BIOL 4640 Mol Micro
Lecture 19 10/9/26
Cell Structures Part 5

- Assignment 12 due on 10/9/26
- Assignment 13 due on 10/9/26 – in class activity
- Assignment 14: In class Activity, Due on 10/12
- Assignment 15: Lecture 19 In class Activity Due on 10/14

Unit 3. Features and Structures of Microbial Cells - C

A. Size and Shape of bacteria
B. Specialized External Structures of bacteria
C. Cell envelope of bacteria
D. Internal structures of bacteria
E. Cell Division in Bacteria
F. Viruses – Chapter 6
G. Atypical bacteria and viruses

Mostly Chapter 3 in book

C. Cell Envelopes (rev)

1. Major Styles/Configurations Observed
   a. Gram-positive (section 3.3)
   b. Mycobacterial (Gm+ actinomycete) - Acid-fast (section 3.3)
   c. Gram-negative (section 3.3)
   d. Mollicutes – *Mycoplasma* – no cell wall (pp. 734-735 6e)
   e. Archaeal (section 19.1)
2. Layers and Components
3. Peptidoglycan Synthesis and inhibition
4. Antibacterial Chemicals and resistance

C. Cell Envelopes - 3

3. Peptidoglycan Synthesis and Antibiotics
   a. Peptidoglycan Synthesis is unique to bacteria
   b. Many antibacterial drugs (antibiotics) interfere with or inhibit peptidoglycan synthesis
      - Antibiotics inhibit different steps
   c. Some enzymes cleave peptidoglycan
      - Lysozyme – defensive enzyme of our body

a. Peptidoglycan Synthesis

- New peptidoglycan needs to be synthesized for both cell growth/division and repair/replacement
- Have to maintain integrity of layer while cutting links between existing peptidoglycan and new peptidoglycan

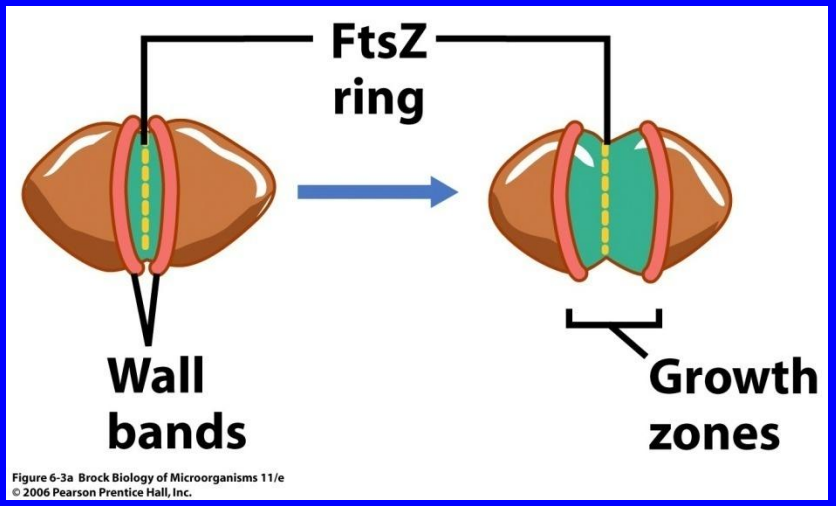


Figure 6-3a Brock Biology of Microorganisms 11/e
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Green is new peptidoglycan

a. Peptidoglycan Synthesis -2

- Site where new peptidoglycan synthesis occurs varies
  - Can occur in middle, one end or dispersed

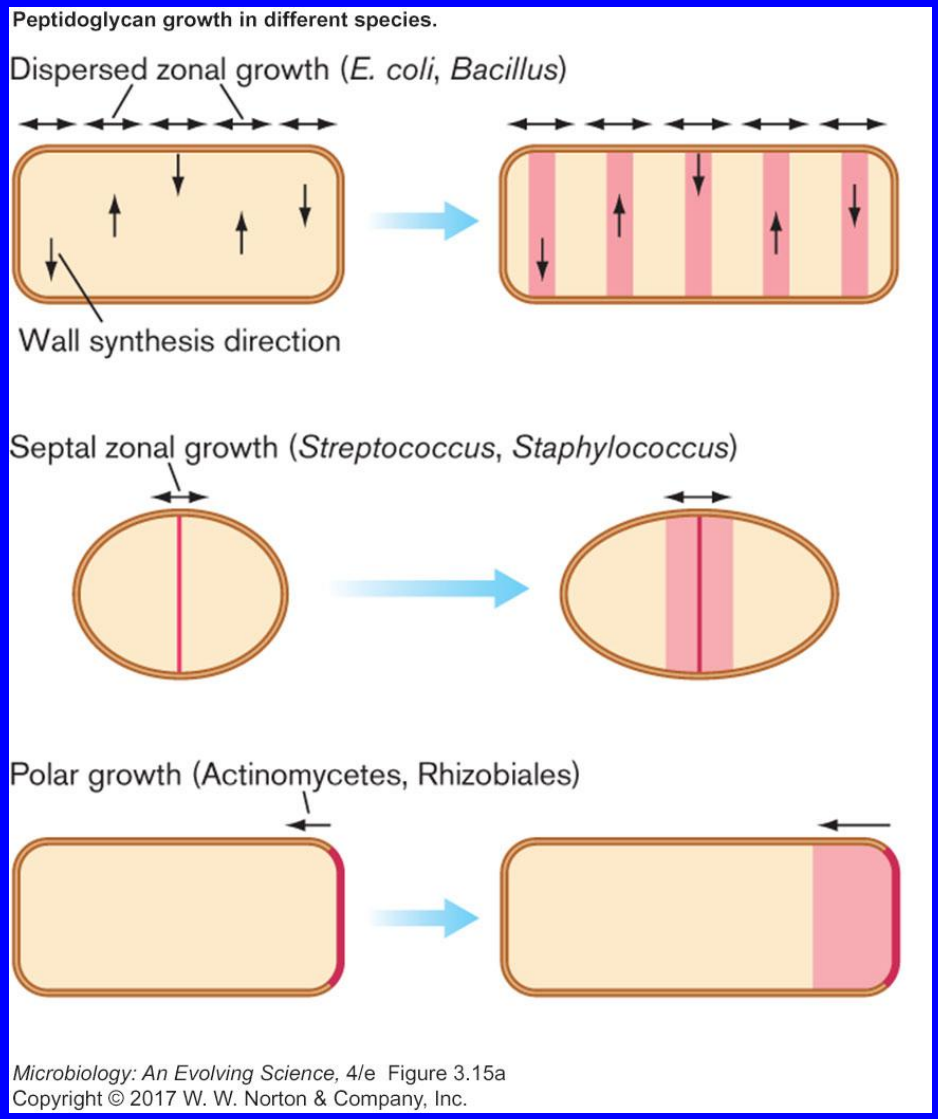


Figure 3.14A
6th edition

a. Peptidoglycan Synthesis - 3

Overview

1. Synthesize two sugar units (NAG and NAM) with peptide off NAM
2. Add 2 sugar unit to an existing chain
3. Then form crosslink to peptide in a different chain

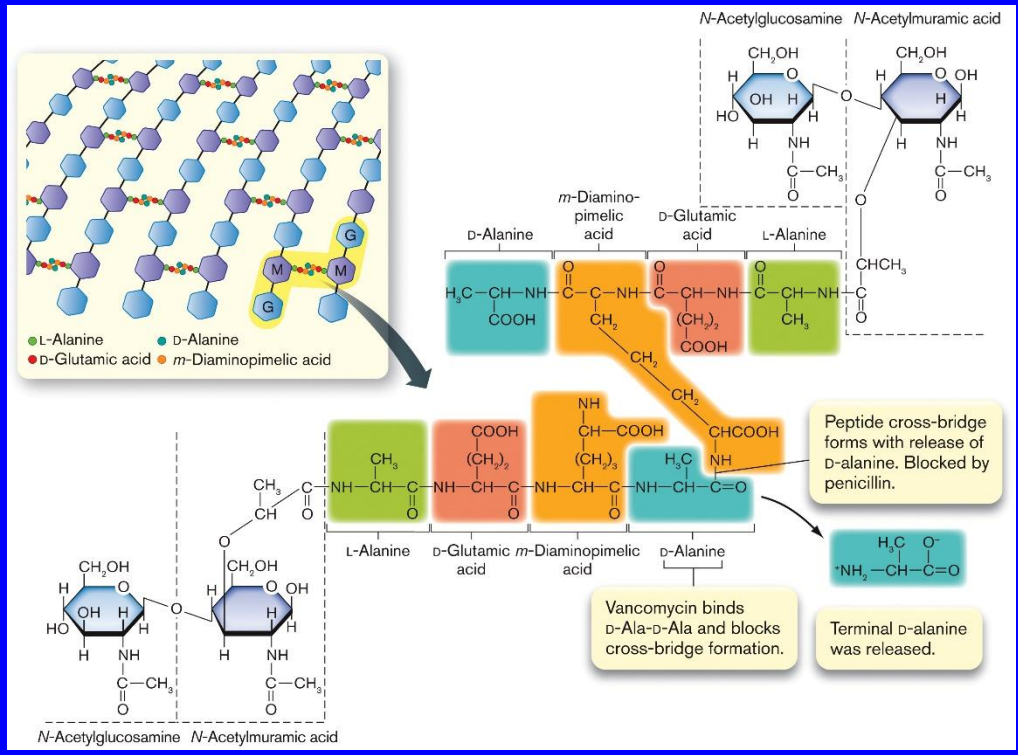
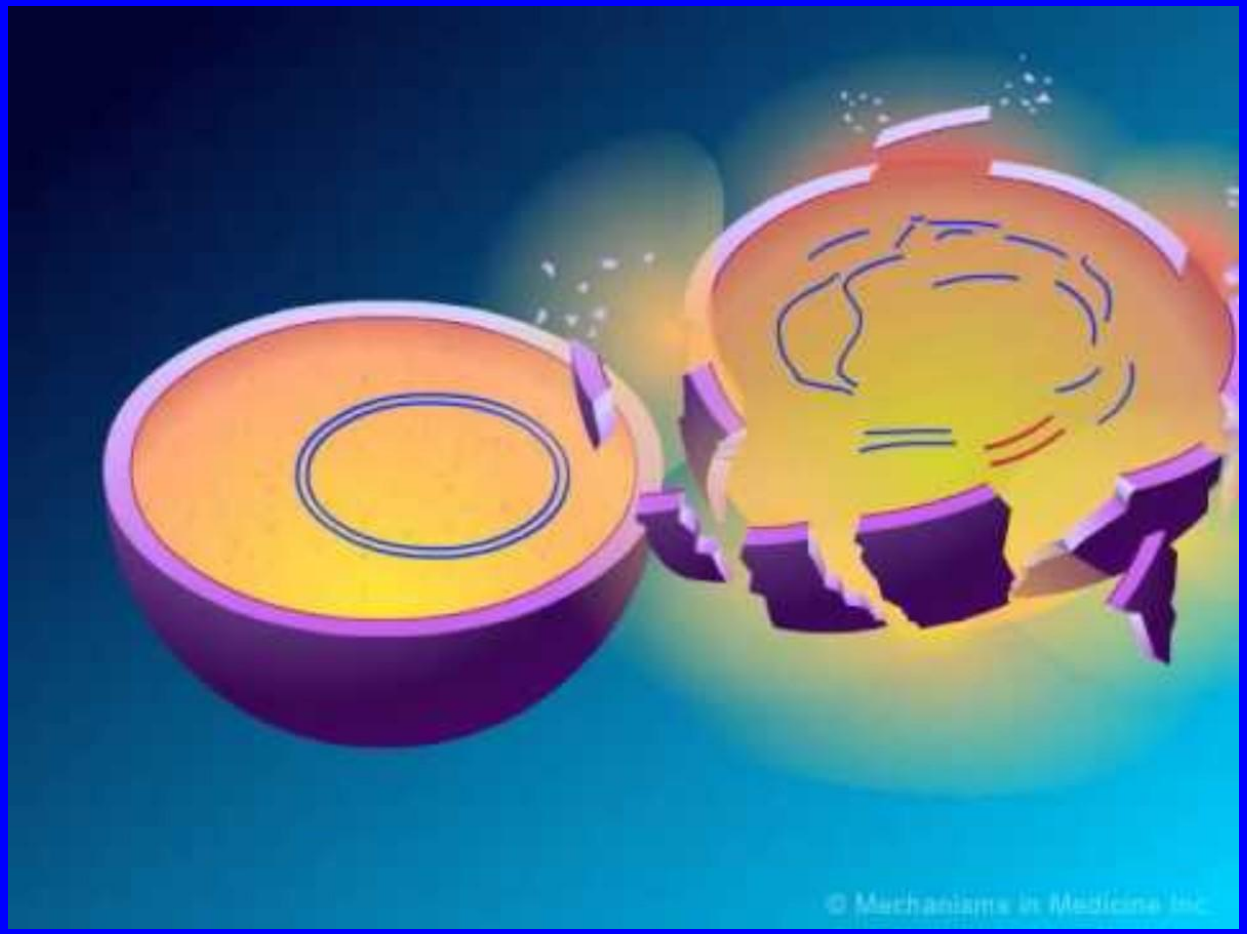


