BIOL 4640 Study Guide for Exam 2 (Wed 10/28/26): Lecture 21 Cell Polarity, Division Proteins and Envelope Review

Source: Dr. Rowen's lecture slides, in the order of the 2025 Exam 2 study guide outline.
Slide blanks still empty in the notes are filled from the textbook and marked "textbook".
Tags like [L16 p08] give the source page of each line.
Textbook to read (Slonczewski 6e, Textbook page on the site): 3.4 Bacterial Cell Division and 3.5 Cell Asymmetry, Membrane Vesicles, and Extensions; the posted review article on cell division proteins. **Also useful:** 3.3 The Envelope and Cytoskeleton and 27.2 Antibiotic Mechanisms of Action (the envelope and cell wall antibiotic review).
(teal italics) = plain meaning of the word before it. ⚠ **Do not mix up = a look-alike pair to keep apart.**

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C. Cell envelope (glycocalyx, outer membrane, cell wall, and cell membrane)

Book: 3.3 The Envelope and Cytoskeleton, Cell Envelope of Bacteria (chapter 3 PDF, p. 49).

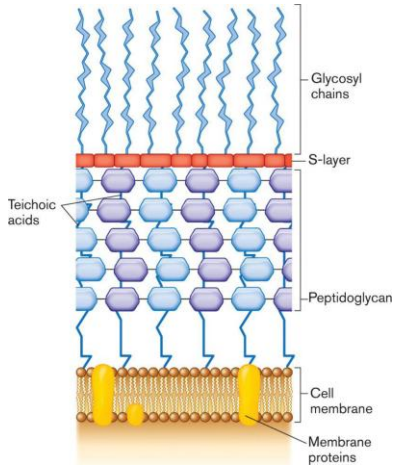
1. Know the layers and components of the envelope in each of the following types of bacteria. How similar and different from each other

Book: 3.3 The Envelope and Cytoskeleton, Cell Envelope of Bacteria (chapter 3 PDF, p. 49).

Envelope *(layers wrapped around the cell)* **types**: **Gram positive**, mycobacterial *(about waxy walled bacteria)*, **Gram negative** *(thin wall plus outer membrane)*, Mollicutes (Mycoplasma), archaeal *(of archaea)*. [L21 p28]

a. Gram positive bacteria

Book: 3.3 The Envelope and Cytoskeleton, Firmicute Cell Envelope: Gram-Positive (chapter 3 PDF, p. 52).



Gram positive envelope: glycosyl chains, S layer, teichoic acids, peptidoglycan and membrane [L21 p29]

On the figure: Glycosyl · teichoic acid · peptidoglycan · cell membrane

Gram positive *(thick wall, stains purple)* **capsule** (some): sugar or proteins. [L21 p29]

Glycosyl (sugar chains): part of the capsule (some?). [L21 p29]

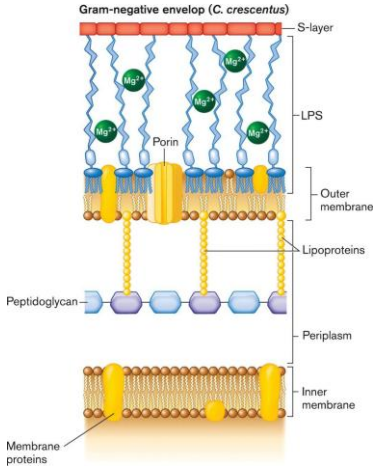
Gram positive S layer (all?): protein. [L21 p29]

Gram positive cell wall (all): thick layer of **peptidoglycan** (all) and **teichoic acid** (some). [L21 p29]

Gram positive cell membrane (all): phospholipids *(membrane fat with a charged head)*, proteins, other lipids. [L21 p29]

b. Gram negative bacteria

Book: 3.3 The Envelope and Cytoskeleton, Proteobacterial Cell Envelope: Gram-Negative (chapter 3 PDF, p. 56).



Gram negative envelope: outer membrane with LPS and porin, periplasm, peptidoglycan, inner membrane [L21 p30]

On the figure: Outer membrane · LPS · peptidoglycan · Periplasm · inner membrane

Gram negative *(thin wall plus outer membrane)* **S layer** (some): protein. [L21 p30]

Gram negative capsule (some): sugar. [L21 p30]

Outer membrane (all): **LPS** *(outer membrane sugar and fat coat)*, proteins, phospholipids *(membrane fat with a charged head)*. [L21 p30]

Gram negative cell wall (all): thin layer of **peptidoglycan** *(sugar and peptide mesh of the wall)*. [L21 p30]

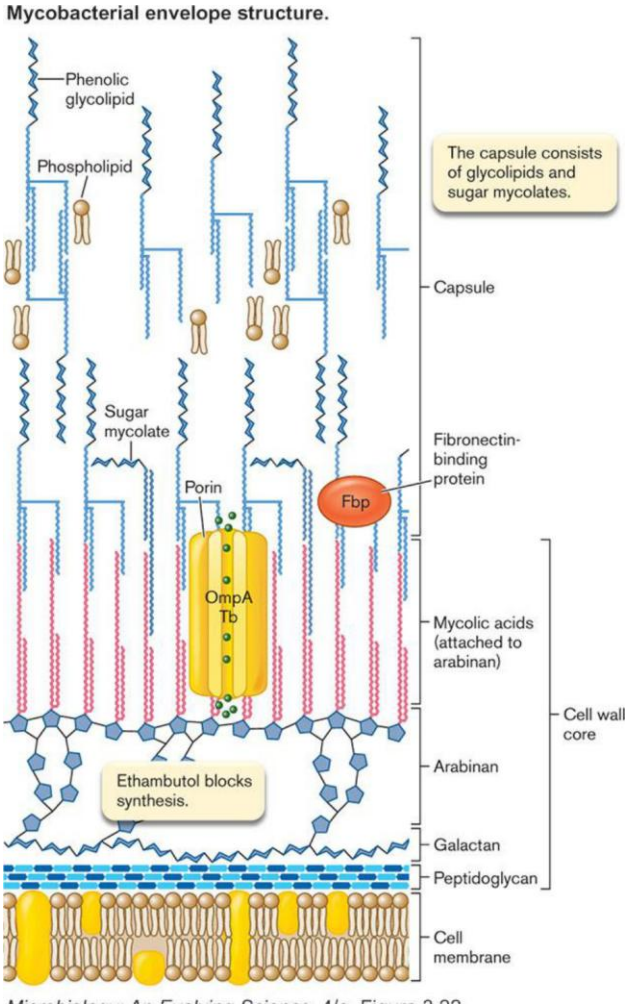
Periplasm (all) *(space between the two membranes)*. [L21 p30]

Cell or inner membrane (all): proteins and phospholipids. [L21 p30]

c. Mycobacteria (3.3 6e)

Book: 3.3 The Envelope and Cytoskeleton, Mycobacterial Cell Envelope (chapter 3 PDF, p. 63).

Mycobacteria *(waxy walled bacteria)*: **Gram positive** actinomycete *(branching thread bacterium)*. [L21 p31]



Mycobacterial envelope: capsule, mycolic acids, arabinan, galactan, peptidoglycan and membrane [L21 p31]

On the figure: capsule · mycolic acids · arabinan · peptidoglycan · cell membrane · Ethambutol

Mycobacterial *(about waxy walled bacteria)* **capsule** (all?): sugars and glycolipids. [L21 p31]

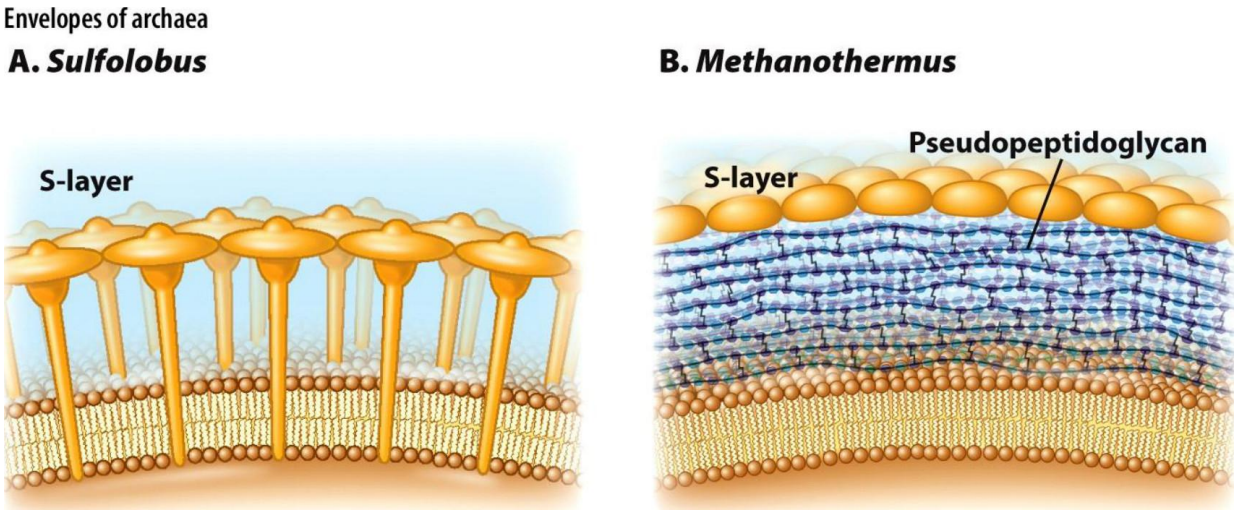
Mycobacterial cell wall (all): **mycolic acids** *(waxy fatty acids of mycobacteria)*, arabinan-galactan (sugars), **peptidoglycan** *(sugar and peptide mesh of the wall)*. [L21 p31]

Mycobacterial cell membrane (all): phospholipids *(membrane fat with a charged head)* and proteins. [L21 p31]

Ethambutol blocks **arabinan** *(five carbon sugar chain)* synthesis. [L21 p31]

d. Archaea (19.1 6e)

Book: 19.1 Archaeal Diversity at a Glance (chapter 19 PDF, p. 5).



