BIOL 4640 Mol Micro
Lecture 21 10/14/26
Cell Structures Part 7

- Assignment 15: Lecture 19 In class Activity Due on 10/14
- Assignment 16 Lecture 21 In-class assignment
- Assignment 17 Microbe Project coming
- Pick up Graded Exam 1 Short answer
- Lab students need to streak plates after class
- Exam 2 on Wednesday, Oct 28
  - Study guide and old exam posted

BIOL 4640
Exam 1, 2026

- 103 pts possible (1 extra question for extra credit)
- (MC correct x 3) + short answer
- Ave 80.1 High 103

Scale	points	# students
A	89-102	15
B	79-88.5	1
C	69-78.5	7
	< 69	6

Unit 3. Features and Structures of Microbial Cells - E

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 Microbes
F. Viruses – Chapter 6
G. Unusual bacteria and viruses

Mostly Chapter 3 in book

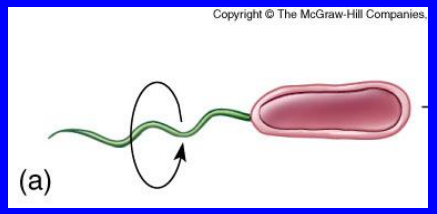
E. Cell Division in Bacteria

1. Types of Cell Division
2. Steps in Cell Division
3. Mechanism of cell division
4. Cell polarity after cell division
5. Proteins involved in Cell Division

- Read sections 3.4 and 3.5 and posted review article

4. Cell polarity and Asymmetry

- The two ends of rod-shaped bacteria may be different – may show cell polarity or asymmetry
- Examples include
  a. Bacteria with a flagellum at one end



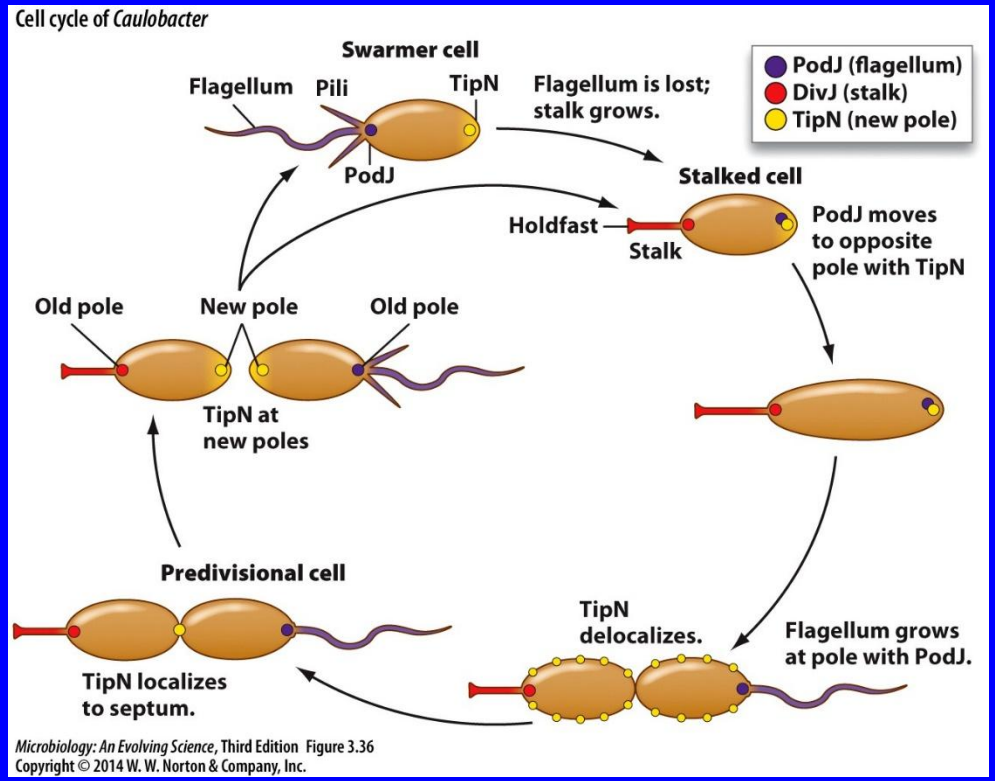
  b. *Caulobauter* –have either a stalk or flagellum on one end
  c. Asymmetry in envelope at poles after typical symmetrical binary cell division
- Can cause properties of cells within a population to differ
- Section 3.5 6th ed

4. Cell Polarity and Asymmetry - 2

b. Caulobauter – Figure 3.32 6/e

1) Swarmer cell has one flagellum on one pole
2) Can lose flagellum and grow a stalk on same end
3) Stalked cells divide and new cell has flagellum on old pole or end.

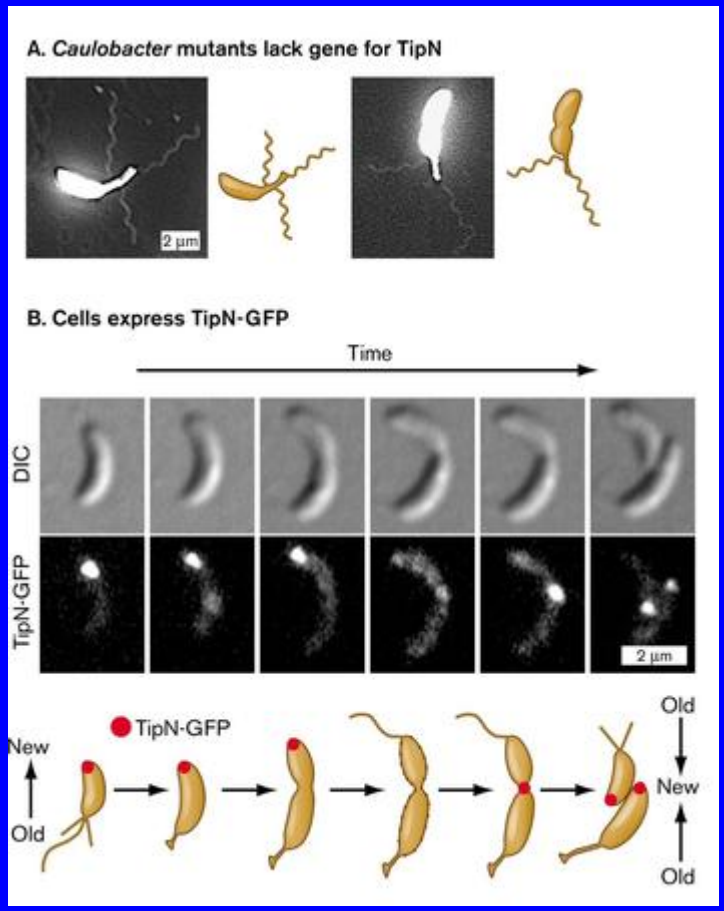
How does the cell know where to put the flagellum (which end is which)?



b. Cell polarity in *Caulobauter*

- Experiments revealed that the TipN protein plays a role in determining cell polarity
- Figure 3.31A shows effect of TipN mutation (cells lacking TipN).
  1) How are these cells different than normal, wild type cells?
- Figure 3.31B shows localization of TipN-GFP protein in normal cells.
  2) Where is TipN localized?
  3) What does these results suggest about the role of TipN

Figure 3.31 6th edition

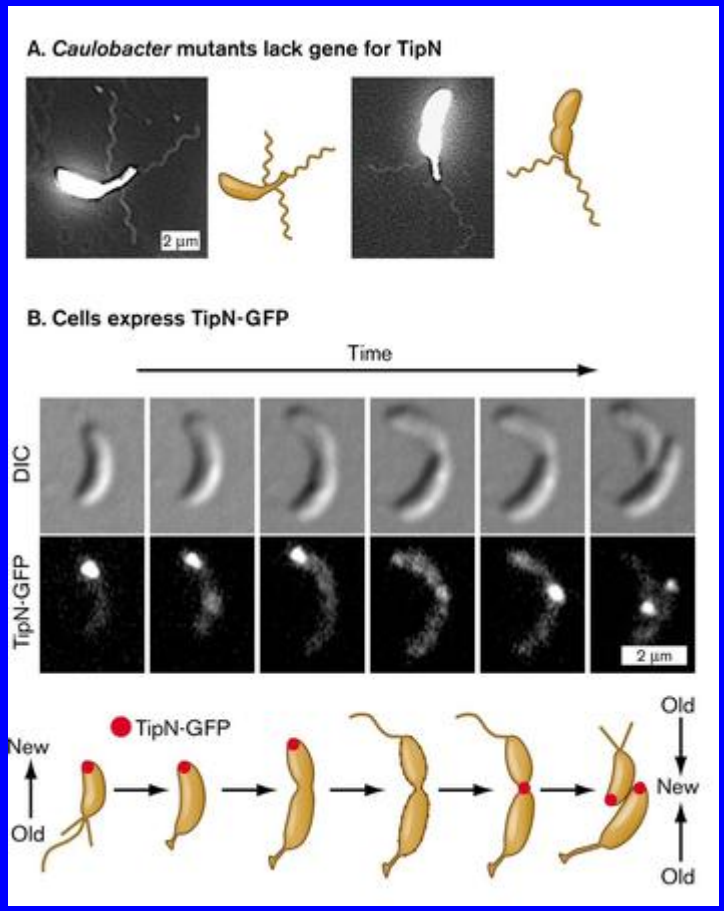


b. Cell polarity in *Caulobauter*

- Figure 3.31A shows effect of TipN mutation (cells lacking TipN).
  1) How are these cells different than normal, wild type cells?
     - Have multiple flagella from stalk end
- Figure 3.31B shows localization of TipN-GFP protein in normal cells.
  2) Where is TipN localized?
  3) What does these results suggest about the role of TipN?

2. TipN localizes to new pole end.
3. Suggests TipN protein may be a marker for the new pole end.

Figure 3.31 6th edition

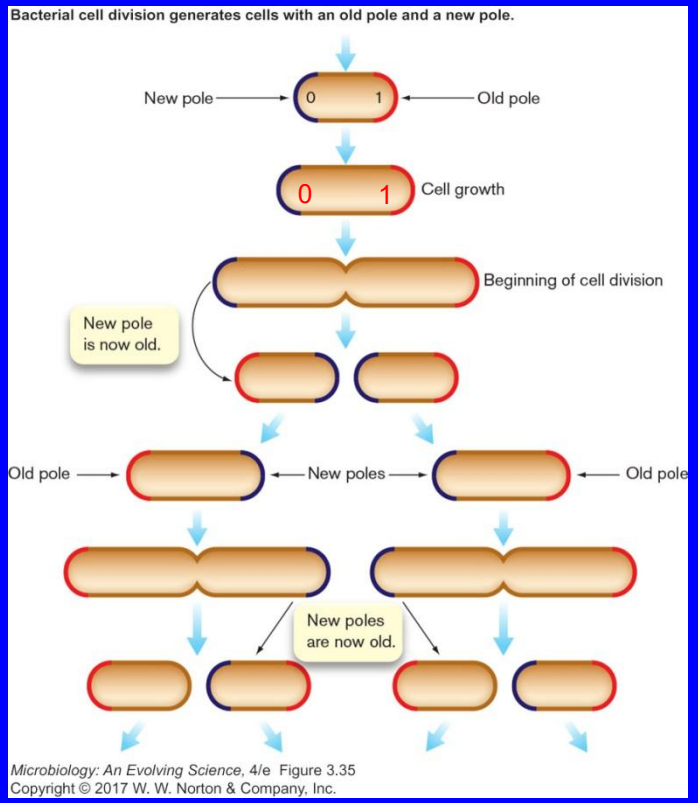


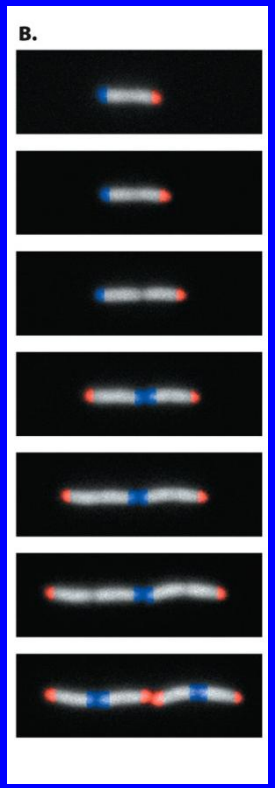
4. Cell Polarity and Asymmetry - 3

c. Typical Binary Fission – Figure 3.33 6th edition

– In bacteria doing apparently symmetrical binary fission, after each cell division, the new cell will have a new pole with newly synthesized cell wall (new pole or 0) and an old pole end (1)

- The old cell wall will be weaker and could lead to cell death
- May explain why some bacterial cells in a population die.
- May affect sensitivity to antimicrobial agents

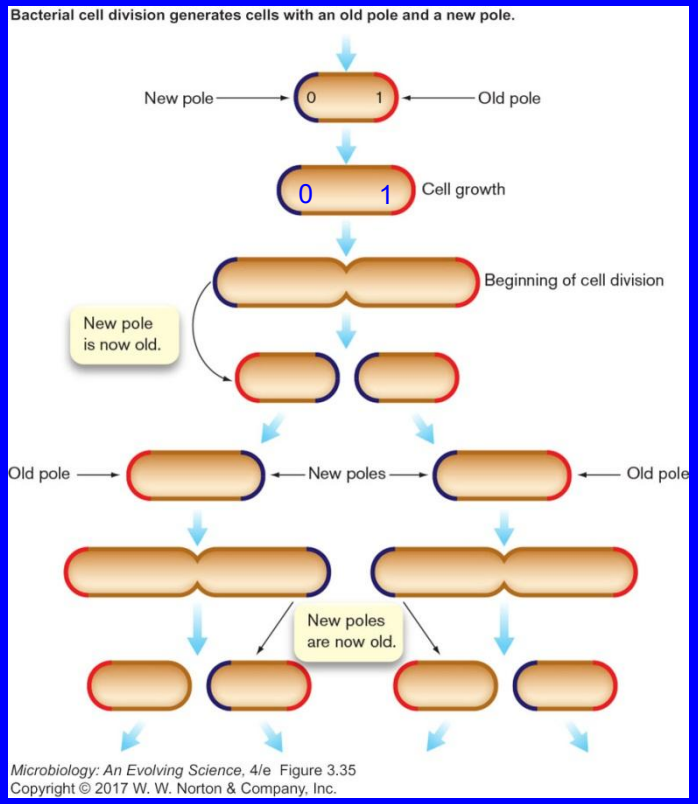


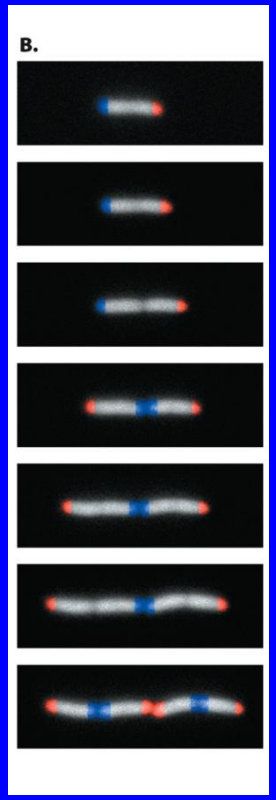


4. Cell Polarity and Asymmetry - 4

c. Typical Binary Fission – Figure 3.33 6th edition

4) Which cell at the bottom has the oldest pole? Track the age of every pole. Label a new pole with a 0 and increase number at a pole following each cell division.