Figure 3.12b
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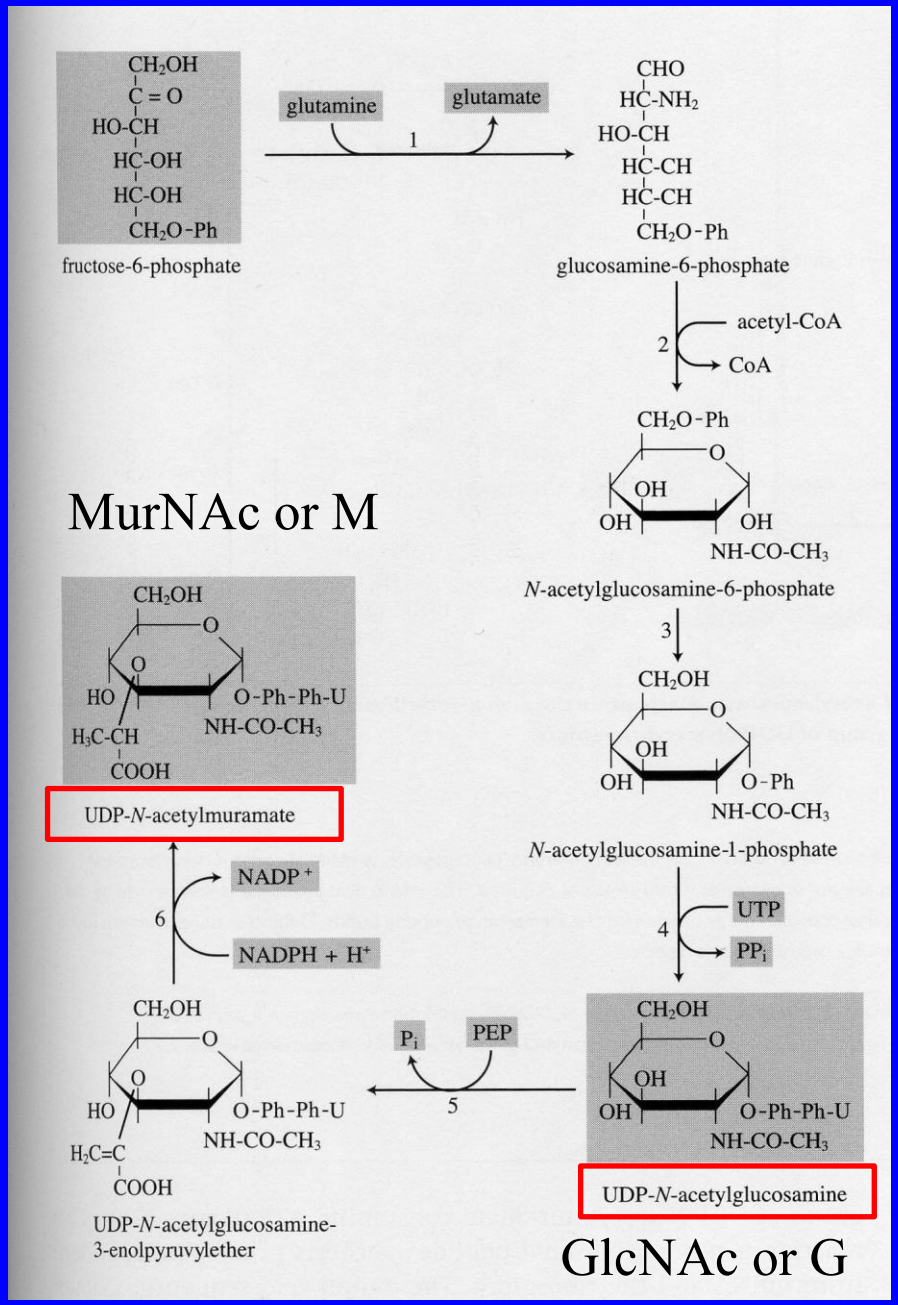
a. Peptidoglycan Synthesis - 4

Video ß-Lactams: Mechanisms of Action and Resistance: 0:01-2:30 covers Peptidoglycan synthesis



a) Peptidoglycan Synthesis

1. Make sugar precursors (NAM and NAG)
   a. Both sugar precursors made from Fructose-6-P in cytosol
   b. Sugars initially attached to UDP



a) Peptidoglycan Synthesis

This is in a Gram-positive bacteria. Similar in Gm neg. but some steps occur in periplasm between membranes.

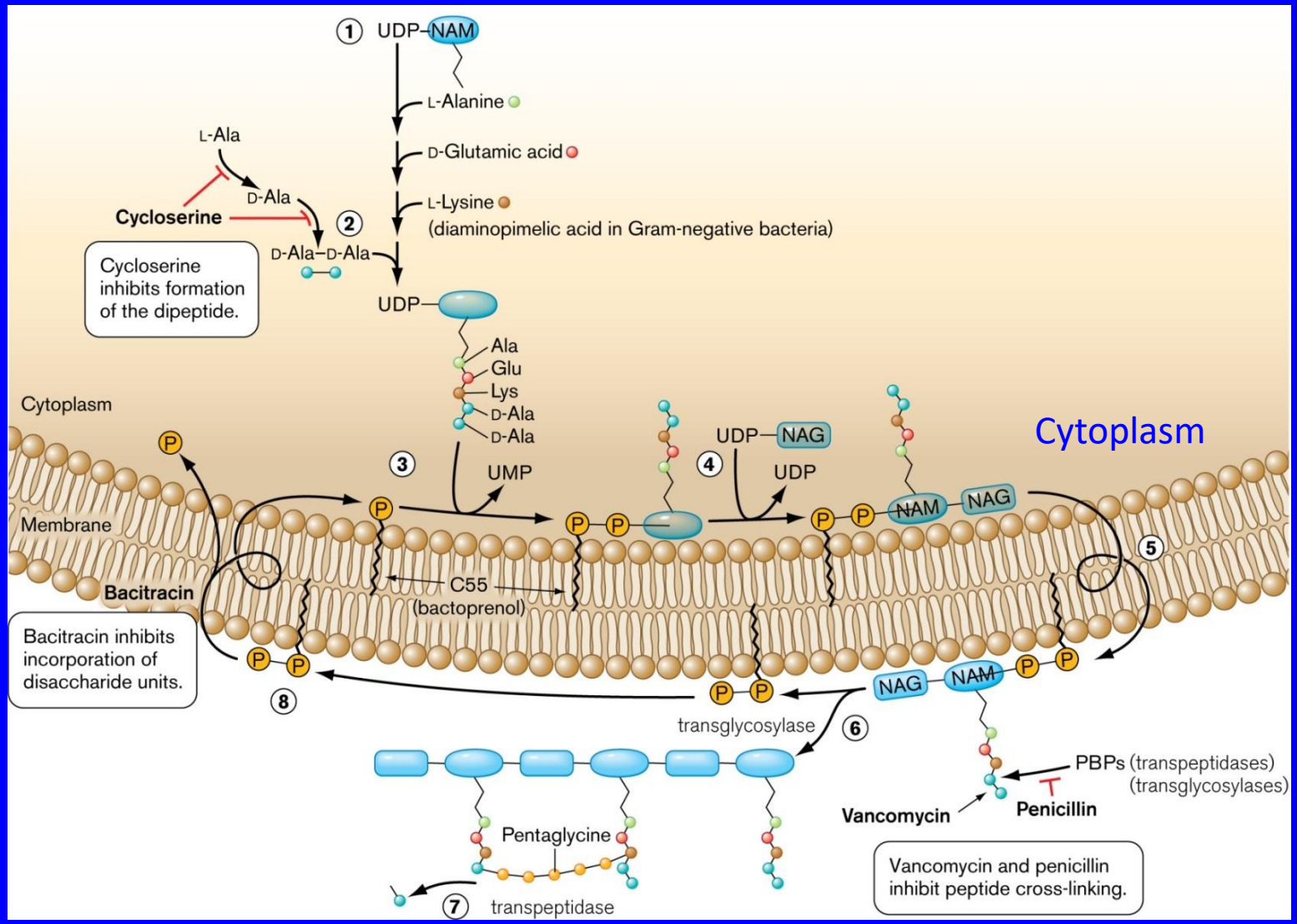


Figure 27.7 of 3rd edition

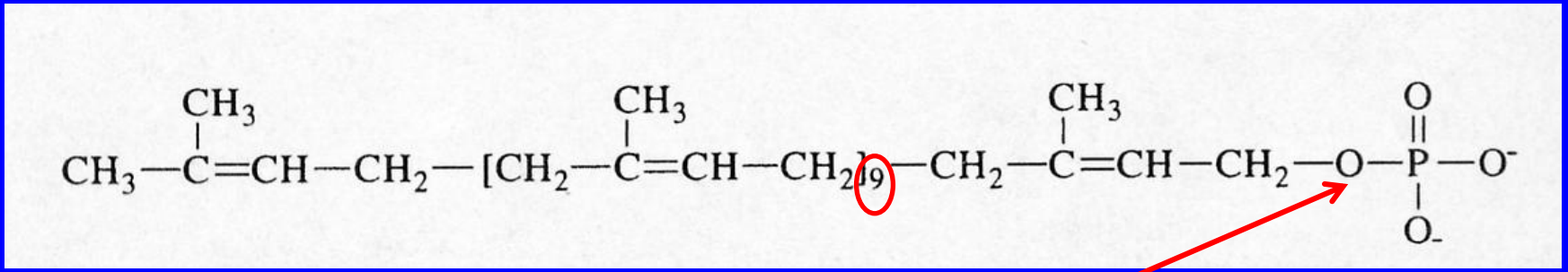
a) Peptidoglycan Synthesis

2. Add 3 amino acids to UDP-NAM to begin peptide
3. Add dipeptide of 2 D-alanine amino acids to peptide
4. Transfer NAM-pentapeptide to bactoprenol (C55) in cell membrane
5. Link NAG to NAM
6. Flip 2 sugar unit to other side of bilayer
7. Transglycosylation - link sugars to a glycan chain
8. Transpeptidase – form cross link
9. Regenerate bactoprenol-P by cleaving off a phosphate group

- Transglycosylation and transpeptidase done by penicillin binding proteins (PBPs)

a). Peptidoglycan Synthesis

4. Sugars transferred to bactoprenol
   - Bactoprenol or undecaprenyl is large isoprenoid phosphate with 55 carbons (C_{55})
   - Bactoprenol helps to carry peptiodoglycan units, LPS and teichoic acid through the cell membrane
   - Transmembrane protein MurJ (flippase) – flips sugars to other side



