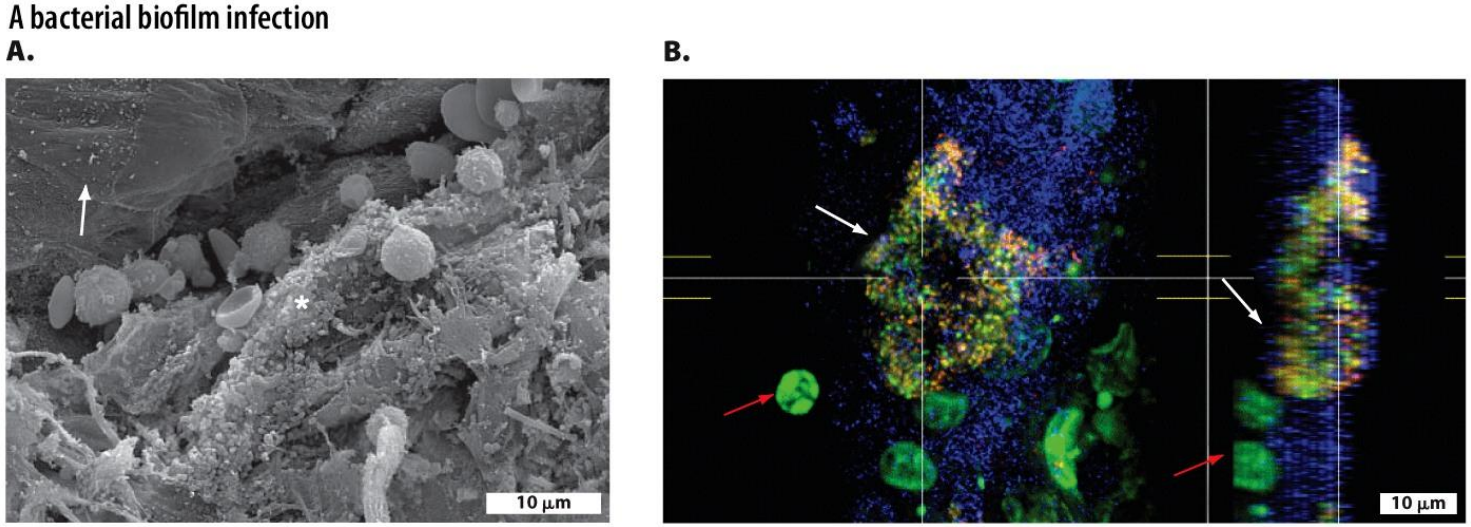
- Infectious agents enter the body through one or more **portals of entry** that are best suited to their mechanism of pathogenesis.
  - **Mouth**
  - **Respiratory tract**
  - **Conjunctiva and mucous membranes**
  - **Wounds, injuries, and skin lesions**
  - **Parenteral route: direct injection into bloodstream (e.g., tick and mosquito bites, needle punctures)**

Effect of Infections on Microbiota

- A pathogen's growth and the host's resulting immune response also will affect the host's normal microbiota.
- Numerous mechanisms affect microbial competition and, ultimately, species diversity within the body.
  - Diarrhea reduces the overall numbers of gut microbiota.
  - Intestinal pathogens occupy host binding sites and alter available nutrients.
  - Inflammation can benefit pathogens more than normal microbiota.

Biofilms and Infections

- Bacteria can attach to surfaces in bulk, forming a **biofilm**.
- Biofilms play an important role in chronic infections by enabling persistent adherence and resistance to bacterial host defenses and antimicrobial agents.



Microbiology: An Evolving Science, Third Edition Figure 25.15

Figure 25.15

Virulence Factors

Virulence Factors and How to Find Them – 1

- To cause disease, all pathogens must . . .
  - Enter a host
  - Find their unique niche
  - Avoid, circumvent, or subvert normal host defenses
  - Multiply
  - Transmit to a new susceptible host
- Pathogens employ **virulence factors**, encoded by virulence genes, to accomplish these goals. Virulence factors include toxins, attachment proteins, capsules, and other devices.

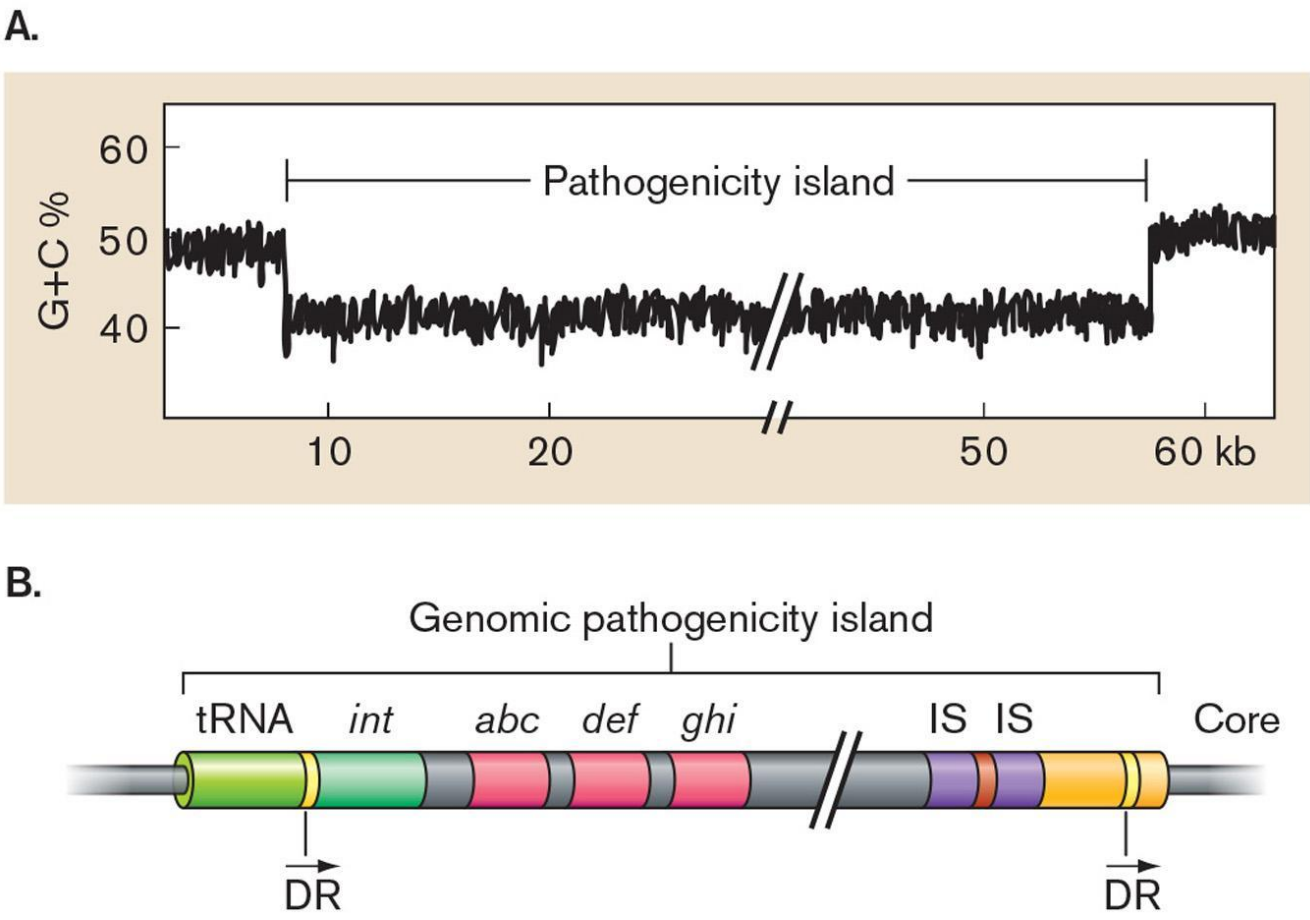
Virulence Factors and How to Find Them – 2

- A suspected virulence gene can be confirmed as having a role in virulence or pathogenicity only if it fulfills a set of **molecular Koch's postulates**.
  1. The phenotype under study should be associated with pathogenic strains of a species.
  2. Specific inactivation of the suspected virulence gene(s) should lead to a measurable loss in virulence or pathogenicity. The gene(s) should be isolated by molecular methods.
  3. Reversion or replacement of the mutated gene should restore pathogenicity.

Pathogenicity Islands – 1

- **Some virulence genes reside on plasmids or in phage genomes.**
- Virulence genes in bacterial pathogens often are clustered into **pathogenicity islands** that encode virulence functions.
- **Most pathogenicity islands appear to have been horizontally transmitted via conjugation or transduction**
  - Unique GC/AT ratio
  - Linkage to a tRNA gene
  - Association with genes homologous to phage/plasmid genes

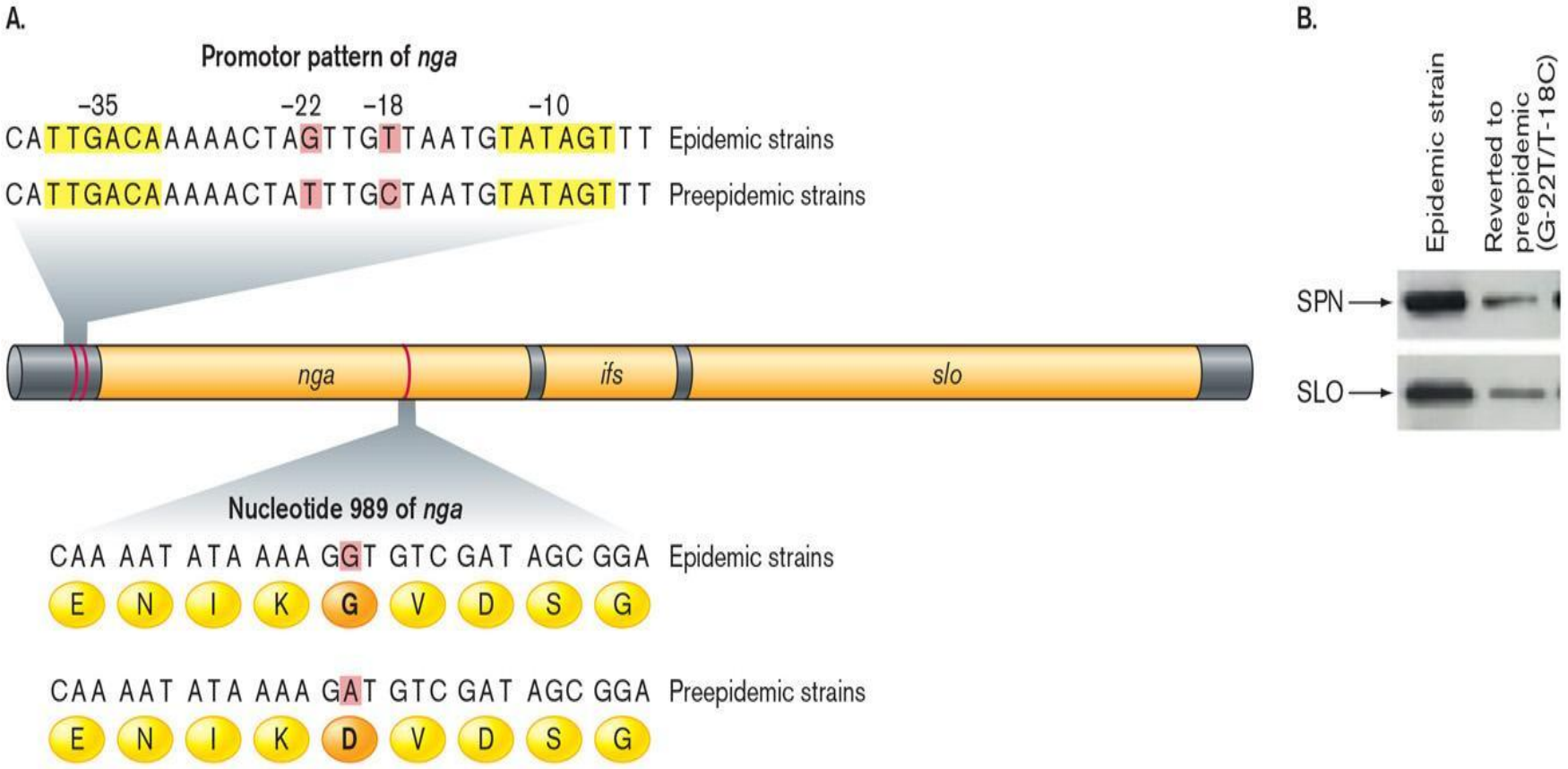
Pathogenicity Islands – 2



Examples of Pathogen Evolution by Horizontal Gene Transfer – 1

- Dangerous pathogens caught in the act of evolving include:
  - *Escherichia coli* O104:H4, which caused a major European outbreak of hemolytic uremic syndrome
  - *Streptococcus agalactiae* and *Staphylococcus aureus.*
  - *Streptococcus pyogenes* (Group A *Streptococcus*)
    - Large-scale genome sequencing data have recently determined that **epidemics are caused by clonal replacement events rather than by reemergence of preexisting clones.**

Examples of Pathogen Evolution by Horizontal Gene Transfer – 2



Microbial Attachment

25.2 Microbial Attachment: First Contact – 1

- **The human body has many ways to exclude pathogens. How do bacteria manage to stick around long enough to cause disease?**
- The first step toward infection is attachment, or adhesion.
  - Any microbial factor that promotes attachment is called an **adhesin**.
  - Viruses attach to host cells through their capsid or envelope proteins.
  - Bacteria use a variety of strategies, **including pili (fimbriae) and other nonpilus proteins,** to bind to specific host cell factors.

Pili – 1

- Many bacteria typically attach to specific host cells using hairlike appendages called **pili** (**fimbriae**). [Note that fimbriae pili are not the same as conjugation, or sex pili used for gene transfer.]
  - **Type I: adhere to carbohydrates on host membranes**
    - Produce a static attachment to host cell
    - Grow from outer membrane of certain Gram-negative bacteria
  - **Type IV: involved in "twitching motility"**
    - Produce a dynamic attachment via assembly and disassembly
    - Grow from inner membrane of many Gram-negative bacteria

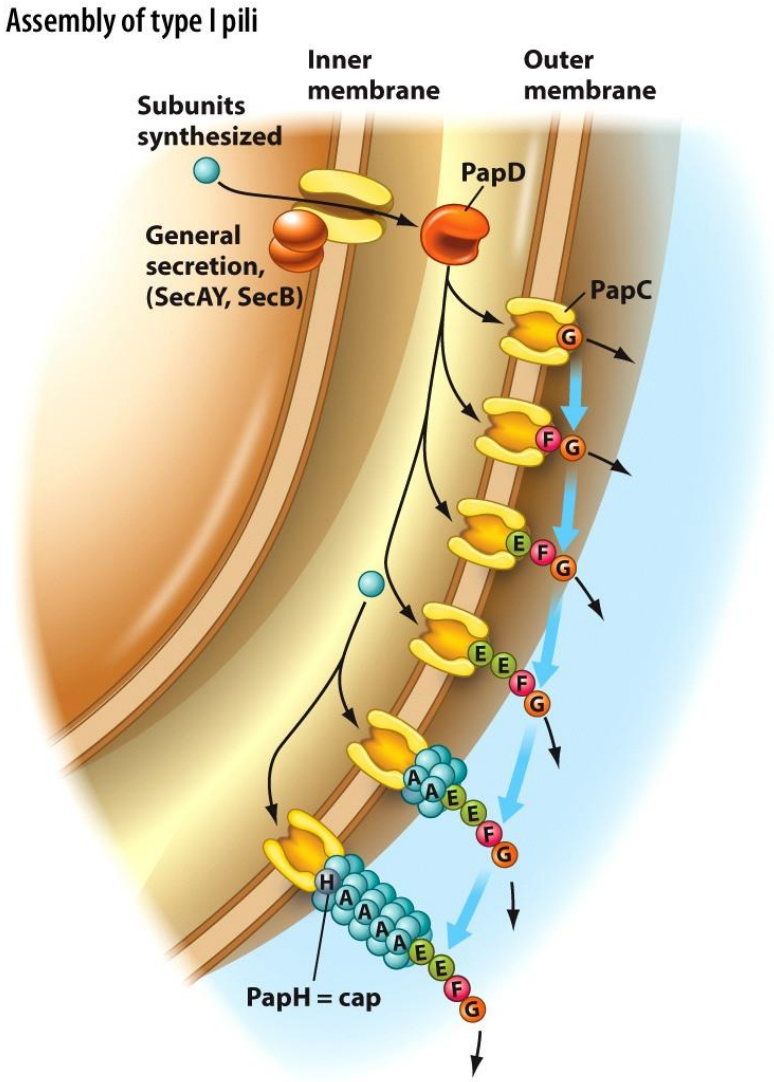


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Figure 25.12

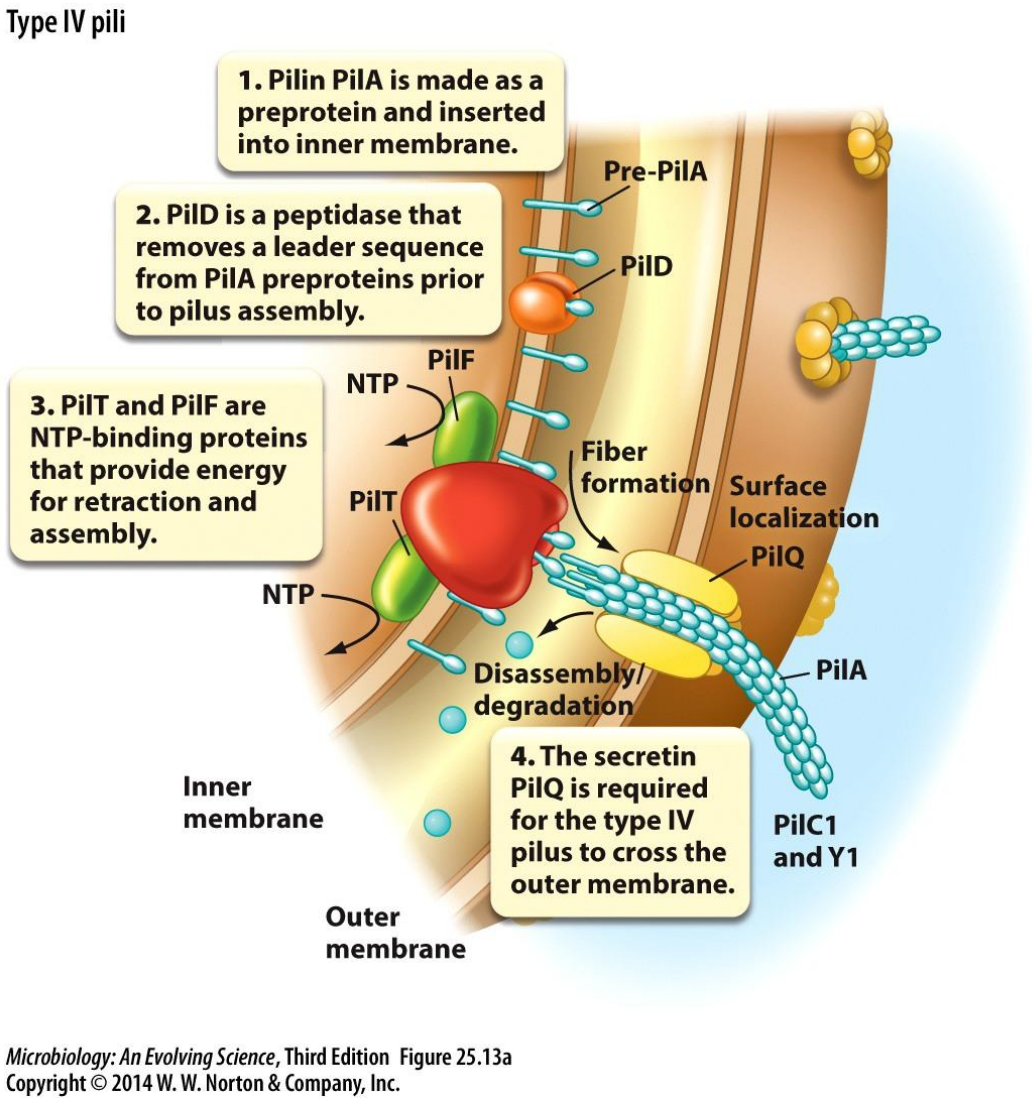

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Figure 25.13

Nonpilus Adhesins – 3

- Why are some people susceptible to certain infections, whereas others are not?
  - **Immunocompetence**
  - **Receptor availability**
- Pathogens rely on very specific surface structures (receptors) to recognize and attach to appropriate host cells.
  - Person-to-person differences in receptor structures are possible.
  - Example: HIV binds C-C chemokine receptor type 5 (CCR5); individuals with a CCR5 mutation are resistant to HIV infection!

Nonpilus Adhesins

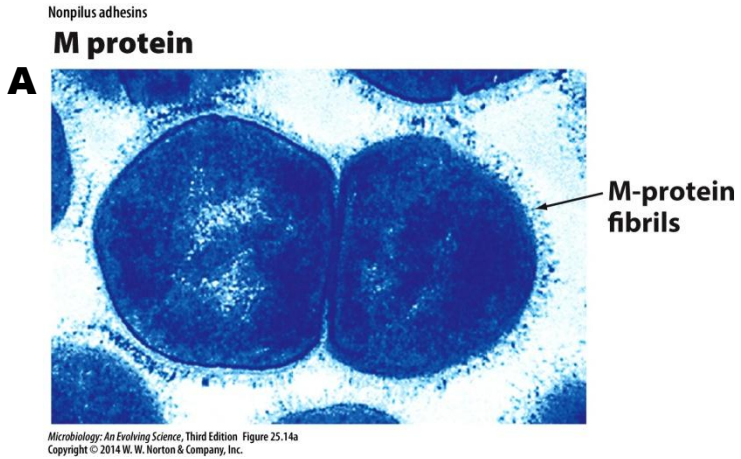
- Bacteria also carry **afimbriate adhesins** that mediate binding to host tissues.

***Streptococcus pyogenes*: M protein**

Binds to fibronectin complement regulatory factor H

***Bordetella pertussis*: Pertactin**

Binds to host cell integrin



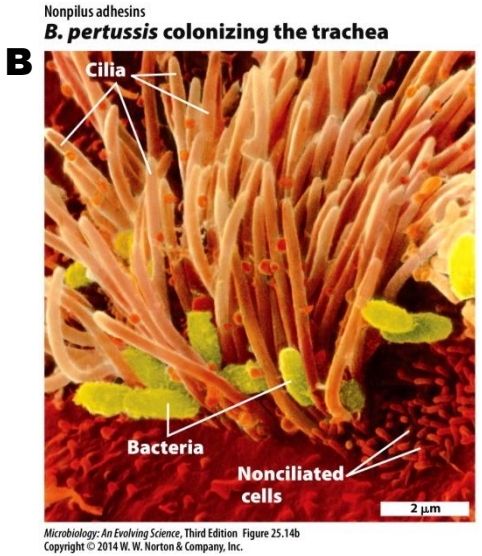


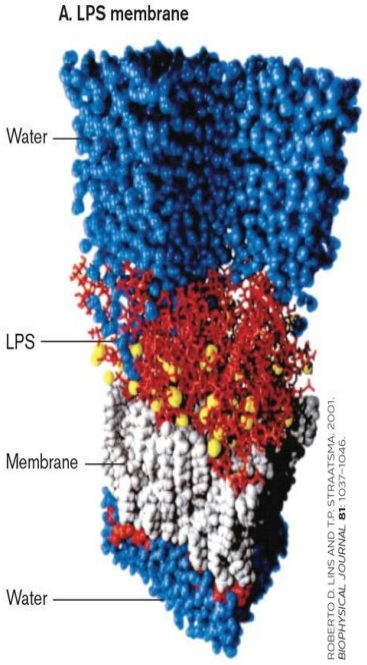
Figure 25.14

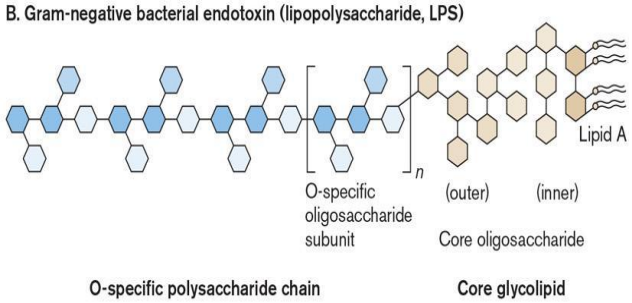
Microbial Toxins

25.3 Toxins Subvert Host Functions – 1

- Bacterial toxins can be divided into two main types.
  1. **Exotoxins**
     - Proteins produced and secreted by various types of bacteria
     - Kill host cells and unlock their nutrients
  2. **Endotoxin**
     - A part of **lipopolysaccharide (LPS)** of Gram-negative bacteria
     - **Hyperactivate host immune systems to harmful levels**

Endotoxin (LPS) Is Made Only by Gram-Negative Bacteria – 2





Made only by Gram-negative bacteria

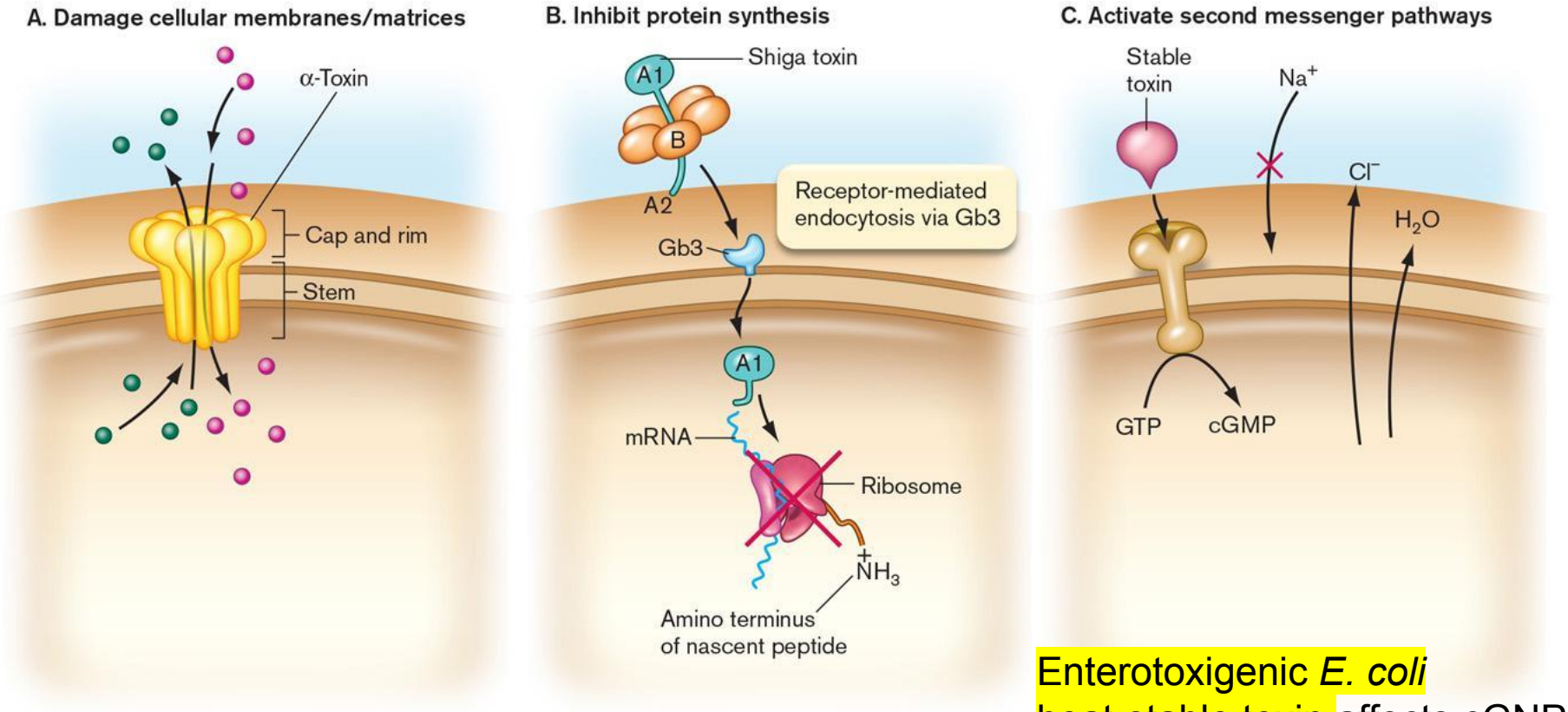
Present in lipopolysaccharide of outer membrane

- **Lipid A** released as bacteria die
  - Causes massive cytokine release from host cells
  - Can trigger fever, shock, and death

Categories of Microbial Exotoxins – 1

- **Microbial exotoxins fall into several categories based on their mechanisms of action.**
  - Plasma membrane disruption
  - Cytoskeleton alterations
  - Protein synthesis disruption
  - Cell cycle disruption
  - Signal transduction disruption
  - Cell-cell adherence
  - Vesicular traffic

Categories of Microbial Exotoxins – 2



Pore-forming toxins assemble in target membranes and cause leakage of compounds into and out of cells

Shiga toxin attaches to ganglioside Gb3, enters the cell, and cleaves 28SrRNA in eukaryotic ribosomes to stop translation

Enterotoxigenic *E. coli* heat-stable toxin affects cGNP production.The results is altered electrolyte transport: inhibition of Na+ uptake and stimulation of Cl- transport in response to the resulting electrolyte imbalance, water leaves the cell

Membrane Disruption – 1

- Some exotoxins disrupt host cell membranes by forming pores that cause leakage of cell constituents (host cell lysis).
  - **Hemolysins** lyse red blood cells (and sometimes other cells).
  - **Leukocidins** lyse white blood cells (leukocytes).
  - Some membrane-disrupting exotoxins function as both hemolysins and leukocidins.
    - Streptolysin S of *Streptococcus pyogenes*

Membrane Disruption – 2

- Two types of exotoxins disrupt host cell membranes.
  - **Pore-forming proteins** insert themselves into membranes by binding cholesterol and membrane receptors
    - **Alpha toxin** of *Staphylococcus aureus*
    - **Panton-Valentine toxin** of MRSA (see **Special Topic 25.1**)
    - **Listeriolysin** O of *Listeria monocytogenes*
  - **Phospholipase enzymes** hydrolyze phospholipids into fatty acids
    - **Phospholipase C** of *Clostridium perfringens*

Microbial Exotoxins

- Microbial exotoxins fall into nine categories based on their mechanisms of action:


Plasma membrane disruption


Cytoskeleton alterations


Protein synthesis disruption


Cell cycle disruption


Signal transduction disruption


Cell-cell adherence


Vesicle traffic


Exocytosis


Superantigens

25.5 Deploying Toxins and Effectors

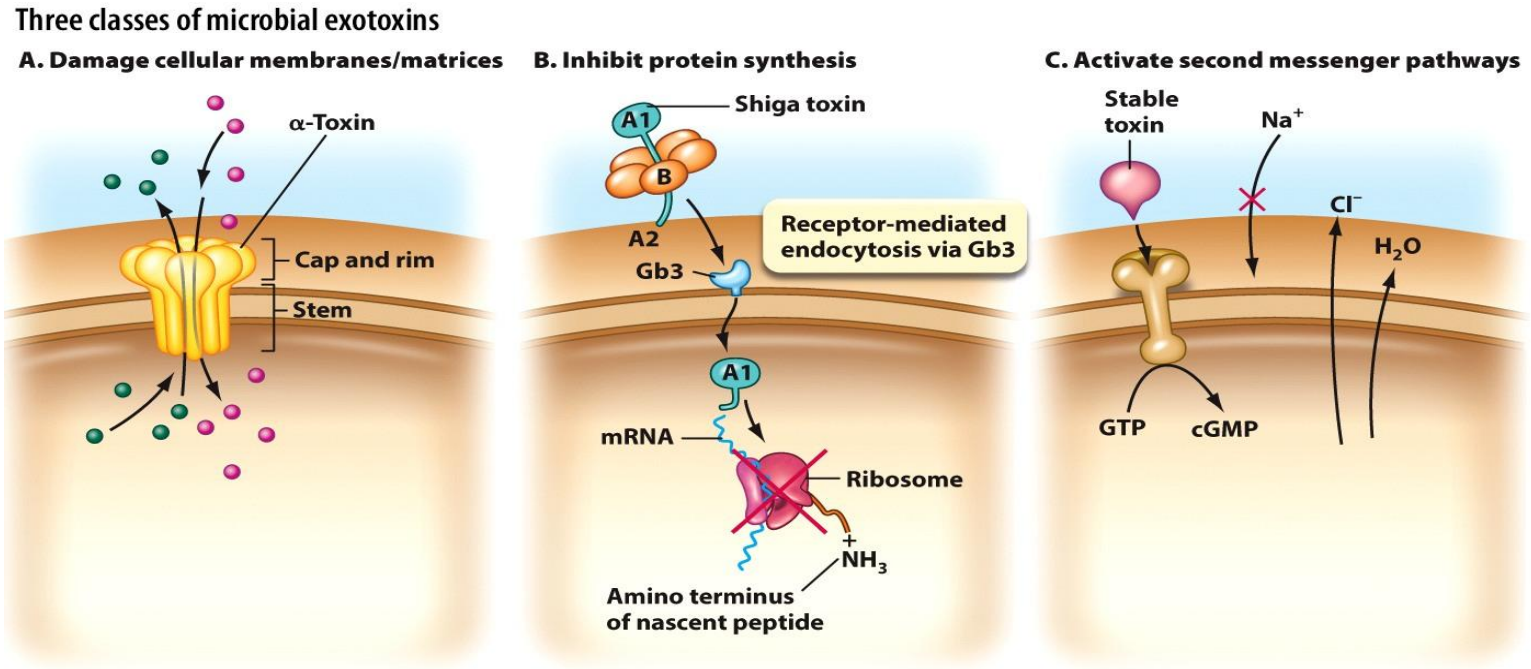
- Many protein secretory systems evolved from, and bear structural resemblance to, other cellular structures that serve fundamental cellular functions.
- The molecular processes that are evolutionarily related to secretion include:

📫 **Type IV pilus biogenesis (homologous to type II protein secretion)**

📫 **Flagellar synthesis (homologous to type III protein secretion)**

📫 **Conjugation (homologous to type IV protein secretion)**

Microbial Exotoxins



Microbiology: An Evolving Science, Third Edition Figure 25.16

Pore-forming toxins assemble in target membranes and cause leakage of compounds into and out of cells

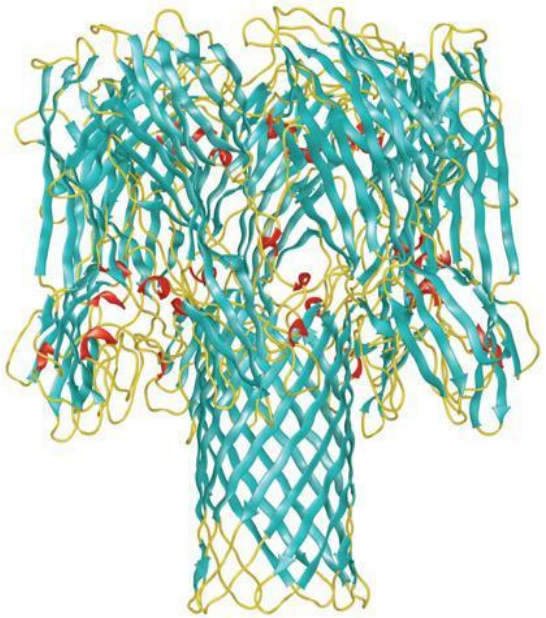
Shiga toxin attaches to ganglioside Gb3, enters the cell, and cleaves 28S rRNA in eukaryotic ribosomes to stop translation.

Enterotoxigenic *Escherichia coli* heat-stable toxin affects cGMP production. The result is altered electrolyte transport—inhibition of Na^+ uptake and stimulation of Cl^- transport. In response to the resulting electrolyte imbalance, water leaves the cell.

Figure 25.16

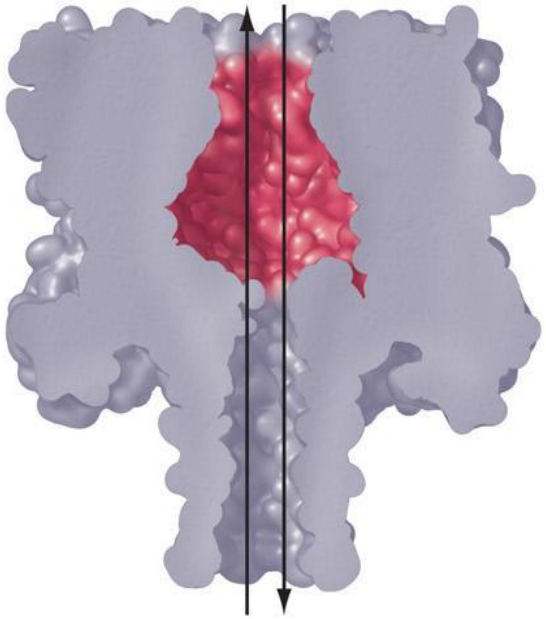
Membrane Disruption – 3

A. Alpha hemolysin



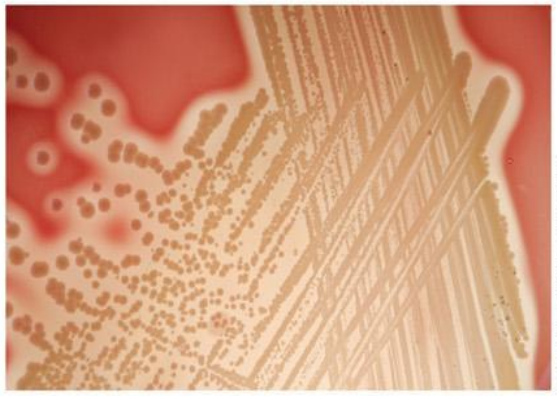
3D image of the pore complex, comprising 7 monomeric proteins.

B. Cross section of alpha hemolysin



Cross section showing the channel. Arrows indicate movement of fluids through the spore.

C. Hemolysis by *S. aureus*

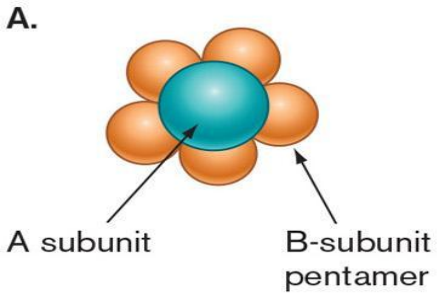


Blood agar plate inoculated with S aureus. The alpha toxin is secreted by the organism and diffuses away from the producing colony. It forms pores in the RBCs embedded in the agar, causing the cells to lyse, thus causing he clear area visible around each colony.

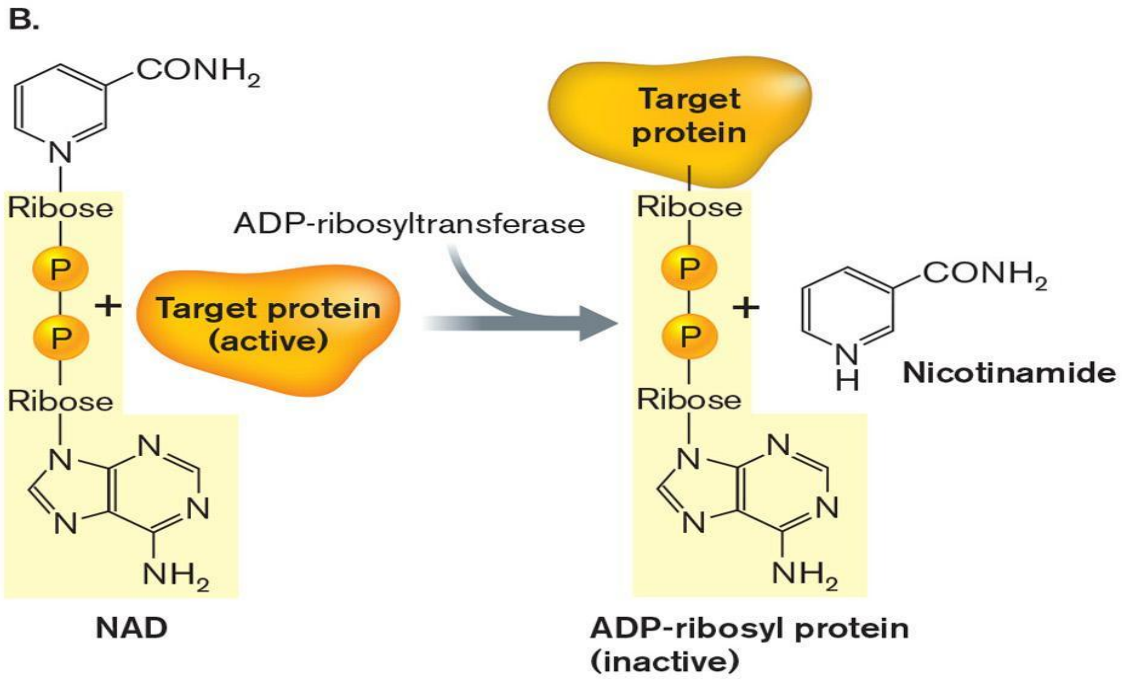
Two-Subunit AB Exotoxins – 1

- **AB exotoxins consist of two subunits, usually called A and B, that work together to disrupt host cell functions.**
  - "A" subunit: toxicity-associated factor
  - "B" subunit: binds host cell, delivers "A" subunit
  - AB5 exotoxins consist of five "B" subunits arranged in a ring with a single "A" subunit nestled in the center.
- **One major subclass of AB exotoxins includes an "A" subunit that has ADP-ribosyltransferase enzymatic activity**

Two-Subunit AB Exotoxins – 2



Typical AB toxin consists of an A subunit and a pentameric B subunit joined noncovalently



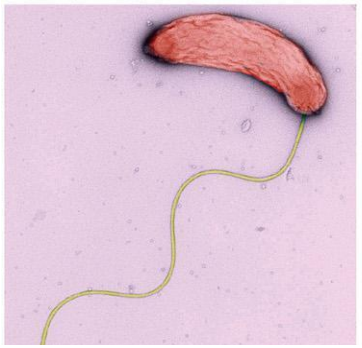
Many AB toxins are ADP-ribosyltransferase enzymes that modify proteins structure and function

Two-Subunit AB Exotoxins – 3

- **Cholera toxin** is an AB5 exotoxin made by *Vibrio cholerae* that disrupts the signaling functions of host cells.
  - The “B” subunits bind to intestinal cell membranes and trigger endocytosis of cholera toxin complex.
  - The “A” subunit ADP-ribosylates a host cell target that leads to a sharp increase in cAMP levels.
  - cAMP activates ion transporters that ultimately cause water to leave the cell, leading to watery stools (diarrhea).