Archaeal envelopes: S layer on stalks and over a pseudopeptidoglycan wall [L21 p35]
On the figure: S layer · Sulfolobus · Methanothermus · pseudopeptidoglycan

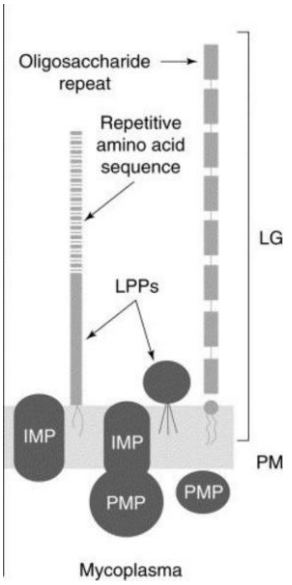
Archaeal *(of archaea)* **S layer** (some to all): protein. [L21 p35]
Archaeal cell wall (some): **pseudopeptidoglycan** *(archaea version of wall mesh)*. [L21 p35]
Archaeal cell membrane *(thin skin around the cell)*: isoprenoids (no phospholipids) and proteins. [L21 p35]
Sulfolobus *(an archaeon)*: S-layer *(crystal like protein coat outside)* proteins on stalks *(stem that anchors the cell)* anchored in the membrane. [L21 p35]
Methanothermus: S-layer over a thick pseudopeptidoglycan wall over the membrane. [L21 p35]

e. Mollicutes (Mycoplasma) (pp 734-5 6e)

Book: 18.3 Firmicutes, Mycoplasma, and Actinobacteria, Mycoplasmas Lack a Cell Wall (chapter 18 PDF, p. 63).

⚠ **Do not mix up: Mycoplasma has NO cell wall *(stiff layer around the cell membrane)*; its outer sugar layer is lipoglycans, not peptidoglycan *(sugar and peptide mesh of the wall)*.** [L21 p32, L21 p33, L21 p34]

Mycoplasma sugar layer (all have one): it may be a capsule *(firm sugar coat outside the cell)*. [L21 p32]
Mycoplasma cell membrane (all): proteins, phospholipids *(membrane fat with a charged head)*, sterols *(stiff ring shaped fat)*. [L21 p32]
Sterols in the membrane *(thin lipid layer)* help stabilize it. [L21 p34]
Mycoplasma: no cell wall. [L21 p32]
Abbreviations: **LG** = lipid-glycans, **LPPs** = lipoproteins *(proteins with fat tails)*, **PM** = plasma membrane. [L21 p32]
More abbreviations: **IMP** = intermembrane protein, **PMP** = peripheral membrane protein. [L21 p32]
Lipoglycans: sugar chains linked to lipids or proteins, on the outside. [L21 p33, L21 p34]
Variable shape: pleomorphic *(many shapes)*; threads, branches and round bodies. [L21 p34]
Parasites *(harm the host)* **or commensals** *(live on a host without harming it)*. [L21 p34]
Parasites or commensals: they live in hosts. [L21 p34]
Mycoplasma can grow in the lab under the right conditions. [L21 p34]



Mycoplasma surface: lipoglycans and lipoproteins anchored in the plasma membrane [L21 p32]
On the figure: LG · LPPs · IMP · PMP · PM

How do scientists determine the structure of the envelope of bacteria?

Book: 3.3 The Envelope and Cytoskeleton, Cell Envelope of Bacteria (chapter 3 PDF, p. 49).

⚠ **Do not mix up: Stains are not the only way: media, electron microscopy, biochemistry and genes also predict the envelope *(layers wrapped around the cell)*.** [L21 p36]

Besides a Gram or acid fast stain, other methods determine or predict the envelope. [L21 p36]

Selective media, like MacConkey agar. [L21 p36]

Electron microscopy. [L21 p36]

Biochemical isolation of unique components found in a specific type of envelope. [L21 p36]

Genes: find genes for envelope components, or for enzymes that make them. [L21 p36]

Gene examples:

Outer membrane *(extra membrane outside the wall)* proteins and LPS *(outer membrane sugar and fat coat)* for **Gram negative** *(thin wall plus outer membrane)*; teichoic acids *(charged chains in Gram positive wall)* for **Gram positive** *(thick wall, stains purple)*.

Mycolic acids *(waxy fatty acids of mycobacteria)* for Mycobacterium; pseudopeptidoglycan *(archaea version of wall mesh)* for Archaea *(single cell microbes without a nucleus, their own domain (also called archaeal bacteria))*. [L21 p36]

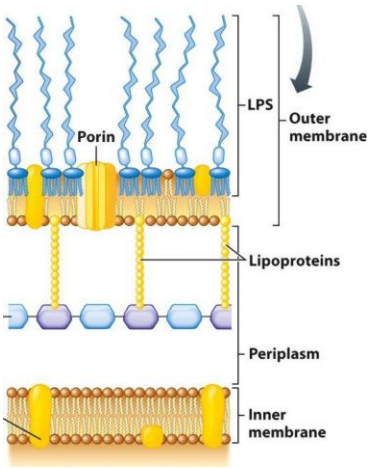
2. Know role, feature and basic composition of each of these layers (3.3 6e)

Book: 3.3 The Envelope and Cytoskeleton, Cell Envelope of Bacteria (chapter 3 PDF, p. 49).

c. Outer membrane

Book: 3.3 The Envelope and Cytoskeleton, Proteobacterial Cell Envelope: Gram-Negative (chapter 3 PDF, p. 56).

⚠ **Do not mix up: Porins *(channels in the outer membrane)* pass HYDROPHILIC *(water loving)* molecules under 700 daltons *(unit of molecule weight)*; LPS *(outer membrane sugar and fat coat)* sugars block HYDROPHOBIC *(water hating)* molecules.** [L21 p51]



Gram negative outer membrane with LPS sugars, porin, lipoproteins and periplasm [L21 p51]

On the figure: outer membrane · LPS · Porins

Gram negative *(thin wall plus outer membrane)* **outer membrane** *(extra membrane outside the wall)*: the membrane *(thin lipid layer)* itself blocks diffusion *(drifting from high to low amount)* of hydrophilic *(water-loving)* molecules. [L21 p51]

Porin *(water filled channel in outer membrane)*: hydrophilic molecules under **700 daltons** can go through. [L21 p51]

▸ LPS

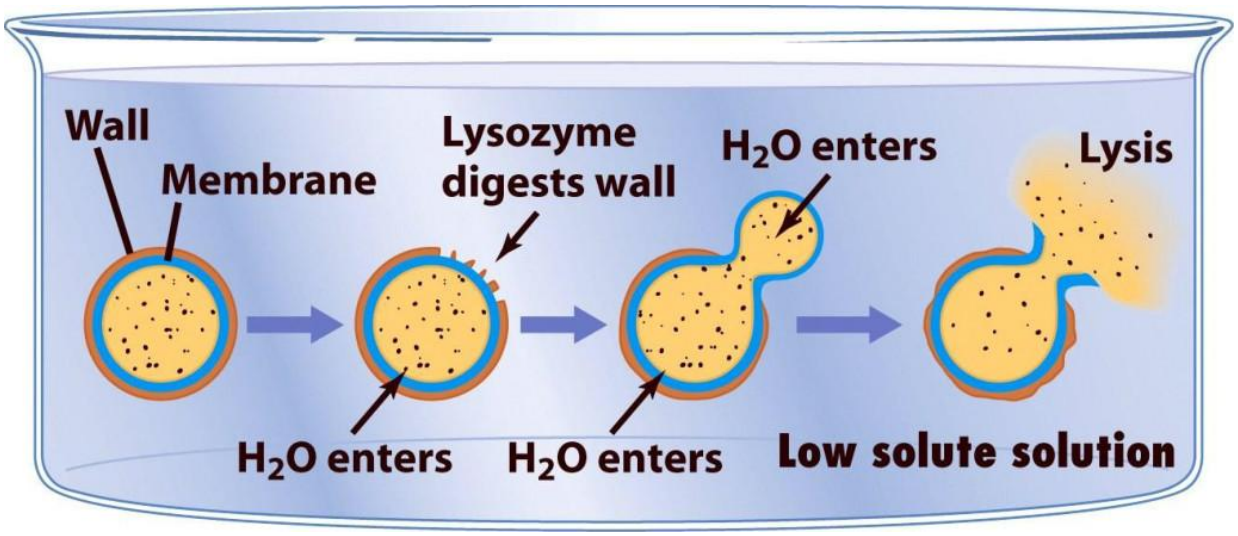
Book: 3.3 The Envelope and Cytoskeleton, Proteobacterial Cell Envelope: Gram-Negative (chapter 3 PDF, p. 56).

LPS *(outer membrane sugar and fat coat)* is tightly packed (**only** saturated fatty acids). [L21 p51]

Sugars of LPS block hydrophobic *(water-fearing)* molecules (dyes, bile salts). [L21 p51]

d. Cell Wall: Role

Book: 3.3 The Envelope and Cytoskeleton, The Cell Wall Is a Single Molecule (chapter 3 PDF, p. 43).



