BIOCHEMISTRY

Bacterial Structure, Chemotherapy & Resistance
March 15th – 24th 2022

Dr. MULINGE
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Course Outline

WEEK	TOPIC	LECTURER (S)
WEEK 1 04-08/10/2021	Tissue metabolism: Differential metabolism in Liver, Muscle, adipose brain and erythrocytes. Integration of metabolism	Prof. Mukuria
WEEK 2 11-15/10/2021	Biochemistry of muscle contraction	,,
WEEK 3 01-05/02/2021	Neurochemistry: Brain energy sources. The blood brain barrier. Neurotransmitters.	,,
WEEK 4 18-22/10/2021	Biotransformation of Xenobiotics: Drug metabolism: Biotransformation of drugs, phase I and phase II reactions	,,
WEEK 5 25-29/10/2021	Pharmacokinetics phase of drug transformations	,,
WEEK 6 01-05/11/2021	Nucleotide metabolism: Overview of structure and function of nucleotides. Purine and Pyrimidine metabolism: degradative and biosynthetic pathways, regulation of nucleotide metabolism.	,,
WEEK 7 08-12/11/2021	Disorders of nucleotide metabolism and drugs targeting nucleotide metabolism: (antifolates, glutamine antagonists, reverse transcriptase inhibitors) and their importance in cancer therapy.	,,
16/11/2021	MID SEMESTER CAT	Prof. Mukuria
WEEK 8 22-26/11/2021	Molecular biology: DNA structure, replication in eukaryotes and prokaryotes	Dr. Mobegi
WEEK 9 29-03/12/2021	Molecular biology: DNA structure, replication in eukaryotes and prokaryotes	,,
WEEK 10 06-10/12/2021	Invitro DNA replication- PCR: principle and applications.	,,
WEEK 11 13-17/12/2021	Transcription in prokaryotes and eukaryotes and post-transcriptional modifications	,,

Course Outline

WEEK	TOPIC	LECTURER (S)
WEEK 12 03-07/12/2022	Translation/genetic code, Pre- & Post-translational modifications.	,,
WEEK 13 10-14/01/2022	Translation: Posttranslational protein modification. Inhibitors of protein synthesis and their role in chemotherapy.	,,
WEEK 14 17-21/01/2022	Parasite biochemistry: Special metabolism in parasitic protozoa and helminths	,,
WEEK 15 24-28/01/2022	Parasite biochemistry: Special metabolism in parasitic protozoa and helminths	,,
WEEK 16 31-04/02/2022	Protein folding and targeting in the cell. Diseases and syndromes associated with the two processes DNA mutations and repair mechanisms: types, effects Mutagens and their effect on DNA and suppressor mutations clinical correlations.	
WEEK 17 07-11/02/2022	Bioinformatics: Introduction to basic bioinformatics and its application in disease detection, drug design and Modern medicine	
WEEK 18 14-18/02/2022	END FIRST SEMESTER EXAMS	Prof. Mukuria Dr Mobegi

Course Outline

WEEK	TOPIC	LECTURER (S)
WEEK 20 21-25/02/2022	**Gene regulation: Structural and transcriptional regulation of gene expression in prokaryotes and eukaryotes repression and induction of transcription of prokaryotic gene, bacterial operon concept, negative vs positive control, other regulatory mechanisms.**	**Dr. Mulinge**
WEEK 21 28-04/03/2022	DNA repair and DNA recombination. Disease/syndromes associated with DNA repair	,,
WEEK 22 07 - 11/03/2022	Molecular virology: Classification and properties of viruses, replication and life cycle of viruses. Interferons, Oncogenes and oncogenic viruses. Viroids and prions. Application –HIV	,,
WEEK23 14 - 18/03/2022	Molecular virology: Classification and properties of viruses, replication and life cycle of viruses. Interferons, Oncogenes and oncogenic viruses. Viroids and prions. Application –HIV	,,
WEEK 24 21 - 25/03/2022	**Bacterial Biochemistry: Bacterial cell structure: Cell envelope; Cell cytoplasm; Cellwall and its biosynthesis. Bacterial toxins, virulence and pathogenesis.**	‘’
WEEK 25 28 - 01/04/2022	Bacterial chemotherapy: Mechanisms of action of antibiotics Bacterial resistance to antimicrobial chemotherapy.	,,
WEEK 26 04 - 08/04/2022	Biochemical endocrinology: Endocrine, paracrine and autocrine mode of secretions. Classification of hormones. Mechanism of hormone action: Signal and signal transduction, Receptors: intracellular and membrane bound receptors. Second messenger role in signal transduction: cAMP, cGMP, lipids, Calcium ions.	,,
WEEK 27 11 - 15/04/2022	Biochemical endocrinology: Synthesis, storage, release, transport, mode of action and degradation of peptide, steroid and prostaglandins derived hormones	,,
12/04/2022	MID 2nd SEMESTER CAT	Dr Mulinge

Course Outline

WEEK	TOPIC	LECTURER (S)
	BREAK	
WEEK 28 25 - 29/04/2022	Molecular genetics: Organization of the human genome, chromosomes and karyotypes. Nuclear and mitochondrial chromosomes.	PROF. NGUU
WEEK 29 02 - 06/05/2022	Gene structure and organization and gene family. Satellite DNA and DNA families. C value of a genome Cot ½ values of DNA and its relation to repetition.	,,
WEEK 30 09 - 13/05/2022	Mendelian laws: Pedigree analysis, Mendelian laws of inheritance (single gene inheritance: autosomal and X-linked inheritance, dominant and recessive inheritance. Extension and exceptions of Mendelian inheritance, nonclassical patterns of single gene inheritance.	,,
WEEK 31 16 - 20/05/2022	Genetic diseases: Single gene (Mendelian) disorders, chromosomal, mitochondrial, multifactorial inheritance and examples of associated disorders.	,,
WEEK 32 23 - 27/05/2022	Population genetics: phenotypes, genotypes and gene and genotype frequencies, Hardy-Weinberg law, equilibrium, frequency of X-linked genes, and genotypes, genetic drift. Genetic polymorphism- factors, molecular basis of mutation, origin of mutation and frequency of new mutations.	,,
WEEK 33 30 - 03/06/2022	Clinical cytogenetics: Numerical chromosomal aberrations, (Euploidy and aneuploidy, sex chromosome aneuploidy and clinical correlations, structural chromosome aberrations-translocations, deletions, trans versions and associated disorders, polymorphic markers and linkage analysis. Genetic diagnosis and therapy: prenatal screening, genetic diagnosis and gene therapy.	,,
WEEK 34 - 36	END YEAR EXAMINATIONS	Prof. Nguu Prof. Mukuria Dr. Mulinge Dr Mobegi

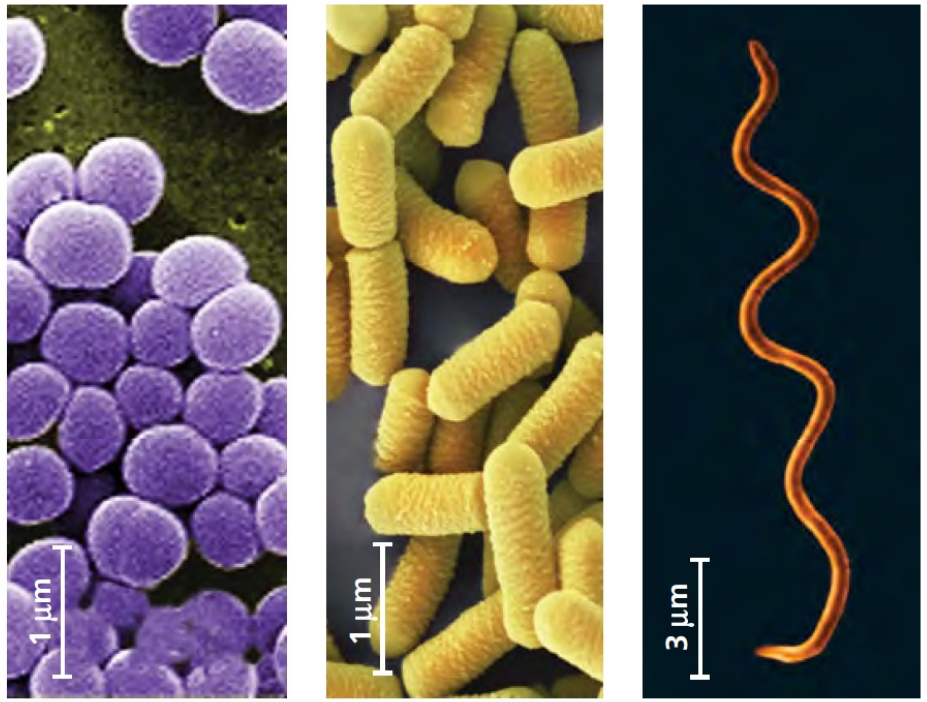
Bacterial Biochemistry

Bacterial structure

A. **Shape.** Along with other properties, shape is used to identify bacteria.

* It is determined by the mechanism of cell wall assembly.

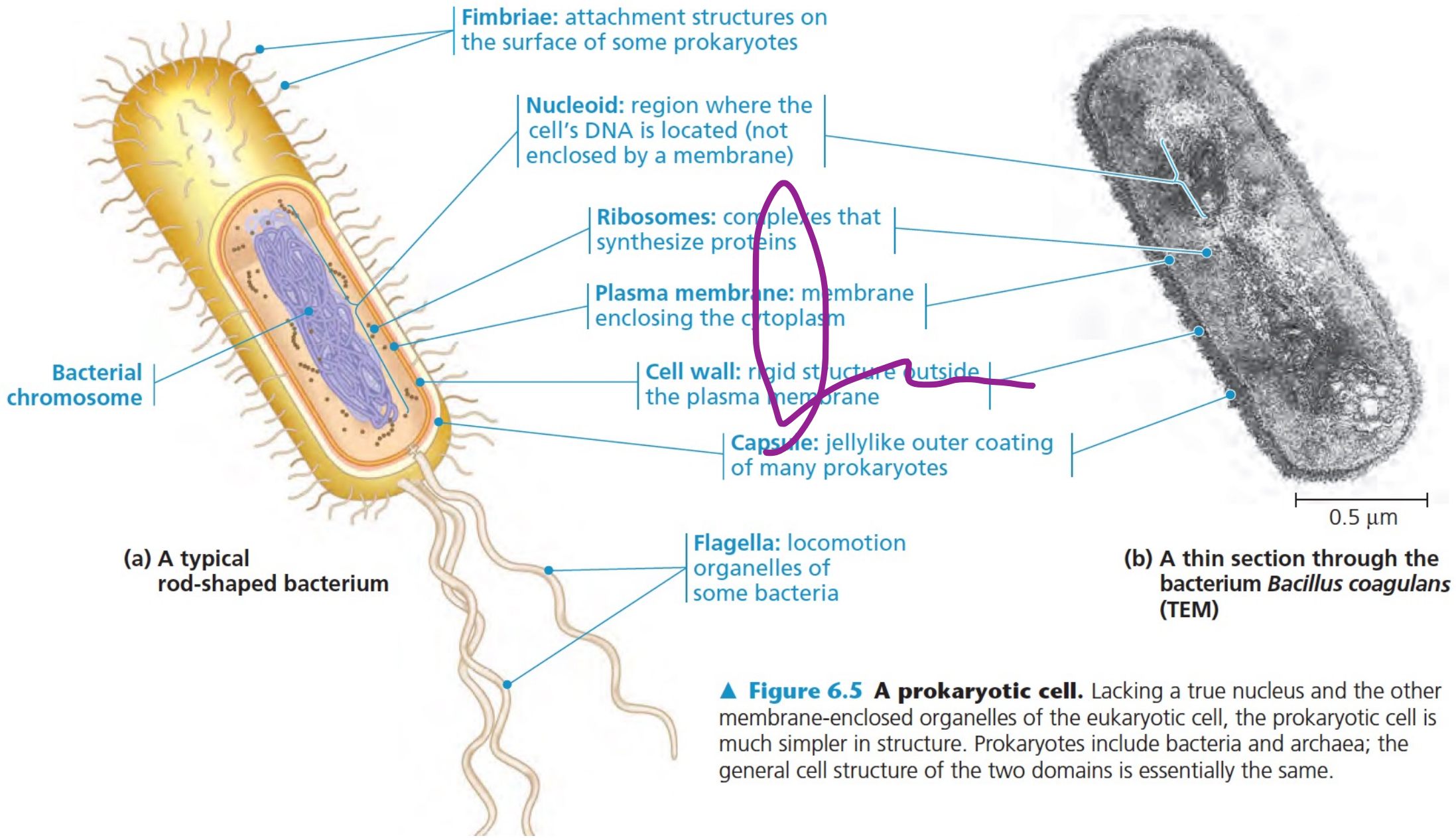
- Bacterial shape usually can be determined with appropriate staining and a light microscope.
- Types
  - a. Round (coccus)
  - b. Rod-like (bacillus)
  - c. Spiral
- Cocci and bacilli often grow in doublets (diplococci) or chains (streptococci). Cocci that grow in clusters are called staphylococci.
- Some bacterial species are pleomorphic, such as Bacteroides.
- Antibiotics that affect cell wall biosynthesis (e.g., penicillin) may alter a bacteria's shape.



(a) Spherical (b) Rod-shaped (c) Spiral

▲ **Figure 27.2 The most common shapes of prokaryotes.** **(a)** Cocci (singular, *coccus*) are spherical prokaryotes. They occur singly, in pairs (diplococci), in chains of many cells (streptococci), and in clusters resembling bunches of grapes (staphylococci). **(b)** Bacilli (singular, *bacillus*) are rod-shaped prokaryotes. They are usually solitary, but in some forms the rods are arranged in chains (streptobacilli). **(c)** Spiral prokaryotes include spirilla, which range from comma-like shapes to loose coils, and spirochetes (shown here), which are corkscrew-shaped (colorized SEMs).

Bacterial structure



▲ **Figure 6.5 A prokaryotic cell.** Lacking a true nucleus and the other membrane-enclosed organelles of the eukaryotic cell, the prokaryotic cell is much simpler in structure. Prokaryotes include bacteria and archaea; the general cell structure of the two domains is essentially the same.

Bacterial structure

B. Nucleus. In bacteria, the nucleus generally is called a nucleoid or nuclear body.

- The bacterial nucleus is not surrounded by a nuclear membrane, nor does it contain a mitotic apparatus.
- Composition. The nucleus consists of polyamine and magnesium Ions bound to negatively charged, circular, supercoiled, double-stranded DNA; small amounts of RNA; RNA polymerase; and other proteins.

C. Cytoplasm

- Bacterial cytoplasm contains ribosomes and various types of nutritional storage granules.

D. Ribosomes. Bacterial ribosomes contain proteins and RNAs that differ from those of their eukaryotic counterparts.

- Types. Bacterial ribosomes have a sedimentation coefficient of 70S and are composed of 30S and 50S subunits containing 16S, and 23S and 5S RNA, respectively.
- Ribosomes engaged in protein biosynthesis are membrane bound.
- **Many antibiotics target ribosomes, inhibiting protein biosynthesis.** Some antibiotics selectively target the 70s ribosomes (e.g., erythromycin), but not 80s ribosomes.

E. Cell (cytoplasmic) membrane

1. Structure. The cell membrane is a typical phospholipid bilayer that contains the following constituents:

- Cytochromes and enzymes involved in electron transport and oxidative phosphorylation.
- Carrier lipids, enzymes, and penicillin-binding proteins (PCP) involved in cell wall biosynthesis.
- Enzymes involved in phospholipid synthesis and DNA replication.
- Chemoreceptors.

2. Functions

- Selective permeability and active transport facilitated by membrane-bound permeases, binding proteins, and various transport systems.
- Site of action of certain antibiotics such as polymyxin.

Bacterial structure

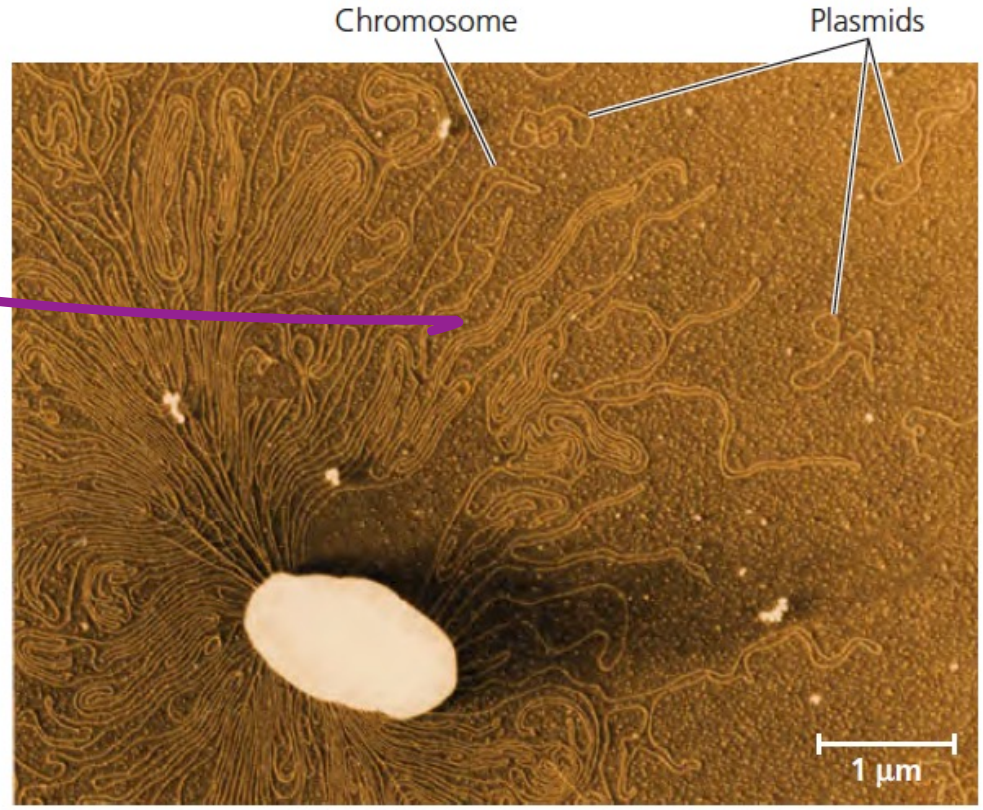
F. Mesosomes are controversial structures that are convoluted invaginations of the plasma membrane.

- ❖ Septal mesosomes occur at the septum (cross-wall); lateral mesosomes are nonseptal.

2. Functions: participate in DNA replication, cell division, and secretion.

G. Plasmids

1. Plasmids are small, circular, nonchromosomal, double-stranded DNA molecules that are:
   - ❖ Capable of self-replication.
   - ❖ Most frequently extrachromosomal but may become integrated into bacterial DNA.
2. Function: contain genes that confer protective properties such as antibiotic resistance or virulence factors or their own transmissibility to other bacteria.