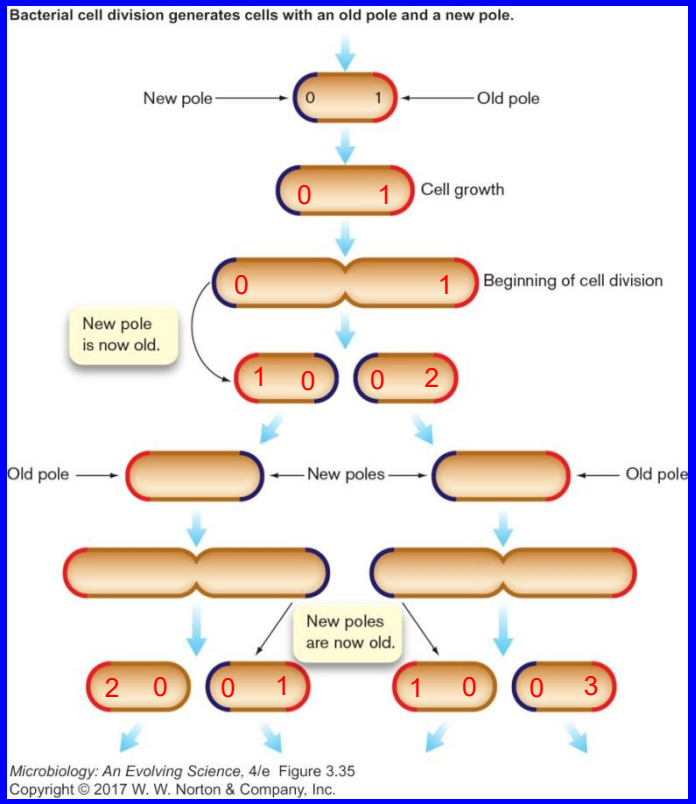


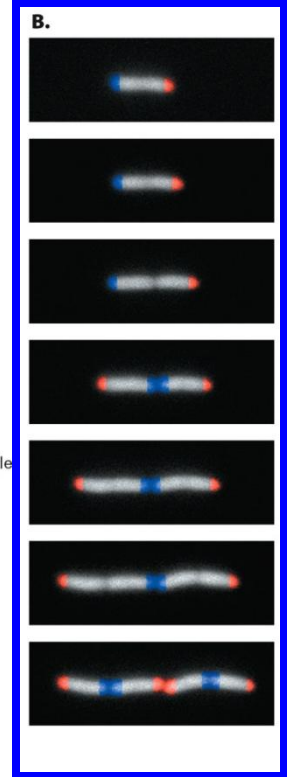
4. Cell Polarity and Asymmetry – 4b

c. Typical Binary Fission – Figure 3.33 6th edition

4) Which cell at the bottom has the oldest pole? Track the age of every pole. Label a new pole with a 0 and increase number at a pole following each cell division.

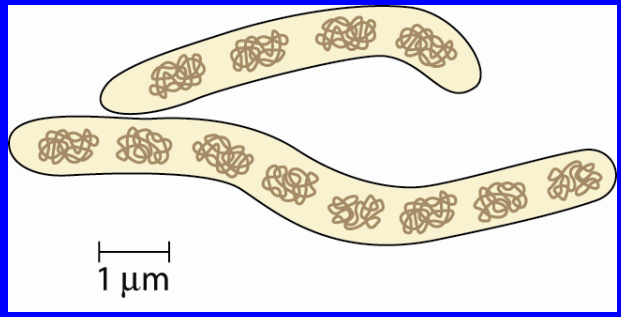
- One on the far right will be at least division 3.





5. Cell Division Proteins

- Have identified protein factors involved in cell division (see posted review article)
- Many identified by isolating temperature sensitive mutations that prevent cell division at non-permissive temperature (usually a higher temperature)
- Cell division is an essential process – can't eliminate completely
- Temperature sensitive mutants grow at permissive temperature
- fts stand for filamentous temperature sensitive



5. Cell Division Proteins - 2

a. FtsZ –
   - Homolog of tubulin (protein that forms microtubules in eukaryotes)
   - Form filaments on inside of cell membrane

a. FtsZ

5) Where does FtsZ protein localize?
   – At the cleavage or division site

A. Phase Micro

B. Blue-DNA

C. Red-FtsZ

D. B + C

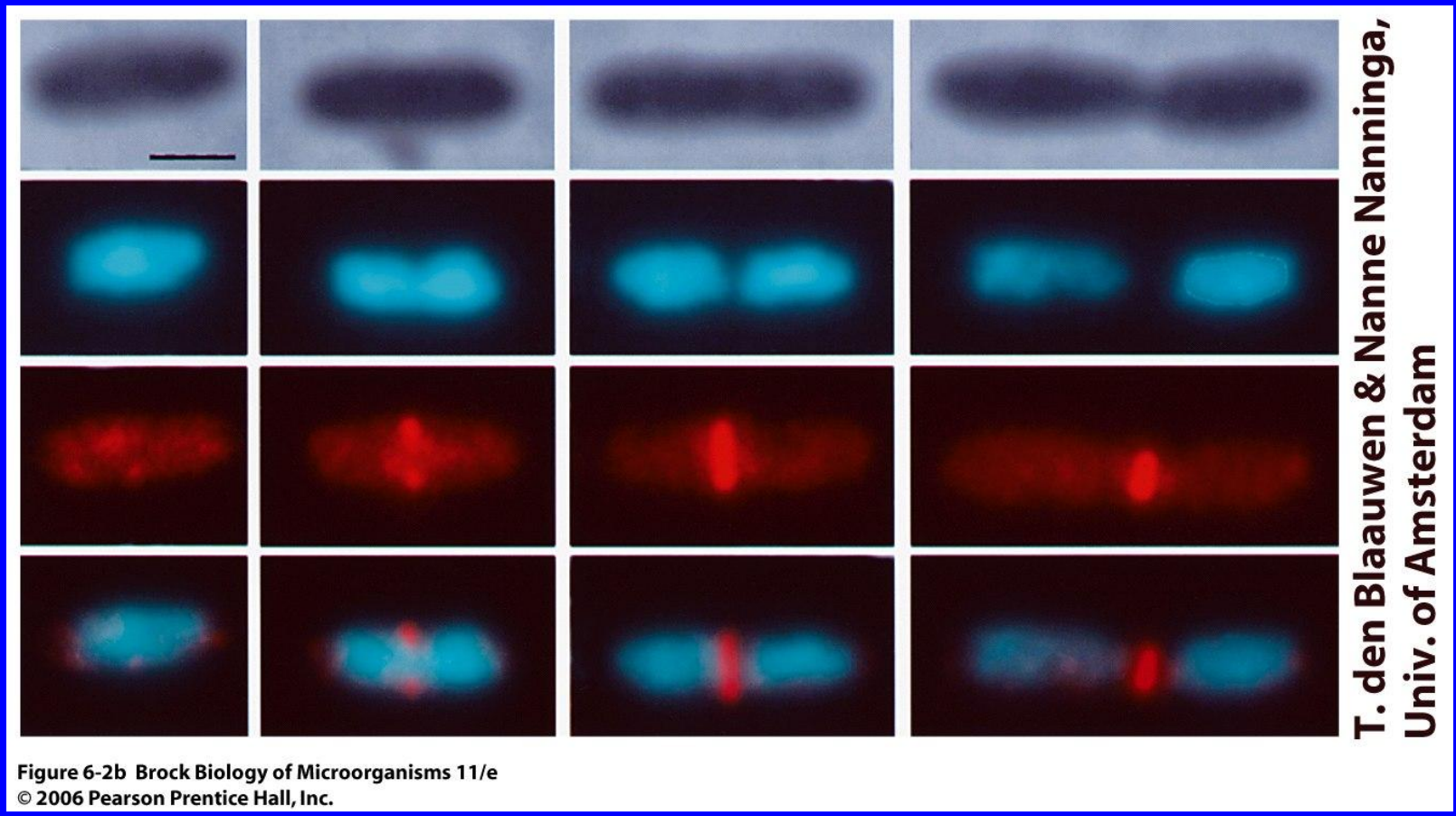
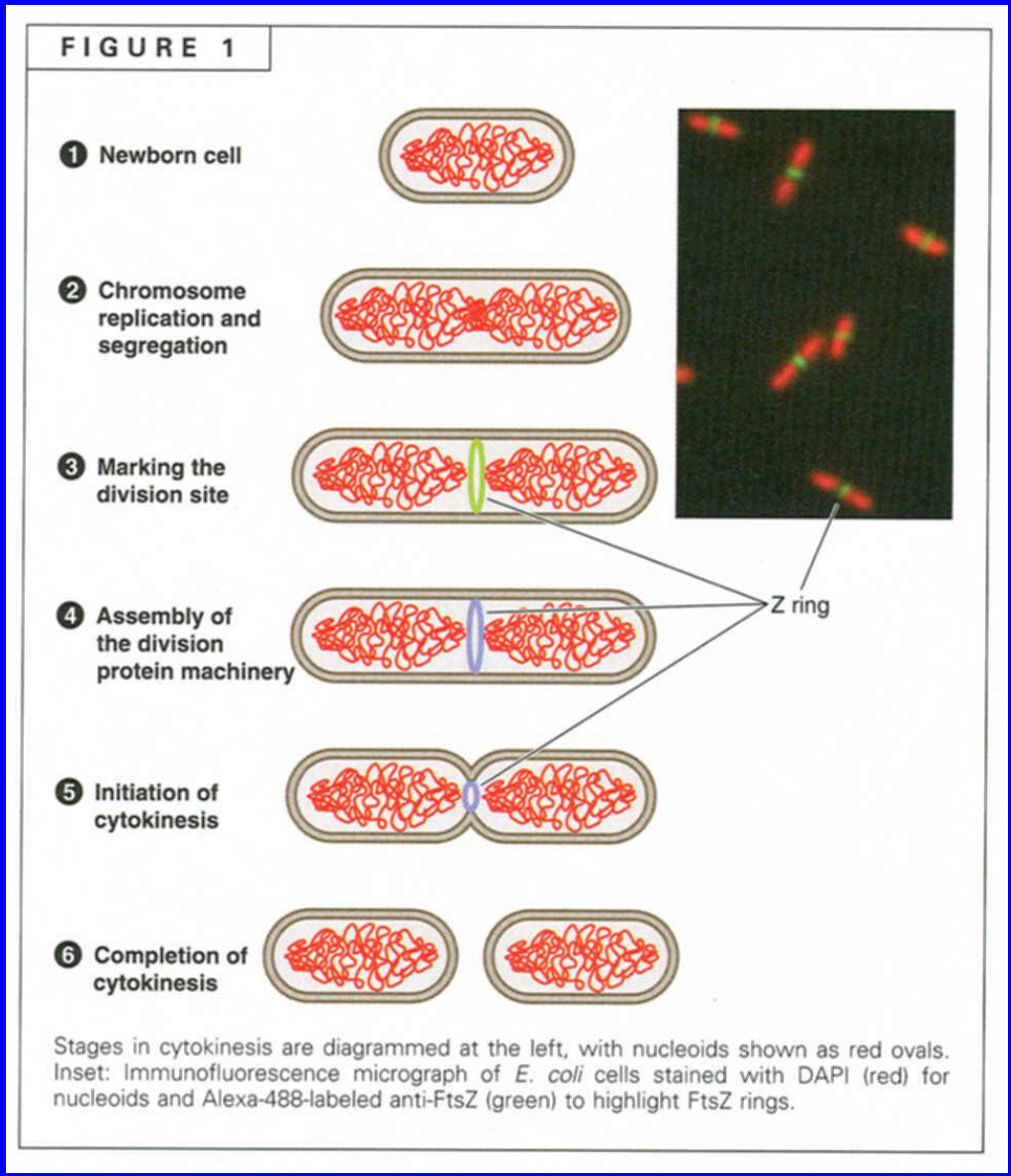


Figure 6-2b Brock Biology of Microorganisms 11/e
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a. FtsZ - 2

- Well studied in *E. coli*
- Forms Z ring at cleavage site
- Ring gets smaller – causes constriction

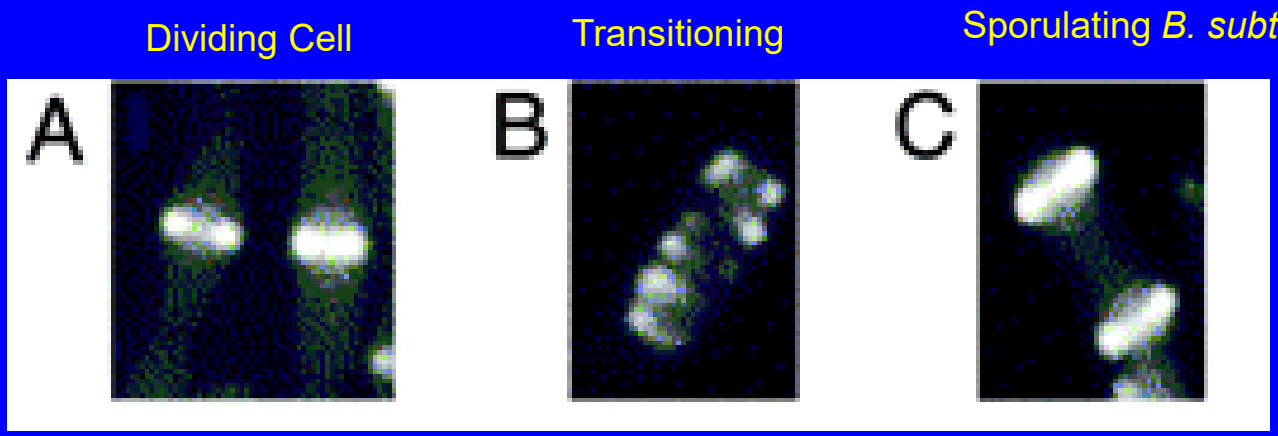


Margolin. 2008. Microbe 3:329-336

a. FtsZ

FtsZ in Gram positive rod *Bacillus*

- In dividing cells, localizes to middle
- In sporulating cells, found at both poles
- In none-dividing or transiting cells, found throughout cell



Shih and Rothfield. 2006. MMBR 70:729-754

a. FtsZ - 2

- A number of other proteins associate with the FtsZ ring
- Build complex (divisome)