   - Sugar is attached via phosphate

a) Peptidoglycan Synthesis - 5

7. Transglycosylation reaction
   - Linking newly synthesized two sugar units to existing peptidoglycan chain

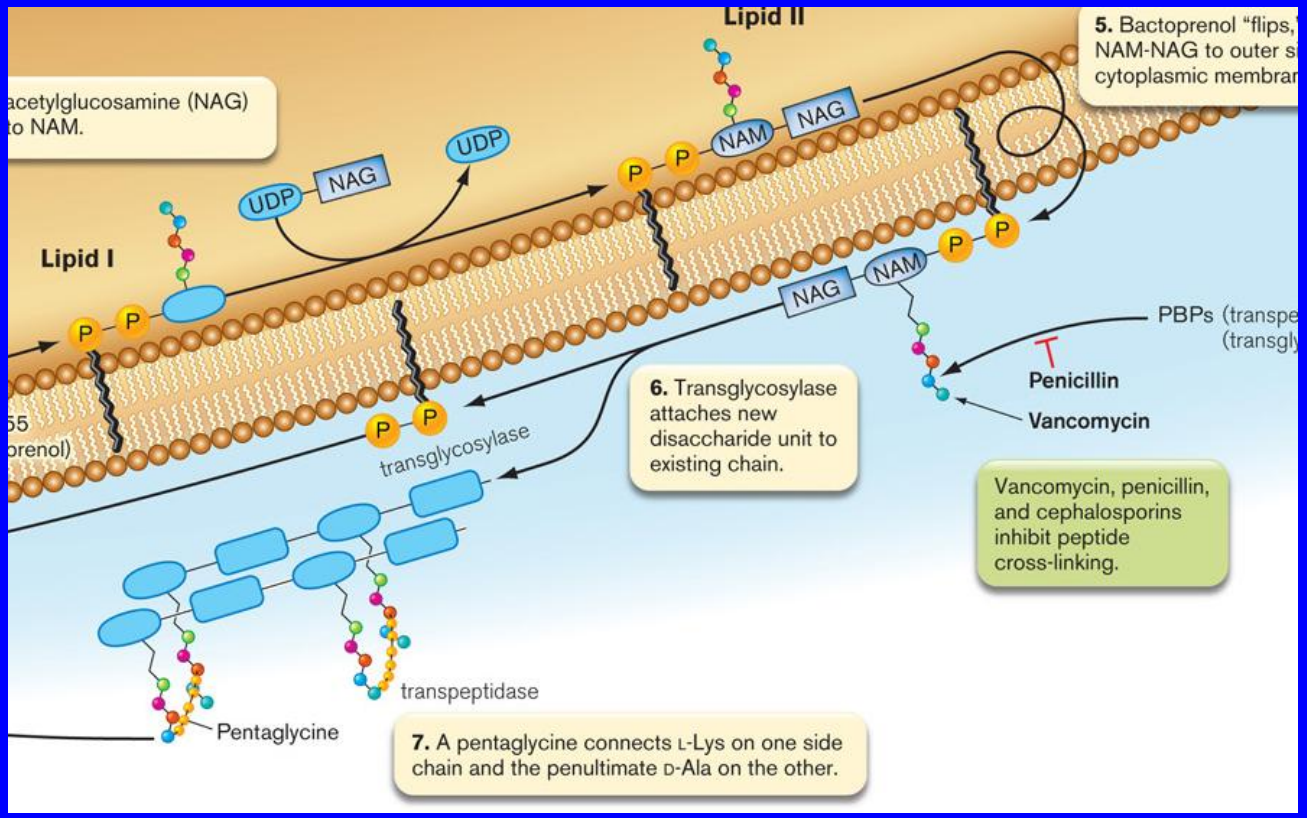


Figure 27.8B
6th ediition

a) Peptidoglycan Synthesis - 6

8. Transpeptidation reaction
   - Crosslinking of peptides
   - In Gm negative, DAP in 3rd position attacks *D*-Ala in 4th position and displaces *D*-Ala in 5th position
   - Done by penicillin binding proteins

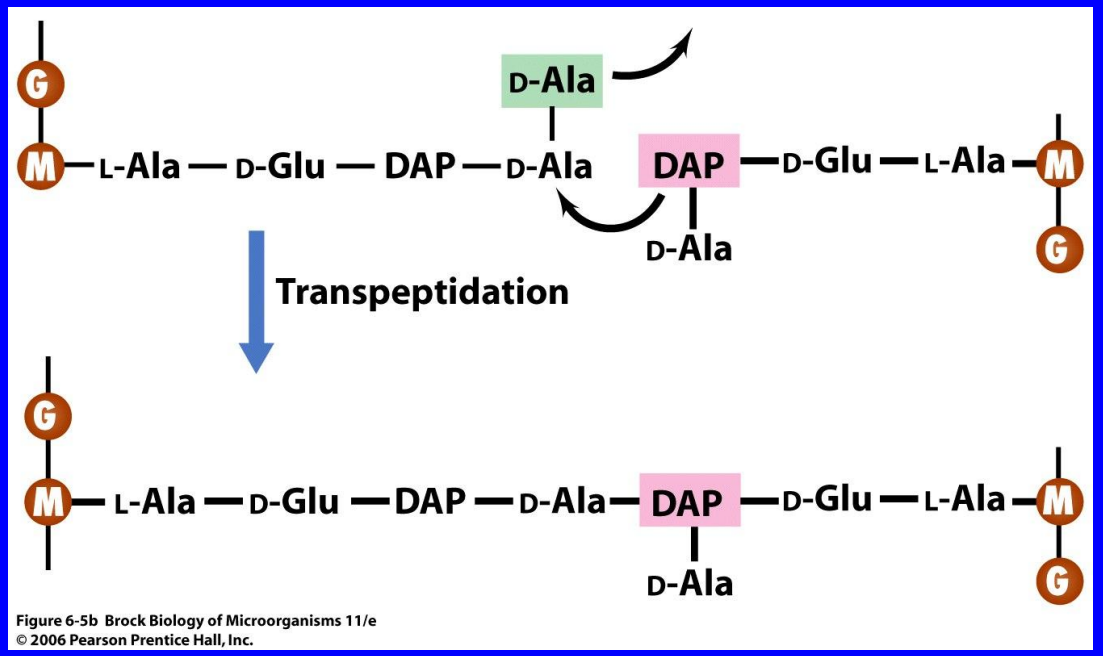


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a) Peptidoglycan Synthesis - 7

- Penicillin binding proteins (PBB)
  - Part of complex involved in synthesis of peptidoglycan
  - Do transglycosylation and transpeptidation reactions

Fig 2.7 White Physiology and Biochem. of Prokaryotes

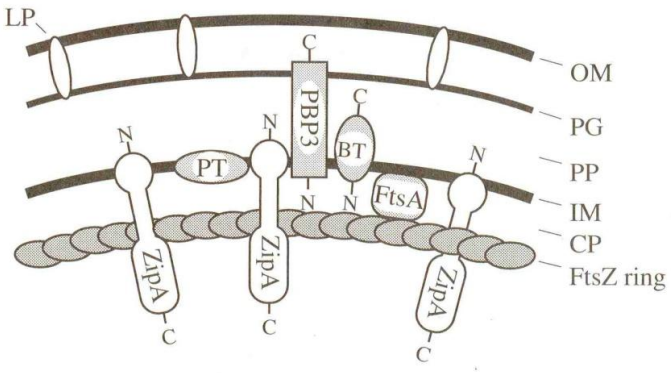


Fig. 2.7 Septal ring model in which a ring of FtsZ proteins is connected to the cell membrane by ZipA. Other abbreviations as follows: PT, FtsK and FtsW division proteins; BT, FtsL, FtsN, and FtsQ division proteins; PP, periplasm; CP, cytoplasm; LP, lipoprotein; OM, outer membrane; PG, peptidoglycan; IM, inner (cell) membrane. *Source*: Hale, C. A., and P. A. J. de Boer. 1997. Direct binding of FtsZ to ZipA, an essential component of the septal ring structure that mediates cell division in *E. coli*. *Cell* 88:175–185.

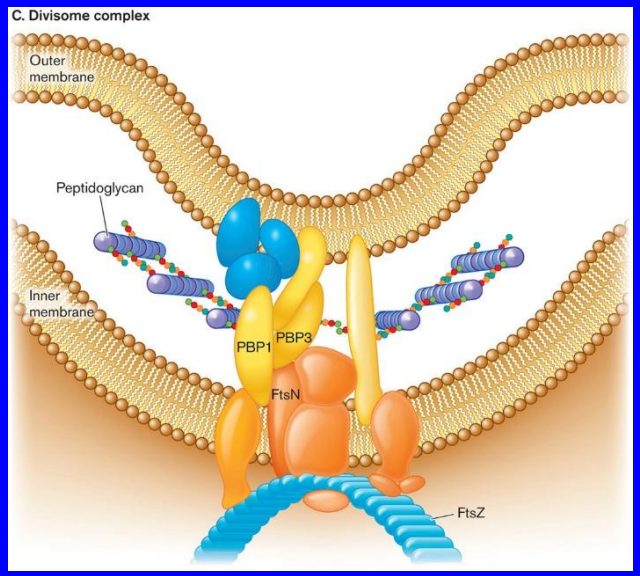


Fig 3.25C in 6th edition

3) Peptidoglycan Synthesis and Antibiotics

b) Antibiotics that interfere with peptidoglycan synthesis

1. Penicillin (β-lactam) family -
2. Cephalosporins -
3. Vancomycin –
4. Bacitracin –
5. D-cycloserine –

c). Enzymes that cleave peptidoglycan

1. Lysozyme
2. Autolysin – produced by bacteria

- Determine mechanism of action for Assignment 12

4. Antibacterial Drugs and Resistance

- Chapter 27 (section 27.1-27.3)

a. Most antibacterial drugs (antibiotics) target unique features of bacteria
   - Most antibiotics inhibit the synthesis of a cell component
   - May be cidal (kill) or static (inhibit growth)

b. Bacterial pathogens are becoming resistant to commonly used antibiotics

c. Tests for Antibiotic Sensitivity

a. Antibacterial Drugs

1) Targets or mechanism of action of Drugs

- Most inhibit an enzyme involved in biosynthesis

TABLE 27.2 Targets of Antimicrobial Agents

Target	Antibiotic examples
Cell wall synthesis	Penicillins, cephalosporins, bacitracin, vancomycin
Protein synthesis	Chloramphenicol, tetracyclines, aminoglycosides, macrolides, lincosamides
Cell membrane integrity	Polymyxin, daptomycin, amphotericin, imidazoles (vs. fungi)
Nucleic acid function	Nitroimidazoles, nitrofurans, quinolones, rifampin; some antiviral compounds, especially antimetabolites
Intermediary metabolism	Sulfonamides, trimethoprim