Two-Subunit AB Exotoxins – 4

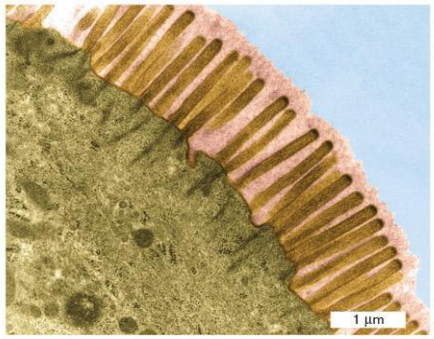
A. *Vibrio cholerae*



AL MURTI/SCIENCE SOURCE

Vibrio cholerae (SEM). Note the slight curve of the cell and the presence of a single polar flagellum

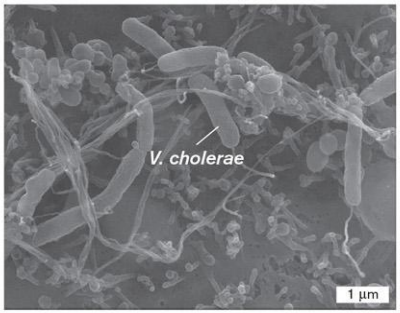
B. Brush border of intestine



DENNIS KUNKEL MICROSCOPY/SCIENCE SOURCE

Brush border of intestine (TEM) V. cholerae binds to the fingerlike villi on the apical surface

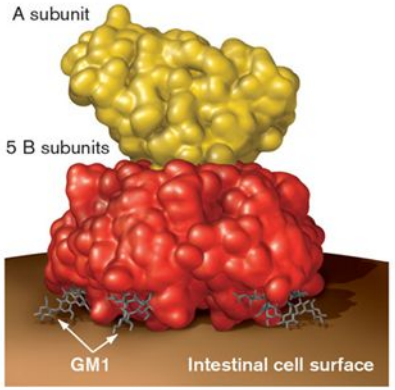
C. *V. cholerae* attachment



KREBS, S. AND R. TAYLOR. 2011. *JOURNAL OF BACTERIOLOGY* **193**: 5260–5270. © 2011 AMERICAN SOCIETY OF MICROBIOLOGY.

V. cholerae, binding to the surface of a host cell (SEM). Note that V. cholerae does not invade the host cells

D. Cholera toxin



3D structure of cholera toxin, binding ganglioside GM1 on the intestinal cell surface

Anthrax Toxin

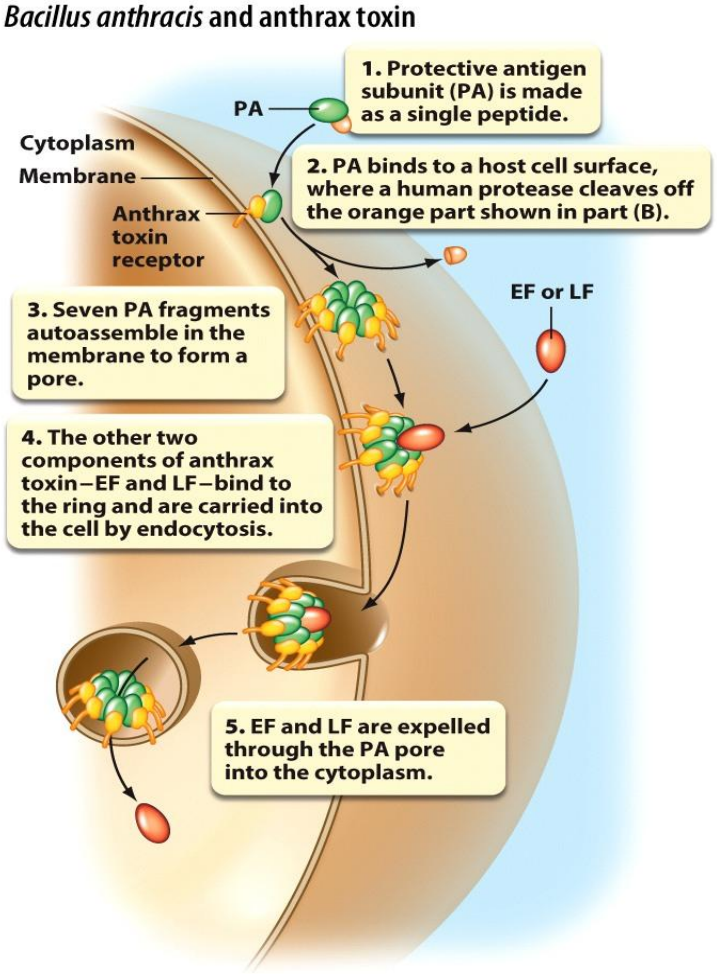
- Made by *Bacillus anthracis*
- Two active toxins:

📫 **Edema factor (EF) raises cAMP levels.**

📫 Causes fluid secretion, tissue swelling

📫 **Lethal factor (LF) cleaves protein kinases**

📫 Blocks immune system from attacking

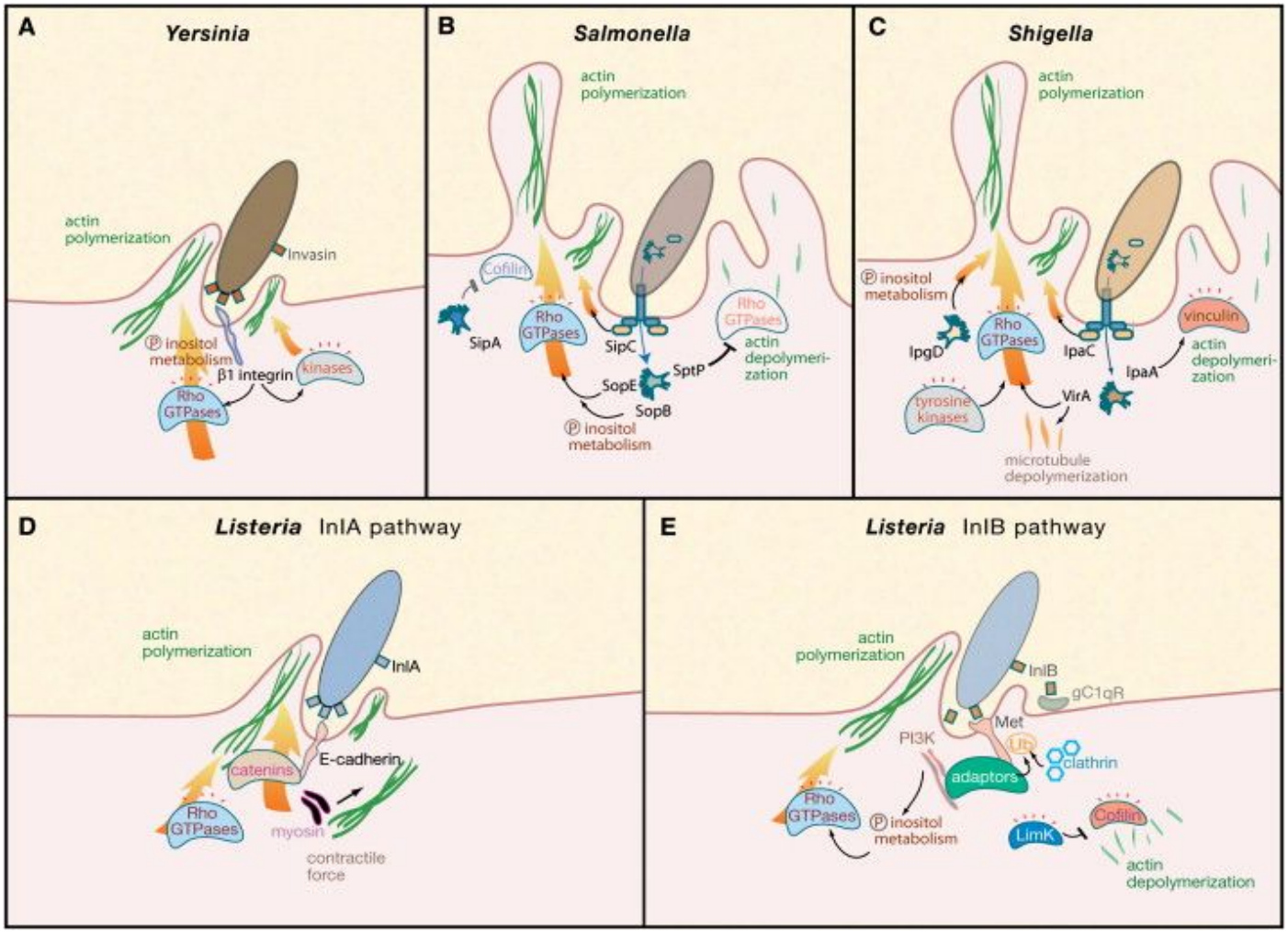


Microbiology: An Evolving Science, Third Edition Figure 25.21c

25.4 Deploying Toxins and Effectors – 1

- Many protein secretory systems evolved from, and bear structural resemblance to, other cell structures that serve fundamental cell functions.
- We will look at several secretion systems and the molecular processes with which they share an evolutionary history.
  - **Type II secretion**: homologous to type IV pilus biogenesis
  - **Type III secretion**: homologous to flagellar synthesis
  - **Type IV secretion**: homologous to conjugation

Secretion Systems for Bacterial Toxins



Type III Secretion Is an Injection Machine – 1

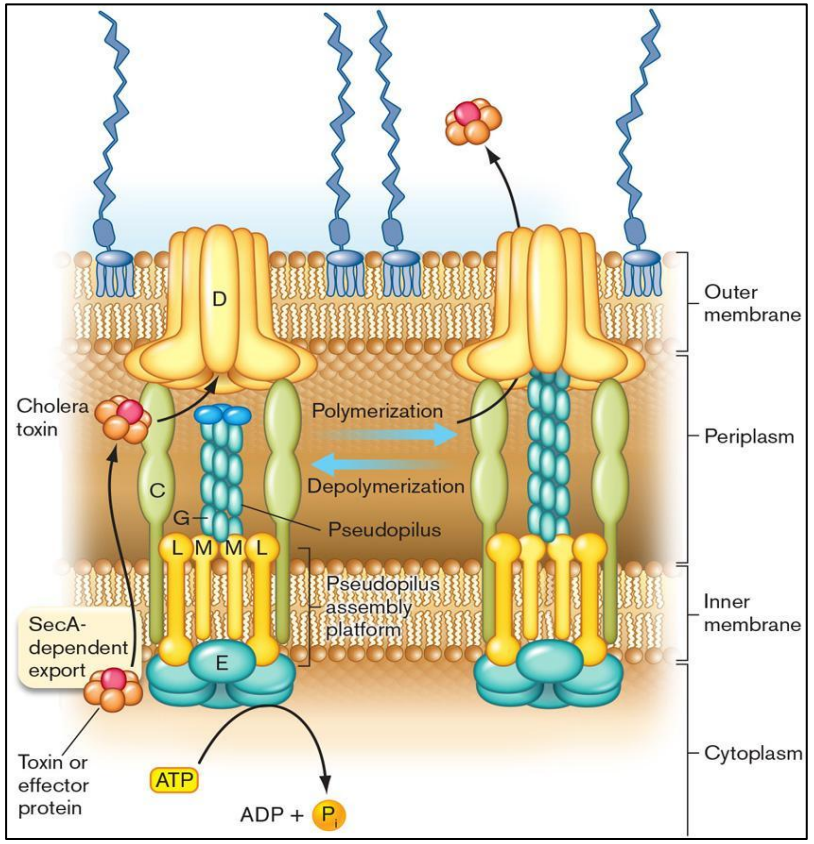
- The **type III secretion system** (**T3SS**) is a reengineered flagellar synthesis mechanism that uses a **molecular syringe to inject proteins** from the bacterial cytoplasm directly into the host cell.
  - Secretion is normally triggered by cell-cell contact between host and bacterium.
  - T3SS genes usually are located within pathogenicity islands inherited via horizontal gene transfer.
  - Found in *Salmonella*, *Yersinia*, *Shigella,* and *Escherichia* species.

Type II Secretion Resembles Type IV Pilus Assembly

- The **type II secretion system** (**T2SS**) is a modification of the same system used for type I pilus biogenesis.

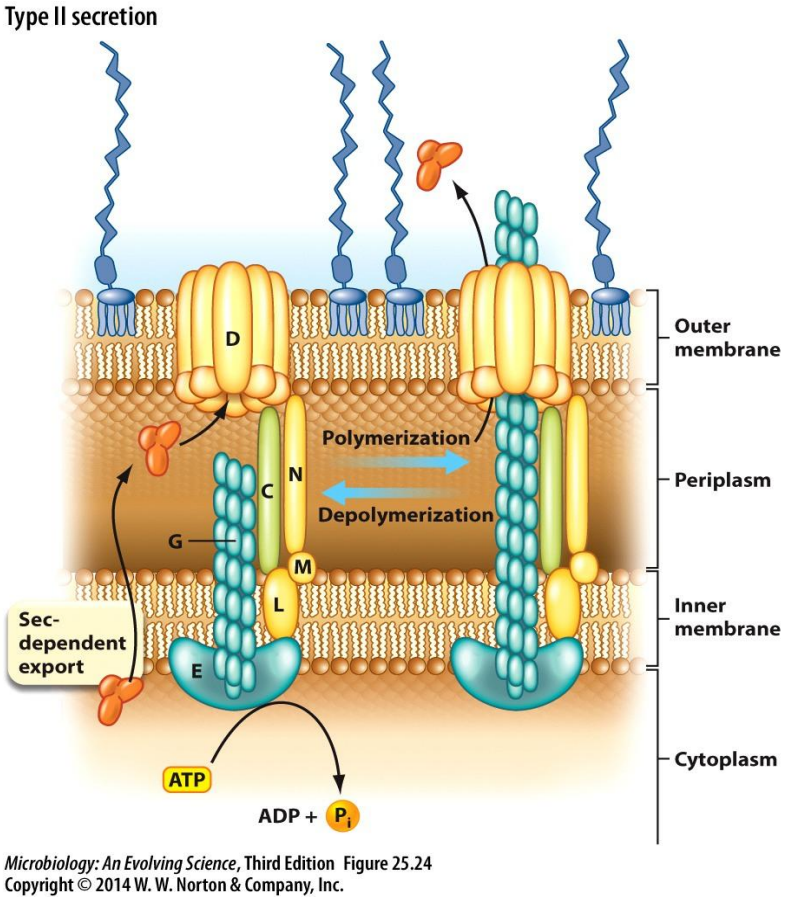
  Secretion structures extend and retract, just like pili.

  Proteins to be secreted first enter the periplasm, then they get folded and secreted via an outer membrane pore.



Type II Secretion

- Similar to Type IV pilus
  - Modified for secreting proteins
- Can extend and retract
- Proteins to be secreted first make their way to the periplasm
  - Are then folded before secretion



Microbiology: An Evolving Science, Third Edition Figure 25.24

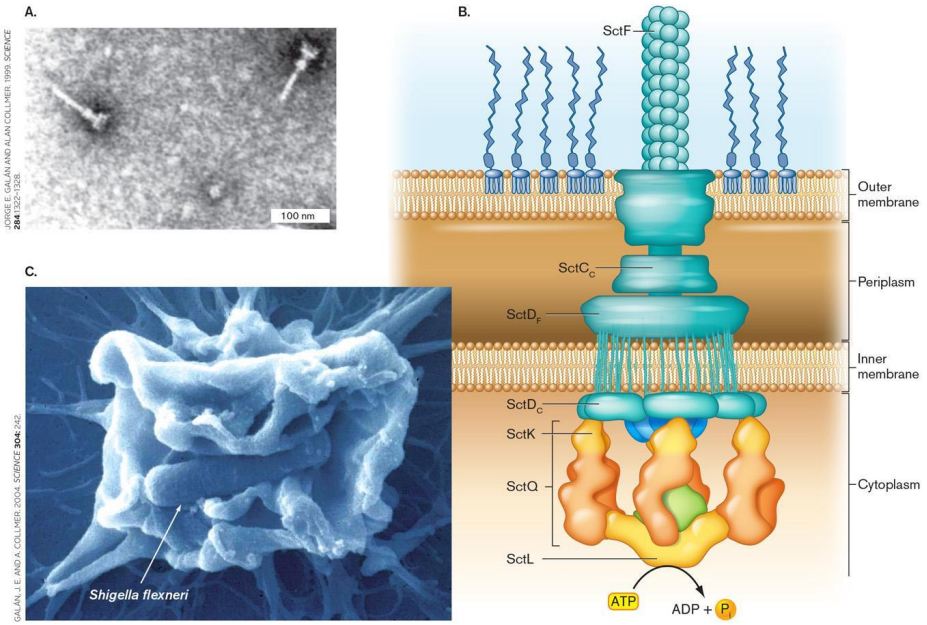
Figure 25.24

Type III Secretion Is an Injection Machine – 1

- The **type III secretion system** (**T3SS**) is a reengineered flagellar synthesis mechanism that uses a molecular syringe to inject proteins from the bacterial cytoplasm directly into the host cell.
  - Secretion is normally triggered by cell-cell contact between host and bacterium.
  - T3SS genes usually are located within pathogenicity islands inherited via horizontal gene transfer.
  - Found in *Salmonella*, *Yersinia*, *Shigella,* and *Escherichia* species.

Type III Secretion Is an Injection Machine – 2

The type III secretion complex from Salmonella enterica serovar Typhimurium type III injectisome. Unlike other secretion systems, the type III mechanism injects proteins directly from the bacterial cytoplasm into the host cytoplasm. The proteins in these systems are related to flagellar assembly proteins.



A. Purified needle complexes (TEM) from *S. Typhimurium*.

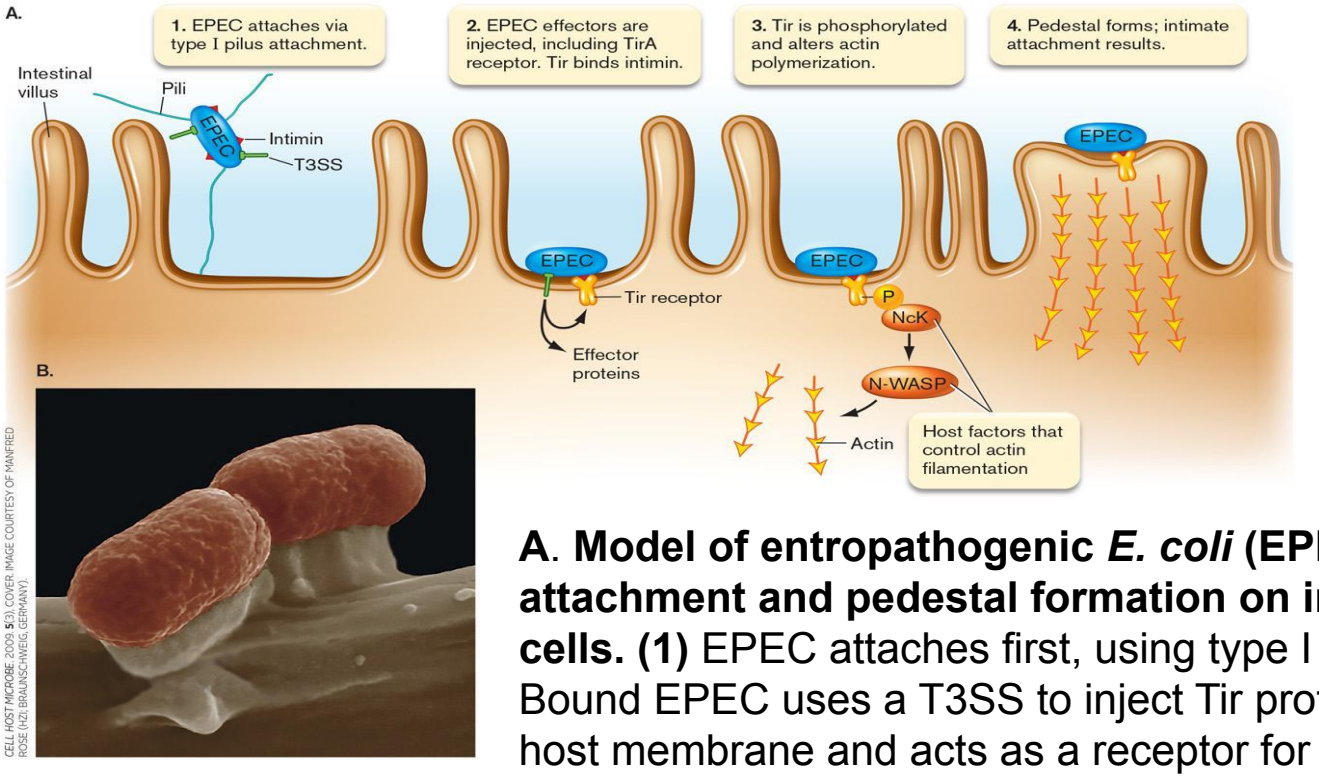
B. Schematic representation of the *S. Typhimurium* needle complex and its putative components

C. *Shigella* invades a host cell ruffle produced as a result of its type III secretion system. *Shigella flexneri* (approx. 2 μm) entering the HeLa cell ruffle (SEM) formed by host actin rearrangements. (Hela cells are immortal cancer cell line). The ruffle engulfs the bacterium and eventually disassembles, internalizing the bacterium.

Type III Secretion Is an Injection Machine – 4

- Some microbes do not rely solely on the natural array of host receptors for attachment.
- Instead, these bacterial pathogens use a T3SS to insert their own receptors into target cells.
  - Bacteria inject Tir proteins into the host cell. These proteins act as receptors for the outer membrane protein **intimin**. Intimin binds to Tir to establish a strong attachment.
  - Used by enteropathogenic *E. coli* (EPEC) and enterohemor-rhagic *E. coli* (EHEC).

Type III Secretion Is an Injection Machine – 5

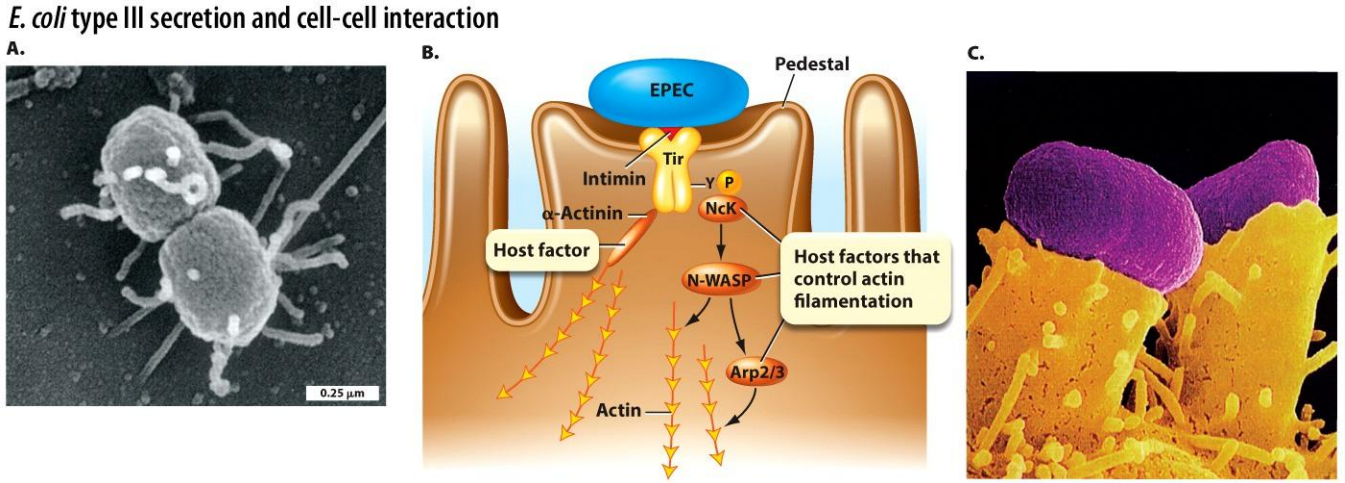


A. Model of entropathogenic *E. coli* (EPEC) attachment and pedestal formation on intestinal cells. (1) EPEC attaches first, using type I pili. **(2)** Bound EPEC uses a T3SS to inject Tir protein into the host membrane and acts as a receptor for the EPEC surface intimin. **(3)** Tir also communicates through phosphorylation with other host factors that control actin filamentation and cytoskeleton formation. Actin polymerization raises the host membrane to produce a pedestal upon which EPEC sits. **B. Pedestal formation (colorized SEM).**

Type III Secretion System

Some microbes do not rely solely on the natural array of host receptors for attachment.

- Instead, these bacterial pathogens use a type III secretion system (T3SS) to insert their own receptors into target cells.
  - One such group of enterprising pathogens is enteropathogenic *Escherichia coli* (EPEC).

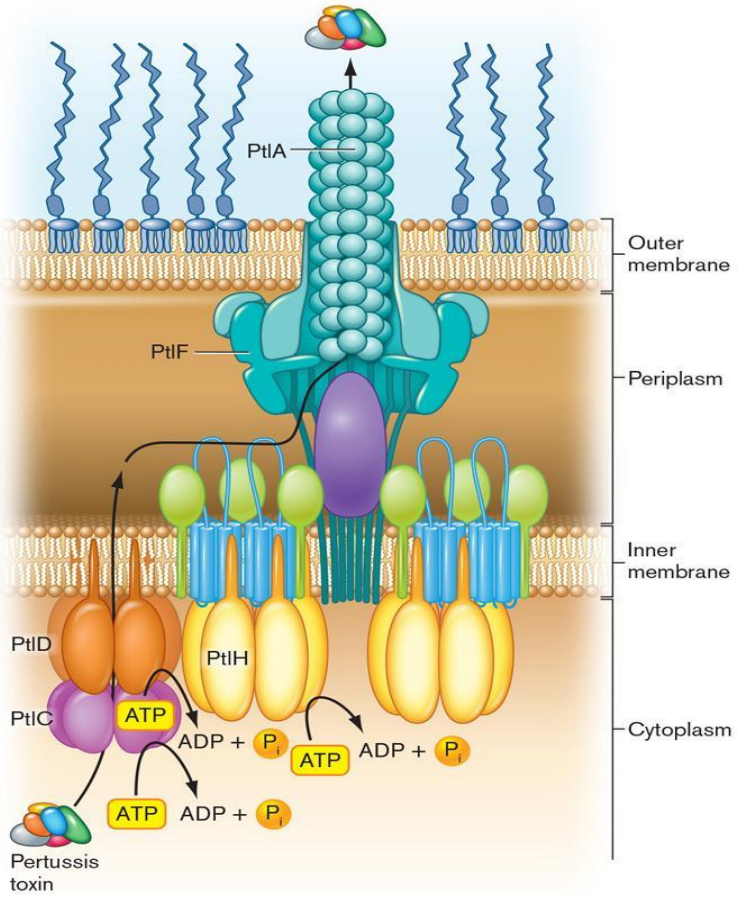


Microbiology: An Evolving Science, Third Edition Figure 25.27

Figure 25.27

Type IV Secretion Resembles Conjugation Systems

- The **type IV secretion system** (**T4SS**) is an evolutionary modification of a conjugation pilus that secretes proteins only, or proteins plus DNA.
  - The T4SS allows bacterial pathogens to secrete proteins directly from their cytoplasms or from their periplasms.
  - Found in *Agrobacterium tumefaciens* and *Bordetella pertussis*



Type III Secretion

- Use a molecular syringe to inject proteins from the bacterial cytoplasm directly into host cell
  - Similar to flagellum
- Genes usually located on pathogenicity island
- Found in *Salmonella*, *Yersinia*, and *Shigella*

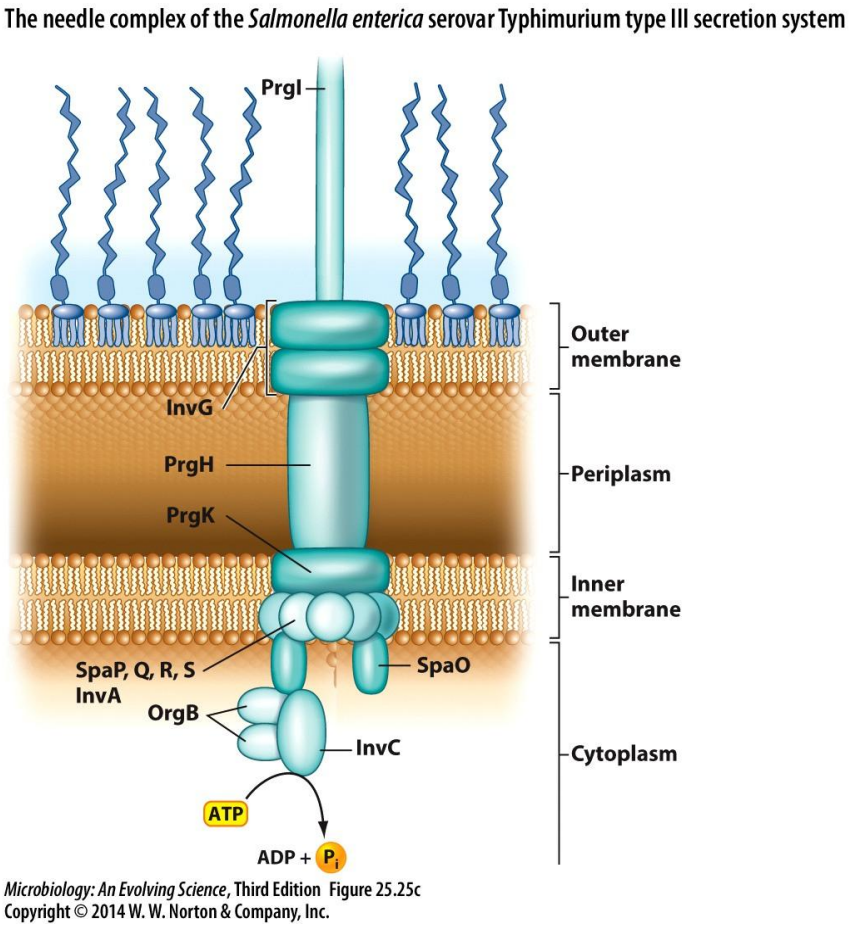


Figure 25.25

Type IV Secretion

- Similar to conjugation pilus
  - Modified to secrete proteins only, or proteins plus DNA
- Can secrete proteins from cytoplasm or periplasm
- Found in *Agrobacterium tumefaciens* and *Bordetella pertussis*

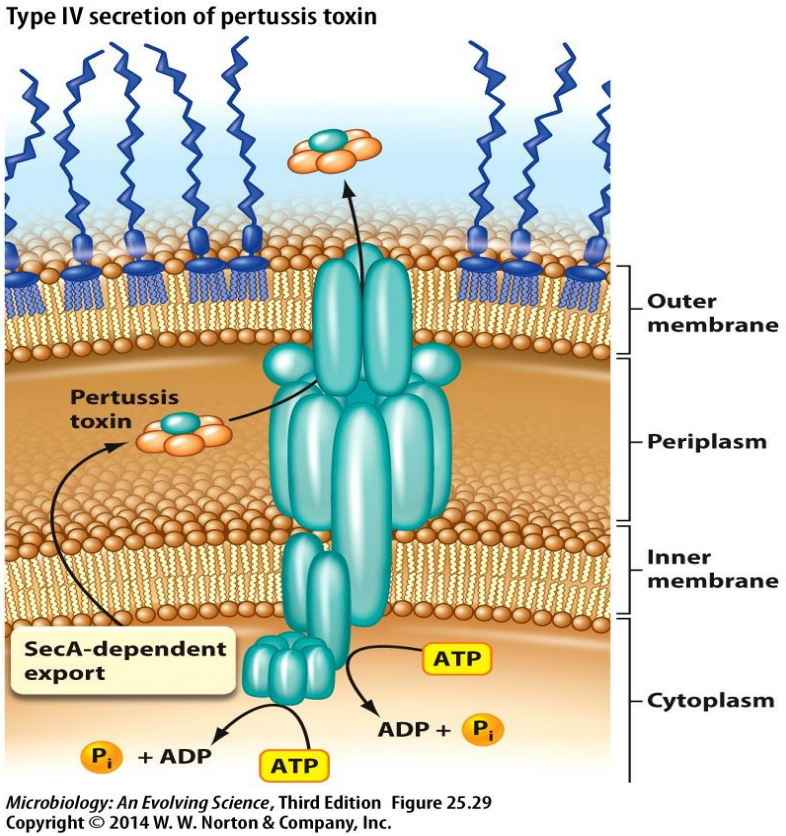


Figure 25.29

Where Am I?

- Various sensing systems act in concert to recognize any specific environmental niche.
  - **Two-component signal transduction systems**
    - Detect magnesium concentration, pH
    - Both low in host cell vacuole
    - Detects exotoxins made by other cells
    - Delays toxin synthesis until many bacteria present
    - Possible pathway for preventing pathogen growth?

Intracellular Pathogens

- **Cell ingests pathogens in phagosome**.
  - Some pathogens use hemolysin to break out.
  - ***Shigella dysenteriae*, *Listeria monocytogenes***

- **Phagosome fuses with acidic lysosome.**

  - Some pathogens secrete proteins to prevent fusion.
  - ***Salmonella*, *Chlamydia*, *Mycobacterium*, *Legionella***

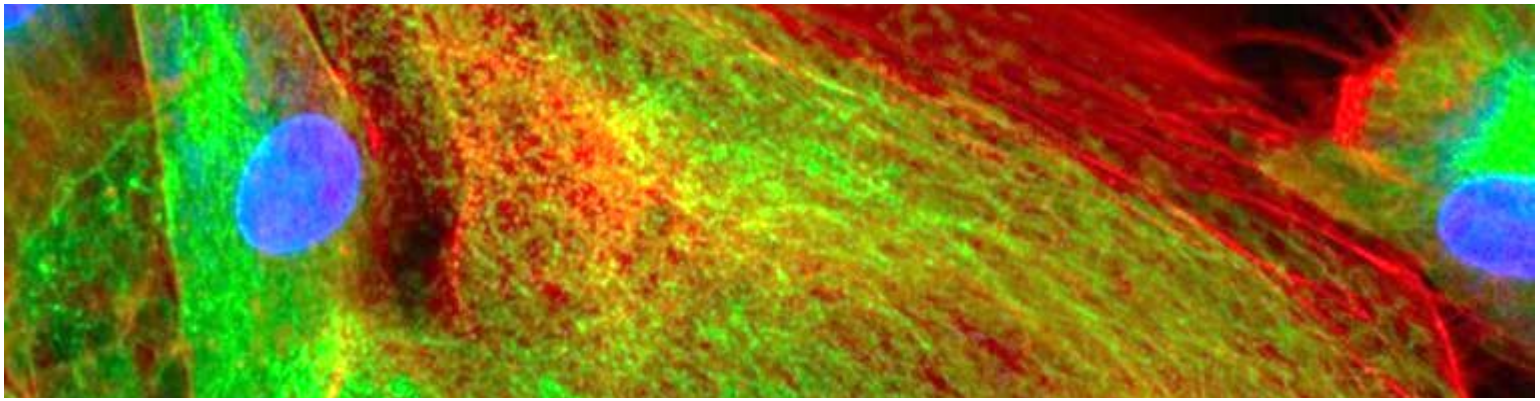
  - Some pathogens mature in acidic environment.
  - ***Coxiella burnetii:* causes Q fever**

Chapter Summary

- **A pathogen is any microbial agent of disease**.
  - Primary pathogens cause disease in normal hosts.
  - Opportunistic pathogens need immunocompromised host.
- **Infection cycles can be direct or indirect.**
- **Virulence factors may be encoded by gene clusters on pathogenicity islands**.
  - Acquired by horizontal transmission
- **Adhesins mediate bacterial attachment to host cells.**
  - Type I pili: static attachment
  - Type IV pili: continually assembled and disassembled
  - Afrimbrial adhesins: pertactin and M protein

MICROBIAL DISEASES

CHAPTER 26



Chapter Overview

- Skin and soft-tissue infections
- Respiratory tract infections
- Gastrointestinal tract infections
- Genitourinary tract infections
- Cardiovascular system infections
- Central nervous system infections
- Systemic infections

Introduction

- Microbial diseases are with us daily and are major contributors to global mortality.
- The need for investigations into microbial disease mechanisms and the body's ability to combat infectious agents have been heightened by:
  - **The emergence of new pathogens, increasing drug resistance, and threats of bioterrorism**
- In addition, effective diagnostic algorithms are needed to quickly identify infectious diseases and prevent their spread.

Characterizing and Diagnosing Microbial Diseases

- Microbial diseases may be classified based on several criteria:
  - **By organism**
  - **By organ system (used in this chapter)**
  - **By portal of entry**
- Each approach has clear benefits and pitfalls.
- Pathogens can be divided into four main groups based on their route of infection:
  - **Food-borne, airborne, blood-borne, and sexually transmitted**

Zoonotic Diseases

- ❑ *Francisella tularensis*
- Diarrheal disease of the travellers
- Tularemia
- ❑ *Coxiella burnetii*
- Q fever
-

- Many infectious diseases display similar symptoms, making diagnosis difficult**.**
- Thus, **knowledge of a patient's history** is vital:
  - Travel information: diarrheal diseases
  - Hobbies: hunters and tularemia (***Francisella tularensis*****)**
  - Occupation: farmers and Q fever (*Coxiella burnetii*)
- Both tularemia and Q fever are **zoonotic diseases**.

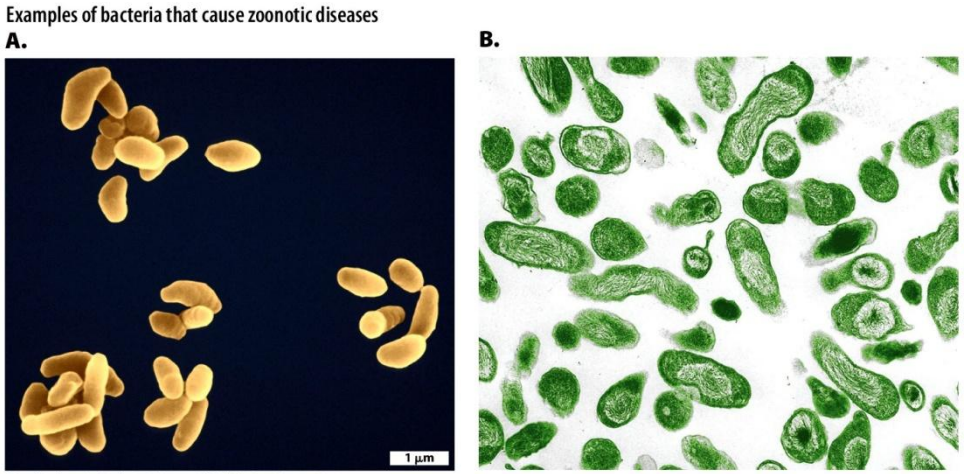


Figure 26.1

Skin and Soft-Tissue Infections

- ❑ *Staphylococcus aureus*
- ❑ *Streptococcus pyogenes*, also called Group A Streptococcus (GAS)
  - Lancefield groups
  - Lancefield grouping is a serological method for classifying streptococci into one of 20 groups (designated by a letter) based on the presence of polysaccharide and teichoic acid antigens in the bacterial

Lancefield groups

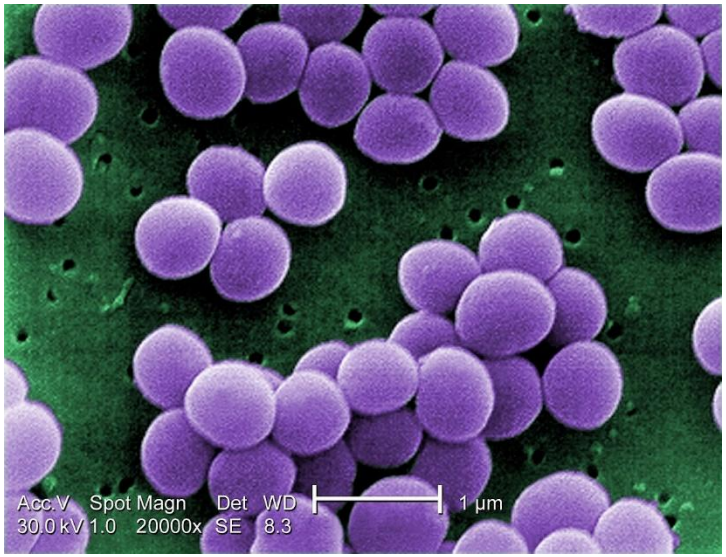
- Lancefield grouping is a serological method for classifying streptococci into one of 20 groups (designated by a letter) based on the presence of polysaccharide and teichoic acid antigens in the bacterial cell wall (Lancefield 1933).
- The technique is now performed using commercial latex agglutination test kits, which allow rapid detection of clinically important streptococcal groups.
- Some streptococci, for example *S. pneumoniae*, have not been assigned to a group because their antigen extracts fail to react with group antisera. With the exception of *S. pneumoniae* all the equine streptococci belong to Lancefield group C.