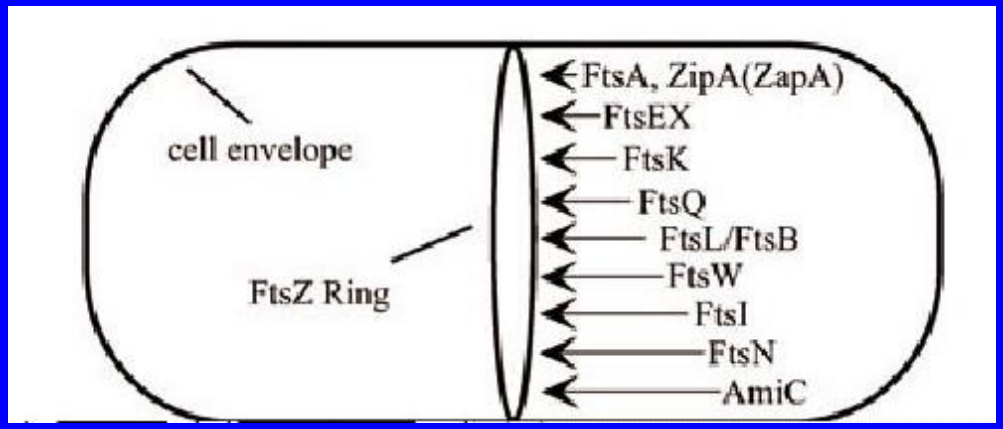


Figure from Weiss. 2004. Molecular Microbiology 54:589

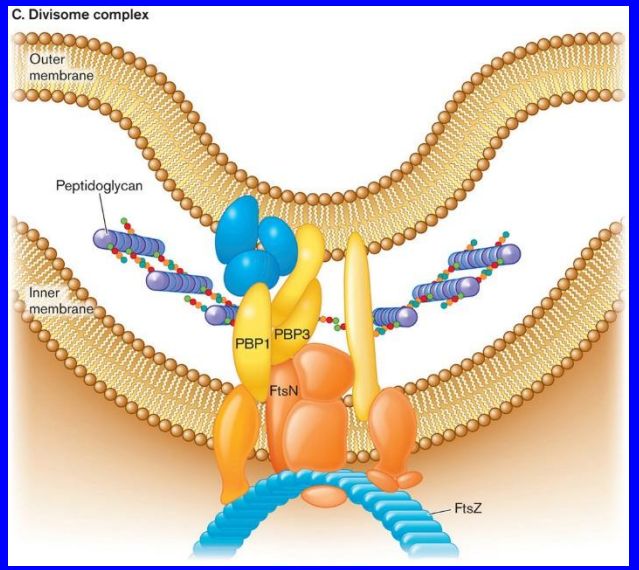


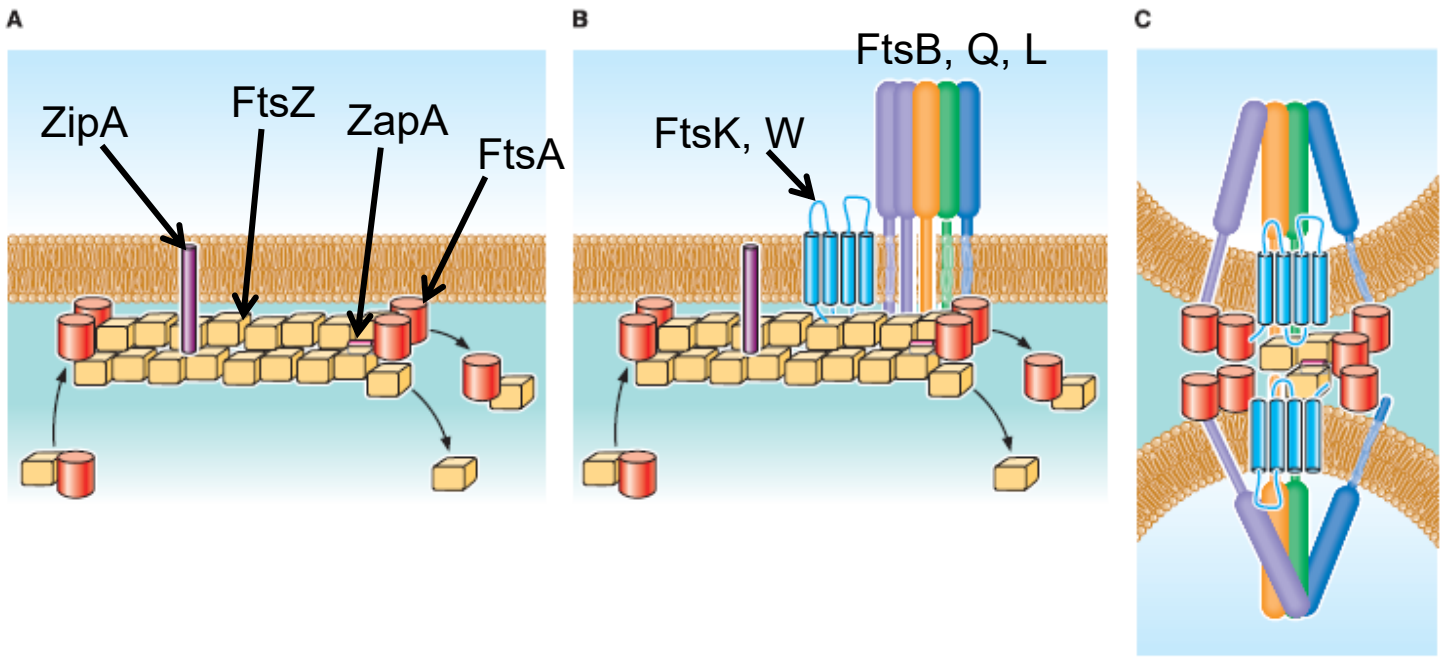
Figure 3.25C
6th edition

a. FtsZ - 3

- FtsZ filaments are dynamic – much turnover of subunits

FtsA and other factors affect turnover

FIGURE 2



Assembly of the cell division machine at the membrane of *E. coli*. (A) With the help of the Min and nucleoid occlusion spatial regulators, FtsZ (yellow boxes) assembles into protofilaments at the future division site. This assembly is dynamic, with FtsZ subunits undergoing constant turnover. Other proteins such as FtsA may associate with FtsZ outside the ring. Interactions between FtsZ and FtsA (red), ZipA (purple), ZapA (magenta) and other proteins help to tether the protofilaments to the inner surface of the cytoplasmic membrane and to promote lateral interactions between protofilaments. (B) Once the FtsZ ring is in place, a number of transmembrane proteins are recruited. Some of these, such as FtsB, FtsQ, FtsL, FtsI, and FtsN, span the membrane only once, while others, such as FtsK and FtsW, span the membrane multiple times. (C) At the last stages of cytokinesis, the cell division machine has lost most of the FtsZ subunits, while many of the other proteins may still persist in subcomplexes.

Margolin. 2008. Microbe 3:329-336

5. Cell Division Proteins - 3

b. FtsA –
   - Homolog of actin
   - Interacts with FtsZ and an associates with membrane
   - Helps bind bundle of FtsZ filaments

c. ZipA
   - Membrane protein
   - Similar role as FtsA

5. Cell division Proteins - 4

d. PBP3 – Penicillin binding protein 3

- A Type of PBP needed for synthesis of new murein (peptidoglycan) at cleavage site
- Does transglycosylation and transpeptidation reactions

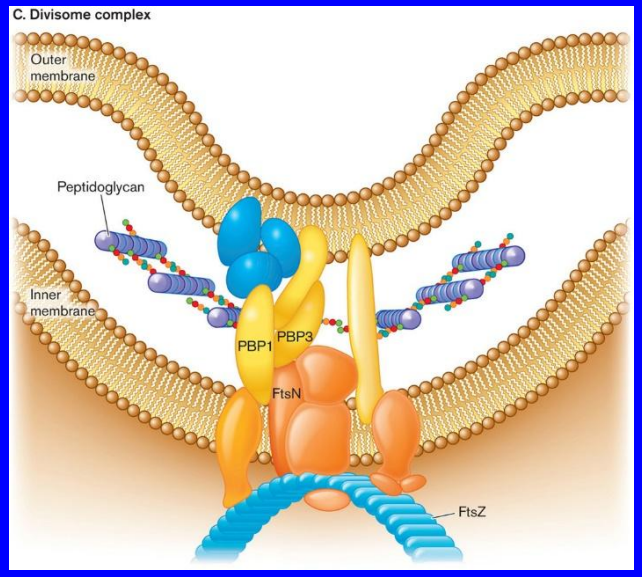


Fig 2.7 White Physiology ar Biochem. of Prokaryotes

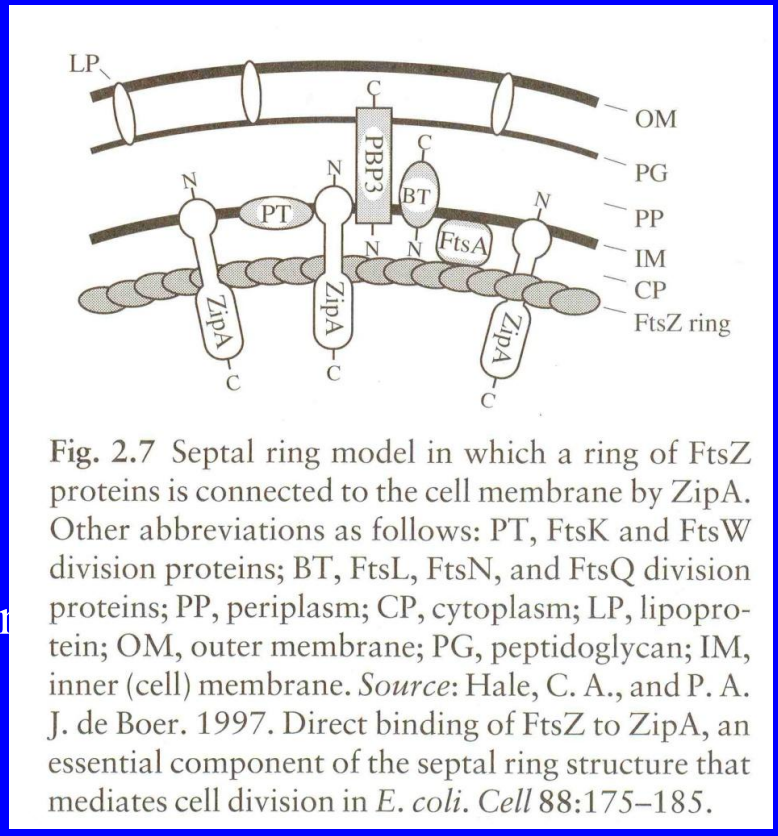


Fig. 2.7 Septal ring model in which a ring of FtsZ proteins is connected to the cell membrane by ZipA. Other abbreviations as follows: PT, FtsK and FtsW division proteins; BT, FtsL, FtsN, and FtsQ division proteins; PP, periplasm; CP, cytoplasm; LP, lipoprotein; OM, outer membrane; PG, peptidoglycan; 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.

5. Cell division Proteins - 5

e. Min proteins

- MinC, MinD, and MinE
- Important for making sure septum formation and cell division occurs in middle of cells
- Mutants form minicells – septation and cytokinesis occurs near one end instead of in middle

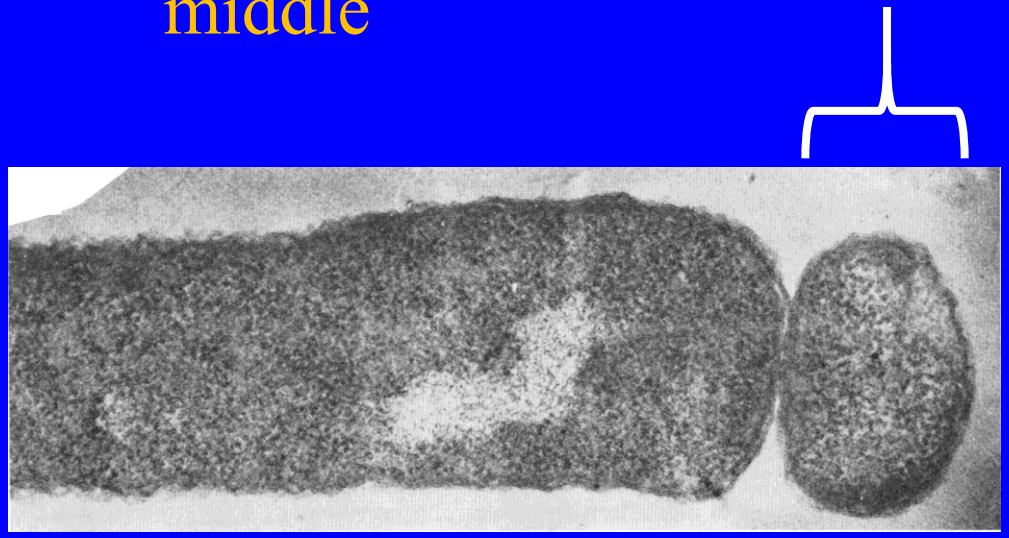


Fig 9.9 Microbe

5. Cell division Proteins - 6

e. MinC, MinD, and MinE –

In E. coli

- MinCD is found in complex along inside of membrane
- MinE is found near center of cell

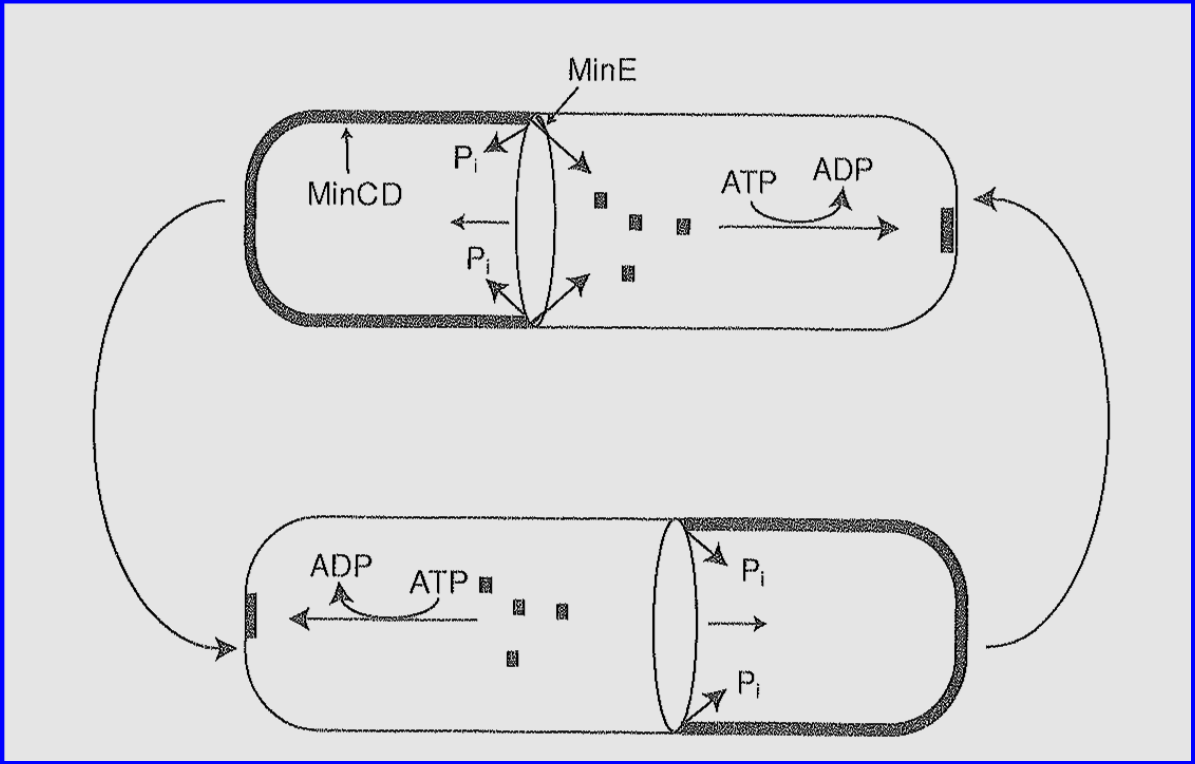
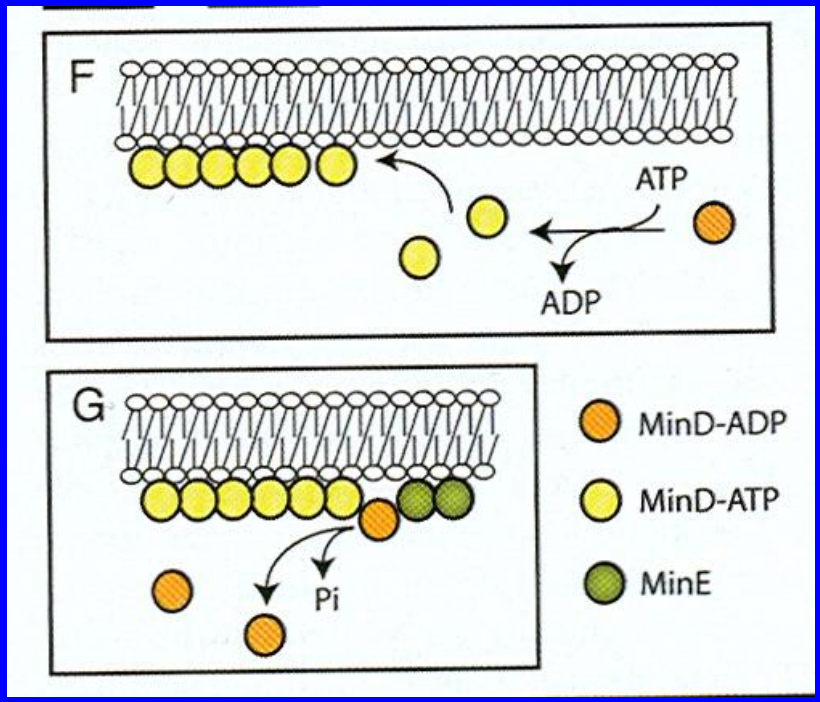