Lysozyme digests the wall in low solute solution: water enters and the cell lyses [L21 p54]

On the figure: wall · membrane · lyse

Cell wall *(stiff layer around the cell membrane)* **role**: keeps the **membrane** *(thin lipid layer)* from expanding out as water comes in by osmosis. [L21 p54, L21 p55]

Holes in the cell wall allow the membrane to bulge out. [L21 p54]

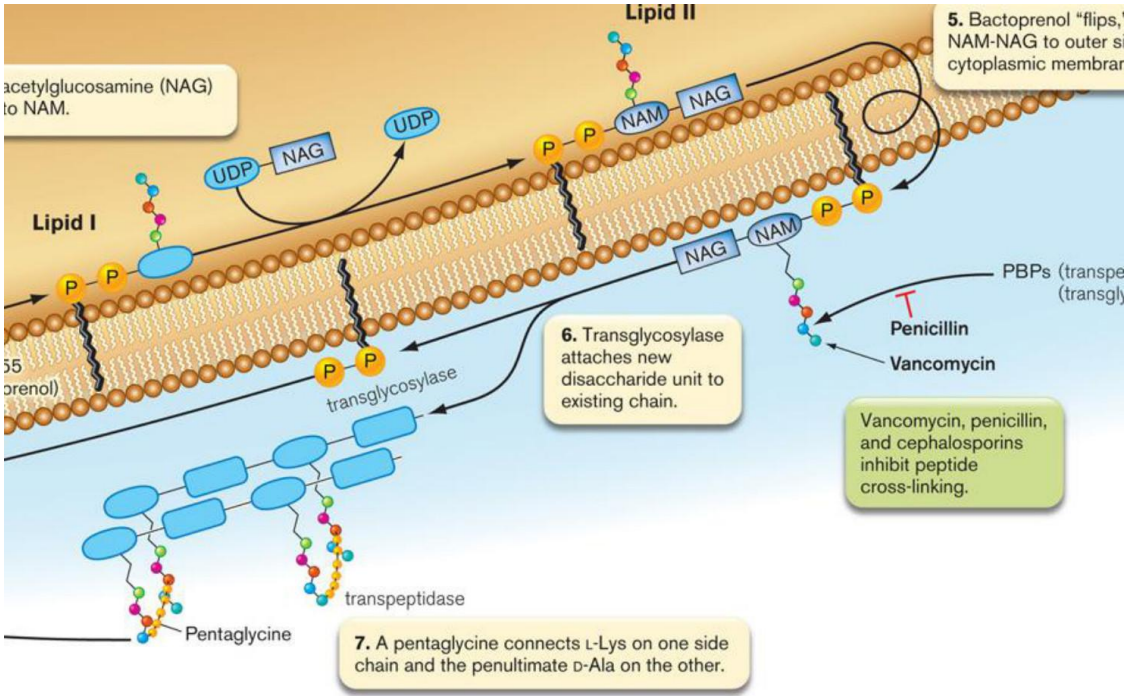
Then the membrane expands out until it lyses *(bursts)*. [L21 p55]

Basic order of events in synthesis

Book: 27.2 Antibiotic Mechanisms of Action, Cell Wall Antibiotics (chapter 27 PDF, p. 29).

⚠ **Do not mix up: Transglycosylation *(joining sugar chains longer)* links SUGARS (two sugar units); transpeptidation *(making peptide bridges between chains)* cross links PEPTIDES *(short chain of amino acids)*.** [L21 p39, L21 p40]

Gram negative *(thin wall plus outer membrane)*: synthesis is similar, but some steps occur in the periplasm *(space between the two membranes)* between membranes *(thin lipid layer)*. [L21 p38]



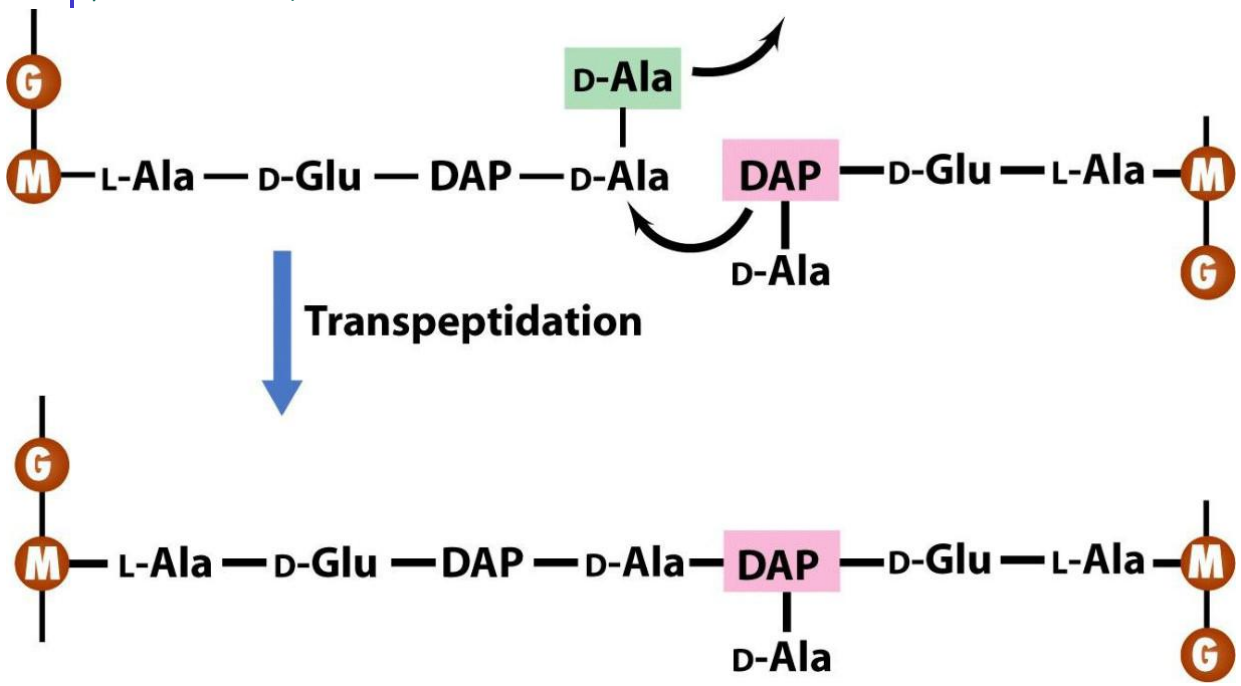
Transglycosylase and transpeptidase steps with the pentaglycine bridge [L21 p39]

On the figure: pentaglycine · L-Lys · D-Ala

Transglycosylation: linking newly synthesized two sugar units to the existing peptidoglycan *(sugar and peptide mesh of the wall)* chain. [L21 p39]

Gram positive *(thick wall, stains purple)* **cross link**: **pentaglycine** connects **L-Lys** on one side chain to the penultimate **D-Ala** *(D form of alanine amino acid)*. [L21 p39]

Transpeptidation: crosslinking of peptides, done by penicillin binding proteins *(wall building enzyme penicillin sticks to)*. [L21 p40]



Gram negative cross link: DAP attacks D-Ala and the terminal D-Ala is released [L21 p40]

On the figure: DAP · D-Ala · transpeptidation

Gram negative cross link: **DAP** *(special amino acid in wall peptide)* in the 3rd position attacks **D-Ala** in the 4th position. [L21 p40]

This displaces the D-Ala in the 5th position. [L21 p40]

Peptidoglycan synthesis and where drugs block it

Pathway step	What happens	Blocked by
Build the NAM peptide	UDP-NAM plus L-Ala, D-Glu, L-Lys (DAP in **Gram negative**)	
Add D-Ala-D-Ala	The dipeptide is added to the NAM peptide	Cycloserine (L-Ala to D-Ala and the dipeptide)
Load the carrier	Transfer to C55 bactoprenol; UMP leaves	
Add NAG	NAG comes from UDP-NAG	
Flip	Bactoprenol flips NAM-NAG (lipid II) to the outer side	
Transglycosylation	Transglycosylase attaches the new disaccharide unit to the existing chain	Penicillin and cephalosporins (PBPs)
Transpeptidation	Peptide cross link (pentaglycine bridge in **Gram positive**)	Penicillin, cephalosporins, vancomycin
Recycle the carrier	Bactoprenol-PP returns to the inside	Bacitracin

Table source: [L21 p38, L21 p39]

4. How do different antibiotics interfere with peptidoglycan synthesis? (section 27.1-3)

Book: 27.2 Antibiotic Mechanisms of Action, Cell Wall Antibiotics (chapter 27 PDF, p. 29).

Know specific mechanism of action of each: a. Penicillin, b. Cephalosporin, c. Bacitracin, d. Vancomycin, e. D-cycloserine, f. Lysozyme

Book: 27.2 Antibiotic Mechanisms of Action, Cell Wall Antibiotics (chapter 27 PDF, p. 29).