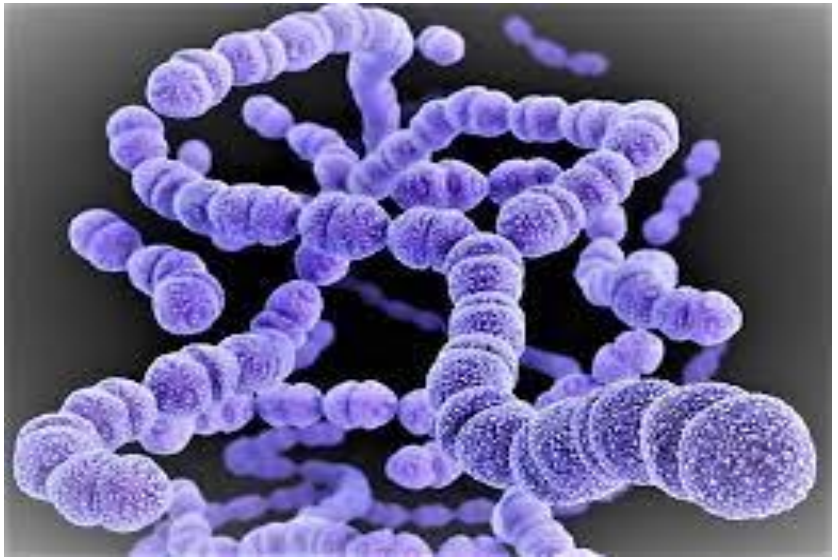
Skin and soft-tissue infections

Beta hemolytic

- **Skin and soft-tissue infections**

Staphylococcus aureus ***Streptococcus pyogenes***

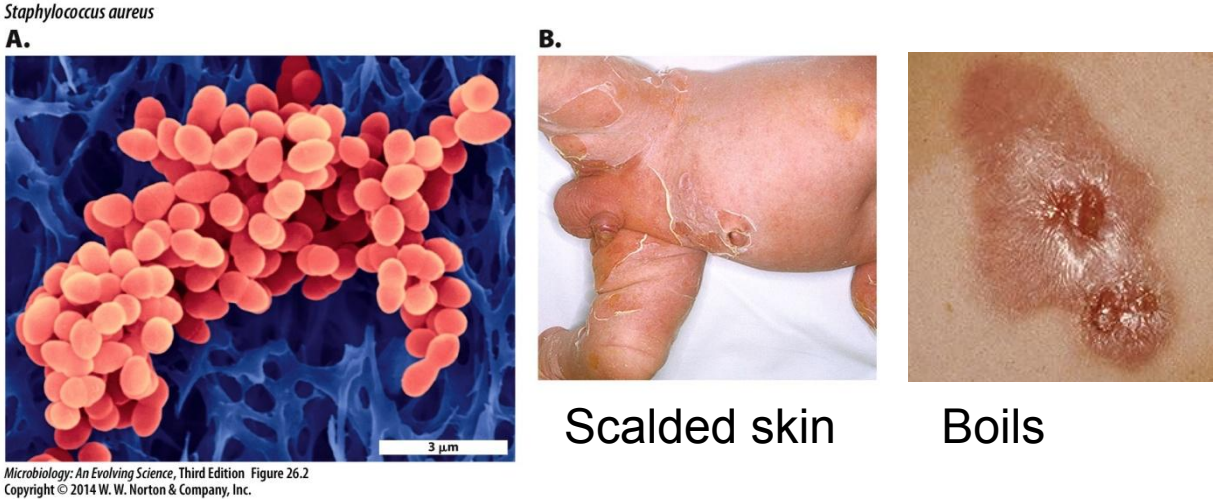




26.2 Skin and Soft-Tissue Infections

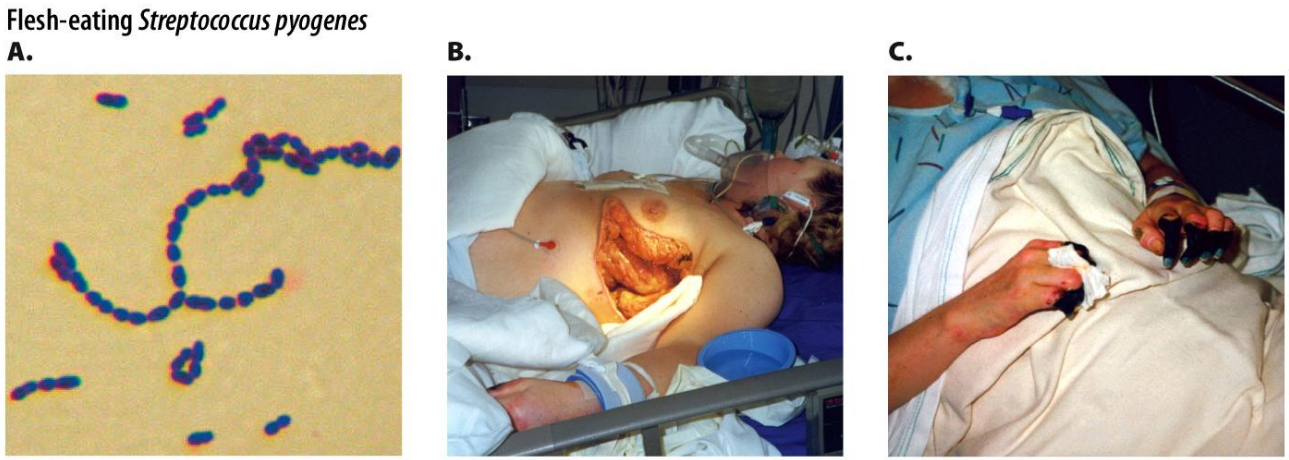
- ***Staphylococcus aureus***
  - Boils: walled off from body with fibrin
  - Can produce toxic shock **superantigen**
  - **MRSA: methicillin-resistant *S. aureus***
    - **Major cause of nosocomial infections (in hospitals)**
  - Some strains make exfoliative toxin (scalded skin syndrome)

Figure 26.2



- ***Streptococcus pyogenes***
  - Best known for causing sore throats and immunological sequelae, such as rheumatic fever
  - Also **necrotizing fasciitis** ("flesh-eating" disease)
    - And a less aggressive but similar skin infection called **cellulitis**
  - Many virulence factors are encoded by prophages

Figure 26.3



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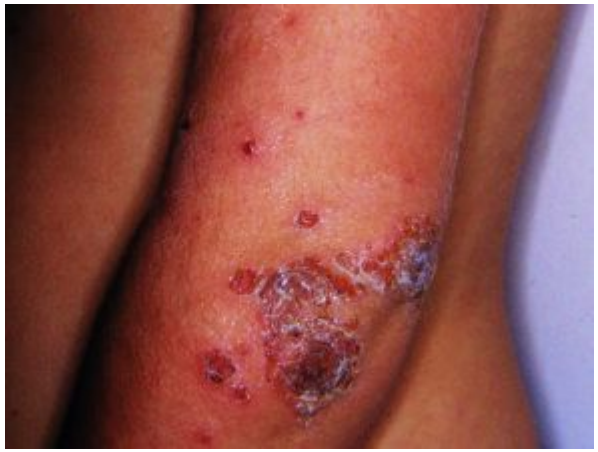
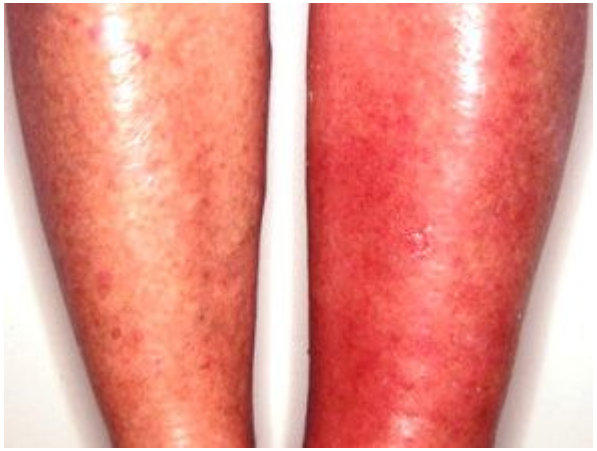
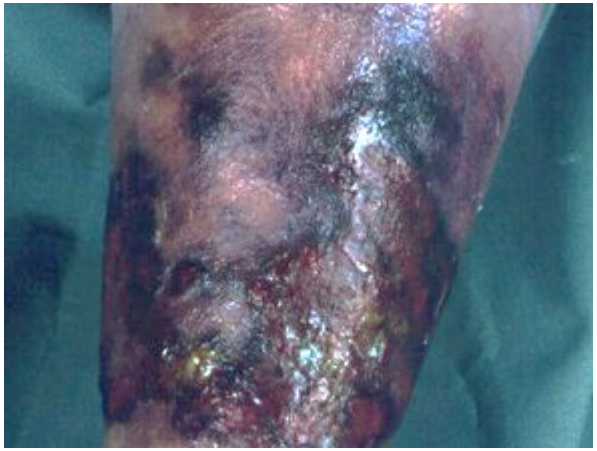
S. pyogenes



Impetigo is a common and highly contagious skin infection that mainly affects infants and children. Impetigo usually appears as red sores on the face, especially around a child's nose and mouth, and on hands and feet. The sores burst and develop honey-colored crusts.



Cellulitis is a common, potentially serious bacterial skin infection. The affected skin appears swollen and red and is typically painful and warm to the touch.
Cellulitis usually affects the skin on the lower legs, but it can occur in the face, arms and other areas.



Necrotizing fasciitis can spread so rapidly that patients often must get surgery done very quickly. Antibiotics are given through a needle into a vein (IV antibiotics) to try to stop the infection. When the bacteria have killed too much tissue and reduced blood flow, multiple surgeries are necessary.

Respiratory Tract Infection

- *Bordetella pertussis*
- *Steptococcus pneumoniae*
- *Mycobacterium tuberculosis*
- *Pneumocystis Jirovecci*

26.3 Respiratory Tract Infections

- The **mucociliary escalator** is primary respiratory defense.
  - ***Bordetella pertussis*** (cause of whooping cough) inhibits it by binding to lung cilia.
  - https://www.youtube.com/watch?v=HMdrhwEnY6M Introduction to Mucociliary Transport Video Microscopy

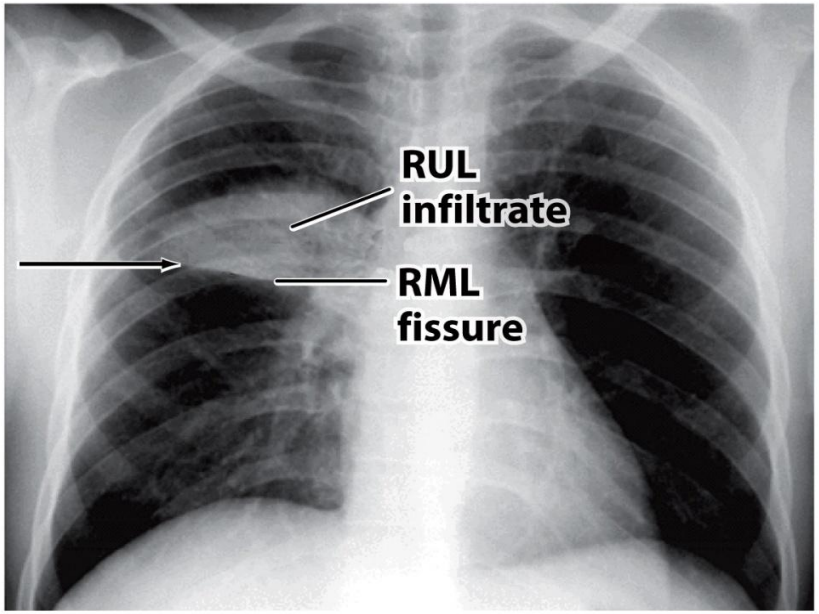
- **Pneumonia is a disease, not a specific infection**.
  - Caused by many different microbes
  - ***Streptococcus pneumoniae*** is the main bacterium:
    - Has capsule that prevents phagocytosis
    - Can invade the bloodstream (bacteremia) and the covering of the brain (meningitis)

Pneumonia caused by *S. pneumoniae*

Pneumonia caused by *Streptococcus pneumoniae*

Lobar pneumonia

A



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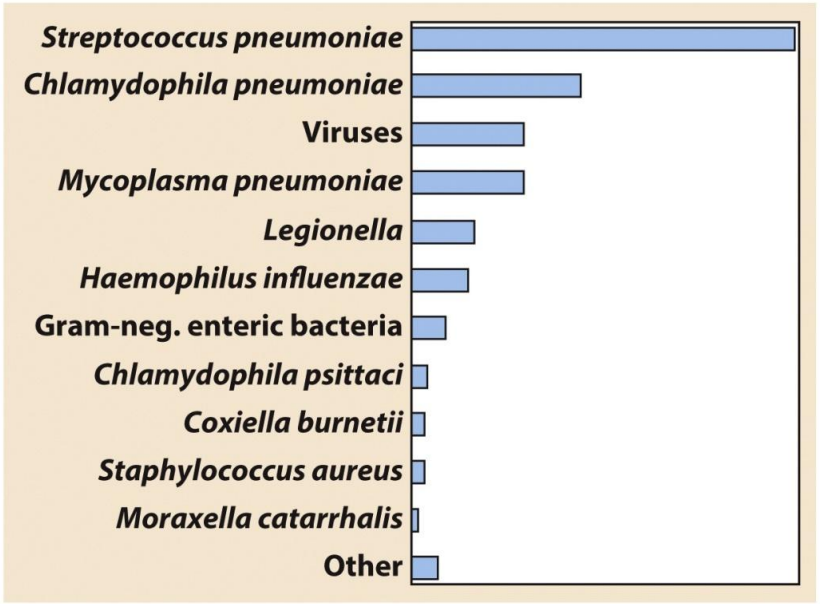
Pneumonia caused by *Streptococcus pneumoniae*

Relative incidence of pneumonia

C



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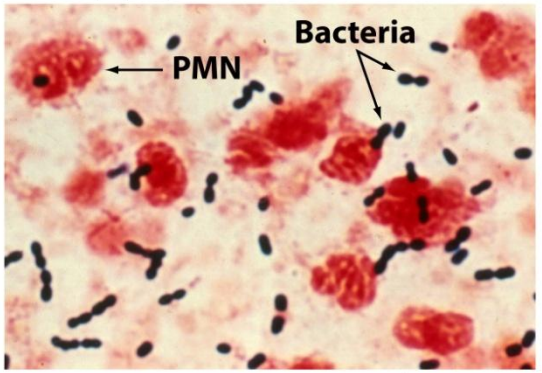
Pneumonia caused by *Streptococcus pneumoniae*

B



Streptococcus pneumoniae

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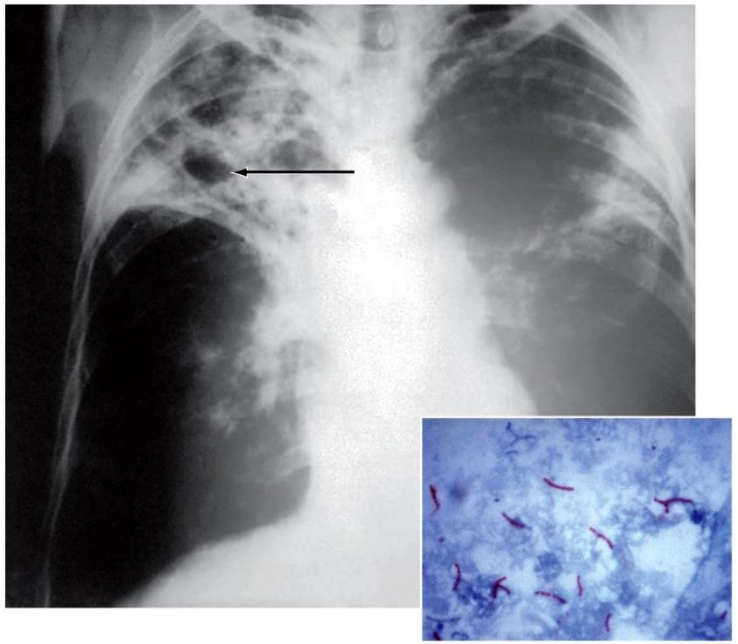
Figure 26.5

- ***Mycobacterium tuberculosis***

- An acid-fast bacillus
- An ancient and reemerging pathogen
- Forms calcified tubercles in the lung
- Can disseminate through the bloodstream



Microbiology: An Evolving Science, Third Edition Figure 26.7

Figure 26.7

- Has high mortality rate due to multidrug-resistant strains and high susceptibility of HIV patients

Gastrointestinal Tract Infection

- *Salmonella enterica* serovar Thyphimurium
- *Campylobacter enteritis*
- *Campilobacter jejuni*
- *Shigella dysenteriae*
- *Vibrio cholerae*
- *Helicbacter pylori*
- Enteroinvasive *E. coli* (EIEC)
- *Other E. coli* remain outside epithelial cells:
  - EHEC, ETEC, EAEC, and E coli 057:H7

Gastrointestinal Tract Infections

- The main symptoms of **gastroenteritis are watery diarrhea and vomiting.** The most frequent causes of self-limiting diarrheal disease include
  - ***Salmonella enterica*** serovar Typhimurium and
  - Bacteria of the genus Campylobacter, such as ***C. enteritis*** *is common cause of intestinal infection*
- A more severe form of gastroenteritis is called **dysentery** (diarrhea with passage of blood or mucus)
  - Bacterial dysentery: ***Shigella* species, including *S. dysenteriae***

- **Remarkably, *Staphylococcus aureus* causes gastrointestinal disease without ever producing infection.**
- Some strains can secrete **enterotoxins** into tainted foods such as pies, turkey dressing, or potato salad, causing **food poisoning**
- The most important treatment for diarrhea is **rehydration therapy**
- **Antibiotics are often inappropriate when treating diarrhea**
  - **Ineffective against viruses; bacterial gastroenteritis resolves spontaneously**
  - **In some cases, antibiotic treatment can actually *trigger* gastrointestinal disease.**
    - Example: clindamycin can kill competing bacteria, thus allowing ***Clostridium difficile*** to thrive
      - Causes pseudomembranous enterocolitis

- **Pseudomembranous colitis** refers to swelling or inflammation of the large intestine (colon) due to an overgrowth of *Clostridium difficile* (C. difficile) bacteria. This infection is a common cause of diarrhea after antibiotic use.

- **Enterobacterial toxin-producing strains**

- **Inject toxin via <u>type III secretion</u>**
- **Bacteria invade epithelial mucosa**.
  - ***Salmonella***
  - ***Shigella***, enteroinvasive ***Escherichia coli*** (EIEC)
    - Produce Shiga toxin
    - Blocks host protein synthesis, damages endothelia
      - Capillary damage, loss of blood, clots
- **Bacteria remaining outside epithelial cells**
  - ***E. coli*: EHEC (O157:H7), ETEC, EAEC**
  - **Entero-hemorrhagic, -toxigenic, -aggrega**tive
  - O157 = serotype of LPS; H7 = serotype of flagella

- **Other bacterial agents of gastrointestinal disease**
  - ***Campylobacter jejuni***
    - Most frequent bacterial cause of diarrhea
  - ***Vibrio cholerae***: cholera
  - ***Helicobacter pylori***: gastric ulcers
    - Secretes urease: urea $\rightarrow NH_4^+$
    - Neutralizes stomach acid
    - Burrows into protective mucous layer
    - Associated with gastric cancer

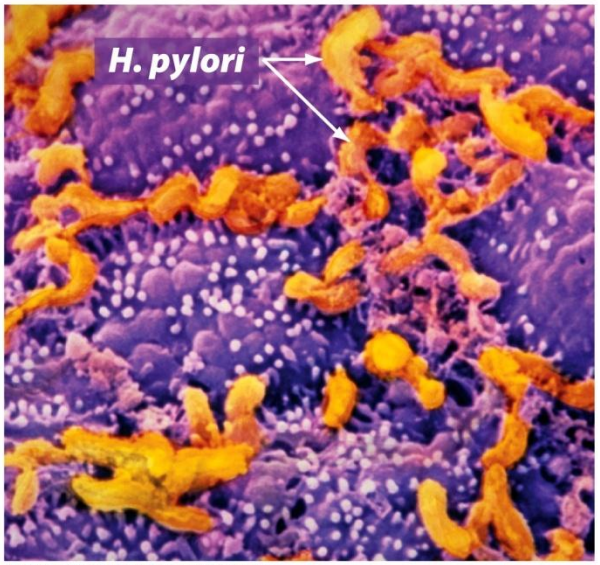


Figure 26.8

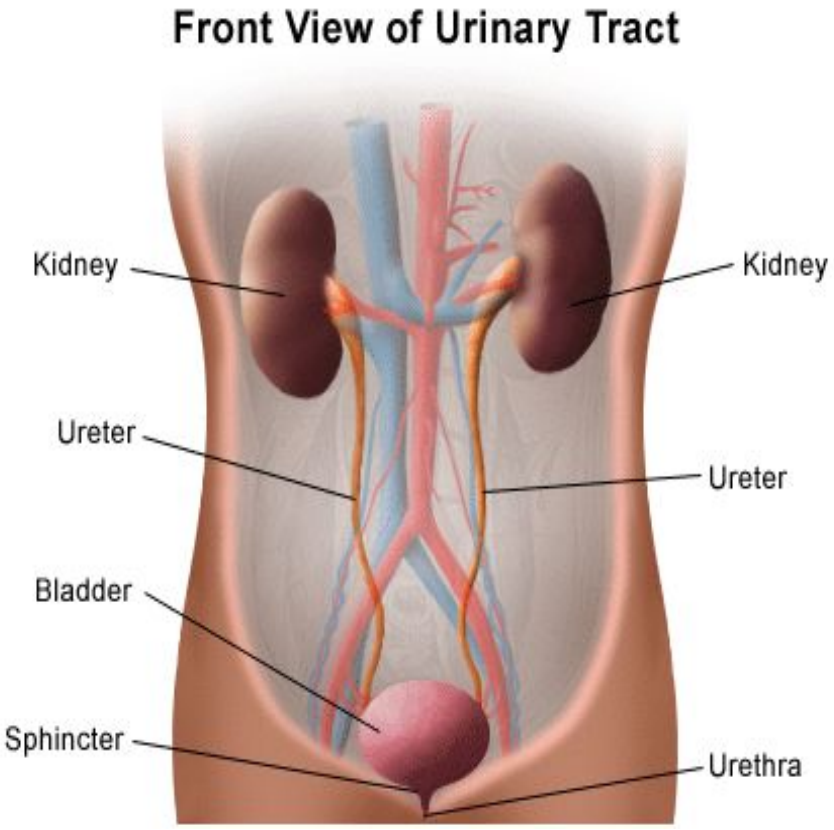
Genitourinary tract infections

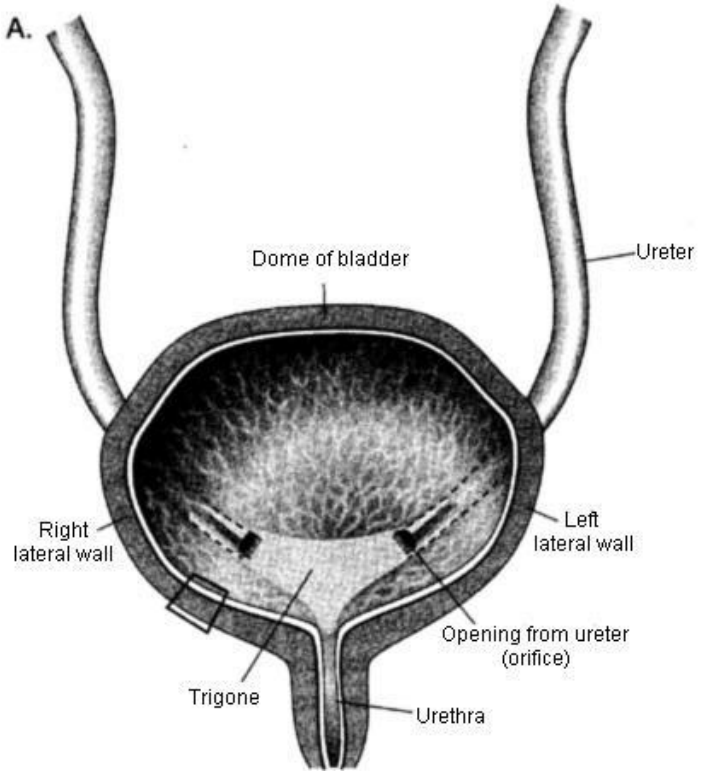
- Uropathogenic *E. coli (UPEC)*

26.5 Genitourinary Tract Infections

- **The urinary tract includes the kidneys, ureters, urinary bladder, and urethra**.
- Active infection of the urinary tract occurs in one of three basic ways:
  - Infection from the urethra to the bladder
  - Descending infection from the kidneys
  - Ascending infection to the kidney
- **Most UTIs are caused by Gram-negative rods from the GI tract.**
  - **Only 5% are caused by Gram-positive bacteria and fungi.**

The urinary system





- **Uropathogenic strains of *Escherichia coli* (UPEC)**
  - Cause about 75% of UTIs
  - Invade the bladder up from the urethra
  - Have P-type pili, with a terminal receptor for the P antigen
  - Have five unique pathogenicity islands

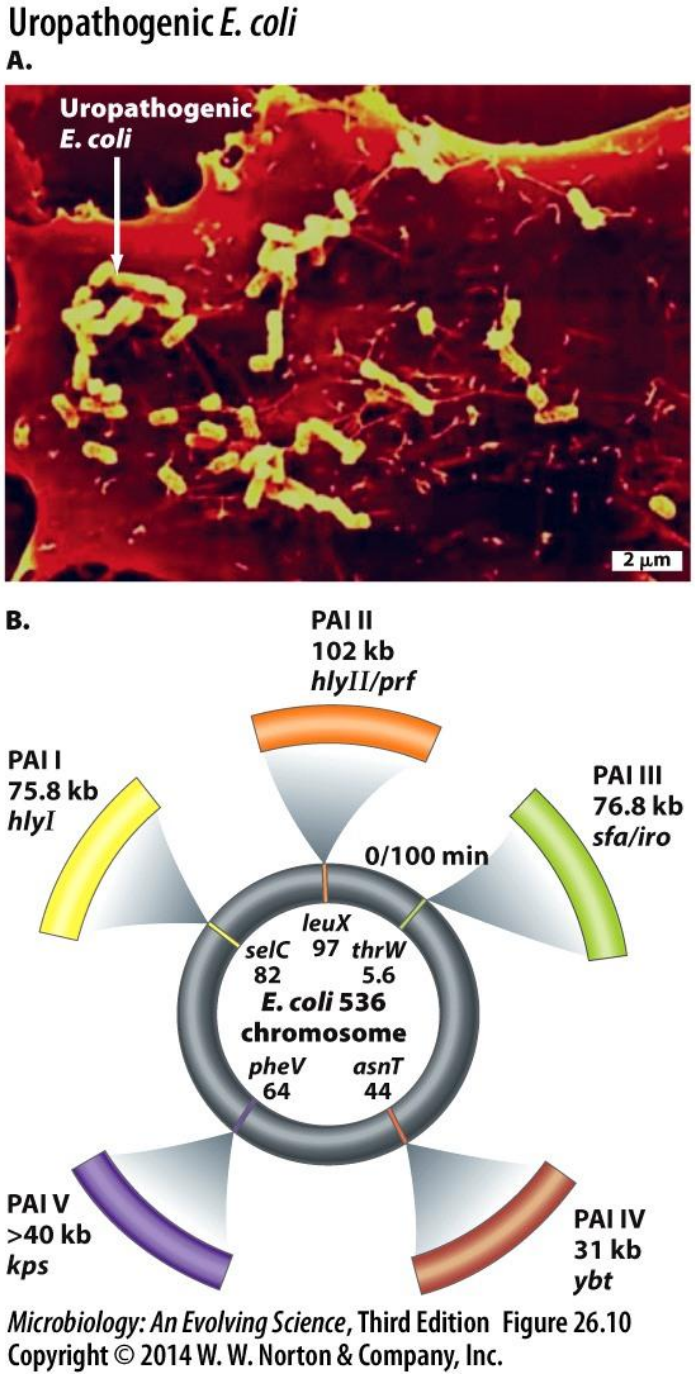


Figure 26.10

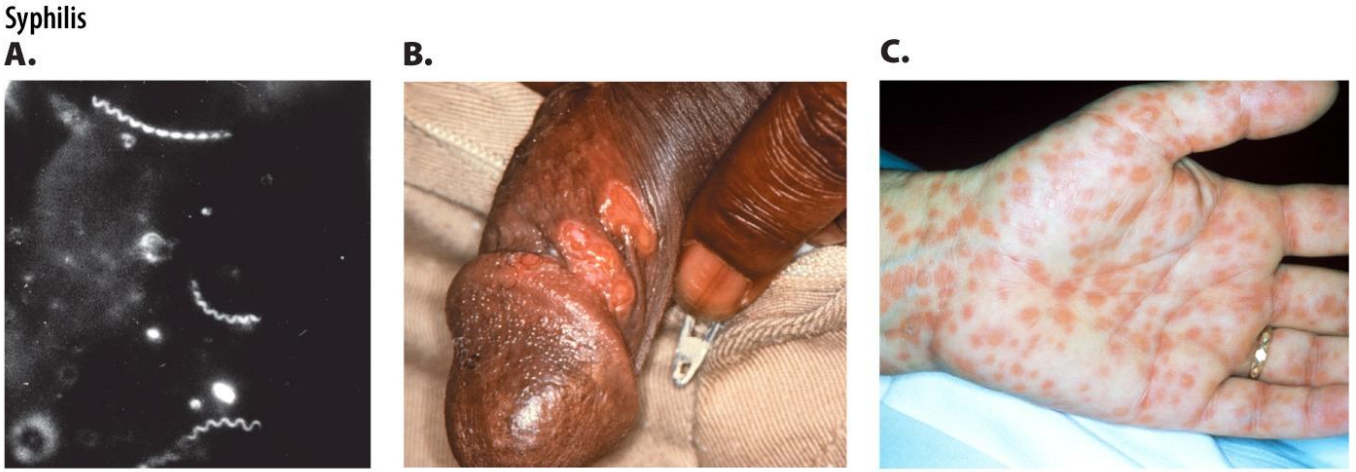
Sexually Transmitted Diseases

- Syphilis by *Treponema pallidum*
- *Chlamydia trachomatis*
- *Chlamydia pneumoniae*

Sexually Transmitted Diseases

- **Syphilis**
  - Caused by the spirochete ***Treponema pallidum***
  - Primary syphilis: **chancre** at site of infection
  - Secondary syphilis: generalized rash
  - Tertiary syphilis: effects on heart and CNS

Figure 26.11



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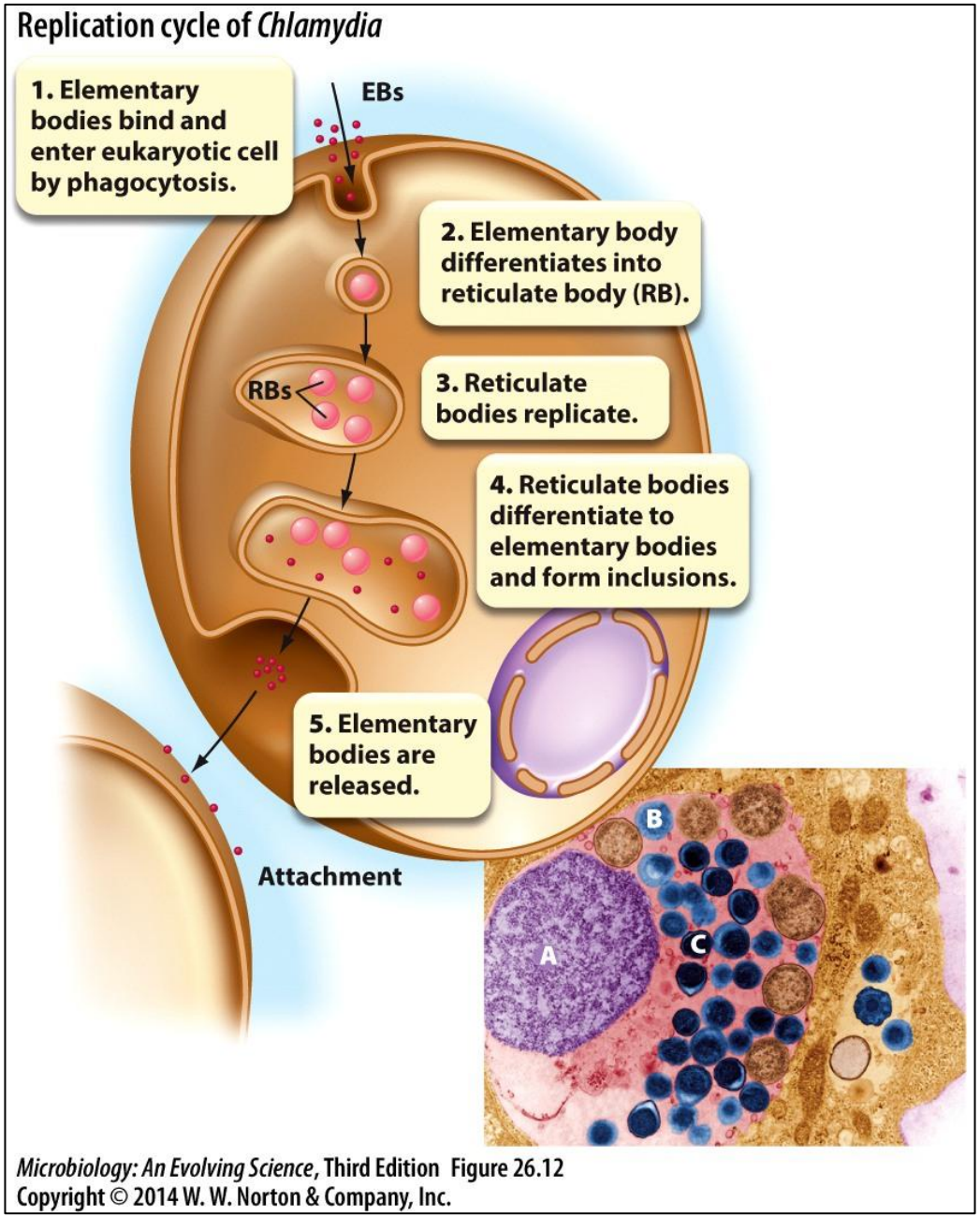
- **Chlamydia**
  - Most frequently reported STD in the United States
  - Caused by unusual Gram-negative bacteria
    - ***Chlamydia trachomatis***
    - ***Chlamydia pneumoniae***
      - Obligate intracellular pathogens
      - Both cause STDs, as well as pneumonia and trachoma of the eye
  - Left untreated, infection can cause serious health problems in both females and males

Trachoma

- Trachoma is a bacterial infection that affects the eyes. It is caused by the bacterium ***Chlamydia trachomatis***. Trachoma is contagious, spreading through contact with the eyes, eyelids, and nose or throat secretions of infected people. It can also be passed on by handling infected items, such as handkerchiefs.
- At first, trachoma may cause mild itching and irritation of your eyes and eyelids. Then you may notice swollen eyelids and pus draining from the eyes. Untreated trachoma can lead to blindness.



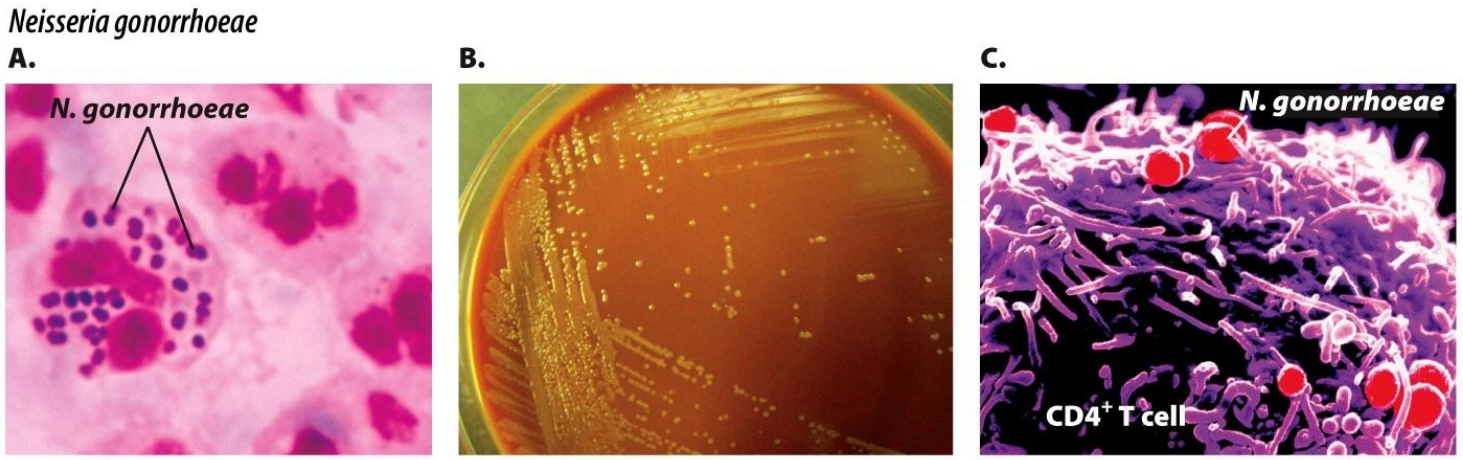
- Elementary bodies are the infective form. They enter eukaryotic cells by endocytosis or phagocytosis
- They differentiate into reticulate bodies, which are the replicative form.
- The reticulate bodies differentiate into elemental bodies that are released

Figure 26.12

Gonorrhea

- Caused by the Gram-negative diplococcus ***Neisseria gonorrhoeae***
  - Over the decades, it has incrementally developed resistance to antibiotics used in its treatment
- Most infected men exhibit symptoms, whereas most women are asymptomatic.
- **Binds to $CD4^+$ T cells, inhibiting T-cell activation**

Figure 26.13

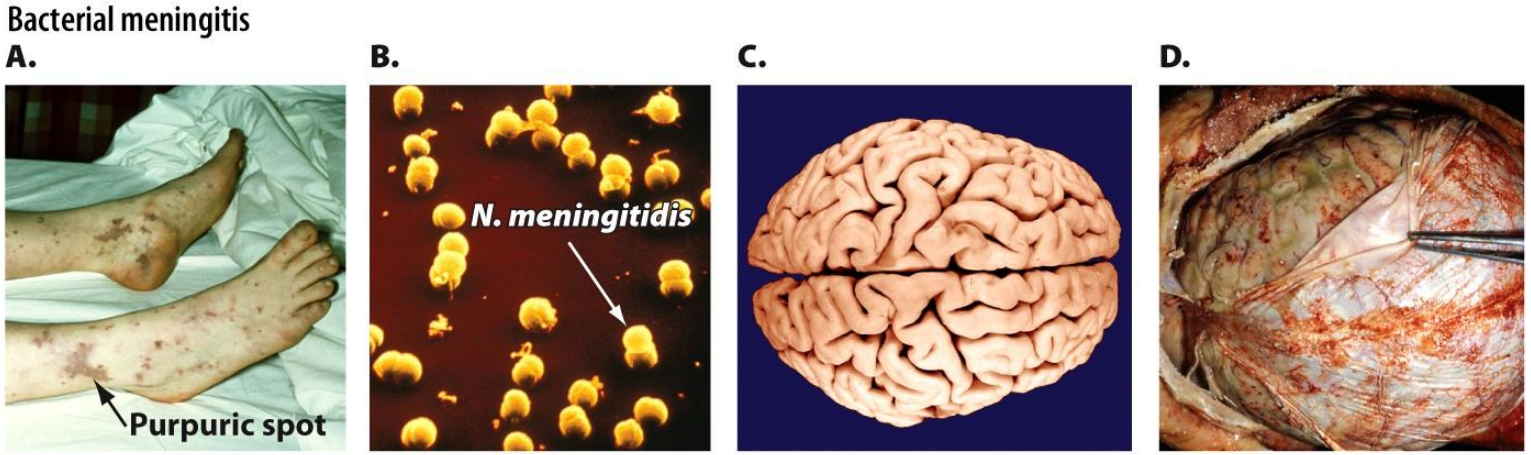


Microbiology: An Evolving Science, Third Edition Figure 26.13

Central Nervous System Infections

- **Meningitis**
  - Infection of membrane surrounding brain
  - **Some bacteria cross blood-brain barrier**
    - ***Streptococcus pneumoniae***
    - ***Haemophilus influenzae***
    - ***Neisseria meningitidis***

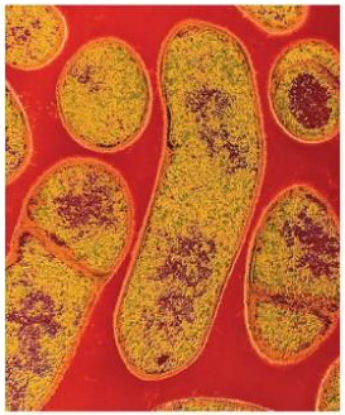
- ***Neisseria meningitidis***
  - Has thick capsule and type IV pili
  - Dangerous if it gets into the bloodstream
  - Crosses from capillary into cerebrospinal fluid
  - Once in meninges, it is very difficult to treat
  - Effective vaccine to capsule components



