BIOL 4640 Study Guide for Exam 2 (Wed 10/28/26)

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 for Flagella and Motility (Slonczewski 6e, Textbook page on the site): 3.6 Specialized Structures (flagella and pili); 25.2 Microbial Attachment: First Contact (pili). **Also useful:** 3.5 Cell Asymmetry, Membrane Vesicles, and Extensions (stalks, nanotubes).

Textbook to read for Pili, External Structures and Envelope Styles (Slonczewski 6e, Textbook page on the site): 3.6 Specialized Structures and 25.2 Microbial Attachment: First Contact (pili); 3.3 The Envelope and Cytoskeleton (the cell envelope). **Also useful:** 19.1 Archaeal Diversity at a Glance (archaea); 18.3 Firmicutes, Mycoplasma, and Actinobacteria (Mycoplasma, pp. 734-735); 3.5 Cell Asymmetry, Membrane Vesicles, and Extensions (stalks, nanotubes).

Textbook to read for Envelope Layers: Outer Membrane, Wall, Periplasm, Membrane (Slonczewski 6e, Textbook page on the site): 3.3 The Envelope and Cytoskeleton (the cell wall); 3.2 Membrane Molecules and Transport (membranes); 4.2 Nutrient Uptake. **Also useful:** 19.1 Archaeal Diversity at a Glance (archaeal lipids, pseudopeptidoglycan).

Textbook to read for Transport, Peptidoglycan Synthesis and Antibiotics (Slonczewski 6e, Textbook page on the site): 4.2 Nutrient Uptake (transport proteins); 27.1 Fundamentals of Antimicrobial Therapy, 27.2 Antibiotic Mechanisms of Action and 27.3 Challenges of Drug Resistance and Discovery (peptidoglycan synthesis and antimicrobial therapy). **Also useful:** 3.4 Bacterial Cell Division (FtsZ ring, septation).

Textbook to read for Internal Structures, Cytoplasm and Ribosomes (Slonczewski 6e, Textbook page on the site): 27.1, 27.2 and 27.3 (antimicrobial therapy); 3.1 The Bacterial Cell: An Overview; part of 3.3 on the cytoskeleton (Bacterial Cytoskeleton); part of 3.6 on thylakoids, carboxysomes and storage granules; the posted source Bacterial Intracellular Structures.

Textbook to read for Internal Structures and Cell Division (Slonczewski 6e, Textbook page on the site): 3.1 The Bacterial Cell: An Overview; part of 3.3 on the cytoskeleton (Bacterial Cytoskeleton); part of 3.6 on thylakoids, carboxysomes and storage granules; 7.2 Genome Organization (nucleoid and DNA packing); the posted source Bacterial Intracellular Structures. **Also useful:** 3.4 Bacterial Cell Division and 3.5 Cell Asymmetry, Membrane Vesicles, and Extensions (cell division, named on the slides); 4.6 Cell Differentiation (endospores, akinetes, myxospores; not assigned).

Textbook to read for Cell Polarity, Division Proteins and Envelope Review (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).

Textbook to read for Viruses and Atypical Bacteria (Slonczewski 6e, Textbook page on the site): Chapter 6 Viruses: 6.1 Viruses in Ecosystems, 6.2 Virus Structure, 6.3 Viral Genomes and Classification, 6.4 Bacteriophages and 6.5 Coronavirus SARS-CoV-2 and Other Viral Pathogens. **Also useful:** the atypical bacteria and giant viruses part is from the notes only.

(teal italics) = plain meaning of the word before it. ⚠ **Do not mix up = a look-alike pair to keep apart.**

Contents

A. Size and shapes of bacteria 14

Size units and ranges 14

Size examples 14

Bacterial shapes 15

B. External Structures of Bacteria 16

What external structures are 16

Flagellum 17

Fimbriae 17

Pilus 17

Glycocalyx, envelope and internal parts 18

What external structures are 18

Main kinds and jobs 18

Pili, fimbriae and motility 19

Appendages and internal structures 19

1. Flagella and motility (3.6 6e) 19

What flagella are 19
Parts and growth 20
Number and location 20
Spirochete flagella 20
Arrangements observed 20
Arrangement names 21
Found in bacteria 21
Never found 21
Seeing flagella 21
Parts and features of flagella 21
Filament and flagellin 22
Basal body parts 22
How the flagellum moves 23
Order of assembly of flagellum 23
Order of assembly steps 24
Short summary of the steps 24
Assembly in plain terms 24
Filament growth 24
Types of motility observed in bacteria: know how each is mediated 25
What motility is 25
Flagella mediated 25
Not flagella mediated 25
- **Swimming motility mediated by flagella** 26
- **Periplasmic flagella in spirochetes** 27
- **Swarming motility** 29
- **Gliding motility** 29
- **Twitching motility** 30

Chemotaxis: what is it 31
Taxis and stimuli 31
Chemotaxis receptors 32
Runs and tumbles 32
Gradient of attractant 32
Reaching food 32
How one could determine if a bacterium is motile and if it has flagella 33
Four assays 33

Motility agar tubes 33
Swarm plate results 33
Agar plate techniques 33
2. Pili and Fimbriae (3.6 and 25.2 6e) 34
What they are 35
Three types of pili 35
Roles and comparison with flagella 35
a. Structure, arrangement in cells and assembly 35
Basic structure and size 35
Number per cell 35
Pilin and tip protein 36
Proteins that form a pilus 36
Changing which pilin is made 37
Assembly compared with flagellum 37
b. Roles of Common pili, Sex pili and Type IV pili 38
Common pili or fimbriae 38
Sex pili 39
Sex pilus steps and F plasmid 39
Type IV pili and twitching 39
Other pilus types 39
Which types are called fimbriae 40
c. Assembly of Type I and Type IV, how it differs from flagellar 40
Type I pili assembly 40
Type IV pili assembly 42
Pili versus flagellum filament 42
Flagellum assembly order 42
3. Other external structures 43
Others as an external structure 43
Stalks and Caulobacter cycle 43
TipN protein 44
Nanotubes 44
C. Cell envelope (glycocalyx, outer membrane, cell wall, and cell membrane) 45
Cell envelope parts 46
Five envelope styles 46
Gram negative layers 46

Archaea and S-layer 47
Layers outside to inside 47
Peptidoglycan, lysozyme and penicillin 47
S layer and archaeal envelope 48
Envelope differences 48
Envelope overview 48
Envelope layers 48
Peptidoglycan sugars and carrier 48
Antibiotics and lysozyme 48
Transport and cell division 48
1. Know the layers and components of the envelope in each of the following types of bacteria. How similar and different from each other 49
Five envelope styles 49
What the styles share 49
How they differ 49
Peptidoglycan differences 50
Electron microscope appearance 50
a. Gram positive bacteria 50
Layers outside to inside 51
Thick wall, no outer membrane 51
Molecules to know 51
b. Gram negative bacteria 51
Layers outside to inside 52
Peptidoglycan thickness 52
Outer membrane 52
c. Mycobacteria (3.3 6e) 53
What mycobacteria are 53
Layers outside to inside 53
Wall sugars and mycolic acid 53
Other parts 53
Acid fast envelope 53
d. Archaea (19.1 6e) 54
S-layer in archaea 54
Cell wall 55
Membrane lipids 55

e. Mollicutes (Mycoplasma) (pp 734-5 6e) 56
No cell wall 57
Membrane proteins 57
Membrane surface 57
How do scientists determine the structure of the envelope of bacteria? 57
Staining techniques 58
Electron microscopy 58
Confirming a Gram positive envelope 58
How the nature of the envelope affects bacterial cells? 59
Why the envelope matters 59
Glycocalyx and S-layer effects 60
Penicillin in stream water 60
2. Know role, feature and basic composition of each of these layers (3.3 6e) 60
Membrane lipids 60
LPS and membrane proteins 61
Layers overview 62
Gram negative layers in order 62
Penicillin and the outer membrane 62
Cell membrane and transport 62
Peptidoglycan in the wall 62
Keeping the layer intact 62
Enzymes that cut peptidoglycan 62
a. Glycocalyx (capsule and slime layer): role and composition 63
What the glycocalyx is 64
Composition 64
Sugar chains and EPS 64
Capsule examples and conclusion 64
Roles 64
b. S-layer: composition and where located 65
What the S-layer is 65
Where it is located 65
Hypothesized roles 67
c. Outer membrane 67
What the outer membrane is 68
Porins 68

Receptors and phage 69
Murein lipoprotein 69
- **LPS** 69
d. Cell Wall: Role 71
Cell wall roles 71
Wall composition 71
Why penicillin may fail 72
- **1) Peptidoglycan: structure, sugars** 72
- **Know how it differs in Gram negative vs Gram positive** 75
- **Basic order of events in synthesis** 76
- **2) Pseudopeptidoglycan: how it differs from peptidoglycan** 81
- **3) Teichoic acids** 82
- **4) Mycolic acids** 83
e. Periplasm 86
What the periplasm is 86
Gram positive contrast 86
f. Cell membrane (3.2 6e) 86
What the cell membrane is 87
Membrane makeup compared 87
Modes of entry 87
- **Role, function** 88
- **Components (types of lipids, etc) observed in bacteria** 90
- **Components observed in Archaea and how different? Isoprenoids** 91
- **How types of fatty acids present change in response to temperature?** 92
3. Movement of Material across Cell membrane (3.2 and 4.2 6e) 93
Why membranes matter 94
Ion levels in mM 94
Transport mechanisms 94
Different types of transport 95
Simple diffusion 95
Weak acids in two forms 95
Drugs that are weak acids or bases 95
Facilitated diffusion 95
Active transport kinds 96
Ion coupled transport 96