⚠ **Do not mix up: Penicillin does NOT damage existing peptidoglycan *(sugar and peptide mesh of the wall)*; it blocks new synthesis, so only growing cells lyse.** [L21 p53]

Penicillin family

Penicillin (beta-lactam) family: structure similar to **D-Ala-D-Ala**, which is cleaved in the transpeptidase *(enzyme making wall cross bridges)* reaction. [L21 p41]

Penicillins bind penicillin binding proteins *(wall building enzyme penicillin sticks to)* that do transpeptidase and transglycosylation *(joining sugar chains longer)*. [L21 p41]

Penicillin rings: ring A is the beta-lactam *(drug family with four member ring)* ring; ring B is the thiazolidine ring. [L21 p41]

Beta-lactamases (penicillinases) cleave the beta-lactam ring. [L21 p41]

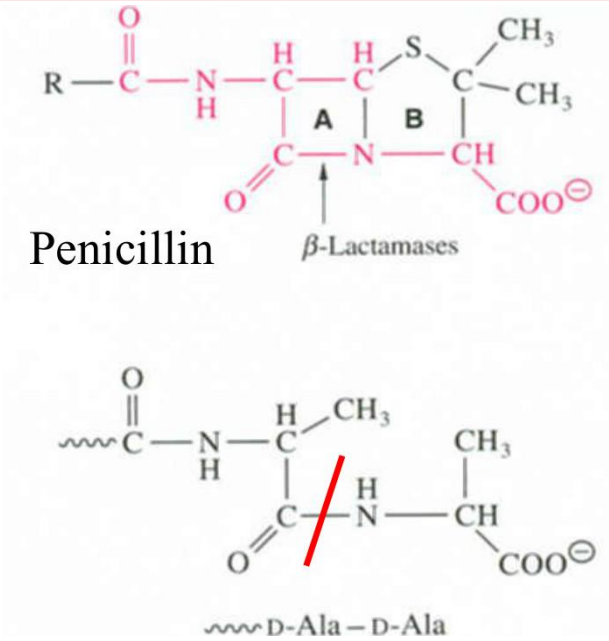
R can be a variety of substituents; in benzylpenicillin it is a benzyl group. [L21 p41]

Cephalosporins

Cephalosporins *(penicillin like wall blocking drugs)*: structure also similar to D-Ala-D-Ala in the peptide *(short chain of amino acids)* cleaved by transpeptidase. [L21 p42]

Cephalosporin generations: first cephalothin; second cefoxitin; third cefoperazone and ceftriaxone. [L21 p42]

All cephalosporins carry the beta-lactam ring; the core is **7**-aminocephalosporanic acid. [L21 p42]



Structures of penicillin and D-Ala-D-Ala, with the beta-lactamase cleavage site [L21 p41]

On the figure: **Penicillins** · **D-Ala-D-Ala**

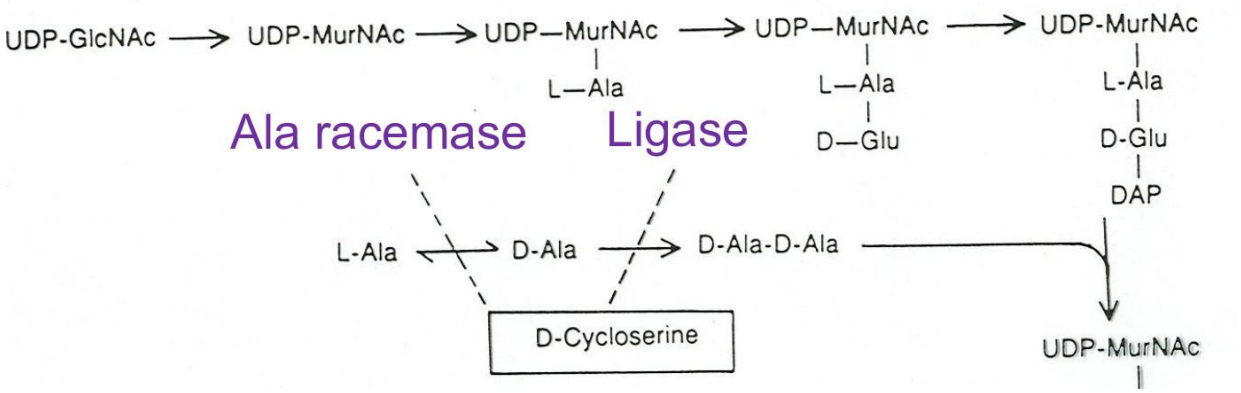
Bacitracin

Bacitracin *(drug blocking carrier recycling)* inhibits hydrolysis *(breaking with water)* of the phosphodiester on bactoprenol-PP (lipid-PP). [L21 p43]

So bacitracin blocks regeneration of bactoprenol-P (lipid-P). [L21 p43]

Blocked step: lipid-PP is not hydrolyzed back to P-lipid for the next unit. [L21 p43]

D-cycloserine



D-cycloserine blocks Ala racemase and D-Ala-D-Ala ligase in the peptide pathway [L21 p44]

On the figure: D-cycloserine · Ala racemase · ligase · L-Ala · D-Ala · D-Ala-D-Ala

D-cycloserine *(drug blocking D alanine steps)*: competitive inhibitor of **Ala racemase** and **ligase**. [L21 p44]

D-cycloserine structure is similar to ligated D-alanine-D-alanine. [L21 p44]

Ala racemase turns **L-Ala** into **D-Ala** *(D form of alanine amino acid)*; **ligase** joins D-Ala to D-Ala. [L21 p44]

D-cycloserine is primarily used to treat mycobacterial *(about waxy walled bacteria)* infections. [L21 p44]

Vancomycin

Vancomycin *(drug blocking wall building)*:

> A large glycopeptide that binds to the pentapeptide.
> Prevents cleavage and transpeptide linkage.
> Wraps around the L-Lys-D-Ala-D-Ala peptide, held by hydrogen bonds. [L21 p45]

Lysozyme

Lysozyme *(enzyme that cuts wall sugars)* breaks the glycosidic bond between sugars M and G (NAM and NAG). [L21 p46]

Low solute solution: lysozyme digests the wall, water enters and the cell lyses. [L21 p46]

Isotonic *(equal solute inside and out)* **solution**: lysozyme gives a protoplast (**Gram positive**) or spheroplast (**Gram negative**). [L21 p46]

Gram positive *(thick wall, stains purple)* **rods with lysozyme**: isotonic gives round protoplasts; low solute leaves lysed *(broken open)* ghosts. [L21 p47]

Look-alikes of D-Ala-D-Ala

Similar in structure to D-alanine-D-alanine: penicillins, cephalosporins, D-cycloserine. [L21 p48]

When penicillin does not work

Some penicillins work better on **Gram positive** than on **Gram negative** *(thin wall plus outer membrane)* bacteria. [L21 p50]

Reason: some do not pass through the outer membrane *(extra membrane outside the wall)* of **Gram negative** bacteria. [L21 p50]

So they do not reach peptidoglycan synthesis in the periplasmic *(in the space between the two membranes)* space. [L21 p50]

Mycoplasma infection: do not use penicillin; Mycoplasma have no peptidoglycan cell wall *(stiff layer around the cell membrane)*. [L21 p52]

Penicillin inhibits synthesis of new peptidoglycan; it does not damage existing peptidoglycan. [L21 p53]

Dormant *(inactive, not growing)*, **non-growing cells** need little new peptidoglycan, so they get no holes. [L21 p53]

Actively growing cells must make new peptidoglycan; without it, holes form and the cell lyses. [L21 p53]

Ampicillin and lysis

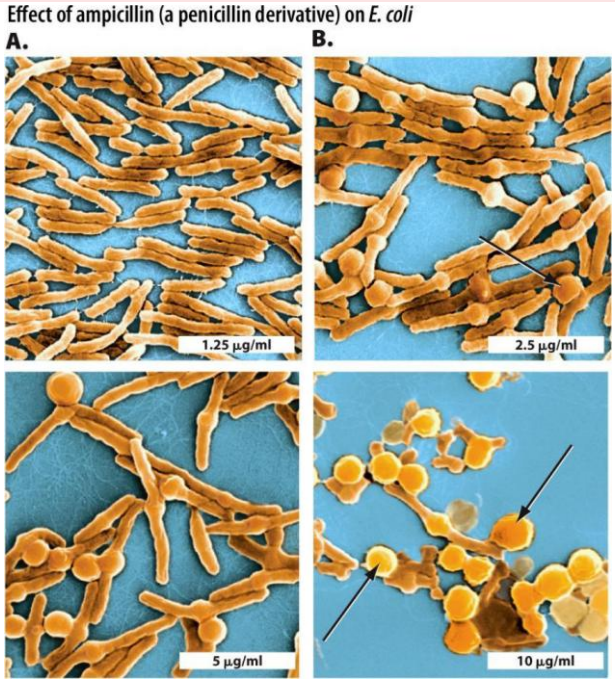
Ampicillin:

Effect similar to the effect of lysozyme.

Causes holes in the cell wall, which allows the membrane *(thin lipid layer)* to bulge out. [L21 p54]

E. coli with ampicillin: **1.25** ug/ml normal rods; **2.5** ug/ml bulges at division sites. [L21 p54]

Higher doses: **5** ug/ml bulging long cells; **10** ug/ml round spheres and lysed cells. [L21 p54]



E. coli with rising ampicillin: rods, bulges, spheres and lysed cells [L21 p54]

On the figure: Ampicillin · E. coli · Penicillins

Why wall drugs kill

Most antibiotics are bacteriostatic *(only stop growth)*: they **only** inhibit or stop growth. [L21 p55]

Peptidoglycan synthesis inhibitors such as penicillin are bactericidal *(kill)* in hypotonic *(low solute outside)* conditions. [L21 p55]

Hypotonic conditions are typically found in the human body or in the lab. [L21 p55]

Why cells die: holes form in the peptidoglycan layer of growing cells. [L21 p55]

Then water comes in by osmosis; the membrane expands out until it lyses. [L21 p55]

The drug may also activate autolysin *(wall-cutting enzyme made by the bacterium itself)*, a bacterial enzyme that cleaves peptidoglycan. [L21 p55]

Mechanism of action of each agent

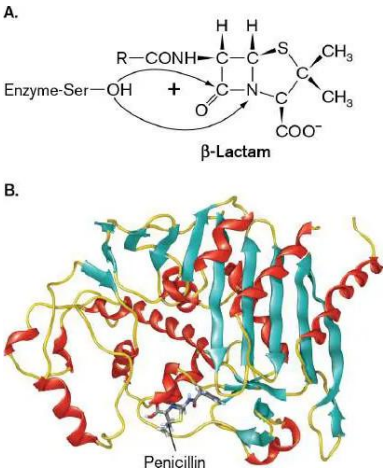
Agent	Mechanism of action
Penicillin (beta-lactam) family	Binds PBPs that do transpeptidase and transglycosylation
Cephalosporins	Inhibit transpeptidase and transglycosylation
Bacitracin	Blocks regeneration of bactoprenol-P (lipid-P)
D-cycloserine	Competitive inhibitor of Ala racemase and ligase
Vancomycin	Binds the pentapeptide; prevents cleavage and transpeptide linkage
Lysozyme	Breaks the glycosidic bond between sugars M and G (NAM and NAG)

Table source: [L21 p37, L21 p43, L21 p44, L21 p45, L21 p46]

How do bacteria resist the effects of antibiotics? (section 27.3 6e)

Book: 27.3 Challenges of Drug Resistance and Discovery, Evolving, and Sharing, Drug Resistance Genes (chapter 27 PDF, p. 83).