1) Target or Site of Action of Antibacterial Drugs - Antibiotics

Most block synthesis of new _____

a. Proteins
b. Cell wall
c. Nucleic acids (DNA or RNA)
d. Metabolites

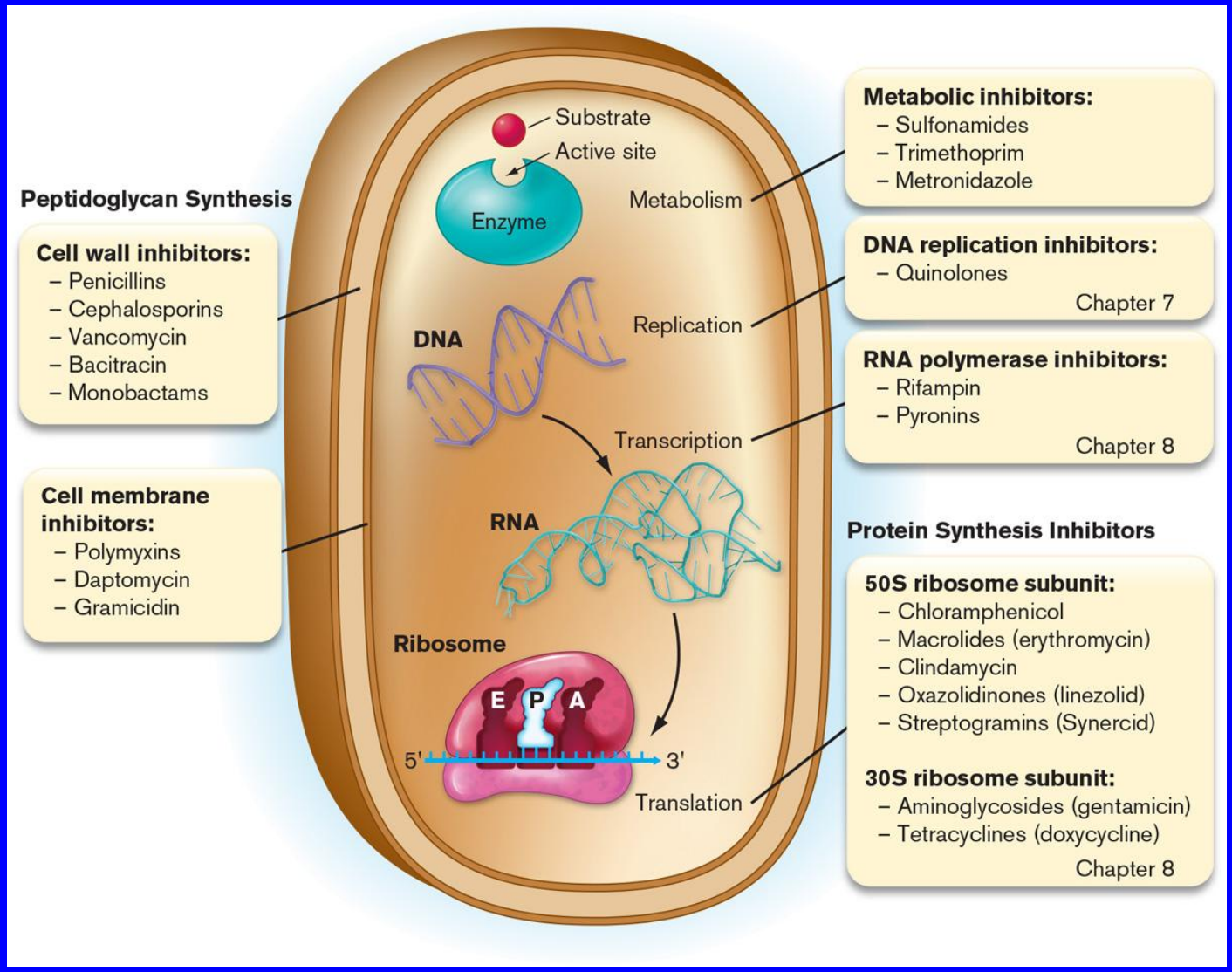
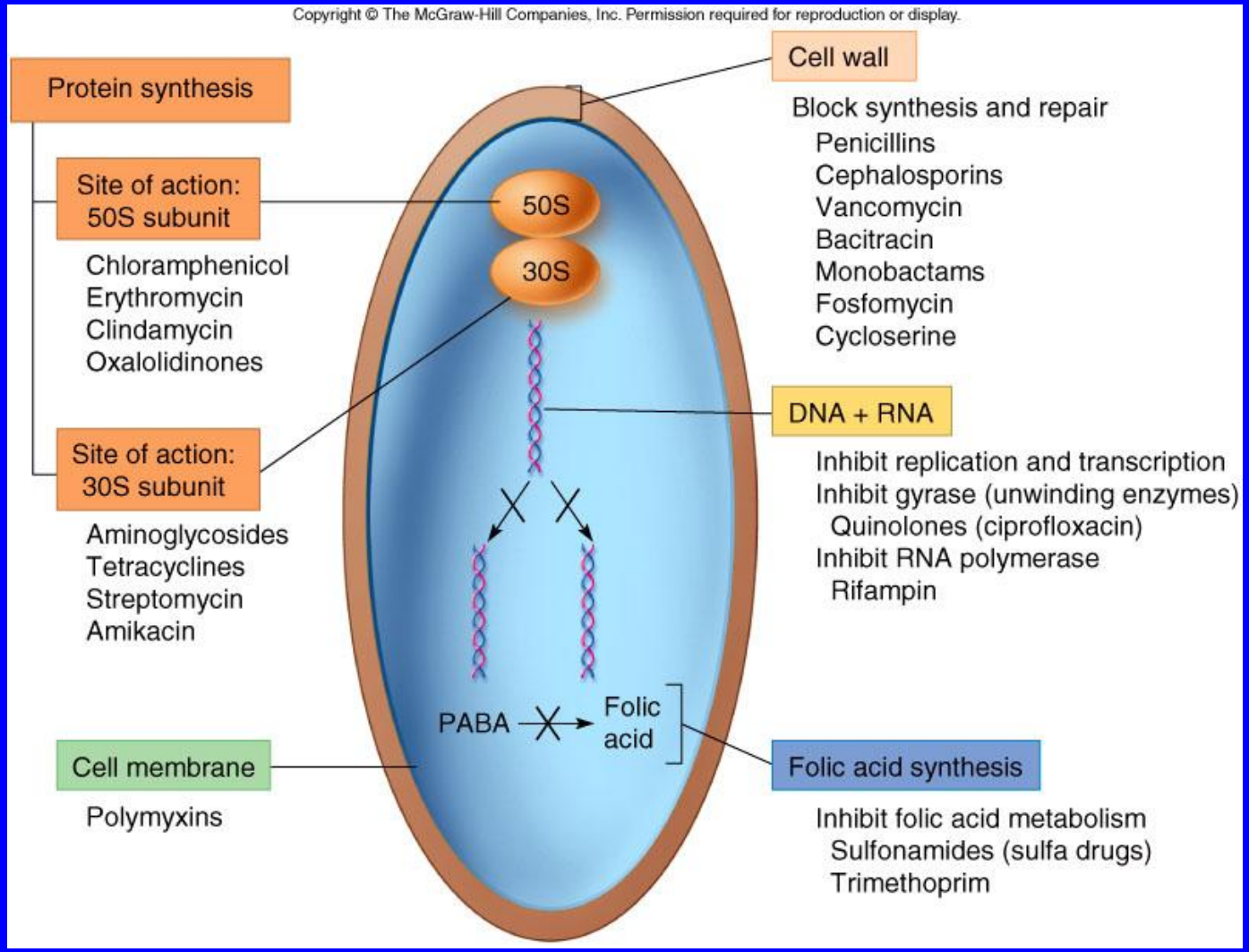


Figure 27.7
6th edition

1) Target or Site of Action of Antibacterial Drugs – Antibiotics - 2

Most block synthesis of new _____

a. Proteins
b. Cell wall
c. Nucleic acids (DNA or RNA)
d. Metabolites



b. Microbial Drug Resistance

- Evolution of pathogenic strains that are resistant to one or more drugs used to treat infections
  - Growing, worldwide problem
  - Complex problem
  - May lead to untreatable infections

1) Timeline of Antibiotic Use and Resistance Part 1

1940

1940
Penicillinase, an enzyme capable of destroying penicillin, identified in bacteria

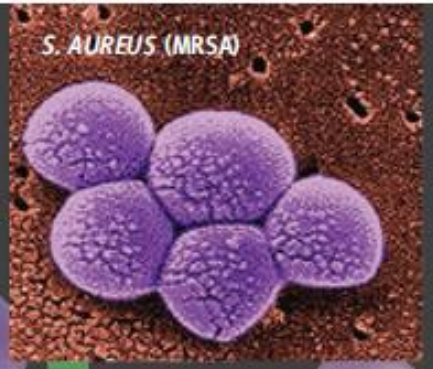
1942
First therapeutic use of penicillin

1943
Penicillin mass-produced

1945
More than 20% of *S. aureus* hospital isolates are penicillin-resistant as penicillinase begins to spread worldwide

1947
Streptomycin approved by FDA

1947
Streptomycin resistance observed



1952
Tetracycline approved by FDA

1956
Tetracycline resistance observed

1958
Vancomycin introduced, although rarely used until the mid-1980s

1959
Methicillin introduced

1961
Methicillin-resistant *S. aureus* (MRSA) observed

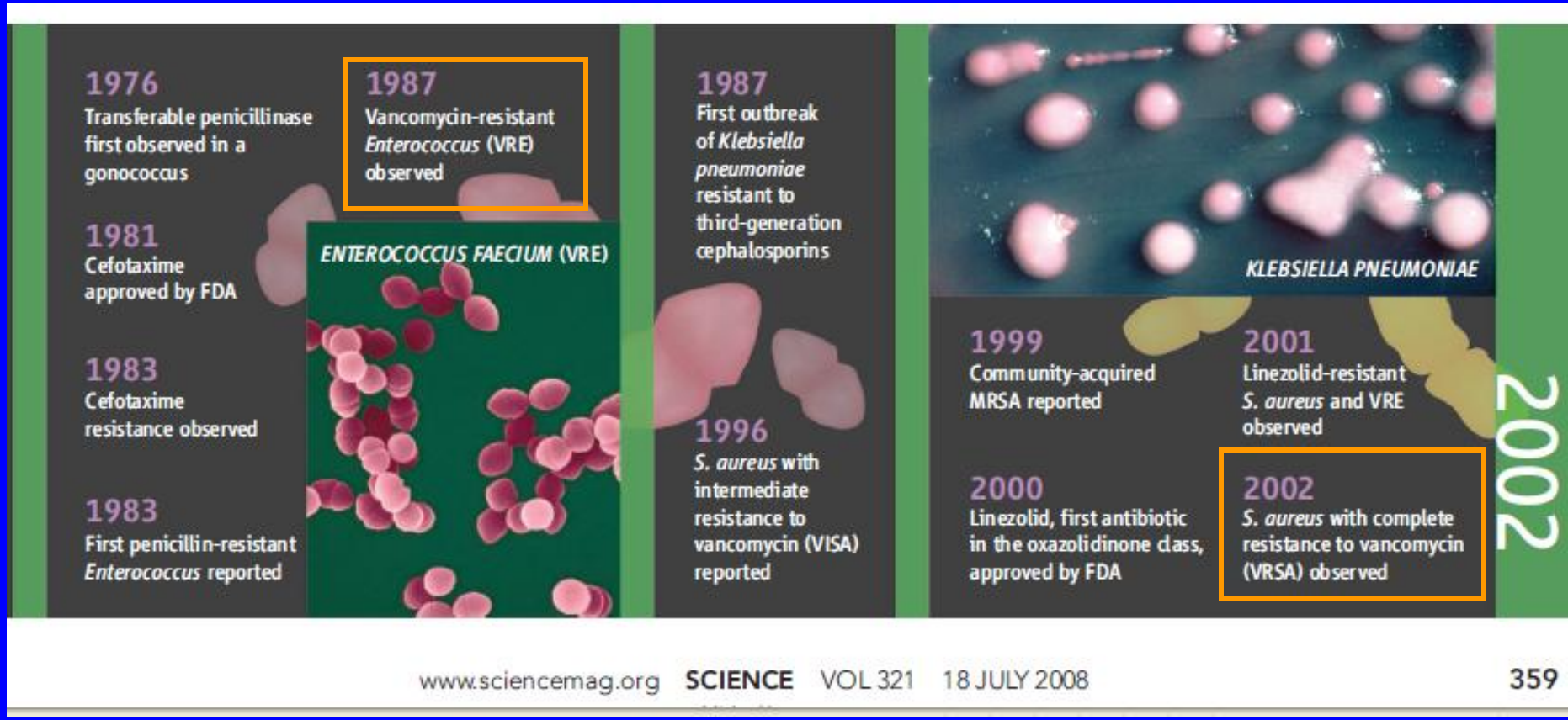
1964
Cephalothin, first antibiotic in the cephalosporin class, introduced