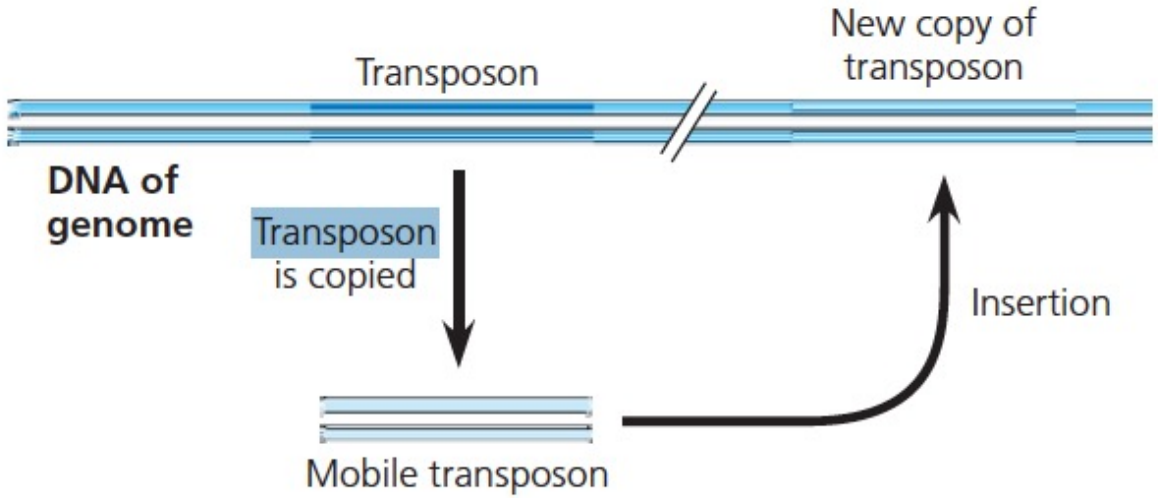
▲ **Figure 27.8 A prokaryotic chromosome and plasmids.** The thin, tangled loops surrounding this ruptured *E. coli* cell are parts of the cell's large, circular chromosome (colorized TEM). Three of the cell's plasmids, the much smaller rings of DNA, are also shown.

H. Transposons

1. Transposons are small pieces of DNA that move between the DNA of bacteria and plasmids; they do not self-replicate.

2. Functions

- ❖ Code for antibiotic resistance enzymes, metabolic enzymes, or toxins.
- ❖ May alter expression of neighboring genes or cause mutations to genes into which they are inserted.



▲ **Figure 21.9 Transposon movement.** Movement of transposons by either the cut-and-paste mechanism or the copy-and-paste mechanism (shown here) involves a double-stranded DNA intermediate that is inserted into the genome.

Bacterial structure

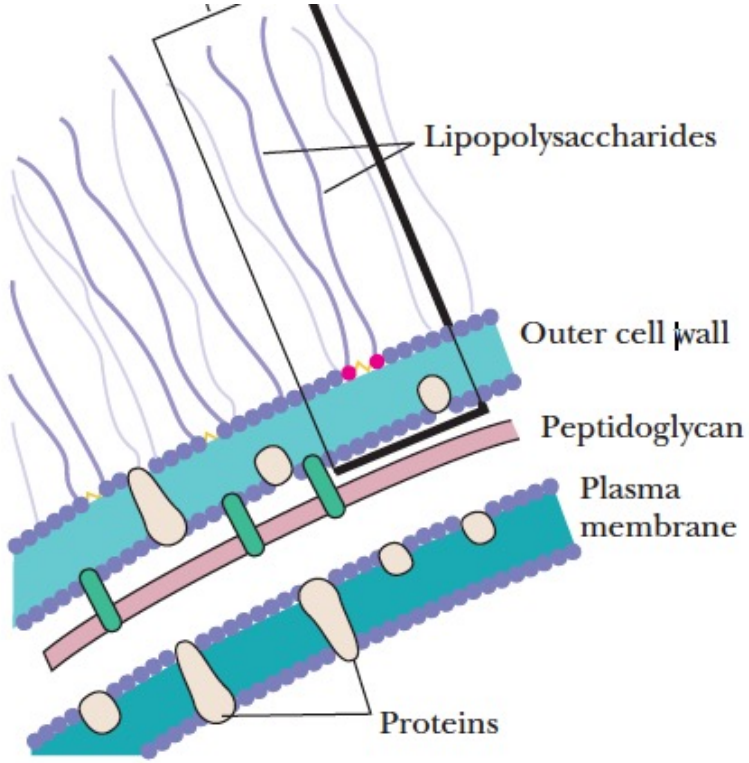
I. Cell envelope (Figs. 2.1 and 2.2 ➔ Next page)

1. General structure. The cell envelope is composed of the macromolecular layers that surround the bacterium. It includes:

- A cell membrane and a peptidoglycan layer except for mycoplasma.
- An outer membrane layer in Gram-negative bacteria.
- A capsule, a glycocalyx layer, or both (sometimes).
- Antigens that frequently induce a specific antibody response.

2. Cell wall

- The cell wall refers to that portion of the cell envelope that is external to the cytoplasmic membrane and internal to the capsule or glycocalyx.
- It confers osmotic protection and Gram-staining characteristics.
- In Gram-positive bacteria it is composed of:
  i. Peptidoglycan
  ii. Teichoic and teichuronic acids
  iii. Polysaccharides
- In Gram-negative bacteria, it is composed of:
  i. Peptidoglycan
  ii. Lipoprotein
  iii. An outer phospholipid membrane that contains lipopolysaccharide



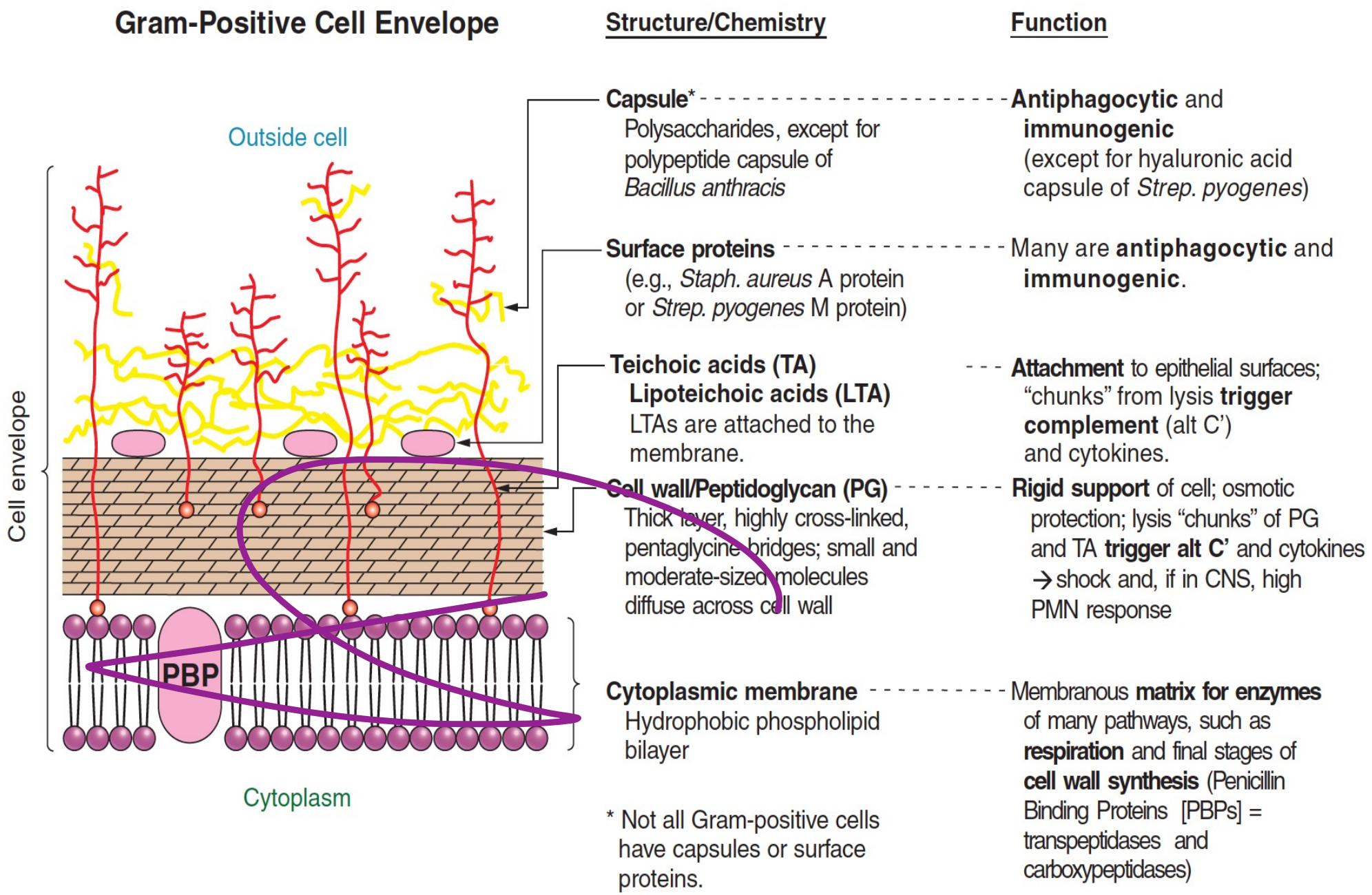


FIGURE 2.1. Gram-positive cell envelope showing structures and describing their chemistry and function. (Modified from Hawley LB. *High-yield microbiology and infectious diseases*. 2nd ed. Baltimore: Lippincott Williams & Wilkins, 2006:2-1.)

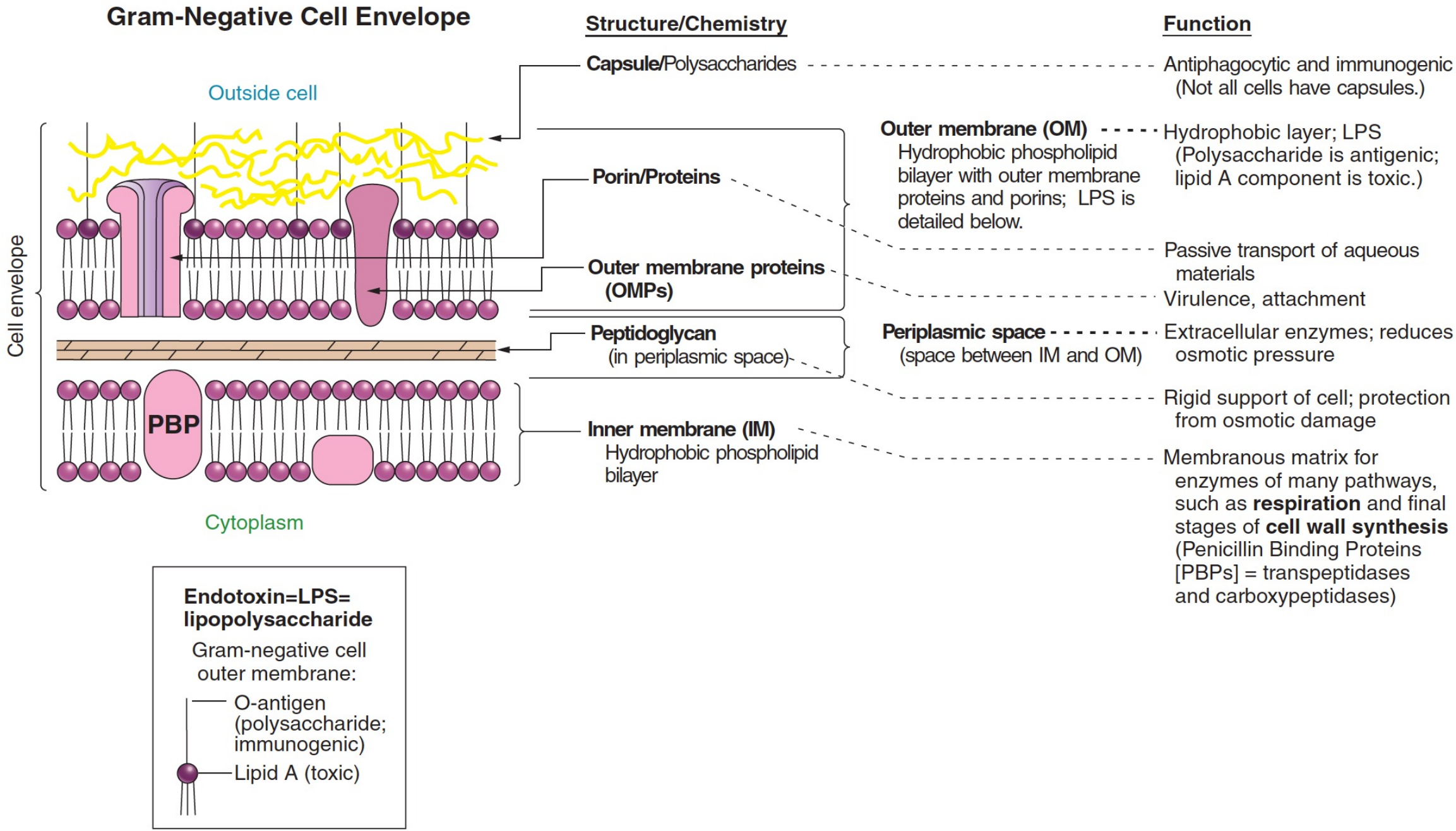


FIGURE 2.2. Gram-negative cell envelope showing structures and describing their chemistry and function. (Modified from Hawley LB. *High-yield microbiology and infectious diseases*. 2nd ed. Baltimore: Lippincott Williams & Wilkins, 2006:2-2.)

Bacterial structure: Peptidoglycan

3. Peptidoglycan (also called mucopeptide or murein) is unique to prokaryotes. It is found in all bacterial cell walls except Mycoplasma.

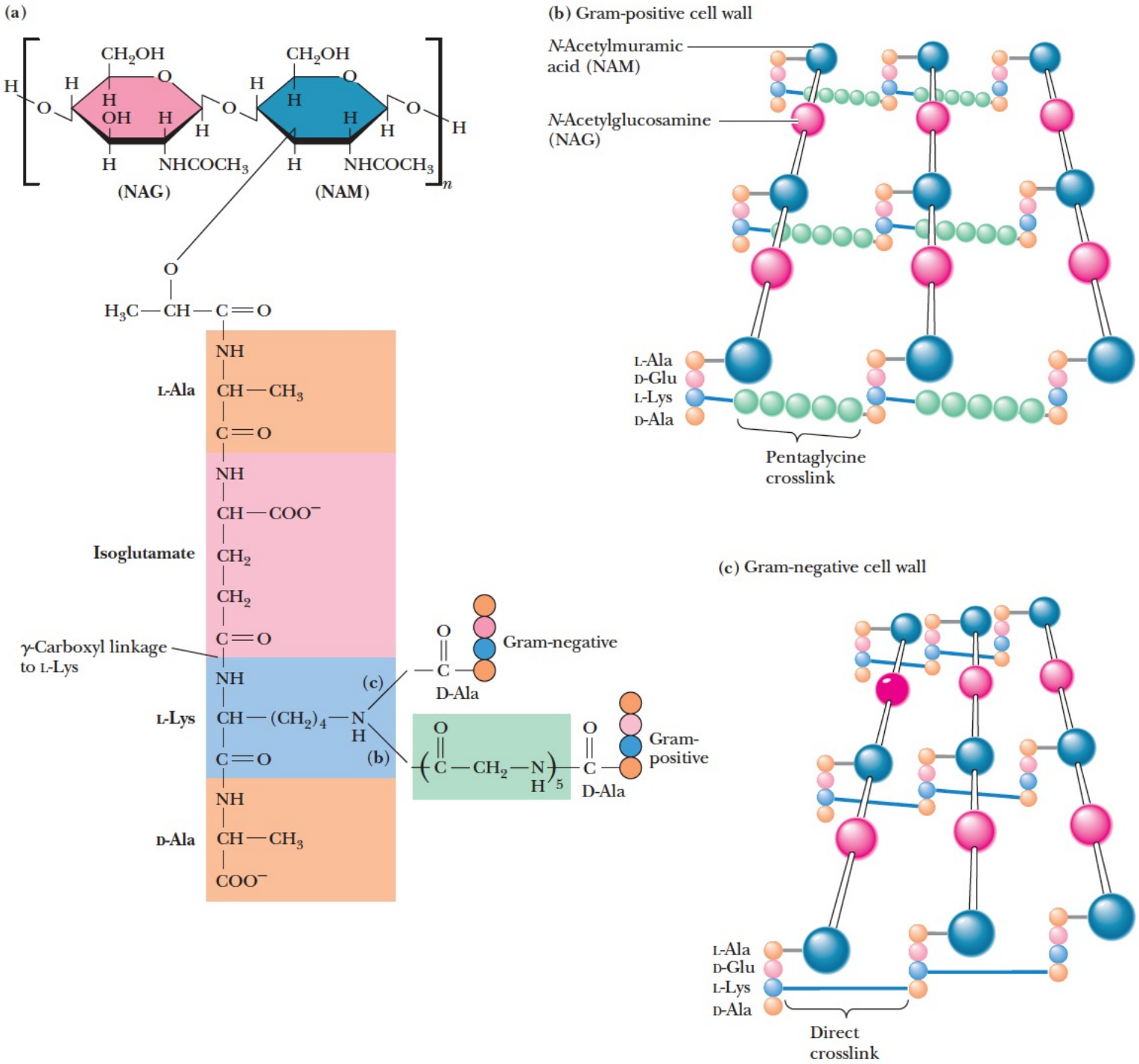
❖ **Structure**

i. This complex polymer consists of a backbone composed of alternating N-acetylglucosamine and N-acetylmuramic acid and a set of identical tetrapeptide side chains.
ii. The tetrapeptide side chains are attached to the N-acetylmuramic acid and are frequently linked to adjacent tetrapeptides by identical peptide cross-bridges or by direct peptide bonds.
iii. The b-1, 4 glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine is cleaved by the bacteriolytic enzyme lysozyme (found in mucus, saliva, and tears).
iv. It may contain diaminopimelic acid, an amino acid unique to prokaryotic cell walls.

❖ Peptidoglycan is the site of action of certain antibiotics such as penicillin and the cephalosporins.

❖ In Gram-positive bacteria, it comprises up to 50% of the cell wall. In Gram-negative bacteria, it comprises only 2% to 10% of the cell wall.