Fig 2.9 White

e. Min Proteins

1) MinD –
   - MinD-ATP (yellow balls) forms dynamic helical filaments on inner surface of membrane
2) MinE
   - Stimulates ATP hydrolysis by MinD and disassemble of MinD filaments

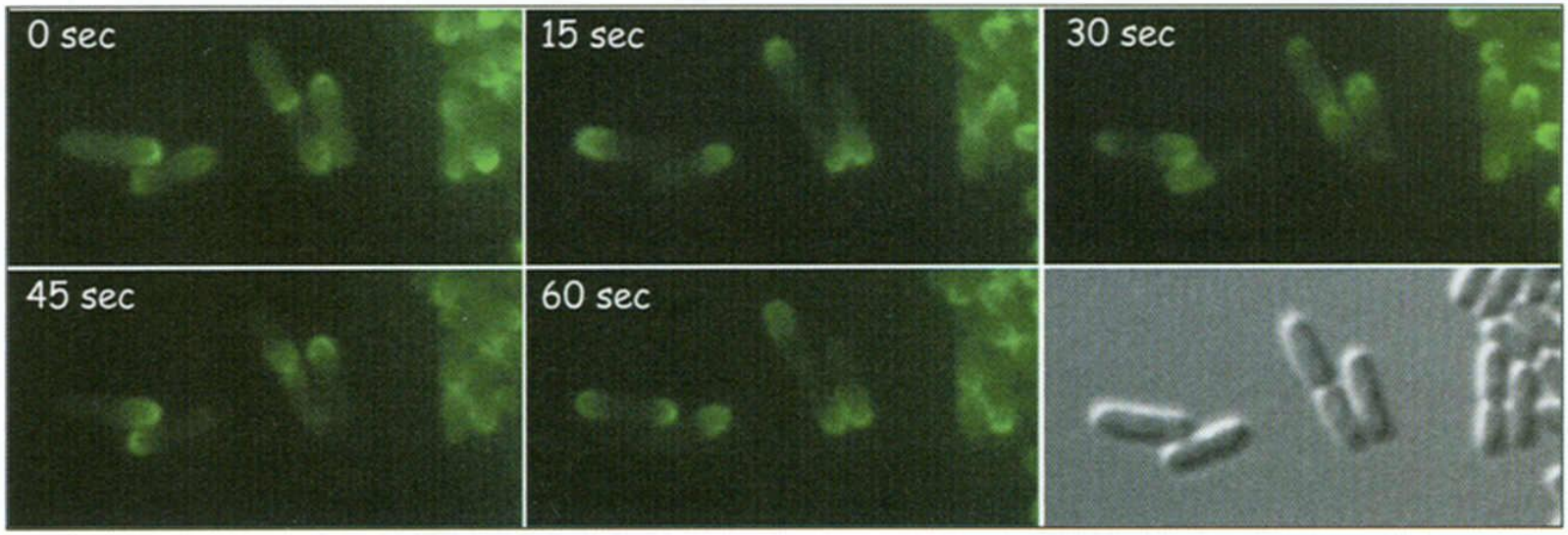


Shih and Rothfield. 2006. MMBR 70:729-754

e. Min Proteins - 2

- Localization of GFP-labeled MinD in growing cells from Margolin. 2008. Microbe 3:329-336
-

FIGURE 3



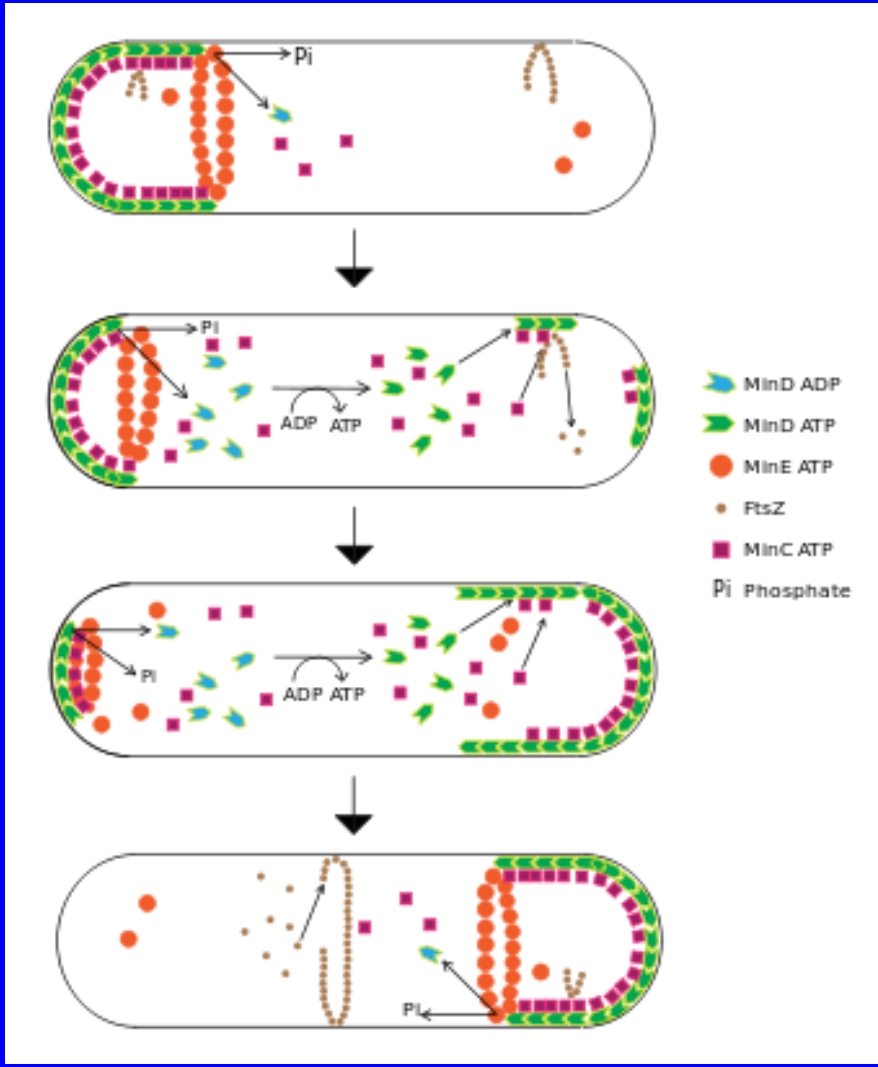
Dynamic assembly of the MinD protein helps to define the division plane. Shown is a time-lapse experiment with a field of *E. coli* cells expressing *gfp-minD*, thus permitting direct visualization of periodic MinD protein localization by fluorescence microscopy. Fluorescence images were captured at 15-second intervals. The last image was taken with differential interference contrast. Note the two separate MinD oscillating systems already in place in the central dividing cell.

e. Min Proteins - 3

- Animation of Min Protein movement
- From paper: James C. Walsh et al (2015), PLoS ONE

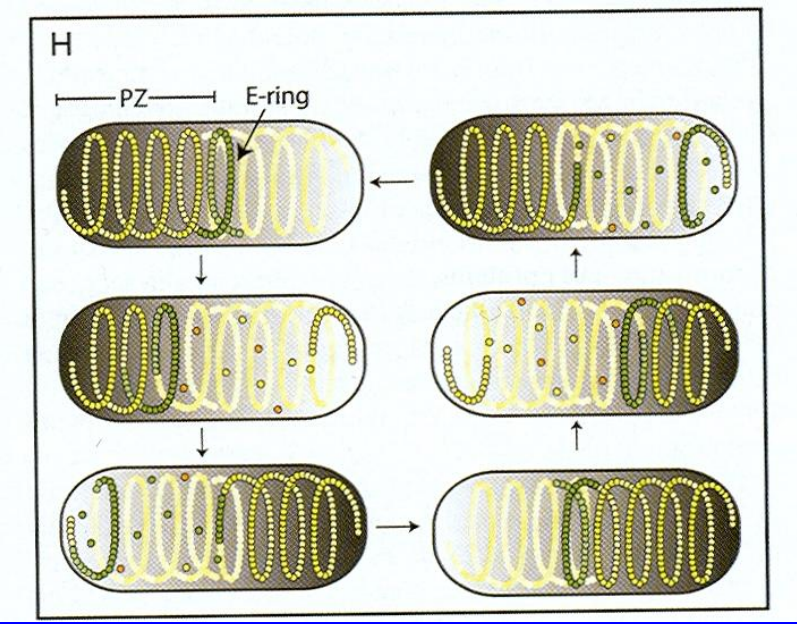
6) What is the movement of MinC and D?

- Oscillates from one pole to other pole



e. Min Proteins - 4

3) MinC
   - Associates with MinD
   - Inhibitor of FtsZ polymerization
   - MinC found at poles but not at the center of the cell
   - Inhibits FtsZ ring formation at poles
   - FtsZ rings can only form in the center of the cell



Shih and Rothfield. 2006. MMBR 70:729-754

Lecture 21 Question

7) What methods were used to study the role of cell division proteins?
   - Looked at the effect of Mutations in the genes on cell division
   - Determined where located in cells using fluorescent microscopy

Material related to Questions on Assignment 12

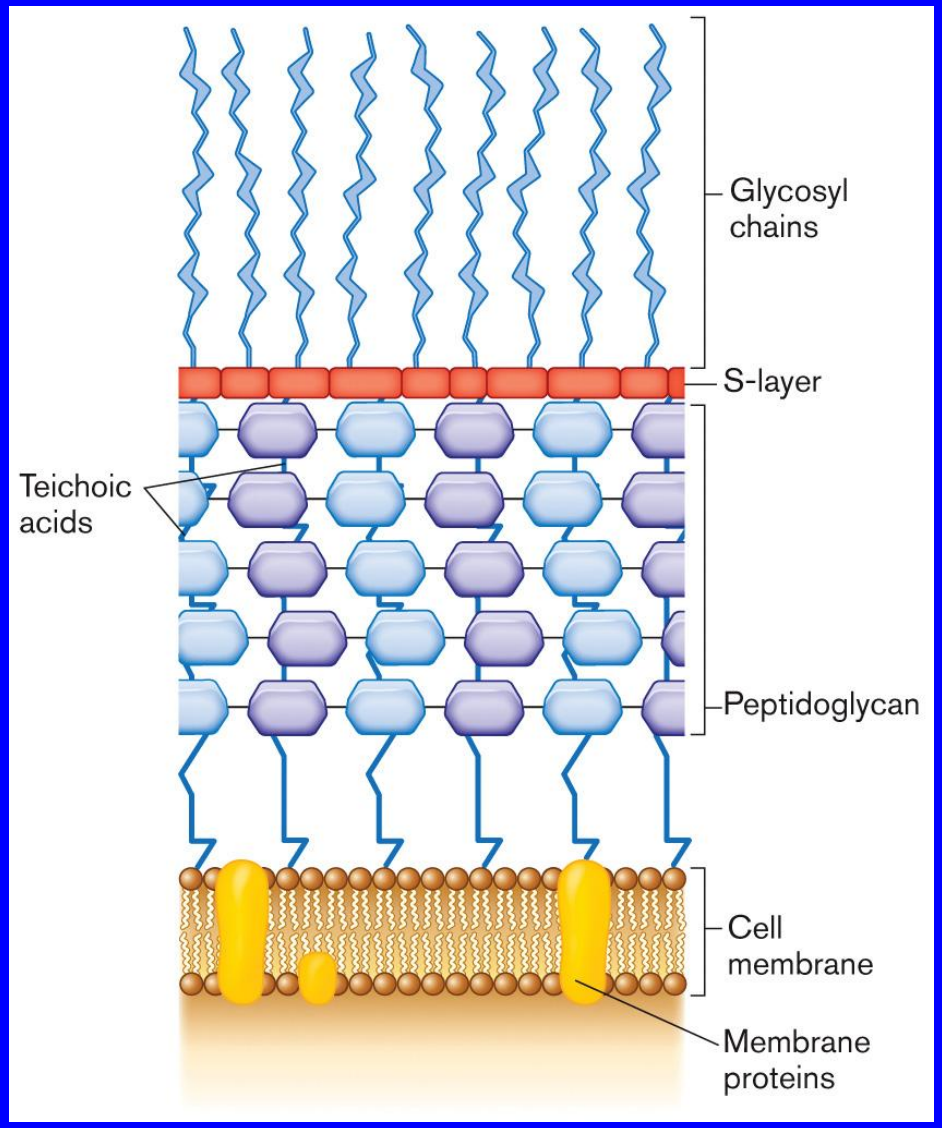
Assignment 12

1. What layers are found in envelope of each of the following types of bacteria?

   What type of molecules are found in each layer?

   a. Gram positive
   b. Mycobacterial
   c. Gram-negative
   d. Mollicutes - *Mycoplasma*
   e. Archaeal

a. Gram-Positive Cell Envelope

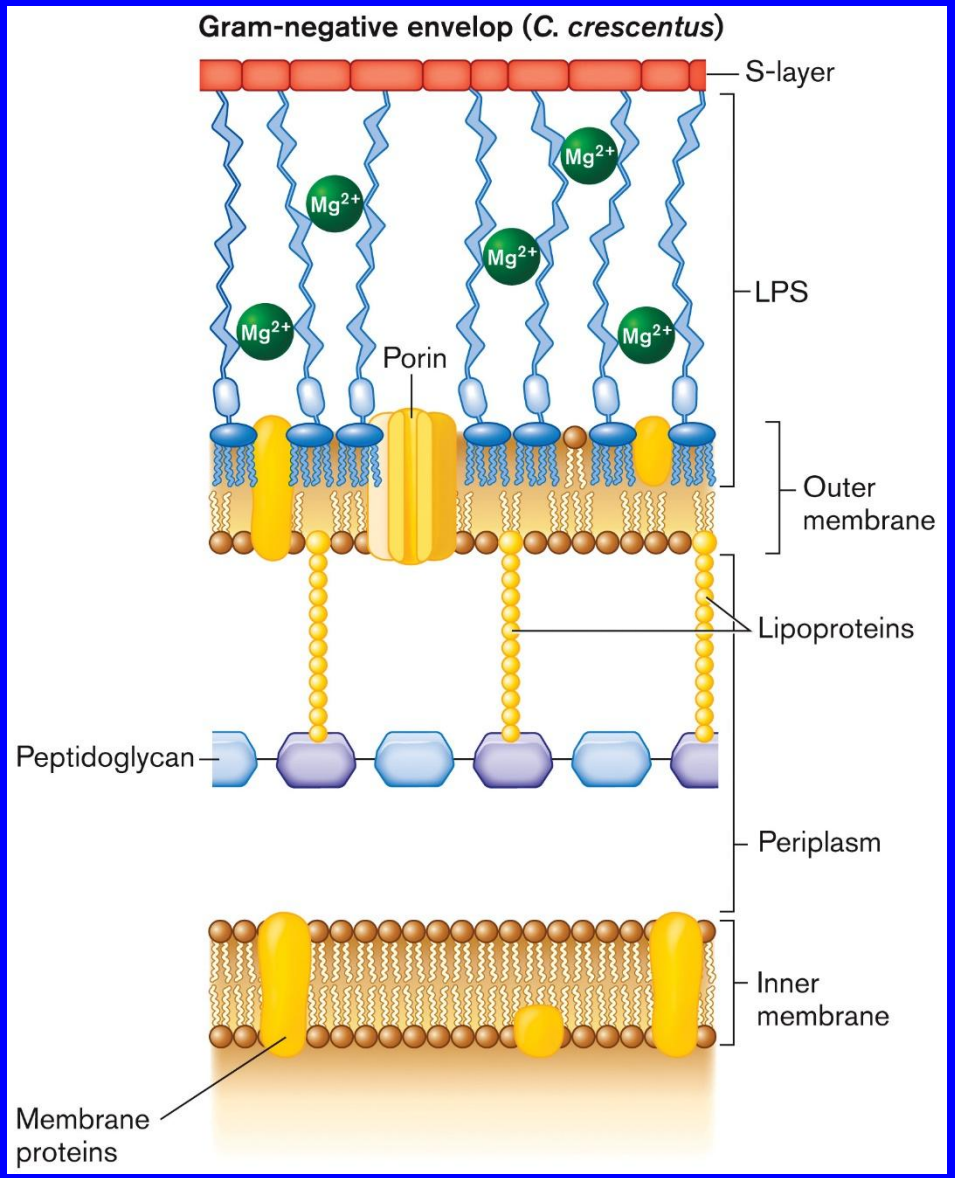


Layers and Components Present

- Capsule (some) – sugar or proteins
- Glycosyl (sugar chains) – part of capsule (some?)
- S layer (all?): protein
- Cell wall (all): Thick layer of peptidoglycan (all) and Teichoic acid (some)
- Cell Membrane (all) with phospholipids, proteins, other lipids