Which ions power transport 96
Proton motive force 97
Na+ motive force 97
ATP driven transport 97
Group translocation (PTS) 98
PTS and glucose 98
Western Sandhill lakes 98
Eastern saline wetlands 99
Type of transport proteins observed in bacteria (section 4.2) 99
Glycerol transporter 99
ABC family basics 99
ABC import steps 100
ABC example, GAG transporter 100
Siderophore mediated iron uptake 100
ABC exporters 100
PTS proteins 101
4. How do different antibiotics interfere with peptidoglycan synthesis? (section 27.1-3) 102
Peptidoglycan as drug target 102
The cell wall and its enzymes 102
Penicillin and growing bacteria 102
Target of antibacterial drugs / antibiotics 103
General rules 103
Processes blocked 103
Drugs by target 103
Know specific mechanism of action of each: a. Penicillin, b. Cephalosporin, c. Bacitracin, d. Vancomycin, e. D-cycloserine, f. Lysozyme 104
Penicillin and cephalosporin 105
Vancomycin 105
Penicillin family structure 105
Lysozyme and autolysin 106
When penicillin may not work 106
Penicillin family 106
Cephalosporins 106
Bacitracin 106
D-cycloserine 107

Vancomycin 107
Lysozyme 107
Look-alikes of D-Ala-D-Ala 107
When penicillin does not work 107
Ampicillin and lysis 108
Why wall drugs kill 108
How do bacteria resist the effects of antibiotics? (section 27.3 6e) 109
Drug resistance overview 109
Prevent entry 110
Multidrug exporter in Gram negative 110
Prevent binding 110
Gram negative and penicillin 110
Approval versus resistance dates 111
Rising resistant strains 111
Date conflicts 112
MIC tetracycline tubes 112
Kirby-Bauer plates 112
Penicillin in stream water 112
Gram positive envelope tests 113
D. Bacterial Internal Structures (Chapter 3): know types of structures seen in bacteria 114
Cell envelopes 114
Peptidoglycan synthesis overview 114
Synthesis steps in order 115
Bactoprenol, flippase and crosslinking 115
Drugs that block wall synthesis 116
Antibiotics in general 116
Antibiotic resistance 116
Resistance mechanisms 117
Beta lactam family 117
Susceptibility tests and MIC 118
Kirby Bauer disk test 118
Antibiotic stewardship 118
Sources for internal structures 118
Internal structures list 119
Compartments and protein-shell inclusions 119

Internal structures outline 119
Surface and wall 119
Cytoplasm contents 120
Ribosome and inhibitors 120
1. Cytoplasm and cytosol 122
Cytoplasm and cytosol 123
Cytoplasm, nucleoid and envelope contents 124
E. coli composition table 124
Cytoplasm makeup 124
Check the two lists 124
2. Ribosomes 125
What ribosomes are 125
Selective inhibition 125
Translation inhibitors by target 125
Penicillin and cephalosporin 126
3. Cytoskeleton: types found and roles 127
FtsZ, MreB and crescentin (CreS): which eukaryotic protein each is like and its role 127
The three filaments 128
Shape-determining proteins 128
4. Nucleoid: DNA and packing proteins 128
a. Nucleoid region: where the DNA is located 129
b. How bacterial DNA is packed: no histones, DNA binding proteins, supercoiling 129
Packing basics 130
DNA binding proteins 130
Supercoiling and loops 130
After the cell is lysed 130
c. Loop domains of about 50 kB 130
What loop domains are 131
Supercoiled loops 131
d. DNA inside a membrane compartment (Gemmata, Planctomycetes) 131
5. Compartments: intracellular membrane structures (know some examples and their role) 132
What compartments are 132
Photosynthesis example 132
a. Thylakoids in cyanobacteria 133
What thylakoids are 133

Compared with chloroplasts 133
Cyanobacteria and examples 134
Prochlorococcus 134
b. Chlorosomes in green bacteria 134
Chlorosome structure 135
Green nonsulfur bacteria 135
Green sulfur bacteria 135
Example names 135
c. Membrane invaginations in purple bacteria 135
Membrane folds inward 135
Purple bacteria types 136
Membrane shapes 136
d. Membrane compartments in methanotrophs 136
6. Inclusions and granules (gas vesicles, carboxysomes, energy and carbon storage polymers, sulfur granules) 137
What inclusions are 137
Not membrane compartments 137
A. Gas vesicles 137
B. Carboxysomes 138
C. Magnetosomes 138
D. Energy and carbon storage granules (PHA, glycogen) 139
E. Sulfur granules 139
7. Endospore: what they are 140
Spores and their types 140
Endospores 140
Endospores of Bacillus: features and the stages of formation 141
Features 141
Stages I and II 141
Stages III to V 141
Stages VI and VII 141
E. Cell division and Differentiation (sections 3.4, 3.5 and posted article) 142
1. Features and Types of Cell division in bacteria 142
a. Binary fission 143
b. Budding 143
c. Multiple fission 144
What multiple fission is 144

Examples 144
d. Asymmetric cell division and differentiation 145
- **Spore types: akinetes, exospores, Chlamydia elementary bodies, endospores, myxospores, fungal spores** 145
2. Basic Steps in process: Replication, cytokinesis, septation, separation 147
The four steps 148
Replication and segregation 148
Division follows 148
3. Mechanism of cell division: how it differs between cell types 148
Autolysins and new peptidoglycan at the division site 149
Three steps at the septum 149
Dividing coccus 149
New peptidoglycan 149
a. Gram negative rod (E. coli) 150
b. Gram positive rod (Bacillus) 150
c. Gram positive cocci (Staphylococcus): septum, planes and arrangements 151
Furrow and septum 151
Arrangements 152
FtsZ ring and where new peptidoglycan is made (Lecture 18 slides) 152
FtsZ ring 152
Divisome proteins 153
PBPs 153
Where new peptidoglycan is made 153
Keeping the layer intact 153
Autolysin 153
4. Cell polarity and asymmetry 153
a. Bacteria with a flagellum at one end 154
b. Caulobacter: swarmer and stalked cells, TipN and cell polarity 154
Swarmer and stalked cells 154
Pole marker proteins 154
TipN mutants and TipN-GFP 155
c. Typical binary fission: new and old poles, pole age 155
New pole and old pole 155
Counting pole ages 156
5. Proteins involved in cell division 156
How division proteins were found: temperature sensitive (fts) mutants 156

a. FtsZ: Z ring, where it localizes, divisome, dynamics 157
What FtsZ is 157
FtsZ in E. coli 157
FtsZ in Bacillus 157
Divisome 157
FtsZ dynamics 158
b. FtsA 159
c. ZipA 159
d. PBP3 (penicillin binding protein 3) 159
e. Min proteins: MinC, MinD and MinE 160
What Min proteins do 160
Where they sit in E. coli 160
MinD and MinE 160
Min oscillation 161
MinC blocks FtsZ at the poles 161
Methods used to study cell division proteins 161
F. Viruses (Chapter 6) 161
1. Size and shape observed 162
Size 162
Shape 162
2. Structural features: components of virus particles (core and covering) 162
Simpler than cells 162
Core and covering 163
a. Nucleic acid 163
b. Enzymes 163
c. Capsid 163
d. Envelope 163
Which components are found in all viruses? 164
Naked vs enveloped viruses 164
3. Features used to classify or group viruses (groups, subgroups, Baltimore Groups I to VII) 164
Groups and subgroups 165
Subgroup examples 165
4. Reproduction 165
a. General features: obligate intracellular parasites, assembled from parts 165
b. Steps in multiplication cycle 166

Animal virus cycle 166
a. Adsorption 166
b. Penetration and c. Uncoating 166
e. Assembly 166
f. Release or escape 167
c. Synthesis stage for the 7 types of viruses and the source of each enzyme 167
Two jobs 167
RNA genomes 167
Group I: dsDNA 168
Group II: ssDNA 168
Group III: dsRNA 168
Group IV: (+) ssRNA 168
Group V: (-) ssRNA 168
Group VI: retroviruses 168
Group VII: DNA reverse-transcribing 168
Enzyme sources compared 168
▸ **Multiplication cycles of SARS-CoV-2, plant viruses and bacteriophage** 169
5. Outcomes of viral infections 170
Different possible outcomes for the host cell 170
Acute and persistent 170
Latent 170
Cytopathic changes 171
Transformation and syncytia 171
Effects on populations of microbes 172
G. Atypical Bacteria and Viruses 172
Typical bacteria vs typical viruses 172
Atypical small bacteria: how they were found 172
1. Rickettsia 173
2. Chlamydia 173
3. Endosymbiotic bacteria 173
Mitochondria and chloroplasts 174
4. Giant viruses 174
Which would you consider a living organism? 174

Click a line to jump to it. The PDF side panel (bookmarks) has the same parts as tabs and topics as subtabs.

A. Size and shapes of bacteria

⚠ **Do not mix up: Viruses are 10-100 nm, not µm; bacteria are 0.4-10 µm.** [L15 p05]

Size units and ranges

Sizes of microbes: the unit is the micrometer (µm), also called micron, which is **10**^-6 m. [L15 p05]

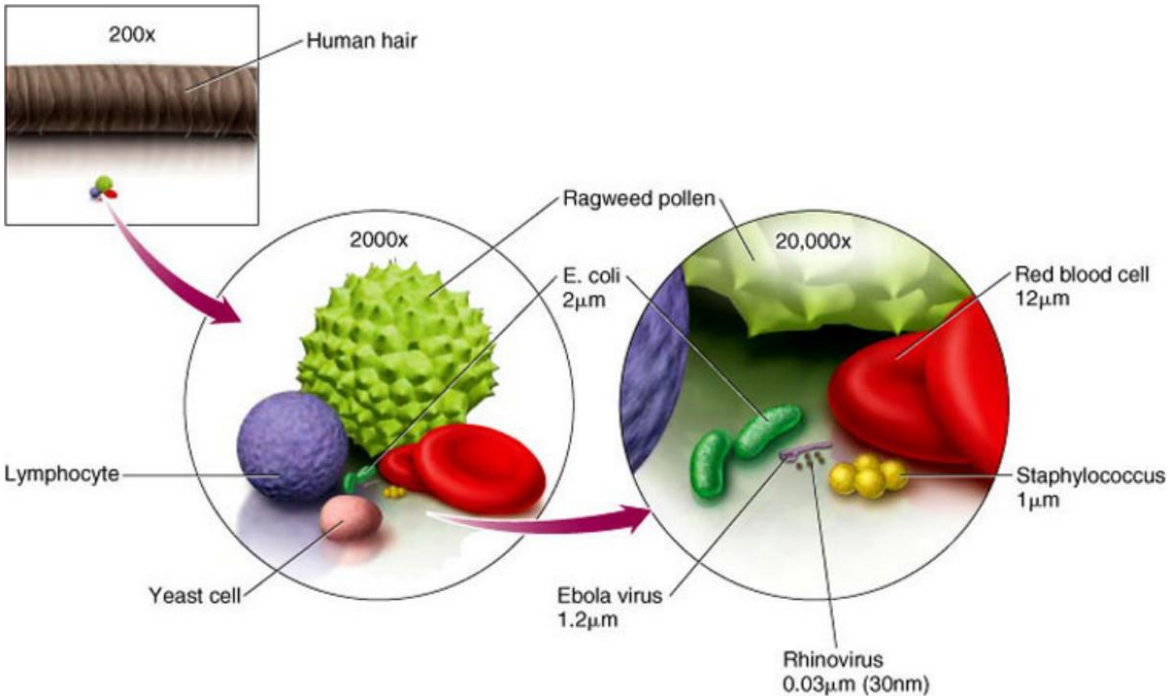
Eukaryotic *(cells with a nucleus)* **microbes** are **10**-**100** µm wide. [L15 p05]