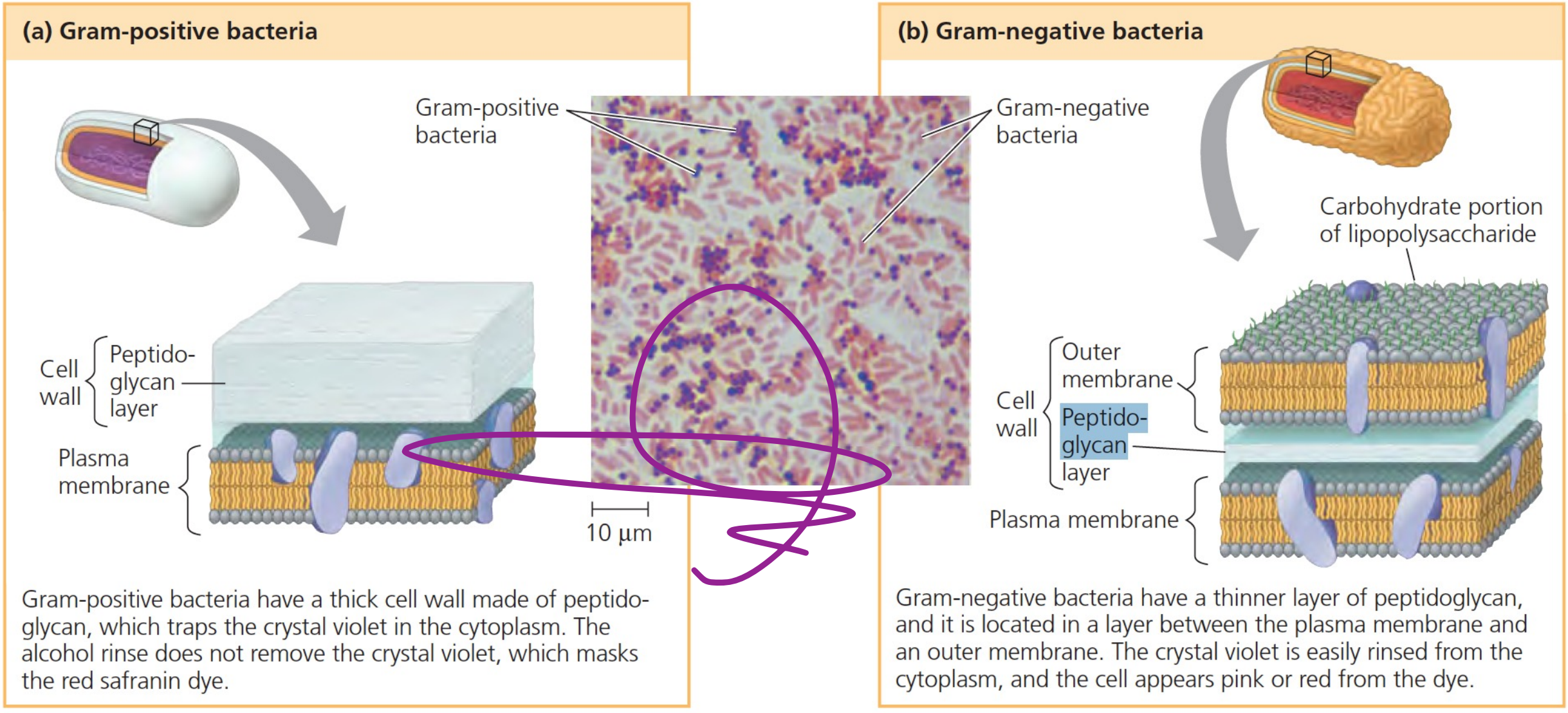
Bacterial structure➔ Peptidoglycan



(a) The structure of peptidoglycan. The tetrapeptides linking adjacent backbone chains contain an unusual y-carboxyl linkage. (b) The crosslink in Gram-positive cell walls is a pentaglycine bridge. (c) In gramnegative cell walls, the linkage between the tetrapeptides of adjacent carbohydrate chains in peptidoglycan involves a direct amide bond between the lysine side chain of one tetrapeptide and D-alanine of the other.

Bacterial structure

▼ Figure 27.3 Gram staining.



Gram-positive bacteria have a thick cell wall made of peptidoglycan, which traps the crystal violet in the cytoplasm. The alcohol rinse does not remove the crystal violet, which masks the red safranin dye.

Gram-negative bacteria have a thinner layer of peptidoglycan, and it is located in a layer between the plasma membrane and an outer membrane. The crystal violet is easily rinsed from the cytoplasm, and the cell appears pink or red from the dye.

Bacterial structure

4. **Teichoic** and **teichuronic acids** are water-soluble polymers, containing a ribitol or glycerol residue linked by phosphodiester bonds.

a. They are found in **Gram-positive** cell walls or membranes.

- ❖ Teichoic acid is found in cell walls and is chemically bonded to peptidoglycan.
- ❖ Lipoteichoic acid is found in cell membranes and is chemically bonded to membrane glycolipid, particularly in mesosomes.

b. Functions

- ❖ Contain important bacterial surface **antigenic determinants**, and lipoteichoic acid helps anchor the wall to the membrane.
- ❖ May account for 50% of the dry weight of a Gram-positive cell wall.

5. Lipoprotein is found in Gram-negative bacteria.

- ❖ Lipoprotein cross-links the peptidoglycan and outer membrane.
- ❖ A peptide bond links the lipoprotein to diaminopimelic acid residues of peptidoglycan tetrapeptide side chains; the lipid portion is noncovalently inserted into the outer membrane.

6. The **periplasmic space** is found in **Gram-negative** cells.

- ❖ It refers to the area between the cell membrane and the outer membrane.
- ❖ Hydrated peptidoglycan, hydrolytic enzymes including b-lactamases, specific carrier molecules, and oligosaccharides are found in the periplasmic space.

7. An **outer membrane** is found in Gram-negative cells.

a. Structure. The outer membrane is a phospholipid bilayer in which the phospholipids of the outer portion are replaced by lipopolysaccharides. It contains:

i. Embedded proteins, including matrix porins (nonspecific pores)
ii. Some nonpore proteins (phospholipases and proteases)
iii. Transport proteins for small molecules

b. Functions

- ❖ Protects cells from harmful enzymes and some antibiotics
- ❖ Prevents leakage of periplasmic proteins.

Bacterial structure

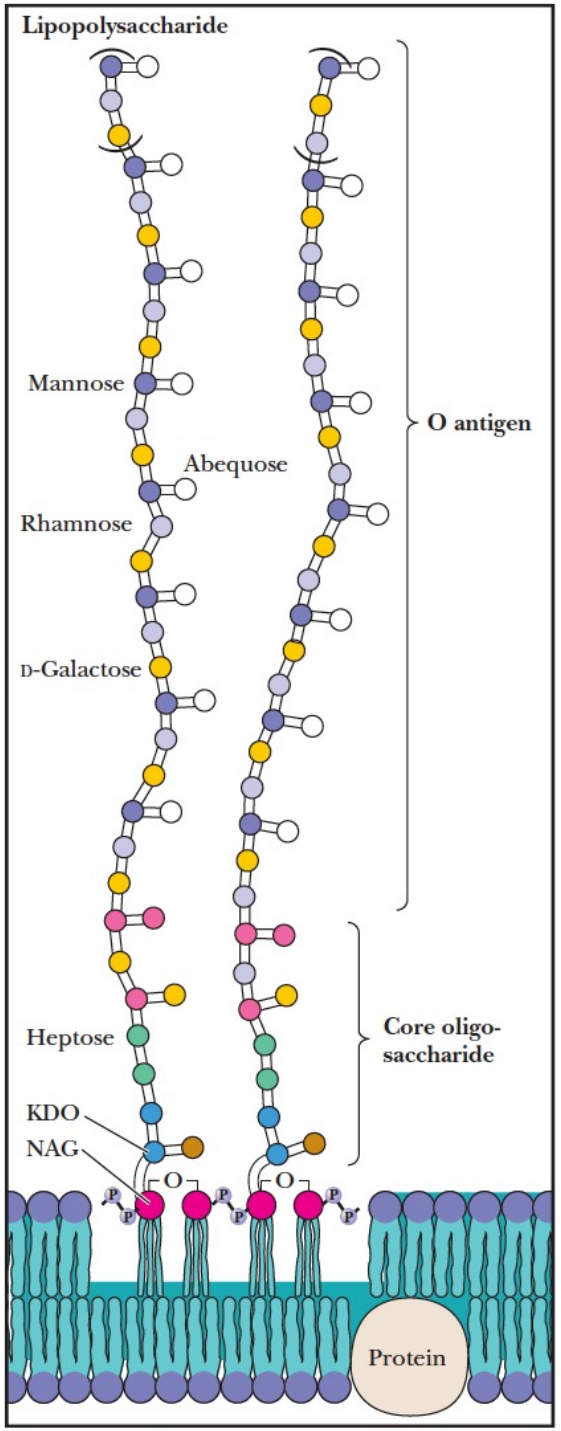
8. Lipopolysaccharide is found in the outer leaflet of the outer membrane of Gram-negative cells.

a. Structure

- Lipopolysaccharide consists of lipid A, several long-chain fatty acids attached to phosphorylated glucosamine disaccharide units, and a polysaccharide composed of a core and terminal repeating units.
- It is negatively charged and noncovalently cross-bridged by divalent cations.

b. Functions

- Also called endotoxin; the toxicity is associated with the lipid A.
- Contains major surface **antigenic determinants**, including O antigen found in the polysaccharide component.



Bacterial structure

J. External layers

1. Surface proteins

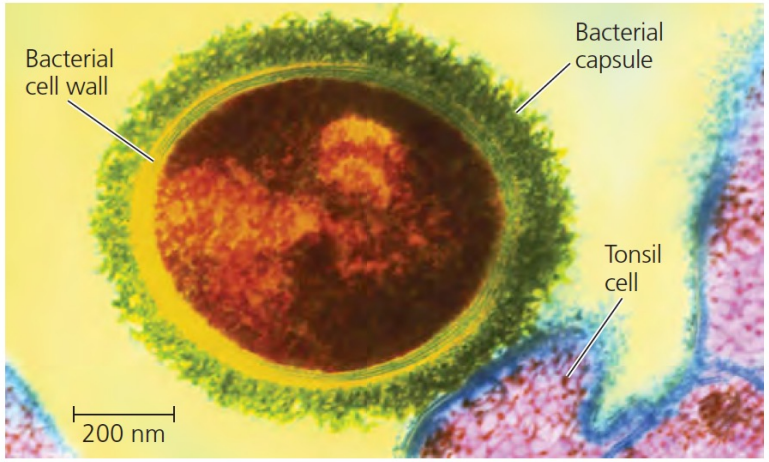
a. These antiphagocytic proteins are external to the cell wall of some gram positive bacteria.

b. Functions: act as adhesins facilitating tissue colonization with several species (e.g., Staphylococcus aureus [fibronectin-binding proteins] and Streptococcus pyogenes [F proteins]).

2. Capsule

a. The capsule is a well-defined structure of polysaccharide surrounding a bacterial cell and is external to the cell wall. The one exception to the polysaccharide structure is the poly-D glutamic acid capsule of Bacillus anthracis.

b. Functions: protects the bacteria from phagocytosis and plays a role in bacterial adherence.



▲ **Figure 27.4 Capsule.** The polysaccharide capsule around this *Streptococcus* bacterium enables the prokaryote to attach to cells in the respiratory tract—in this colorized TEM, a tonsil cell.

3. Glycocalyx

a. The glycocalyx refers to a loose network of polysaccharide fibrils that surrounds some bacterial cell walls.
   - ➢ It is sometimes called a slime layer.
   - ➢ It is synthesized by surface enzymes.

b. Functions: associated with adhesive properties of the bacterial cell and contains prominent antigenic sites.

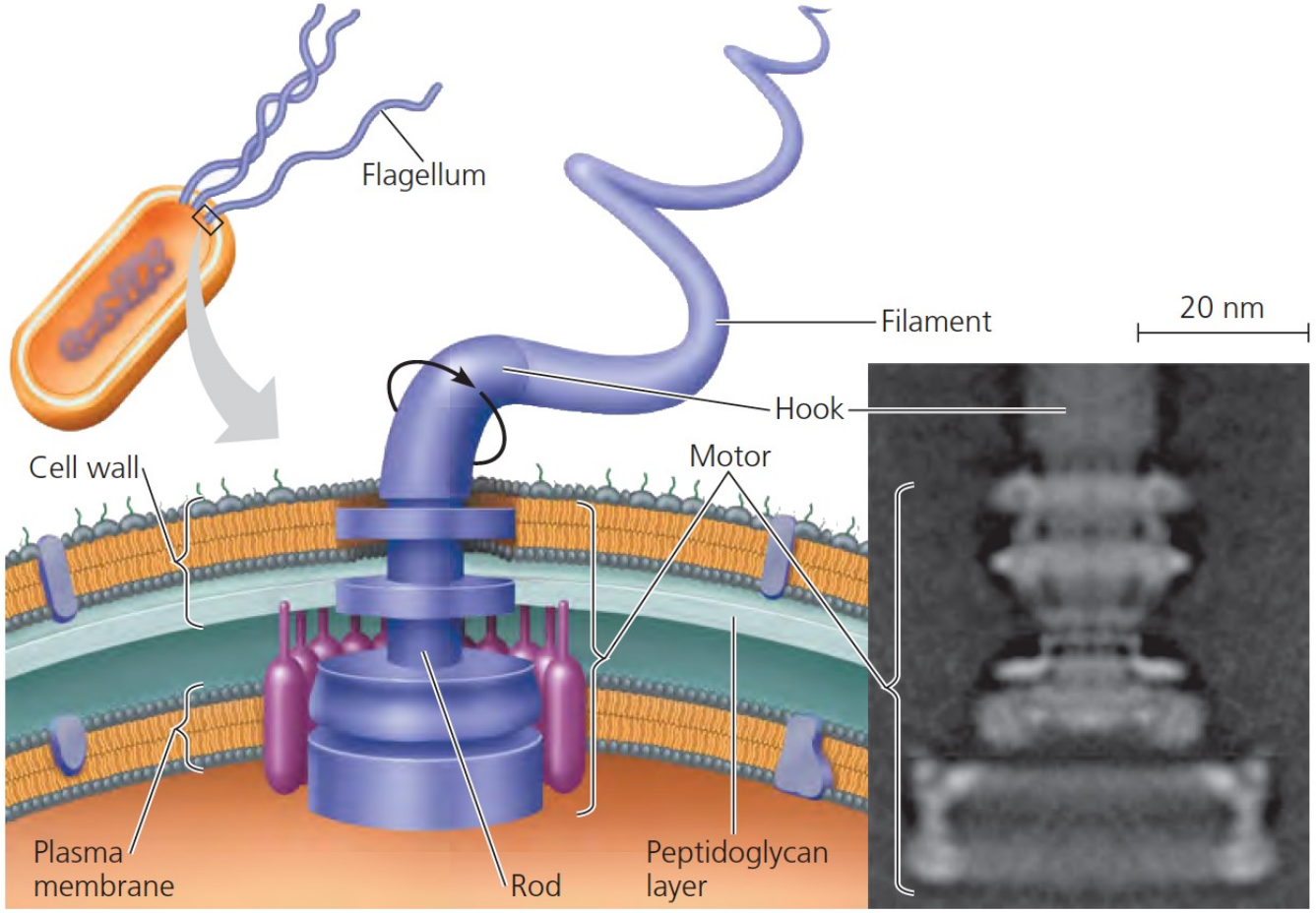
Bacterial structure

K. Appendages

1. Flagella are protein appendages for locomotion and contain prominent antigenic determinants.

a) They consist of a basal body, hook, and a long filament composed of a polymerized protein called flagellin.

b) Flagella may be located in only one area of a cell (polar) or over the entire bacterial cell surface (peritrichous).



▲ **Figure 27.6 A prokaryotic flagellum.** The motor of a prokaryotic flagellum consists of a system of rings embedded in the cell wall and plasma membrane (TEM). ATP-driven pumps in the motor transport protons out of the cell. The diffusion of protons back into the cell provides the force that turns a curved hook and thereby causes the attached filament to rotate and propel the cell. (This diagram shows flagellar structures characteristic of gram-negative bacteria.)

Bacterial structure

K. Appendages

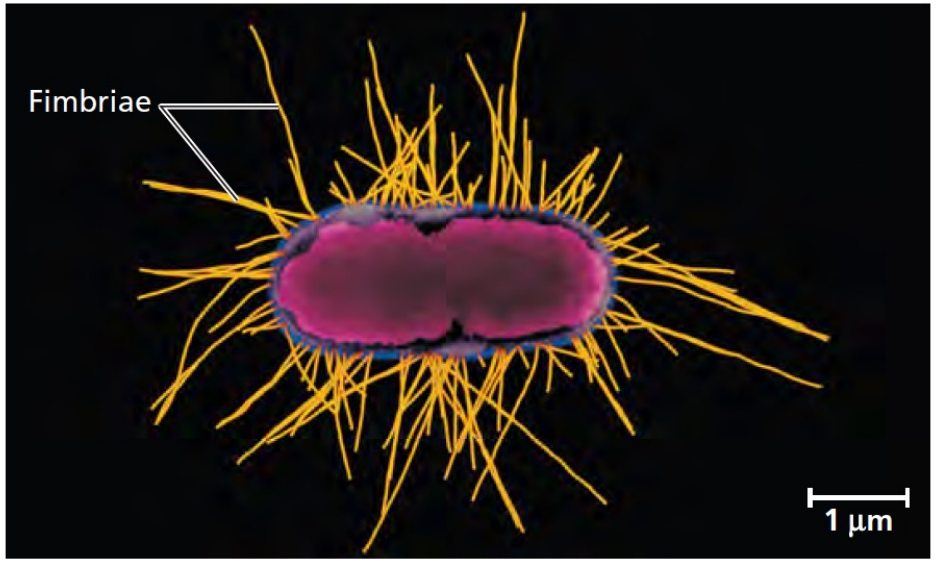
2. Pili (fimbriae) are rigid surface appendages composed mainly of a protein called pilin.

a. Types

- Ordinary pili (adhesins) are involved in bacterial adherence and Gram-positive cell conjugation.
- Sex pili are involved in attachment of donor and recipient bacteria in Gram-negative cell conjugation.

b. Functions

- Ordinary pili are the **colonization antigens or virulence factors** associated with some bacterial species such as S. pyogenes and Neisseria gonorrhoeae.
- They also may **confer antiphagocytic properties**, such as the M protein of S. pyogenes



▲ **Figure 27.5 Fimbriae.** These numerous protein-containing appendages enable some prokaryotes to attach to surfaces or to other cells (colorized TEM).

▲ **Figure 27.12 Bacterial conjugation.** The *E. coli* donor cell (left) extends a pilus that attaches to a recipient cell, a key first step in the transfer of DNA. The pilus is a flexible tube of protein subunits (TEM).

Bacterial structure

L. Endospores

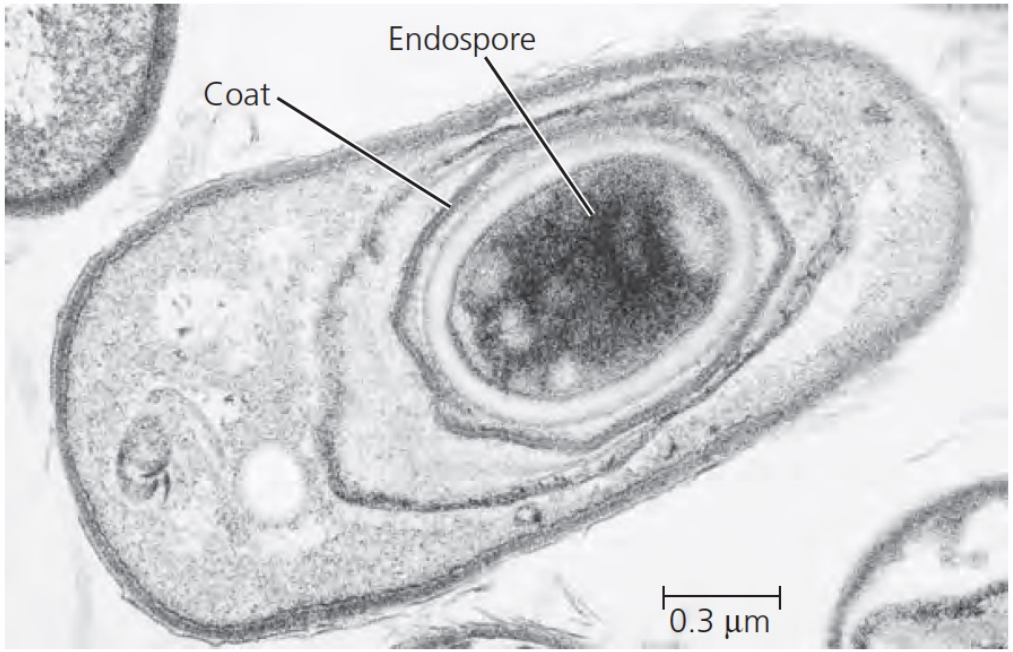
1. General characteristics. Endospores are formed as a survival response to certain adverse nutritional conditions, such as depletion of a certain resource. These metabolically inactive bacterial cells are highly resistant to desiccation, heat, and various chemicals. They are helpful in identifying some species of bacteria (e.g., Bacillus and Clostridium).