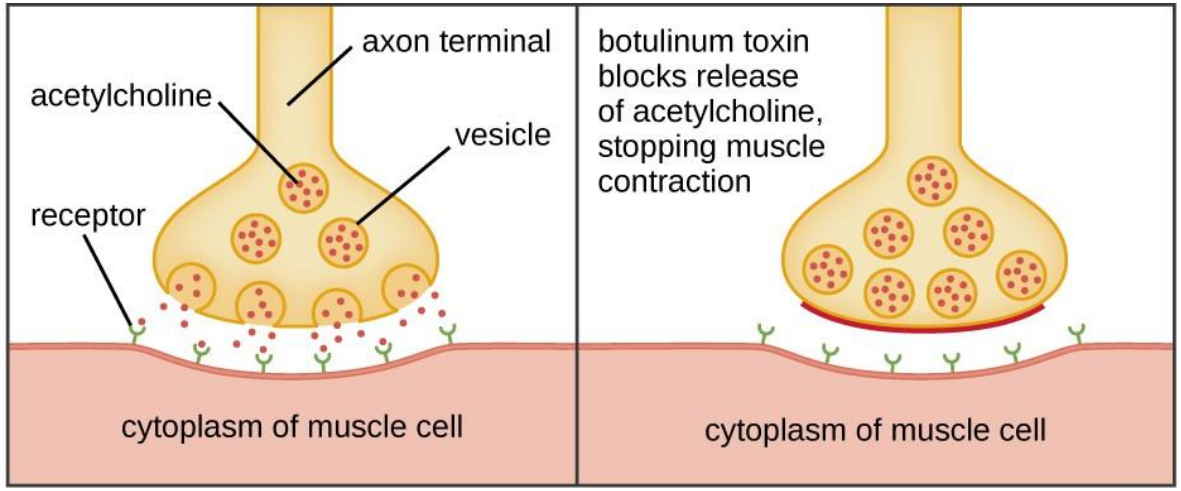
Microbiology: An Evolving Science, Third Edition Figure 26.16

Figure 26.16

- ***Clostridium* toxins**
  - ***C. botulinum***: botulism toxin ("Botox")
    - Anaerobe, grows in canned food
    - Spores survive unless autoclaved
    - Toxin blocks the release of acetylcholine
    - Causes **flaccid paralysis**
  - ***C. tetani***: tetanus toxin
    - Anaerobe, grows in puncture wounds
    - Blood flow interrupted; tissue becomes anaerobic
    - Toxin blocks release of GABA, a primary inhibitory transmitter for the central nervous system. Its function is to reduce neuronal excitability
    - By inhibiting nerve transmission
    - Causes **spastic paralysis**

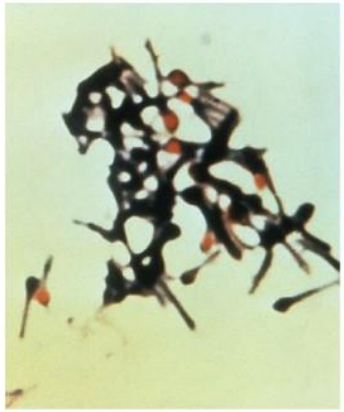


botulinum toxin
(flaccid paralysis: stops muscle contraction)

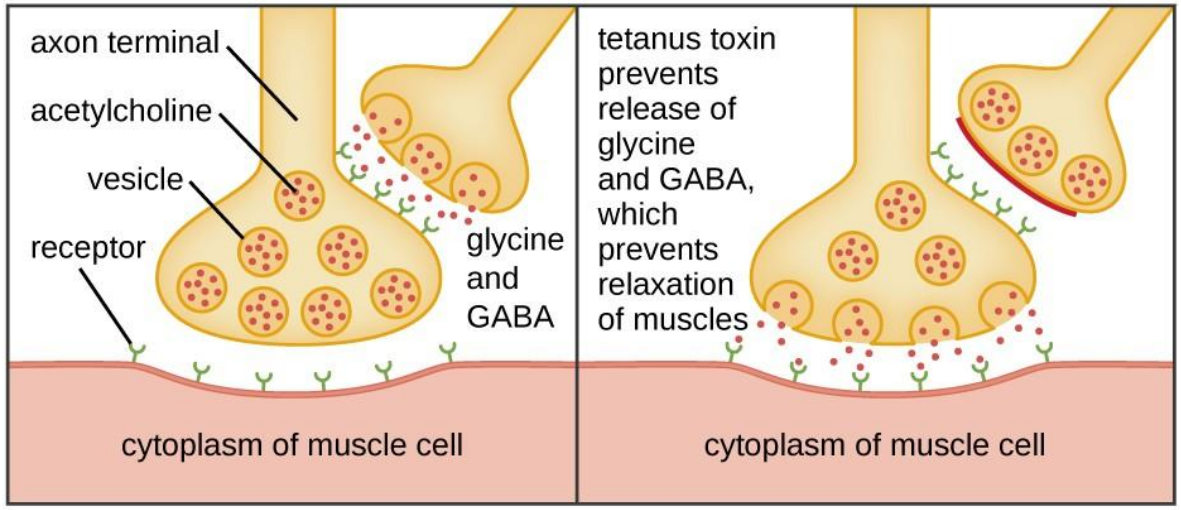


normal mechanism

abnormal mechanism



tetanus toxin
(spastic paralysis: stops uncontrollable muscle contraction)



normal mechanism

abnormal mechanism

Cardiovascular Diseases

- Infections of the cardiovascular system include:
  - **Endocarditis:** inflammation of the heart's inner lining
  - **Septicemia:** presence of microbes in the blood
  - **Bacteremia:** presence of bacteria in the blood
    - Can develop from a local infection situated anywhere in the body
    - Caused by Gram-positives, Gram-negatives, aerobes, and anaerobes

- **Bacterial Endocarditis**
  - Bacterial causes are usually viridans streptococci from the oral microbiota: ***Streptococcus mutans***
    - Enters the bloodstream following a dental procedure
    - Grows on damaged heart valves
    - Forms biofilm
    - Difficult to treat

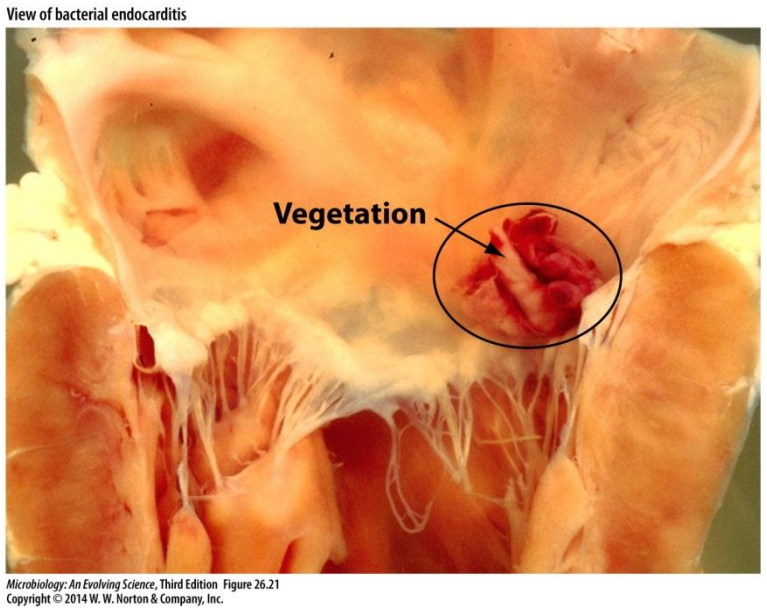


Figure 26.21

Systemic Infections

- Septicemia disseminating throughout body
- **Plague**
  - Caused by the bacterium ***Yersinia pestis***
  - Bite of flea introduces organism
    - **Moves to lymph nodes: bubonic plague**
    - **Moves to bloodstream: septicemic plague**
    - **Inhaled: pneumonic plague**
      - Highly infectious
  - Virulence factors inhibit phagocytosis
  - Type III secretion system injects virulence proteins

Zoonotic disease

- A zoonosis is another name for zoonotic disease. This type of disease passes from an animal or insect to a human. Some don't make the animal sick but will sicken a human.
- Zoonotic diseases range from minor short-term illness to a major life-changing illness. Certain ones can even cause death.
- Zoonosis include those caused by a **virus, bacteria, fungus, and parasites.**
- Zoonotic diseases spread by mosquitos and ticks are some of the most serious of these diseases (Healthline)

- **Lyme disease**
  - Caused by ***Borrelia burgdorferi***, a spirochete
  - Transmitted by ticks
  - Bacterium can travel to any part of the body
  - Has three stages:
    - **Stage 1: a bull's-eye rash (erythema migrans)**
    - **Stage 2: joint, muscle, and nerve pain**
    - **Stage 3: arthritis, with WBCs in the joint fluid**
  - Treatment with antibiotics is recommended for all stages

Lyme disease

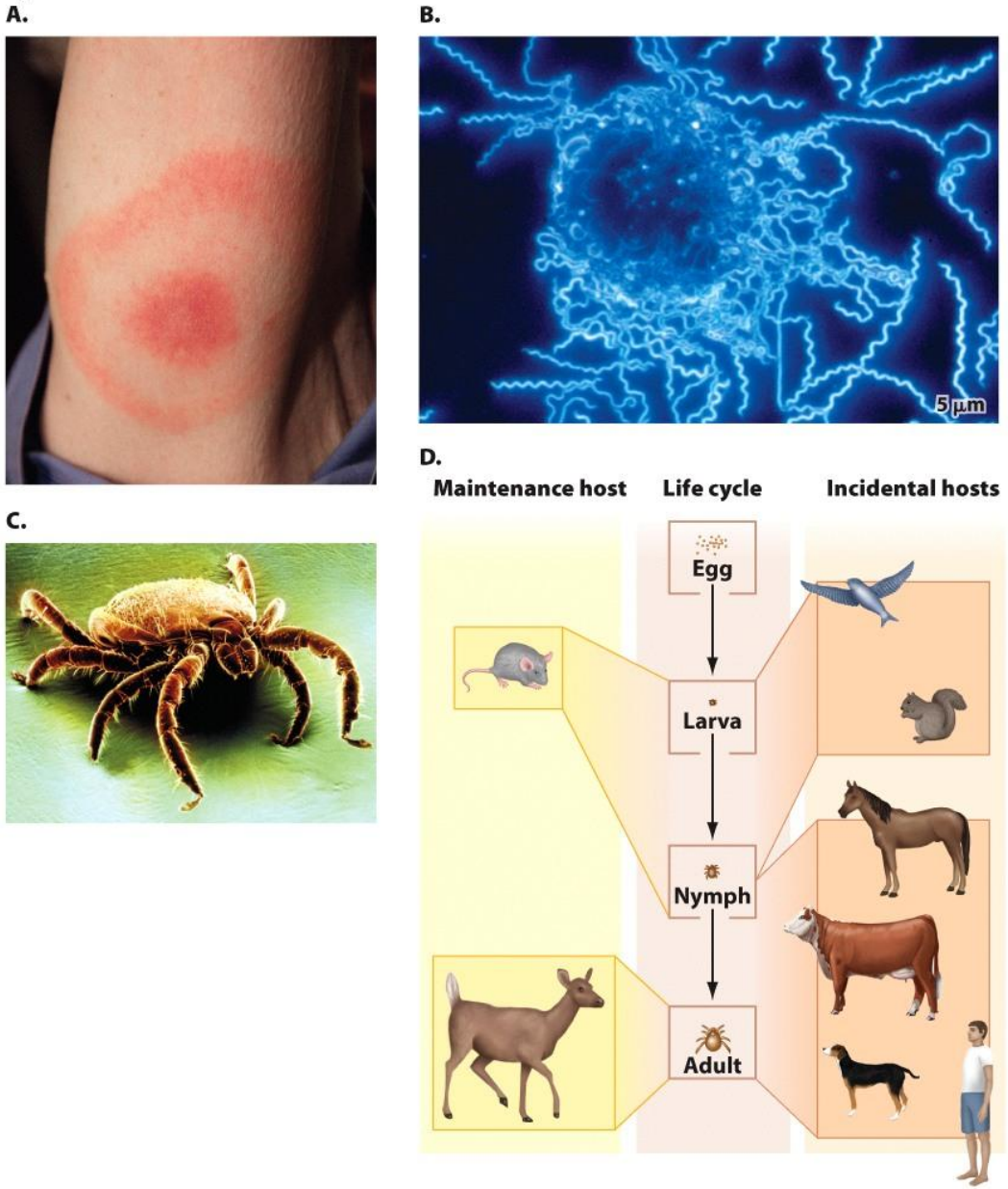


Figure 26.25

Microbiology: An Evolving Science, Third Edition Figure 26.25

Immunization

- Vaccines are typically given in childhood
  - Most are administered as multiple booster doses
    - Except influenza: new vaccine every year
  - Serious side effects are very rare
- **Herd immunity**
  - Vaccinating a large percentage of a community effectively conveys herd immunity by interrupting transmission of contagious diseases
    - Example: gardasil—human papillomavirus vaccine
  - **Only works for diseases spread person to person**

Chapter Summary

- Pathogens can be classified as food-borne, airborne, blood-borne, or sexually-transmitted.
- Patients histories are vital in diagnosing diseases.
- Skin and soft-tissue infections include:
  - Boils; scalded skin syndrome (*Staphylococcus aureus*)
  - Necrotizing fasciitis (*Streptococcus pyogenes*)
  - Measles: rubella and rubeola (viral infections)
- Respiratory tract infections include pneumonia (caused by a variety of organisms) and tuberculosis.
- The main causes of GI tract infections include:
  - Bacteria: EHEC, *Salmonella*, *Shigella*, *H. pylori*
  - Protozoa: *Entamoeba*, *Cryptosporidium*, *Giardia*
  - Viruses: rotavirus (single greatest cause of gastroenteritis)

Chapter Summary

- The main cause of UTIs is uropathogenic ***Escherichia coli*** (UPEC)
- Sexually transmitted diseases include:
  - Syphilis, gonorrhea; **chlamydia** (bacterial diseases)
  - Trichomoniasis (protozoan disease), AIDS (viral disease)
- Pathogens that cause CNS infections include:
  - ***Neisseria meningitidis*** (meningitis), ***Clostridium botulinum*** toxin (flaccid paralysis), ***C. tetanus*** toxin (spastic paralysis)
- Cardiovascular system infections include:
  - Septicemia, endocarditis, malaria
- Pathogens that cause systemic infections include:
  - ***Yersinia pestis*** (plague), ***Borrelia burgdorferi*** (Lyme disease)
- Herd immunity can protect unimmunized people.

FYI

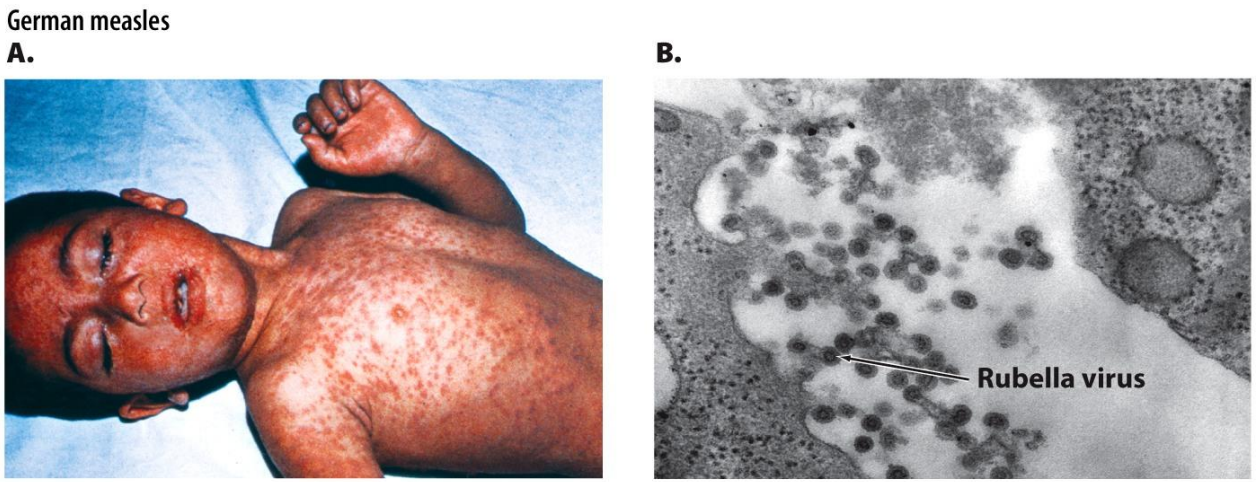
Viral diseases

- Paramyxovirus
- Herpes virus
- Togavirus
- Influenza virus and rhinovirus
- Rotavirus
- Hepatitis virus
- Human immunodeficiency virus

Viral Diseases Causing Skin Rashes

- Viruses cause a maculopapular skin rash.
  - Usually infect through respiratory tract
  - **Paramyxovirus**: rubeola (“measles”)
  - **Herpes virus**: chickenpox, shingles
  - **Togavirus**: rubella (“German measles”)

Figure 26.4



Microbiology: An Evolving Science, Third Edition Figure 26.4

Viral Diseases of the Lung

- Numerous viruses can cause lung infections
  - **Influenza virus and rhinovirus**
  - **SARS (severe acute respiratory syndrome)**
  - **Respiratory syncytial virus (RSV)**
    - A negative-sense, single-stranded RNA, enveloped virus
    - The most common cause of pneumonia among infants and children under 1 year of age
    - Remains localized in the lung

Gastroenteritis caused by viruses

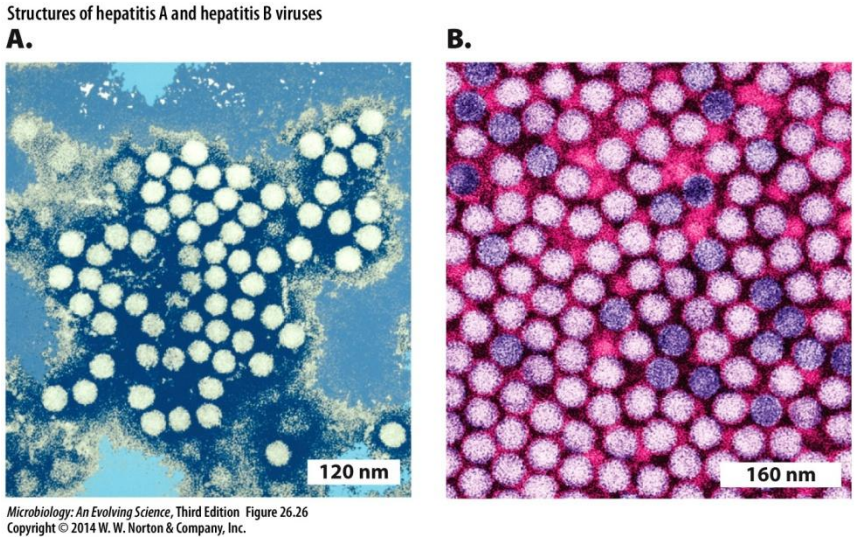
- **Rotavirus is the single greatest cause of gastroenteritis**
- Double-stranded RNA viruses
- Highly infectious, spreading by the fecal-oral route
- Endemic around the globe; affects all age groups

Hepatitis Viruses

- **Hepatitis** is a term meaning inflammation of the liver.
  - Caused by several blood-borne viruses, including:
    - **HAV: hepatitis A—picornavirus (ssRNA)**
    - **HBV: hepatitis B—hepadnavirus (dsDNA)**
    - **HCV: hepatitis C—flavivirus (ssRNA)**

Viral infections
- Adenovirus
- Enteroviruses

Figure 26.26

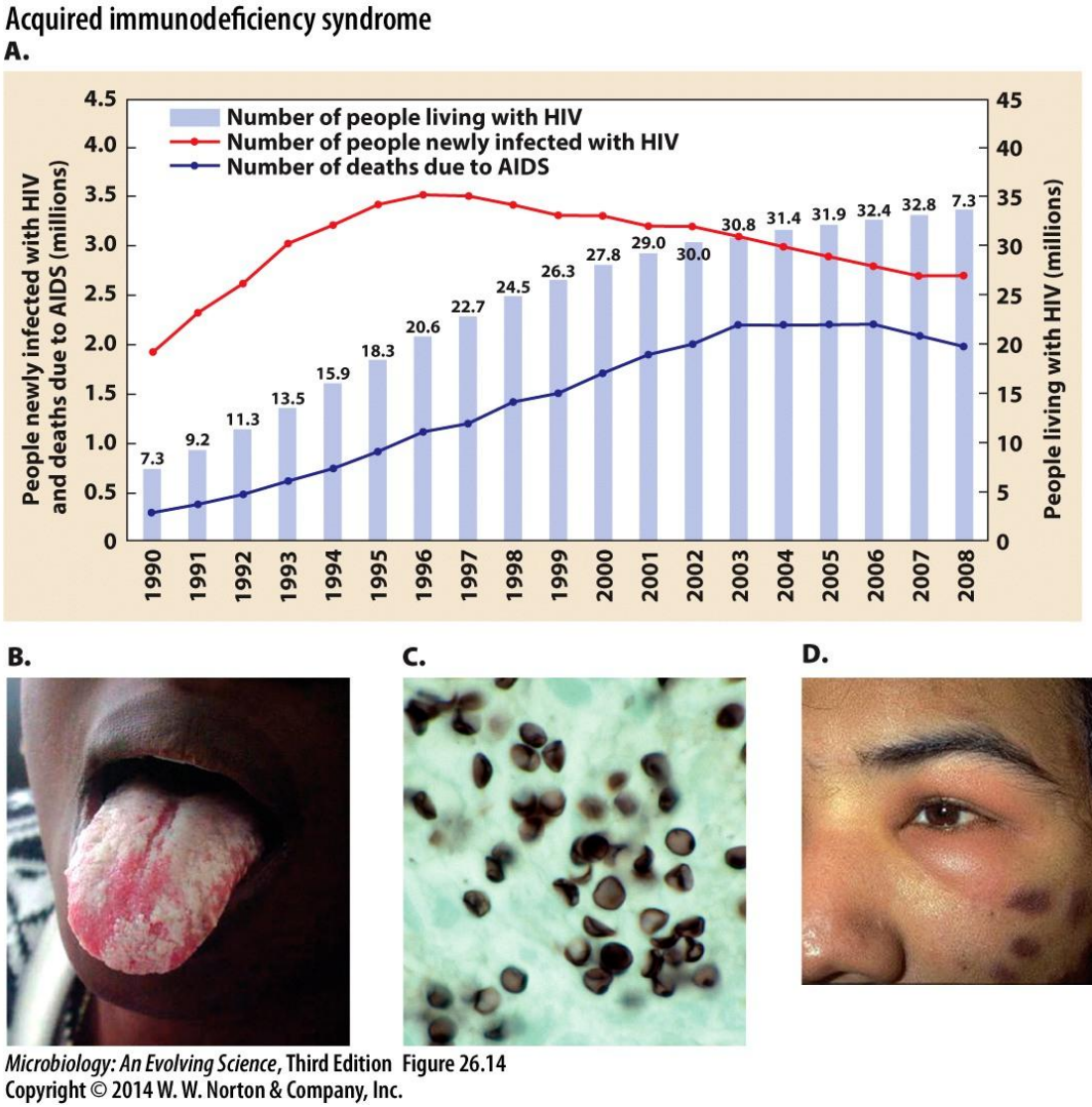


Viral endocarditis

- Association between **enterovirus** endomyocardial infection and late severe cardiac events in some adult patients receiving heart transplants.

Human Immunodeficiency Virus

ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)



Microbiology: An Evolving Science, Third Edition Figure 26.14

Figure 26.14

- **Acquired immunodeficiency syndrome**
  - **HIV: a lentiviral retrovirus**
    - **Attacks $CD4^+$ T cells, glial cells**
    - **First stage**: AIDS-related complex
      - Fever, headache, rash
    - **Second stage**: AIDS
      - Depletion of T cells
      - Opportunistic infections
        - Oral **candidiasis**
        - **Pneumocystosis**
    - **Third stage**: AIDS-related dementia
    - **Fourth stage**: rare cancers
      - **Kaposi's sarcoma via herpes virus type 8** infection

Fungal Diseases

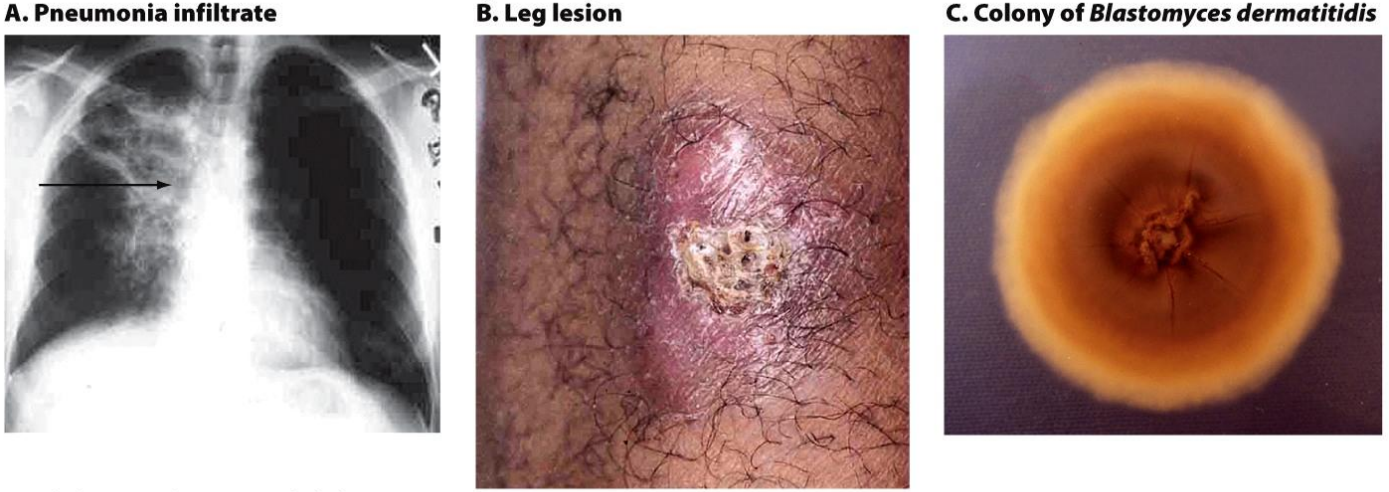
- Most fungi are not dangerous: Mild fungal skin diseases can look like a rash and are very common. Ex. ***Trichophyton rubrum*** (ectopathogen) causes Athtete's foot.
- Fungal diseases in the lungs are often similar to other illnesses such as the flu or tuberculosis.
- ***Blastomyces dermatitidis*** can cause skin, and bone lesions, and metastatic or disseminating lesions in the lung, causing acute pneumonia
- Some fungal diseases like **fungal meningitis** and bloodstream infections are less common than skin and lung infections but can be deadly. It can be caused by ***Candida albicans***, ***Cryptococcus neoformans*** and ***Histoplasma.***

Blastomyces dermatitidis

- Dimorphic fungus found in the soil
- Infection usually associated with occupational and recreational activities
- Does not usually cause an increase in WBCs
- Can cause **metastatic lesions**

Figure 26.6

Pneumonia and metastatic disease caused by *Blastomyces dermatitidis*



Microbiology: An Evolving Science, Third Edition Figure 26.6

Pathogenic Monocellular Eukaryotes

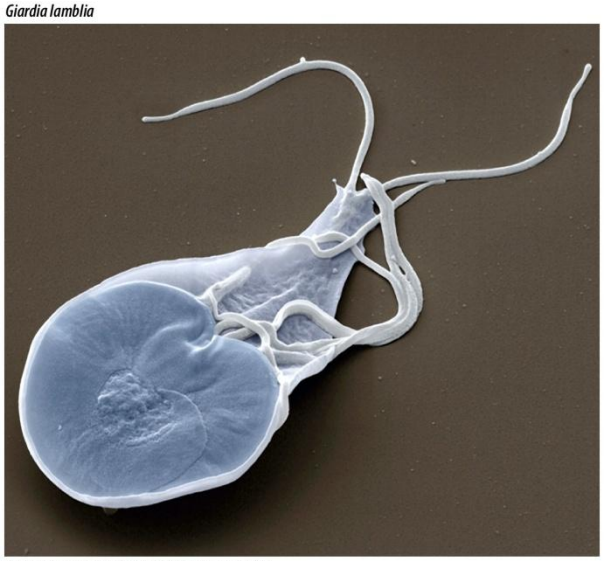
- Amoeba
- Entamoeba
- Cryptosporidium
- Naegleria
- Acanthamoba
- Giarda lamblia
- Trichomonas

Pathogenic Ameba

- Unicellular eukaryotic organism
- ***Entamoeba histolytica*** causes amebic dysentery
- Which is a more severe form of gastroenteritis,
- e.g. diarrhea with passage of blood or mucus.

Protozoal infections

- ***Entamoeba***
- ***Cryptosporidium***
- ***Naegleria***
- ***Acanthamoeba***
- ***Giardia lamblia*** attaches to the intestinal wall



Microbiology: An Evolving Science, Third Edition Figure 26.9b

Figure 26.9

- **Trichomoniasis**
  - ~ 2–3 million infections per year in the United States
  - Caused by ***Trichomonas vaginalis***, a flagellated protozoan
    - No cyst; transmitted via trophozoite stage
  - **Reservoirs are the male urethra and female vagina**
  - Feeds on bacteria in the vagina
    - pH increases
  - Treated with **metronidazole**

Figure 26.15

Microbiology: An Evolving Science, Third Edition Figure 26.15

The plague

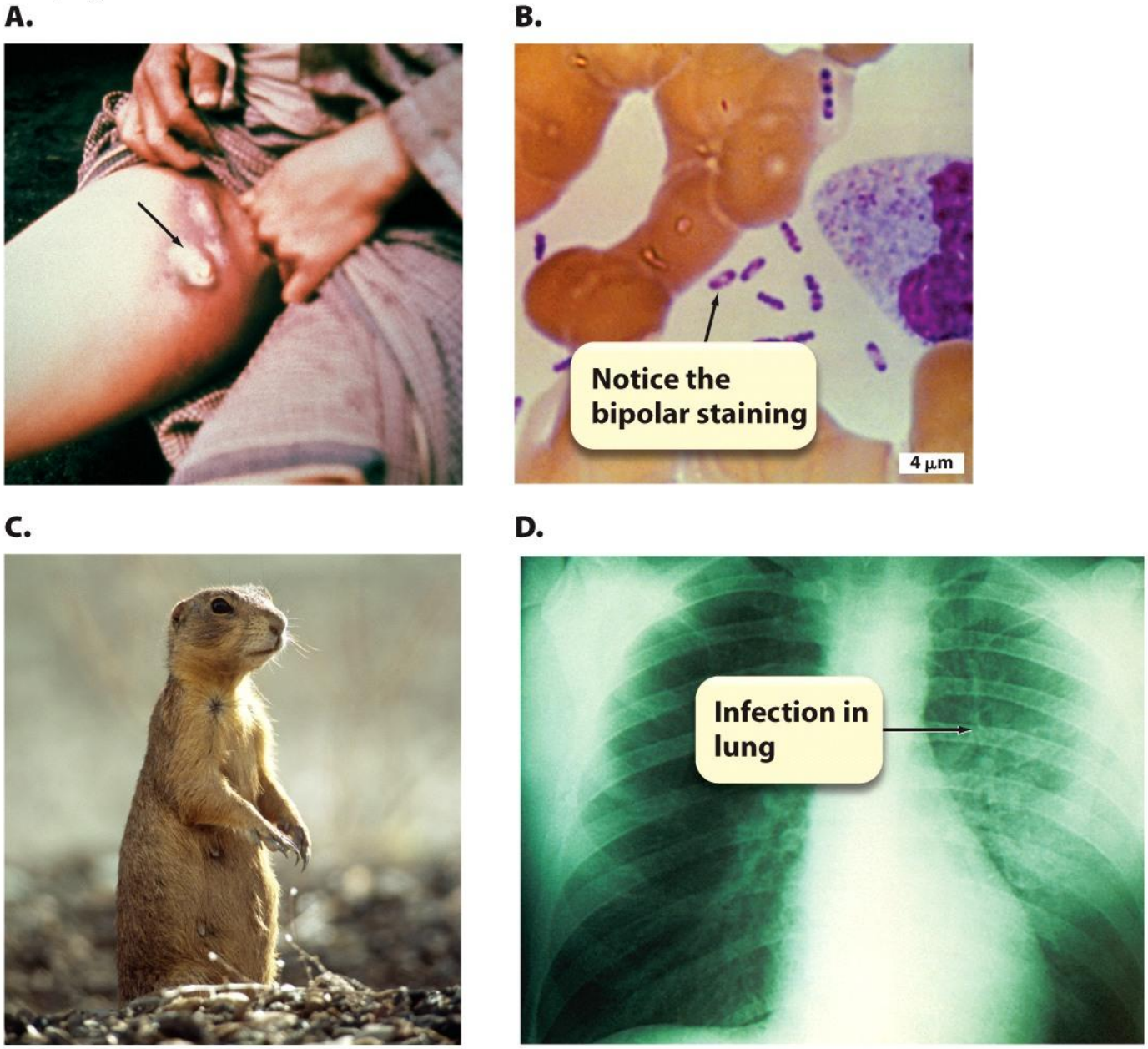
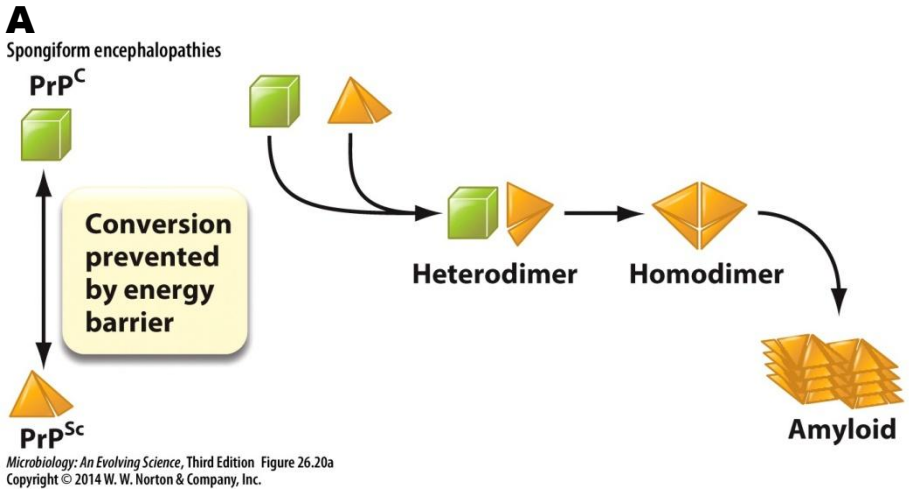


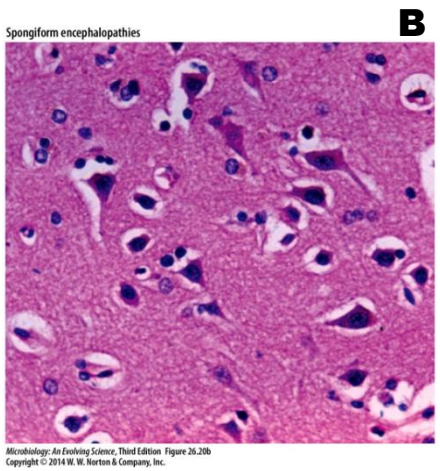
Figure 26.23

Microbiology: An Evolving Science, Third Edition Figure 26.23

Prions

- Proteinaceous infectious particles
- Cause **spongiform encephalopathies**
  - Improperly folded proteins form aggregates that damage the brain
  - Most mammals suffer from these diseases





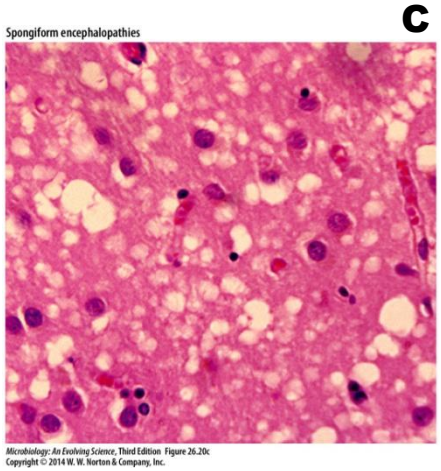


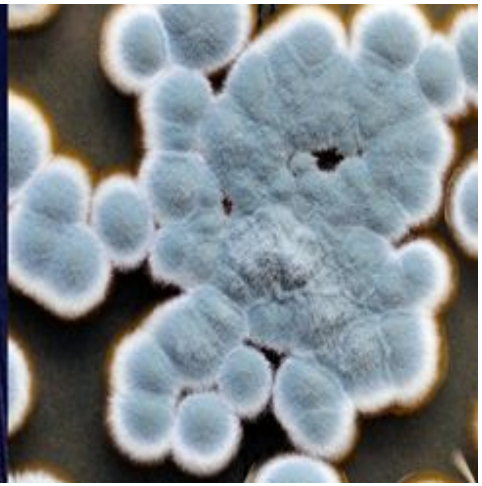
Figure 26.20

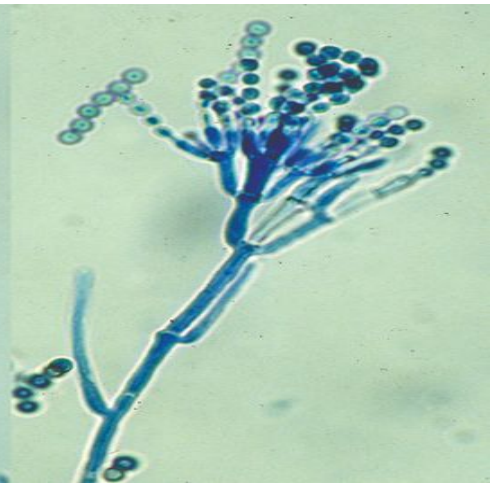
Malaria

- Causes 1–3 million deaths per year.
- Four protozoan ***Plasmodium* species: *P. falciparum*, *P. malariae*, *P. vivax*, and *P. ovale*.**
  - *P. falciparum* is the most deadly of all.
    - Infects liver, red blood cells (RBCs).
    - New merozoites are released every 48–72 hours.
    - Many parasites are killed in each generation.
    - Others switch protein placed on RBC surface.
      - 60 *var* genes encode different surface proteins; thus parasite constantly eludes immune system.
    - Chloroquine resistance is a problem.

Antimicrobial Chemotherapy and Discovery

Lecture 22 (Ch. 27)



Chapter Overview

- The golden age of antibiotic discovery
- Basic concepts of antimicrobial therapy
- Measuring drug susceptibility
- Mechanisms of action
- Challenges of drug resistance
- The future of drug discovery
- Antiviral agents
- Antifungal agents

Introduction

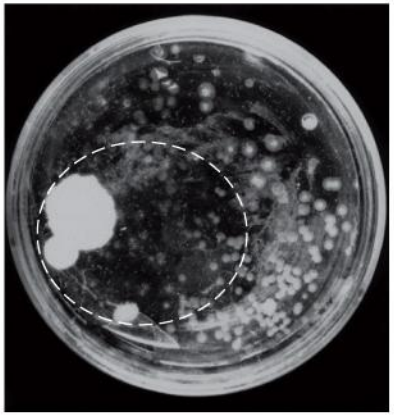
- The discovery of antibiotics about 80 years ago has played a major role in increasing life expectancy throughout the world.
  - From 45 to 50 years (prior to 1918) to nearly 79 years now
- **But antibiotics may soon become useless**.
  - Their overuse and misuse have led to the development of antibiotic-resistant strains

The Golden Age of Antibiotic Discovery

- **Antibiotics** are compounds produced by one microbe that adversely affect other microbes.
- The modern antibiotic revolution began in 1928 with the discovery of penicillin by **Alexander Fleming**.
  - A contaminating mold had inhibited the growth of ***Staphylococcus aureus*** colonies on a plate.
    - The mold was identified as ***Penicillium notatum*.**
  - Penicillin was purified in the early 1940s by **Howard Florey and Ernst Chain.**
    - Has saved millions of lives since!

The dawn of antibiotics

A.



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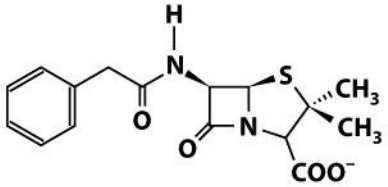
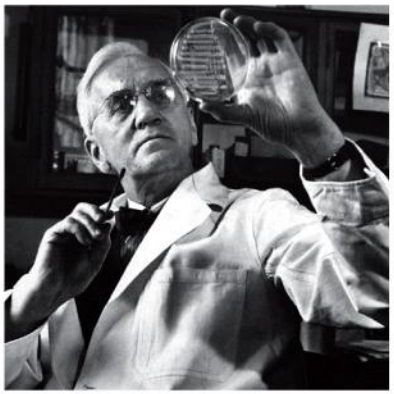


Penicillin G

C.