⚠ **Do not mix up: Beta-lactamase *(enzyme that destroys beta lactam drugs)* destroys penicillin; an altered PBP *(penicillin binding wall builder enzyme)* simply does not bind it.** [L21 p49]



Beta-lactamase attacks the beta-lactam ring of penicillin [textbook 27.21]

On the figure: penicillin

Resisting the penicillin family, way 1: express and secrete a beta-lactamase, which cleaves penicillin. [L21 p49]

Way 2: express an altered penicillin binding protein *(wall building enzyme penicillin sticks to)* that penicillin does not bind. [L21 p49]

Altered PBP source: a mutation of a chromosomal gene, or an acquired gene. [L21 p49]

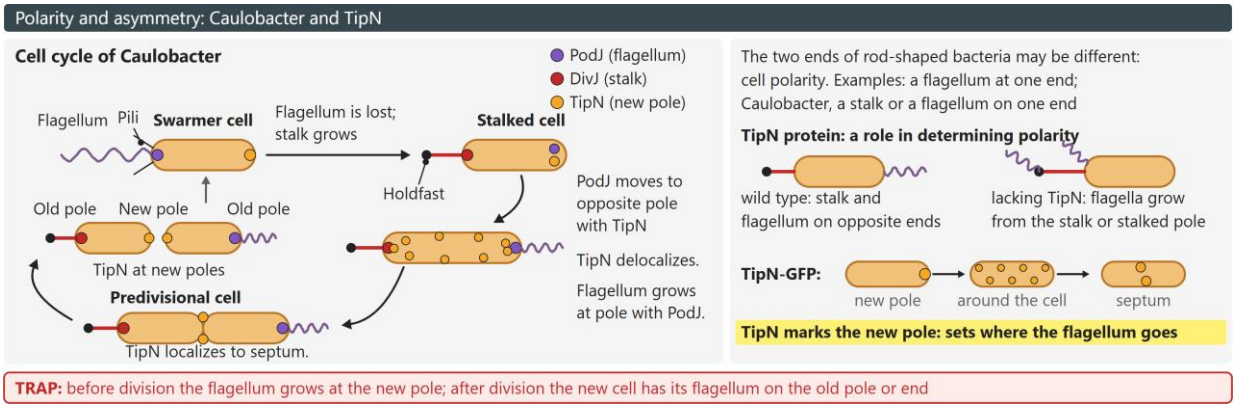
E. Cell division and Differentiation (sections 3.4, 3.5 and posted article)

Book: 3.4 Bacterial Cell Division (chapter 3 PDF, p. 78); 3.5 Cell Asymmetry, Membrane Vesicles, and Extensions (chapter 3 PDF, p. 99).

4. Cell polarity and asymmetry

Book: 3.5 Cell Asymmetry, Membrane Vesicles, and Extensions, Growth Asymmetry and Polar Aging (chapter 3 PDF, p. 103); 3.5 Cell Asymmetry, Membrane Vesicles, and Extensions, Bacterial Cell Differentiation (chapter 3 PDF, p. 99).

⚠ **Do not mix up: Polarity *(which end is which)* is not only Caulobacter: symmetric binary fission also gives an old and a new pole.** [L21 p05, L21 p09]



Cell polarity: the two ends of a rod differ, as in Caulobacter [L21 p06]

On the figure: Cell polarity · flagellum · Caulobacter · stalk

Cell polarity or asymmetry: the two ends of rod-shaped bacteria may be different. [L21 p05]

Examples: a **flagellum** *(whip tail that spins)* at one end; **Caulobacter**, with a **stalk** *(stem that anchors the cell)* or a flagellum on one end. [L21 p05]

Also: asymmetry in the envelope *(layers wrapped around the cell)* at the poles after typical symmetrical binary fission. [L21 p05]

Effect: asymmetry can cause properties of cells within a population to differ. [L21 p05]

a. Bacteria with a flagellum at one end

Book: 3.5 Cell Asymmetry, Membrane Vesicles, and Extensions, Bacterial Cell Differentiation (chapter 3 PDF, p. 99).

Polar flagellum *(whip tail that spins)*: a rod-shaped cell with one flagellum at one end. [L21 p05]

b. Caulobacter: swarmer and stalked cells, TipN and cell polarity

Book: 3.5 Cell Asymmetry, Membrane Vesicles, and Extensions, Bacterial Cell Differentiation (chapter 3 PDF, p. 99).

⚠ **Do not mix up: TipN marks the NEW pole; the flagellum *(whip tail that spins)* grows at the PodJ pole, not the stalk *(stem that anchors the cell)*.** [L21 p06]

Swarmer and stalked cells

Swarmer *(swimming daughter cell)* **cell**:

> Has one **flagellum** on one pole.
>
> Can lose its flagellum and grow a stalk on the same end. [L21 p06]

Stalked cells divide; the new cell has a flagellum on the old pole or end. [L21 p06]

Swarmer cell parts: flagellum, **pili** *(thin hairs on the cell)* and **TipN**. [L21 p06]

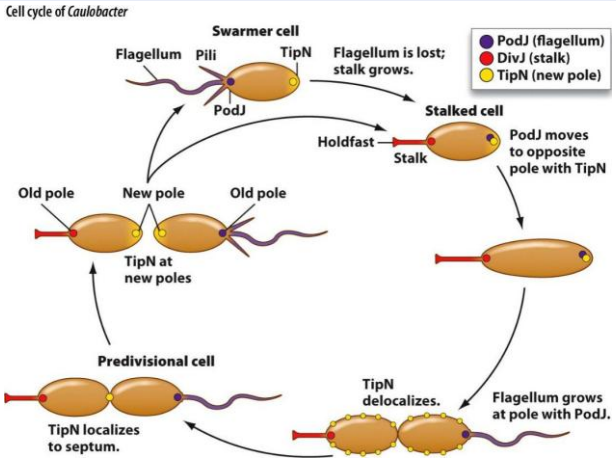
Stalked cell: a stalk with a **holdfast** *(sticky foot)* at its end. [L21 p06]

Pole marker proteins

Cell cycle: flagellum is lost, stalk grows; **PodJ** moves to the opposite pole with TipN. [L21 p06]

Next: TipN delocalizes *(spreads out)*; the flagellum grows at the pole with PodJ. [L21 p06]

Predivisional cell: TipN localizes to the septum *(cross wall)*. [L21 p06]

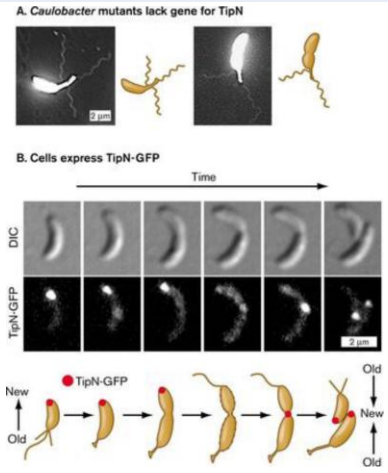


Caulobacter cell cycle with PodJ, TipN, old poles and new poles [L21 p06]

On the figure: PodJ · TipN · holdfast · Swarmer cell · stalked cells · Predivisional cell · flagellum · pili

After division: TipN at the new poles; each cell has an old and a new pole. [L21 p06]

TipN mutants and TipN-GFP



Cells lacking TipN and TipN-GFP moving from the new pole to the septum [L21 p08]

On the figure: TipN · TipN-GFP · Caulobacter

TipN: experiments revealed it plays a role in determining cell polarity *(which end is which)*. [L21 p07]

Cells lacking TipN have multiple flagella *(whip tails that spin)* from the stalk end. [L21 p07, L21 p08]

Normal swarmer cells: one flagellum on one pole. [L21 p06]

TipN-GFP *(TipN tagged with a glowing green protein)*: TipN localizes to the new pole end. [L21 p07, L21 p08]

Role of TipN: it may be a marker for the new pole end. [L21 p07, L21 p08]