Figure 3.15 6th edition

b. Gram-Negative Envelope



Layers and Components present

- S layer (some): protein
- Capsule (some): sugar
- Outermembrane (all): LPS, proteins, phospho-lipids,
- Cells wall (all): Thin layer of peptidoglycan
- Periplasm (all)
- Cell or inner Membrane (all): proteins and phospholipids
-

Figure 3.15
6th edition

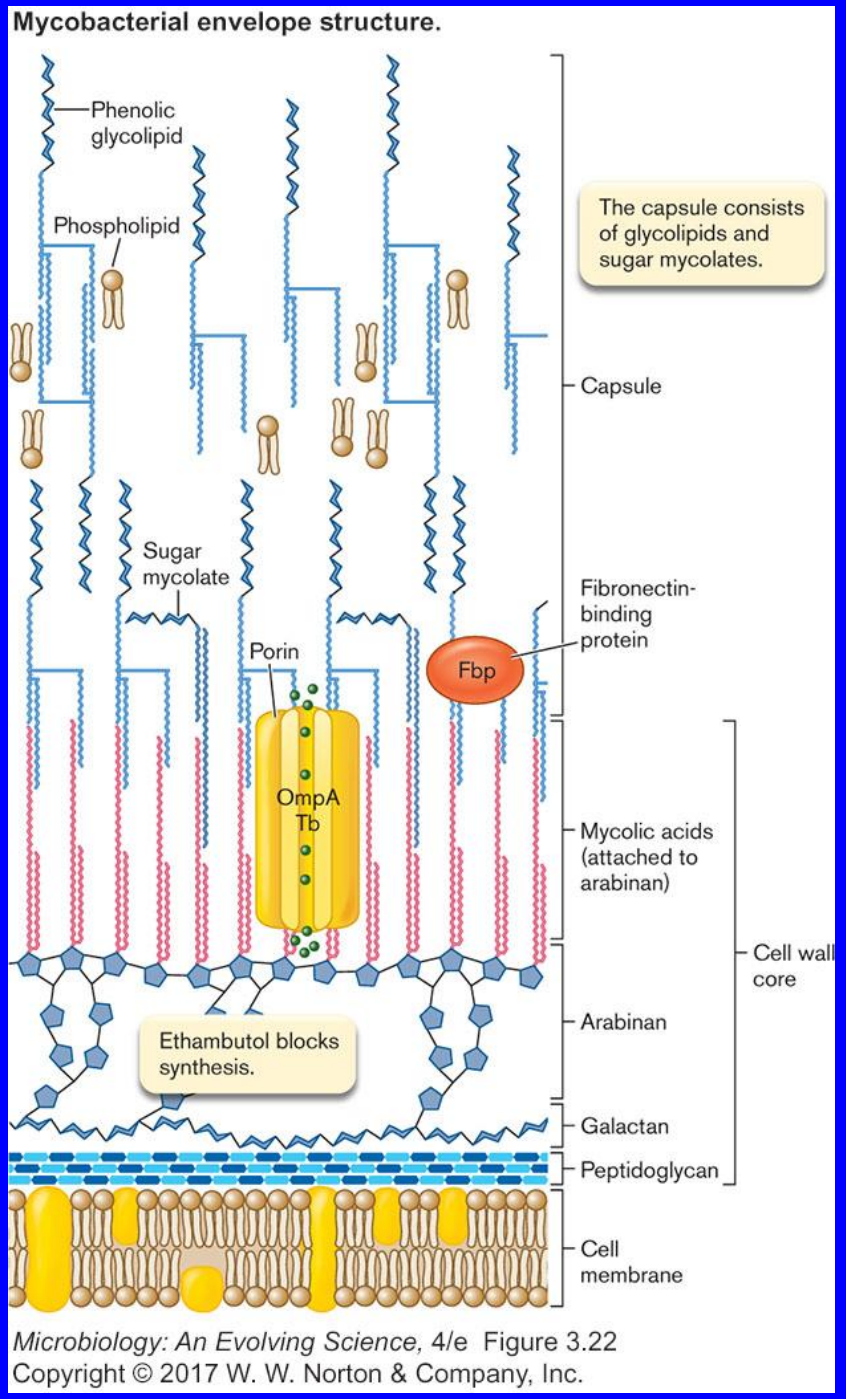
b. Mycobacterial Envelope

- Gram positive actinomycete

Layers and Components present

- Capsule (all?) – sugars and glycolipids
- Cell wall (all): Mycolic acids, Arabinan-galactan (sugars), Peptidoglycan
- Cell membrane (all) with phospholipids and proteins

Figure 3.21A
6th edition



d. Envelopes of Mollicutes - *Mycoplasma*

Layers and Components

- Sugar layer = Capsule? (all)
- Cell membrane (all): proteins, phospholipids, sterols
- No cell wall

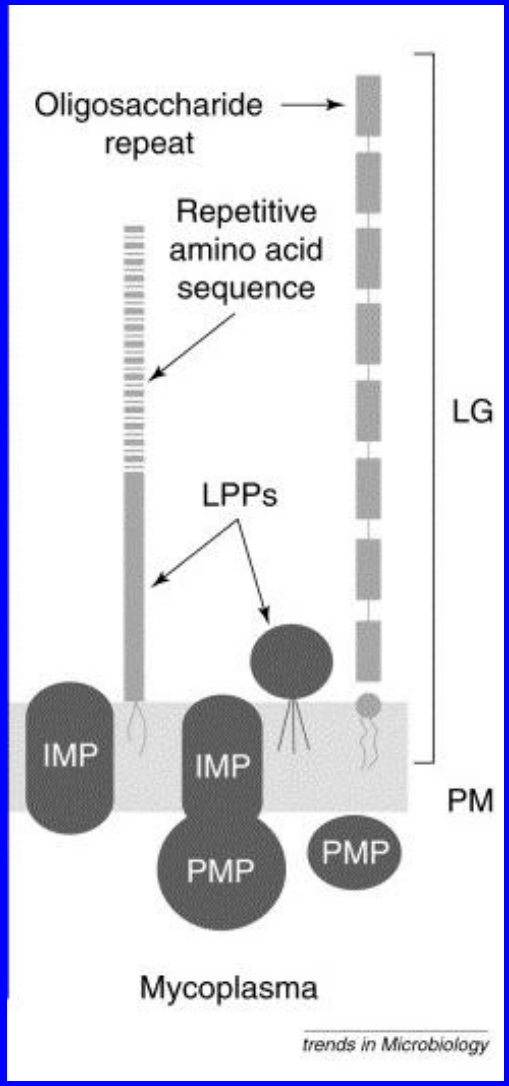
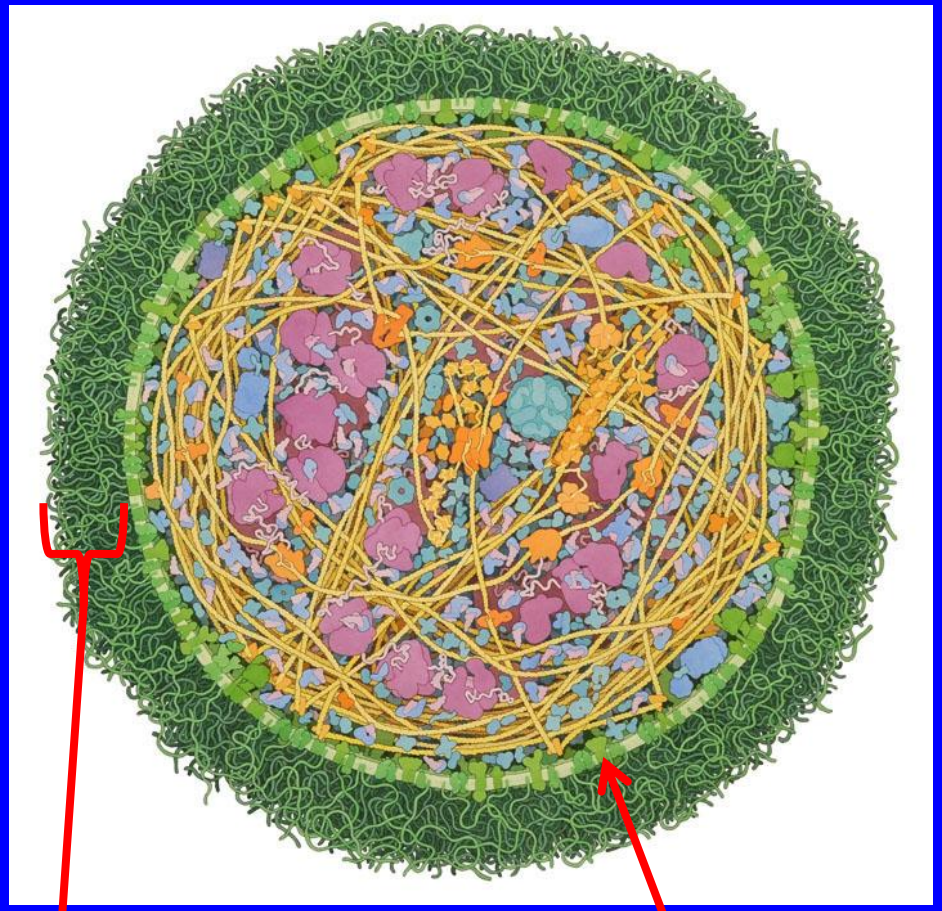


Figure Legend: LG = lipid-glycans?, LPPs = lipoproteins, PM = plasma membrane, IMP= intermembrane protein, PMP = peripheral membrane protein,

d. Envelopes of Mollicutes – *Mycoplasma* - 2

Components

1. Lipolgycans (sugar chain linked to lipids or proteins)
2. Phospholipid cell membrane with sterols and proteins



Lipoglycans – sugars linked to lipids or proteins

Cell membrane with proteins

d. Mollicutes - Mycoplasma

- No cell wall
- Membrane contains sterols and proteins - sterols help stabilize
- Sugar chains (glycans) on outside linked to lipids or proteins
- Have a variable shape - pleomorphic
- Parasites or commensals –

  Live in hosts
- Can grow in lab under right conditions


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Figure showing pleomorphic (variable) shape of Mycoplasma

e. Envelopes of Archaea

Layers and Components Observed

1. S layer (some-all): protein
2. Cell wall (some): pseudopeptidoglycan
3. Cell membrane with isoprenoids (no phospholipids) and proteins

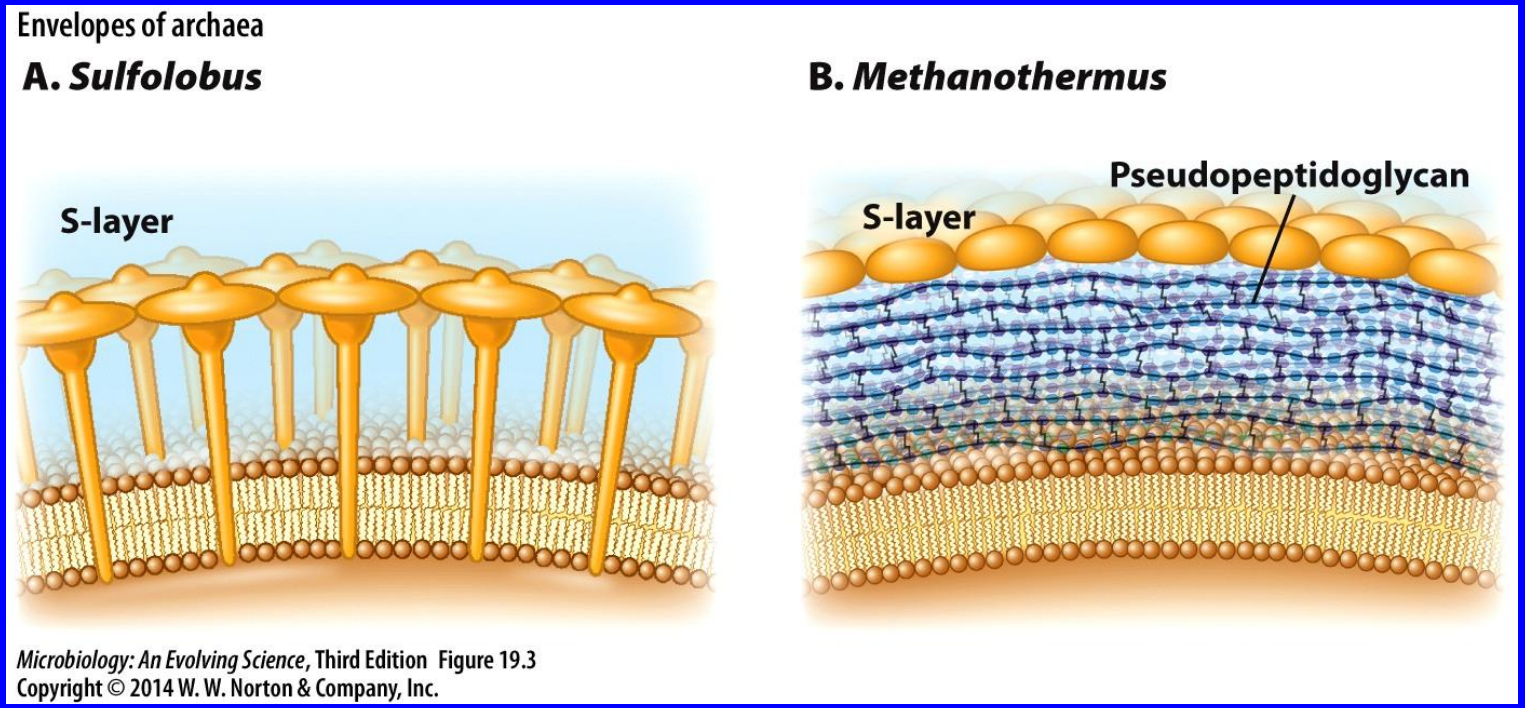


Figure 19.3 3rd edition

Assignment 12 Answers - 2

2. Beside a Gram or acid-fast stain, list two other methods one could use to determine or predict the nature of the envelope of a newly isolated bacterium?