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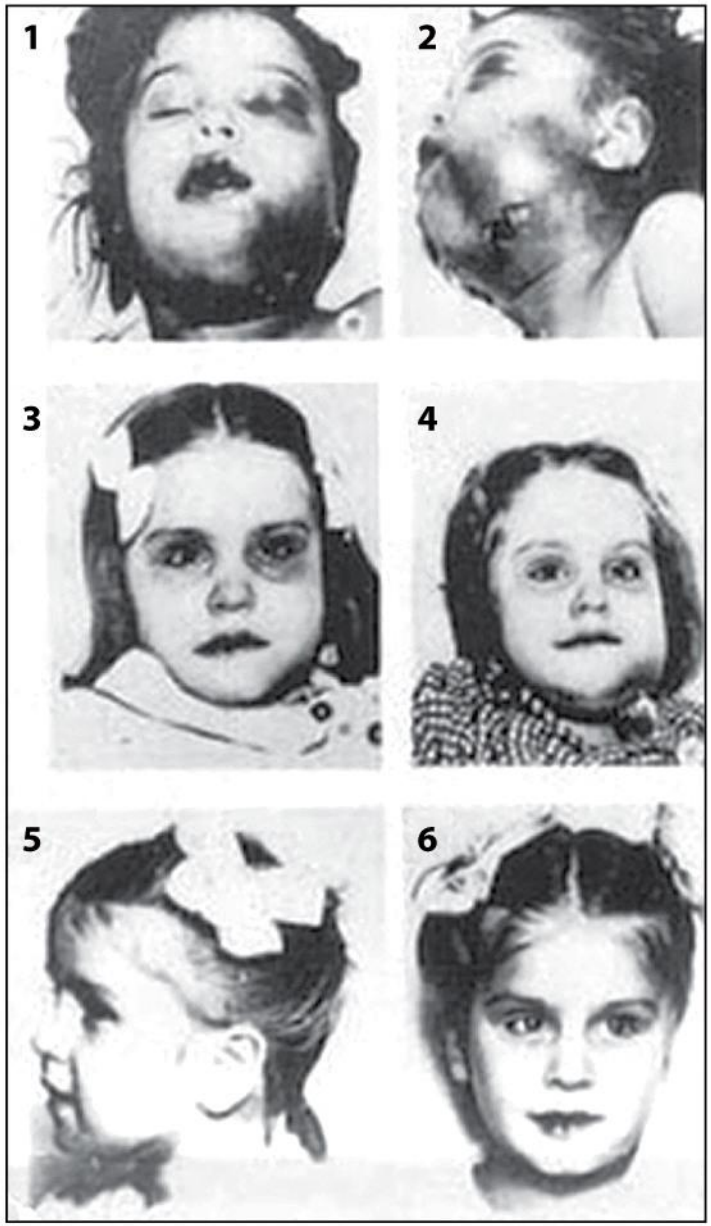


Figure 27.1

Microbiology: An Evolving Science, Third Edition Figure 27.1

- **Gerhard Domagk (1930s)**
  - Discovered *sulfa drugs*
  - Inactive until converted by the body to active agents
  - Analogs of **PABA (Para-aminobenzoic acid), a precursor of a vitamin needed for DNA synthesis**
- **Selman Waksman (1940s)**
  - Discovered **streptomycin**
  - Antibiotic produced by an actinomycete bacterium found in the soil

Streptomyces griseus

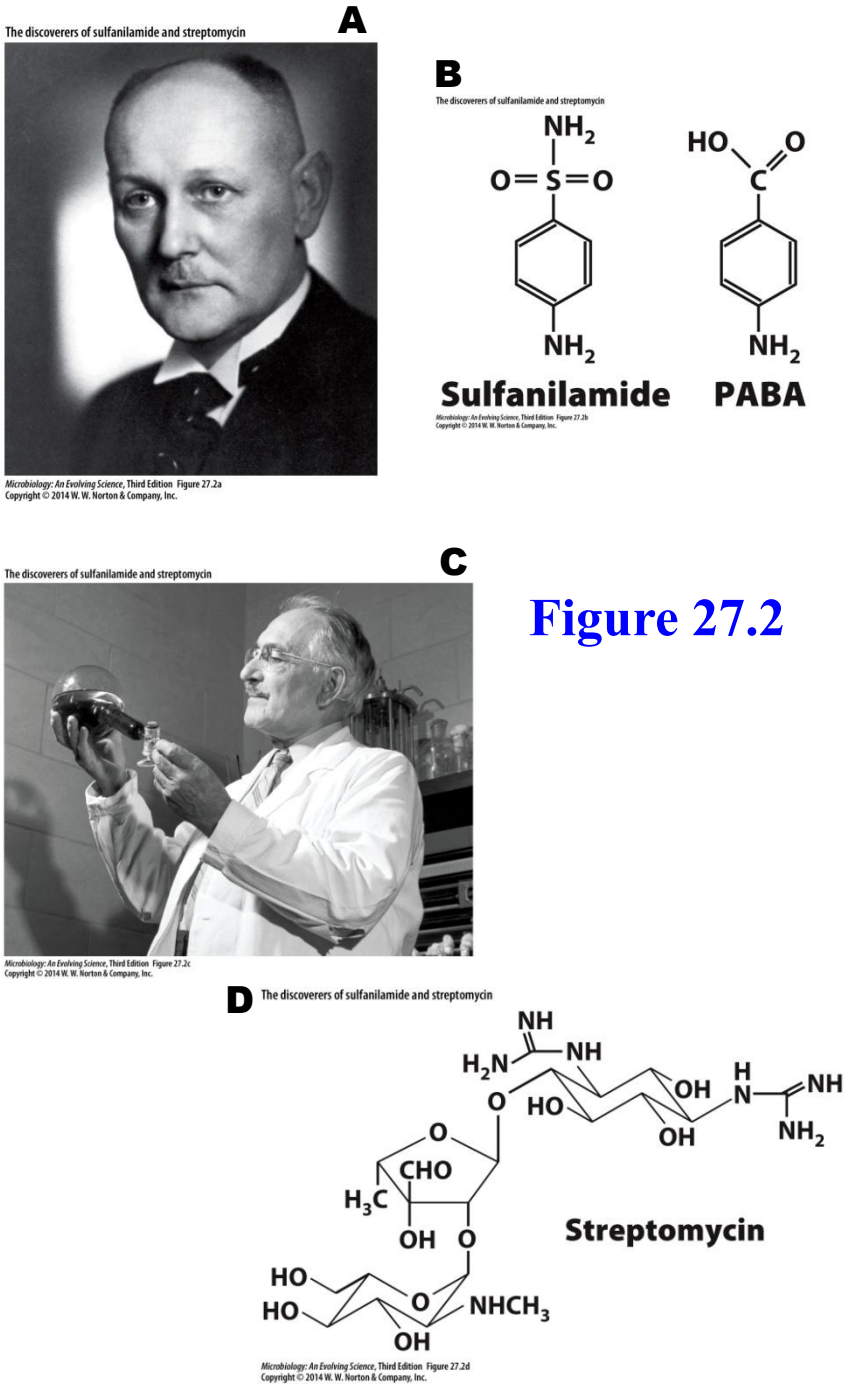


Figure 27.2

Fundamentals of Antimicrobial Therapy

- Antibiotics comprise mostly of chemotherapeutic agents used to treat microbial diseases.
- The term "**antibiotic**" originally referred to any **compound produced by one microbial species that could kill or inhibit the growth of other microbes.**
  - Today the term "antibiotic" is also used for **synthetic chemotherapeutic agents, such as sulfonamides,** that are clinically useful but chemically synthesized.
- Many natural and synthetic compounds affect microbial growth, but their utility in a clinical setting is dictated by certain key characteristics.

Antibiotics Exhibit Selective Toxicity

- **Antibiotic must affect target organism.**
  - But it must *not* affect humans.
  - Many have side effects at high concentration.
    - **Chloramphenicol** interferes with our ribosomes.
      - At high levels, it interferes with RBC development.
      - Some may cause allergic response.
      - Antibiotics are foreign substances in our bodies.
  - **Drug should affect microbial physiology.**
    - That does not exist, or is greatly modified, in humans
      - **Peptidoglycan**
      - **Differences in ribosome structure**
      - **Biochemical pathway missing in humans**

Antimicrobials Have a Limited Spectrum of Activity

- **Broad spectrum**
  - Effective against many species
- **Narrow spectrum**
  - Effective against few or a single species
- **Source of antibiotics**
  - Most discovered as natural products
  - Often modified by artificial means to:
    - Increase efficacy
    - Decrease toxicity to humans

Antibiotics Are Classified as Bacteriostatic or Bactericidal

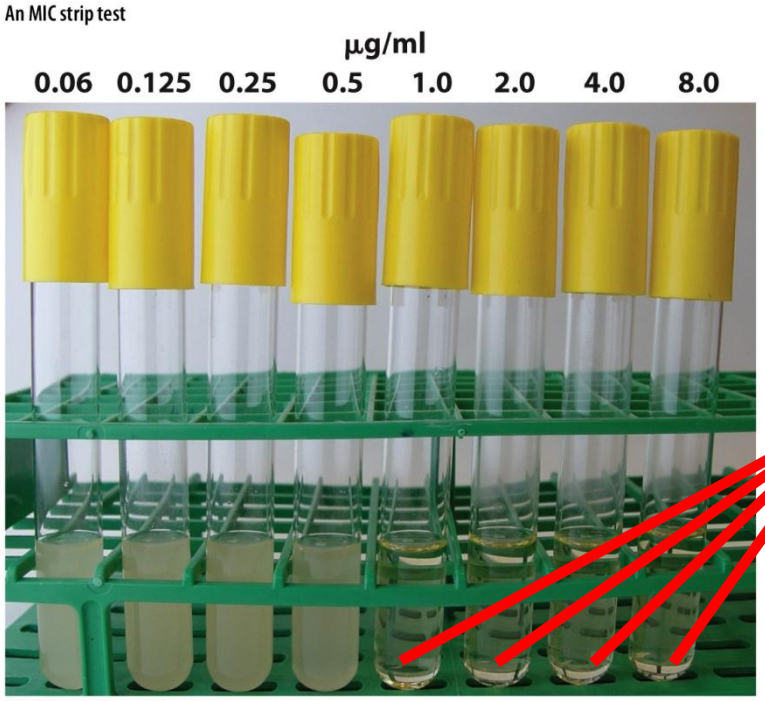
- **Bactericidal antibiotics** kill target organisms.
  - Many drugs only affect growing cells.
  - Inhibitors of cell wall synthesis
  - Only effective if organism is building new cell wall
  - Example: penicillin
- **Bacteriostatic antibiotics** prevent growth of organisms.
  - Cannot kill organism
  - Immune system removes intruding microbe

Measuring Drug Susceptibility

- One critical decision a clinician must make when treating an infection is which antibiotic to prescribe for the patient.
- There are several factors to consider, including:
  - **The relative effectiveness of different antibiotics on the organism causing the infection**
  - **The average attainable tissue levels of each drug**

Minimal Inhibitory Concentration

- The **MIC** is the lowest concentration that prevents growth
  - Varies for different bacterial species
  - Test by diluting antibiotic



Microbiology: An Evolving Science, Third Edition Figure 27.3
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Figure 27.3

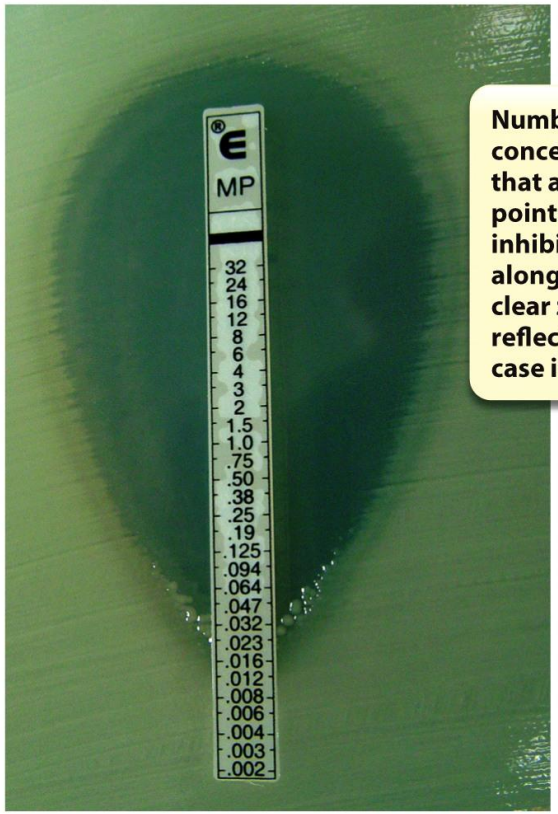
- Lowest concentration with no growth: MIC
  - May still have living (but nongrowing) organisms
  - Plate liquid without antibiotic: Do colonies form?
    - No colonies: minimal lethal concentration (MLC)
    - MLC always lower than MIC

Minimal Inhibitory Concentration

- The time required to evaluate antibiotic effectiveness can be reduced by using a strip test that avoids the need for dilutions.

The MIC is the point at which the elliptical **zone of inhibition** intersects with the strip.

An MIC strip test



Numbers reflect the relative concentrations of antibiotic that are present at various points within the zone of inhibition. The concentrations along the periphery of the clear zone are equal and reflect the MIC, which in this case is 0.047 μg/ml.

Microbiology: An Evolving Science, Third Edition Figure 27.4

Figure 27.4

Kirby-Bauer Disk Susceptibility Test

- Clinical labs can receive up to 100 or more isolates in one day, so individual MIC determinations are impractical.
- The **Kirby-Bauer assay** tests strain sensitivity to multiple antibiotics.
  - Uses a series of round filter paper disks impregnated with different antibiotics.
  - A dispenser delivers up to 12 disks simultaneously to the surface of an agar plate covered by a bacterial lawn.
  - During incubation, the drugs diffuse away from the disks into the surrounding agar and inhibit growth of the lawn.
    - Size of cleared zones reflects relative sensitivity

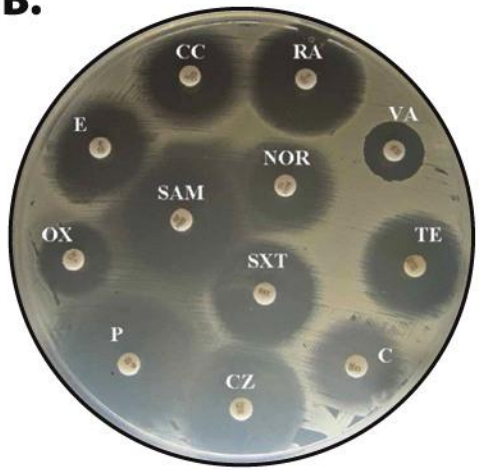
Kirby-Bauer Disk Susceptibility Test

- The following are standardizations used to make the test reproducible and easier:
  - Size of the agar plate: 150 mm
  - Depth of the media
  - Media composition: **Mueller-Hinton agar**
  - The number of organisms spread on the agar plate
  - Size of the disks: 6 mm
  - Concentrations of antibiotics in the disks
  - Incubation temperature: 37ºC

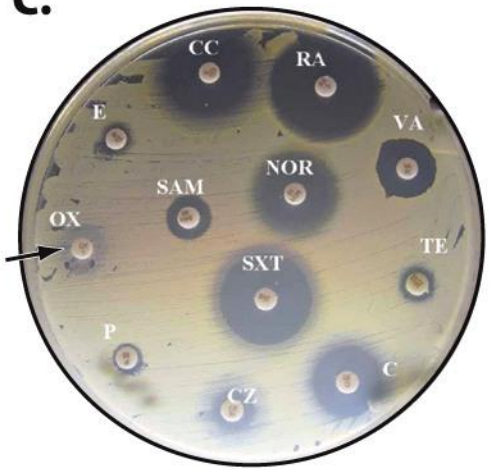
The Kirby-Bauer disk susceptibility test

A.

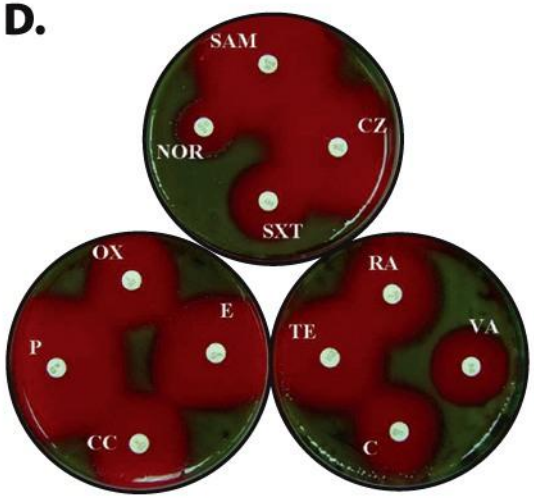
B.



C.



D.

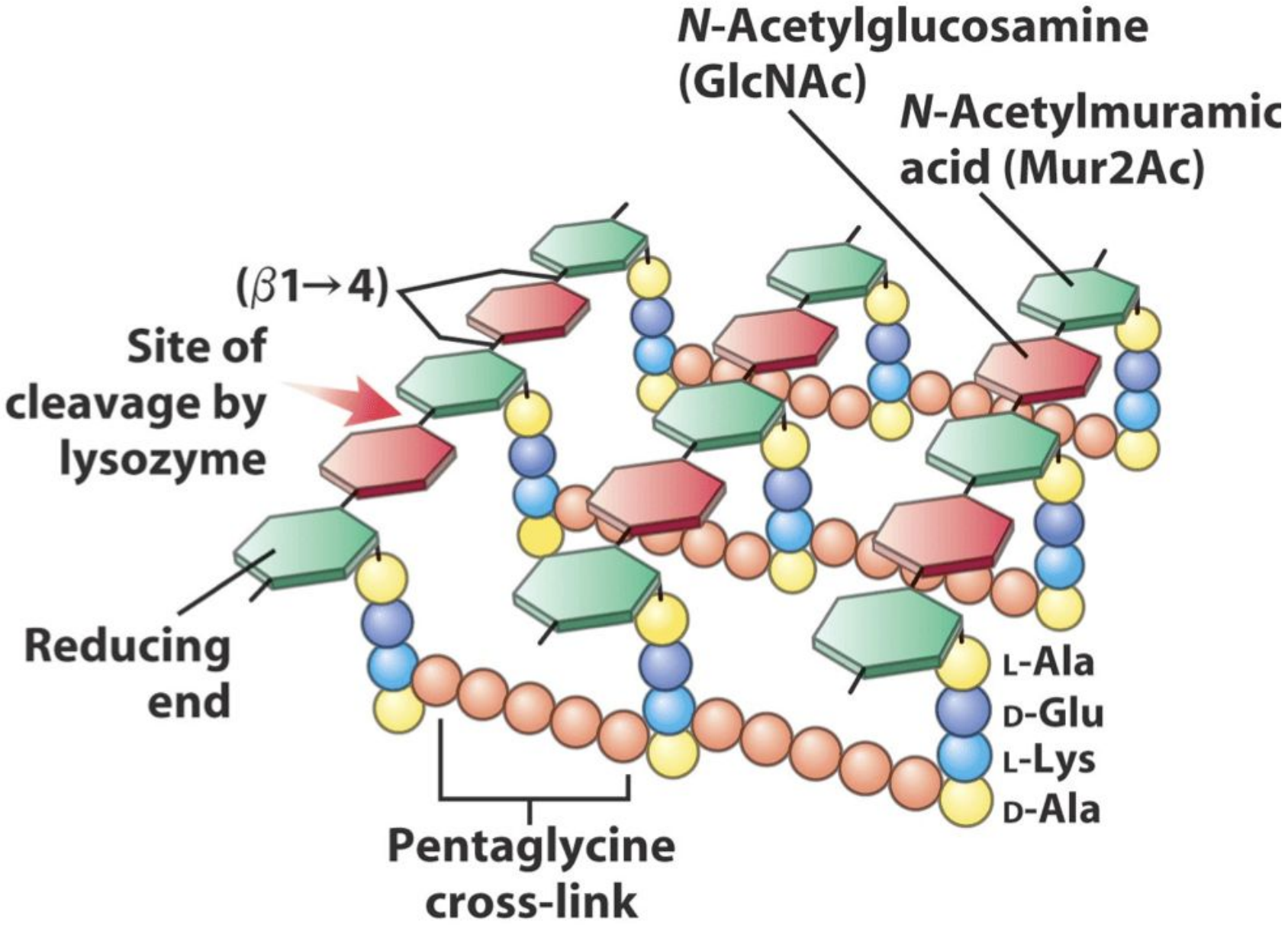


Microbiology: An Evolving Science, Third Edition Figure 27.5

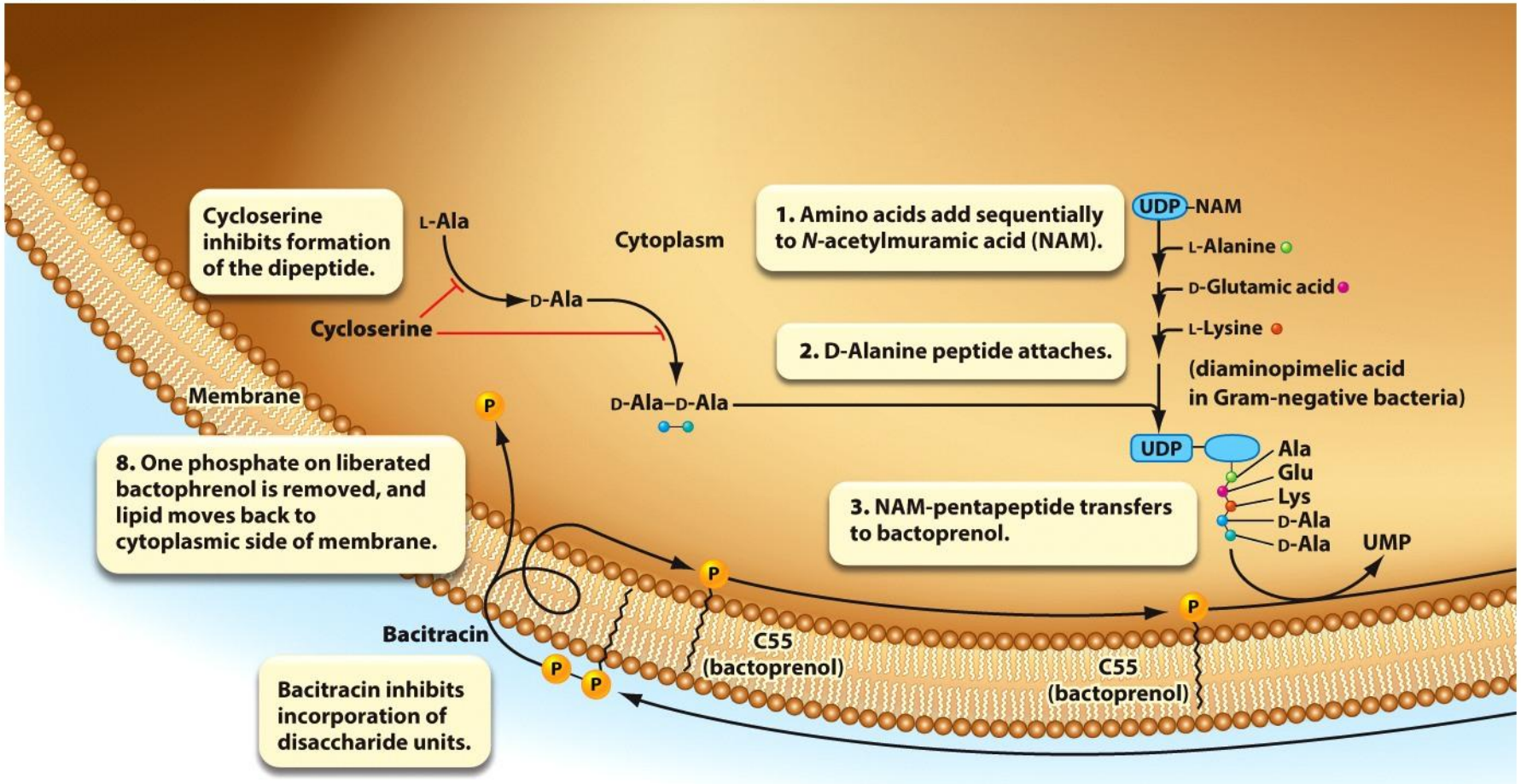
Figure 27.5

Cell Wall Antibiotics

- Peptidoglycan synthesis is rather complex.
  - However, it may be summarized in these four steps:
    1. Precursors are made in the cytoplasm.
       - **UDP-NAG and UDP-NAM-peptide**
    2. They are carried across the cell membrane by a lipid carrier: **bactoprenol**.
       - The carrier is then recycled.
    3. The precursors are polymerized to the existing cell wall structure by **transglycosylases**.
    4. The peptide side chains are cross-linked by **transpeptidases**.


N-Acetylglucosamine
(GlcNAc)
N-Acetylmuramic
acid (Mur2Ac)
(β1→4)
Site of
cleavage by
lysozyme
Reducing
end
L-Ala
D-Glu
L-Lys
D-Ala
Pentaglycine
cross-link

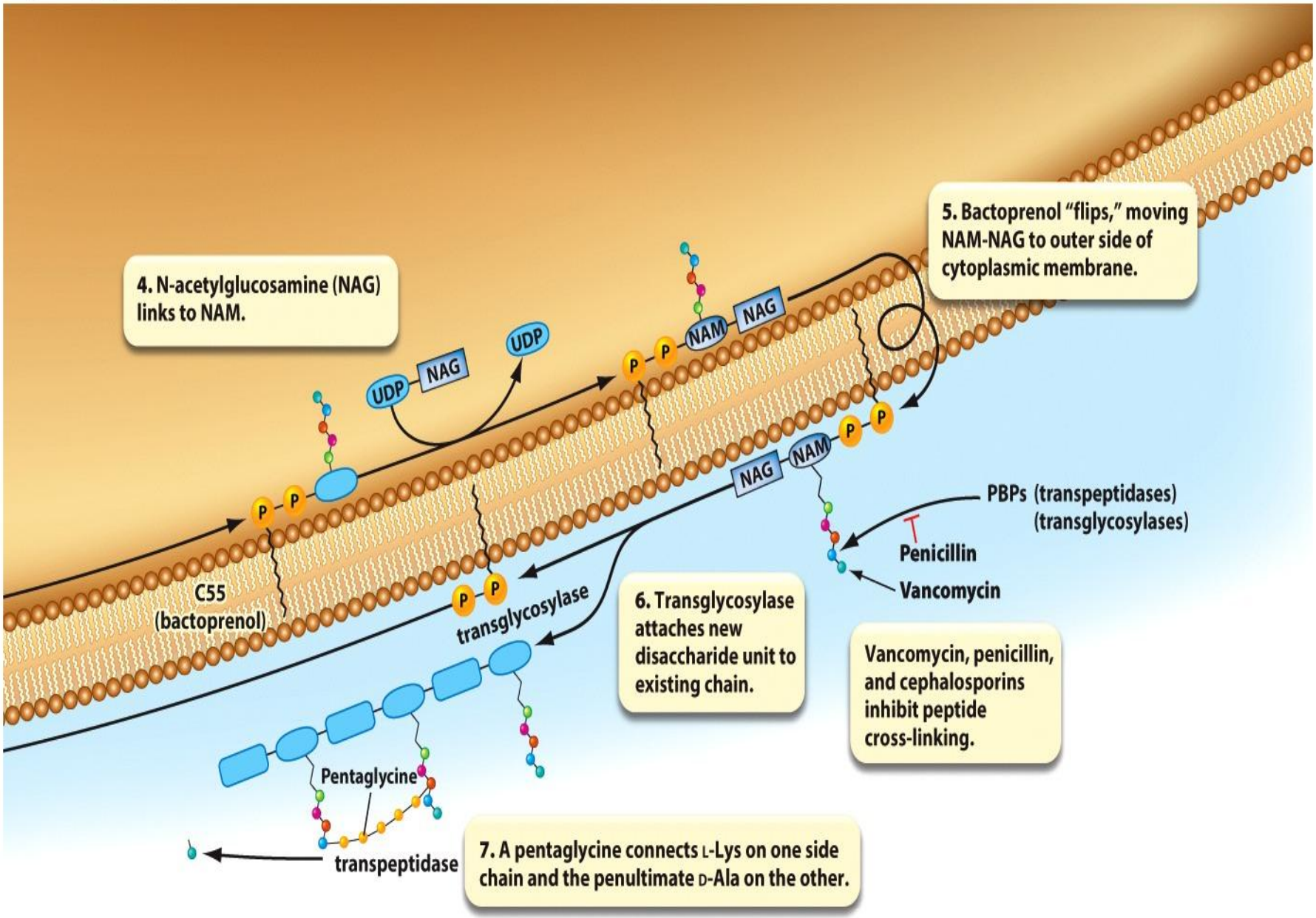
Peptidoglycan synthesis in a Gram-positive bacterium, and targets of antibiotics



Microbiology: An Evolving Science, Third Edition Figure 27.7a

Figure 27.7

Peptidoglycan synthesis in a Gram-positive bacterium, and targets of antibiotics



Microbiology: An Evolving Science, Third Edition Figure 27.7b

Beta-Lactam Antibiotics

- **Penicillins, cephalosporins**
  - The beta-lactam ring chemically resembles the D-Ala-D-Ala piece of peptidoglycan.
  - **This molecular mimicry allows the drug to bind transpeptidase and transglycosylase (which is why the proteins are called penicillin-binding proteins**).
    - Thus, preventing their activities and halting synthesis of the chain
  - R groups can be modified to generate a number of semisynthetic drugs.

Other Antibiotics That Inhibit Synthesis of the Cell Wall

- **Vancomycin:** binds ends of peptides
  - 📫 Prevents action of t**ransglycosylases** and **transpeptidases**
  - 📫 Same step as penicillin, but different activity
- **Cycloserine:** inhibits formation of the D-ala-D-ala dipeptide precursor
- **Bacitracin:** blocks the lipid carrier
  - 📫 Disaccharide subunits do not reach periplasm

Drugs That Disrupt Cell Membranes

- **Gramicidin**
  - Cyclic peptide produced by ***Bacillus brevis***
  - Forms a cation channel, through which ions leak

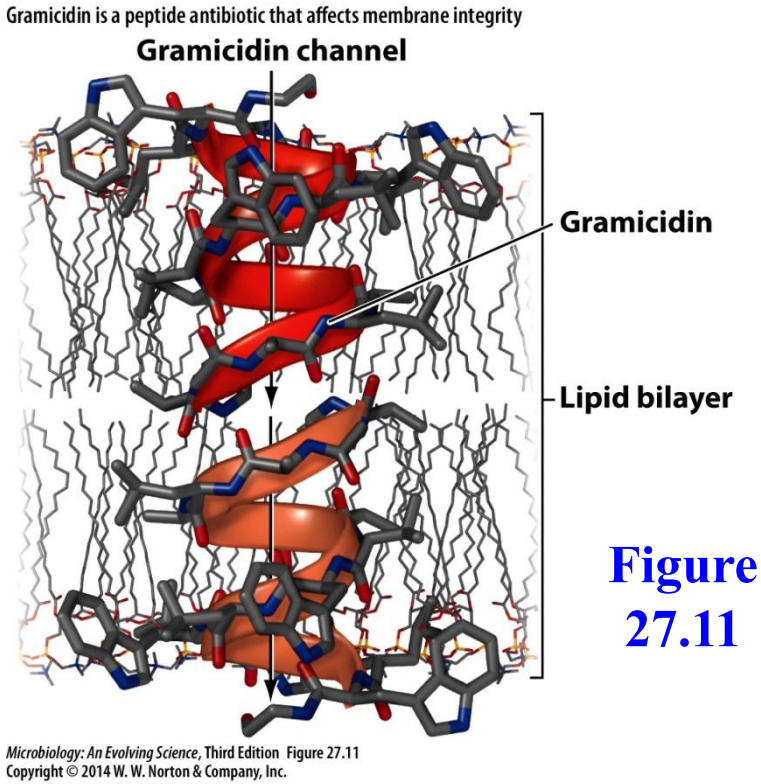
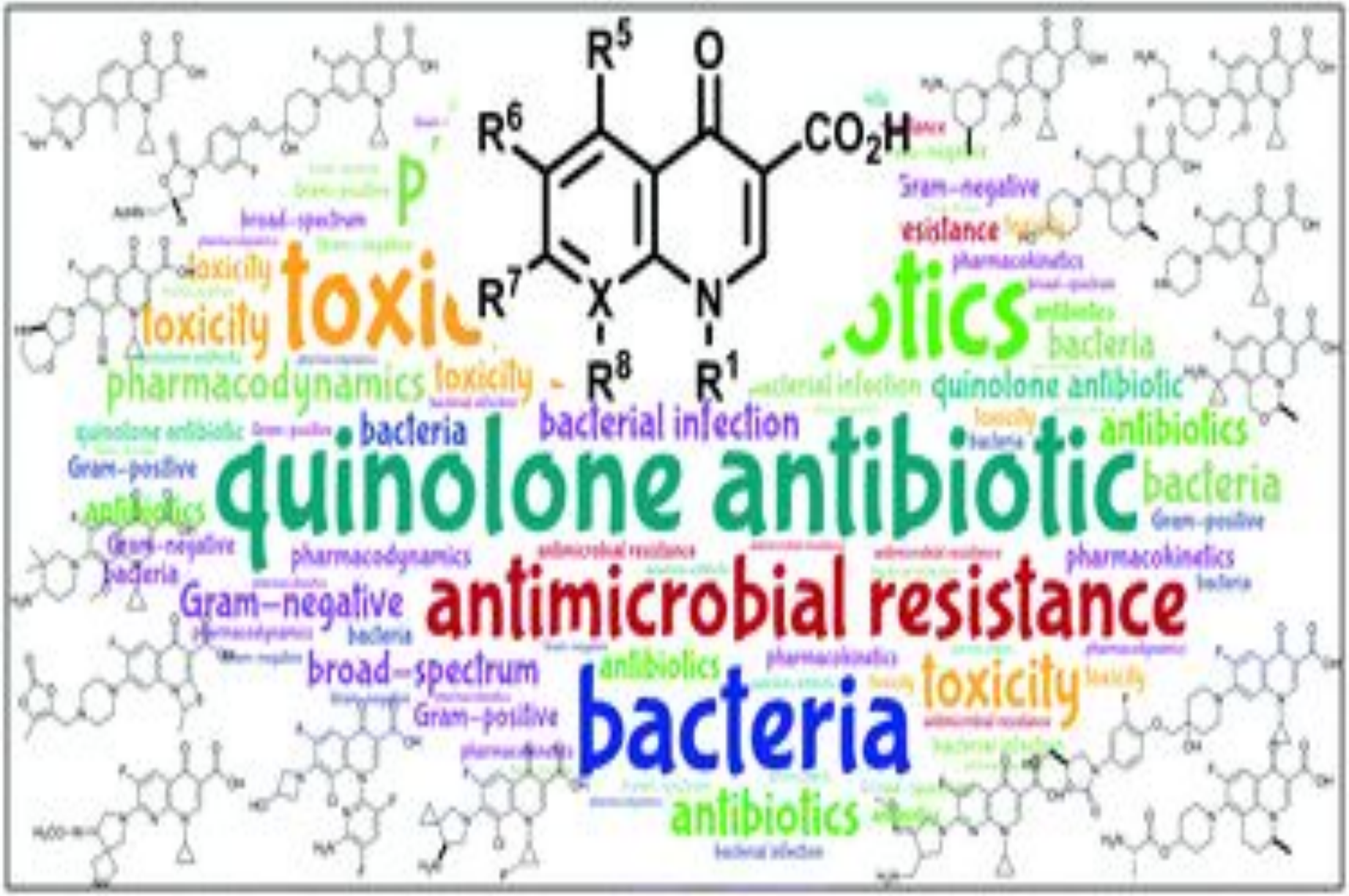


Figure 27.11

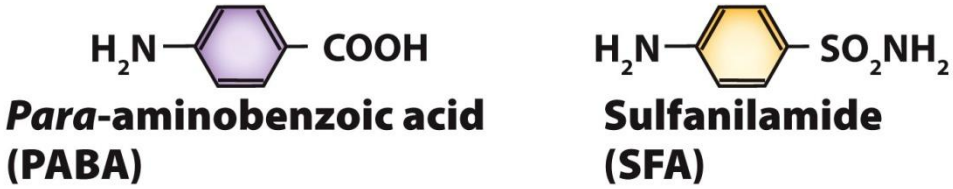
- **Polymyxin**
  - Produced by ***Bacillus polymyxa***
  - Destroys cell membrane, just like a detergent
  - Used only topically

Drugs That Affect DNA Synthesis and Integrity

- **Quinolones: nalidixic acid, ciprofloxacin**
  - **Block bacterial DNA gyrase**, and so prevent DNA replication
- **Metronizadole**
  - Nontoxic, unless metabolized by **anaerobe ferredoxin**
- **Sulfa drugs**
  - **Analogs of PABA, a precursor of folic acid**
    - Needed for DNA synthesis
    - Supplied in our diet, thus no folic acid synthesis to inhibit

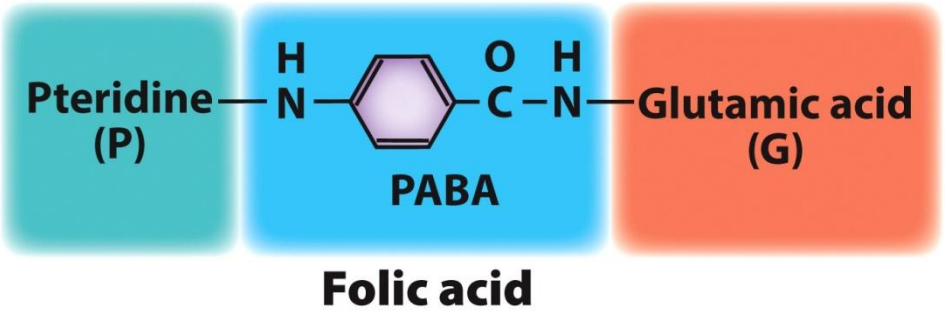

R5
O
R6
CO2H
R7
X
N
R8
R1
quinolone antibiotic
antimicrobial resistance
bacteria
toxicity
antibiotics
pharmacodynamics
pharmacokinetics
bacterial infection
Gram-negative
Gram-positive
broad-spectrum
quinolone antibiotic
bacteria
antibiotics
toxicity
Gram-negative
Gram-positive
broad-spectrum
antimicrobial resistance
pharmacodynamics
pharmacokinetics
bacterial infection

Mode of action of sulfanilamides



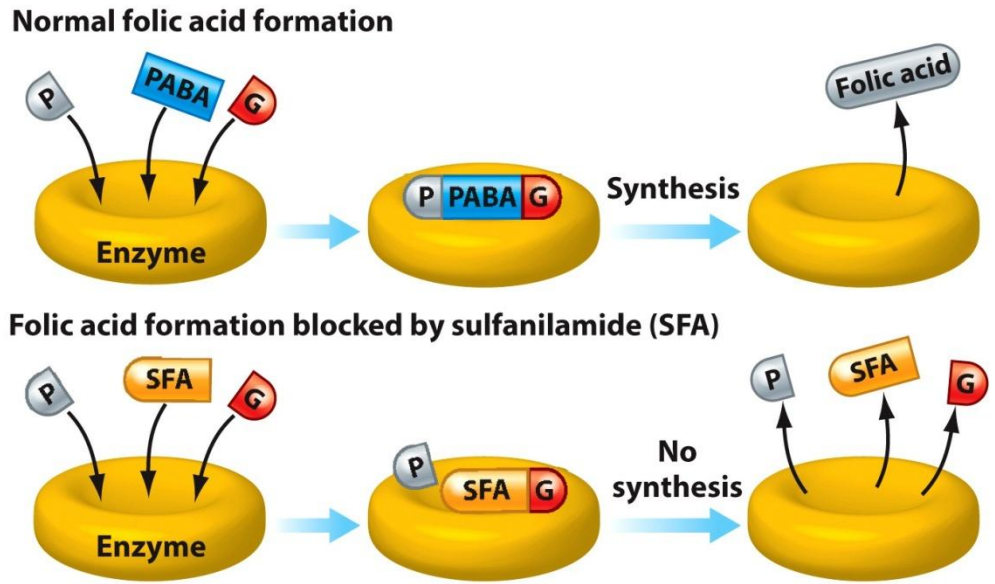
Microbiology: An Evolving Science, Third Edition Figure 27.12a

Mode of action of sulfanilamides



Microbiology: An Evolving Science, Third Edition Figure 27.12b

Mode of action of sulfanilamides



Microbiology: An Evolving Science, Third Edition Figure 27.12c

Figure 27.12

FOLIC ACID FAST FACTS

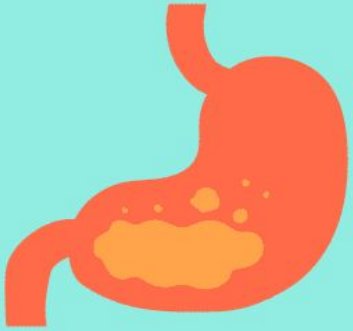
Folic acid is a B vitamin that our body uses to make new cells. Multivitamins are a great source of folic acid.

It is recommended that women take 400 micrograms (mcg) of folic acid every day.

To meet the daily recommended amount of folic acid, women can eat a bowl of breakfast cereal that has 100% of the daily value of folic acid per serving.