2. Structure

- ❖ Endospores possess a core that contains many cell components, a spore wall, a cortex, a coat, and an exosporium.
- ❖ The core contains calcium dipicolinate, which aids in heat resistance within the core.

3. Function: endospores germinate under favorable nutritional conditions after an activation process that involves damage to the spore coat. They are not reproductive structures.

M. Biofilms are aggregates of bacterial cells that form in soil and marine environments and the surface of medical implants devices (e.g., prostheses). They enhance nutrient uptake and often exclude antimicrobials.



▲ **Figure 27.9 An endospore.** *Bacillus anthracis*, the bacterium that causes the disease anthrax, produces endospores (TEM). An endospore's protective, multilayered coat helps it survive in the soil for years.

Bacterial structure

Cell wall synthesis

1. Cell wall synthesis involves the cytoplasmic synthesis of peptidoglycan subunits, which are translocated by a membrane lipid carrier and cross-linked to existing cell wall by enzymes associated with the plasma membrane of Gram-positive bacteria or found in the periplasmic region of Gram-negative bacteria.
2. In Gram-positive cells, it involves the covalent linkage of teichoic acid to N-acetylmuramic acid residues.
3. In Gram-negative cells, three components (lipoprotein, outer membrane, lipopolysaccharide) are added, whose constituents or subunits are synthesized on or in the cytoplasmic membrane and assembled outside of the cell.

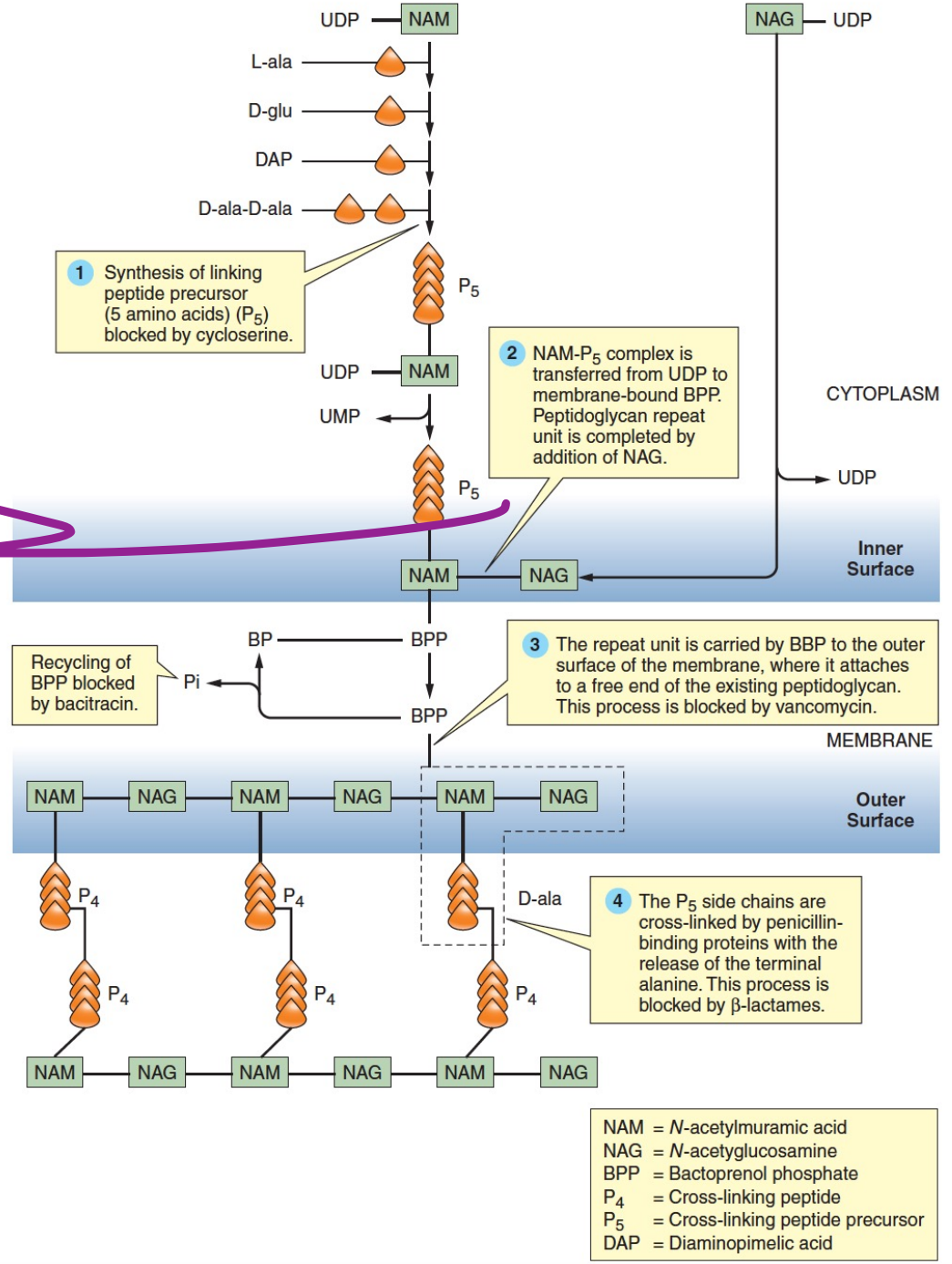


FIGURE 2.4. Synthesis of peptidoglycan.

BACTERIAL PATHOGENESIS

BACTERIAL PATHOGENESIS

- The pathogenesis of a bacterium depends on its virulence properties and the capabilities of the host's defense mechanism.
- Normal flora may become pathogenic if they gain access to normally sterile body areas or their environmental conditions allow them to multiply to a level not controlled by the host.

B. Virulence factors.

These features may be genetically encoded on the bacterial chromosome or located on plasmids.

❖ Structural bacterial components. These virulence factors include:

i. Antiphagocytic surface proteins and capsules
ii. Adhesins that promote colonization
iii. Endotoxins of Gram-negative bacteria
iv. Immunoglobulin G (IgG) antibody binding surface proteins
v. Antigenic switching of surface antigens due to phase variation or antigenic variation processes

❖ Extracellular gene products. These include:

i. Degradative enzymes like collagenase and hyaluronidase that facilitate tissue invasion
ii. IgA antibody-degrading proteases
iii. Exotoxins

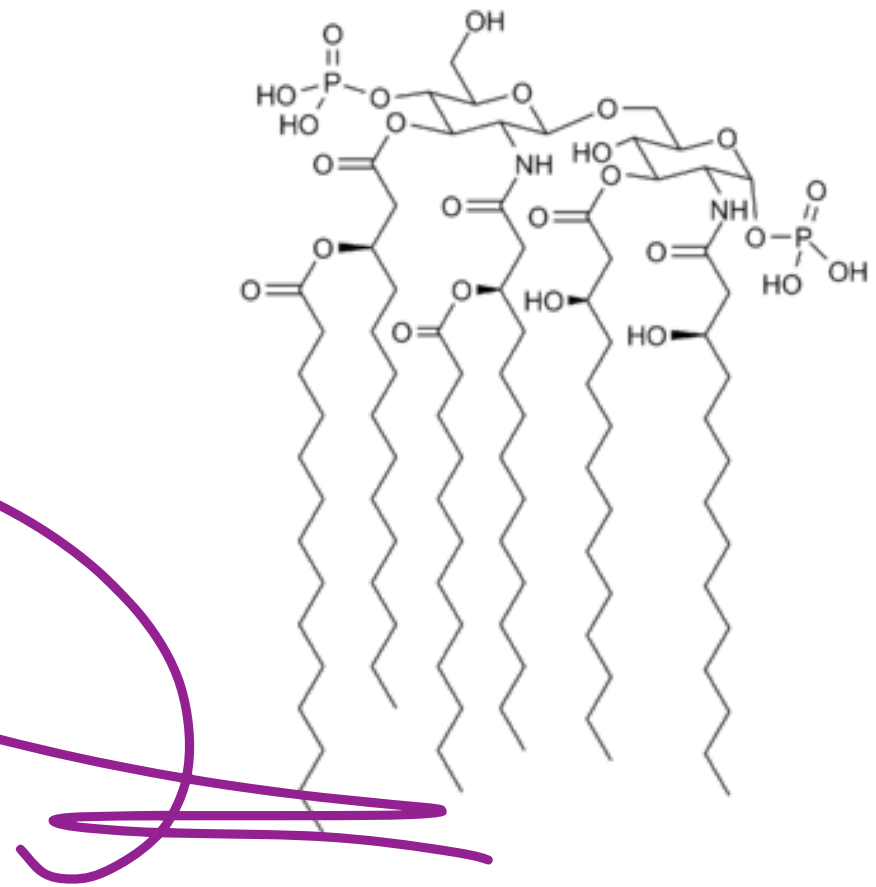
❖ Growth properties include the capacity for intracellular growth and the ability to form biofilms.

C. Toxins

- Pathogenic prokaryotes usually cause illness by producing poisons, which are classified as exotoxins or endotoxins.

1. Endotoxins consist of the lipid-A component of Gram-negative bacteria. Endotoxins have the following actions:

- Induce the release of endogenous pyrogens (e.g., interleukin 1 [IL-1], tumor necrosis factor [TNF], prostaglandins, etc.).
- Increase vascular permeability.
- Initiate complement and blood coagulation cascades.
- Cause fever, hypotension, disseminated intracellular coagulation, and shock.



Chemical structure of lipid A as found in *coli*

C. Toxins

2. Exotoxins are secreted by Gram-positive and Gram-negative bacteria; they may be genetically encoded in the bacterial chromosome, a plasmid, or a phage.

❖ **Actions.** They have the following five mechanisms of action (see Table 2.2):

i. Alter cellular components
ii. Act as superantigens that cause inappropriate release of cytokines
iii. Inhibit protein synthesis
iv. Increase cAMP
v. Alter nerve impulse transmission

Examples

❖ Some exotoxins (e.g., Shiga toxin and cholera toxin) have an A-B subunit structure in which one or more B subunits are involved in binding and the A subunit possesses the biological activity inside the cell.

❖ Others are a single polypeptide with:

✓ Enzymatic activity (e.g., a toxin of Clostridia perfringens, which has phospholipase-C activity)
✓ Other biological activities (e.g., superantigens like toxic shock syndrome toxin 1 [TSST-1] of S. aureus)

Toxoids are chemically altered forms of toxins that may be used as immunization agents. Toxoids induce antibodies that minimize the toxin's biological affects (e.g., diphtheria and tetanus toxins).

Bacterial Exotoxins

table 2.2 Examples of Bacterial Exotoxins

Biological Effect	Toxin Name	Organism	Gene Location	Mechanism
Alter Cellular Components	α toxin	Staphylococcus	Bacterial Chromosome	Forms pore
	Streptolysin O	Streptococcus pyogenes	Bacterial Chromosome	Forms pore
	α toxin	Clostridia perfringens	Bacterial Chromosome and Plasmid	Disrupts Membranes
	Type III Cytotoxin	Pseudomonas aeruginosa	Phage	Cytoskeletal Changes
	Type III Cytotoxin	Salmonella species	Bacterial Chromosomes	Alters Actin Cytoskeleton
Superantigens	TSST-1	Staphylococcus aureus	Bacterial Chromosome	Release of cytokines
	Enterotoxin	Staphylococcus aureus	Phage	Release of cytokines
	Erythrogenic Toxins A and C	Streptococcus pyogenes	Phage	Release of cytokines
Inhibition of Protein Synthesis	Diphtheria Toxin	Corynebacterium diphtheria	Phage	ADP Ribosylates Elongation Factor 2
	Exotoxin A	Pseudomonas	Bacterial Chromosome	ADP Ribosylates Elongation Factor 2
	Shiga Toxin	Shigella dysenteriae	Plasmid	Inactivates 60S Ribosomes
	Vero Toxin	Enterohemorrhagic E. coli	Bacterial Chromosome or Phage	Inactivates 60S Ribosomes
Increased Synthesis of cAMP	Cholera Toxin	Vibrio cholera	Bacterial Chromosome	Turns on Stimulatory G Protein
	Enterotoxigenic ST Toxin	E. coli	Plasmid	Turns on Stimulatory G Protein
	Anthrax Toxin	Bacillus anthrax	Plasmid	Adenylate Cyclase Activity
	Pertussis Toxin	Bordetella pertussis	Bacterial Chromosome	Turns off inhibitory G Protein
Altered Nerve Impulse Transmission	Tetanus Toxin	Clostridia tetani	Plasmid	Inhibits Inhibitory Neuro-transmitter Release
	Botulinun Toxin	Clostridia botulinun	Phage	Inhibits Acetylcholine Release

Antimicrobial Chemotherapy

ANTIMICROBIAL CHEMOTHERAPY

A. General characteristics

- Antimicrobial chemotherapy is based on the **principle of selective toxicity**, which implies that a compound is harmful to a microorganism but innocuous to its host.

- The drugs used in antimicrobial therapy have the following properties (Fig. 2.12):
  i. Are antimetabolites
  ii. Inhibit cell wall biosynthesis
  iii. Inhibit protein synthesis
  iv. Inhibit nucleic acid synthesis
  v. Alter or inhibit cell membrane permeability or transport

- Antimicrobial drugs can be either bacteriostatic (inhibit growth) or bactericidal (kill).

- Synergistic combinations of bacteriostatic drugs (e.g., trimethoprim and sulfamethoxazole) are sometimes used in antimicrobial therapy.

- Antimicrobial activity can be quantitated and may be modified in certain situations.
  - Dilution or diffusion test is used to determine antimicrobial activity, which is quantitated by determining the minimal inhibitory concentration.
  - Antimicrobial activity may differ in vitro and in vivo.
  - Drug stability, pH, microbial environment, number of microorganisms present, length of incubation with drug, and metabolic activity of microorganisms can alter antimicrobial actions of certain drugs.
  - Genetic or nongenetic drug resistance may modify the antimicrobial activity of a drug for a specific bacterium.

Antimicrobial Chemotherapy

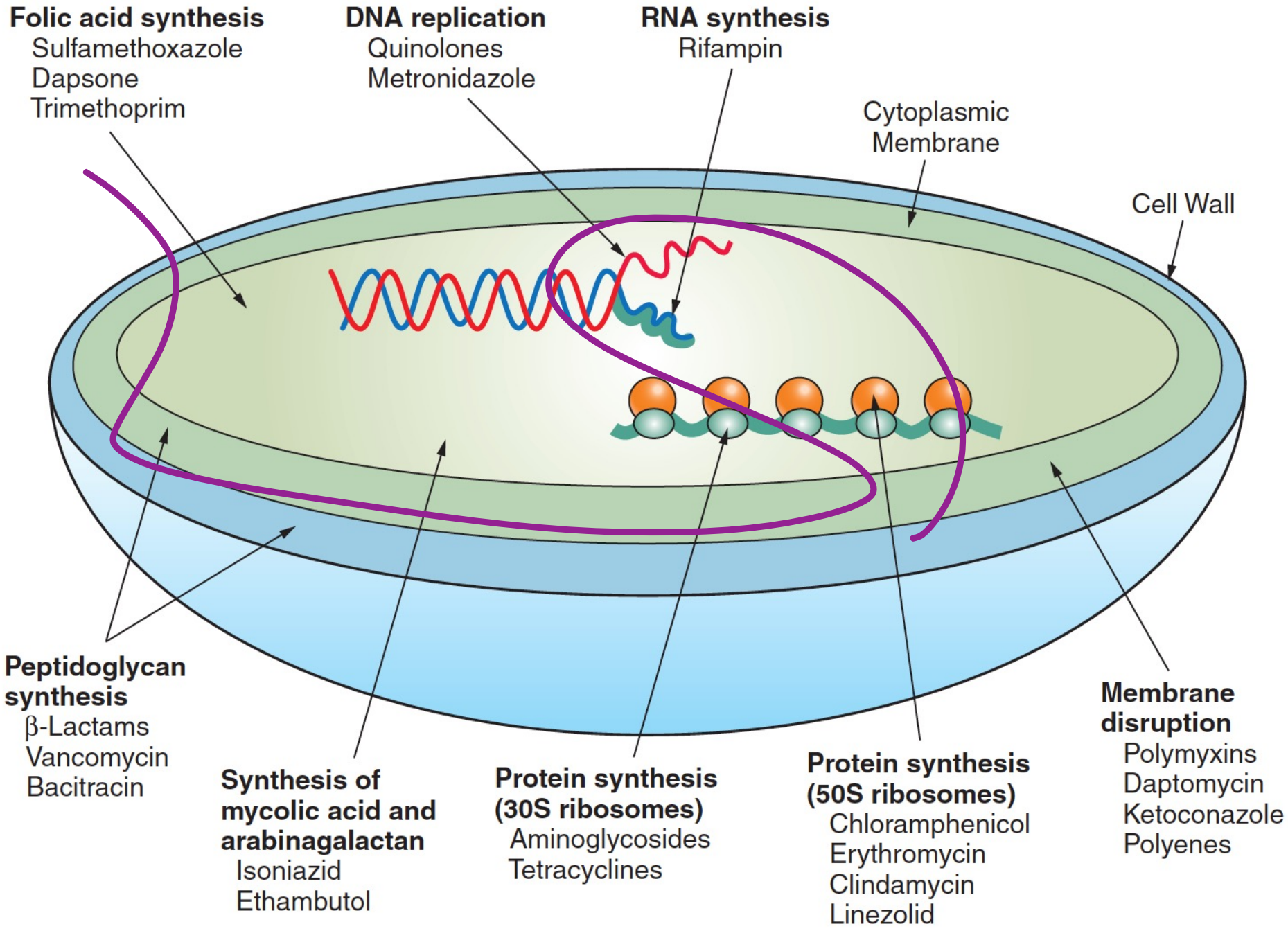


FIGURE 2.12. Sites of antibiotic activity.