Bacteria are **0.4**-**10** µm. [L15 p05]

Viruses are **0.01**-**0.1** µm, which is **10**-**100 nm**. [L15 p05]

Nanometer (nm): **1** µm is **1,000 nm**; the virus range **0.01**-**0.1** µm is **10**-**100 nm**, and rhinovirus at **0.03** µm is **30 nm**. [L15 p05]

Size examples



Sizes of microbes beside blood cells and pollen at three scales [L15 p05]

On the figure: Ebola virus · E. coli · Staphylococcus · red blood cell · rhinovirus

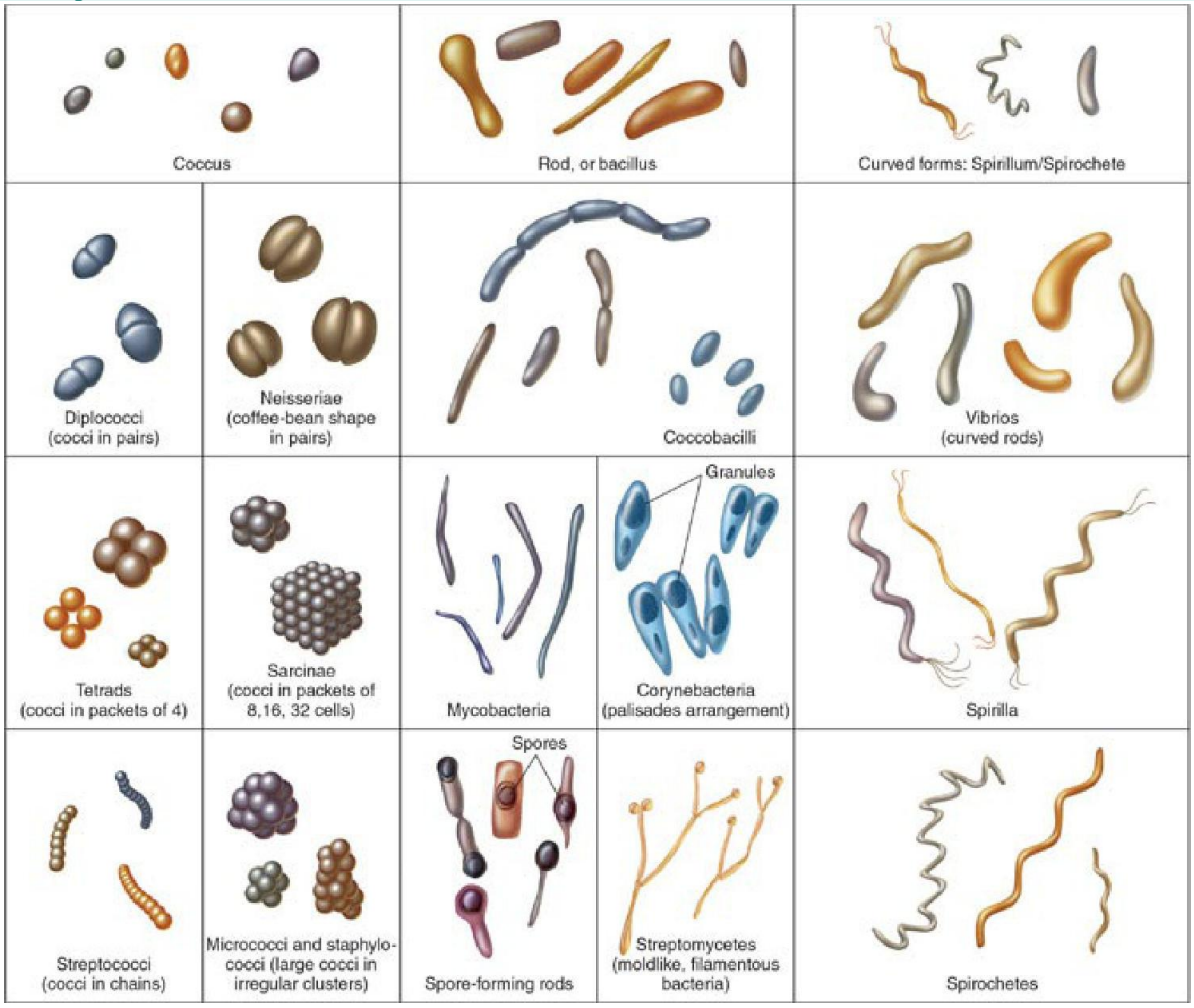
Sizes: E. coli is **2** µm, Staphylococcus is **1** µm. [L15 p05]

More sizes: red blood cell **12** µm, rhinovirus **0.03** µm (**30 nm**). [L15 p05]

Ebola virus (**1.2** µm) is longer than Staphylococcus (**1** µm). [L15 p05]

Watch out: the size list gives viruses as **0.01**-**0.1** µm, but the size figure shows Ebola virus at **1.2** µm, so a virus is not always smaller than a bacterium. [L15 p05]

Bacterial shapes



Bacterial shapes and groupings: coccus, rod, vibrio and spiral forms [L15 p06]

On the figure: Coccus · bacillus · vibrio · spirilla · spirillum · cocci

Four shapes of bacteria: **cocci** *(round cells)*, **bacillus** *(rod cells)*, **vibrio** *(curved rods)*, spiral. [L15 p06]

Coccus is a sphere and **bacillus** (plural bacilli) is a rod. [L15 p06]

Vibrio is a curved rod and **spirillum** (plural **spirilla**) is a spiral. [L15 p06]

Some bacteria grow in clusters or filaments. [L15 p06]

Group	Size
Eukaryotic microbes	**10-100** µm wide
Bacteria	**0.4-10** µm
Viruses	**0.01-0.1** µm (**10-100 nm**)

Table source: [L15 p05]

Item in the size comparison	Size	Magnification where it appears
Human hair	no size given	**200**x
Ragweed pollen	no size given	**2000**x and **20,000**x
Lymphocyte	no size given	**2000**x
Yeast cell	no size given	**2000**x
E. coli	**2** µm	**2000**x and **20,000**x
Red blood cell	**12** µm	**20,000**x (also at **2000**x)
Staphylococcus	**1** µm	**20,000**x (also at **2000**x)
Ebola virus	**1.2** µm	**20,000**x
Rhinovirus	**0.03** µm (**30 nm**)	**20,000**x

Table source: [L15 p05]

Shape or arrangement	Description
Coccus	sphere
Diplococci	cocci in pairs
Neisseriae	coffee-bean shape in pairs
Tetrads	cocci in packets of **4**
Sarcinae	cocci in packets of **8, 16, 32** cells
Streptococci	cocci in chains
Micrococci and staphylococci	large cocci in irregular clusters
Rod or bacillus	rod
Vibrios	curved rods
Spirilla and spirochetes	spiral forms
Mycobacteria, corynebacteria, spore-forming rods	mycobacteria: thin, slightly curved rods; corynebacteria: palisades arrangement, granules; spore-forming rods: spores at the end or middle of the rod
Streptomycetes	moldlike, filamentous bacteria

Table source: [L15 p06]

Shape	What it looks like
Mycobacteria	thin, slightly curved rods
Corynebacteria	palisades arrangement; granules
Spore-forming rods	spores at the end or middle of the rod
Coccobacilli	short oval rods
Streptomycetes	moldlike, filamentous bacteria
Spirilla	spiral with flagella in tufts at the ends
Spirochetes	a tight, flexible spiral shape; flagella in the periplasm

Table source: [L15 p06]

B. External Structures of Bacteria

Book: 3.6 Specialized Structures (chapter 3 PDF, p. 119); 3.6 Specialized Structures, Pili and Stalks (chapter 3 PDF, p. 123).