Caulobacter pole marker proteins

Protein	Marks
PodJ	Flagellum pole
DivJ	Stalk
TipN	New pole

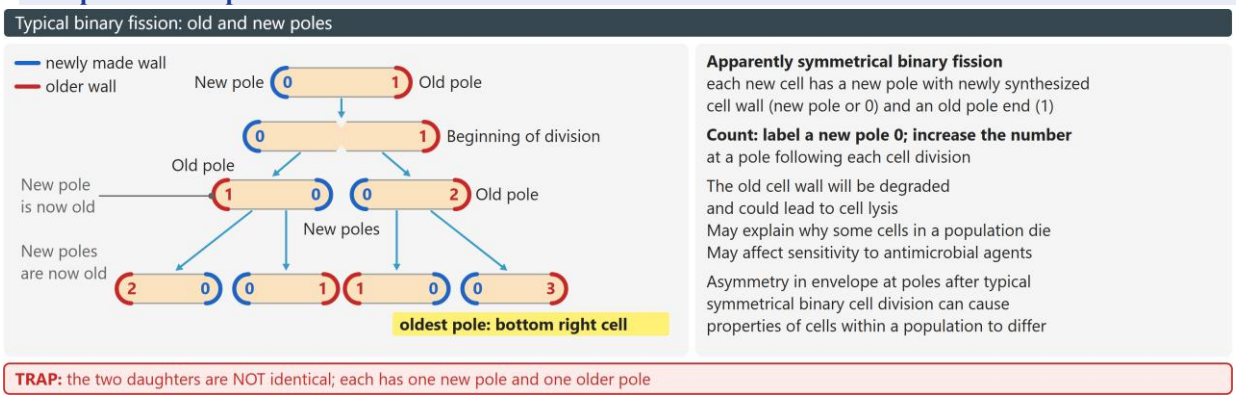
Table source: [L21 p06]

c. Typical binary fission: new and old poles, pole age

Book: 3.5 Cell Asymmetry, Membrane Vesicles, and Extensions, Growth Asymmetry and Polar Aging (chapter 3 PDF, p. 103).

⚠ **Do not mix up: A NEW pole is age 0; every cell division adds 1 to each existing pole.** [L21 p10]

New pole and old pole



Pole ages over two divisions: new pole 0, older poles count up [L21 p10]

On the figure: NEW pole · Old poles · Oldest pole · binary fission · cell wall · lysis

Typical binary fission looks symmetrical, but each new cell has a new and an old pole. [L21 p09]

New pole (0): newly synthesized **cell wall** *(stiff layer around the cell membrane)*; **old pole (1)**: the other end. [L21 p09]

Each division adds **1** to the age of every existing pole. [L21 p09]

Old cell wall will be weaker. [L21 p09]

Weaker old wall could lead to cell death. [L21 p09]

Old poles:

> May explain why some bacterial cells in a population die.
> May also affect sensitivity to antimicrobial agents. [L21 p09]

Counting pole ages

Rule: label a new pole **0**; add **1** at a pole after each cell division. [L21 p10]

Oldest pole: the original old pole; after **2** divisions it is age **3.** [L21 p10]

After 2 divisions the far right cell has the oldest pole, at least division **3.** [L21 p10]

Next oldest: the original new pole, age **2** after **2** divisions. [L21 p10]

Pole ages over two divisions

Stage	Pole ages of each cell
Start, 1 cell	0, 1
After 1 division, 2 cells	1, 0 and 0, 2
After 2 divisions, 4 cells	2, 0 and 0, 1 and 1, 0 and 0, 3

Table source: [L21 p09, L21 p10]

5. Proteins involved in cell division

Book: 3.4 Bacterial Cell Division, Cell Division by Septation (chapter 3 PDF, p. 78).

⚠ **Do not mix up: FtsA, ZipA and ZapA help tether FtsZ *(protein ring for cell splitting)*; MinC inhibits FtsZ polymerization and is not a tether.** [L21 p17, L21 p19, L21 p25]

Protein factors involved in cell division have been identified. [L21 p11]

Cell division proteins

Protein	What it is	Role in division
FtsZ	Homolog of tubulin	Forms filaments on the inside of the cell membrane; Z ring at the cleavage site; the ring gets smaller and causes constriction
FtsA	Homolog of actin	Interacts with FtsZ and associates with the membrane; helps bind bundle of FtsZ filaments; affects FtsZ turnover
ZipA	Membrane protein	Similar role as FtsA; connects the FtsZ ring to the cell membrane
PBP3	Penicillin binding protein **3**	New murein at the cleavage site: transglycosylation and transpeptidation
MinC	Associates with MinD	Inhibitor of FtsZ polymerization; inhibits FtsZ ring formation at the poles
MinD	MinD-ATP forms dynamic helical filaments	Associates with MinC; oscillates from one pole to the other pole
MinE	Found near the center of the cell	Stimulates ATP hydrolysis by MinD and disassembly of MinD filaments

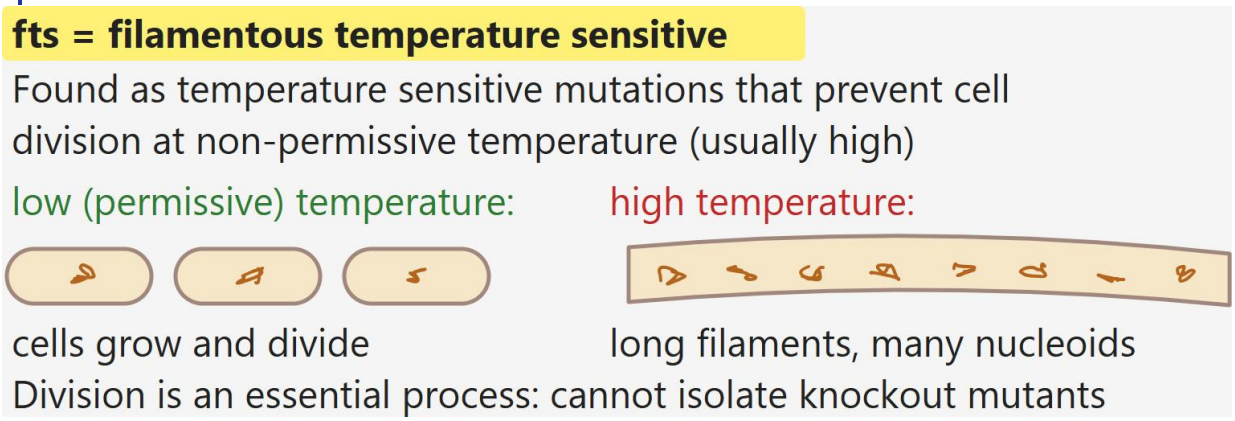
Table source: [L21 p12, L21 p14, L21 p17, L21 p18, L21 p19, L21 p21, L21 p22, L21 p25]

How division proteins were found: temperature sensitive (fts) mutants

Book: Not in the assigned textbook sections; see the posted review article on cell division proteins.

⚠ **Do not mix up: fts = filamentous temperature sensitive: at the higher temperature cells form filaments instead of dividing.** [L21 p11]

> **Many division proteins** were identified by isolating temperature sensitive mutations. [L21 p11]
> **These mutations** prevent cell division at the non-permissive temperature, usually a higher temperature. [L21 p11]
> **Temperature sensitive mutants** grow at the permissive temperature. [L21 p11]
> **Cell division is an essential process**: its genes cannot be eliminated completely. [L21 p11]
> **fts** stands for filamentous temperature sensitive. [L21 p11]



fts mutants: normal cells at low temperature, long filaments at high temperature [L21 p11]

On the figure: filamentous temperature sensitive · permissive · long filaments · many nucleoids · knockout

> **fts mutant cells**: long filaments with many nucleoids *(DNA regions)* spaced along them. [L21 p11]

a. FtsZ: Z ring, where it localizes, divisome, dynamics

Book: 3.4 Bacterial Cell Division, Cell Division by Septation (chapter 3 PDF, p. 78); 3.3 The Envelope and Cytoskeleton, Bacterial Cytoskeleton (chapter 3 PDF, p. 67).

⚠ **Do not mix up: FtsZ *(protein ring for cell splitting)* is the tubulin *(protein of eukaryote tube filaments)* homolog; FtsA is the actin *(protein of eukaryote thin filaments)* homolog. Do not swap FtsZ and FtsA.** [L21 p12, L21 p18]

What FtsZ is

> **FtsZ**:
>> Homolog of tubulin, the protein that forms microtubules in eukaryotes.
>> Forms filaments on the inside of the cell membrane *(thin skin around the cell)*. [L21 p12]

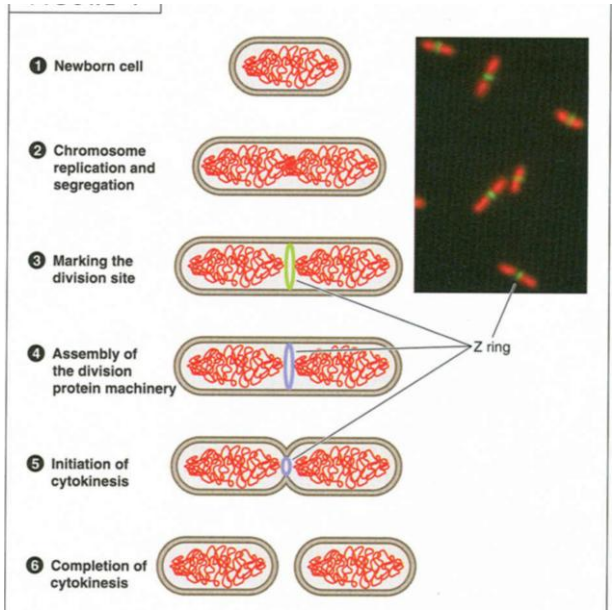
FtsZ in E. coli

Where FtsZ localizes: at the cleavage or **division site**, in the middle of the cell. [L21 p13]