Antimicrobial Chemotherapy

table 2.3 Properties of Antibacterial Agents

Mechanism of Action	Agent	Site of Action	Effect	Resistance*
Inhibitors of cell wall biosynthesis	Cycloserine	Peptidoglycan tetrapeptide side chain	Bactericidal	2
	Bacitracin	Membrane carrier molecule	Bactericidal	2
	β-Lactams			
	Penicillins	Peptidoglycan cross-linking	Bactericidal	1
	Cephalosporins	Peptidoglycan cross-linking	Bactericidal	1,2, 3
	Carbapenems	Peptidoglycan cross-linking	Bactericidal	2, 3
	Vancomycin	Translocation of cell wall intermediates	Bactericidal	2, 3

*1 Drug inactivation
2 Target site mutation
3 ↓ Uptake
4 ↑ Efflux
5 New plasmid-coded enzyme

Antimicrobial Chemotherapy

table 2.3 Properties of Antibacterial Agents

Mechanism of Action	Agent	Site of Action	Effect	Resistance*
Inhibitors of protein biosynthesis	Aminoglycosides			
	Streptomycin	30S ribosomal subunit	Bactericidal	1, 2, 3
	Kanamycin	30S ribosomal subunit	Bactericidal	1, 2, 3
	Gentamicin	30S ribosomal subunit	Bactericidal	1, 2, 3
	Tetracycline	30S ribosomal subunit	Bacteriostatic	1, 2, 3, 4
	Spectinomycin	30S ribosomal subunit	Bacteriostatic	1, 2
	Chloramphenicol	50S ribosomal subunit	Bacteriostatic	1, 2, 3
	Erythromycin	50S ribosomal subunit	Bacteriostatic	1, 2, 3, 4
	Clindamycin	50S ribosomal subunit	Bacteriostatic	2, 3
	Linezolid	50S ribosomal subunit	Bacteriostatic	2

*1 Drug inactivation
2 Target site mutation
3 ↓ Uptake
4 ↑ Efflux
5 New plasmid-coded enzyme

Antimicrobial Chemotherapy

table 2.3 Properties of Antibacterial Agents

Mechanism of Action	Agent	Site of Action	Effect	Resistance*
Inhibitors of nucleic acid synthesis	Quinolones	DNA gyrase and Topoisomerase IV	Bactericidal	2, 4
	Novobiocin	DNA gyrase and Topoisomerase IV	Bacteriostatic	
	Rifampin	DNA-dependent RNA polymerase	Bactericidal	2
	Metronidazole	Disrupts DNA	Bactericidal	2

*1 Drug inactivation
2 Target site mutation
3 ↓ Uptake
4 ↑ Efflux
5 New plasmid-coded enzyme

Antimicrobial Chemotherapy

table 2.3 Properties of Antibacterial Agents

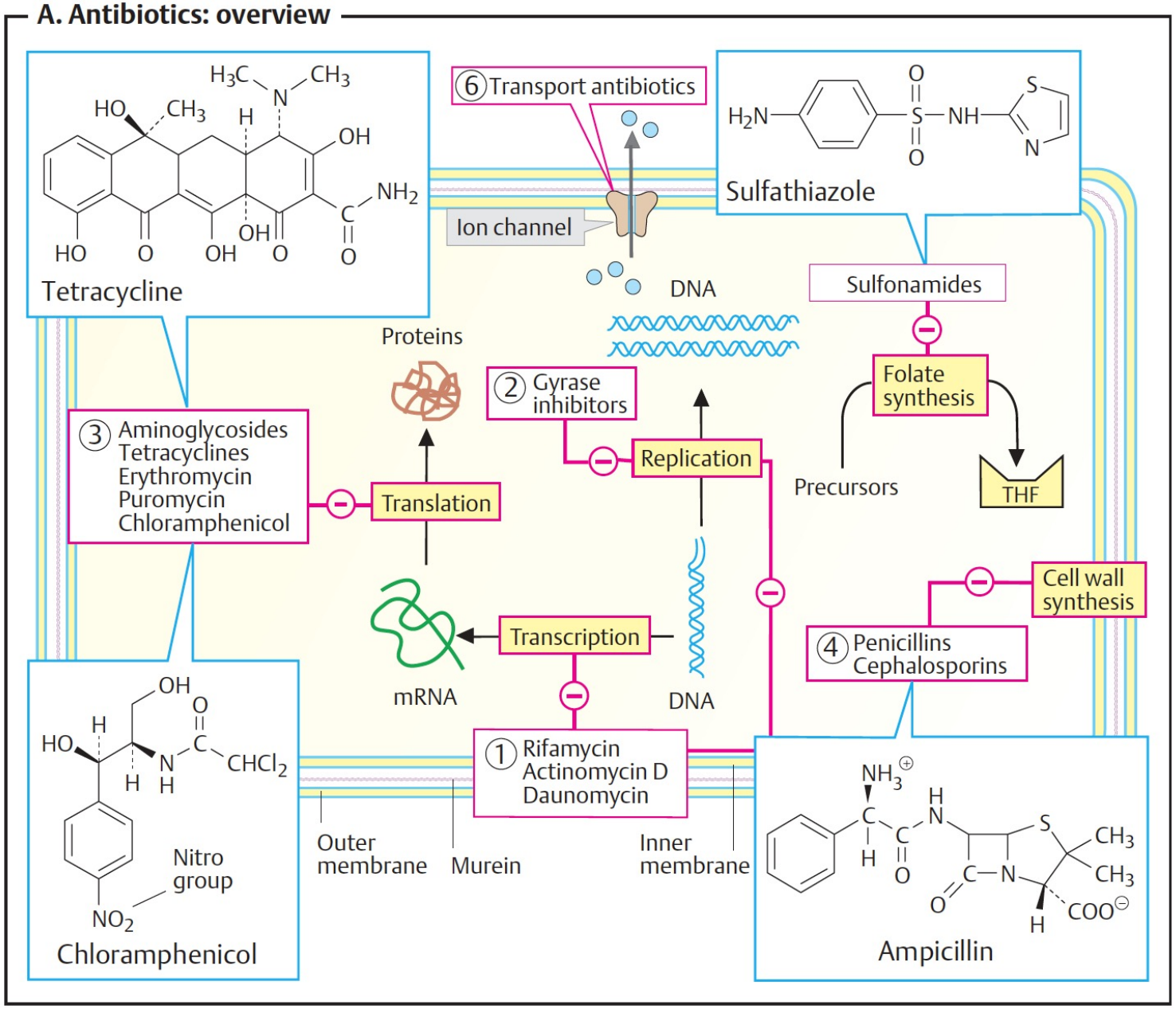
Mechanism of Action	Agent	Site of Action	Effect	Resistance*
Inhibitor of mycolic acid synthesis	Isoniazid	Mycobacterial mycolic acid biosynthesis	Bactericidal	2
Inhibitor of arabinogalactan synthesis	Ethambutol	Arabinogalactan synthesis	Bacteriostatic	2
Alteration of cytoplasmic membrane	Polymyxins	Bacterial membrane permeability	Bactericidal	
	Colistin	Bacterial membrane permeability	Bactericidal	
	Daptomycin	Depolarization of membrane	Bactericidal	

*1 Drug inactivation
2 Target site mutation
3 ↓ Uptake
4 ↑ Efflux
5 New plasmid-coded enzyme

Mechanisms of action of Antimetabolites

1. Antimetabolites are structural analogs of normal metabolites that inhibit the action of specific enzymes.

- ❖ They include bacteriostatic (sulfonamide, trimethoprim, para-aminosalicylic acid) and bactericidal (isoniazid) drugs.
- ❖ Some combinations of individual antimetabolites are bactericidal (e.g., trimethoprim and sulfamethoxazole).



Mechanisms of action: Cell wall synthesis inhibitors

2. Cell wall synthesis inhibitors are bactericidal.

a. General characteristics

Mechanisms of action:

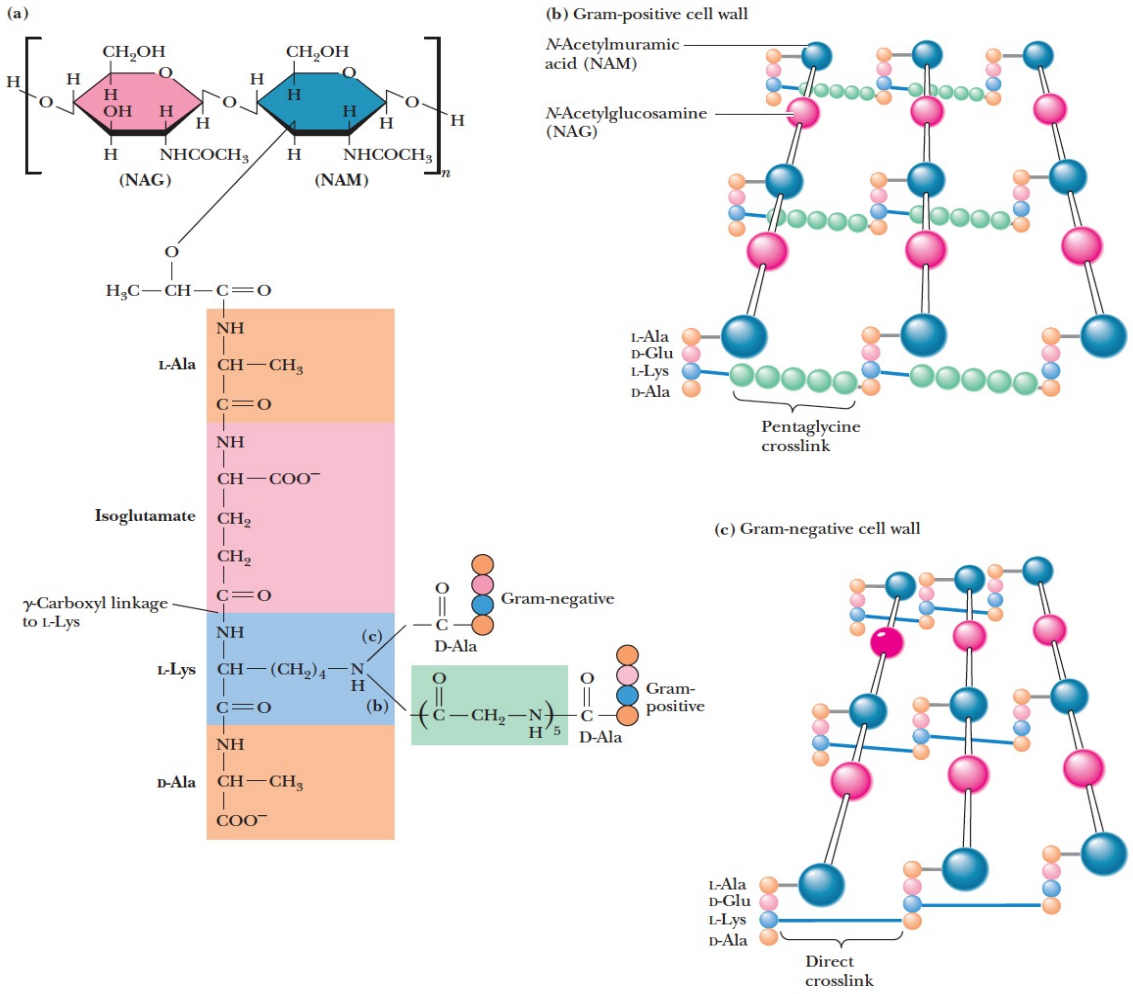
- b-lactam drugs **inhibit transpeptidation (cross-linking) of peptidoglycan** (e.g., penicillins, cephalosporins, and carbapenems).
- Others **inhibit the synthesis of peptidoglycan** (cycloserine, bacitracin, vancomycin).

Location of action:

- They may act in the cytoplasm (cycloserine);
- in the membrane (bacitracin, penicillins, and cephalosporins); or
- in the cell wall (vancomycin).

*** Cell wall synthesis is required for these drugs to be effective.

- Bacteria may take on aberrant shapes or become spheroplasts when exposed to these drugs.



Mechanisms of action: Cell wall synthesis inhibitors

b. Penicillins

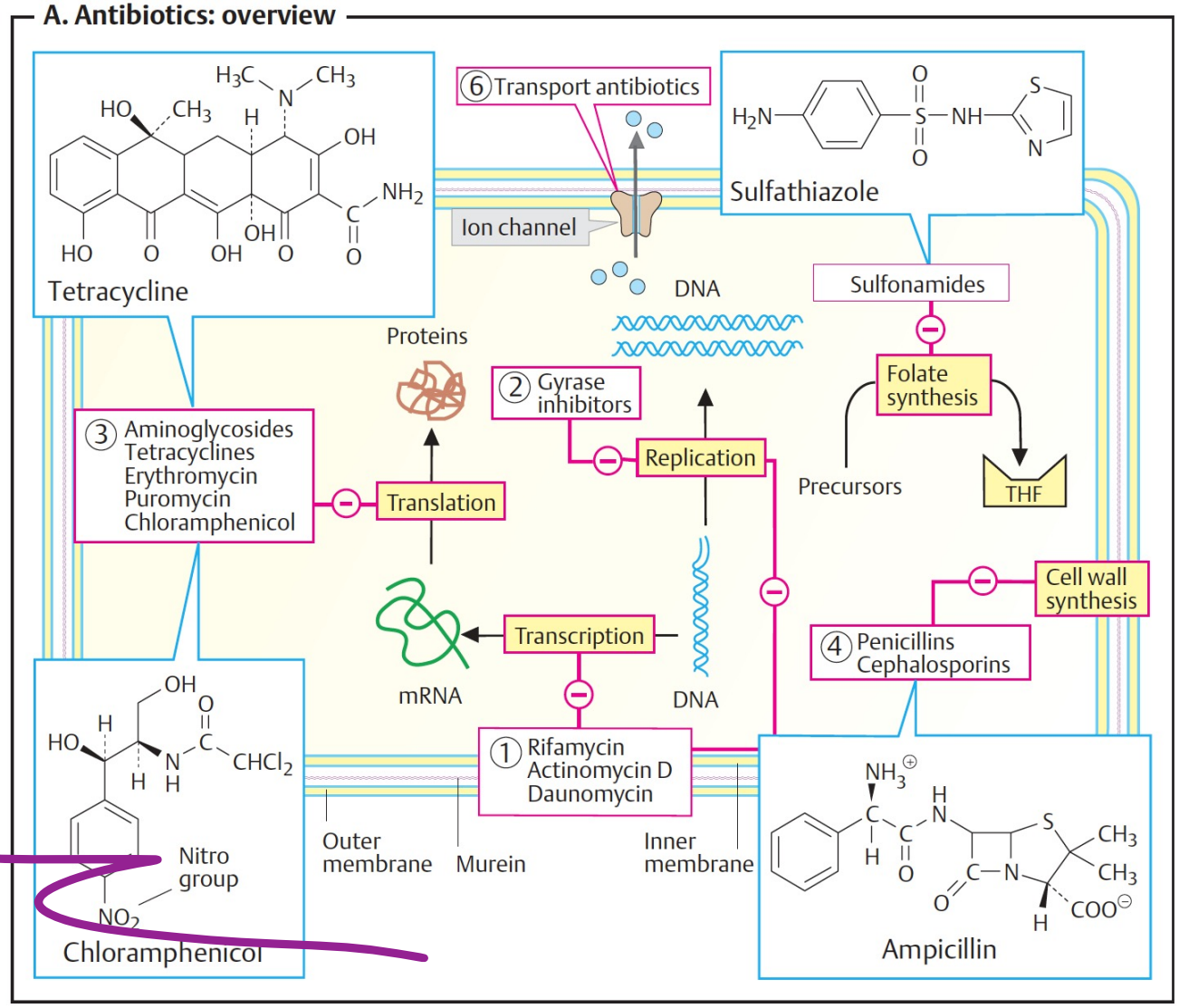
- Inhibit the transpeptidation enzymes involved in cell wall synthesis. Additionally, they:
  i. Are active against Gram-positive bacteria and Gram-negative bacteria
  ii. React with penicillin-binding proteins
  iii. Have a b-lactam ring structure that is inactivated by b-lactamases (penicillinases), which are genetically coded in some bacterial DNA or some R plasmids.

c. Cephalosporins

- They have a mechanism of action similar to that of penicillin. They also:
  i. Are active against both Gram-positive and Gram-negative bacteria
  ii. Contain a b-lactam ring structure that is inactivated by some b-lactamases
  iii. Are frequently used to treat patients who are allergic to penicillins

d. Carbapenems

- They have a mechanism similar to that of penicillin; they have a b-lactam ring fused to a five-carbon ring and are resistant to b-lactamases.



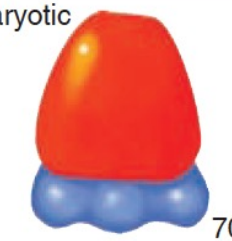
Mechanism of Action: Protein Synthesis inhibitors

3. Protein synthesis inhibitors are frequently known as broad-spectrum antibiotics and require bacterial growth for their effect.

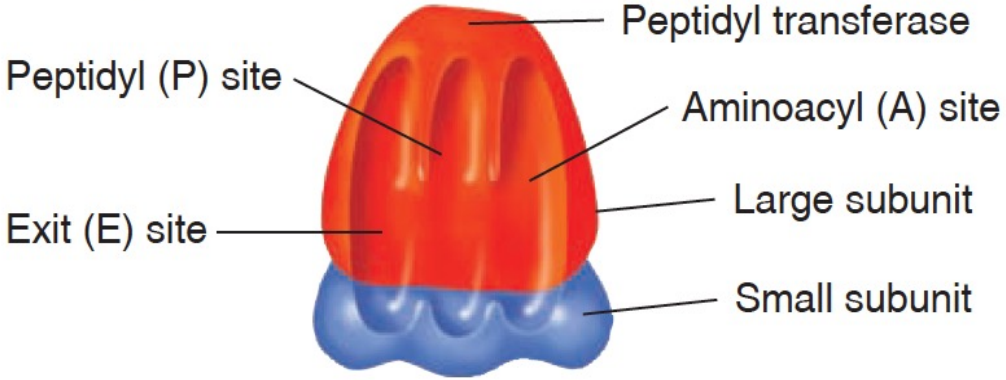
a. Aminoglycosides include streptomycin, neomycin, kanamycin, and gentamicin.

- Mechanism of action. These drugs are bactericidal for Gram-negative bacteria and bind to the 30S ribosomal subunit, irreversibly blocking initiation of translation or cause mRNA misreading (or both).
  - They are not active against anaerobes or intracellular bacteria.
- Their effective concentration range is narrow before toxicity occurs;
  - toxic effects include renal damage and eighth cranial nerve damage (hearing loss).
- Acetylation may modify their action; they also can be rendered inactive by enzymes contained in R plasmids.

(a) A ribosome has two subunits composed of RNA and protein.