1966
Cephalothin resistance observed

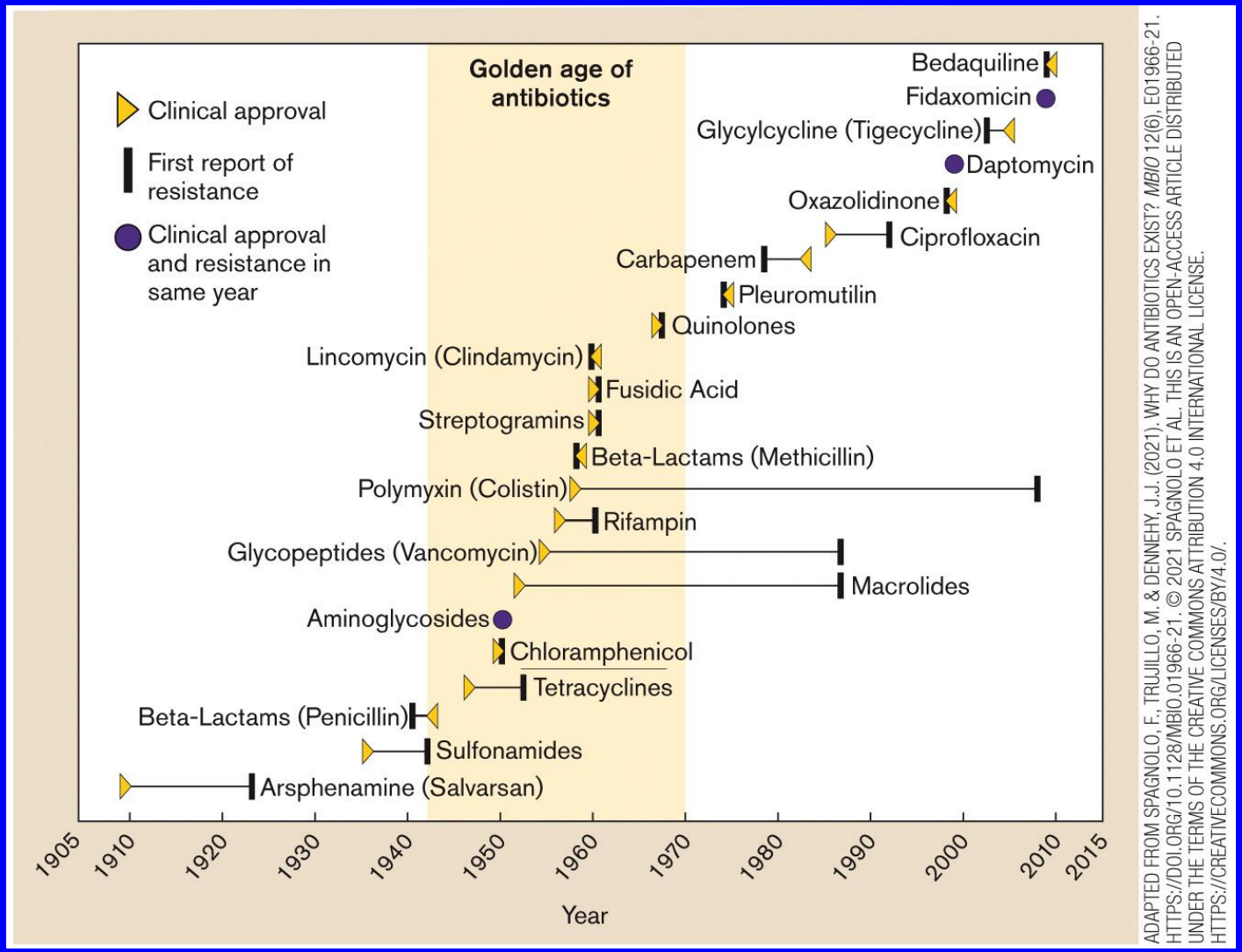
1967
Gentamicin approved by FDA

1970
Gentamicin resistance observed

1) Timeline of Antibiotic Use and Resistance Part 2

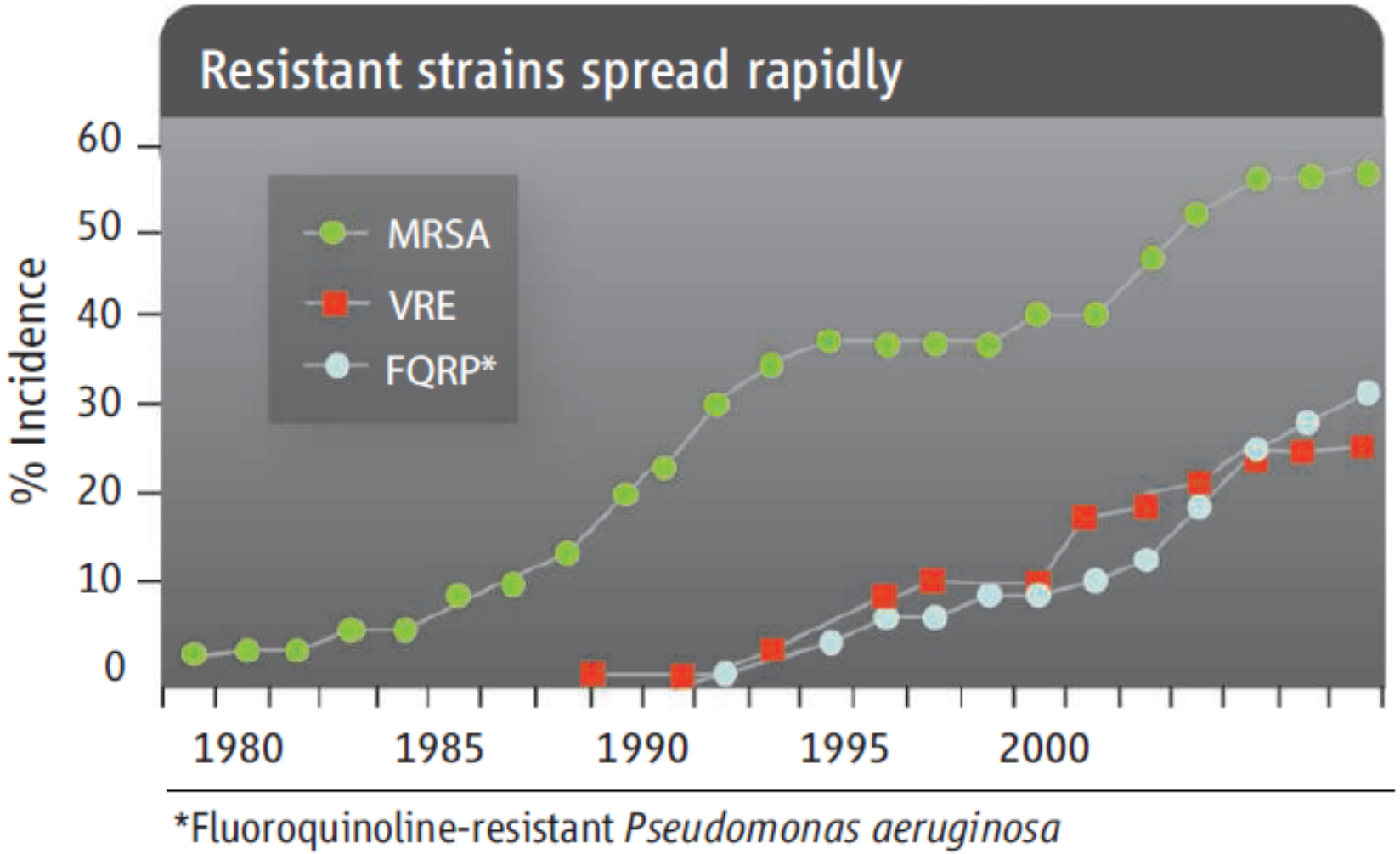


1) Timeline of Antibiotic Use and Appearance of Resistance Figure 2



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2) Timeline of Occurrence of Antibiotic Resistance Strains



2) Mechanisms of Drug Resistance

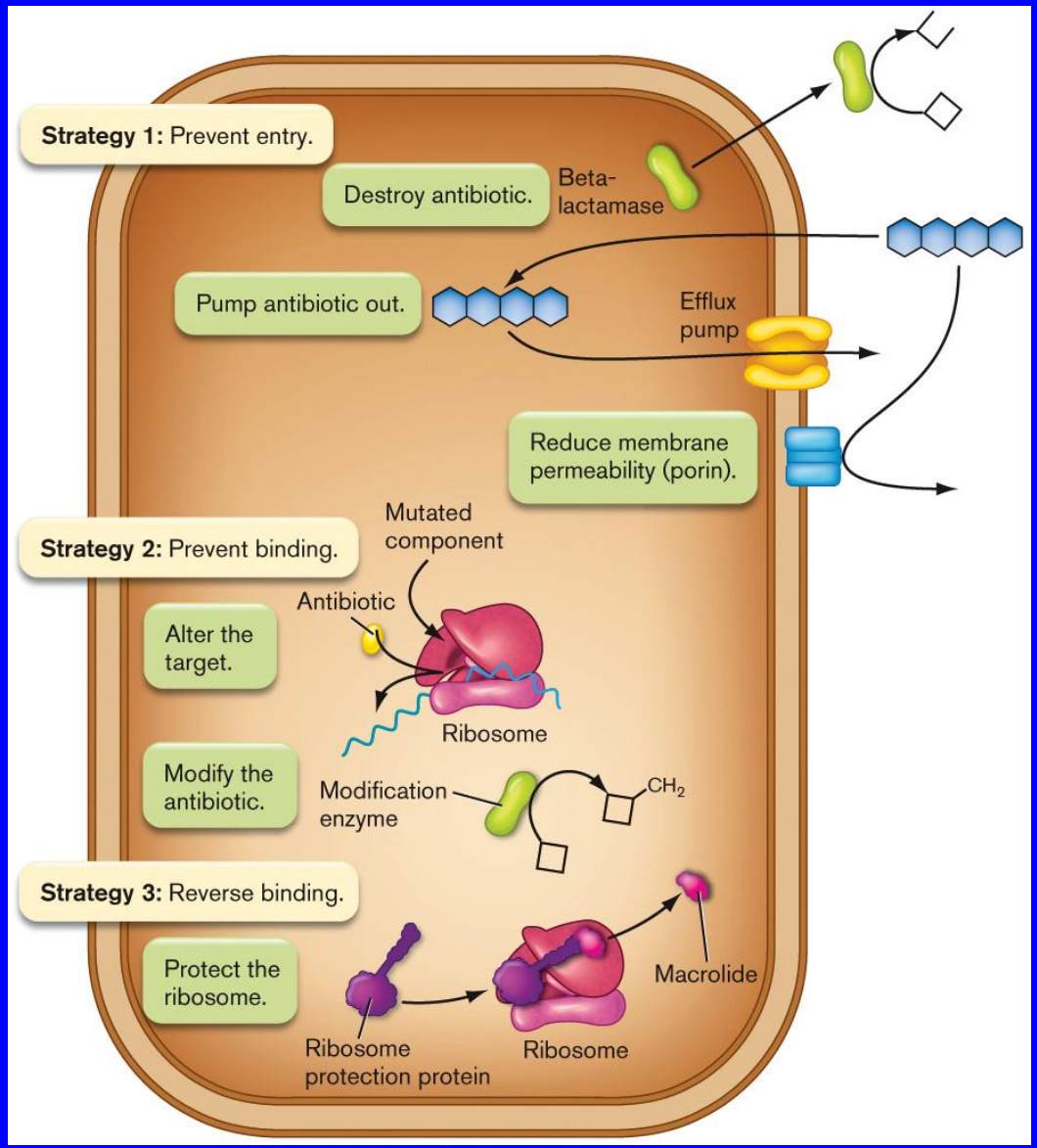


Figure 27.20
6th edition

2) Mechanisms of Drug Resistance - 2

a) Prevent entry – lower levels
   1. Degrade drug
   2. Elimination of drug increased –
      – Make a factor that pumps drug out
      – Multidrug exporter
   3. Uptake of drug reduced –
      – Eliminate transport protein

b) Prevent binding
   1. Target of drug changes –
      • Drug no longer binds to or inhibits target enzyme
   2. Drug modified –
      • Makes a factor that cleaves or modifies the drug