⚠ **Do not mix up: Fimbriae *(short sticky hairs)* help adhesion *(sticking to a surface)*; a pilus *(thin hair for sticking or DNA swap)* is hollow and transfers DNA *(cell genetic instruction molecule)*; flagella *(whip tails that spin)* give motility *(ability to move)*.** [L15 p04]

What external structures are

External structures:

> Protein filaments that project out from the cell.
>
> Are not enclosed by the cell membrane *(thin skin around the cell)* or cell wall *(stiff layer around the cell membrane)*. [L15 p07]

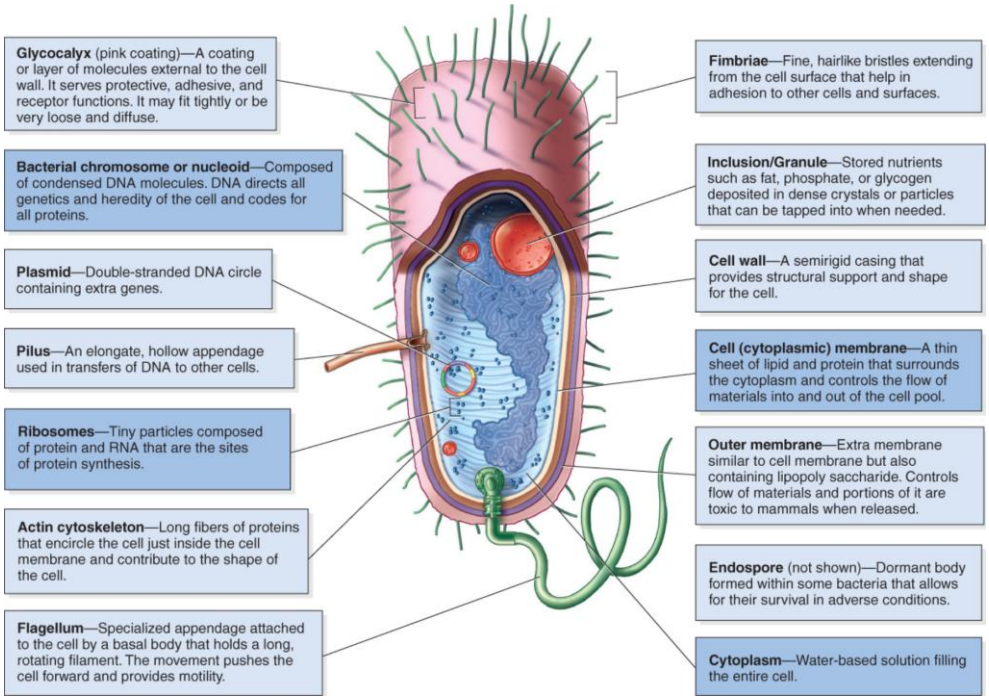
Appendages *(parts that stick out)* are flagella, pili *(thin hairs on the cell)* and fimbriae. [L15 p03]

Flagella (type **1** of **2** main types): used for motility. [L15 p07]

Pili and fimbriae (type **2** of **2** main types): used for adherence. [L15 p07]

Other external structures are in textbook section 3.5. [L15 p07]

Flagellum



Bacterial cell with each external and internal part named and described [L15 p04]

On the figure: Flagellum · Fimbriae · pilus · Glycocalyx · outer membrane · cell wall · ribosomes · actin cytoskeleton

> **Flagellum** *(whip tail that spins)*: attached to the cell by a basal body *(anchor of the whip in the envelope)* that holds a long, rotating filament. [L15 p04]
>
> The **flagellum** provides motility; its movement pushes the cell forward. [L15 p04]

Fimbriae

> **Fimbriae**:
>
> > Fine, hairlike bristles extending from the cell surface.
> >
> > Help in adhesion to other cells and surfaces. [L15 p04]
> >
> > And common pili are thinner than flagella: **3-10 nm** wide versus **14-20 nm** for a flagellum.

Pilus

> **Pilus**: an elongate, hollow appendage used in transfers of DNA to other cells. [L15 p04]
>
> A pilus is built of straight filaments of protein monomers called pilin *(protein that builds a hair)*; short attachment pili are also called **fimbriae** (textbook).

Glycocalyx, envelope and internal parts

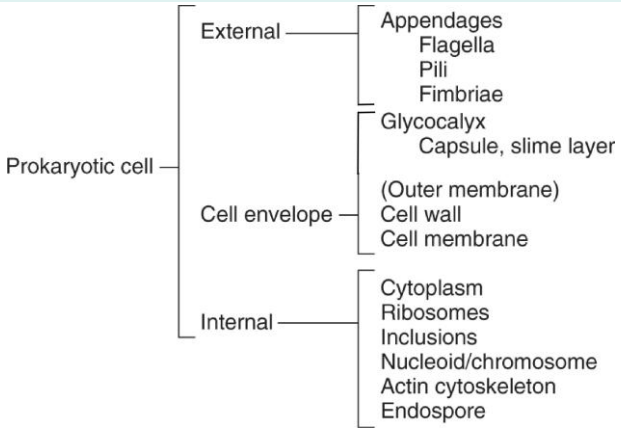
Glycocalyx *(sugary coat)* has two forms: capsule *(firm sugar coat outside the cell)* and **slime layer** *(loose sticky coat outside)*. [L15 p03]

Cell envelope *(layers wrapped around the cell)* parts: **outer membrane** (**Gram-negative only**), **cell wall**, cell membrane. [L15 p03]

Outer membrane holds **lipopolysaccharide** (LPS) *(sugar and fat molecule)*; portions of it are toxic to mammals when released. [L15 p04]

Internal parts: cytoplasm *(jelly inside the cell membrane)*, **ribosomes** *(protein building machine)*, inclusions *(clumps of protein and other material)*, nucleoid *(DNA region without a membrane)* or chromosome *(one long DNA molecule)*, actin *(protein of eukaryote thin filaments)* cytoskeleton *(inner scaffold of the cell)*, endospore *(tough dormant survival form)*. [L15 p03]

Watch out: the structure chart puts the glycocalyx (capsule, slime layer) with the external structures, but the cell envelope list includes cell membrane, cell wall, outer membrane and glycocalyx/capsule. [L15 p03]



Prokaryotic cell parts sorted into external, cell envelope and internal [L15 p03]

On the figure: Appendages · Glycocalyx · Cell envelope · internal · slime layer

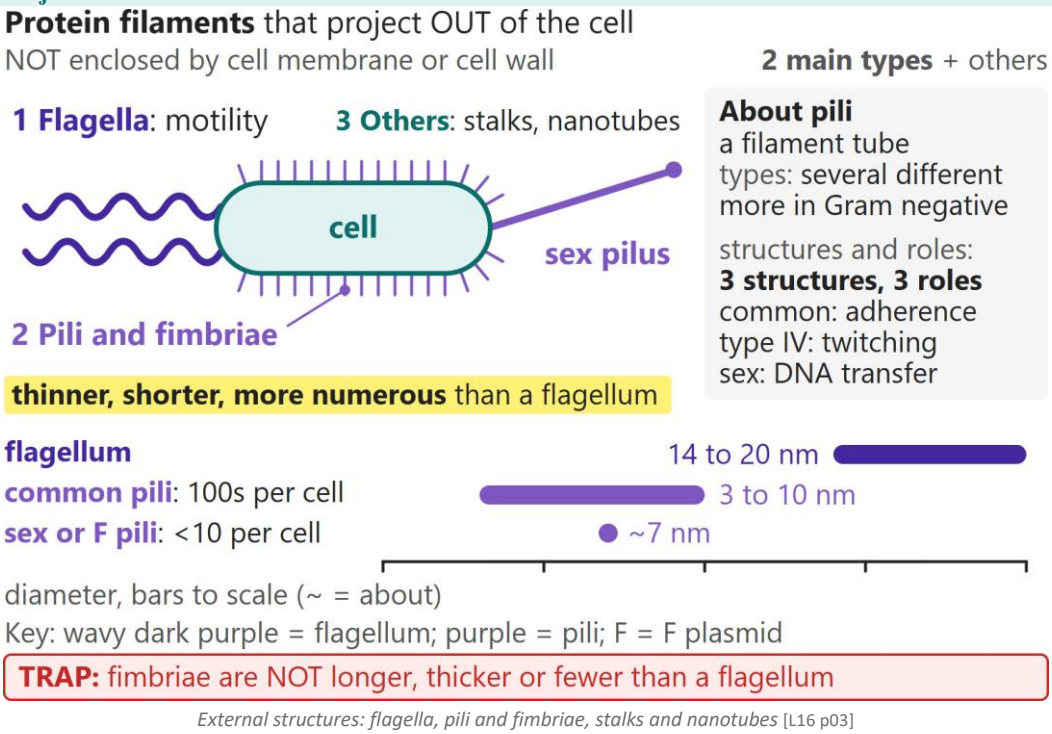
What external structures are

External structures: protein filaments that project out from the cell. [L16 p03, L16 p20, L16 p21]

They are **NOT** enclosed by the cell membrane or cell wall. [L16 p03, L16 p20, L16 p21]

Watch out: This describes the protein filaments; nanotubes *(tiny tube joining two cells)*, listed under Others, are an extension of the cytoplasm or periplasm *(space between the two membranes)* between two cells. [L16 p03, L16 p20, L16 p21]

Main kinds and jobs



External structures: flagella, pili and fimbriae, stalks and nanotubes [L16 p03]

On the figure: flagella · pili and fimbriae · stalks · nanotubes · thinner

There are **two main types** of external structure: **flagella**, and **pili and fimbriae**. [L16 p03]

Flagella: motility. [L16 p03, L16 p20, L16 p21]

Pili and fimbriae: adherence. [L16 p03, L16 p20, L16 p21]

Others: **stalks** *(stem that anchors the cell)*, **nanotubes**. [L16 p03, L16 p20, L16 p21]

Pili, fimbriae and motility

Fimbriae are **thinner** than **flagella**.

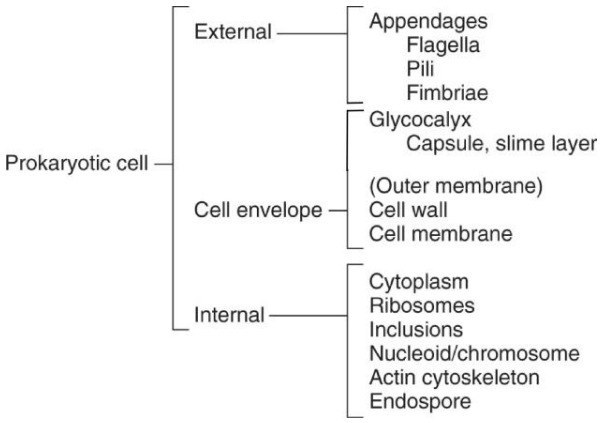
Pili mediate attachment, DNA transfer and a form of motility.

Twitching *(jerky crawl using pili)*, **gliding** *(smooth crawl on a surface)* **and sliding** *(passive spreading by pushing)* are motility not mediated by flagella.