Complete Ribosomes	Subunits	Nucleotides	Proteins
Prokaryotic  70S	 50S	23S rRNA 3000 nucleotides 5S rRNA 120 nucleotides	31
	 30S	 16S rRNA 1700 nucleotides	21

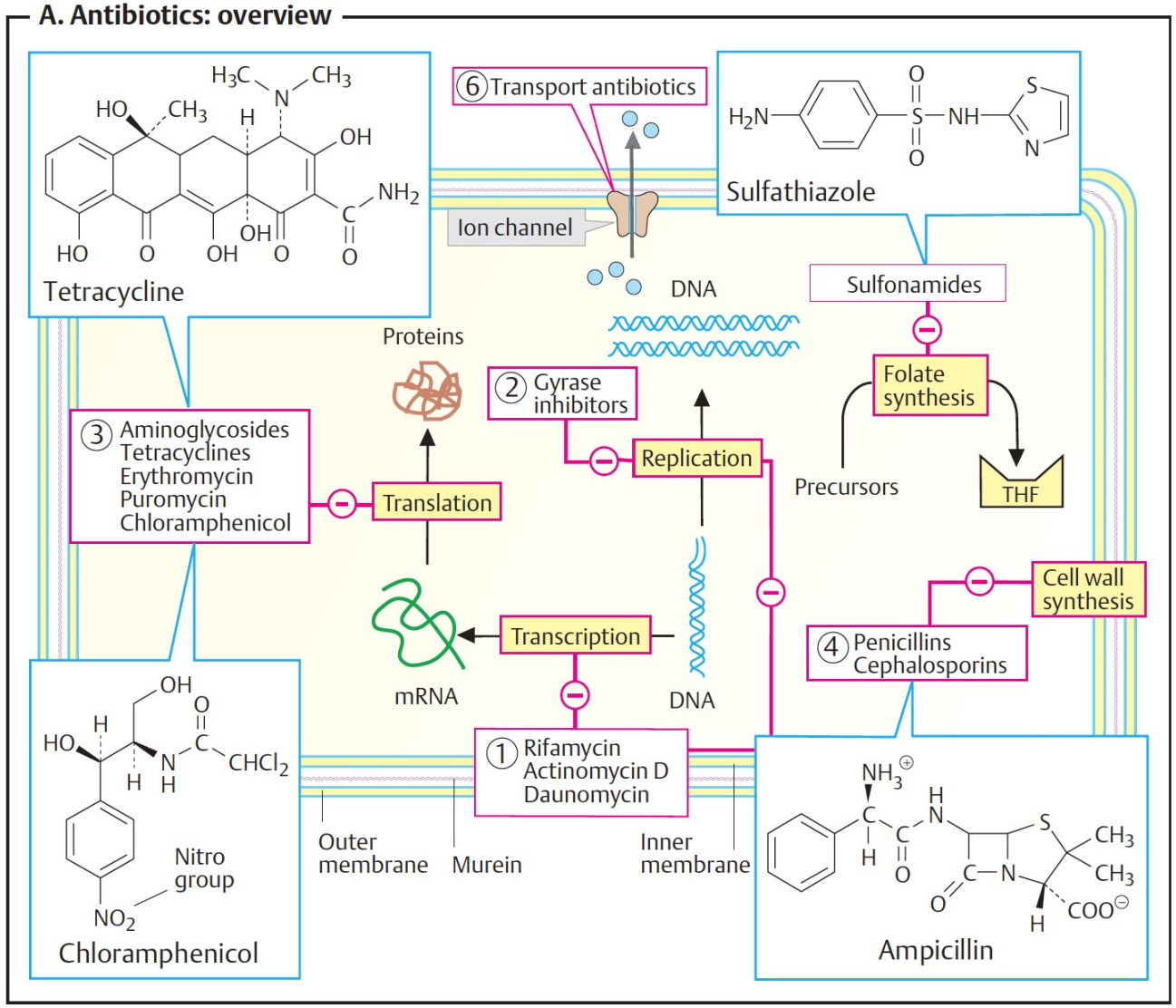
(b) Different parts of a ribosome have different functions.



Mechanism of Action: Protein Synthesis inhibitors

b. Tetracyclines include tetracycline, oxytetracycline, and chlortetracycline.

- Mechanism of action. These drugs are bacteriostatic, bind to the 30S ribosomal subunit, and prevent binding of aminoacyl tRNA to the acceptor site. They are transported out of or bound to a plasmid-derived protein in cells containing specific tetracycline R plasmids.
- They may be deposited in teeth and bones, which can cause tooth staining and structural problems in the bones of children.

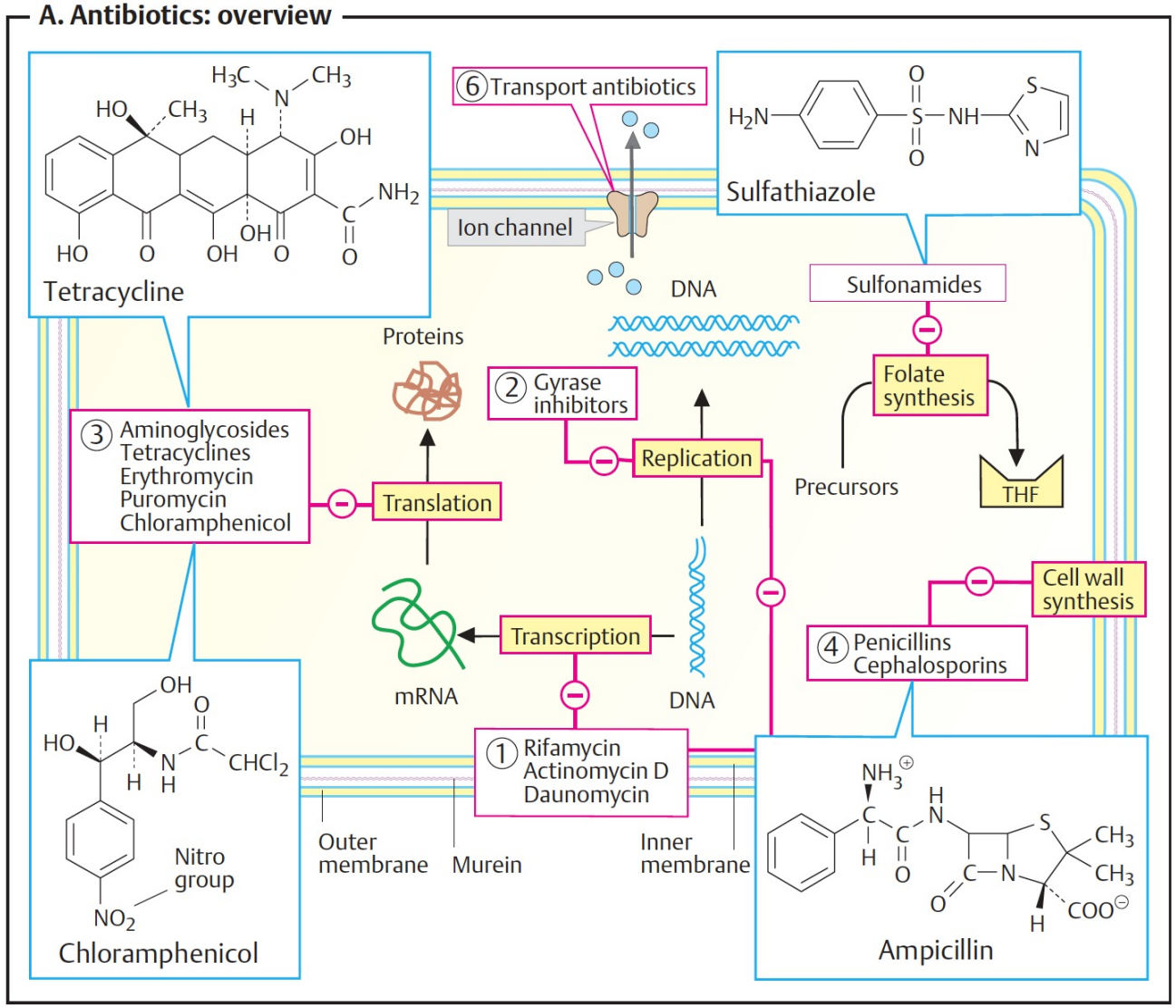


Mechanism of Action: Protein Synthesis inhibitors

c. Chloramphenicol

(1) Mechanism of action. This drug is bacteriostatic for Gram-positive and Gram-negative bacteria, rickettsia, and chlamydia; it binds to the 50S ribosomal subunit and inhibits peptide-bond formation.

(2) The enzyme chloramphenicol acetyltransferase, which is carried on an R plasmid, inactivates chloramphenicol.

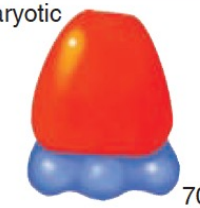


Mechanism of Action: Protein Synthesis inhibitors

d. Macrolides and lincomycins include erythromycin (macrolide) and lincomycin and clindamycin (lincomycins).

- Mechanism of action. These drugs are bacteriostatic and bind to the 23S RNA in the 50S ribosomal subunit, blocking translocation.
- In bacteria that have a mutation in a 50S ribosomal protein or that contain an R plasmid with genetic information, methylation of 23S RNA occurs, rendering these drugs ineffective by preventing the drug from binding.

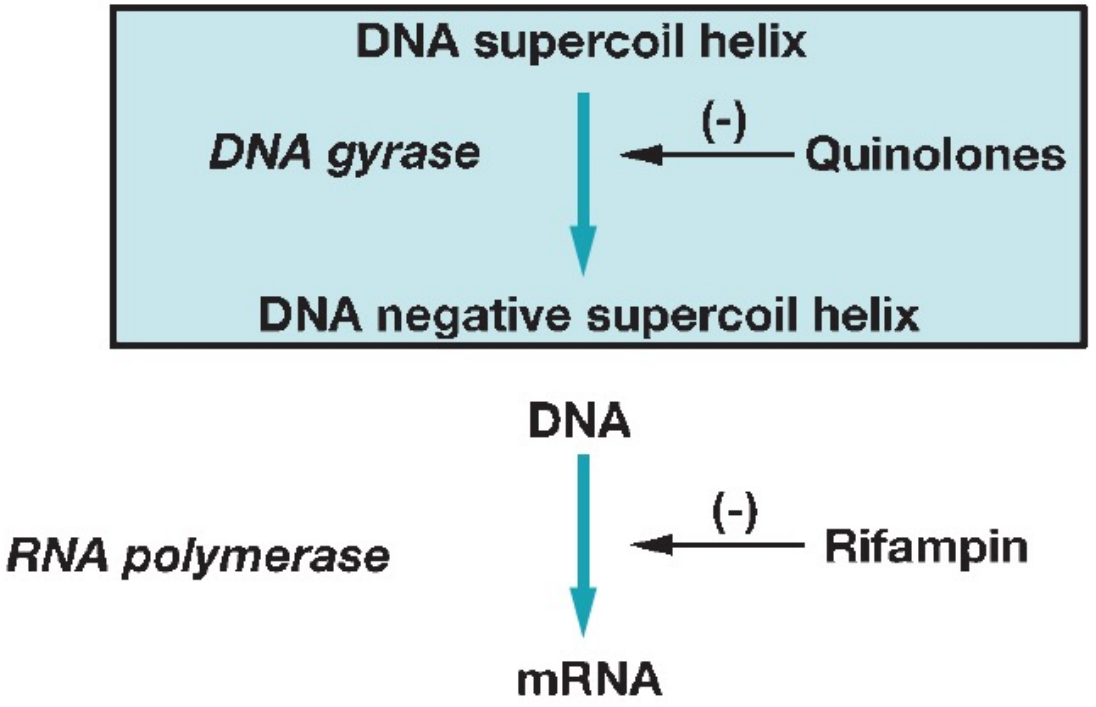
(a) A ribosome has two subunits composed of RNA and protein.

Complete Ribosomes	Subunits	Nucleotides	Proteins
Prokaryotic  70S	 50S	 23S rRNA 3000 nucleotides 5S rRNA 120 nucleotides	31
	 30S	 16S rRNA 1700 nucleotides	21

ANTIMICROBIAL CHEMOTHERAPY: Nucleic acid synthesis inhibitors

4. Nucleic acid synthesis inhibitors

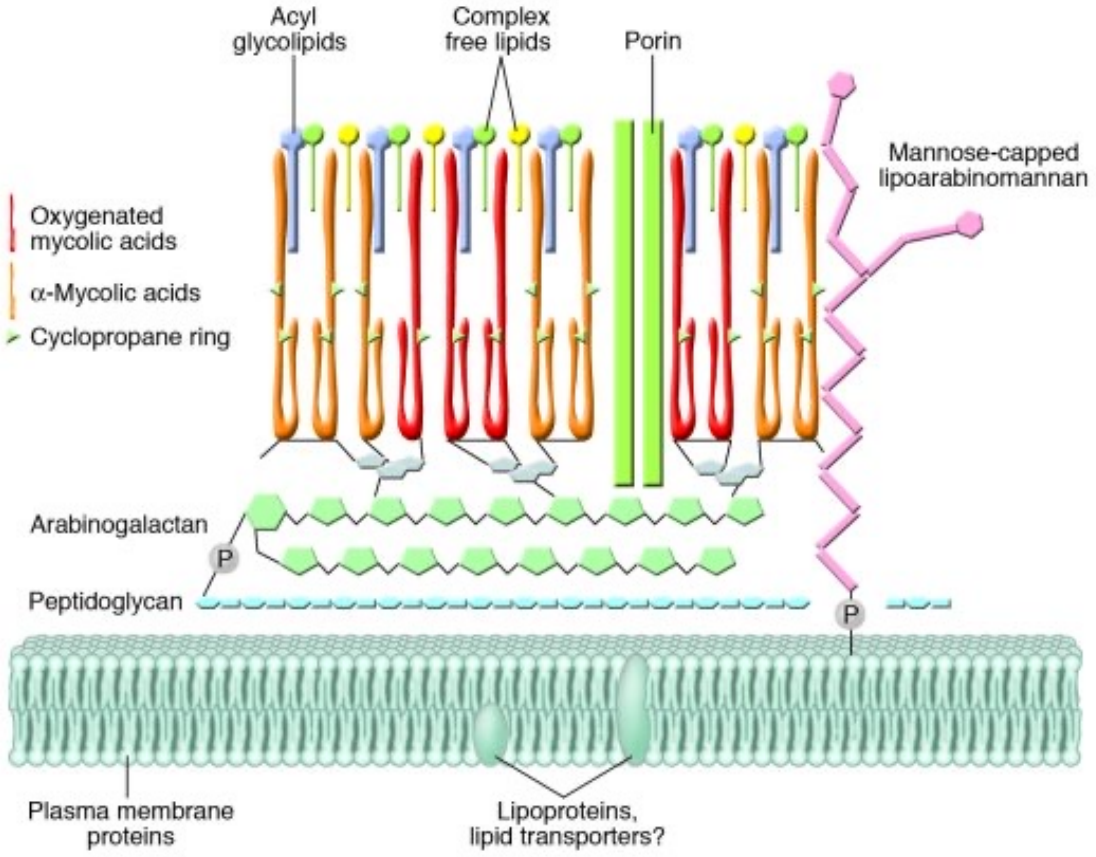
- Mechanism of action. These drugs inhibit DNA (quinolones, derivatives of nalidixic acid) or RNA (rifampin) synthesis. They are generally bactericidal and are quite toxic to mammalian cells.
- b. Examples
  - i. Actinomycin and mitomycin bind to strands of DNA or inhibit replication enzymes.
  - ii. Nalidixic acid inhibits DNA gyrase activity.
  - iii. Rifampin inhibits DNA-dependent RNA polymerase by binding to the β- subunit of the polymerase.



ANTIMICROBIAL CHEMOTHERAPY: Nucleic acid synthesis inhibitors

5. Mycolic acid synthesis inhibitor (isoniazid) is a bactericidal drug that inhibits mycobacterial mycolic acid biosynthesis.

- ❖ Isonizid is an analogue of pyridoxine (vitamin B6).
- ❖ Isoniazid is a prodrug whose active metabolite inhibits synthesis of the mycobacterial cell wall.
- ❖ It does so by inhibiting the enzyme enoyl-ACP reductase required for the synthesis of mycolic acid which is unique to mycobacteria.



The outermost layer of mycobacteria consists of phospholipids and mycolic acids that make a waxy layer that resists penetration from antibiotics.

7. Cytoplasmic membrane inhibitors

- Mechanism of action. These drugs are bacteriocidal and alter the permeability properties of the plasma membrane polymyxin and polyenes or inhibit fungal membrane lipid synthesis (azoles: miconazole and ketoconazole).
- They are effective against Gram-negative (polymyxin) and sterol-containing mycoplasma and fungal (polyenes: nystatin and amphotericin B) infections; used primarily as topical treatment or with severe infections.
- They can react with mammalian cell membranes and are therefore toxic.

Antibiotic Resistance

Antibiotic resistance Mechanisms

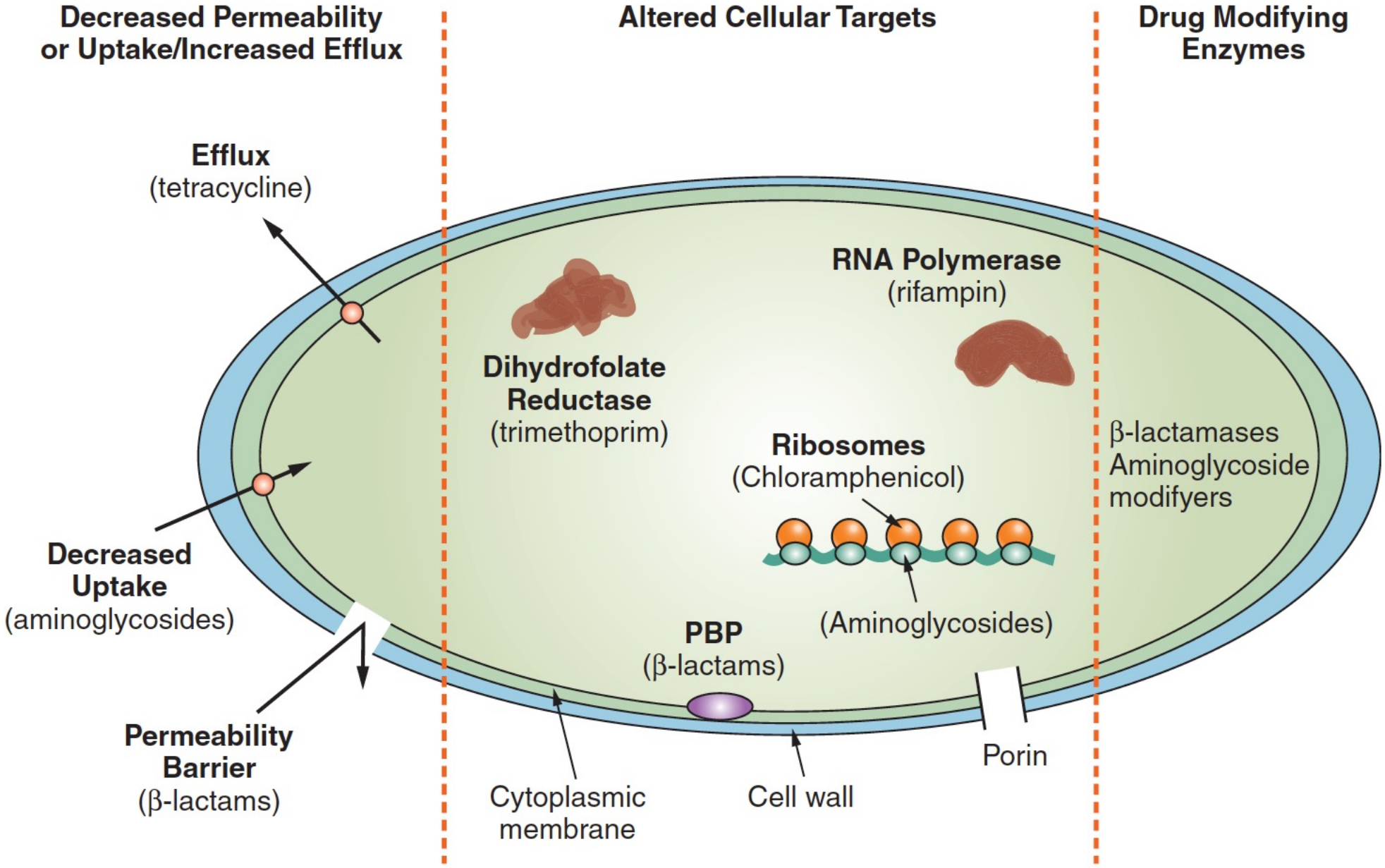


FIGURE 2.13. General mechanisms of bacterial resistance to antibacterial drugs.