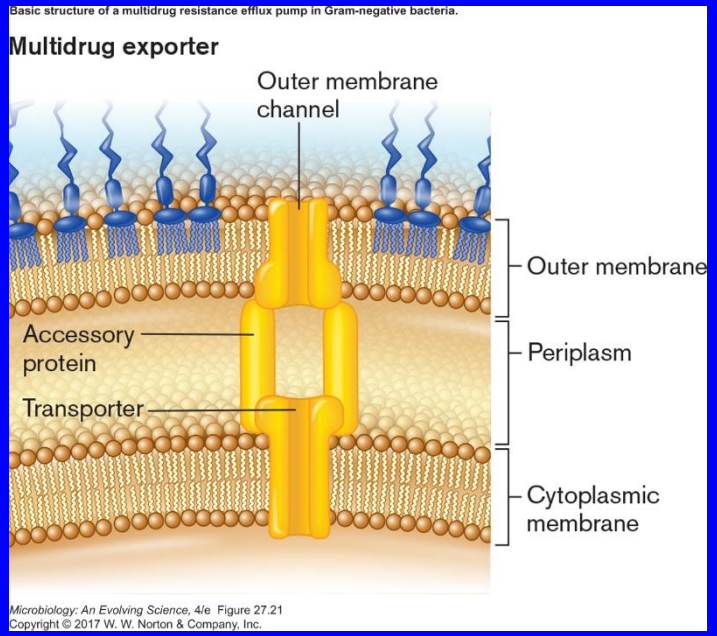
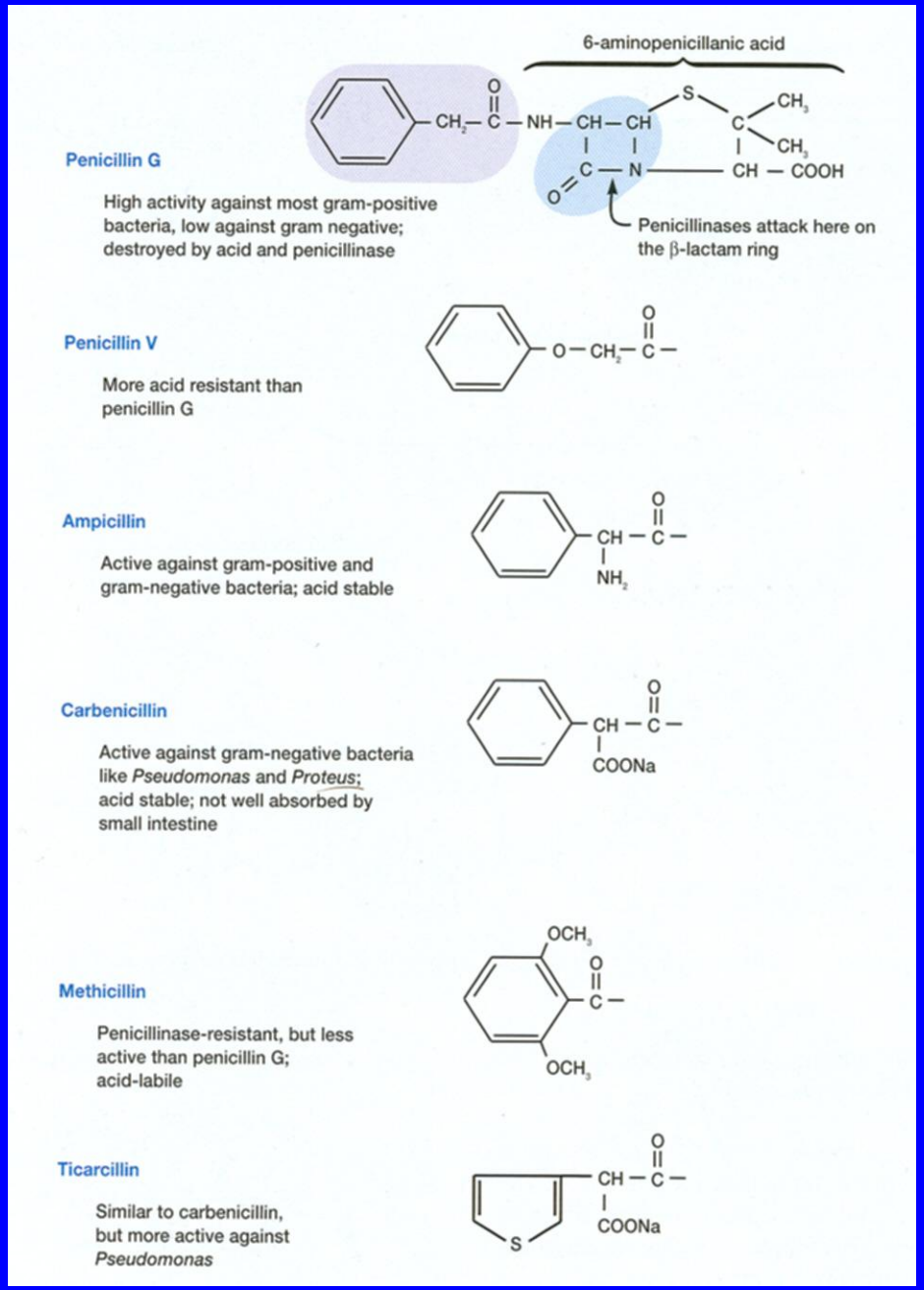


Figure 27.23
6th edition

Penicillin or β-lactam Family

- Functional groups off ring vary
- Affects spectrum of activity, properties, and sensitivity to particular β-lactamases



c. Tests for Antibiotic Susceptibility

- Methods to determine if an isolate is resistant or sensitive to an antibiotic

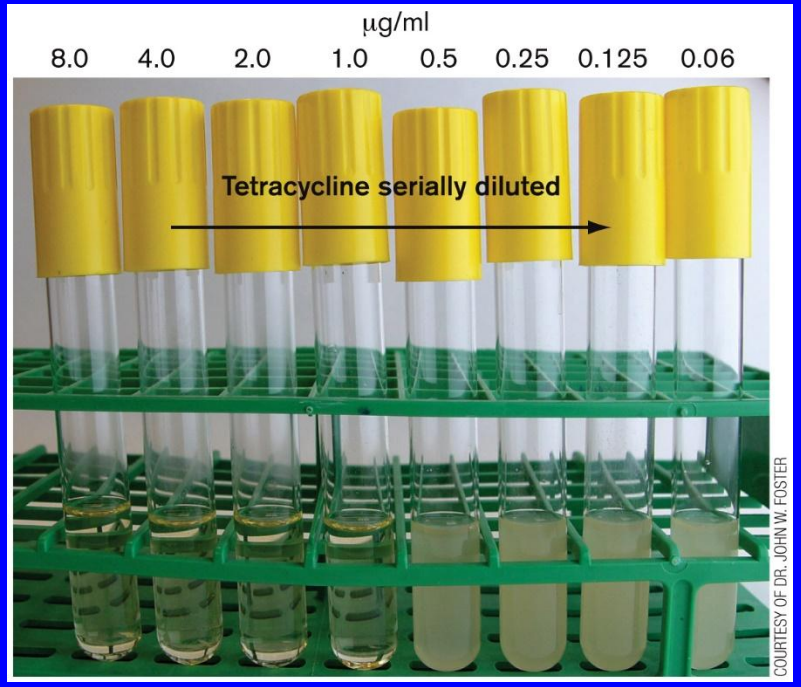
1) Minimal Inhibitory Concentration (MIC)
2) Kirby-Bauer Disk Diffusion assay
3) Genetic methods

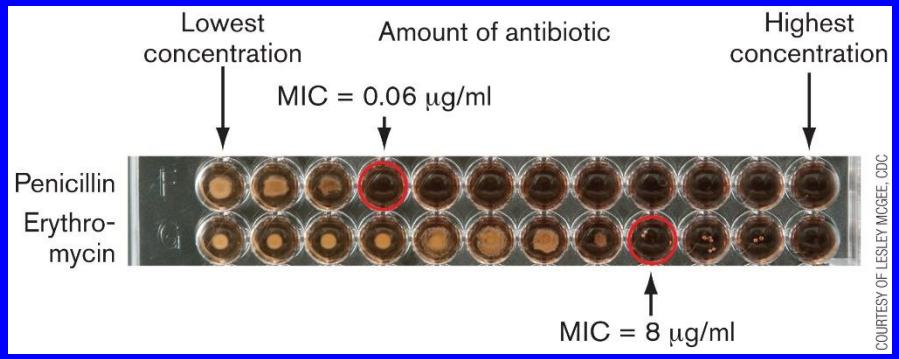
- Covered in section 27.1 in 6th edition

1) Minimal Inhibitory Concentration (MIC)

Figure 27.3 6th edition

1. Serially dilute drug
2. Add small number of cells (10^5) to tube with media and drug
3. Incubate
4. Determine lowest concentration that prevents growth
   - 1.0 ug/ml)





2. Kirby Bauer Disk Diffusion Assay

1. Spread cells over surface of plate
2. Place one or more disks impregnated with a drug on plates
3. Measure zone of no growth around disk. Compare to chart

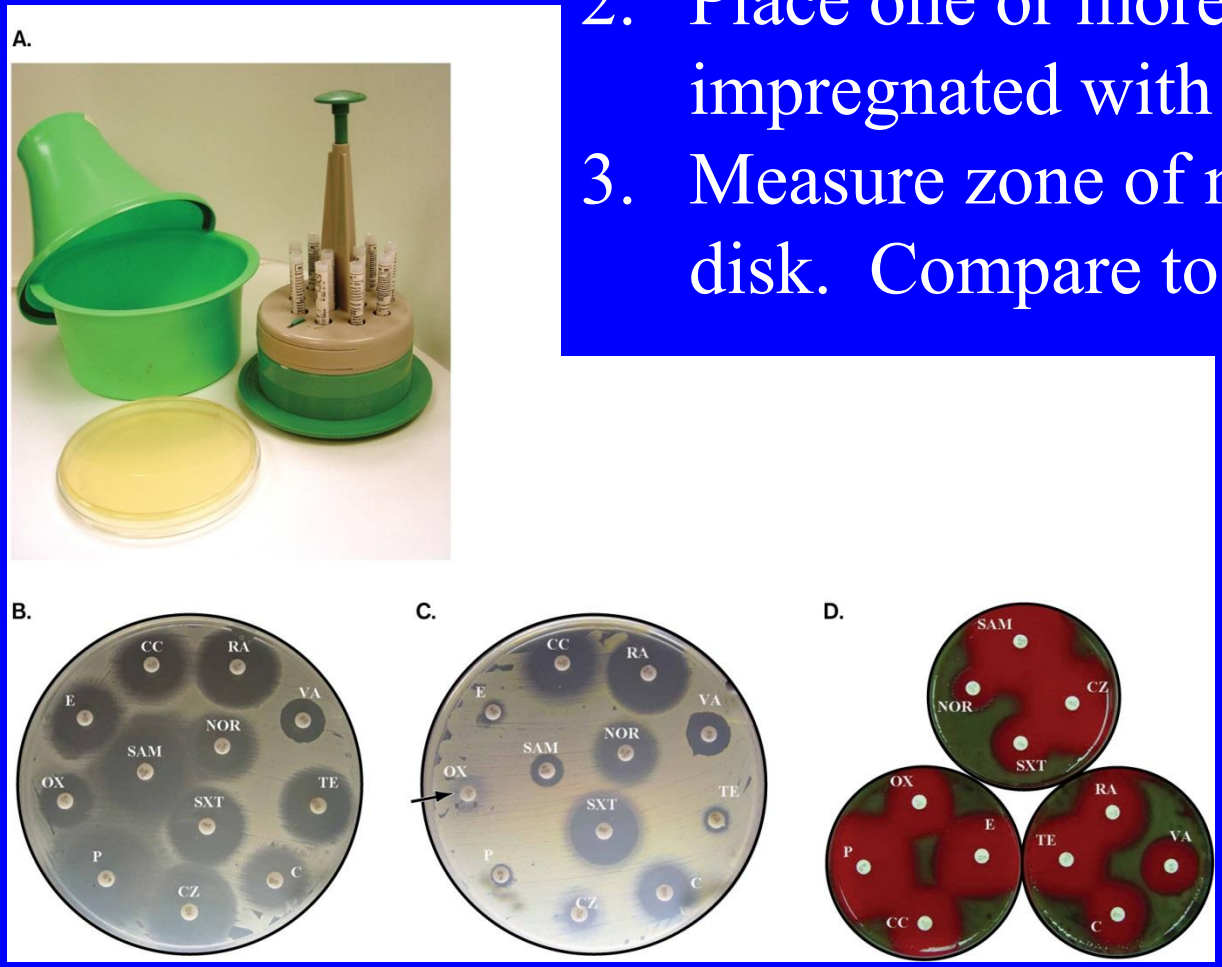


Figure 27.5
6th edition

b. Microbial Drug Resistance - 2

Assignment 15 10/9 In class Group Activity

1. Many of you are hoping to be a doctor. What could a doctor and medical center do to help mitigate the problem of antibiotic resistance increasing in pathogenic strains of bacteria?