Appendages and internal structures

Prokaryotic cell: **appendages** *(parts that stick out)* are external structures: flagella, pili and fimbriae. [L16 p24]

Prokaryotic cell: **internal structures** are cytoplasm, **ribosomes**, **inclusions** *(stored material in the cell)*, nucleoid or chromosome, **actin cytoskeleton** and **endospore** *(tough dormant body formed inside some bacteria)*. [L16 p24]



Prokaryotic cell parts sorted into external, cell envelope and internal [L16 p24]

On the figure: Appendages · flagella · pili · fimbriae · ribosomes · inclusions · actin cytoskeleton · endospore

Part	Description
Glycocalyx	coating or layer of molecules external to the cell wall; serves protective, adhesive and receptor functions; may fit tightly (capsule) or be very loose and diffuse (slime layer)
Outer membrane	extra membrane similar to the cell membrane but also containing lipopolysaccharide; controls flow of materials; portions of it are toxic to mammals when released
Cell wall	semirigid casing that gives structural support and shape
Cell membrane	thin sheet of lipid and protein that surrounds the cytoplasm and controls the flow of materials into and out of the cell
Cytoplasm	water-based solution filling the entire cell
Ribosomes	tiny particles of protein and RNA; sites of protein synthesis
Inclusions (granules)	stored nutrients such as fat, phosphate or glycogen, in dense crystals or particles that can be tapped when needed
Nucleoid (chromosome)	condensed DNA; directs all genetics and heredity; codes for all proteins
Plasmid	double-stranded DNA circle containing extra genes
Actin cytoskeleton	long fibers of proteins that encircle the cell just inside the cell membrane and contribute to the shape of the cell
Endospore	dormant body formed within some bacteria; lets them survive adverse conditions

Table source: [L15 p04]

1. Flagella and motility (3.6 6e)

Book: 3.6 Specialized Structures, Rotary Flagella (chapter 3 PDF, p. 126).

⚠ **Do not mix up: Flagellin *(protein that builds the whip)*** is added at the filament TIP, NOT the base (the rings, rod and hook are built first).

What flagella are

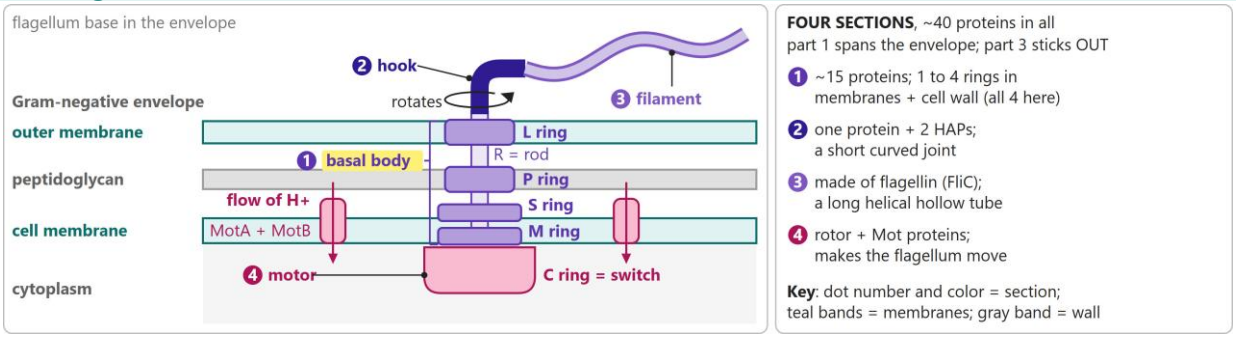
Flagella *(whip tails that spin)*: a complex structure of about **40 proteins**. [L15 p07, L15 p12]

Some sections of the flagellum *(whip tail that spins)* sit in the membrane *(thin lipid layer)*. [L15 p07, L15 p12]

The filament portion sticks out from the cell. [L15 p07, L15 p12]

Flagellar motility *(ability to move)* makes the population disperse, decreasing competition, and lets cells swim toward a favorable habitat (textbook).

Parts and growth



Flagellum base in the envelope with its four sections and about 40 proteins [L15 p13]

On the figure: basal body · hook · filament · motor · Flagellin

Four major sections: **basal body** *(anchor of the whip in the envelope)*, **hook**, **filament**, **motor**. [L15 p13]

Filament growth: **flagellin** subunits *(building block piece)* are added at the **tip**, not the **base**. [L15 p18]

Number and location

Number per cell is **0** to many. [L15 p08, L15 p09]

Number and location are a characteristic of a species. [L15 p08, L15 p09]

Location: one end, both ends or all over. [L15 p10]

Some cells can lose and regain their flagella. [L15 p08, L15 p09]

Spirochete flagella

Spirochetes *(long corkscrew shaped bacteria)* have their flagella in the periplasm *(space between the two membranes)*. [L15 p11]

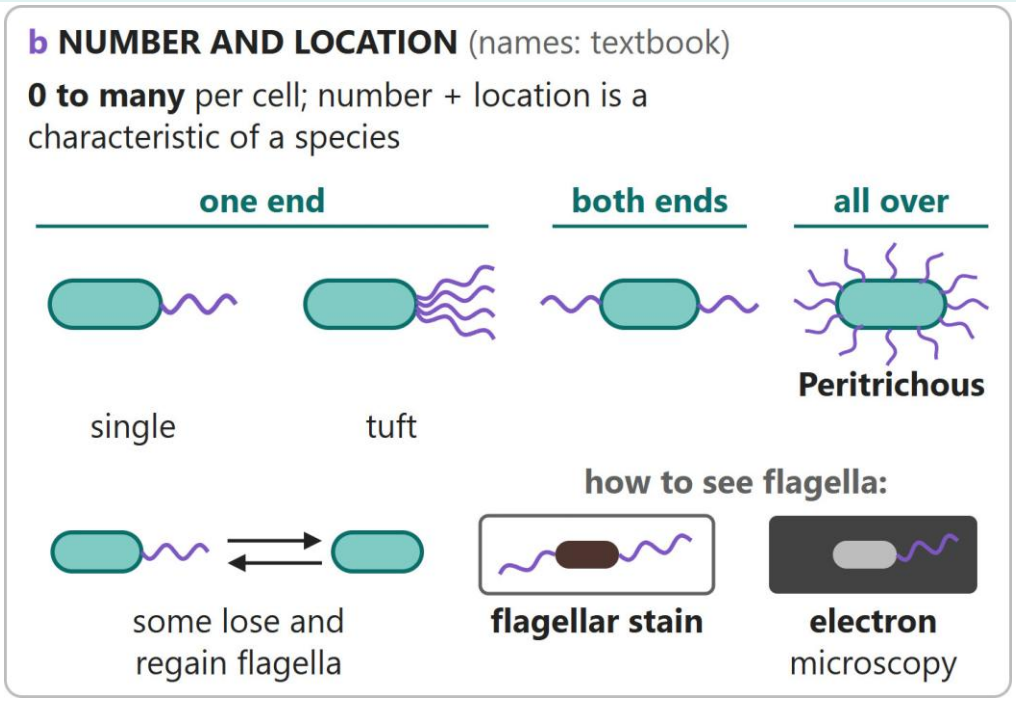
In spirochetes the flagella extend from each pole and wrap around the cell. [L15 p11]

Arrangements observed

Book: 3.6 Specialized Structures, Rotary Flagella (chapter 3 PDF, p. 126).

⚠ **Do not mix up: Polar means flagella *(whip tails that spin)* at an end of the cell (one or several at one end, or one at each end); peritrichous *(flagella all over the cell)* means flagella all over the cell.** [L15 p10]

Arrangement names



Flagella at one end, both ends or all over, and how to see them [L15 p09]

On the figure: Single · peritrichous · Flagellar stain · electron microscopy

Single polar *(one flagellum at one end)* is one flagella arrangement. [L15 p27]

Peritrichous *(flagella all over the cell)* is the name for the many flagella type. [L15 p28]

Several flagella at one end form a **tuft** *(small cluster)* at one pole. [L15 p09]

Found in bacteria

Found in bacteria:

- A single polar flagellum *(whip tail that spins)*.
- One flagellum on each end.
- Multiple polar flagella all out of one end.
- Many flagella over the whole body.

Never found

Never found: rod shaped bacteria with all their flagella enclosed inside the cell or inner membrane *(the skin around the cytoplasm)*.

This differs from spirochetes *(long corkscrew shaped bacteria)*, whose flagella sit in the periplasm *(space between the two membranes)*, beneath the outer sheath.

Seeing flagella

Flagellar stain and electron microscopy are used to see the flagella. [L15 p08, L15 p40]

Arrangement	What it looks like
Single flagellum at one end (polar)	a single polar flagellum (at one end)
Several flagella at one end	multiple polar flagella (all stick out of one end)
Flagella at both ends	a single flagellum on each end
Flagella all over the cell (peritrichous)	multiple flagella spread over the entire body
Periplasmic (spirochetes)	located in the periplasm; flagella extend out from each pole and wrap around the cell

Table source: [L15 p09, L15 p10, L15 p11, L15 p28]

Parts and features of flagella

Book: 3.6 Specialized Structures, Rotary Flagella (chapter 3 PDF, p. 126).