Mechanisms of Antimicrobial Resistance

1. Phenotypic
2. Genotypic

Phenotypic Mechanisms of Antimicrobial Resistance

Mechanism	Example
Enzymatic inactivation/inactivation of the drug	Beta-lactamases Aminoglycoside-modifying enzymes
Impermeable cell wall	Glycopeptides vs. Gram-negatives Imipenem-resistance in *P. aeruginosa*
Efflux	Most tetracycline resistance in Gram-negatives Multi-drug efflux in *P. aeruginosa* Quinolone resistance in *S. aureus*
Target modification	Glycopeptide resistance in enterococci Penicillin-resistance in pneumococci
Target by-passing	Methicillin-resistance in *S. aureus* Most anti-folate resistance

Phenotypic Mechanisms of Antimicrobial Resistance

Mechanism	Example
Protection of target site	Tetracyclines
Overproduction of target	Sulfonamides, Trimethoprim, Glycopeptides
Bind-up antibiotic	Glycopeptides

Genotypic Mechanisms of Antimicrobial Resistance

2 key mechanisms:

Intrinsic (Primary)

Acquired (Secondary)

Genotypic Mechanisms of Antimicrobial Resistance

1. Intrinsic Resistance:

– Usually related to structural features

e.g. permeability of cell wall or target modification

– Chromosomally mediated

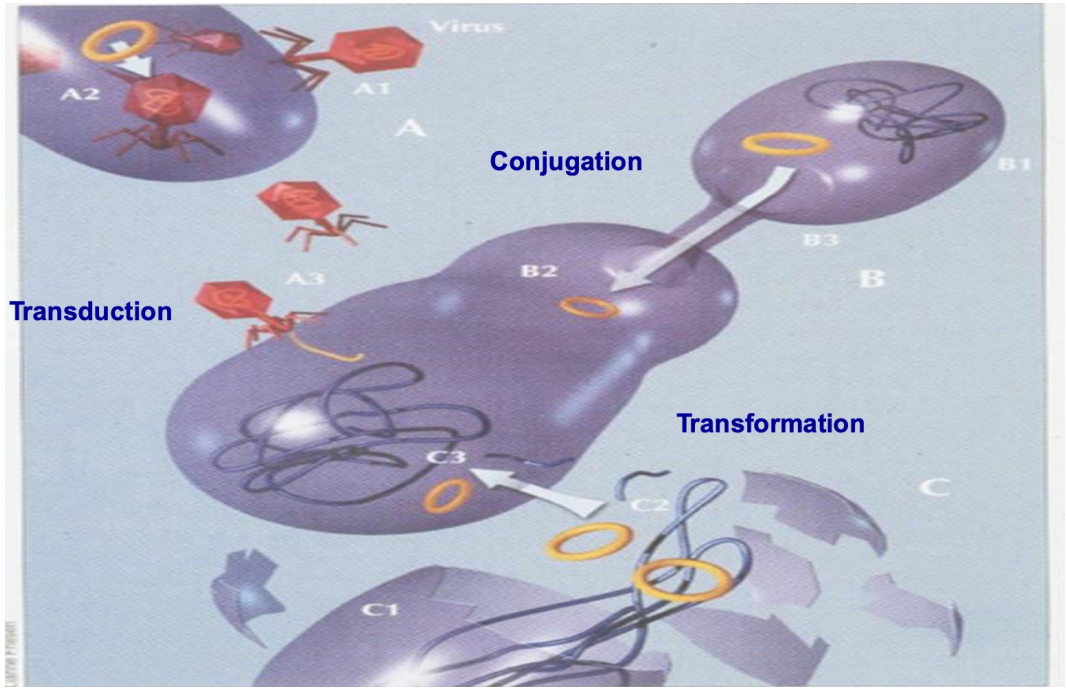
e.g. *Pseudomonas aeruginosa*, *S. maltophilia*, Enterococci, others

- ❖ A chromosomal mutation alters the structure of the receptor of the drug or the permeability of the drug.

Genotypic Mechanisms of Antimicrobial Resistance

2. Acquired Resistance:

- ❖ Acquisition of resistance genes [Plasmids, Transposons, other DNA]
  - Transduction
  - Transformation
  - Conjugation



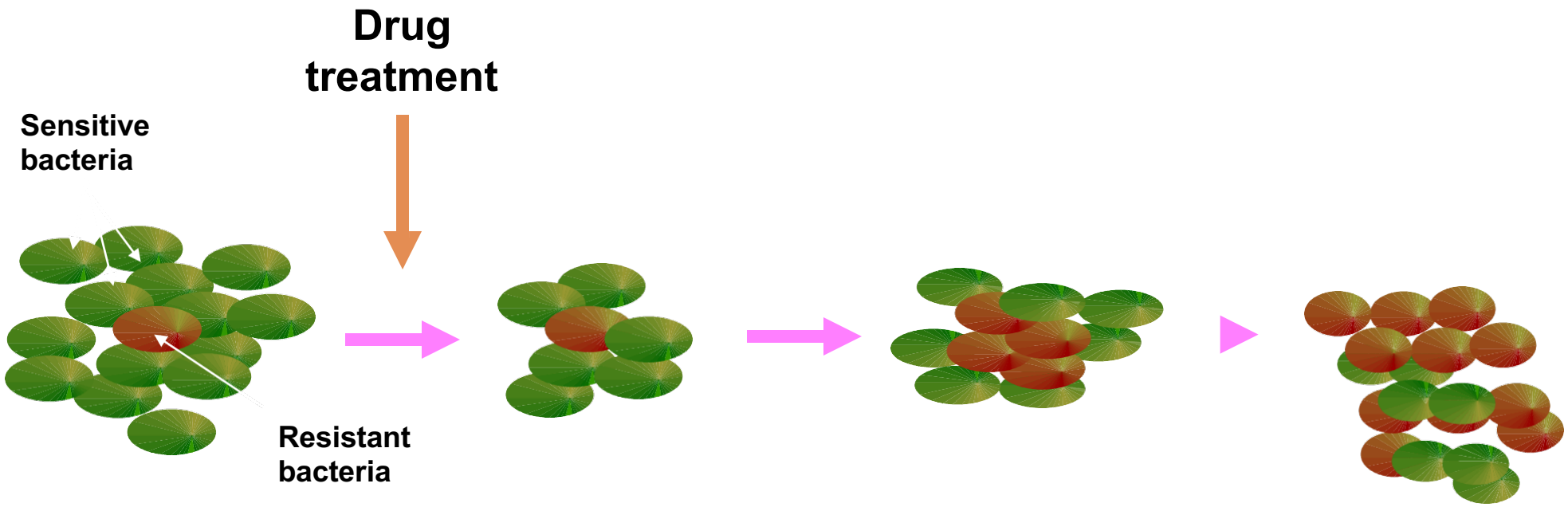
A plasmid (R factor or R plasmid) that codes for enzymes is introduced; these enzymes degrade the drug (b-lactamase) or modify it (acetyltransferase). The plasmid may also code for proteins that pump the drug out of the cell in an energy-dependent fashion.

a. The R factor or R plasmid:
   i. Contains insertion sequences and transposons.
   ii. Can acquire additional resistance genes by plasmid fusion or from transposons.
   iii. Can consist of two components, the resistance transfer factor (RTF), which codes for replication and transfer, and the r or resistance determinant, which contains genes for replication and resistance.
   iv. Can be transmitted from species to species.
   v. Is responsible for the rapid development of multiple drug-resistant bacteria over the past 30 years.

Selected Issues With Acquired Antimicrobial Resistance

Antibiotic Class	Mechanism of Resistance
Cephalosporins	Extended-Spectrum β-lactamases (ESBL) Chromosomal Cephalosporinases
Vancomycin/Teicoplanin	Modified cell wall precursors with decreased affinity for vancomycin (e.g. VRE)
Quinolones	Alterations in DNA topoisomerase, efflux mechanisms, permeability changes
Macrolides	Methylation of bacterial ribosome (MLS phenotype); Efflux (M phenotype)
Aminoglycosides	Aminoglycoside-modifying enzymes
Penicillin / β-lactam	Altered Penicillin-binding proteins (eg. MRSA, PRSP)

Resistance Due to Antibiotic Selection



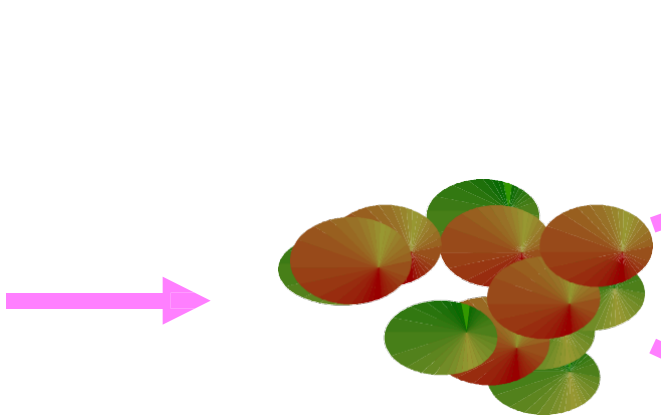
Spontaneous mutation occurs in the absence of drug selection in a sensitive population

Mutant is selected for by drug treatment as sensitive strains die off

Resistant clone grows within what used to be a sensitive population

Resistant clone becomes dominant (may be multi-drug resistant)

Dissemination of Resistant Bacteria



Resistant clone spreads to other patients & contacts

Resistant clone spreads to the environment

Resistant clone results in failure of therapy or re-infection/relapse in the same patient

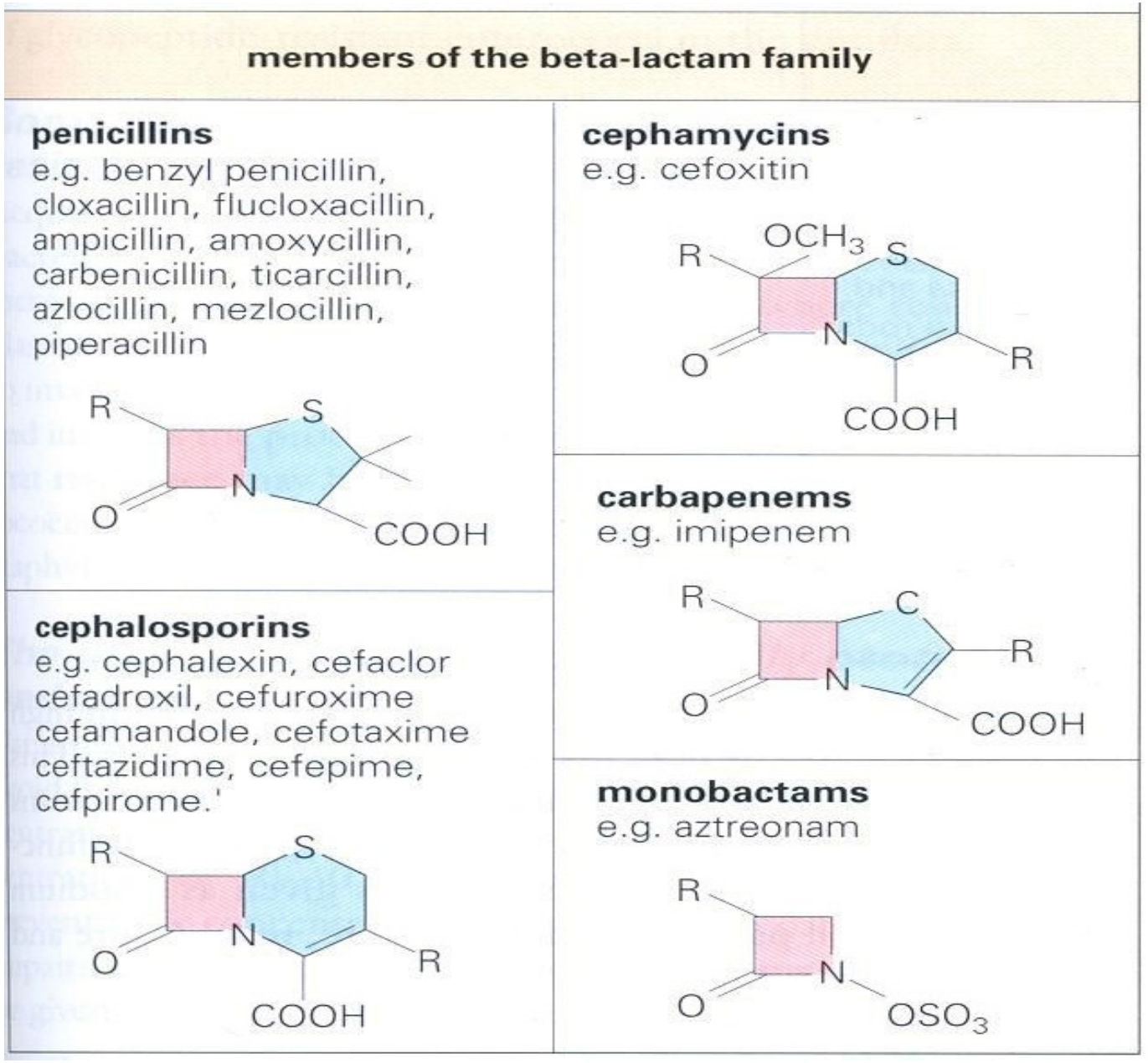
Mechanisms of Antibiotic Resistance

1. Enzymatic inactivation:

- Beta-lactamases
- Aminoglycoside-modifying enzymes

Most common β-lactamases found in *E. coli* and *K. pnuemoniae* are plasmid mediated TEM-1, TEM-2, SHV-1

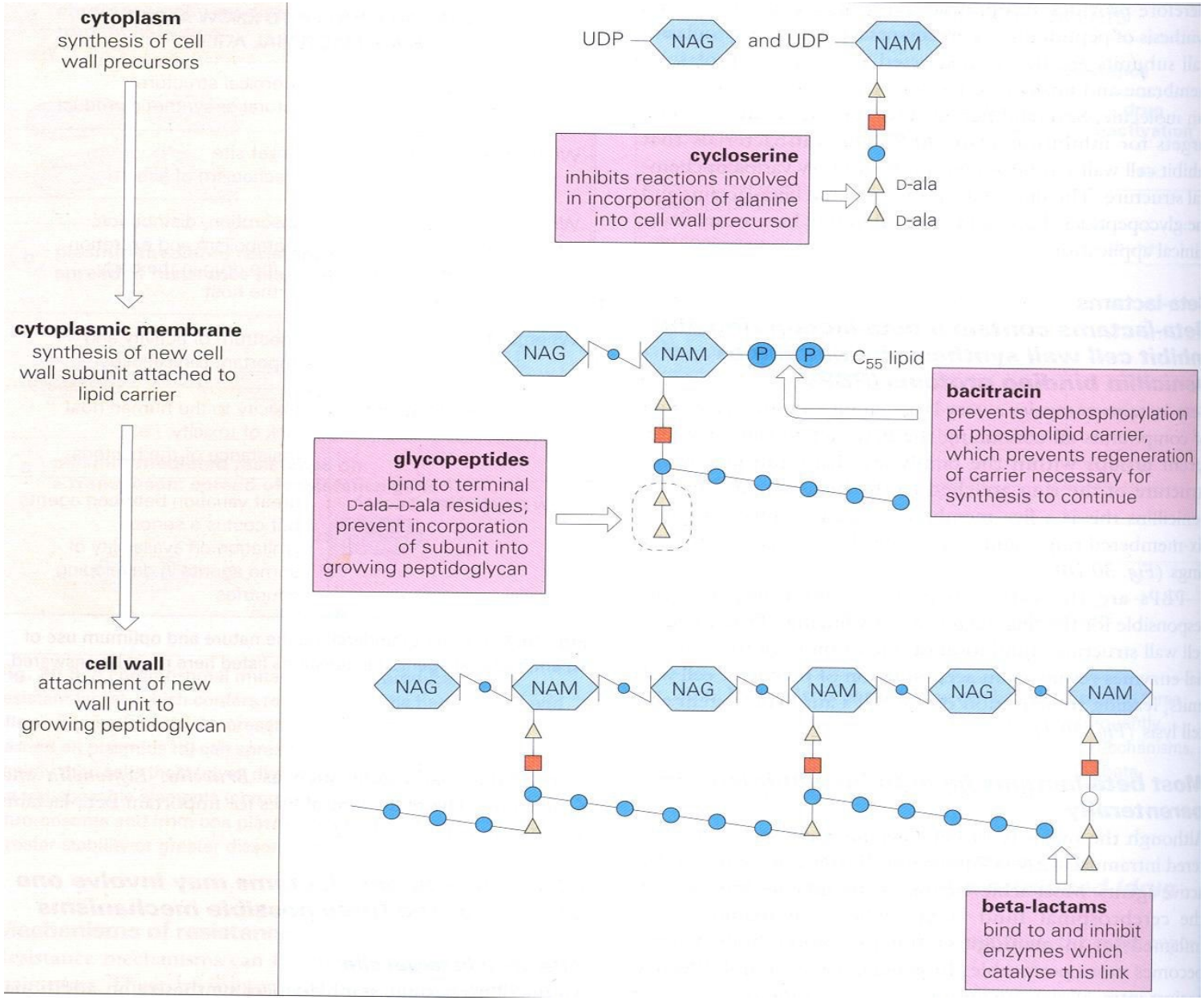
Responsible for ampicillin resistance in *E. coli* & ampicillin-cephalothin resistance in *K. pnuemoniae*



β-lactam Antimicrobials

Mechanism of Action:

- Target: penicillin binding proteins (PBP)
- PBPs are essential enzymes (carboxypeptidases, transpeptidases & transglycosylases) for building the bacterial cell wall
- Multiple PBPs in different organisms



β-lactams Antimicrobials

2. Altered Target (Remodeling):

S. pneumoniae: slow remodeling of PBP**s** (3 of the 6 PBPs are altered - 1a, 2x, & 2b)

Due to transformation of PBP genes via scavenging of genetic material

- Gradual increase in MICs (<0.06 to 0.5 / 1.0)
  - » 3 of 6 altered PBPs for Pen Resistance
  - » 2 of 6 altered PBPs for Ceph Resistance