- Selective media like MacConkey agar
- Electron microscopy
- Biochemical Isolation of unique components found in a specific type of envelope
- Find genes encoding components or enzymes for synthesis of components of an envelope layers (outer membrane proteins and LPS for Gm negative, teichoic acids for Gm positive, mycolic acids for Mycobacterium, pseudopeptidoglycan for Archaea)

Assignment 12 Answers - 3

3. What is the mechanism of action of each
   a. Penicillin (β-lactam) family - inhibits transpeptidase (peptide cross linking) and transglycosation -
   b. Cephalosporins - inhibit transpeptidase and transglycolysation
   c. Bacitracin – recycling of bactoprenol
   d. D-cycloserine – synthesis of D-alanine
   e. Vancomycin – transpeptidase
   f. Lysozyme– cleave peptidoglycan

a) Peptidoglycan Synthesis

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

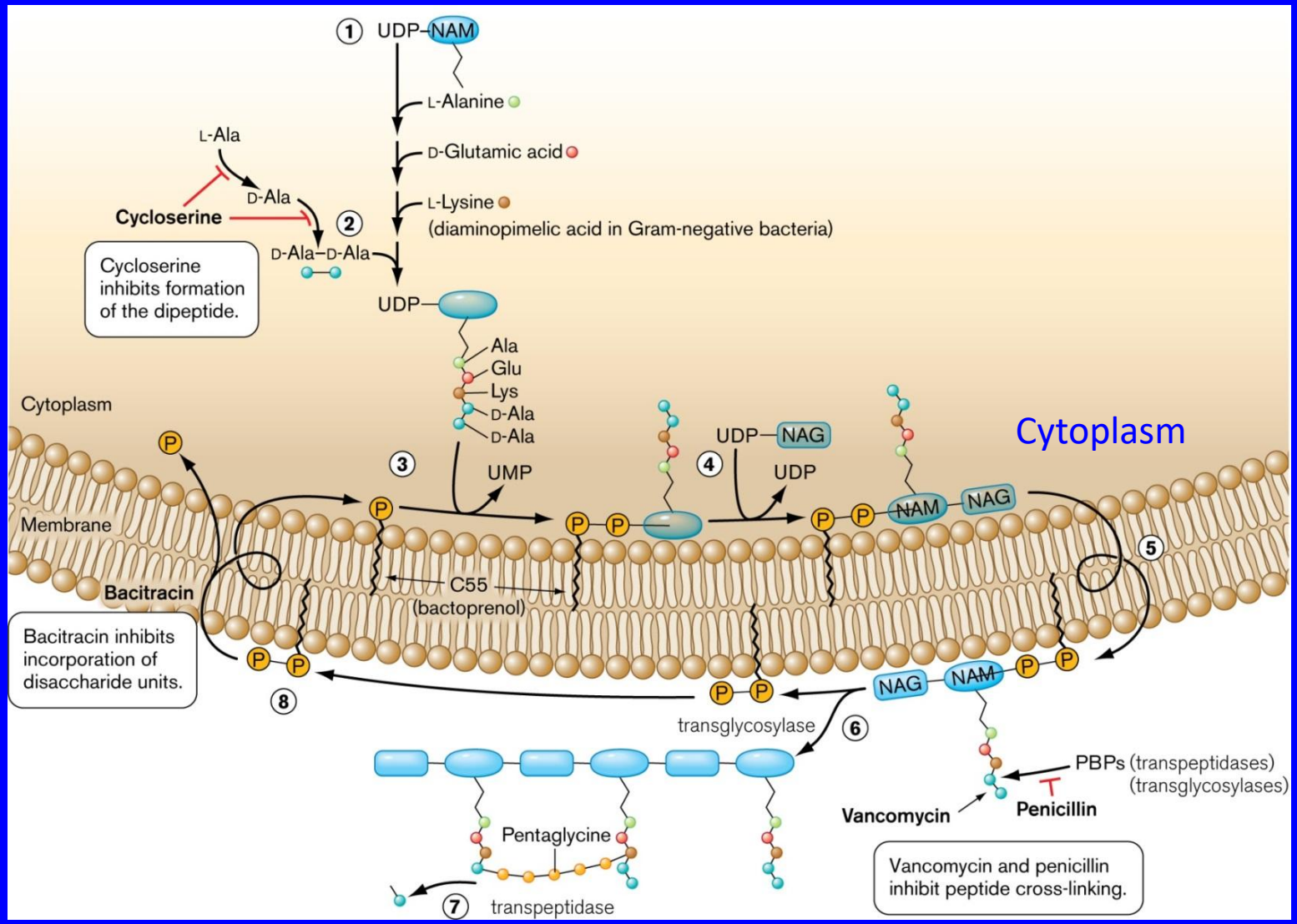


Figure 27.7 of 3rd edition

a) Peptidoglycan Synthesis - 5

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

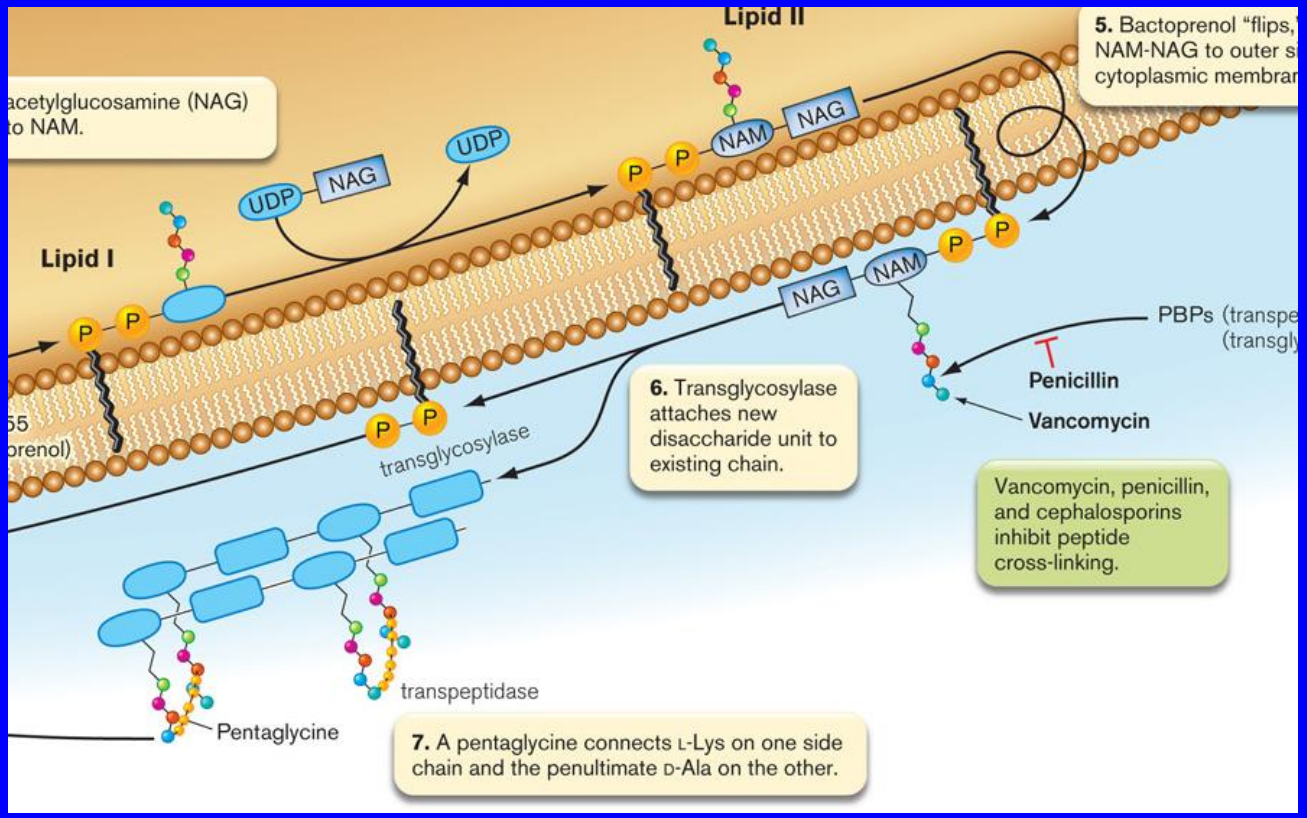


Figure 27.8B
6th ediition

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

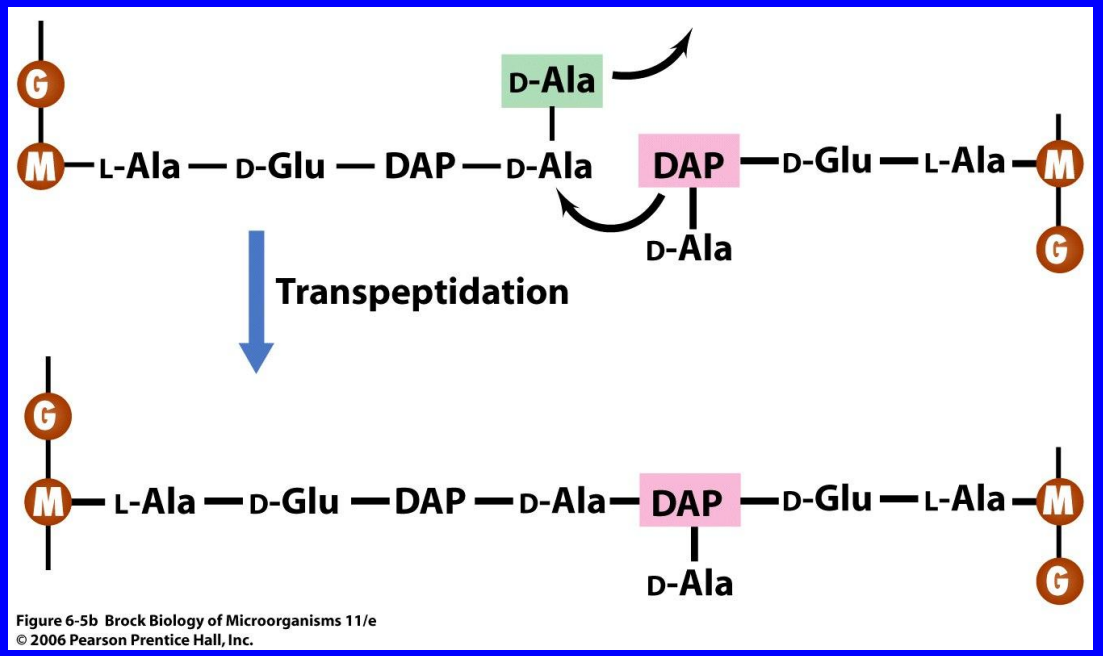


Figure 6-5b Brock Biology of Microorganisms 11/e
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a. Penicillin (β-lactam) family

- Structure similar to D-Ala-D-Ala that is cleaved in transpeptidase reaction.
- Bind to penicillin binding proteins that do transpeptidase and transglycosylation

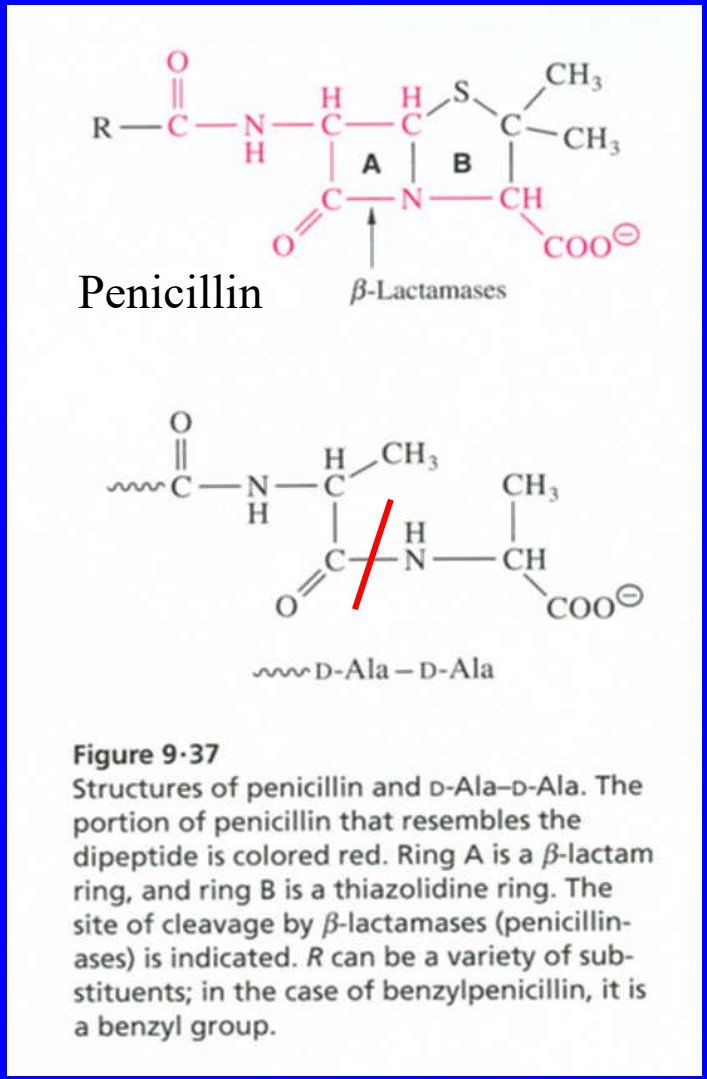
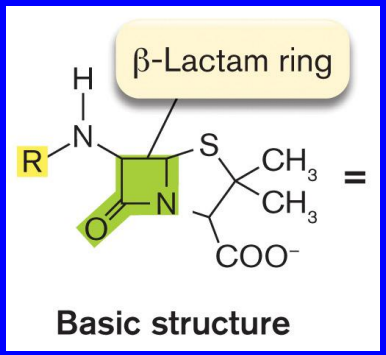
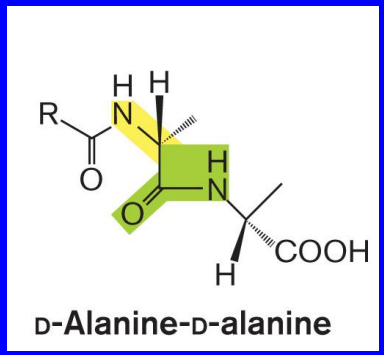


Figure 9·37
Structures of penicillin and D-Ala–D-Ala. The portion of penicillin that resembles the dipeptide is colored red. Ring A is a β-lactam ring, and ring B is a thiazolidine ring. The site of cleavage by β-lactamases (penicillinases) is indicated. *R* can be a variety of substituents; in the case of benzylpenicillin, it is a benzyl group.