⚠ **Do not mix up: Filament is flagellin *(protein that builds the whip)*; hook is hook protein plus 2 HAPs *(hook associated proteins)*; motor has Mot proteins *(motor proteins that turn the flagellum)*.** [L15 p14, L15 p15, L15 p16]



Flagellin filament: helical tube of subunits, 14 nm across, hollow core [L15 p15]

On the figure: Salmonella typhimurium · helical · 14 nm · 40,000 daltons · flagellin

Filament and flagellin

Flagellin subunits *(building block piece)*:

- Self polymerize *(assemble on their own)* in a **helical** or spiral arrangement.
- Form a hollow tube. [L15 p15]

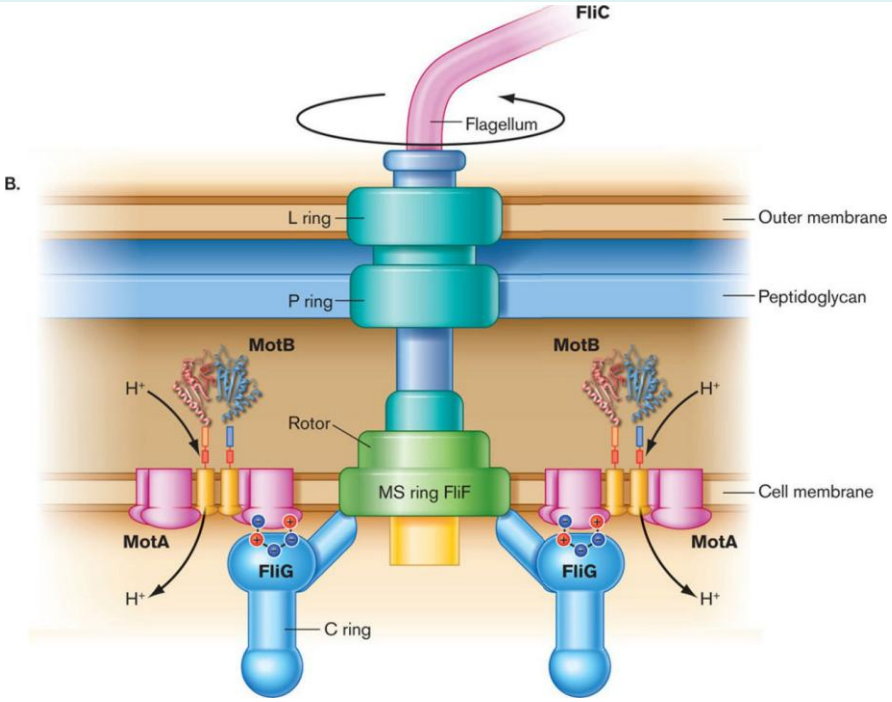
Filament:

- A cell may have several different **flagellin** genes.
- The cell can change the flagellin gene it uses. [L15 p15]

Salmonella typhimurium filament: helical, **14 nm** in diameter (within the **14-20 nm** range). [L15 p15]

Salmonella typhimurium flagellin: **40,000 daltons** *(unit of molecule weight)*. [L15 p15]

Basal body parts



Motor: rotor, MS ring, C ring, MotA and MotB with proton flow [L15 p16]

On the figure: L ring · P ring · MS ring · C ring · MotA · MotB · H+ · Rotor

Position of the rings (**Gram-negative** flagellum): **L ring** at the outer membrane *(extra membrane outside the wall)*. [L15 p12, L15 p14, L15 p16]

P ring is at the peptidoglycan *(sugar and peptide mesh of the wall)*. [L15 p12, L15 p14, L15 p16]

Rotor and MS ring (FliF) are in the cell membrane *(thin skin around the cell)*. [L15 p12, L15 p14, L15 p16]

C ring (with FliG) is below the MS ring, inside the cell; **FliG** *(switch protein in the C ring)* sits at the top of the C ring under **MotA** *(motor protein in the membrane)*, and its + and - charges make contact with MotA. [L15 p12, L15 p14, L15 p16]

The **C ring** is also called the **switch** (C ring/switch). [L15 p14]

Hook (H): hook protein and **2** HAPs; the filament (F) comes after it. [L15 p13, L15 p14]

MotA is in the cell membrane, and **MotB** sits beside it in the cell membrane and reaches into the periplasm *(space between the two membranes)*. [L15 p12, L15 p14, L15 p16]

How the flagellum moves

How the flagellum *(whip tail that spins)* **moves**: the motor section mediates movement of the flagellum. [L15 p19, L15 p20, L15 p16]

The motor section is the rotor plus the Mot proteins. [L15 p19, L15 p20, L15 p16]

H+ flows from the periplasm side through MotA and MotB into the cytoplasm *(jelly inside the cell membrane)*; this proton flow powers the rotation. [L15 p16]

The motor is driven by the transmembrane proton current, the same proton potential that drives the membrane-embedded ATP *(cell energy molecule)* synthase (textbook).

The **filament** rotates by means of the motor at the **base** (rotor plus Mot proteins). [L15 p16]

The motor is embedded in the layers of the cell envelope *(layers wrapped around the cell)* and has an axle and rotary parts made of specific proteins (textbook).

Stuck flagellum: when the flagellum is stuck to the glass slide, the motor turns the whole cell. [L15 p19]

A cell turned by a stuck flagellum spins in place. [L15 p19]

Section	Parts and numbers
Basal body	spans the membrane; about **15** different proteins; **1**-**4** rings in the membranes and cell wall (L, P, S, M); central rod (R)
Hook	hook protein and **2** HAPs
Filament	**14**-**20 nm** wide; up to **5**-**10** µm long; usually a single protein called flagellin; may have a different protein on the **tip** (cap protein)
Motor	rotor section plus Mot proteins; mediates movement of the flagellum

Table source: [L15 p13, L15 p14, L15 p15, L15 p16]

Part	Where it sits in the flagellum
MotA	in the cell membrane
MotB	in the cell membrane and reaching into the periplasm; forms part of the ion channel whose flux of hydrogen ions powers rotation (textbook)
H+ arrows	flow through MotA and MotB across the cell membrane
FliG	part of the C ring; forms part of the device that generates torque (rotary force) (textbook)
FliC	flagellin, the protein of the filament, which sticks out from the cell
Rotation	the whole flagellum rotates

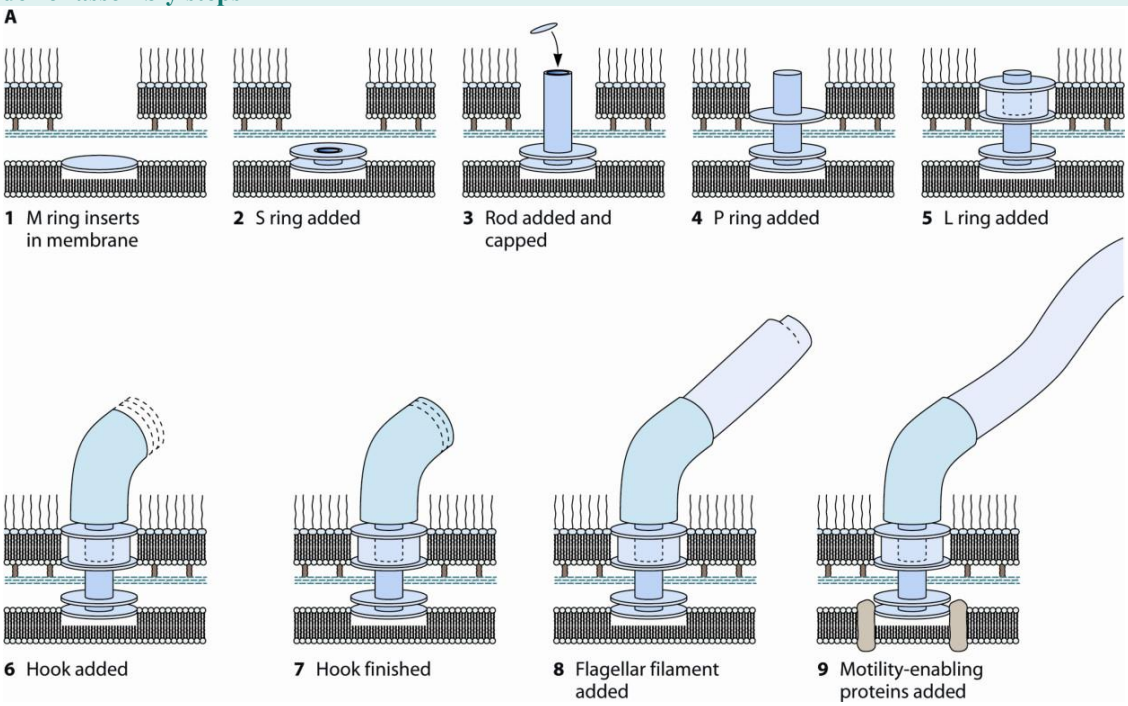
Table source: [L15 p12, L15 p16]

Order of assembly of flagellum

Book: 3.6 Specialized Structures, Rotary Flagella (chapter 3 PDF, p. 126).

⚠ **Do not mix up: Order: M, S, rod, P, L, hook, filament, Mot proteins *(motor proteins that turn the flagellum)* last.** [L15 p18]

Order of assembly steps



Nine assembly steps from M ring to motility proteins [L15 p17]

On the figure: M ring · S ring · rod · P ring · L ring · hook · filament · motility enabling

Order of assembly (steps **1**-**9**): **1** **M ring** inserts in the membrane. [L15 p17, L15 p18]

Where each ring goes: the M ring inserts into the cell membrane *(thin skin around the cell)*, the **S ring** stacks on top of it; the **P ring** sits at the peptidoglycan *(sugar and peptide mesh of the wall)* above the S ring; the **L ring** sits in the outer membrane *(extra membrane outside the wall)*. [L15 p16, L15 p18]

Order of assembly:

2 **S** ring added. **3** **rod** added and capped. **4** P ring added.
5 L ring added. **6** **hook** added. **7** hook finished.
8 flagellar **filament** added. **9** motility *(ability to move)* enabling (Mot) proteins added. [L15 p17, L15 p18]

Short summary of the steps

In short: rings of the basal body *(anchor of the whip in the envelope)* go into the cell membrane (**1** and **2**). [L15 p18]

In short: then the rod (**3**), then two outer membrane rings (**4** and **5**). [L15 p18]

Watch out: This summary calls both outer membrane rings, but the flagellum *(whip tail that spins)* diagram puts the P ring at the peptidoglycan and the L ring at the outer membrane. [L15 p18]

In short: then the hook (**6** and **7**), then the filament (**8**). [L15 p18]

In short: last, the motility proteins in the cell membrane (**9**). [L15 p18]

Assembly in plain terms

Assembly:

Two ring structures form in the cell membrane.
Two more rings (the P ring and the L ring) are then added.
The rings go in the cell wall *(stiff layer around the cell membrane)* layer or outer membrane.
The hook section is added.
The filament is formed.