Unit 3. Features and Structures of Microbial Cells - D

A. Size and Shape of bacteria
B. Specialized External Structures of bacteria
C. Cell envelope of bacteria
D. Internal structures of bacteria
E. Cell Division in Bacteria
F. Viruses – Chapter 6
G. Unusual bacteria and viruses

Mostly Chapter 3 in book

D. Internal Structures

- Not covered much in textbook
- Other sources

1. Bacterial Intracellular Structures

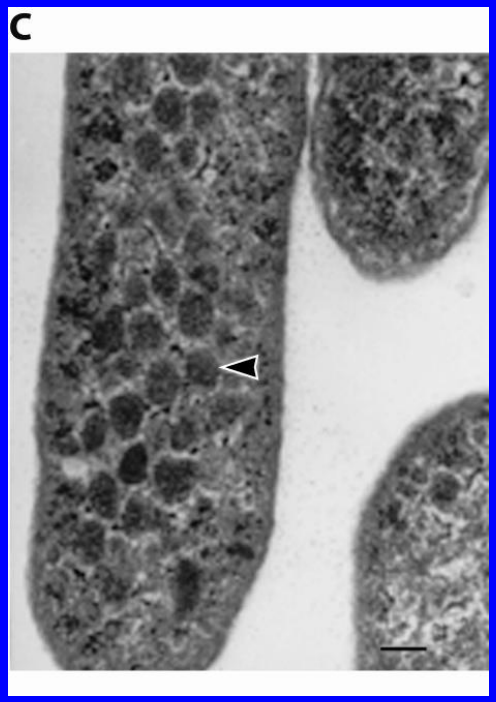
Assignment 15 Group Activity

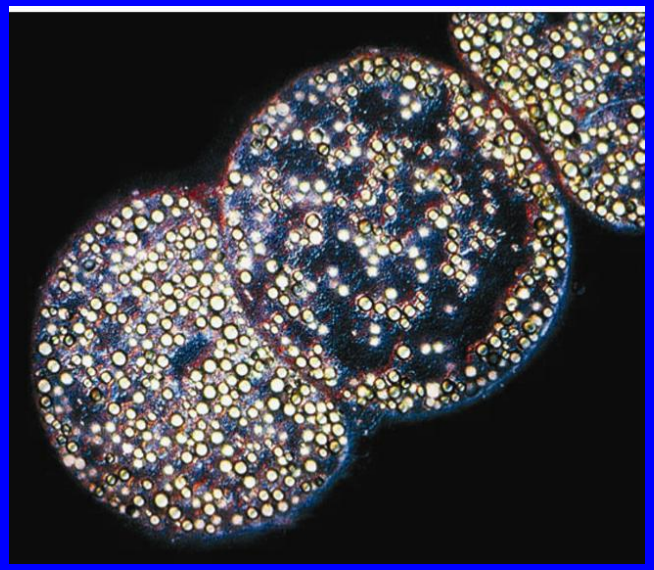
2. Off the top of your head, create a list of structures you think have been observed inside a bacterial cell? Work in groups

4. Which of the following has been observed in the cytoplasm of some bacterial cells?

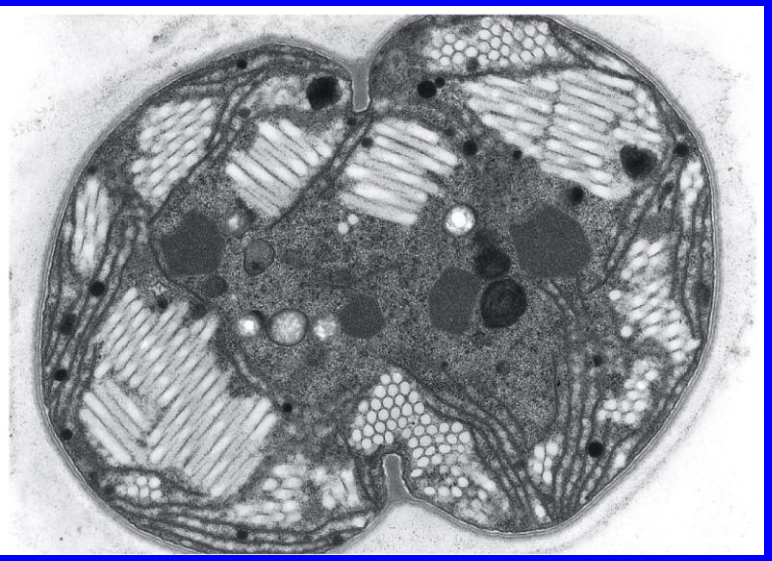
a.	Membrane bound compartments	Yes/No
b.	DNA within an intracellular membrane	Yes/No
c.	Three types of cytoskeleton	Yes/No
d.	DNA wrapped around proteins	Yes/No
e.	Aggregates of metabolites or glycogen	Yes/No
f.	Clumps of iron and sulfur	Yes/No
g.	UFOs (Unidentified floating objects)	Yes/No

Additional Images of Bacteria



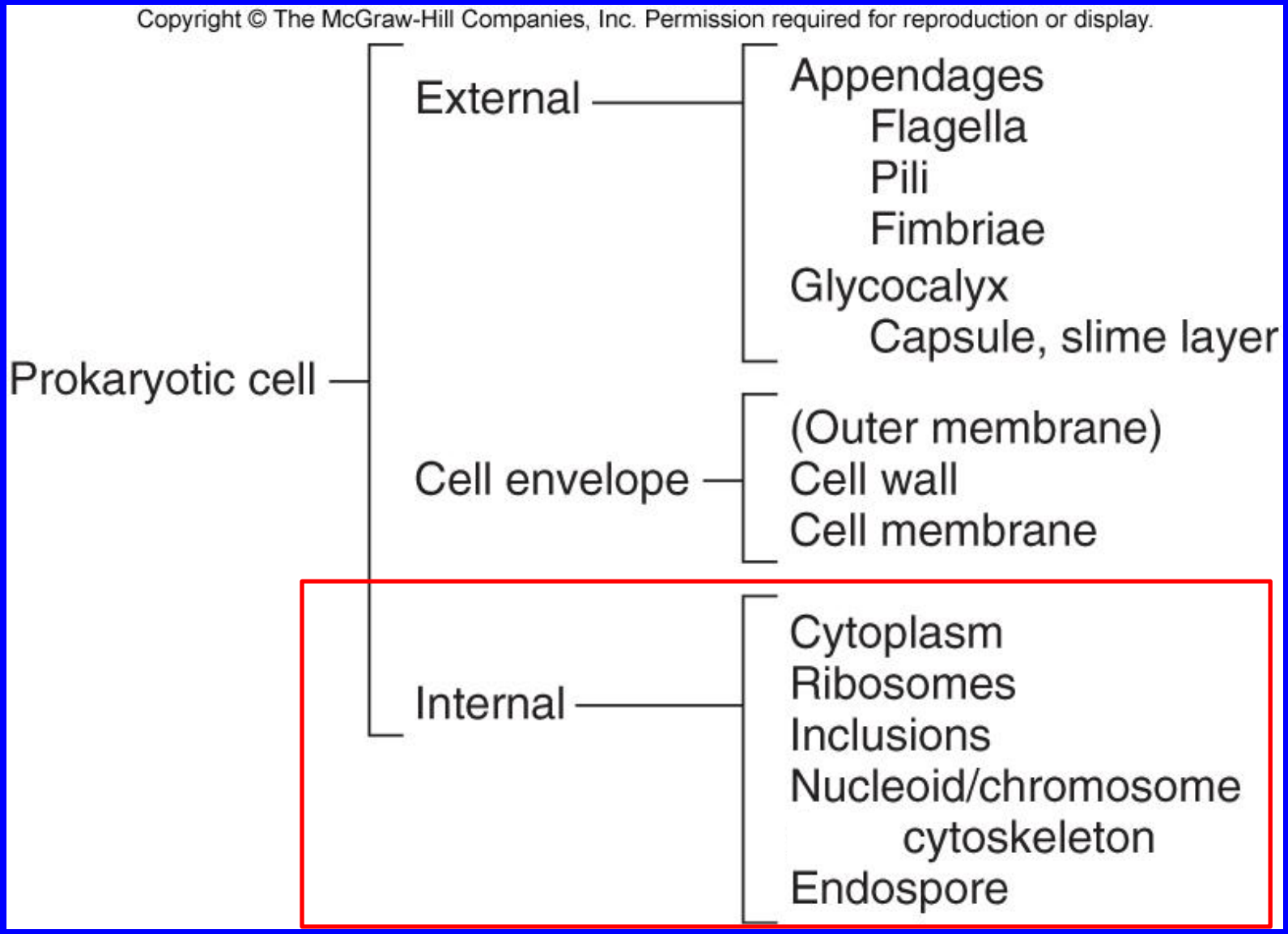






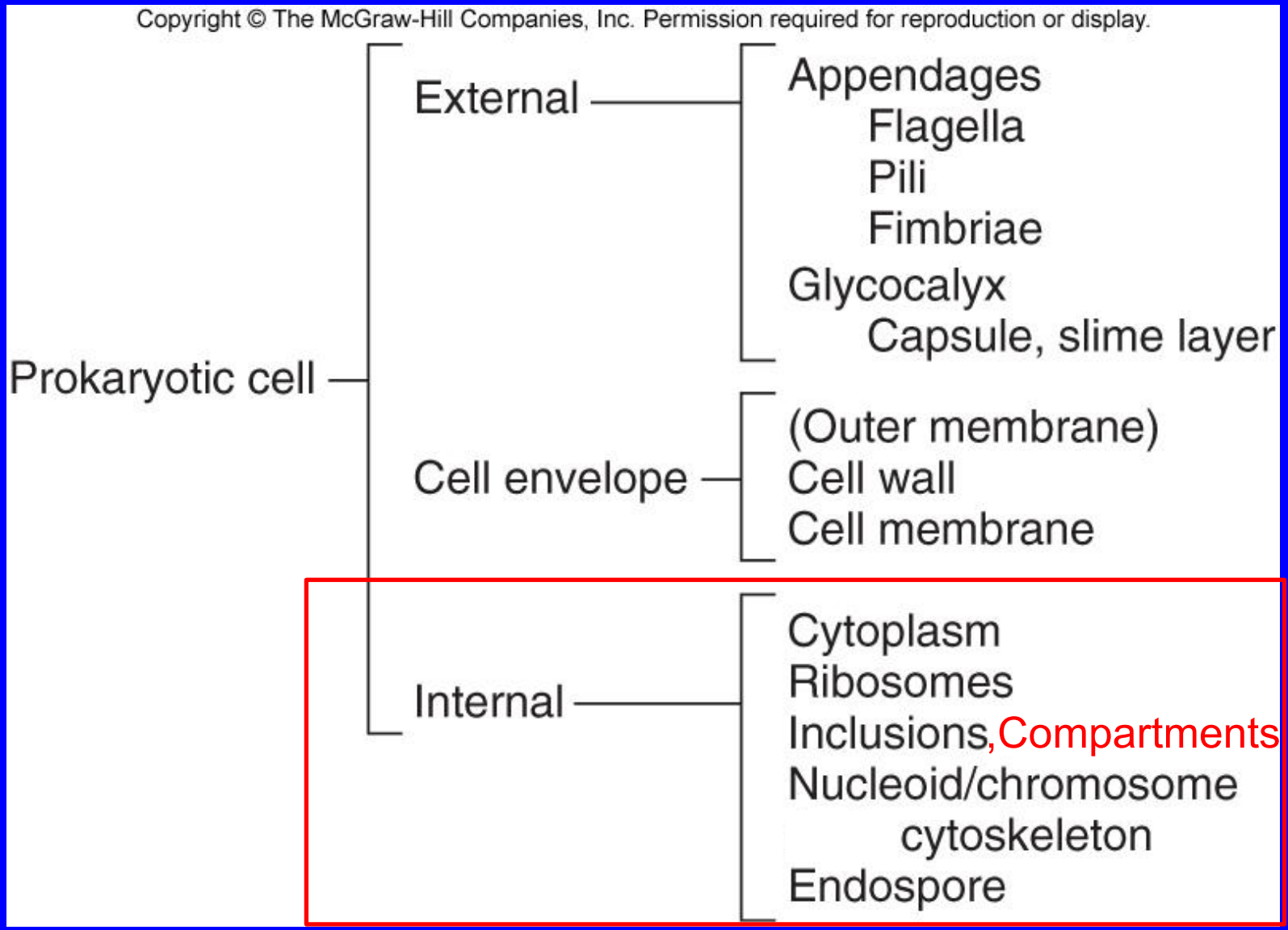
3. Internal Structures

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3. Internal Structures revised