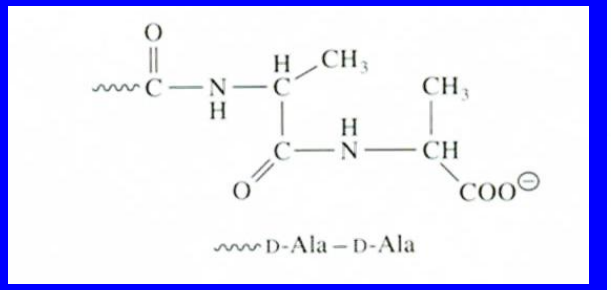
Figure 27.9A 6th edition Penicillin

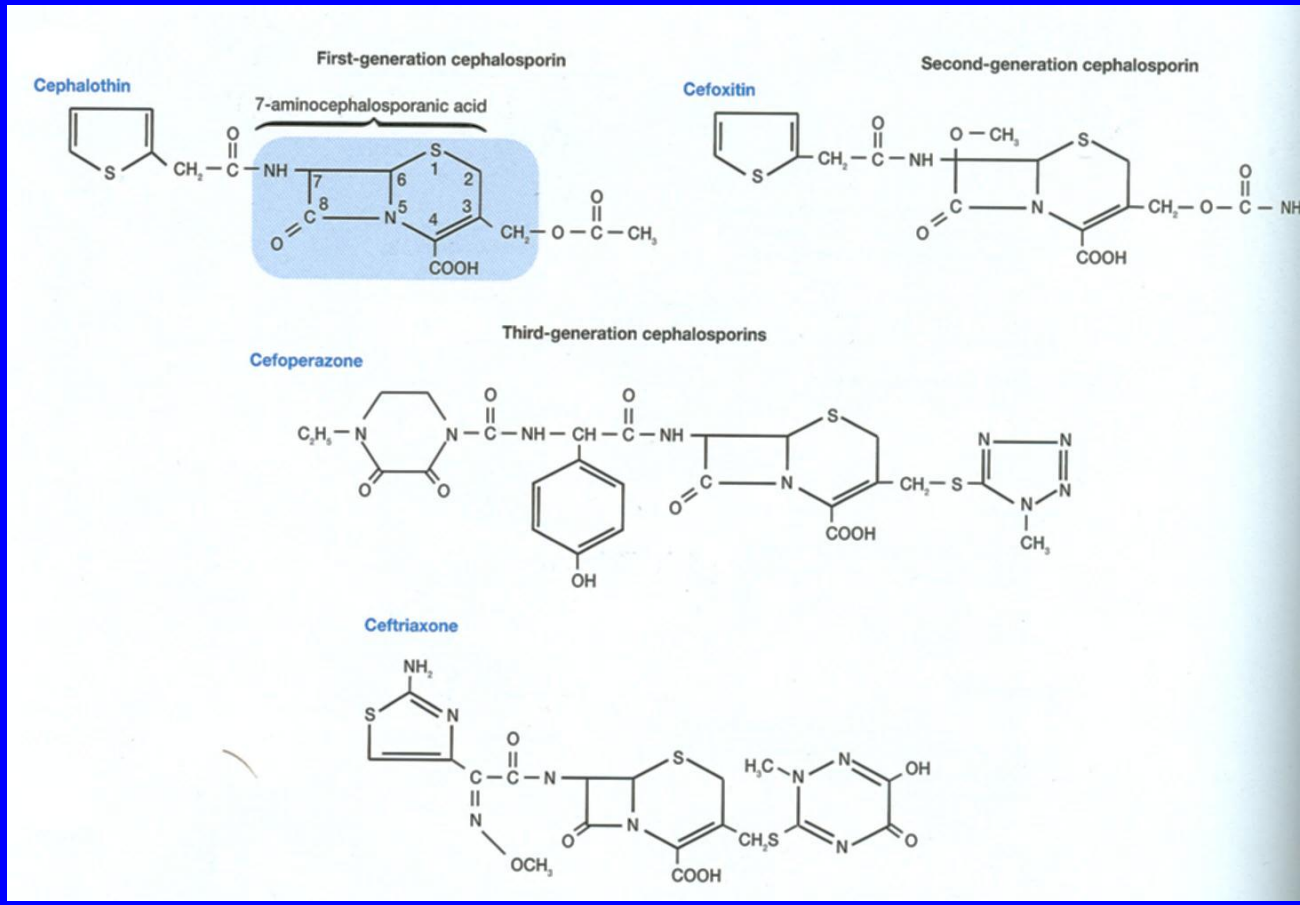




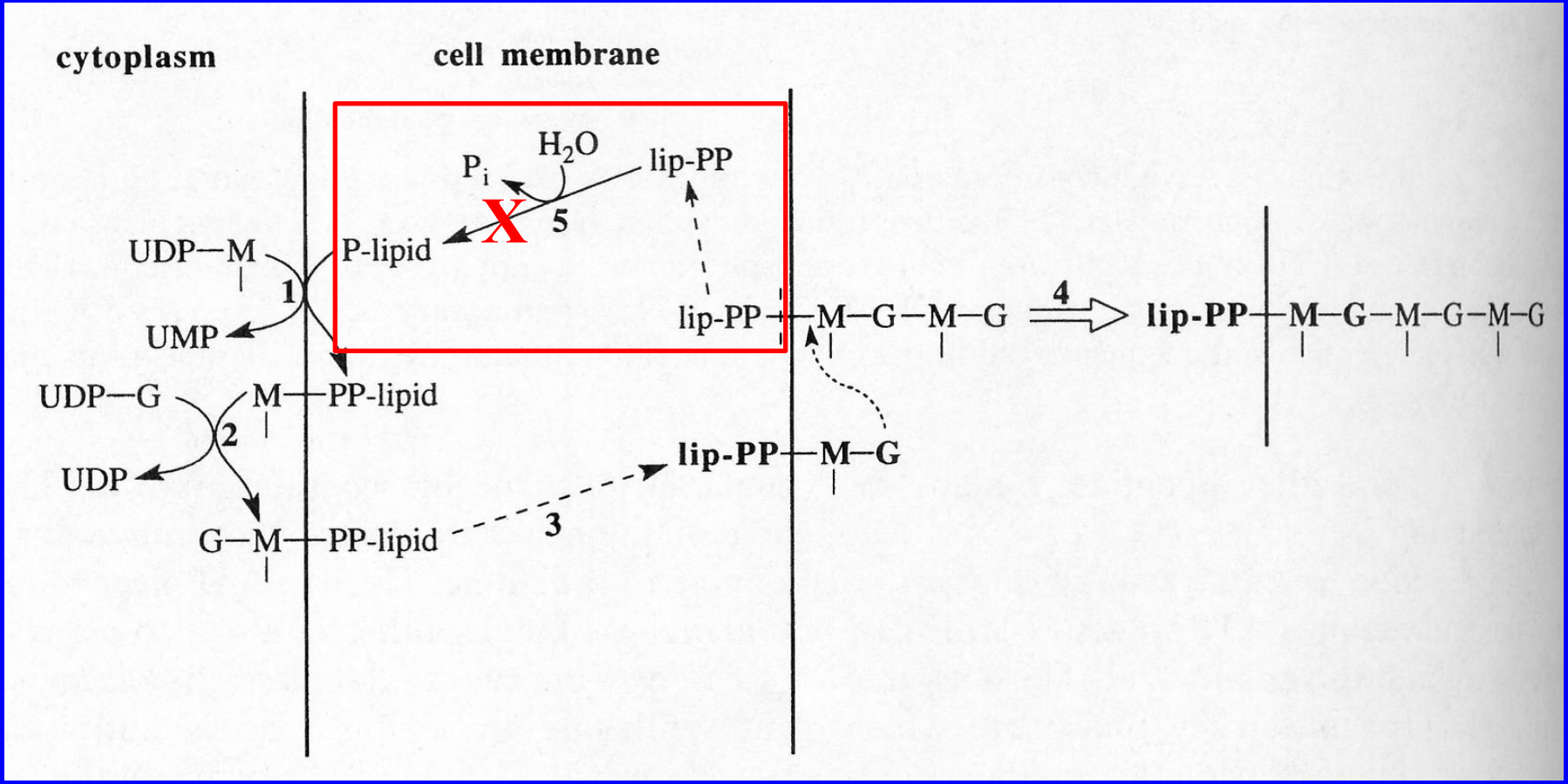
b. Cephalosporins

- Structure is also similar to D-Ala-D-Ala in peptide that is cleaved in transpeptidase reaction





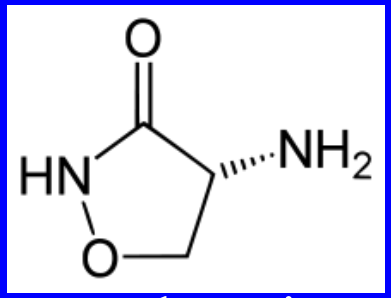
c. Bacitracin



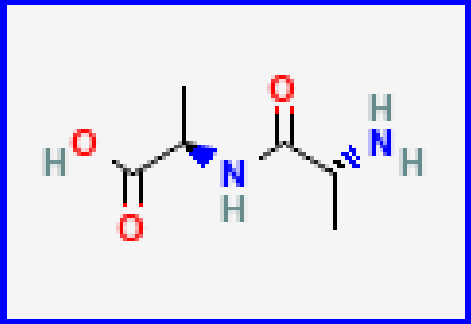
- Inhibits hydrolysis of phosphodiester on bactoprenol-PP (lipid-PP in figure) and thus regeneration of bactoprenol-P (lipid-P)

d. D-cycloserine

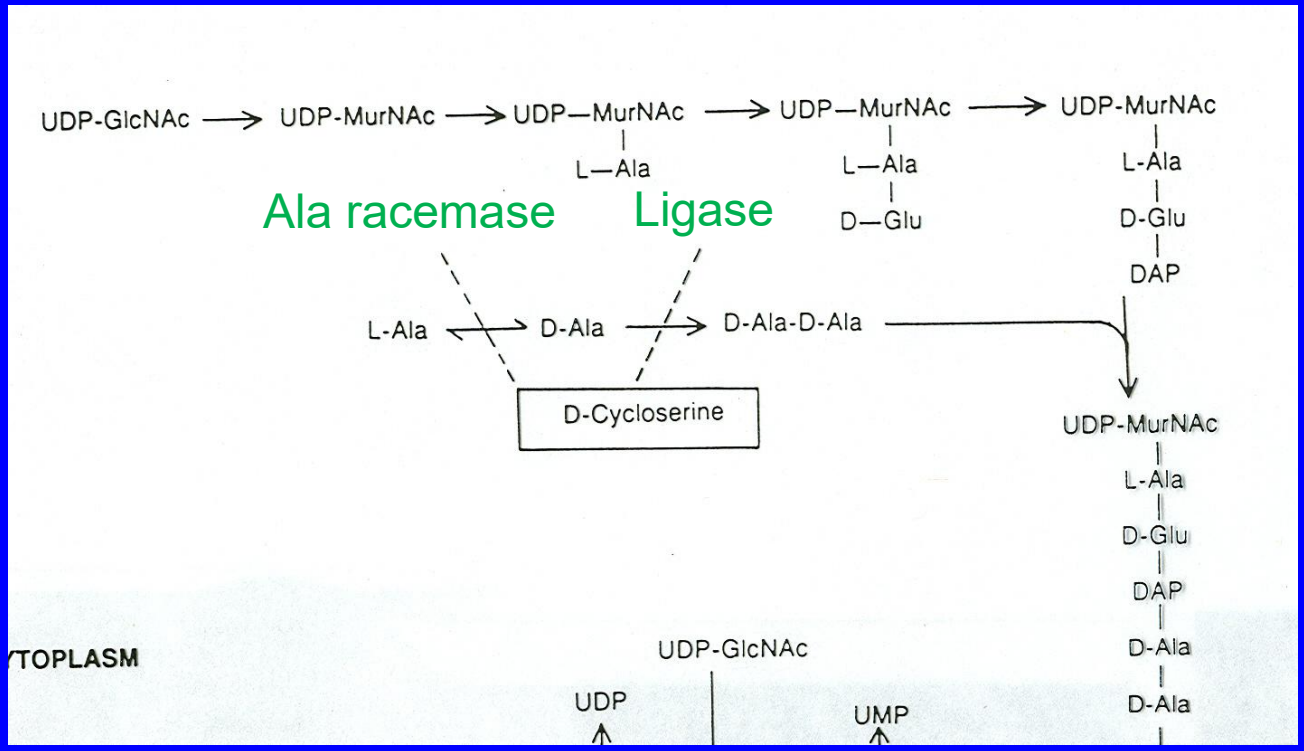
- Competitive inhibitor of Ala racemase and ligase
- Structure similar to ligated D-Alanine-D-alanine



D-cycloserine

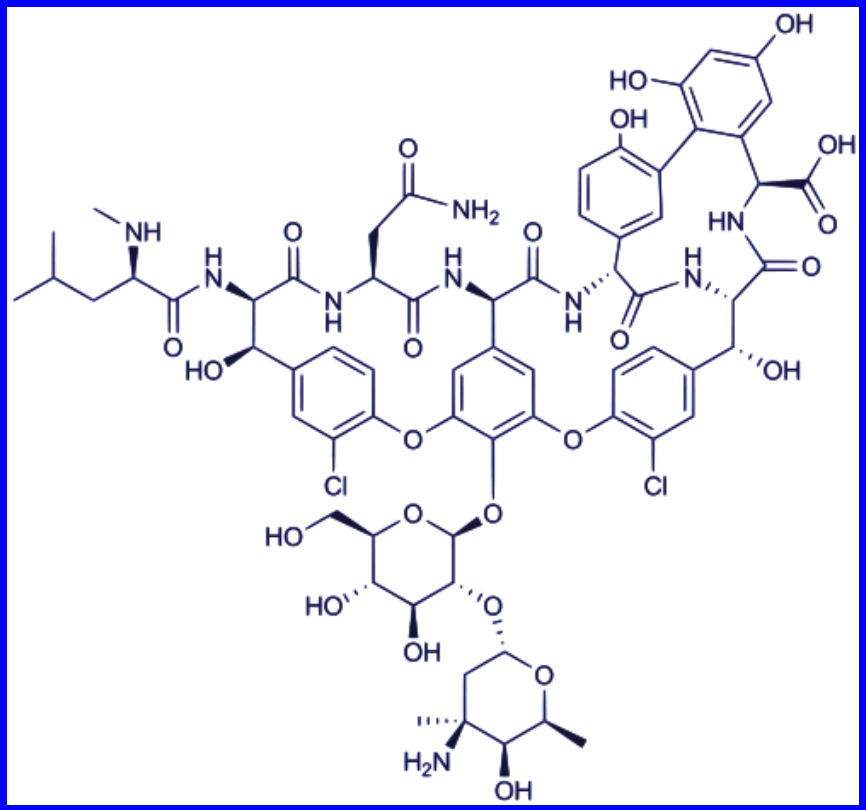


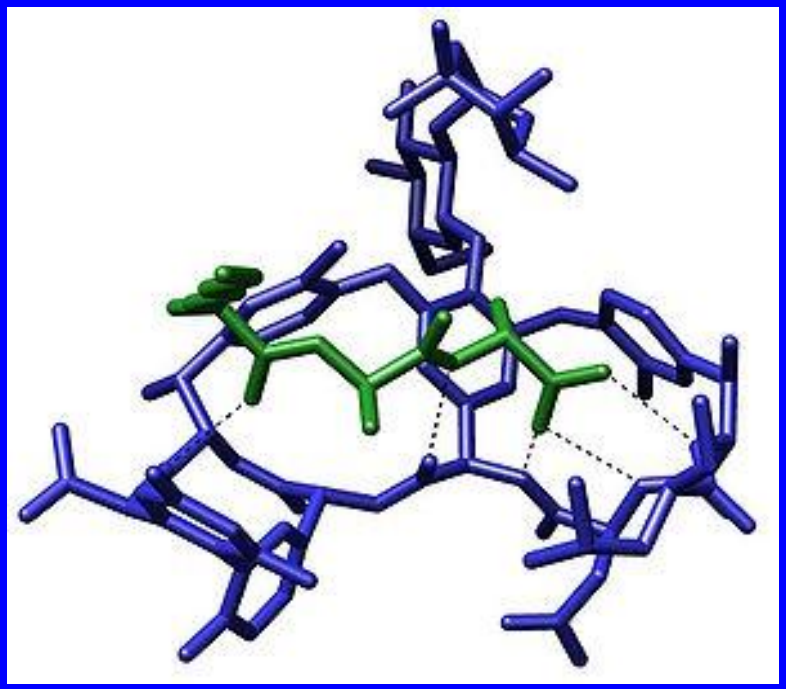
D-Ala-D-Ala



e. Vancomycin

- Large glycopeptide that binds to pentapeptide and prevents cleavage and transpeptide linkage