Filament growth

Filament growth:

Flagellin *(protein that builds the whip)* subunits *(building block piece)* travel up through the core (hollow center) of the flagellum and are added at the **tip**.
Is by **extrusion** *(pushing out)* of flagellin subunits through the core of the flagellum. [L15 p18]

Assembly step	What happens
1 M ring	M ring inserts in membrane
2 S ring	S ring added
3 rod	rod added and capped
4 and 5 P ring, L ring	P ring added, then L ring added
6 and 7 hook	hook added, then hook finished
8 filament	flagellar filament added
9 Mot proteins	motility enabling (Mot) proteins added in the cell membrane, last

Table source: [L15 p17, L15 p18]

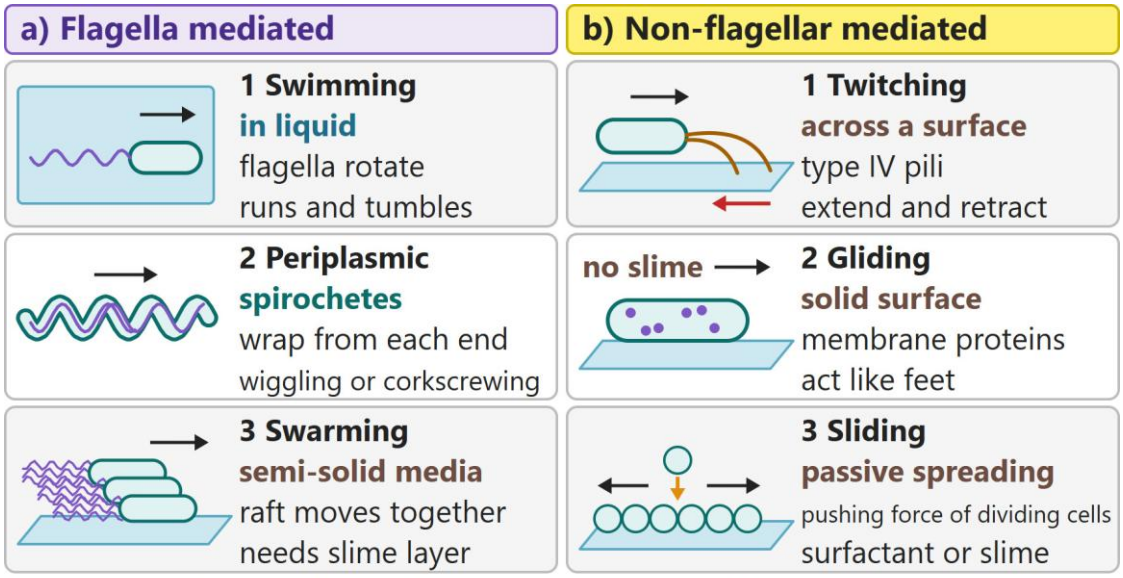
Types of motility observed in bacteria: know how each is mediated

Book: 3.6 Specialized Structures, Rotary Flagella (chapter 3 PDF, p. 126); 3.6 Specialized Structures, Pili and Stalks (chapter 3 PDF, p. 123).

⚠ **Do not mix up: Twitching *(jerky crawl using pili)*, gliding *(smooth crawl on a surface)* and sliding *(passive spreading by pushing)* are NOT flagella *(whip tails that spin)* mediated; swarming *(group moving on a surface using flagella)* and periplasmic *(in the space between the two membranes)* flagella are.**

What motility is

Motility *(ability to move)*: purposeful movement in a liquid or across a surface. [L15 p22, L15 p25]



Six motility types split into flagella mediated and non-flagellar mediated [L15 p25]

On the figure: flagella mediated · non-flagellar mediated · swimming · swarming · Twitching · gliding · sliding · periplasmic

Motility splits into **flagella mediated** and **non-flagellar mediated** types. [L15 p22, L15 p25]

Flagella mediated

Flagella mediated: (**1**) typical flagellar **swimming**, (**2**) **periplasmic** flagella, (**3**) **swarming** on a surface. [L15 p25, L15 p34]

Corkscrew swimming of spirochetes *(long corkscrew shaped bacteria)* is flagella mediated.

Not flagella mediated

Non-flagellar mediated:

(**1**) **twitching** on a surface.

(**2**) **gliding** on a surface, (**3**) **sliding** on a surface. [L15 p25, L15 p34]

Not flagella mediated: twitching, gliding and sliding.

Type	Key feature
Swimming	in a liquid; typical flagella
Swarming	on a surface; flagella; a group (raft) moves together
Twitching	on a surface; pilus retraction
Gliding	on a surface; focal-adhesion complexes
Sliding	on a surface; spreading by growth

Table source: [L15 p25]

Type	Mediated by	Where and key facts
Swimming	typical flagella rotating	liquid; runs and tumbles; direction controlled by length of runs
Periplasmic flagella	flagella in the periplasm	spirochetes; flagella out from each end; wiggling or corkscrewing; good for burrowing into thick mucus
Swarming	flagella (position may change to lateral)	surface or semi-solid media; a group (raft) moves together; requires a slime layer
Twitching	type IV pili	surface; type IV pili thought to extend, attach and then shorten to pull the cell forward; several **Gram-negative** species
Gliding	thought to be motility proteins in the membrane that act like feet (mechanism not understood in all genera)	solid surface; over **12** genera; Myxococcus, Flavobacterium; no slime involved in Flavobacterium
Sliding	powered by the pushing force of dividing cells	passive spreading across a surface; needs surfactants or exopolysaccharide or protein slime layer; also described as spreading by growth

Table source: [L15 p25, L15 p29, L15 p31, L15 p33, L15 p35, L15 p36, L15 p37, L15 p38, L15 p39]

▸ Swimming motility mediated by flagella

Book: 3.6 Specialized Structures, Rotary Flagella (chapter 3 PDF, p. 126).

What swimming is

Swimming motility *(ability to move)*:

> Directional movement (taxis *(moving toward or away from a stimulus)*); examples of swimming cells are Escherichia coli and Pseudomonas aeruginosa.
>
> Is mediated by controlling the length of runs (flagella on). [L15 p26]

Cells sense a change in concentration. [L15 p26]

Then cells decide to keep the flagella *(whip tails that spin)* on or turn them off. [L15 p26]

How flagella turn

Motility comes from the turning or spinning of the flagellum *(whip tail that spins)*.

Most flagella can turn in either direction.

Single polar flagellum

Single polar flagellum:

> The cell turns it counterclockwise (**CCW**) or clockwise (**CW**) for runs.
>
> To stop (tumble), it either stops turning or turns in the opposite direction. [L15 p27]

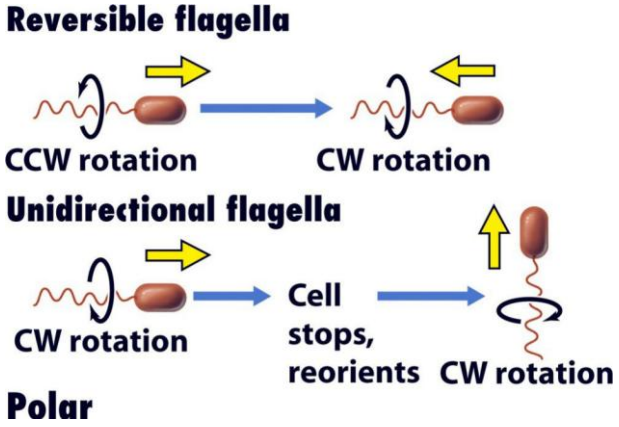
Reversible flagella: run **CCW** then **CW** (cell moves back the other way). [L15 p27]

Unidirectional flagella

Unidirectional flagella: one polar flagellum that turns one way **only**. [L15 p27]

It runs **CW**, the cell stops, reorients, then runs again with **CW**. [L15 p27]

This differs from multiple flagella, where **CCW** is the run. [L15 p27]



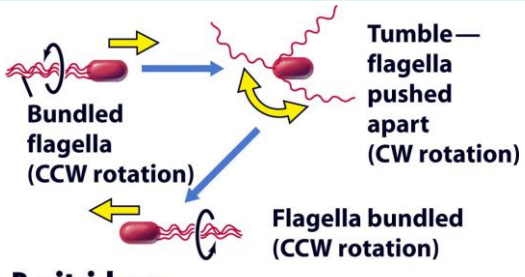
Single polar flagellum: reversible runs, or one direction then reorient [L15 p27]

On the figure: Reversible flagella · Unidirectional flagella · CCW · CW

Multiple flagella

Multiple flagella:

> Turning is coordinated.
>
> **CCW** gives forward movement (slower than a cell with a single flagellum).
>
> The opposite direction (**CW**) gives a stop (**tumble**).
>
> **CCW rotation** bundles the flagella for a run.
>
> **CW rotation** pushes them apart for a tumble *(stop and turn to a new direction)*. [L15 p28]



Many flagella: bundled for a run, pushed apart for a tumble [L15 p28]

On the figure: tumble · CCW rotation · CW rotation

▸ Periplasmic flagella in spirochetes

Book: 3.6 Specialized Structures, Rotary Flagella (chapter 3 PDF, p. 126).

⚠ **Do not mix up: Opposite rotation at the two ends gives translational swimming; same rotation gives nontranslational forms.** [L15 p30]

Spirochetes and their flagella

Periplasmic *(in the space between the two membranes)* **flagella** *(whip tails that spin)*: motility *(ability to move)* seen in spirochete bacteria. [L15 p11, L15 p29, L15 p30]

Spirochetes *(long corkscrew shaped bacteria)* have a **tightly coiled** spiral shape. [L15 p29]

Outer membrane *(extra membrane outside the wall)* **sheath** (**outer sheath**): surrounds the plasma membrane *(thin lipid layer)* and cell wall *(stiff layer around the cell membrane)* of a spirochete. [L15 p30]

The flagella sit in the periplasm *(space between the two membranes)* and extend out from each pole. [L15 p11, L15 p29, L15 p30]

Spirochete flagella wrap around the cell from each end.

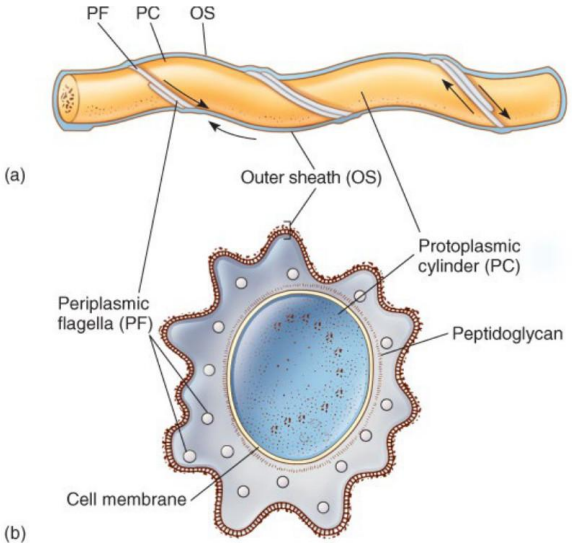
How the flagella move the cell

Turning of the flagella causes a wiggling or corkscrewing locomotion. [L15 p31]

Spirochete flagella produce a wiggling swimming motility.