Over time: FtsZ is diffuse, then a band at midcell between two nucleoids *(DNA region without a membrane)*, then a dot. [L21 p13]

Well studied in E. coli: FtsZ forms the **Z ring** at the cleavage site. [L21 p14]

The ring gets smaller, which causes constriction. [L21 p14]



Z ring stages in E. coli from newborn cell to completed cytokinesis [L21 p14]

On the figure: Z ring · cytokinesis · division site

FtsZ in Bacillus

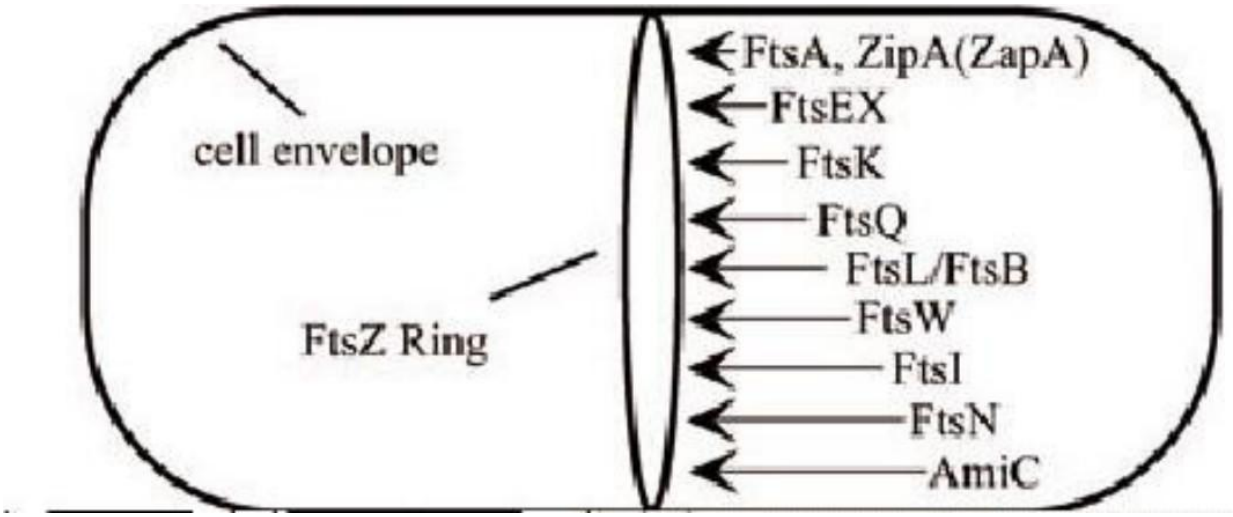
Bacillus (**Gram positive** rod), dividing cells: FtsZ localizes to the middle of the cell. [L21 p15]

Sporulating cells: FtsZ is found at both poles. [L21 p15]

Non-dividing or transitioning cells: FtsZ is found throughout the cell. [L21 p15]

Divisome

A number of other proteins associate with the FtsZ ring and build a complex, the divisome *(cell splitting machine)*. [L21 p16]



Proteins added to the FtsZ ring at the division site [L21 p16]

On the figure: FtsA · ZipA · ZapA · FtsEX · FtsK · FtsQ · FtsN · AmiC

Proteins added to the ring: **FtsA**, **ZipA**, **ZapA**, **FtsEX**, **FtsK**, **FtsQ**, FtsL/FtsB, FtsW, FtsI, **FtsN**, **AmiC**. [L21 p16]

Divisome complex: FtsZ under the inner membrane *(the skin around the cytoplasm)*; FtsN, PBP1 and PBP3 reach the peptidoglycan *(sugar and peptide mesh of the wall)*. [L21 p16]

FtsZ dynamics

FtsZ filaments are dynamic: much turnover of subunits *(building block piece)*. [L21 p17]

FtsA and other factors affect turnover. [L21 p17]

FtsZ subunits assemble into protofilaments at the future division site. [L21 p17]

FtsA, ZipA and ZapA help tether the protofilaments to the inner surface of the cytoplasmic membrane. [L21 p17]

Once the FtsZ ring is in place, transmembrane proteins are recruited (FtsK, W; FtsB, Q, L). [L21 p17]

FtsB, FtsQ, FtsL, FtsI, FtsN span the membrane once; FtsK and FtsW span it many times. [L21 p17]

Last stages of cytokinesis *(splitting the cell in two)*: the division machine has lost most of the FtsZ subunits. [L21 p17]

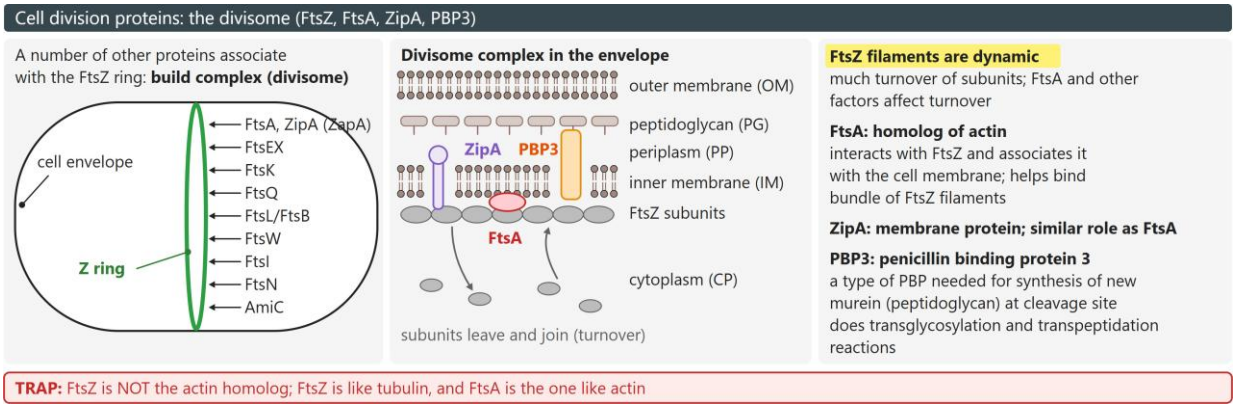
Z ring stages in E. coli

Stage	What happens
1	Newborn cell
2	Chromosome replication and segregation
3	Marking the division site: the Z ring forms
4	Assembly of the division protein machinery
5	Initiation of cytokinesis: the ring gets smaller
6	Completion of cytokinesis: two cells

Table source: [L21 p14]

b. FtsA

Book: Not in the assigned textbook sections; see the posted review article on cell division proteins.



Divisome proteins: FtsA like actin, ZipA, PBP3 and the FtsZ ring turnover [L21 p18]

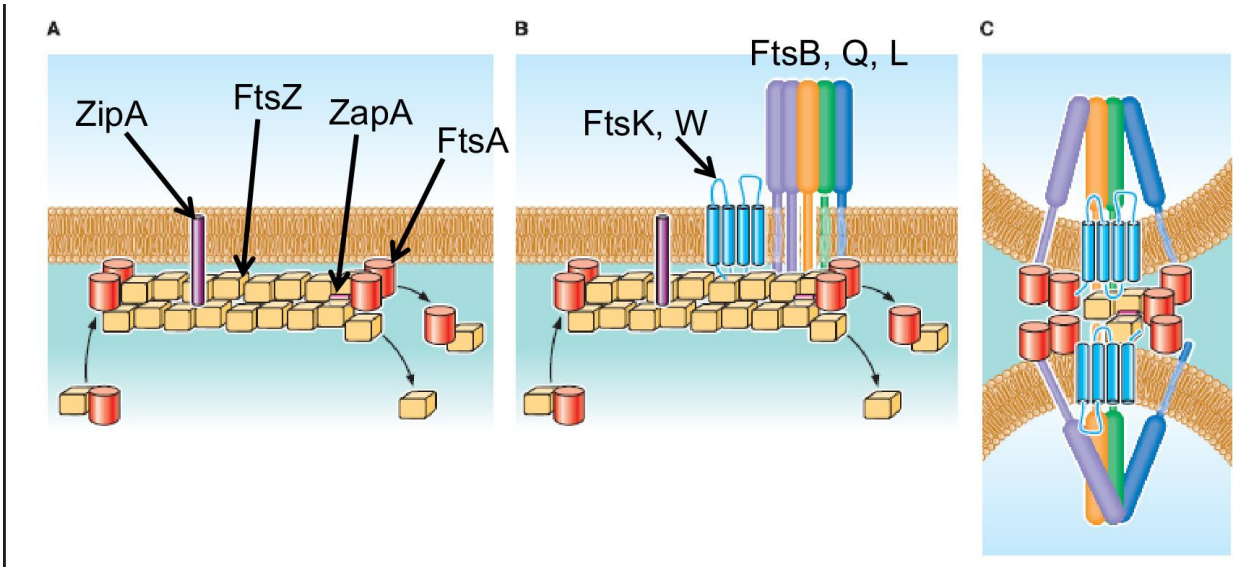
On the figure: FtsA · FtsZ · actin · FtsZ filaments

FtsA:

Homolog of **actin** *(protein of eukaryote thin filaments)*.
Interacts with **FtsZ** *(protein ring for cell splitting)* and associates with the membrane *(thin lipid layer)*.
Helps bind bundle of **FtsZ filaments**. [L21 p18]
May associate with FtsZ outside the ring. [L21 p17]

c. ZipA

Book: Not in the assigned textbook sections; see the posted review article on cell division proteins.



FtsZ protofilaments tethered to the membrane by ZipA and FtsA [L21 p17]

On the figure: ZipA · FtsZ · FtsA

ZipA:

A membrane *(thin lipid layer)* protein.