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D. Internal Structures outline

1. Cytoplasm
2. Ribosomes
3. Cytoskeleton
4. Nucleoid
5. Compartments
6. Inclusions and Granules
7. Endospores

Bacterial Internal Structures

1. Cytoplasm – everything inside cell membrane

   Contains

   – Cytosol – liquid portion of cytoplasm
   – A variety of minerals, salts and proteins, sugars, etc. dissolved in water
   – Nucleoid – tightly coiled DNA
   – Inclusions - Aggregates of proteins and other material - most
   – Membrane-bound compartments – a few have

1. Cytoplasm - 1

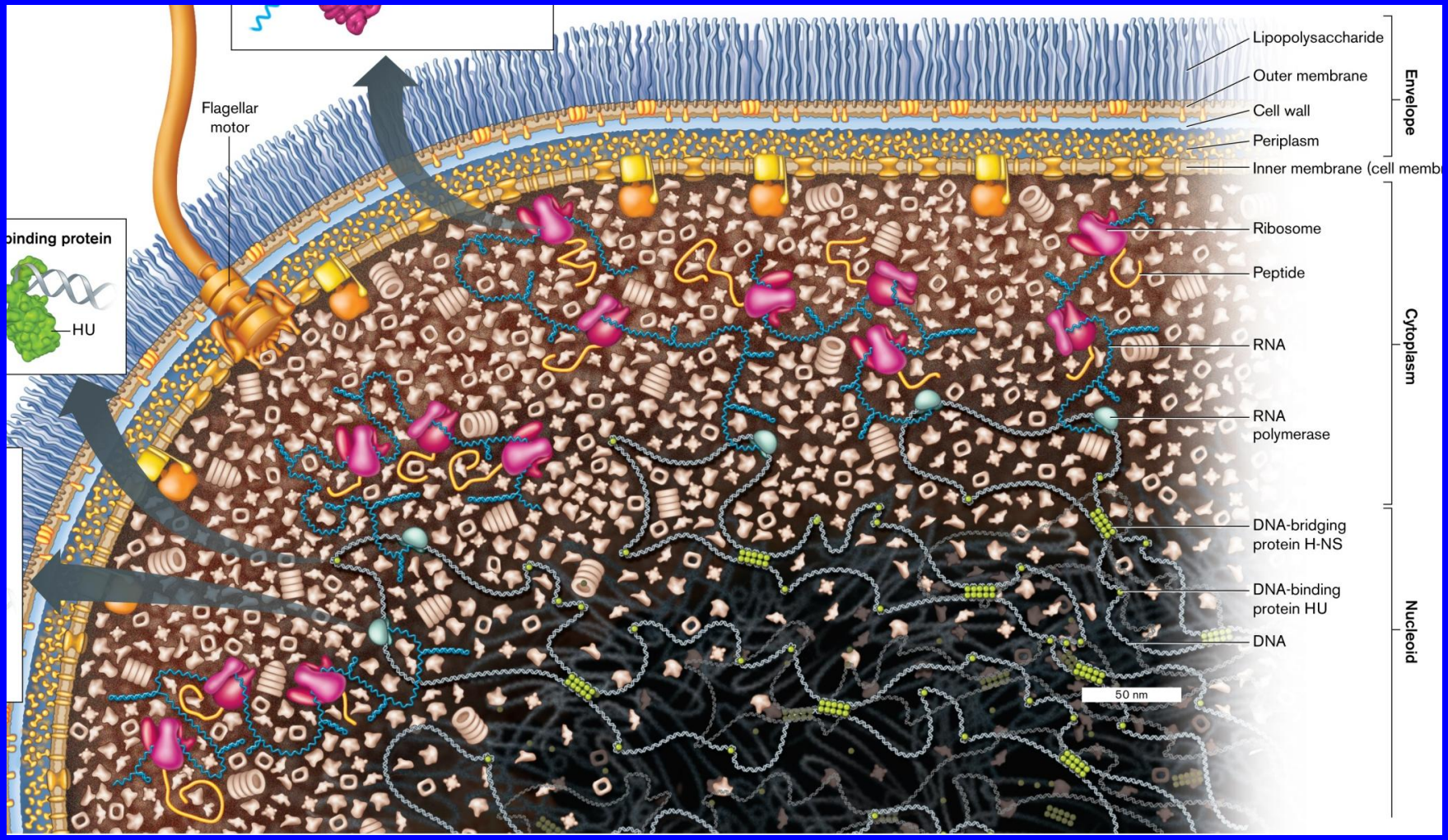


Figure 3.1c 6th edition S F Micro

1. Cytoplasm - 2

- Here they tried to estimate the number of each component per cell

TABLE 3.1 Molecules of a Bacterial Cell, *Escherichia coli*, during Balanced Exponential Growth[a]

Component	Percentage of total weight[b]	Approximate number of molecules/cell	Number of different kinds
Water	70	20,000,000,000	1
Proteins	16	2,400,000	4,000[c]
RNA:			
rRNA, tRNA, and other small RNA (sRNA) molecules	6	250,000	200
mRNA	0.7	4,000	2,000[c]
Lipids:			
Phospholipids (membrane)	3	25,000,000	50
Lipopolysaccharides (outer membrane)	1	1,400,000	1
DNA	1	2[d]	1
Metabolites and biosynthetic precursors	1.3	50,000,000	1,000
Peptidoglycan (murein sacculus)	0.8	1	1
Inorganic ions	0.1	250,000,000	20
Polyamines (mainly putrescine and spermidine)	0.1	6,700,000	2

[a]Values shown are for a hypothetical "average" cell cultured with aeration in glucose medium with minimal salts at 37°C.

[b]The total weight of the cell (including water) is about 10^{-12} gram (g), or 1 picogram (pg).

[c]The number of different kinds is difficult to estimate for proteins and mRNA because some genes are transcribed at extremely low levels and because proteins and RNA include kinds that are rapidly degraded.

[d]In rapidly growing cells, cell fission typically lags approximately one generation behind DNA replication; hence, two identical DNA copies per cell.

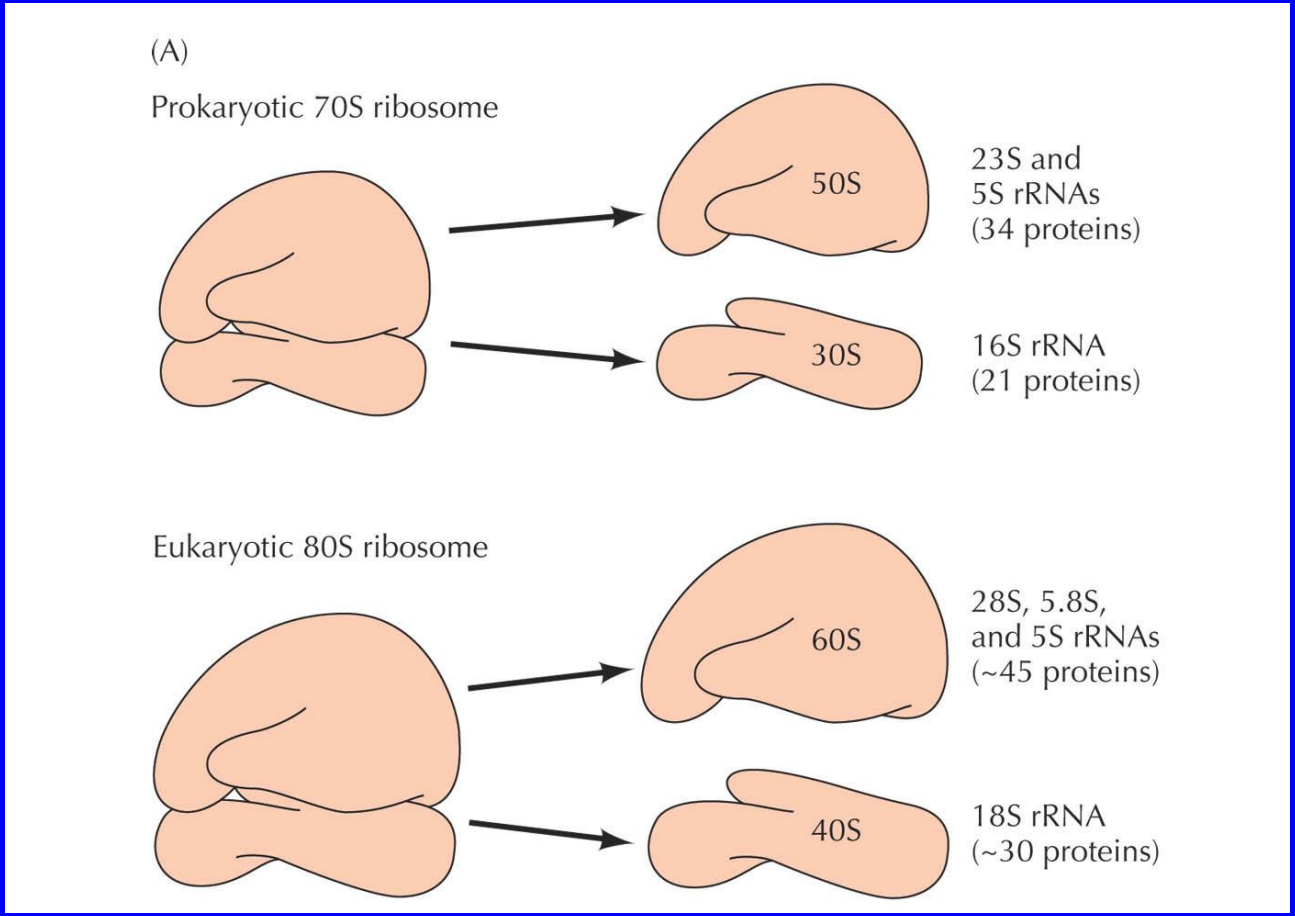
Source: Modified from F. Neidhardt and H. E. Umbarger. 1996. Chemical composition of *Escherichia coli*, p. 14. In F. C. Neidhardt (ed.), *Escherichia coli and Salmonella: Cellular and Molecular Biology*, 2nd ed. ASM Press, Washington, DC.

1. Cytoplasm - 3

- Crowded with macromolecules, particularly ribosomes (~15,000 per cell)
  - 60-70% of wet weight of cytoplasm is water
  - 20% of weight protein
  - 7-20% RNA (rRNAs – 65% of ribosomes)
  - 3% lipid
  - 1-2 % DNA
- Viscous –
  - Rate of diffusion is reduced 10 fold

2. Ribosomes

- Differs in size and components from eukaryotic
- Selectively inhibited by some antibiotics



Inhibitors of Translation

Inhibitor	Action
Chloramphenicol	Inhibits peptidyl transferase on the prokaryotic large subunit
Cycloheximide	Inhibits peptidyl transferase on the eukaryotic large subunit
Erythromycin	Inhibits translocation by the prokaryotic large subunit
Fusidic acid	Inhibits elongation in prokaryotes by binding to EF-G · GDP in a way that prevents its dissociation from the large subunit
Paromomycin	Increases the ribosomal error rate
Puromycin	An aminoacyl–tRNA analog that causes premature chain termination in prokaryotes and eukaryotes
Streptomycin	Causes mRNA misreading and inhibits chain initiation in prokaryotes
Tetracycline	Inhibits the binding of aminoacyl–tRNAs to the prokaryotic small subunit
Diphtheria toxin	Catalytically inactivates eEF2 by ADP-ribosylation
Ricin/abrin/ -sarcin	**Ricin** and **abrin** are poisonous plant glycosidases that catalytically inactivate the eukaryotic large subunit by hydrolytically depurinating a specific highly conserved A residue of the 28S RNA, which is located on the so-called **sarcin–ricin loop** that forms a critical part of the ribosomal factor-binding center; **α-sarcin** is a fungal protein that cleaves a specific phosphodiester bond in the sarcin–ricin loop

Some are antibiotic drugs used for bacterial infections
Some are toxins produced by organisms
Some are used for research purposes.