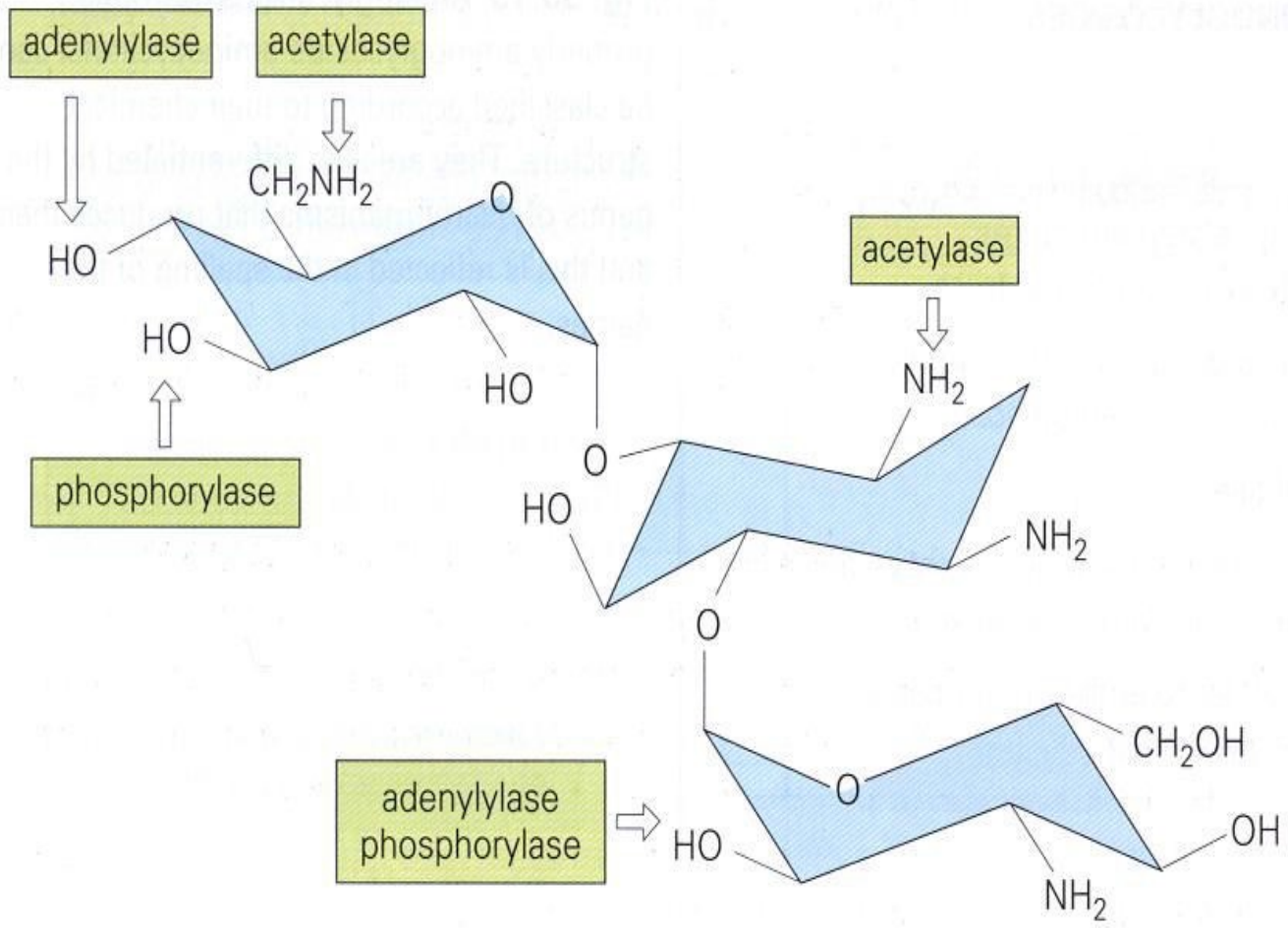
High Level resistance when MIC ≥4 ug/ml

Concomitant resistance to other unrelated classes of antibiotics (~10 to 15% are MDR)

Mechanisms of Antibiotic Resistance

1. Enzymatic inactivation:

- Beta-lactamases
- Aminoglycoside-modifying enzymes



Mechanisms of Antibiotic Resistance

2. **Target modification:**

   Penicillin resistance in pneumococci (PRSP)

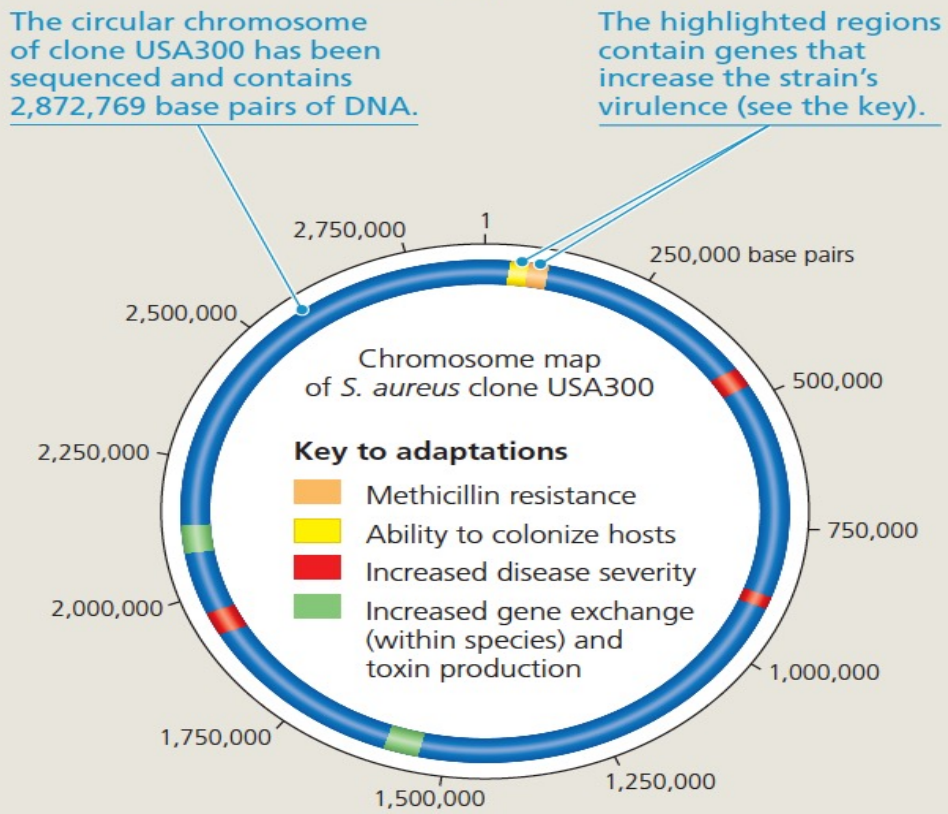
   Vancomycin resistant enterococci (VRE)

3. **Target by-passing:**

   Methicillin-resistance in *S. aureus* (MRSA)

The Rise of MRSA

Most methicillin-resistant *Staphylococcus aureus* (MRSA) infections are caused by recently appearing strains such as clone USA300. Resistant to multiple antibiotics and highly contagious, this strain and its close relatives can cause lethal infections of the skin, lungs, and blood. Researchers have identified key areas of the USA300 genome that code for its particularly virulent properties.



WHY IT MATTERS MRSA infections have proliferated dramatically in the past few decades, and the annual death toll in the United States is in the tens of thousands. There is grave concern about the continuing evolution of drug resistance and the resulting difficulty of treating MRSA infections. Ongoing studies of how MRSA strains colonize their hosts and cause disease may help scientists develop drugs to combat MRSA.

β-lactams Antimicrobials

3. **Target By-passing (Novel PBP):**

S. aureus- synthesis of a novel PBP 2a (capable of continuing cell wall synthesis) → MRSA (encoded for by chromosomal mecA gene)

Hospital MRSA (multi-drug resistant) vs Community MRSA (less drug resistant)

Mechanisms of Antibiotic Resistance

4. Target modification and efflux:

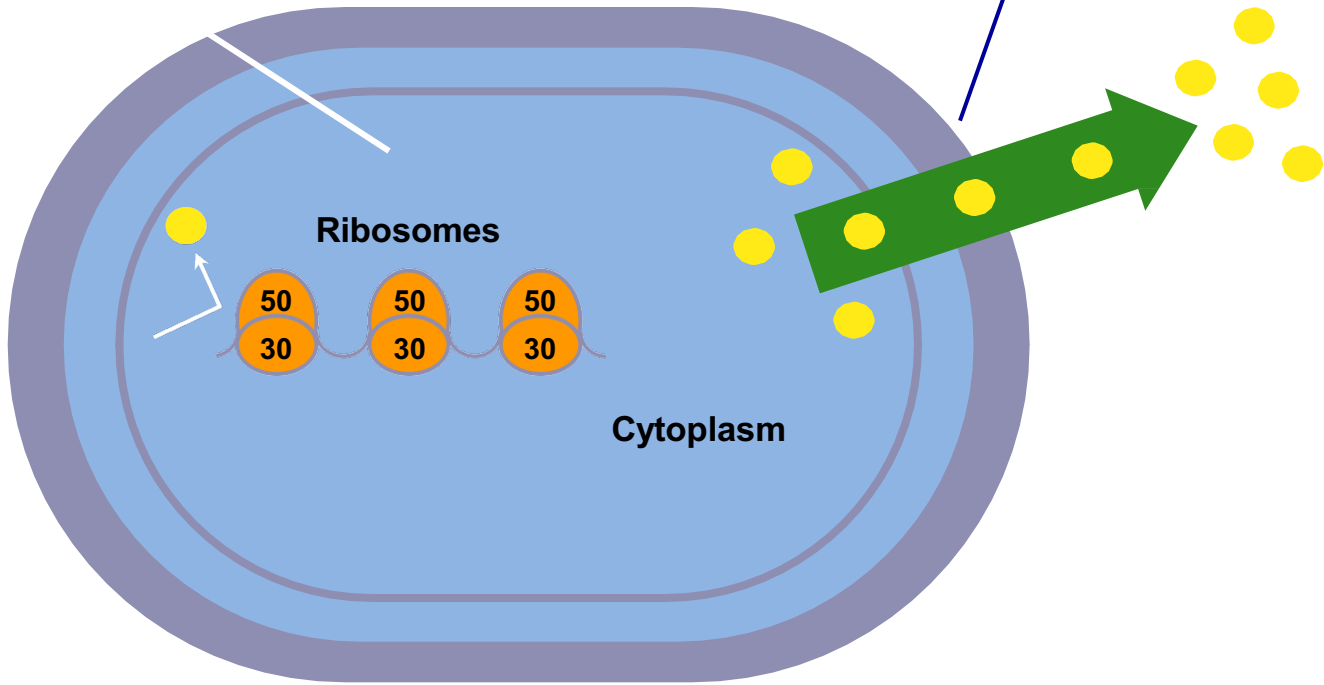
MLS antibiotics

Macrolides	-	Erythromycin
	-	Azithromycin
	-	Clarithromycin
Ketolides	-	Telithromycin
Lincosamides	-	Clindamycin
Streptogramins	-	Quinipristin / Dalfopristin

Mechanisms of Macrolide Action & Resistance

Bacteria alter macrolide binding site
(*erm*AM gene, MLS_B phenotype)
↓
Macrolide unable to block protein synthesis

Bacteria activate efflux pumps
(*mefE* gene, M phenotype)
↓
Macrolide excreted from cell



Macrolide

MLS Antibiotics – Mechanism of Resistance

1. **Target modification (erm genes):**

   Inducible - *S. pneumo* - all MLS

   - *S. aureus* - only M

   Constitutive

   Acquisition of a gene; one step

   MIC increases from < 0.5 to > 8.0 mg/L

MLS Antibiotics – Mechanism of Resistance

2. **Efflux pump (mef genes):**
   - *S. aureus* - MS phenotype
   - *S. pneumo* - MLS phenotype
   - Rare in *S. aureus*
3. **Inactivation:**
   - Rare in *S. aureus*
   - Not seen in *S. pneumoniae*

Mechanisms of Antibiotic Resistance

5. Target Modification:

- Fluoroquinolone resistance in *S. pneumoniae*

Fluoroquinolones

Mechanism of Action:

- Bind to 2 essential enzymes
  - DNA gyrase (topoismerase II)
  - Topoisomerase IV
- Results in termination of nucleic acid synthesis and replication
- Bactericidal - kill both multiplying and resting bacteria

Mechanism of Resistance:

Decreased permeability - low level resistance

Energy dependent efflux - low level resistance

Target modification - high level resistance

Point mutations of gyr A & par C

No plasmid (transferable) resistance until recently

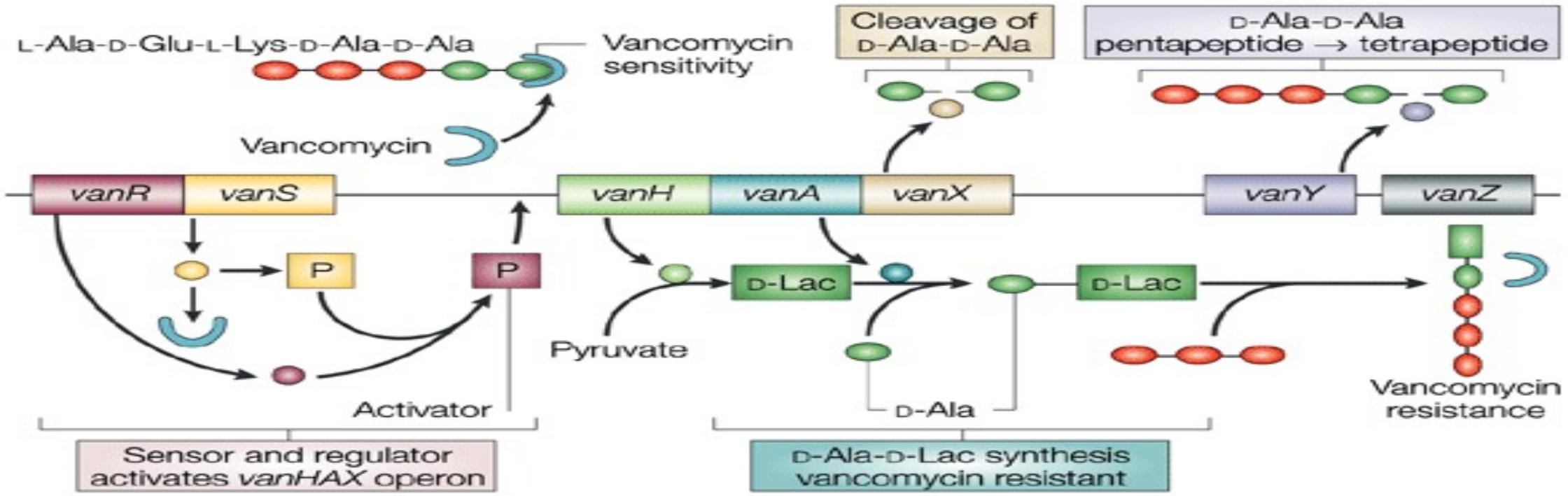
Fluoroquinolones

Mechanism of Resistance:

- 1st / 2nd generation agents
  - single mutations required for clinically relevant resistance
- 3rd / 4th generation agents
  - two mutations required for clinically relevant resistance

VRSA: Vancomycin-Resistant *S. aureus*

First identified in US 2002
High-level resistance (MIC >16 µg/mL)
vanA and associated genes from VRE

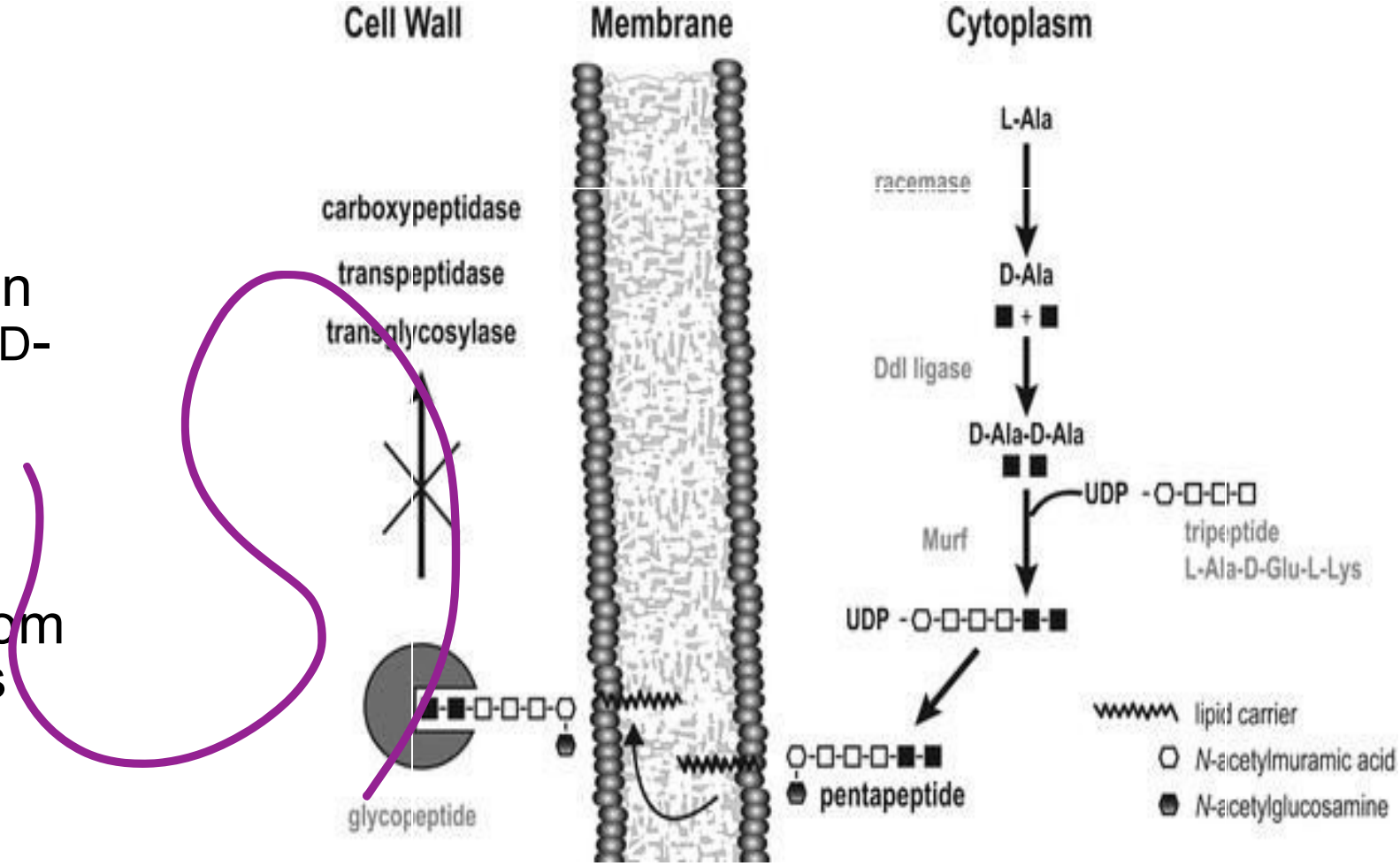


hVISA and VISA: Mechanism of Resistance

Overexpression of the *vraSR* two-component system

Thickened cell walls contain an increased number of cell wall D-Ala-D-Ala residues

Creates a reservoir that effectively sequesters vancomycin and prevents it from reaching the lethal target sites



hVISA is **the precursor of VISA and is composed of cell subpopulations with various degrees of vancomycin resistance**. They were initially named vancomycin-resistant S. aureus (VRSA) and hetero-VRSA (hVRSA), respectively, because both of them caused infection that was clinically refractory to vancomycin therapy

Why it is important to study Antibiotic -resistance

- Understanding mechanism of action / resistance should allow selection of appropriate empiric therapy
- Pharmacokinetic / Pharmacodynamic properties important in understanding how antibiotics work and implications of resistance
- Low level resistance may be overcome with higher doses (e.g. PRSP) or combination therapy (e.g. amp + gent)
- Co-resistance may or may not be predictable
- Susceptibility does not predict clinical success, but resistance may increase likelihood of failure

QUESTIONS..