Similar role as **FtsA**. [L21 p18]

Septal *(at the splitting wall)* **ring**: a ring of **FtsZ** *(protein ring for cell splitting)* proteins is connected to the cell membrane *(thin skin around the cell)* by **ZipA**. [L21 p19]

d. PBP3 (penicillin binding protein 3)

Book: 3.4 Bacterial Cell Division, Cell Division by Septation (chapter 3 PDF, p. 78).

⚠ **Do not mix up: PBP3 does BOTH reactions at the cleavage site: transglycosylation *(joining sugar chains longer)* and transpeptidation *(making peptide bridges between chains)*.** [L21 p19]

PBP3:

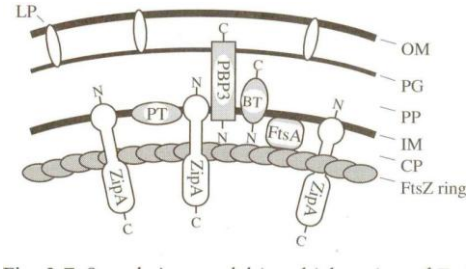
Penicillin binding protein *(wall building enzyme penicillin sticks to)* **3.**

A type of PBP *(penicillin binding wall builder enzyme)* needed for synthesis of new murein (peptidoglycan) at the cleavage site.

Does transglycosylation and transpeptidation reactions. [L21 p19]

Septal *(at the splitting wall)* **ring parts**: FtsZ *(protein ring for cell splitting)* ring, **ZipA**, **FtsA**, **FtsK** and **FtsW**, FtsL, **FtsN** and **FtsQ**, **PBP3**. [L21 p19]

Layers across the septal ring: outer membrane *(extra membrane outside the wall)*, peptidoglycan *(sugar and peptide mesh of the wall)*, periplasm *(space between the two membranes)*, inner membrane *(the skin around the cytoplasm)*, cytoplasm *(jelly inside the cell membrane)*. [L21 p19]



Septal ring parts across the outer membrane, periplasm and inner membrane [L21 p19]

On the figure: PBP3 · FtsA · ZipA · FtsZ ring · FtsK · FtsW · FtsN · FtsQ

e. Min proteins: MinC, MinD and MinE

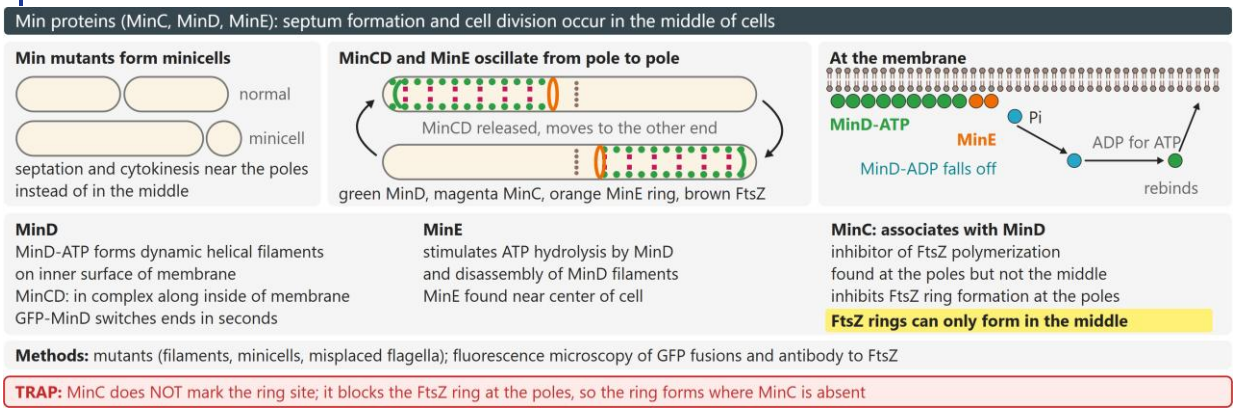
Book: Not in the assigned textbook sections; see the posted review article on cell division proteins.

⚠ **Do not mix up: MinC sits at the POLES and blocks FtsZ *(protein ring for cell splitting)* there; FtsZ rings form only in the center.** [L21 p25]

What Min proteins do

Min proteins: MinC, MinD and MinE. [L21 p20]

Role: make sure septum *(cross wall)* formation and cell division occur in the middle of cells. [L21 p20]



Min mutants make minicells; MinCD and MinE oscillate from pole to pole [L21 p20]

On the figure: minicells · MinC · MinD · MinE · MinD-ATP · MinD-ADP · FtsZ · oscillates

Min mutants form minicells: septation *(building the cross wall)* and cytokinesis *(splitting the cell in two)* occur near one end instead of the middle. [L21 p20]

Minicell: a small round cell pinched off at one end of a rod. [L21 p20]

Where they sit in E. coli

MinCD: found in a complex along the inside of the membrane *(thin lipid layer)*. [L21 p21]

MinE: found near the center of the cell. [L21 p21]

The MinE ring sits at the MinCD zone edge and moves with it. [L21 p21]

Cycle: MinCD covers half the cell; ATP *(cell energy molecule)* becomes ADP *(used up energy molecule)*; MinCD moves to the other end. [L21 p21]

MinD and MinE

MinD-ATP forms dynamic helical filaments on the inner surface of the membrane. [L21 p22]

MinD-ADP exchanges ADP for ATP, then binds the membrane again. [L21 p22]

MinE stimulates ATP hydrolysis *(breaking with water)* by **MinD** and disassembly of MinD filaments. [L21 p22]

MinE at the filament end: MinD-ADP falls off the membrane and Pi is released. [L21 p22]

Min oscillation

GFP-MinD *(MinD tagged with a glowing green protein)* switches from one end of the cell to the other. [L21 p23]

Images every 15 seconds show this; dynamic MinD assembly helps define the division plane. [L21 p23]

Central dividing cell: two separate MinD oscillating systems are already in place. [L21 p23]

MinC and MinD movement: they oscillate from one pole to the other pole. [L21 p24]

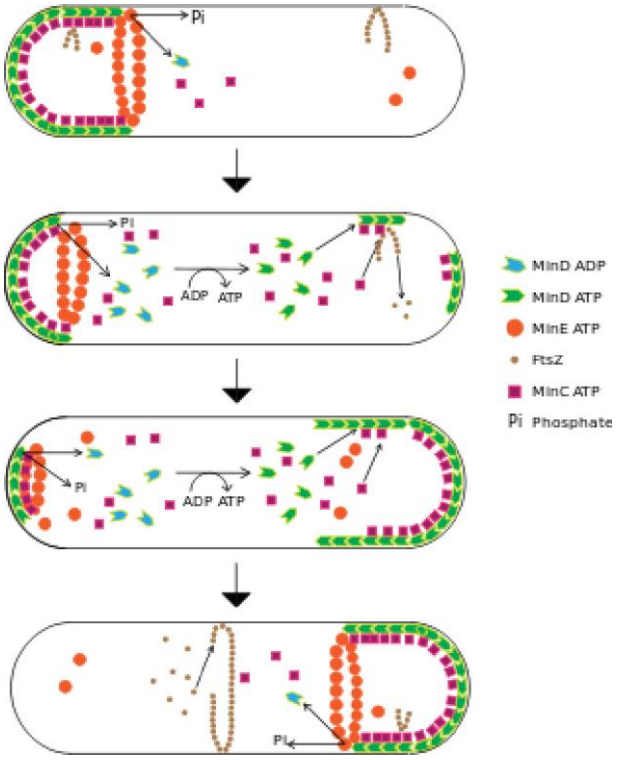
Step 1: **MinD-ATP** with MinC coats the membrane at one pole. [L21 p24]

Step 2: the **MinE** ring at its edge triggers ATP hydrolysis; **MinD-ADP** and MinC fall off. [L21 p24]

Step 3: MinD swaps ADP for ATP and rebinds at the opposite pole, over and over. [L21 p24]

Result: MinC spends the least time in the middle of the cell. [L21 p24]

FtsZ forms where MinC is absent. [L21 p24]



Min oscillation: MinD, MinC and MinE switch poles so FtsZ forms in the middle [L21 p24]

On the figure: MinD-ATP · MinD-ADP · MinE · MinC · FtsZ

MinC blocks FtsZ at the poles

MinC:

- Associates with MinD.
- Inhibitor of FtsZ polymerization.
- Is found at the poles but not at the center of the cell.
- Inhibits FtsZ ring formation at the poles. [L21 p25]

So FtsZ rings can **only** form in the center of the cell. [L21 p25]

Polar zone (PZ): the MinD and MinC coil fills one pole; MinE ring at its edge. [L21 p25]

Methods used to study cell division proteins

Book: Not in the assigned textbook sections; see the posted review article on cell division proteins.

⚠ Do not mix up: fts genes are studied with temperature sensitive mutants because division genes cannot be eliminated completely. [L21 p11, L21 p26]

Method 1: looked at the effect of mutations in the genes on cell division. [L21 p26]

Mutant results: fts mutants form filaments; Min mutants form minicells; TipN mutants misplace flagella *(whip tails that spin)*. [L21 p26]

Method 2: determined where the proteins are located in cells using fluorescent microscopy. [L21 p26]

Fluorescent tags: GFP fusions (TipN-GFP, GFP-MinD) and a fluorescent antibody to FtsZ *(protein ring for cell splitting)*. [L21 p26]

DNA *(cell genetic instruction molecule)* **stain**: DAPI shows the nucleoids *(DNA region without a membrane)*; images are often taken as a time lapse. [L21 p14, L21 p26]