Learn more at NBDPN.org

How Folic Acid Is Used in the Body

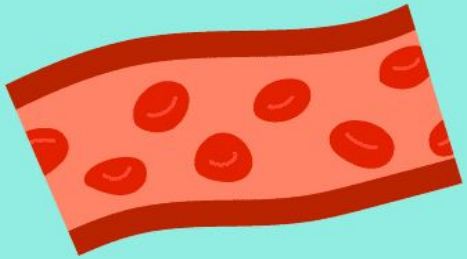


Digestion

Brain and nerve function

Liver, heart, and other organ health

DNA creation and repair



Proper blood cell function

Healthy hair, skin, and nails

RNA Synthesis Inhibitors

- **Antibiotics that inhibit transcription are bactericidal and most active against growing bacteria**
- **Rifampin**
  - Binds to the beta subunit of RNA polymerase
  - Prevents the elongation step of transcription
- **Actinomycin D**
  - Prevents the initiation step of transcription
  - Binds to DNA from any source
    - Thus, not selectively toxic

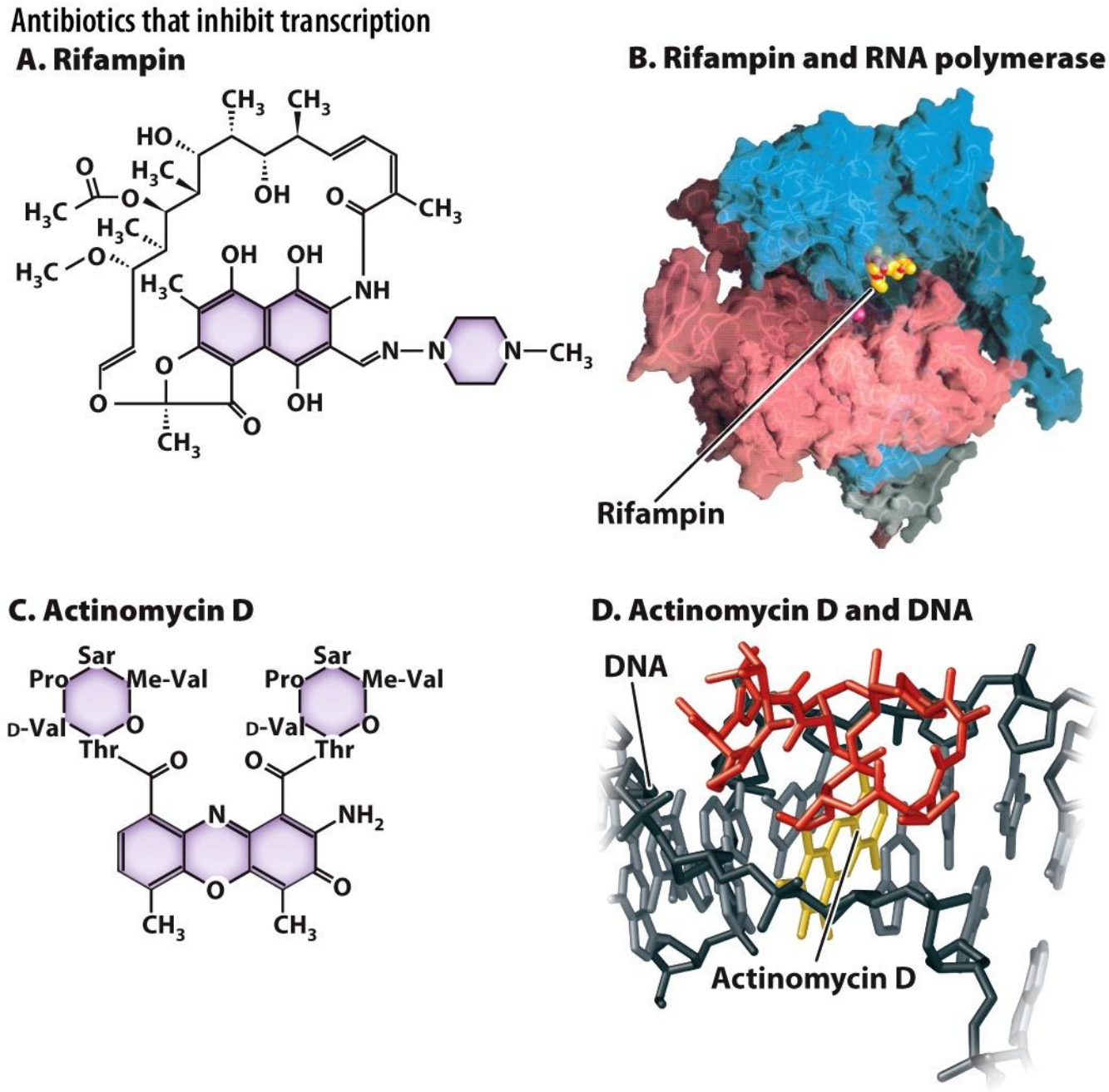


Figure 27.14

Microbiology: An Evolving Science, Third Edition Figure 27.14

Protein Synthesis Inhibitors

- **Drugs that affect the 30S subunit**
  - **Aminoglycosides** cause the translational misreading of mRNA
    - **Are bactericidal**
    - **Include streptomycin**
  - **Tetracyclines:** block the binding of charged tRNAs to the A site of the ribosome
    - **Are bacteriostatic**
    - **Include doxycycline**

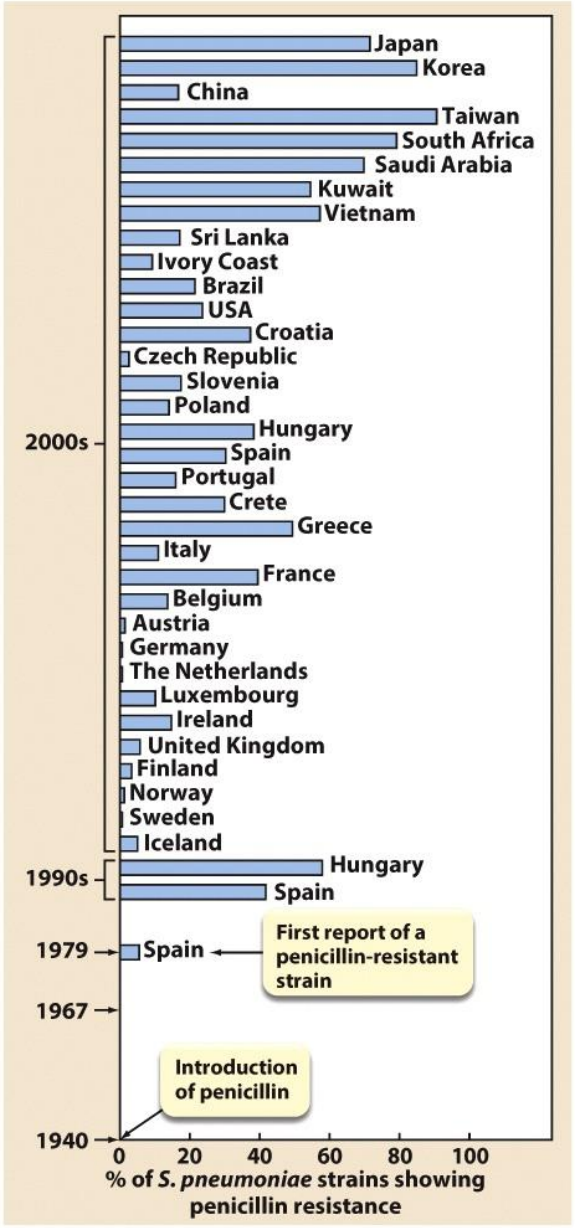
Protein Synthesis Inhibitors

- **Drugs that affect the 50S subunit**
  - **Macrolides:** inhibit translocation
  - **Lincosamides:** inhibit translocation
  - **Chloramphenicol:** inhibits peptidyl transferase activity
  - **Oxazolidinones:** prevent formation of the 70S ribosome initiation complex
  - **Streptogramins**
    - Streptogramin A: blocks tRNA binding
    - Streptogramin B: blocks translocation

Challenges of Drug Resistance

- Antibiotics are considered **secondary metabolites** because they often have no apparent primary use in the producing organism.
  - Not essential for survival
  - But enhance ability to survive competition
- Microbes prevent self-destruction by means of various **antibiotic resistance mechanisms**.
  - **Example: make enzymes to disable antibiotics**
  - **Genes encoding some of these drug-resistance mechanisms have been transferred to pathogens.**

The rise of penicillin-resistant *Streptococcus pneumoniae* throughout the world



Microbiology: An Evolving Science, Third Edition Figure 27.17

- **Antibiotic resistance is a growing problem worldwide**
  - Antibiotics are overused
  - Overprescribed; used in farm animal feed
- This exerts selective pressure for drug-resistant strains
  - ***Streptococcus pneumoniae***
  - ***Acinetobacter baumanii***
    - **Resistant to multiple drugs**

- There are four basic forms of antibiotic resistance

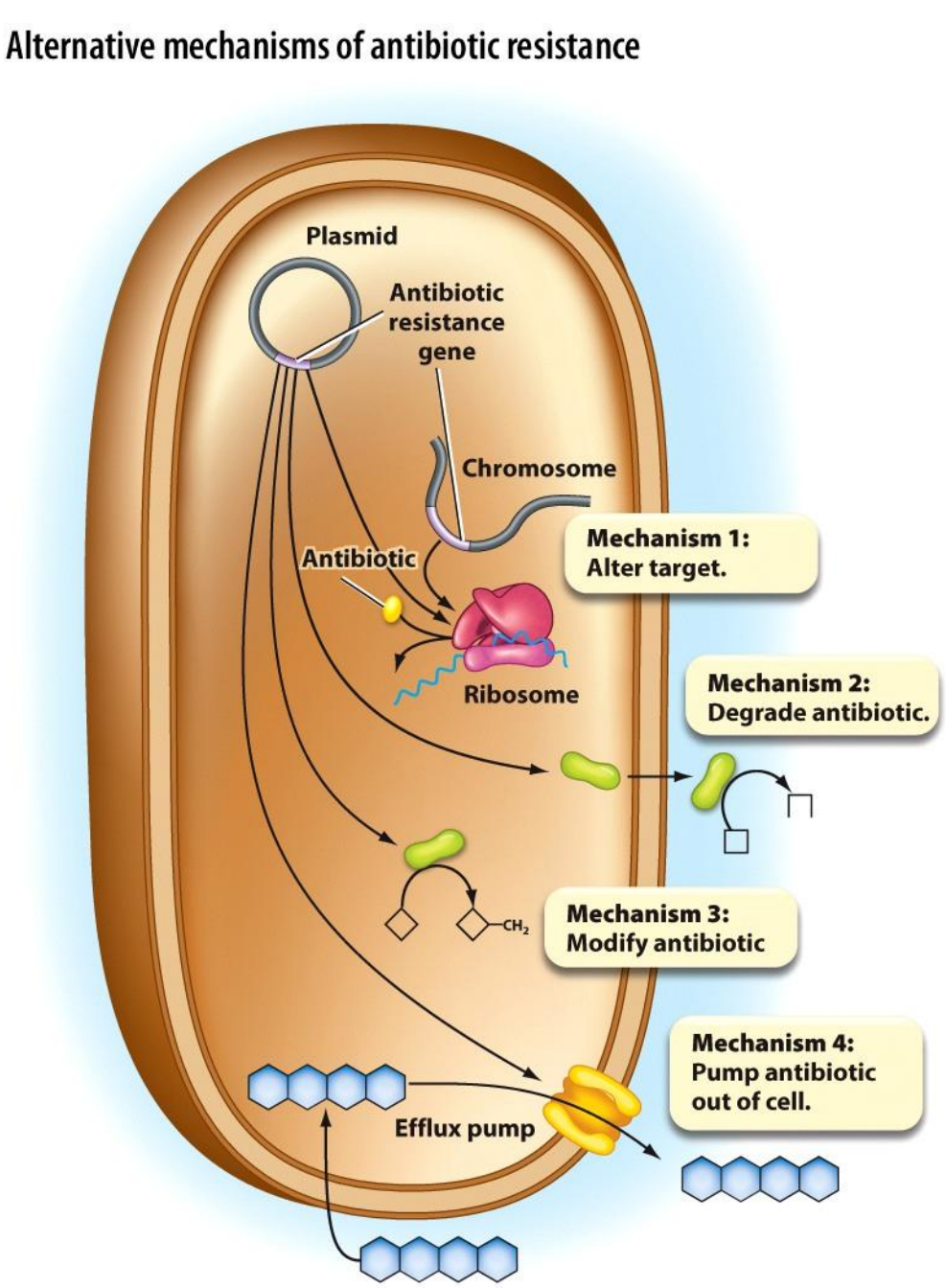


Figure 27.18

Antibiotic-Resistance Mechanisms

- **Modify the target so that it no longer binds the antibiotic.**
  - Mutations in ribosomal proteins confer resistance to streptomycin.
- **Destroy the antibiotic before it gets into cell.**
  - The beta-lactamase enzyme specifically destroys penicillins.

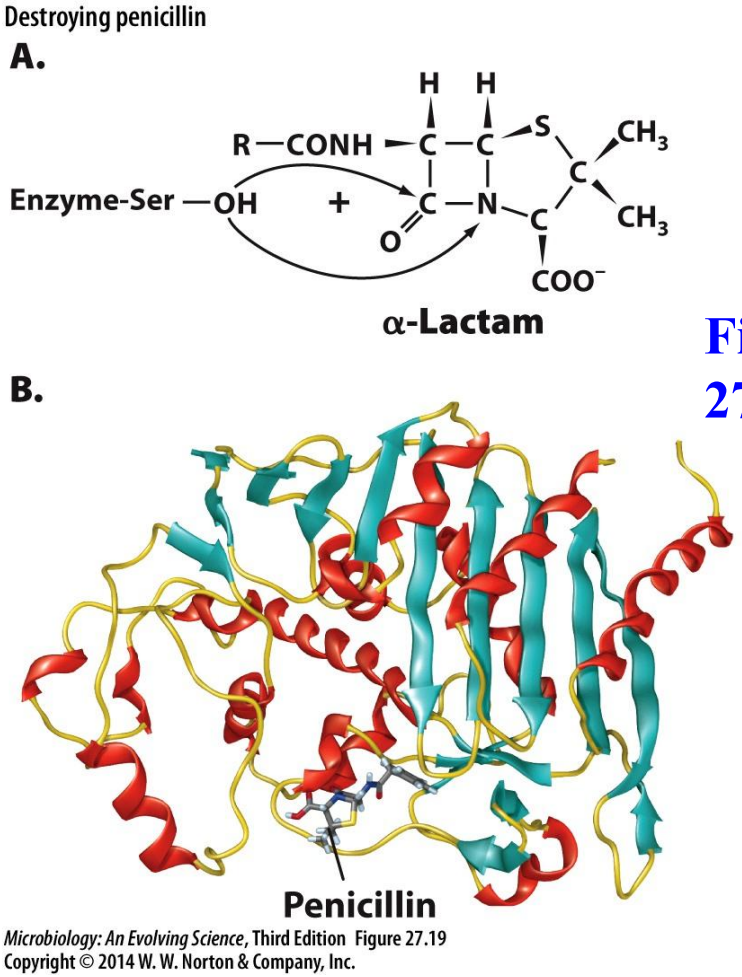


Figure 27.19

Microbiology: An Evolving Science, Third Edition Figure 27.19

Antibiotic Resistance Mechanisms

- Add modifying groups that inactivate the antibiotic.
  - Three classes of enzymes are used to modify and inactivate the aminoglycoside antibiotics.

- **Pump the antibiotic out of the cell.**
  - Specific and nonspecific transport proteins
  - Similar strategy is used in cancer cells.

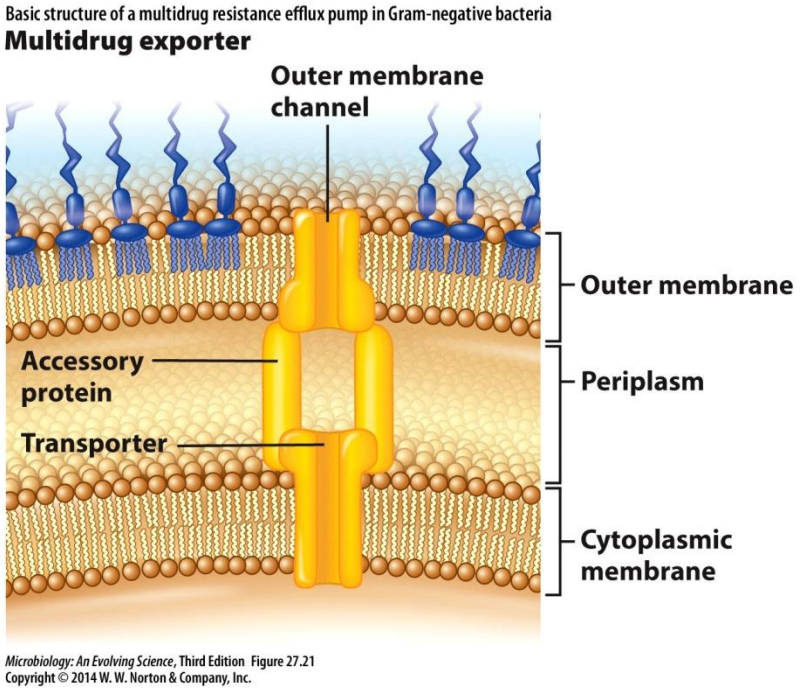


Figure 27.21

How Does Drug Resistance Develop?

- De novo antibiotic resistance develops through gene duplication and/or mutations.
- Can be acquired via horizontal gene transfer:
  - **Conjugation**
  - **Transduction**
  - **Transformation**
- Recently, multidrug resistance has also been attributed to the presence of integrons.

Integrons

- **Integrons** are genetic mechanisms that allow bacteria to adapt and evolve rapidly through the stockpiling and expression of new genes. These genes are embedded in a specific genetic structure called gene cassette that generally carries one promoterless open reading frame together with a recombination site.

The Future of Drug Discovery

- Evolutionary pressure is constant.
  - Requires constant search for new antibiotics
- The modern drug discovery process is outlined as such:
  - **Identify new targets using genomics.**
  - **Design compounds to inhibit targets.**
  - **Alter compound structure to optimize MIC.**
  - **Determine spectrum of compound.**
    - **Narrow or broad?**
  - **Determine pharmaceutical properties.**
    - **Not toxic to animals; persistence in body**

Antibiotics from the sea

The hero marine **bacteria**, Planctomycetes, naturally produce antibiotic compounds to fight against other **bacteria**. Thanks to Jogler's lab work, a whopping 79 new cultures of Planctomycetes could pave the way for a new source of antibiotics and help those who suffer from antibiotic-resistant infections.Dec 13, 2019

- Potential targets for rational antimicrobial drug design include proteins expressed only in vivo or proteins expressed both in vivo and in vitro.
- Candidate antimicrobial compounds can be designed to bind and inhibit the active site of a known enzyme
- Combinatorial chemistry is used to make random combinations of compounds that can be tested for enzyme inhibitory activity and antimicrobial activity.
- Intriguing ideas that may lead to novel antimicrobial therapies include:
  - Nanotubes to poke holes in bacterial cell membranes
  - Molecules that “cork” the type III secretion apparatus
  - Interfering with the quorum-sensing mechanisms

Methods to Identify Drug-Resistant Pathogens

- The proportion of antibiotic-resistant infections has doubled since 2002, rising from 5.2% to 11% of all infections.
- The faster a clinical lab can identify a pathogen's antibiotic susceptibility, the more quickly a clinician can prescribe an appropriate narrow-spectrum antibiotic.
  - **Traditional MIC tests take up to 3 days to complete; using automated methods cuts this down to 2 days.**
  - **Multiplex PCR platforms can detect pathogen-specific or drug resistance gene DNA sequences within an hour.**

Evolving, and Sharing, Drug Resistance Genes

- De novo antibiotic resistance develops through gene duplication and/or mutations.
- Antibiotic resistance also can be acquired via horizontal gene transfer (conjugation, transduction, and transformation).
  - A study in 2015 found that antibiotic-resistant microbes occur naturally in uncontacted Amazon communities.
  - **Recently, multidrug resistance has been attributed to the presence of highly-mobile gene expression elements called integrons.**

How Did We Get into This Mess? – 2

- Another proposed source of antibiotic resistance is the widespread practice of adding antibiotics to animal feed.
  - **Giving animals subtherapeutic doses of antibiotics in their food makes for larger, and therefore more profitable, animals.**
  - **Some estimates suggest that 80% of all antibiotics used in the United States (up until 2017) were fed to healthy livestock.**
  - **Feeding growth-promoting antibiotics to cattle can stimulate the spread of pathogenicity genes between bacteria.**

How Did We Get into This Mess? – 3

- Many of these situations have conspired to produce incredibly dangerous bacteria resistant to almost every antibiotic known.
- **ESKAPE pathogens**
  - Term coined by the Infectious Diseases Society of America
  - Six highly resistant bacterial species that collectively cause about two-thirds of all U.S. nosocomial infections

Enterococcus faecium
Staphylococcus aureus
Klebsiella pneumoniae
Acinetobacter baumannii
Pseudomonas aeruginosa
Enterobacter sp.

How Did We Get into This Mess? – 4

SIRIRAT SHUTTERSTOCK

How Did We Get into This Mess? – 5

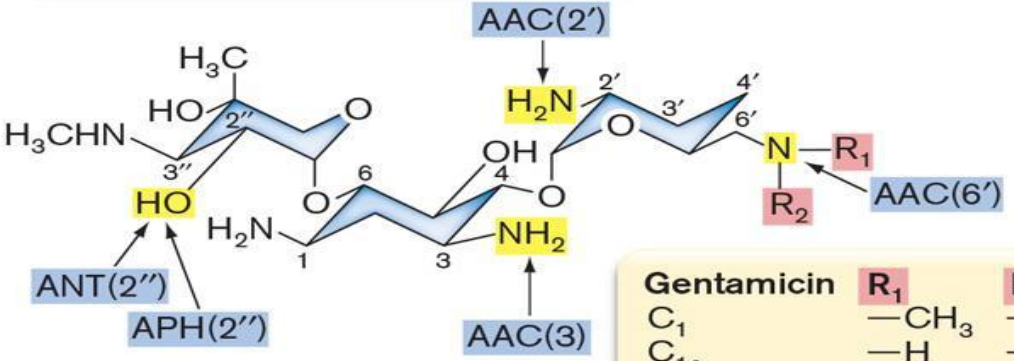
- When should antimicrobials be used? **Antibiotic stewardships** are coordinated interventions that improve and measure antibiotic use.
  - Do not use antibiotics to treat viral infections.
  - Do not use an antibiotic if a patient's microbiome includes a strain that is resistant to the drug.
  - Know which antibiotic resistant strains are prevalent in the community or hospital before prescribing.
  - Consider how long the patient needs to take the antibiotic.
  - De-escalate antibiotic usage whenever possible.

Biofilms, Persisters, and the Mystery of Antibiotic Tolerance

- Why do some infections return after bactericidal antibiotic treatment is discontinued?
- A subpopulation of dormant organisms, called **persister cells**, often arises within a population of antibiotic-susceptible bacteria.
  - The stalled metabolism of persisters renders them **tolerant to bactericidal antibiotics during treatment**.
  - Persister cells can be **found in any biofilm or population of late-exponential-phase cells.**
  - Tolerance **provides antibiotic resistance at the price of not growing.**

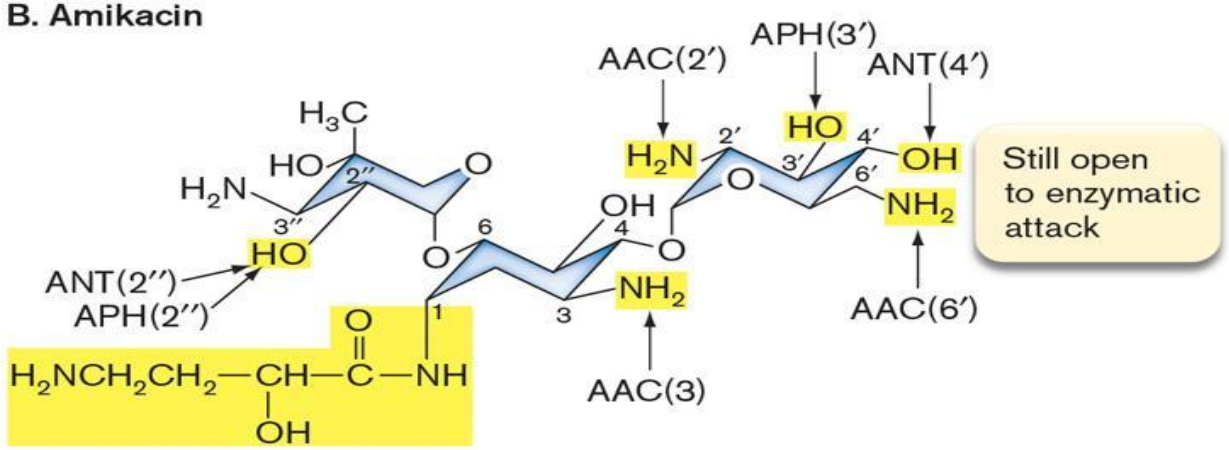
A. Gentamicin

The yellow-highlighted sites are all open to attack from aminoglycoside-modifying enzymes.



Gentamicin	R_1	R_2
C_1	$-CH_3$	$-CH_3$
C_{1a}	$-H$	$-H$
C_2	$-CH_3$	$-H$

B. Amikacin



Still open to enzymatic attack

This side chain protects amikacin from attack by AAC(3,2′), APH(3′,2″), and ANT(2″) by steric hindrance.

Fighting Resistance and Finding New Drugs – 1

- The prudent use of current antibiotics and innovative strategies for finding new ones hopefully will enable us to continue to control evolving pathogens.

1. Directly countering drug resistance

   — Dummy target compounds inactivate resistance enzymes (e.g., clavulanic acid)

   — Alter antibiotic's structure so that it sterically hinders access of bacterial modifying enzymes

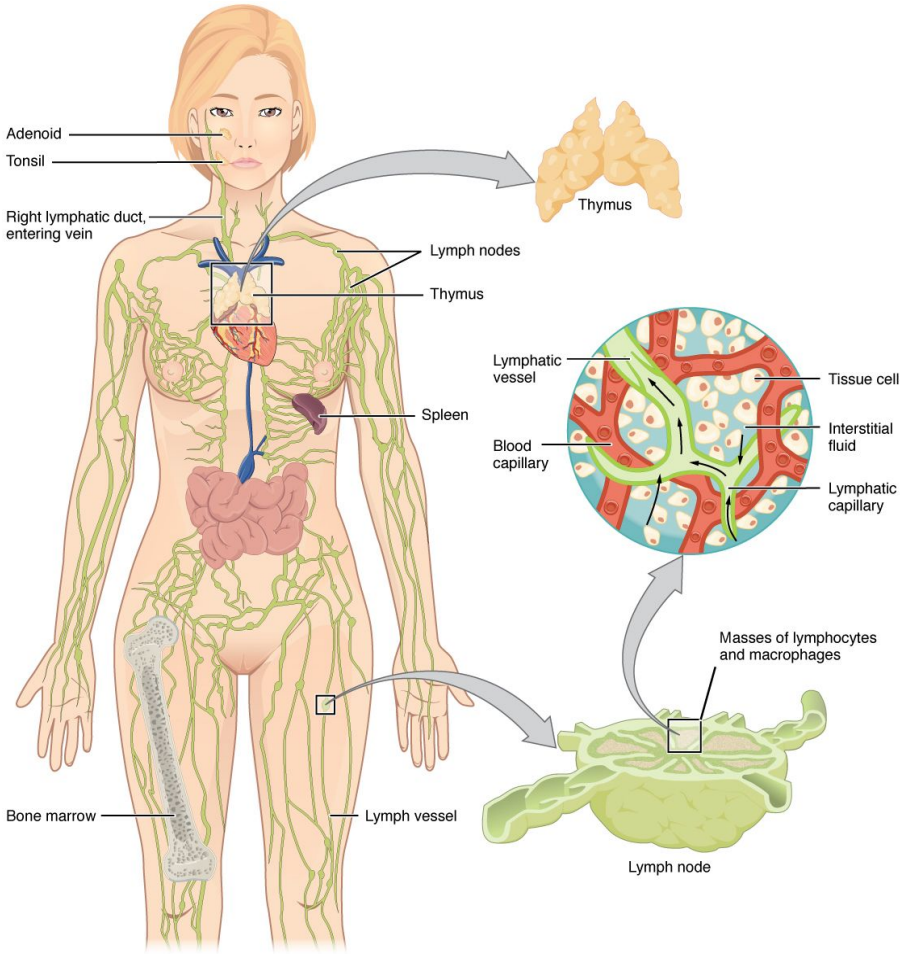
Fighting Resistance and Finding New Drugs – 2

2. Finding new antibiotics
   - — Brute-force screening of microbes, plants, and animals
   - — Combinatorial chemistry
   - — Genome sequence analysis to identify potential bacterial molecular targets
   - — Photosensitive chemicals
   - — Interfering with quorum-sensing mechanisms
   - — CRISPR-based strategies for reversing antibiotic resistance

Fighting Resistance and Finding New Drugs – 3

3. Antipersister and antibiofilm approaches
   - Kill persisters directly
   - Prevent persister formation
   - Interfere with biofilm formation
   - Induce biofilm dispersal

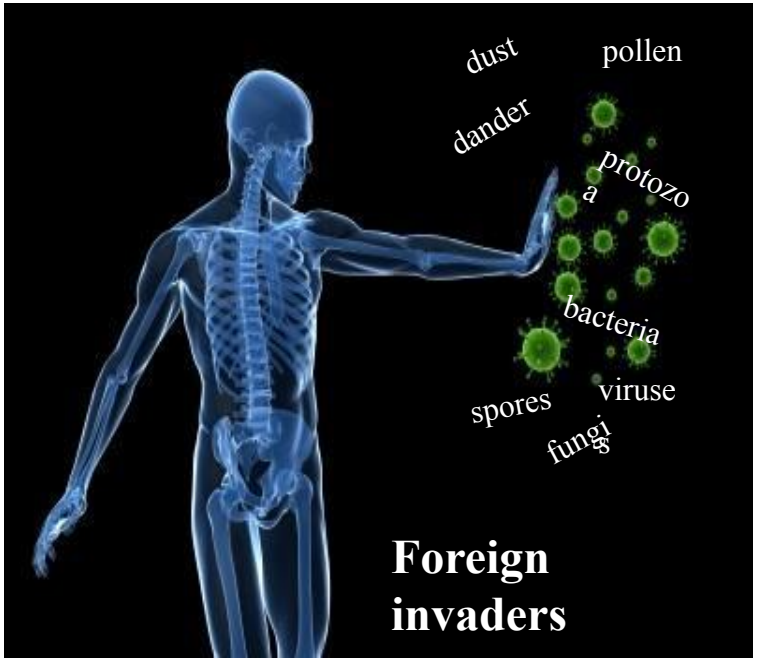
- The progression of these strategies has been slow because the road to FDA approval, though necessary for safety reasons, is long (8 to 10 years) and expensive, and it can discourage some companies from investing in antibiotic discovery.



Introduction to the Immune System

The Innate and Adaptive Immunity

What is the immune system?



The immune system is a system of defense to protect the body against foreign invaders (foreign antigens)

- **Exterior defense**: Physiological barriers, chemical barriers, mechanical barriers, microbiological

Exterior Defense

Barrier types	Example of external barriers
Example of external barriers	Skin covering the body, cilia in the lining of the lungs, nose and throat.
Chemical barriers	Lysozyme in tears, pH, and mucin at mucosal surface
Mechanical barriers	Sneezing, coughing, tear flow, and flushing of urinary tract
Microbiological barriers	Normal microflora, commensal microorganisms in the gut and vagina

Interior or Systemic defense

- In the body there are **cells** and **soluble factors** that deal with foreign antigens, which have come into the body.
- In humans and higher vertebrates there are two lines of internal defense, and distinct cells and soluble factors are involved in each type of immunity, and hence they are divided into:
  1. **Cells and factors of the innate immunity**
  2. **Cells and soluble factors of the adaptive immunity**

Components of the immune system

The immune system is composed of specialized **organs**, **tissues**, **cells** and **soluble factors** that work in concert in order to mount an adequate immunological response.

Organs	Tissues
Thymus **Spleen** **Lymph nodes**	**Bone marrow** **Lymphoid tissues**
Cells	**Soluble Factors**
White blood cells **Red blood cells and platelets are also involved in the inflammation**	**Complement system** **Antimicrobial peptides** **Antibodies** **Cytokines** **Chemotactic factors**

Components of the Immune System

The immune system is composed of lymphoid organs, tissues, cells and soluble factors.

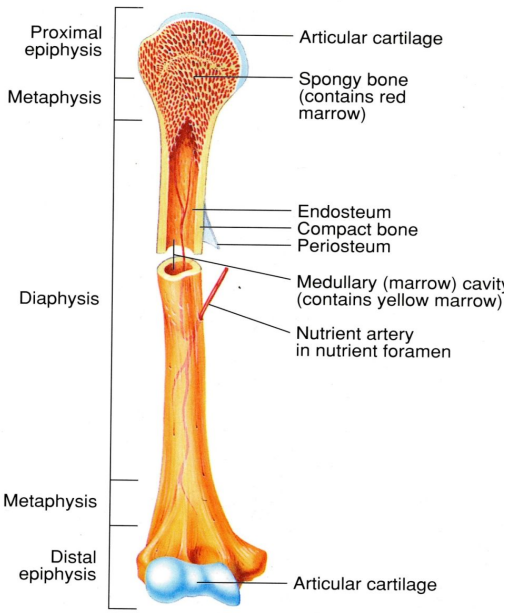
Organs of the immune system and their functions

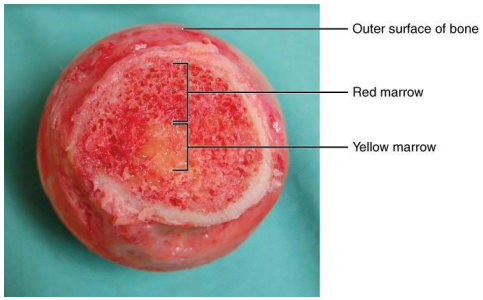
- **Primary lymphoid organ**: **The thymus** is a bilobed organ where immature T cells (also called progenitor T cells) undergo maturation
- **Secondary lymphoid organs include the spleen and lymph nodes.** These organs contain B cells, T cells and macrophages.
  - **The spleen** is the largest secondary lymphoid organ where antigens from the blood are trapped
  - **Lymph nodes** are encapsulated organs that are located at the junction of lymphatic vessels

The Bone Marrow

The bone marrow consists of connective tissue that is contained in the cavity of most bones in the body. There are two types of bone marrow:

- **Yellow marrow rich in fat cells**
- **Red marrow containing developing blood cells**. Red bone marrow is primarily a site of development of all blood cells from a common precursor, the **hematopoietic stem cell** by a process called **hematopoiesis**.
- All blood cell types mature in the bone marrow except for the progenitor T cells, which undergo maturation in the thymus.

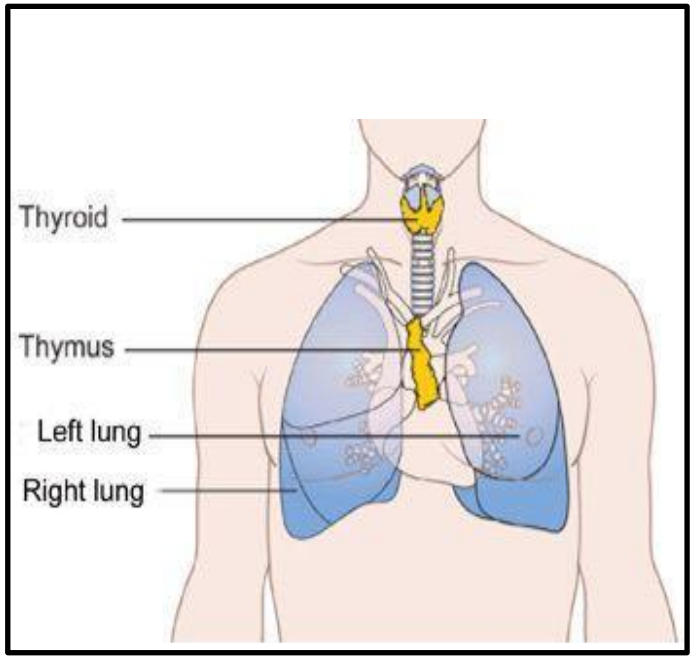


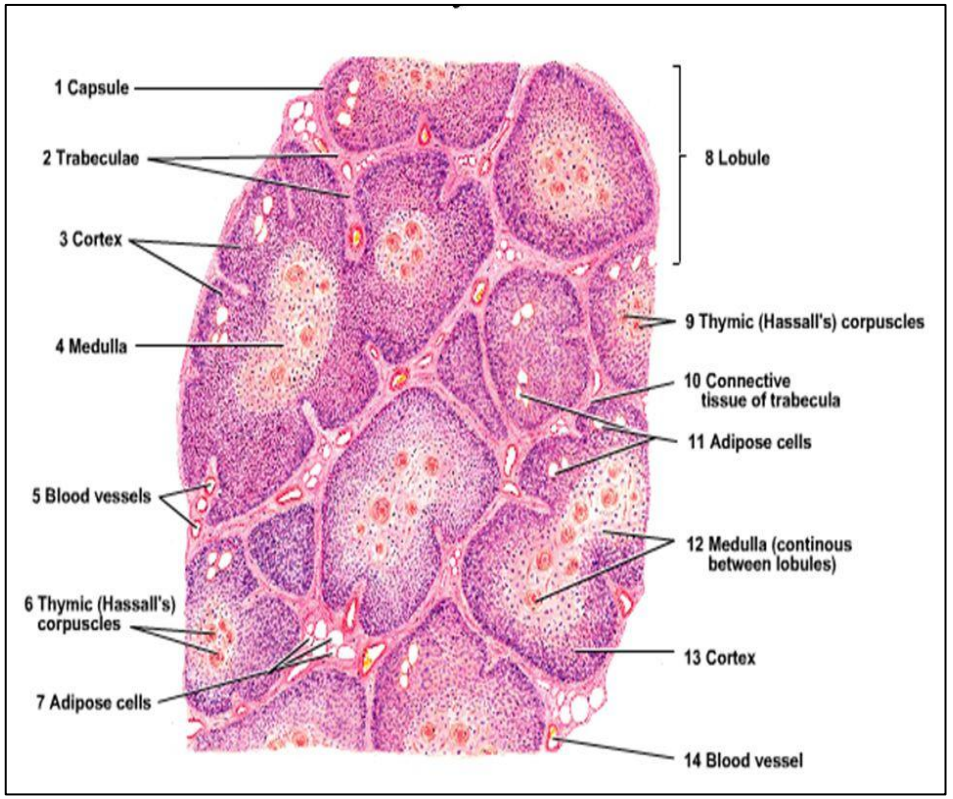


The Thymus

The thymus is an encapsulated bilobed organ that is located in the thoracic cavity, between the chest bone and the heart. The thymus contains lobules, which are organized into 2 regions: a cortex and a medulla. **The cortex contains immature T cells, whereas mature T cells can be found in the medulla**. T cell maturation occurs in the cortical medullary region. It is noteworthy that only around 10 % of the progenitor T cells could reach maturation, indicating that around 90% of progenitor T cells are eliminated during the maturation process. The thymus increases in size after birth, reaching its largest size at puberty, after which time it progressively decreases in size with age. **Thymic involution is associated with reduced thymopoiesis and immunosenescence.**

The thymus





The thymus is a bilobed organ that is divided into lobules. **Immature thymocytes are localized in the vortex, and T cell maturation occurs in the cortical medullary region**

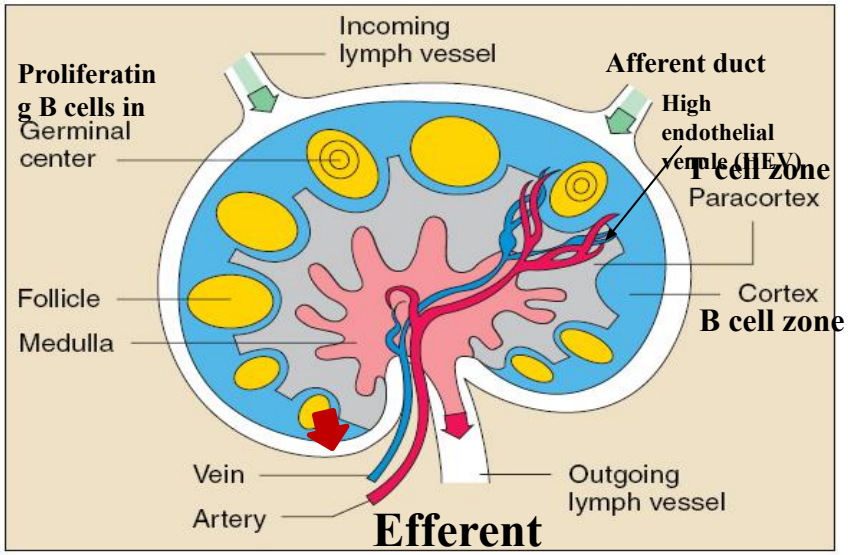
The lymph nodes

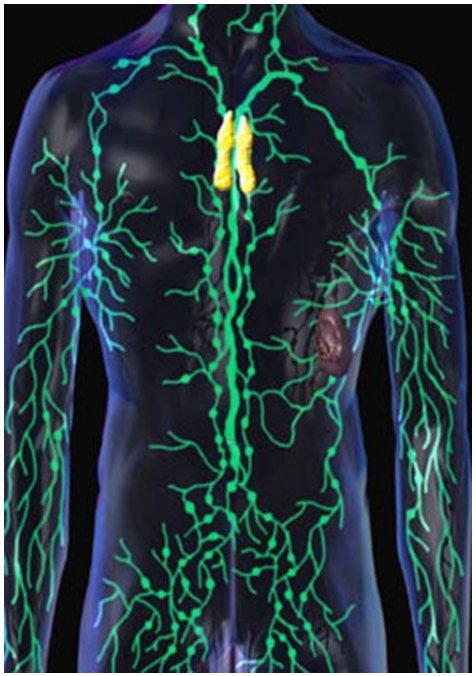
The lymph nodes are small encapsulated organs that are located at major junctions of lymphatic vessels. The lymph node is organized into 3 regions, including the **cortex or B cell zone** that contains **resting B cells in primary follicles and proliferating B cells in germinal centers, the paracortex or T cell zone, and the medulla, where T, B cells, and macrophages can be found.**

Lymph nodes are irrigated by both the blood circulatory system and the lymphatic system. **Blood enters the lymph node via the artery to the arterioles and comes out through the venules and vein.**

The **lymph comes into the lymph node via the afferent vessels or ducts, and out via the efferent duct.** Therefore, lymph nodes trap antigens from both the blood circulatory and lymphatic systems.