Blue: Vancomycin
Green: L-Lys-D-Ala-D-Ala peptide

f. Lysozyme

Breaks glycosidic bond between sugars M and G and thus hole in wall will appear

1. Lysis in low solute solution

2. Protoplast (Gm+) or spheroplast (Gm-) in isotonic solution

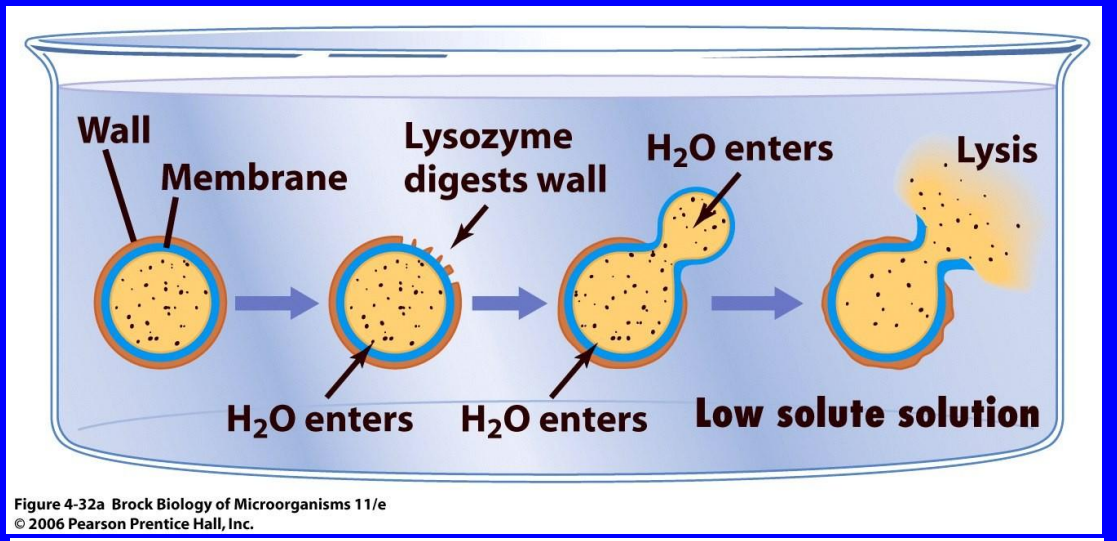


Figure 4-32a Brock Biology of Microorganisms 11/e
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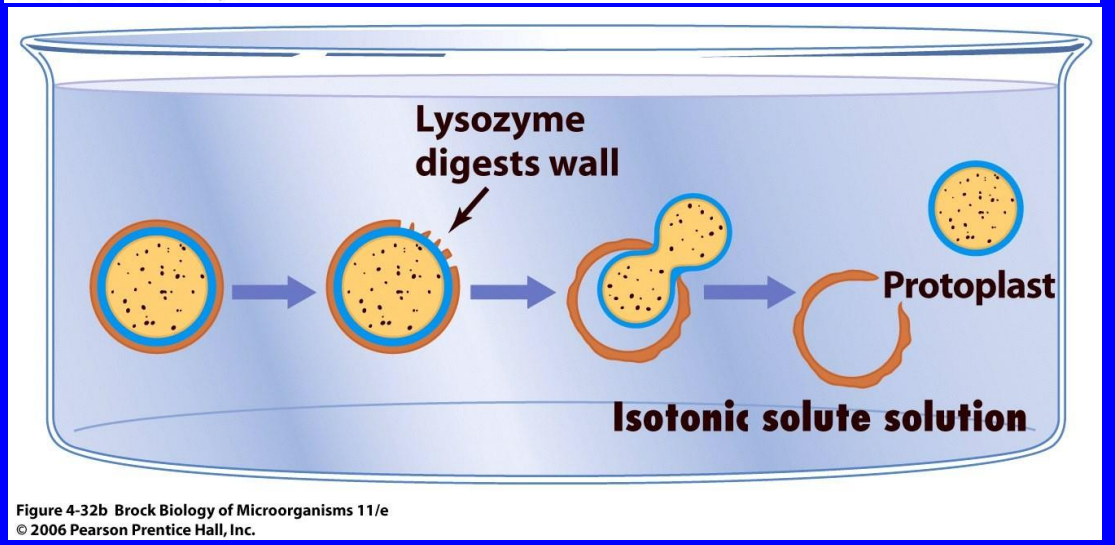


Figure 4-32b Brock Biology of Microorganisms 11/e
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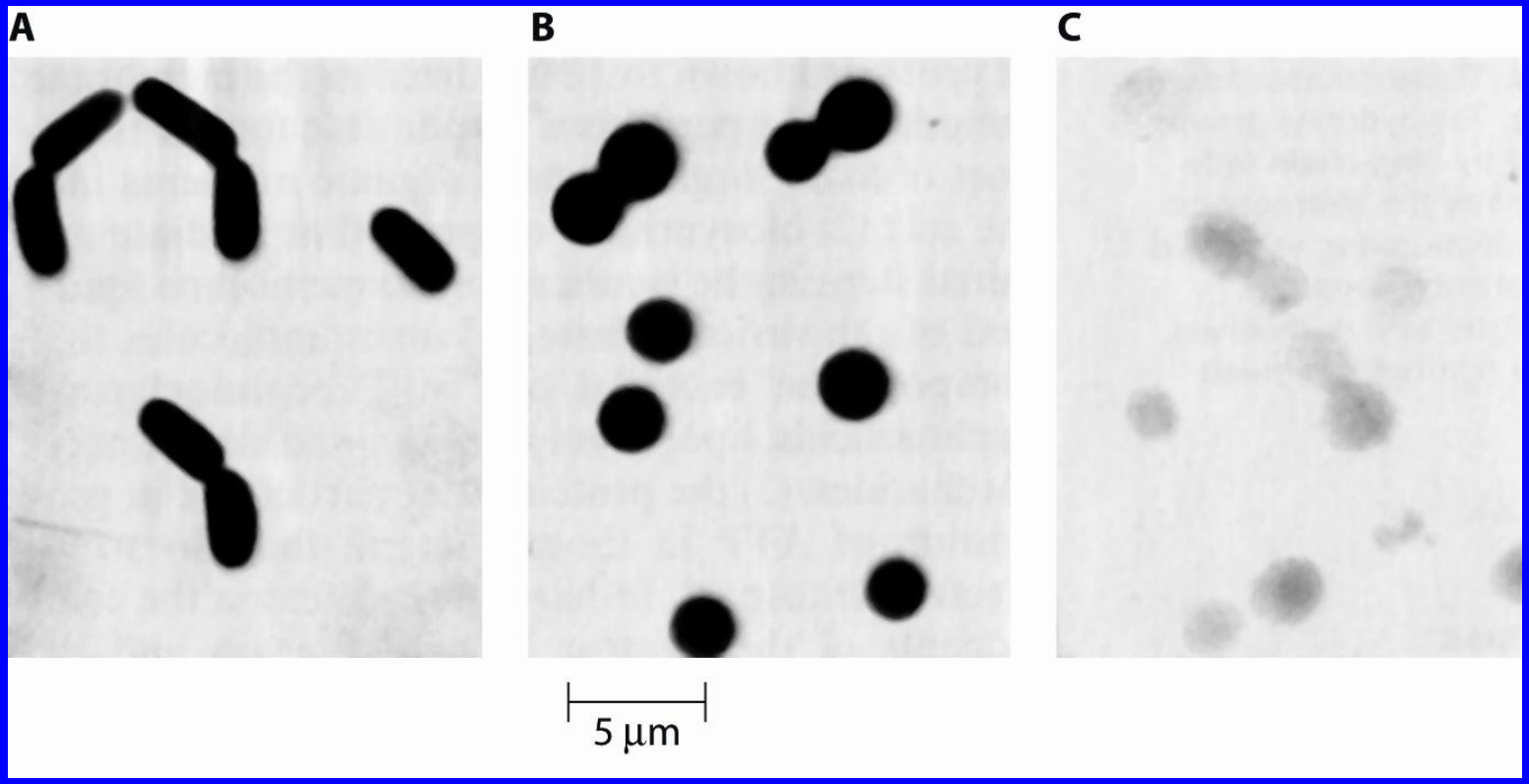
f. Lysosyme Part 2

2. Lysozyme treatment of Gm+ rod

Untreated

Lysozyme and isotonic solution

Lysozyme and low solute solution



Lecture 21 questions

8. Which antibiotics have a region that is similar in structure to D-Alanine-D-alanine?
   - Penicillins
   - Cephalosporins
   - D-cyclserine

Assignment 12 - 4

4. Describe two ways bacterial cells can resist members of the penicillin β-lactam family of antibiotics.
   1. Express and secrete a beta-lactamase which cleaves penicillin
   2. Expression of an altered version of a penicillin binding protein to which penicillin does not bind. Altered version can be due to a mutation of a chromosomal gene or from an acquired gene.

Assignment 12 - 5

5. Some penicillin antibiotics are more effective against Gram-positive bacteria than Gram-negative? Come up with a reason? Hint: where is the peptidoglycan located in Gm neg. bacteria?

Some do not pass through the outer membrane of Gram-negative bacteria and so do not reach where the peptidoglycan synthesis is occurring in periplasmic space in Gram negative bacterial cells.

Gram Negative Outer Membrane

Outer membrane

- LPS tightly packed (only sat. fatty acids)
- Sugars of LPS blocks hydrophobic molecules (dyes, bile salts)
- Membrane blocks diffuse of hydrophilic molecules
- Hydrophilic molecules < 700 daltons can go through porin

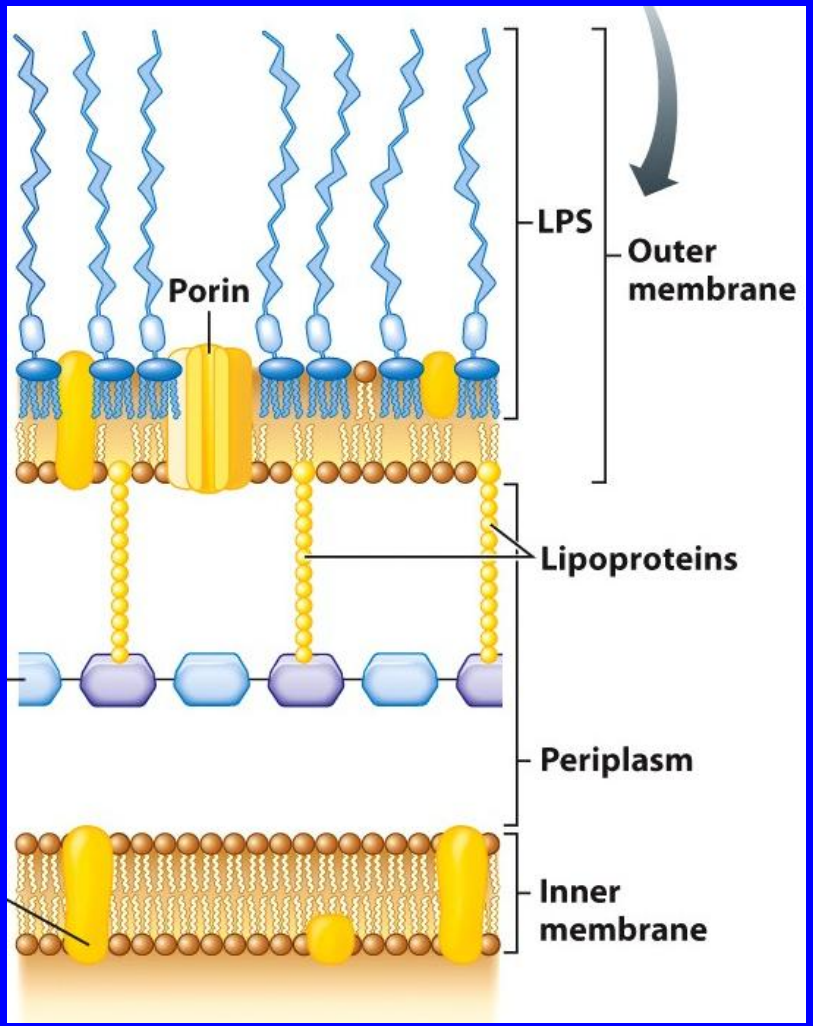


Fig 3.15 Gm-neg Envelope

Assignment 12 - 6

6. Should penicillin be used to treat an infection caused by *Mycoplasma* bacteria (a Mollicute)? Why?

   No, Mycoplasma have no peptidoglycan cell wall

Assignment 12 - 7

7. Why would penicillin only be effective against cells that are actively growing?

- Penicillin inhibits the synthesis of new peptidoglycan and does not damage existing peptidoglycan. Dormant non-growing cell would not need to make much new peptidoglycan and thus would not get holes in their peptidoglycan layer. Actively growing cells would develop need to synthesize new peptidoglycan and if unable then holes in their peptidoglycan layer will develop which would cause the cell to lyse.

Ampicillin

Effect similar to effect of lysozyme

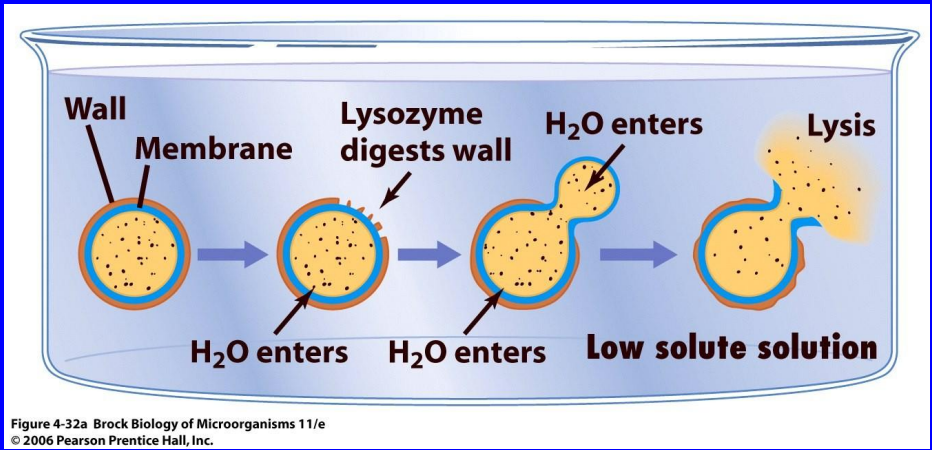
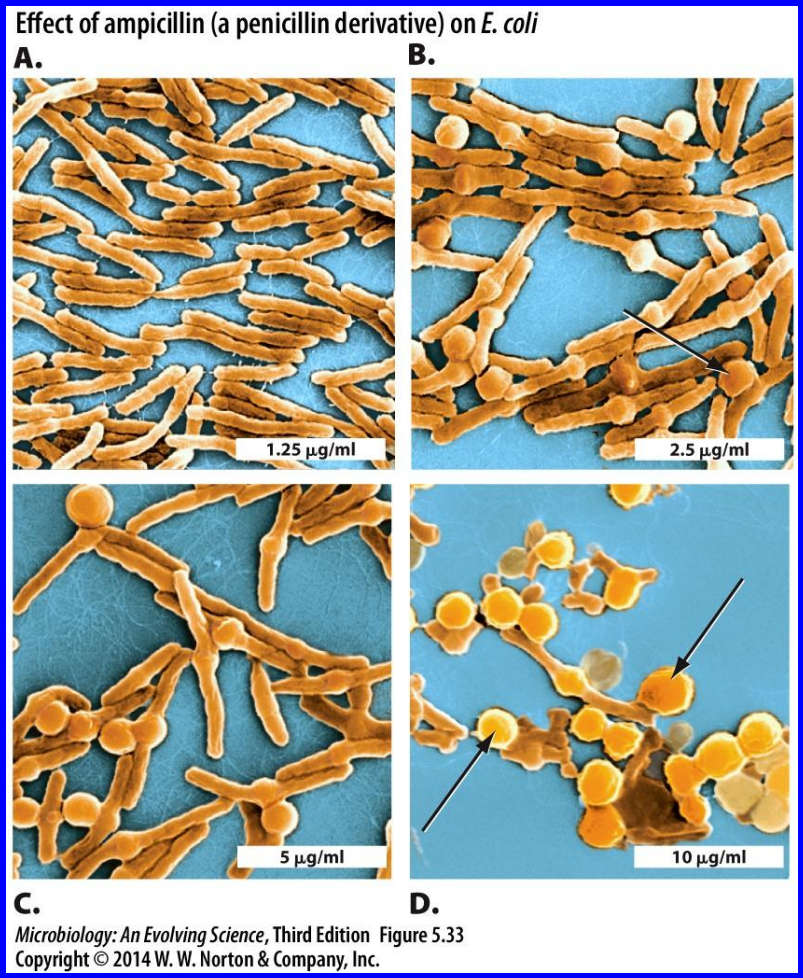


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Causes holes in cell wall which allows membrane to bulge out



Microbiology: An Evolving Science, Third Edition Figure 5.33
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Assignment 12 - 8

8. Most antibiotics are only bacteriostatic *i.e.* they only inhibit or stop the growth of bacterial cells. Antibiotics that interfere with peptidoglycan synthesis such as penicillin are bactericidal or can lead to the death of bacterial cells if the cells are in hypotonic conditions typically found in the human body or in the lab. Why would the blocking of new peptidoglycan synthesis cause the death of bacterial cells? Hint: what is the role of the cell wall?

The inhibition of the synthesis of new peptidoglycan would eventually lead to holes in the peptidoglycan layer of growing cells which would lead to cell lysis because the membrane would then be able to expand out until it lyses due to water coming in due to osmosis.

Drug may also activate bacterial enzyme autolysin that cleaves peptidoglycan