Lymph nodes



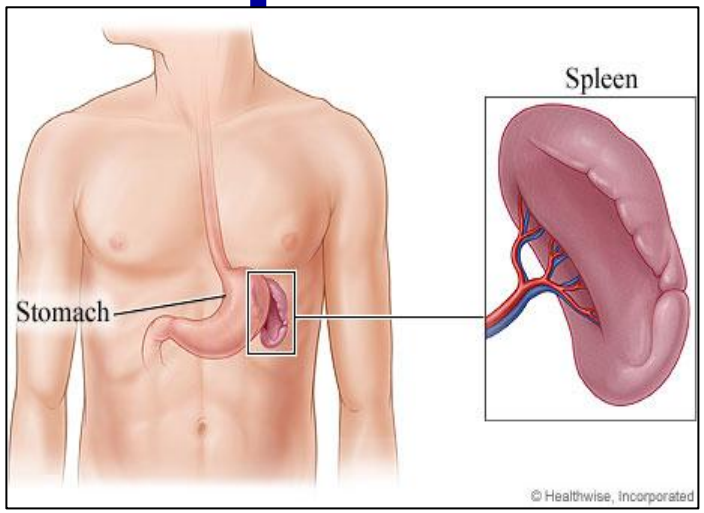


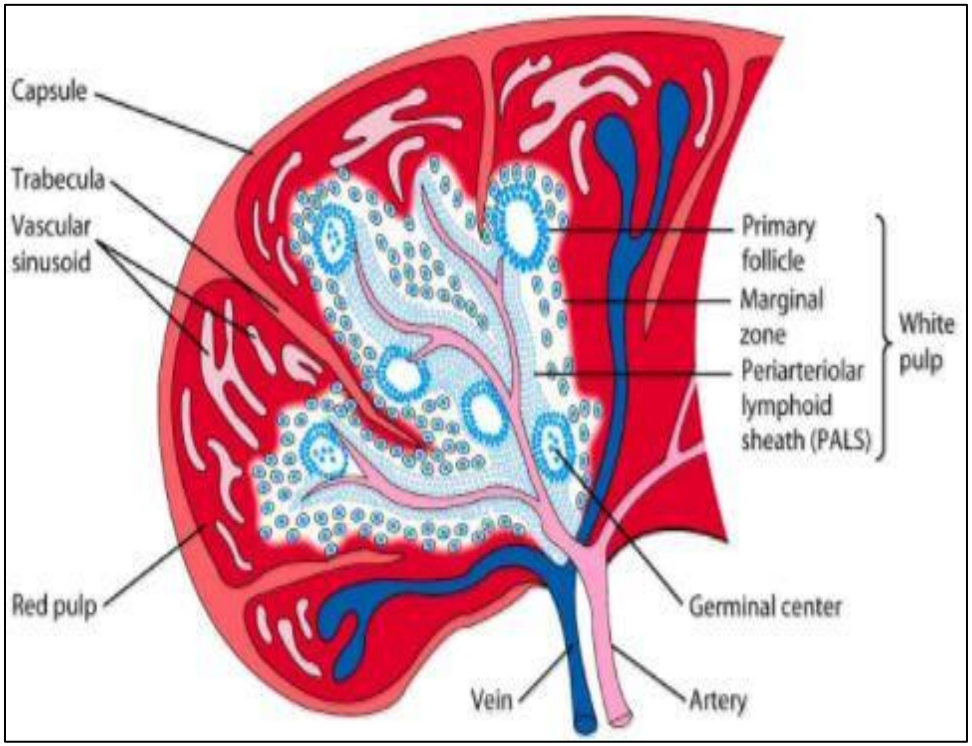
Lymph nodes are encapsulated organs where antigens in both the blood and the lymph are trapped and where immune cells (B and T cells, macrophages, dendritic cells and follicular dendritic cells) are located and ready to interact with each other in order to mount an adequate immunological response

The Spleen

The spleen is the largest encapsulated secondary lymphoid organ. In humans, the spleen is located below the rib cage and to the left of the abdomen. The spleen contains two regions that are designated as the red pulp and white pulp. The red pulp contains worn out red blood cells that are destined for destruction, hence it is referred to as a cemetery for RBCs. The process of removing worn out and dead RBCs is called **hemocatharsis,** meaning cleansing of the blood. The white pulp is rich in white blood cells and it forms a sheath around the central arteriole. The sheath is designated as **periarteriolar lymphoid sheath (PALS),** which is a tissue rich in T cells (mostly CD4+ T cells and CD8+ T cells, and in smaller numbers B cells that are located in follicles and germinal centers, and macrophages that are located in the marginal zone. .

The spleen

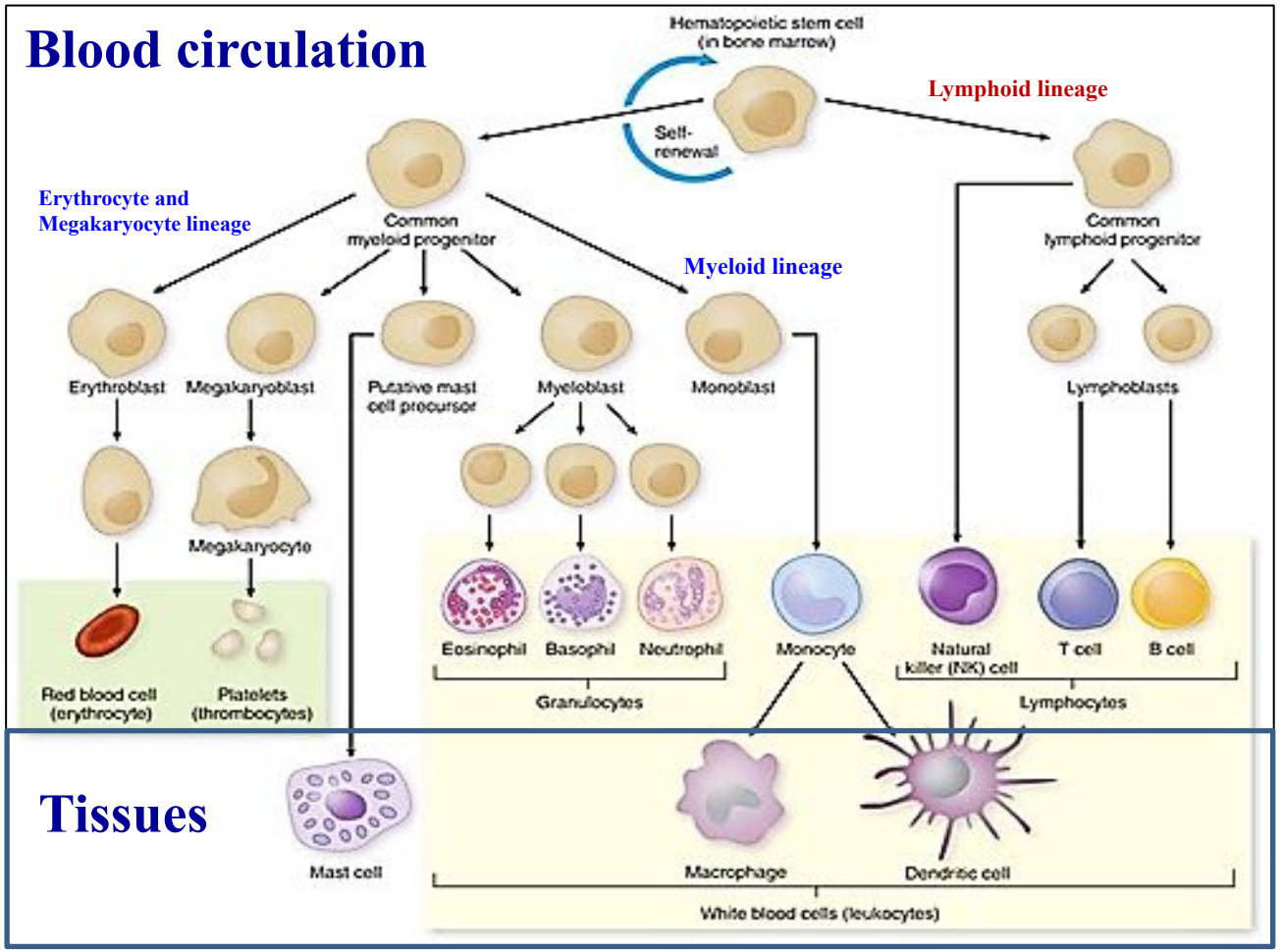




The spleen is the largest secondary lymphoid organ, in which antigens in the blood are trapped Blood circulation gets into the spleen via the artery and arterioles and come out via venules and the vein, and hence the spleen serves as a trap of foreign antigens that are coming from the blood circulation

Hematopoiesis

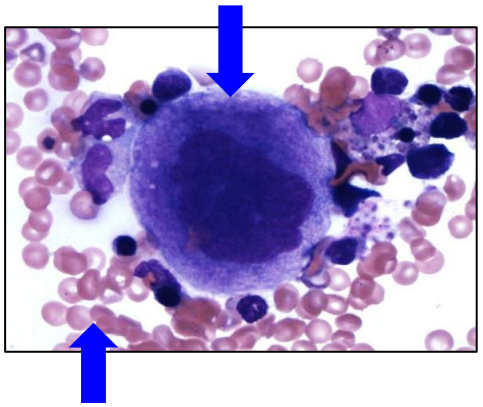
All blood cells are derived from a common precursor, the **hematopoietic stem cell** via three lineages of differentiation: **The lymphoid lineage, the myeloid lineage, and the erythrocyte/megakaryocyte lineage**



- **Non-Immune cells: Erythrocytes & Megakaryocytes.**

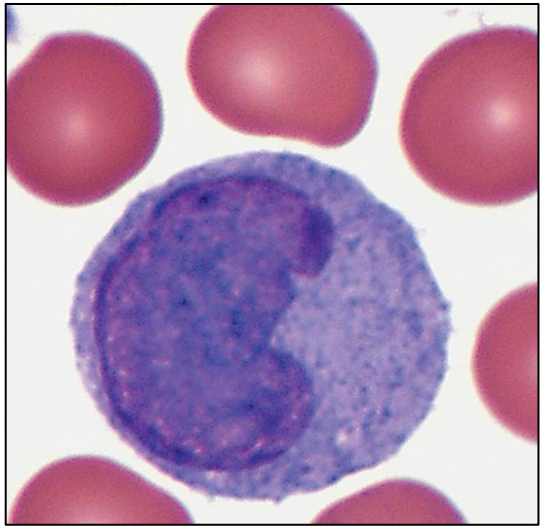
Erythrocytes, also referred to as red blood cells (RBCs) are the most abundant cells in peripheral blood. RBCs do not have a nucleus. These cells are not immune cells, but they play a role in the binding and removal of immune complexes from the blood circulation.

Megakaryocytes are large cells (at least 20 X larger than the RBCs). These cells are the precursors to platelets, and around 4000 platelets can be generated by the fragmentation of one megakaryocyte. Platelets are necessary for blood clotting, and they also play an important role in the inflammatory response.



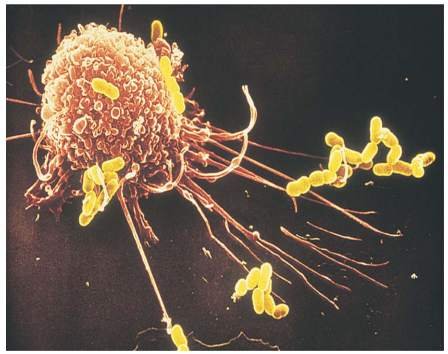
Cells of the myeloid lineage

Monocytes are the largest white blood cells, which constitutes 3–7% of the leukocytes in the circulating blood. They are characterized by a kidney-shaped nucleus, grey cytoplasm and small red-blue granules upon staining by the Giemsa stain. Pictures of monocytes and macrophages are available at. **Monocytes can leave the blood circulation and differentiate into tissue macrophages and dendritic cells.**



Macrophages

- Macrophages are larger than the monocytes, with larger cytoplasm t hat is heavily vacuolated. Macrophages assume different shapes and are given different names based on their location. Ex., Alveolar macrophages in the lung, peritoneal macrophages, Kupffer cells that are resident macrophages in the liver, brain microglia (resident macrophages in the brain), spleen sinus macrophages, lymph node sinus macrophages, Kidney intraglomerular mesangial cells…etc. Macrophages display abundant dendrites when exposed to bacteria (Fig.2.7A). Macrophages have the ability to perform phagocytosis of foreign antigens, and hence like the neutrophils, they are designated as "professional"phagocytes.

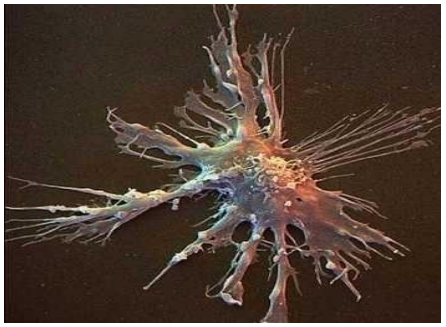


Monocyte-Derived Dendritic cells

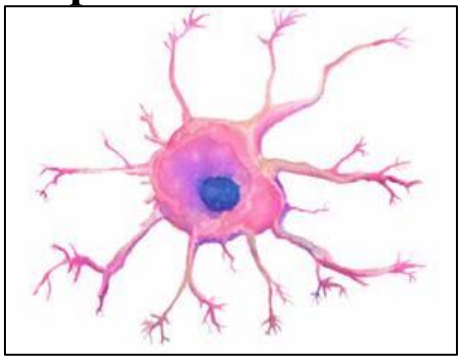
Monocyte-Derived Dendritic cells belong to a heterogeneous population of **professional antigen-presenting cells (APCs).**

Monocyte-derived DCs (mDCs), which are also called ~~myeloid DCs are the best~~ understood types of DCs.

The mDCs are characterized by a



Dendritic cell exposed to virus

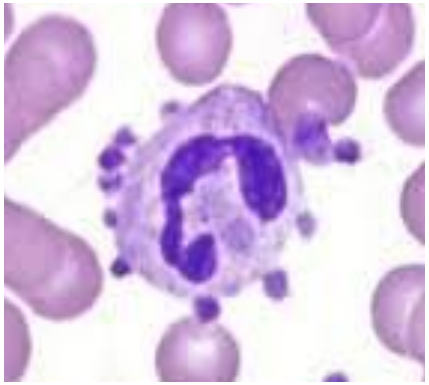


Polymorphonuclear granulocytes

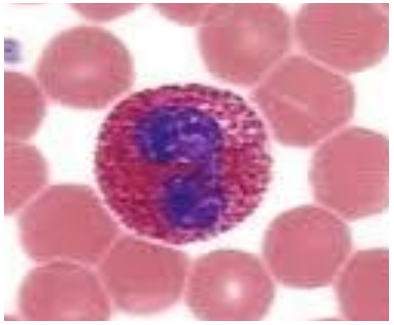
Neutrophils (50-70% of leukocytes) have a multilobed nucleus and cytoplasmic granules that are small and stained in pale blue with the neutral Giemsa stain.

The primary function of neutrophils is to perform phagocytosis of foreign antigens in the blood circulation.

These cells will also respond to chemotactic factors and chemokines generated at the site of injury and/or inflammation. The **chemokine CXCL8** produced by activated macrophages facilitate neutrophil extravasation, and the **chemotactic factor complement C5a** direct them to the site of the infection

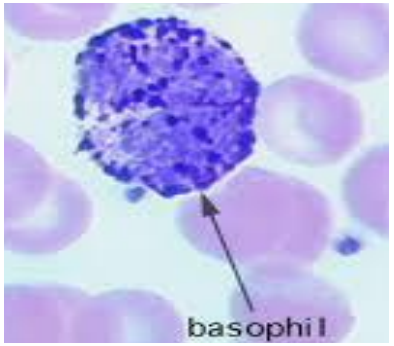


- **Eosinophils** (>0-5% of leukocytes) have a bilobed nucleus and cytoplasmic granules that are stained in red with the acidic dye eosin. They are pro-inflammatory white blood cells, which play a role in the immune defense against small parasites, such as helminth worms.



Eosinophil

- **Basophils** (>0-2% of leukocytes) have a bilobed nucleus and cytoplasmic granules stained in blue with the basic dye Alcian blue. Basophils are also involved in the immune response to parasitic infection.



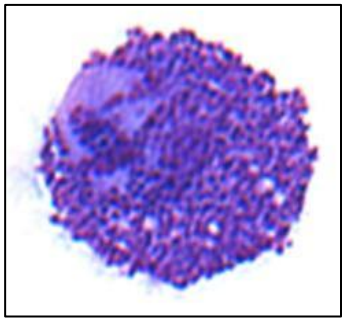
Basophil

Mast Cells

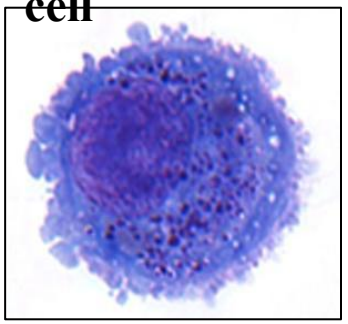
Mast cells are only found in tissues, in both connective tissue (Connective tissue mast cells or CTMCs) and mucosa (mucosa-associated mast cells or MMCs).

Mast cells show some similar morphological characteristics to basophils, such as the presence of a blue cytoplasm with deep purple granules by histochemical staining method using combined Alcian Blue-Safranin O.

A direct precursor to mast cells has not yet been identified.

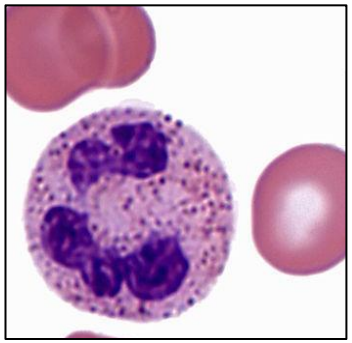


Inactive mast cell

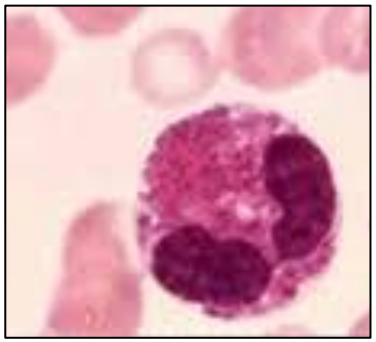


Degranulated mast cell

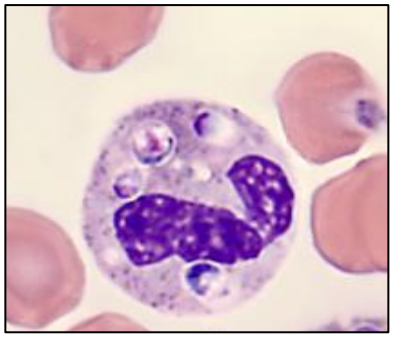
Cells of the innate immunity



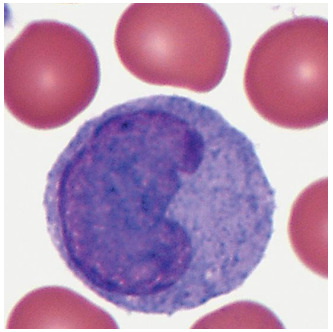
Neutrophil



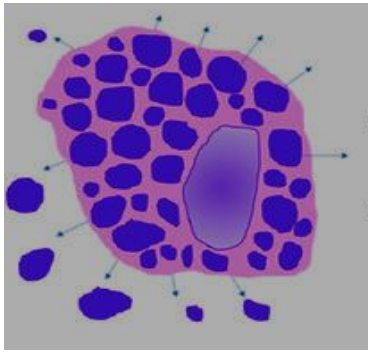
Eosinophil



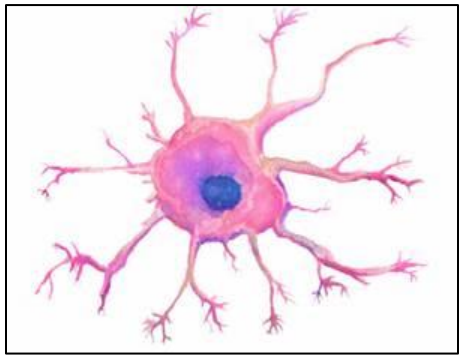
Basophil



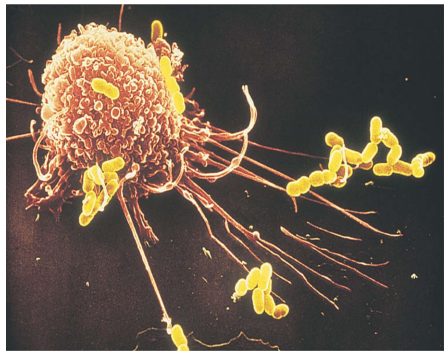
Monocyte



Mast cell



Dendritic cell



Macrophage

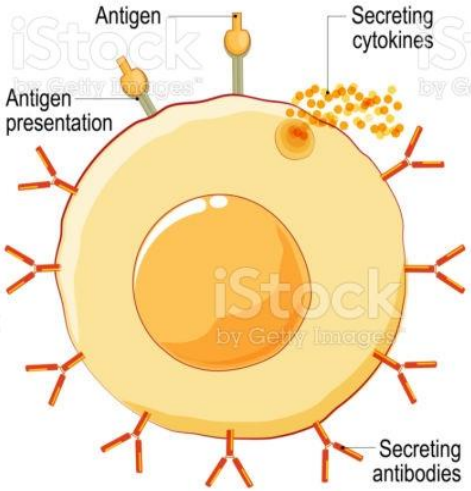
Recap

Cells of the innate immunity

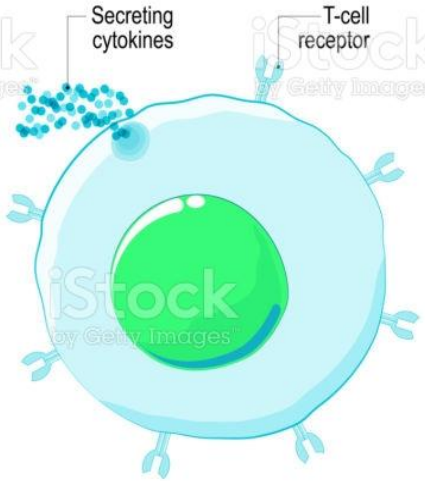
- Polymorphonuclear granulocytes (Neutrophils, Eosinophils and Basophils)
- Monocytes that differentiate into tissue Macrophages and Denditic cells
- Mast cells in connective tissue and mucosa-associated tissues
- Natural Killer cells (NK cells).
- Note: Megakaryocytes and Erythrocytes are not immune cells, but they do contribute to the immune system, Megakaryocytes being precursors to platelets, and Erythrocytes help in the removal of immune complexes from the blood circulatory system.

Cells of the Adaptive Immunity

Cells of the adaptive immune system

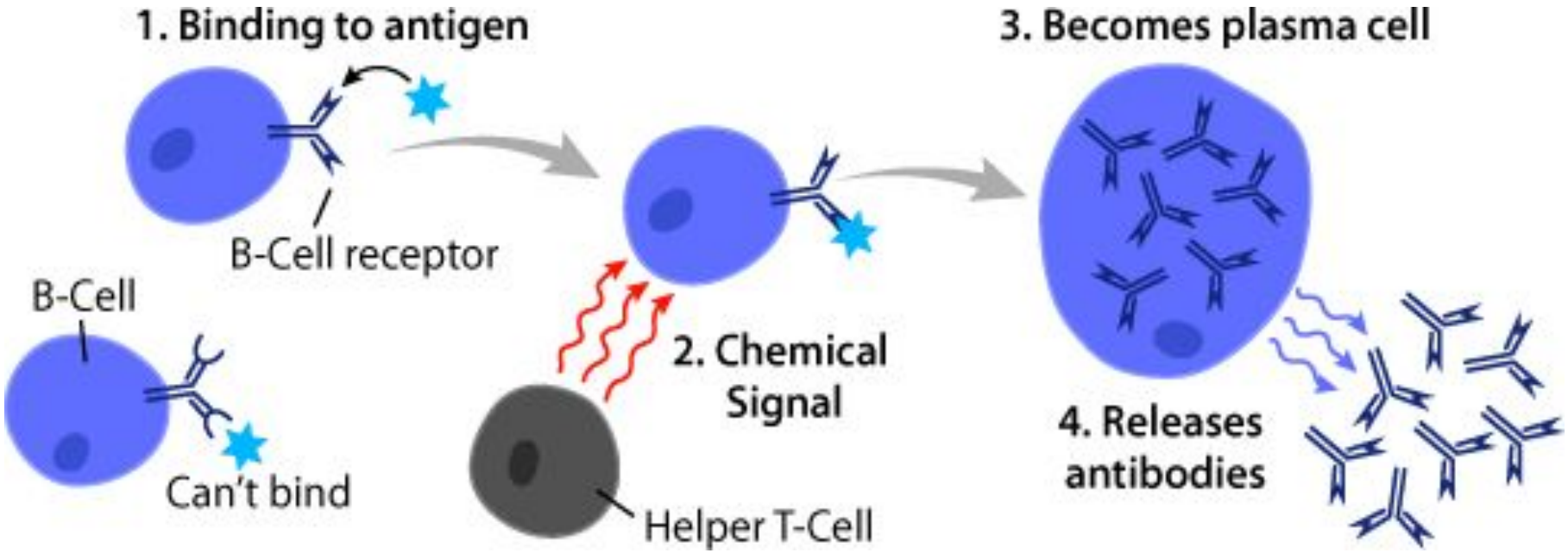


B-cell

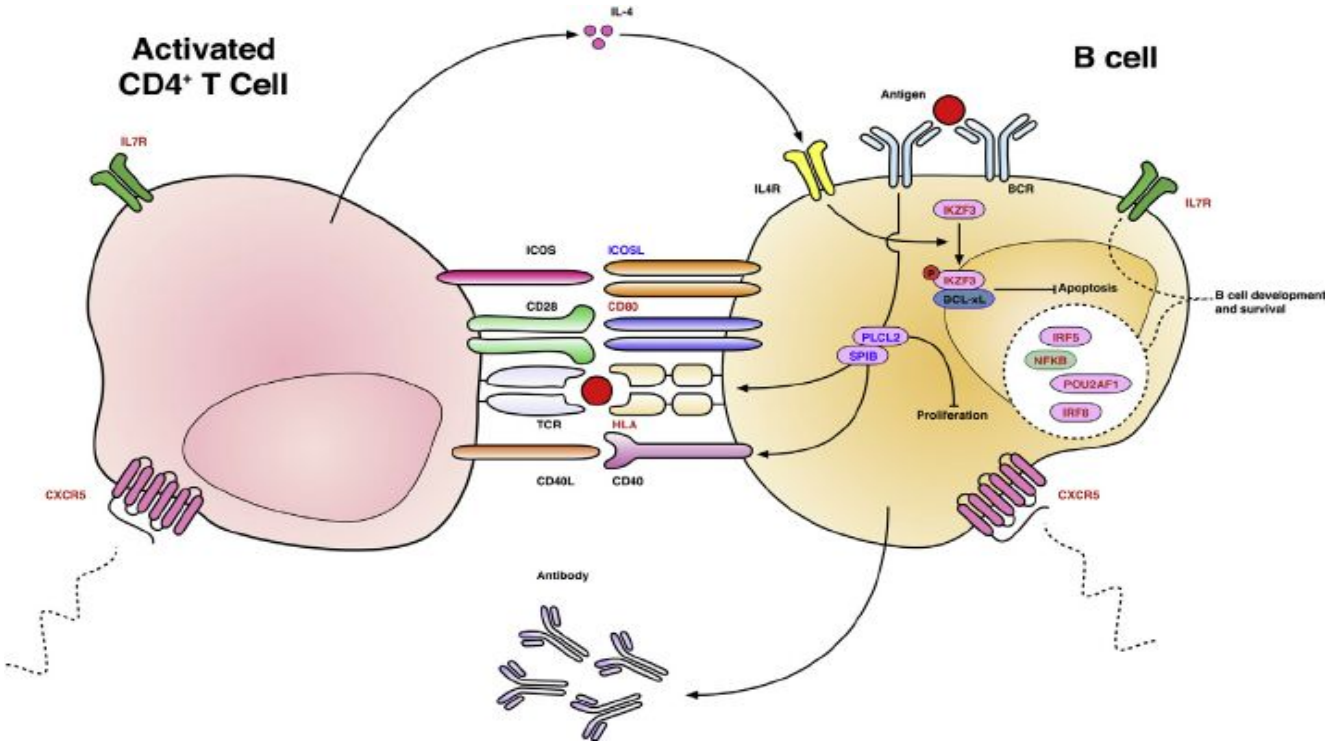


T-cell

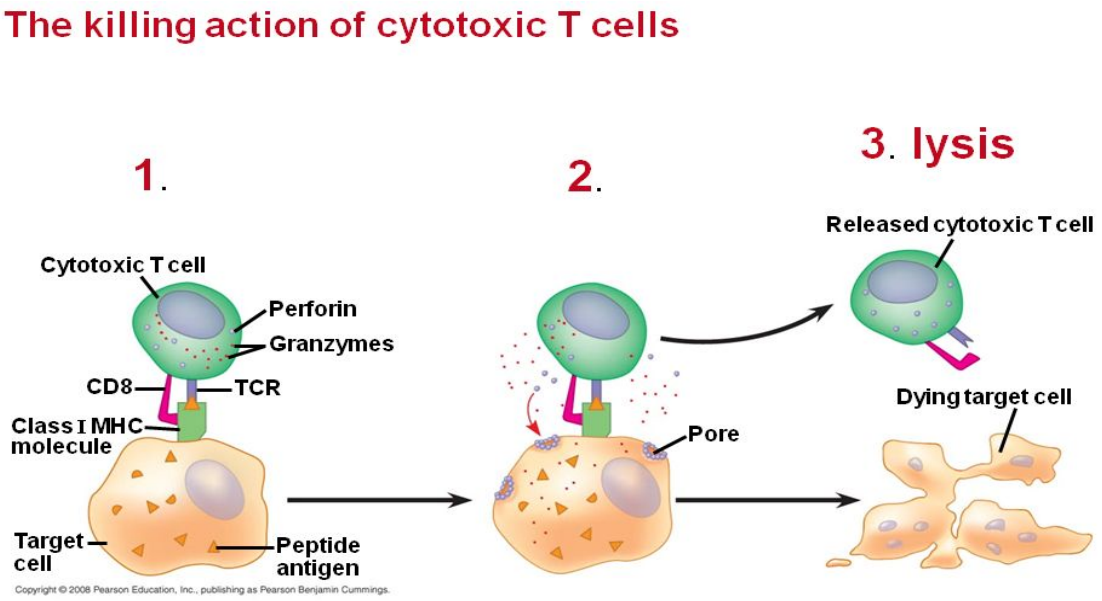
Clonal selection of B cell by antigen and differentiation into plasma cells producing antibodies



T-helper cell and B-cell Interaction



The Killing Action of Cytotoxic T Cells



Recap

The cells of the adaptive immunity include B and T cells

- **B cells** which differentiate into plasma cells producing antibodies
- **T cells** which include

  TCR-1 T cells

  TCR-2 T cells, which are divided into
  - CD4+ T helper cells by producing cytokines
  - CD8+ Cytotoxic T cells which kill infected cells by induction of apoptosis

The Immune Responses

Lecture contents

The systemic Immunological response to foreign antigens involves two lines of defense

First line of defense

- Involves cells and soluble factors of the innate immunity
- Immediate response

Second line of defense

- Delayed response
- Specific response
- Interaction between innate and adaptive components

Innate Immunity or First Line of Defense

The importance of the innate immunity is the readiness of innate immune cells and soluble factors in the elimination of foreign antigens and infected cells.

Our focus is on the following:

1. Phagocytosis of foreign antigens by neutrophils and macrophages.
2. The inflammatory response to an infection and/or injury.
3. Lysis of Gram-negative bacteria ny the complement system.
4. Detection and killing of infected or transformed cells by NK cells.
5. Antimicrobial factors.

Phagocytosis

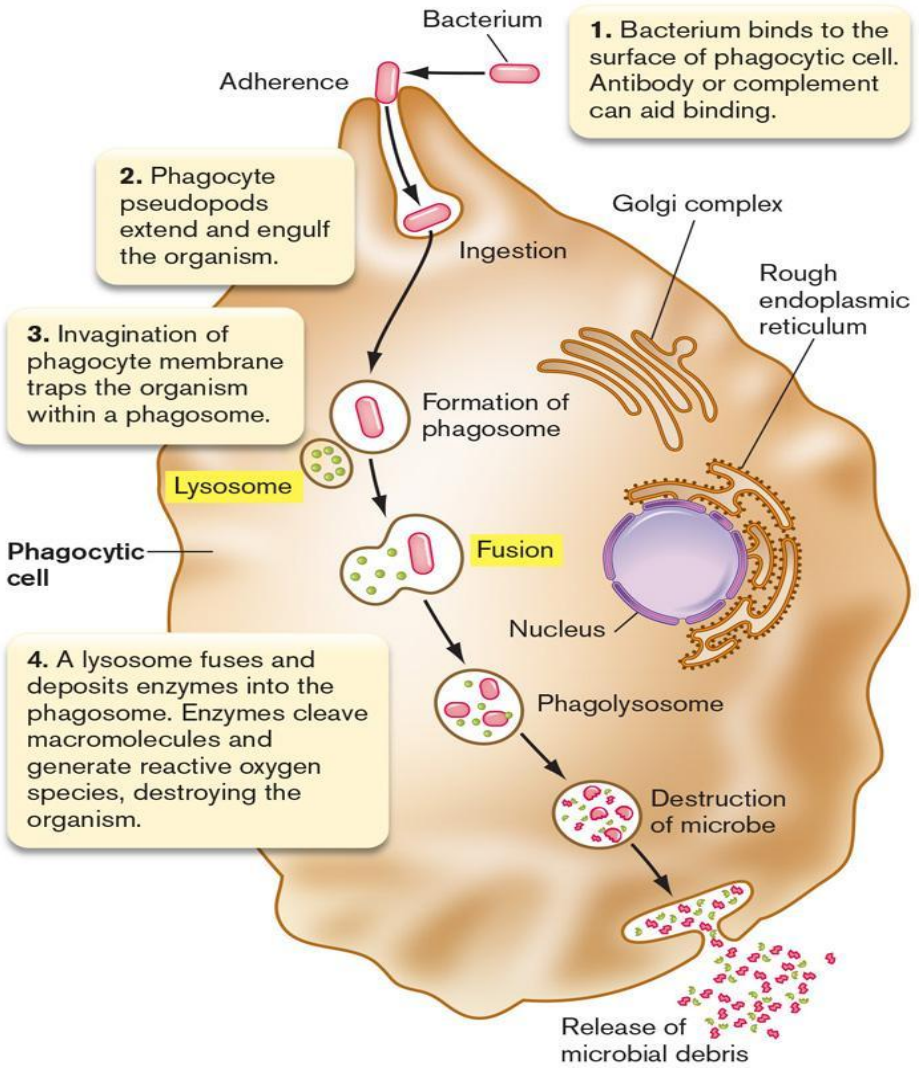
Neutrophils and macrophages are professional phagocytic cells

- Macrophages are the main phagocytes in tissues.
- Neutrophils are the main phagocytes in the blood circulatory system and, when called by macrophages, they extravasate and migrate to the site of an infection and/or inflammation.

The next slide will show the main four steps in phagocytosis

Phagocytosis

Macrophages and neutrophils are referred to as professional phagocytic cells or phagocytes. Phagocytosis is facilitated by the coating of the antigen with **opsonins**, such as antibodies or complement component **C3b**



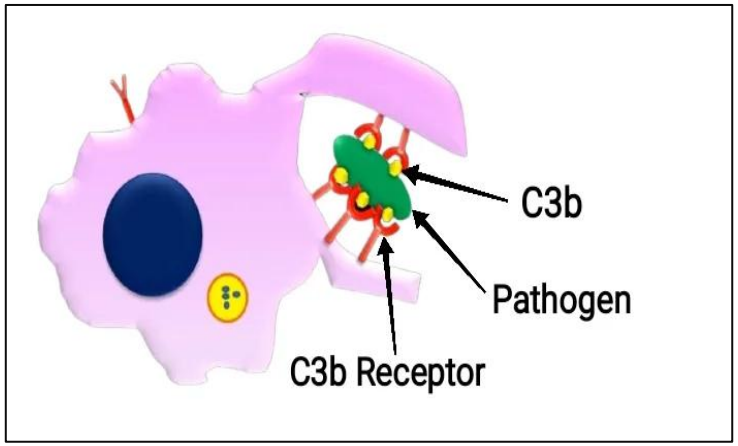
Steps in phagocytosis

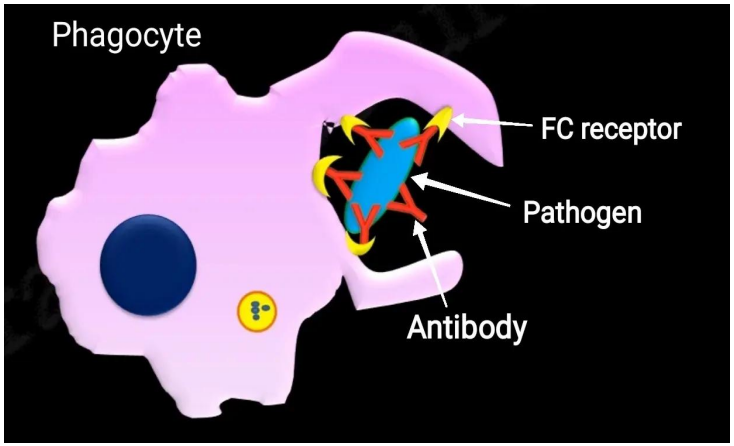
Step 1

- Binding of bacterium by surface receptors on phagocytes. Ex. CD14 binding LPS on gram-negative bacteria, and receptors for opsonins which are proteins that can bind both to bacteria and the corresponding receptors on phagocytes, thus facilitating phagocytosis. Examples of opsonins include **antibodies** and the complement component **C3b**.

Step 2

- Internalization of the bacterium is facilitated by the cross-linking of the bacterium and the phagocyte.





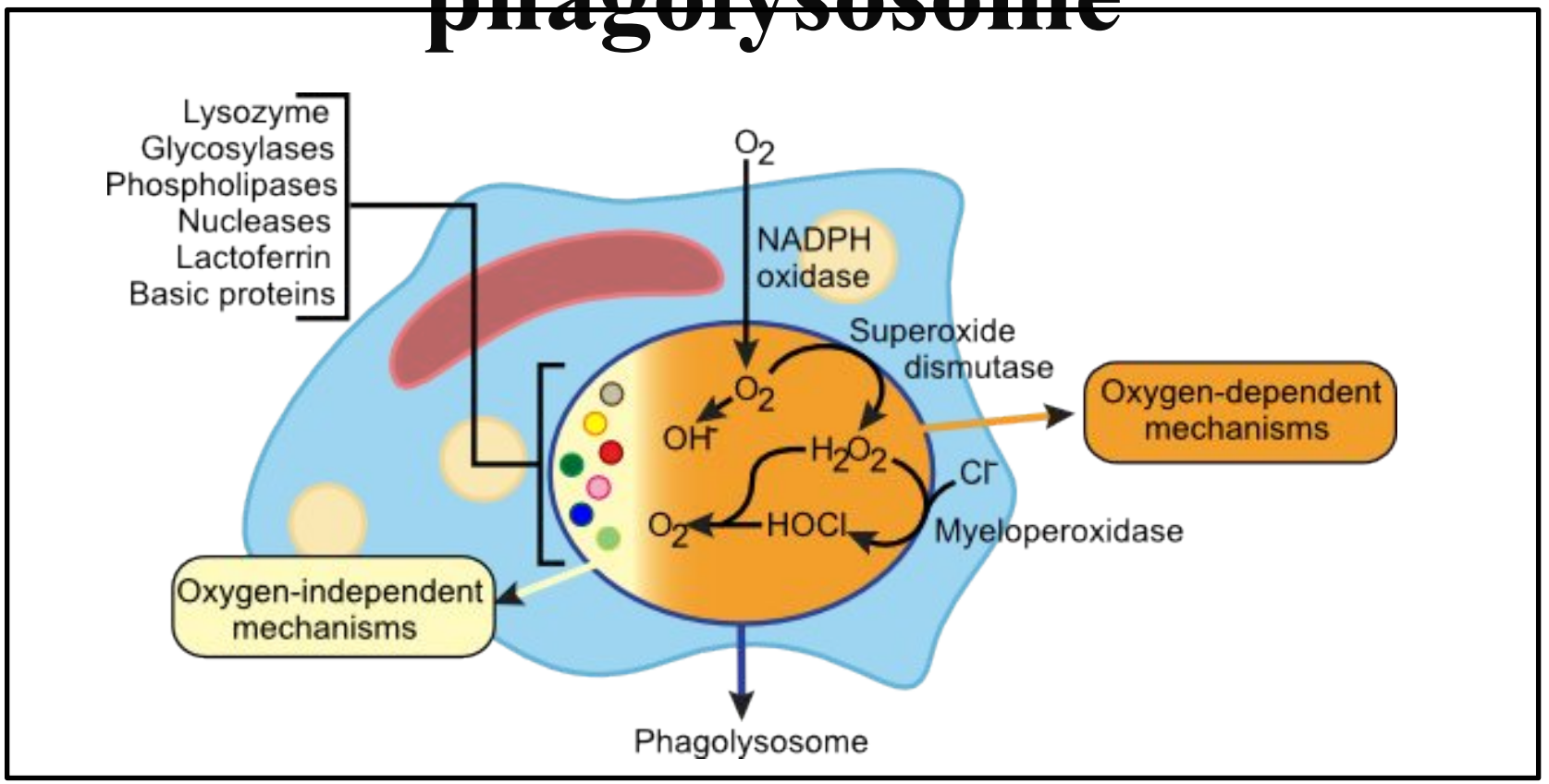
Step 3

- Internalization of the bacterium in a phagosome

Step 4

- Fusion of the lysosome with the phagosome, thus forming a phagolysosome, where the bacterium is destroyed by up to 40 acidic lysosomal enzymes, such as **lysozyme** digesting peptidoglycan cell wall, hydrolytic enzymes or acid hydrolases such as **nucleases**, **proteases**, **lipases**, **phosphatase**, etc., and protein such as lactoferrin that binds iron and deprive iron-dependent bacteria.
- Oxygen-dependent killing of bacteria. The bacterium is also destroyed by **reactive oxygen species** (ROS), such as **superoxide anion** (O_2^-), **hydrogen peroxide** (H_2O_2), and **hydroxyl radical** (HO•), consist of radical and non-radical oxygen species formed by the partial reduction of oxygen. Cellular ROS are generated endogenously as in the process of mitochondrial oxidative phosphorylation,

Killing of bacteria in the phagolysosome



The beneficial acute inflammatory response

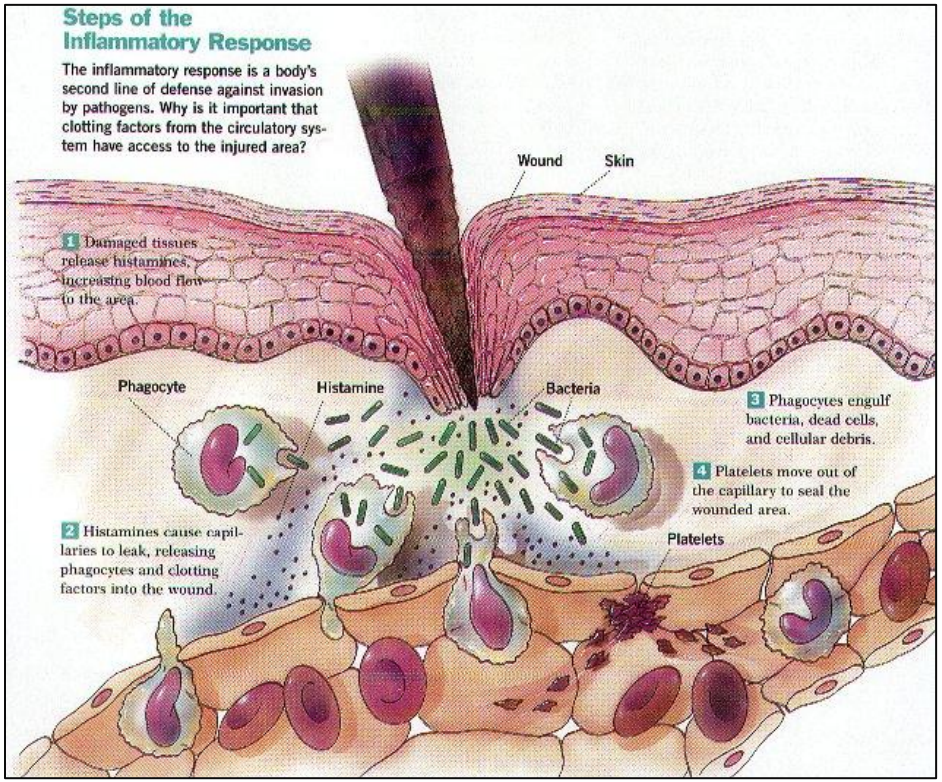
Inflammation

The five cardinal signs of inflammation

- Redness
- Heat
- Pain
- Swelling
- Loss of function

Question: What can cause these signs?

The Inflammatory Response



Beneficial acute inflammatory response to injury and infection

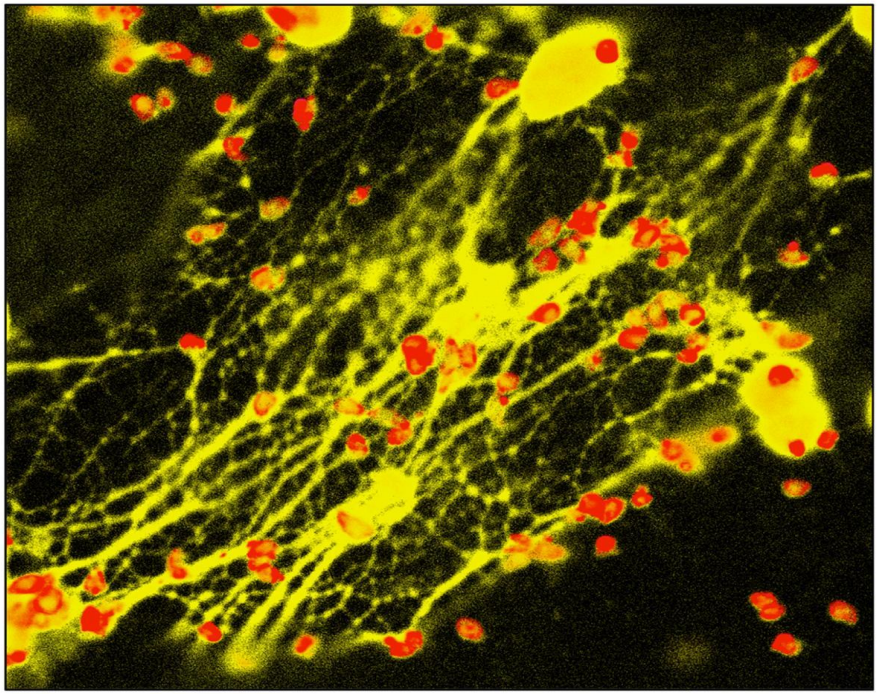
- Macrophages performing phagocytosis of bacteria
- Activated macrophages producing cytokines to activate other immune cells in tissue, to mobilize circulatory neutrophils and platelets, to induce fever by pyrogens, etc.

The 5 signs of inflammation

1. **Redness** resulted from vasodilation caused by histamine and prostaglandin produced by mast cells.
2. **Swelling** resulted from exudates caused by vasodilation and smooth muscle contraction.
3. **Heat** resulted from the effect of pyrogens (produced by activated macrophages: IL-1 beta and IL-6 on the hypothalamus.
4. **Pain** resulted from the stimulation of nerve ending by bradykinin and prostaglandin.
5. **Loss of function** caused by disruption of tissue structure.

Neutrophil undergoing NETosis

- Neutrophils can extravasate (get out of blood vessel) in response to the **chemokine CXCL8** produced by macrophage and the **chemotactic factor C5a** from complement activation.
- CXCL8 induces the expression of **adhesion molecules** on neutrophils (LFA-I) and on endothelial cells (ICAM-I).
- C5a is a chemotactic factor that attracts neutrophils to the site of the infection/inflammation.
- **Neutrophils do not recirculate, and they die by apoptosis or NETosis at the site of the infection/inflammation.**



Functions of the complement system

- **Lysis of foreign cells** for the insertion of on of membrane attack complexes (MAC) into the membrane of Gram-bacteria
- **Production of reactive proteins**:

  C3b: Opsonin

  C3a and C5a at low concentration: chemotactic factors

  C3a and C5a at higher concentration, are anaphylatoxins that bind to their respective receptors on mast cells, triggering the release of factors of anaphylaxis

Complement activation

- The complement system is composed of over 30 heat-labile proteins that are produced mainly by the liver. These proteins circulate in the blood and extracellular fluid in an inactivated state until a complement component encounters a foreign cell, leasing to a sequence of enzymatic reaction on the surface of the foreign cell, such as *E. coli* (Gram- bacteria).
- Complement activation occurs by three pathways:
  - The **Alternative pathway** which is also called the **properdin pathway**
  - The **Lectin pathway**
  - The **Classical pathway**

Initiation or Activation of the Complement

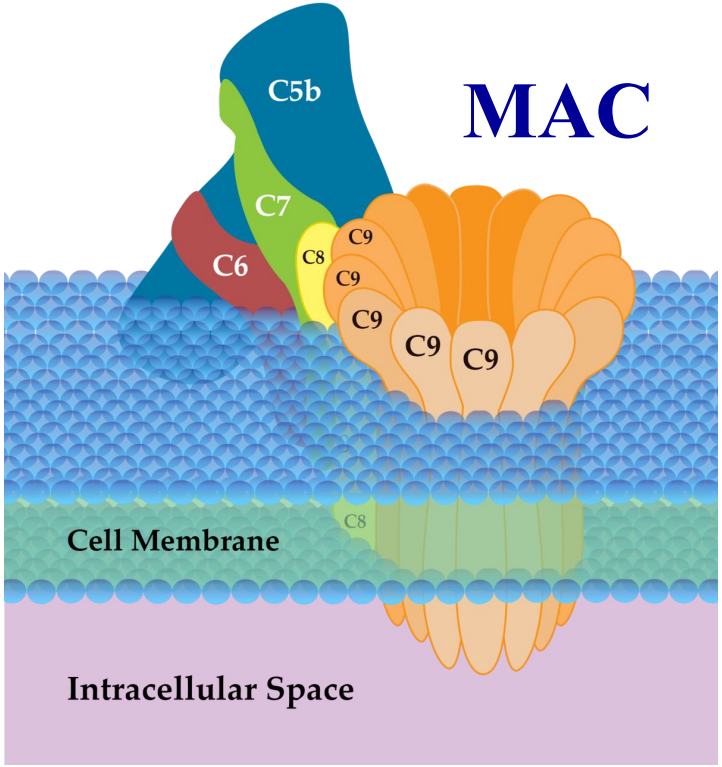
- The **Alternative pathway** is initiated by the binding of a fluid phase C3 convertase that consists of iC3 associated with Factor Bb (iC3Bb), which cleaves complement C3 into 2 fragments C3a and C3b that binds to the bacterial surface, followed by the **formation of the C3 convertase C3bBb on bacterial surface**. Binding of the protein properdin to C3bBb extends the half life of this C3 convertase .
- The **Lectin pathway** is initiated by the binding of a Mannan-binding lectin (MBL) complex to mannose residues on the bacterial surface. Mannan-binding lectin complex consists of one molecule of Mannan-binding lectin associated with two molecule of MBL associated serine protease 1 (MBL-SP1) and two molecule of MBL-associated serine protease 2 (MBL-SP2), which results in the activation of a serine protease. Cleavage of complement C4 and C2 by this enzyme results in the **formation of C4bC2b (C4b2b), which is a C3 convertase.**
- The **Classical pathway** is initiated by the binding of antibodies of the IgG and IgM classes to the bacterial surface, which is followed by the binding of C1 complex (C1q associated with two C1r and two C1s to the antibodies), which results in the activation of a serine protease. Like in the Lectin pathway, cleavage of complement C4 and C2 by this enzyme results in the **formation of C4bC2b (C4b2b), which is a C3 convertase**.

Formation of C5 convertase and MAC

Formation of C5 convertase. This step is similar in the three pathways: C3 convertase cleaves C3 into C3a and C3b fragments. Binding of C3b to C3 convertase results in the formation of **C5 convertase: C3bBb3b in the Alternative pathway, and C4b2b3b in the Lectin and Classical pathways.**

Formation of the membrane attack complex (MAC). This step is identical in all three pathways. C5 convertase cleaves C5 into two fragments C5a and C5b, which gets associated sequentially with C6, C7 and C8, forming the complex C5b678 which inserts into the membrane of the bacteria. Finally, C9 binds to C8 of C5b678, followed by C9 polymerization **(C5b678(C9)n** in the membrane of the bacteria, thus creating a channel or pore, leading to bacterial lysis.

Membrane attack complex



https://www.youtube.com/watch?v=vbWYz9XDtLw

The Classical Pathway of the complement system

Antimicrobial soluble factors

Alpha and beta defensins
Alpha-2 macroglobulins
Lysozyme
Polyamines
Kinins
Pentrexins

Antimicrobial Factors

Antimicrobial factors include:

- **Antimicrobial peptides:** Alpha and beta defensins that disrupt microbial membranes.
- **Alpha-2 macroglobulin** inhibits potentially damaging proteases
- **Pentraxins:** Pentameric plasma proteins of the innate immunity that bind microorganisms and host phagocytes, thus facilitating phagocytosis.
- **Lysozyme:** Enzyme digesting bacterial cell wall.
- **Polyamines:** Molecules causing the sequestration of lipoteichoic acid of Gram+ bacteria.
- **Kinins:** plasma proteins of the blood coagulation with antifungal and anti bacterial properties.

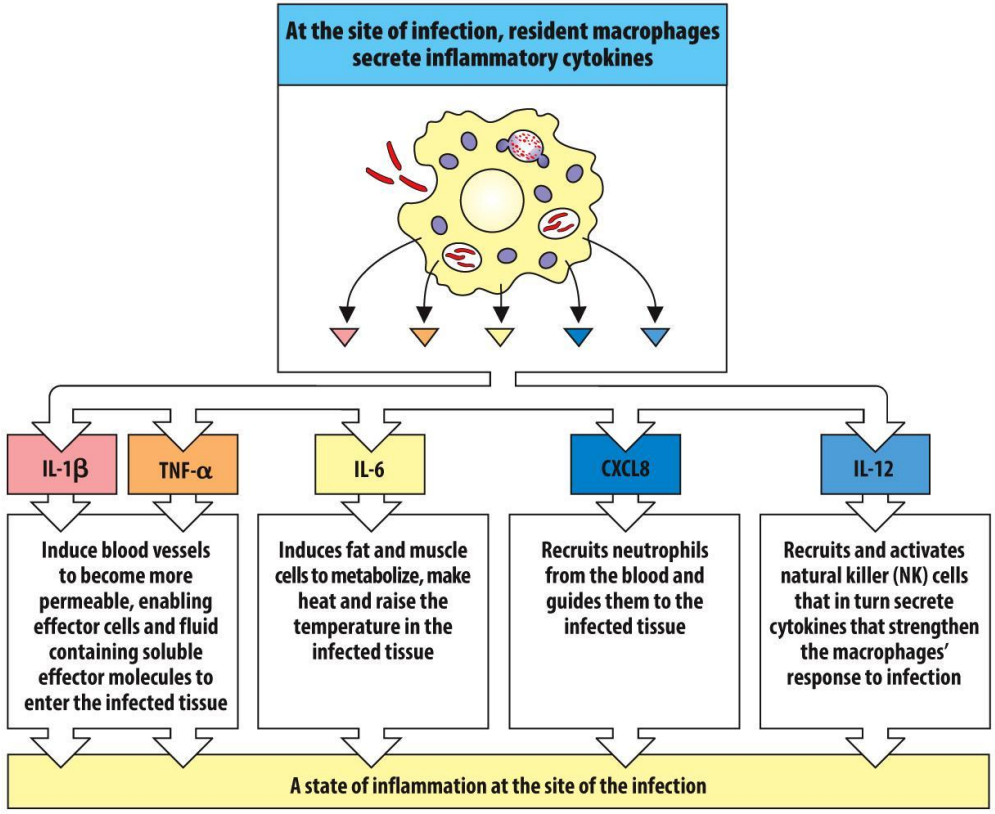
Natural Killer Cells

- NK cells have the natural (or innate) ability to detect and kill infected, stressed out and abnormal cells, such as virus- or bacteria-infected cells, or cancer cells. NK cells are derived from the lymphoid lineage, but **they do not have the characteristics of the cells of the adaptive immunity, hence they are considered as members of the innate immunity**. A distinctive feature in NK cells is the expression of **inhibitory receptors and activating receptors.**
- The killer inhibitory receptors that recognize MHC-I expressed on cells in the body contain a region designated as **Immunoreceptor Tyrosine-based Inhibition Motif (ITIM)** in their cytoplasmic tail.
- In contrast NK cells have the **activating receptors**, such as **NKG2D** that binds to **MICA** and **MICB** that are expressed by cells that are under stress, such as tumor cells or infected cells. The activating receptors contain a region in their cytoplasmic tail designated as **Immunoreceptor Tyrosine-based Activation Motif (ITAM)**

Cells of the Lymphoid lineage

- **B lymphocytes or B cells.** These cells express **B cell receptors for antigens (BCRs),** which are membrane-associated surface immunoglobulins (Igs) or antibodies (Abs).
- The surface Abs expressed by naïve mature B cells (mature B cells that have not yet encountered the corresponding antigens) are of the **IgM and IgD classes**. There are around 10^5 surface immunoglobulins on naïve B cells.
- Upon binding antigen B cell get activated and proliferate, and the cells generated undergo **differentiation into effector cells**, which are in this case antibody forming cells or plasma cells, and memory B cells as described previously.

Cytokines Produced by Macrophages in the Inflammatory Response



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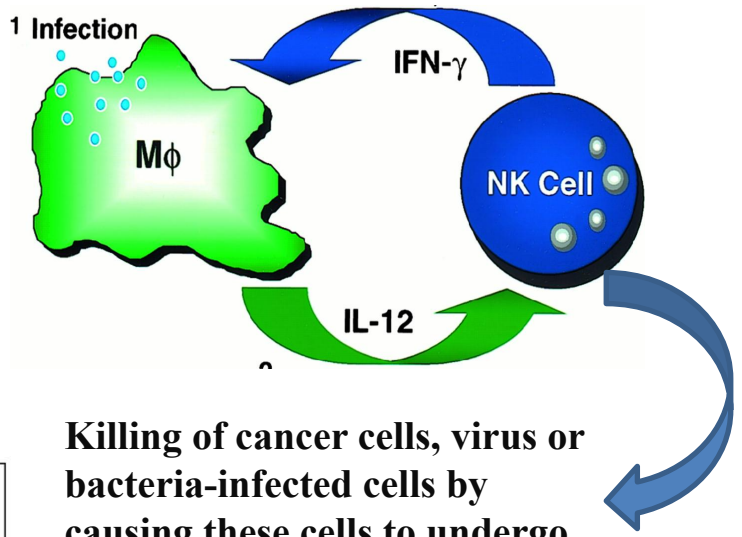


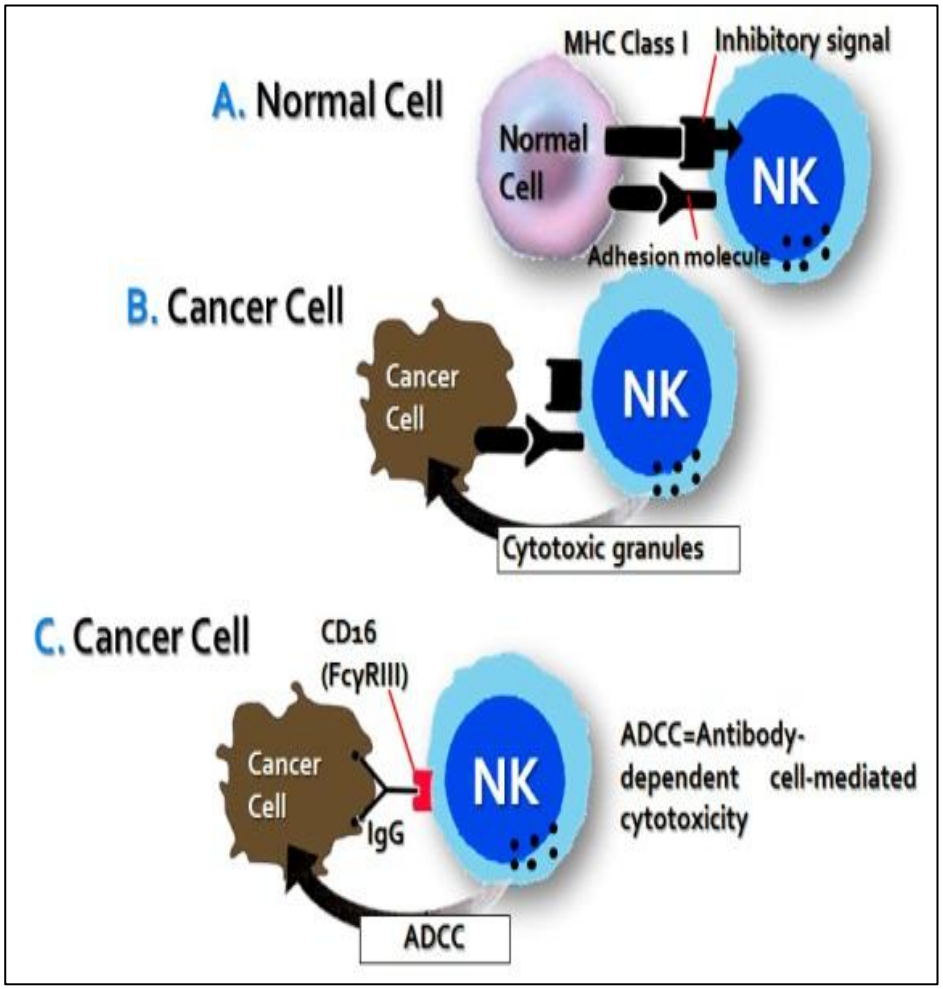
Killing of cancer cells, virus or bacteria-infected cells by causing these cells to undergo apoptosis, similarly to cytotoxic T cells (described in later slides)

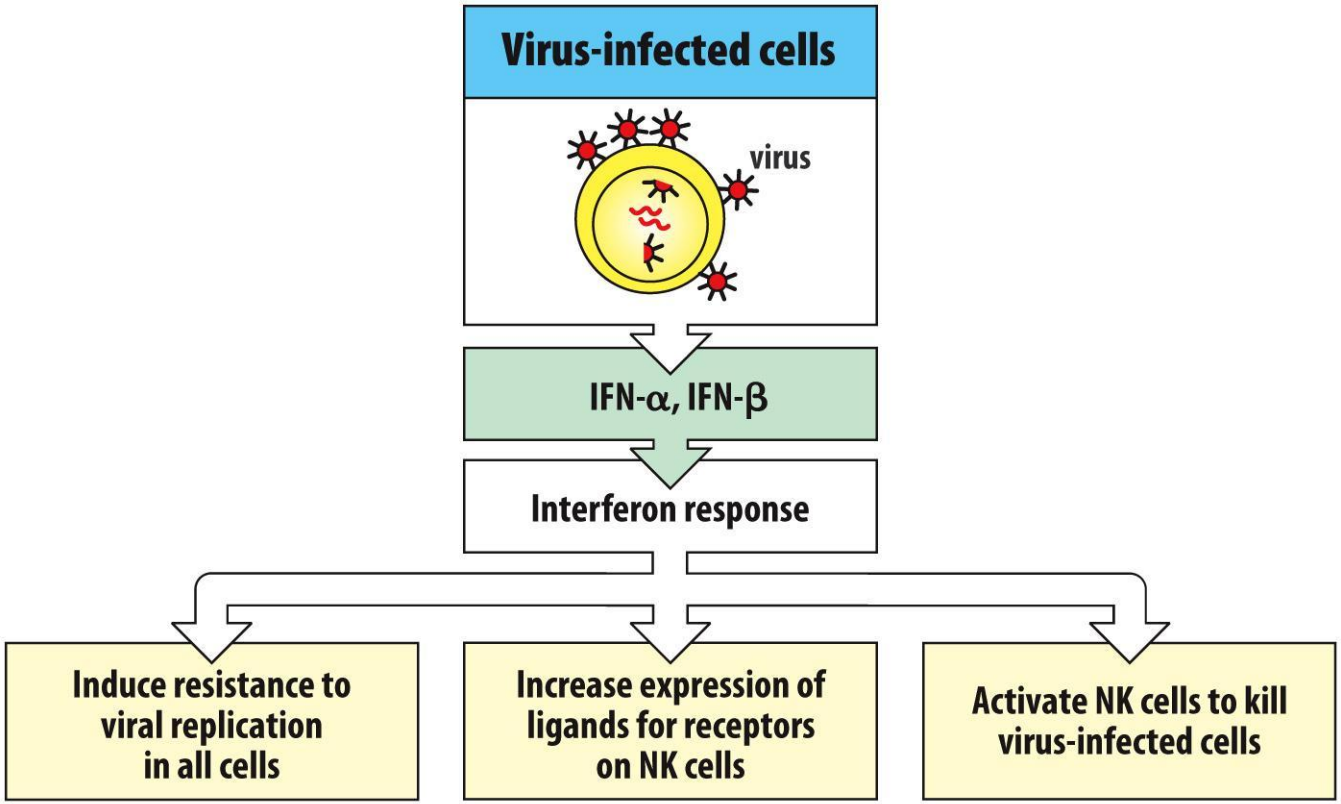
Function of NK cells

A. **NK cell has inhibitory receptors that bind to body cell expressing MHC-I.** This is a mechanism to prevent the killing of body cell by NK cells.

B. **Some cancer cells that fail at expressing MHC-I** are killed by NK cells.

C. **Cancer cells are also killed by NK cells if they are coated by antibodies to cancer antigens**. This process is called ADCC (Antibody-Dependent Cellular Cytotoxicity).





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The adaptive immune response

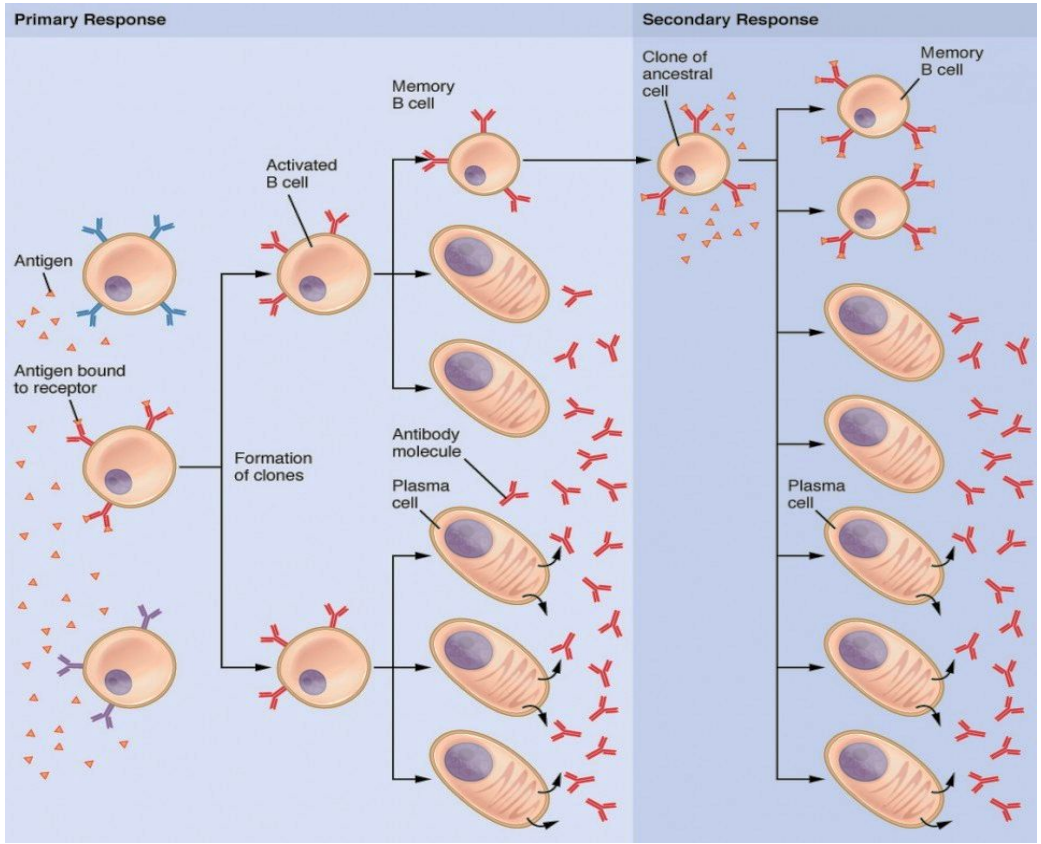
The Adaptive Immunity

- The Adaptive Immunity is also called Acquired Immunity. This form of immunity is characterized by **specificity in binding antigen and memory in response upon subsequent encounter with the same antigen.**
- **The cells of the acquired immunity are the B cells and T cells**. Common characteristics between these cells are their high specificity in the recognition of antigens. Only a few cells can recognize a given antigen, and these cells get activated and then proliferate and differentiate into effector cells and memory cells
- Our focus is on **B cells, T helper cells (Th), and cytotoxic T (Tc) cells**. The effector B cells are plasma cells that produce specific antibodies against the antigen, whereas the effector Th cells produce cytokines that activate other immune cells, and effector Tc specifically kill altered or infected cells in the body. While B cell receptor (BCR) can bind antigen directly, **TCR-2 of Th can only bind antigen peptide in association with MHC class II, and TCR-2 of Tc can only bind antigen peptide in association with MHC-I.**

B lymphocytes

As illustrated, there is a repertoire of B cells that differ from each other in antigen binding specificity. BCRs consist of cell surface antibody molecules that are represented in different colors.

A. Clonal selection by antigen
B. Clonal activation
C. Clonal proliferation and differentiation into plasma cells and memory cells
D. Secondary immune response is mediated by activation of memory cell which responds much faster and at much higher levels compared to the initial B cell



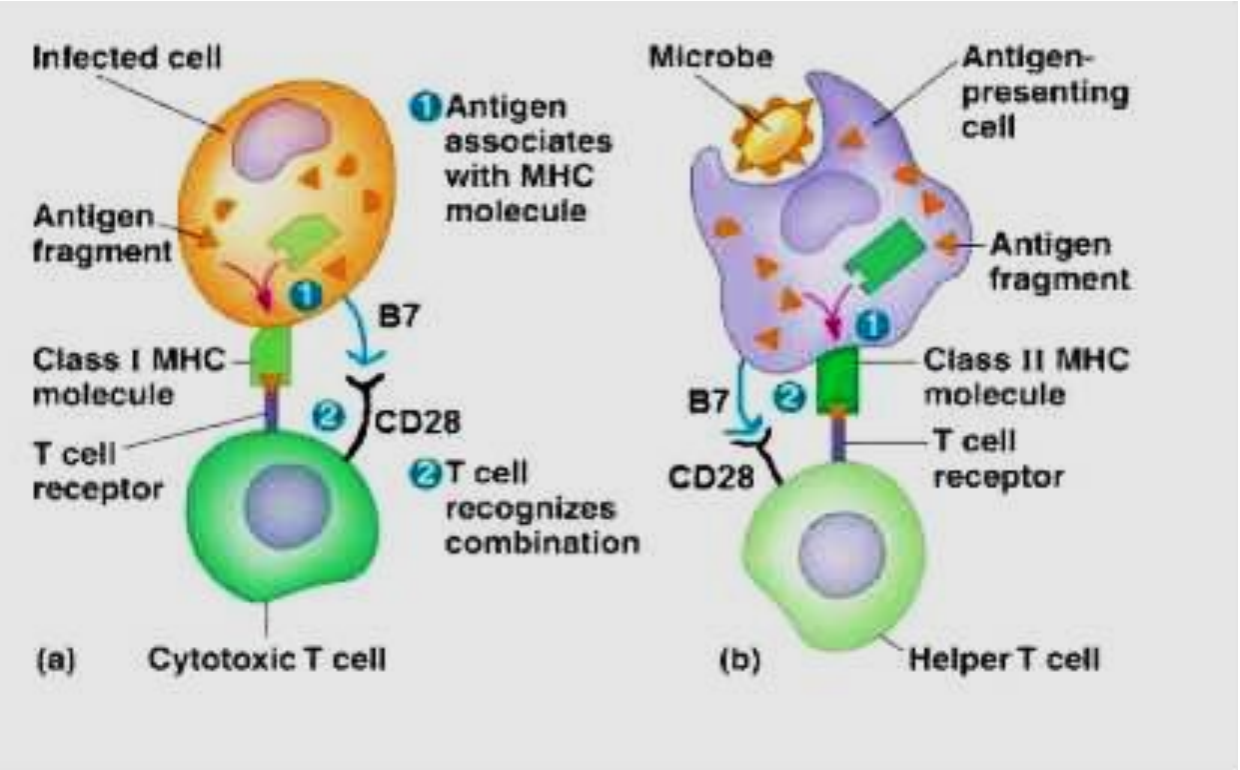
T Lymphocytes or T cells.

- These cells are cells of the adaptive cell-mediated immunity.
- There are two types of T cells, which differ in T cell receptors for antigen (abbreviated as TCRs): These cells include the **TCR-1 T cells and TCR-2 T cells** based on the type of TCRs.
- **TCR-1 is a dimer composed of a γ and a δ chains** and, like BCR, it can bind directly foreign antigen. These cells do not have the characteristics of cells of the adaptive immunity, and hence will not be addressed in this lecture.
- **TCR-2 is a dimer composed of a α and a β chains**. TCR-2 T cells cannot bind directly antigens and they are divided into two groups of cells based on the presence of CD4 (example Th cells) or CD8 (example Tc cells) surface molecules.

CD4+ T cells, also called CD4 T cells include T helper cells (Th), follicular T helper cells (Tfh), and regulatory T cells or Tregs. We will focus first on Th cells. As mentioned above, these cells cannot bind directly protein antigens, but only foreign antigen peptide that is associated with MHC class II molecule (abbreviated as Agp:MHC-II complex) that are expressed on antigen-presenting cells (APCs : Macrophages, B cells and Dendritic cells). TCR-2 T cells undergo clonal selection upon binding the corresponding Agp:MHC complexes, they get activated and undergo clonal proliferation and differentiation into effector cells that can carry out their specific functions, and memory cells.

CD8+ T cells that are also referred to as cytotoxic T lymphocytes (CTLs) bind to Agp:MHC-I complex that are expressed on infected or altered cells in the body and kill these cells by the induction of apoptosis.

Functions of T-helper (Th) cells vs cytotoxic T (Tc) cells



Elimination of infected body cells

Elimination of extracellular antigens

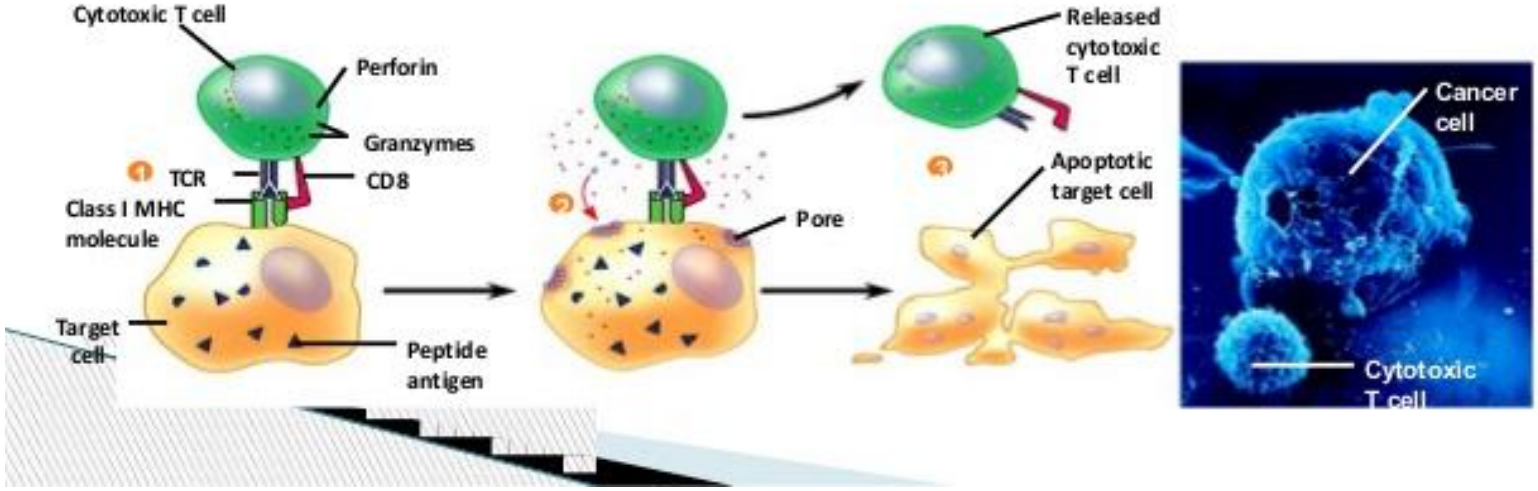
Killing of Target cells by Cytotoxic T cells

- Tc recognizes target cell by **TCR-2 of Tc binding to Agp:MHC-I** complex that are expressed on the surface of the host infected cells
- Activated Tc expresses **perforin** molecules, which polymerize in the target cell membrane and create a **polyperforin channel** through which serine protease granzymes are transferred from Tc to the target cells
- **Granzymes** induce the apoptosis of the target cell.

The activated cytotoxic T cell

- Secretes proteins that destroy the infected target cell

1. A specific cytotoxic T cell binds to a class I MHC–antigen complex on a target cell via its TCR with the aid of CD8. This interaction, along with cytokines from helper T cells, leads to the activation of the cytotoxic cell.
2. The activated T cell releases perforin molecules, which form pores in the target cell membrane, and proteolytic enzymes (granzymes), which enter the target cell by endocytosis.
3. The granzymes initiate apoptosis within the target cells, leading to fragmentation of the nucleus, release of small apoptotic bodies, and eventual cell death. The released cytotoxic T cell can attack other target cells.



Antigen Processing & Presentation

There are two pathways of antigen processing and presentation.

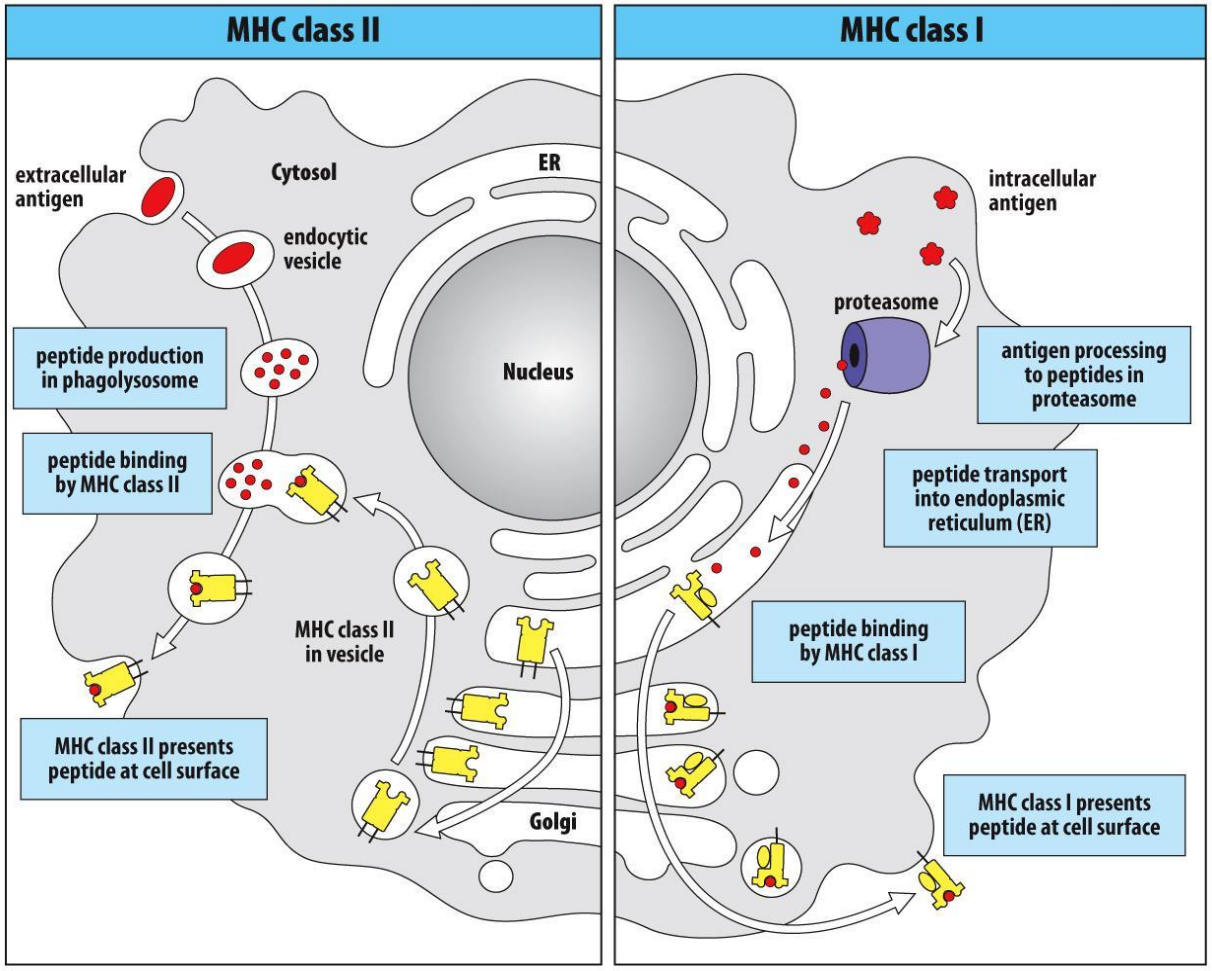
1. **The exogenous or endocytic pathway**
   - The protein antigen is extracellular.
   - Endocytosis of extracellular antigen.
   - Processing in an endosome by proteases into antigen peptides.
   - Association of peptide antigen (Agp) in association with MHC-II.
   - Presentation of Agp: MHC-II complex on the antigen processing . cell (APC: dendritic cells, B cells & macrophages) to cognate CD4+ helper T cell.

2. The endogenous or cytosolic pathway of antigen processing.

- The foreign or worn-out protein antigen is inside of a host cell.
- Processing of the protein antigen occurs in the proteasome in the cytoplasm
- Antigen peptides enter the RER through the membrane-associated TAP1 and TAP2.
- The antigen peptide is loaded onto the antigen loading complex (which is composed of MHC-I associated with calreticulin, ERP57, and Tapasin which bins to TAP-1 and TAP-2

Endocytic or exogenous pathway of antigen and presentation to Th cells

Only Antigen Presenting cells (APCs) can present antigen peptide:MHC-II comlex to Th cells. APCs include Macrophages, B cells and dendritic cells



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Endogenous pathway of antigen and presentatio n to Th cells

All cells in the body can process intracellular antigen for presentation to Tc

Principles of Innate and Adaptive Immune Response

- https://www.ncbi.nlm.nih.gov/books/NBK27090/
- https://www.birmingham.ac.uk/Documents/college-mds/facilities/cis/.../Chapter1.pdf
- https://www.youtube.com/watch?v=skPtWocTKdU
- https://www.youtube.com/watch?v=sYjtMP67vyk