This motility is good for burrowing into thick mucus. [L15 p31]

Direction of rotation of the flagella at each end determines the direction of motility. [L15 p30]

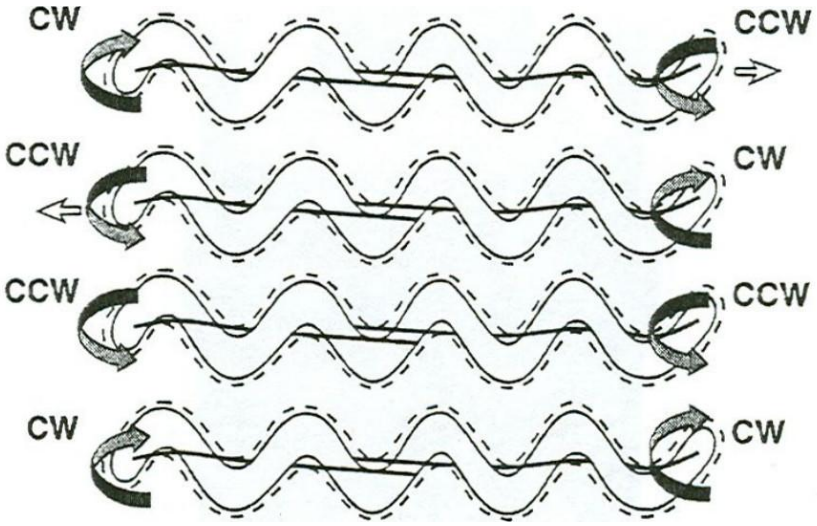


Spirochete with outer sheath and periplasmic flagella around the cell [L15 p11]

On the figure: outer sheath · Periplasmic flagella

B. burgdorferi

B. burgdorferi has between **7** and **11** **periplasmic flagella** attached at each end of the cell; the diagram shows **only** one per end, for simplicity. [L15 p30]



Rotation at each end and the direction the spirochete swims [L15 p30]

On the figure: CW · CCW

B. burgdorferi: opposite rotation at the two ends gives translational *(moving forward)* swimming. [L15 p30]

Opposite rotation means **CW** at one end with **CCW** at the other. [L15 p30]

Translational swimming goes toward the **CCW** end; the reverse pattern swims the other way. [L15 p30]

Same rotation at both ends (**CCW** and **CCW**, or **CW** and **CW**) gives nontranslational forms. [L15 p30]

Part of a spirochete	What it is
Outer sheath (OS)	outer sheath that surrounds the plasma membrane and cell wall
Periplasmic flagella (PF)	located in the periplasm, beneath the outer sheath; wrap around the cell
Protoplasmic cylinder (PC)	inner part of the cell (PC); the flagella wrap around the cell
Peptidoglycan	layer next to the cell membrane
Cell membrane	the cell membrane of the spirochete

Table source: [L15 p11, L15 p31]

Rotation (left end, right end)	Direction of swimming	What the cell does
CW, CCW	right	swims toward the **CCW** end (right)
CCW, CW	left	swims toward the **CCW** end (left)
CCW, CCW	none	nontranslational: no net swimming
CW, CW	none	nontranslational: no net swimming

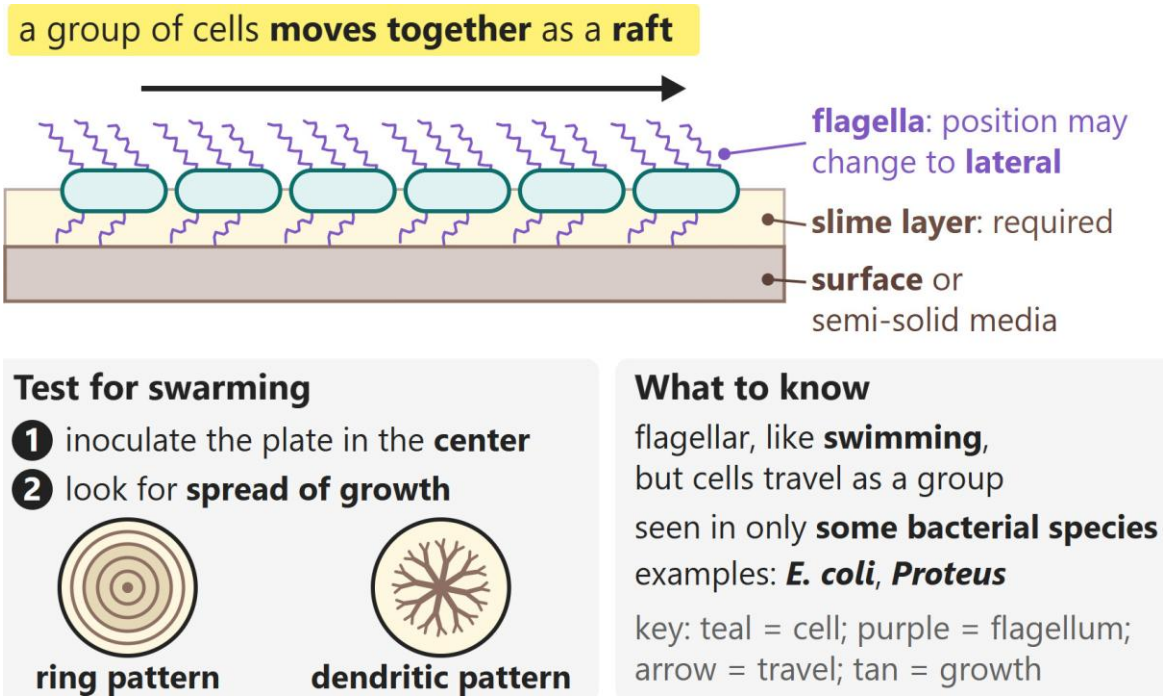
Table source: [L15 p30]

Swarming motility

Book: Not in the assigned sections (3.6, 25.2).

⚠ **Do not mix up: Swarming *(group moving on a surface using flagella)* is a group (raft) moving on a surface and needs a slime layer *(loose sticky coat outside)*.** [L15 p33]

What swarming is



Swarming raft on a slime layer, and ring and dendritic plate patterns [L15 p33]

On the figure: raft · lateral · slime layer · ring pattern · dendritic pattern · semi-solid media

Swarming:

Movement across a surface or **semi-solid media**.
Is mediated by flagella *(whip tails that spin)*, and their position may change to **lateral** *(along the side of the cell)*.
A group (**raft**) of bacteria moves together.
Requires a **slime layer**.
Is observed in some bacterial species. [L15 p33]
Examples: Escherichia coli and Proteus. [L15 p32]

Swarming Proteus

Swarming E. coli

Swarming test

Test: inoculate a plate in the center and look for spread of growth. [L15 p33]
Patterns shown: **ring pattern** and **dendritic pattern**. [L15 p33]

Gliding motility

Book: Not in the assigned sections (3.6, 25.2).

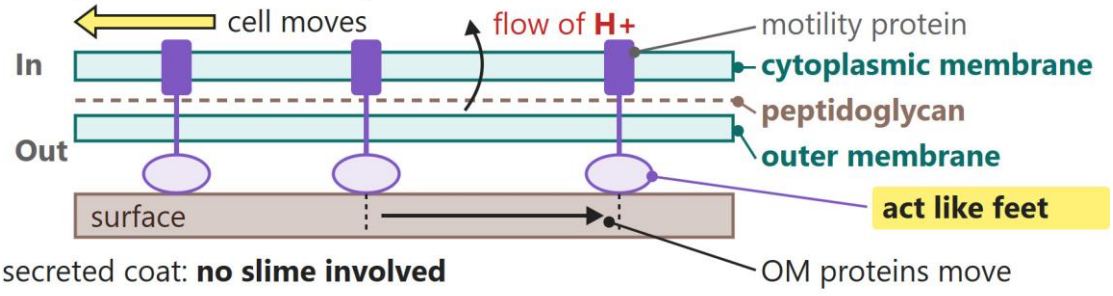
⚠ **Do not mix up: Gliding *(smooth crawl on a surface)* is NOT flagella *(whip tails that spin)* mediated, and Flavobacterium uses no slime.** [L15 p37, L15 p38]

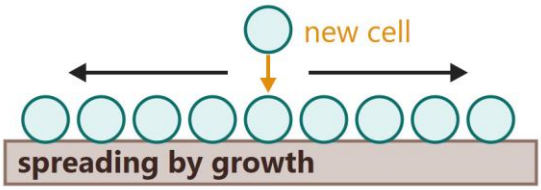
What gliding is

Gliding: across a **solid surface**; seen in over **12 genera**

example: *Myxococcus*; mechanism **not understood** in all genera

example: ***Flavobacterium*** **(Bacteroidetes)**





Sliding: a **passive** spreading powered by the pushing force of **dividing cells**; needs surface factors: **surfactants** or **exopolysaccharide** or **protein slime layer**

key: teal = cell parts; purple = proteins

Flavobacterium gliding on membrane proteins, and sliding by growth [L15 p38]

On the figure: Flavobacterium · cytoplasmic membrane · peptidoglycan · outer membrane · H+ · feet · surfactants · exopolysaccharide

Gliding:

- Motility *(ability to move)* across a solid surface. [L15 p37]
- Is not flagella mediated. [L15 p25]
- Is observed in over **12** genera of bacteria. [L15 p37]

The mechanism is not understood in all genera. [L15 p37]

Gliding examples

Gliding examples: Myxococcus and the Bacteroidetes **Flavobacterium**. [L15 p37, L15 p38]

Flavobacterium layers from inside to outside: **cytoplasmic membrane** *(thin lipid layer)*, **peptidoglycan** *(sugar and peptide mesh of the wall)*, **outer membrane** *(extra membrane outside the wall)*, then the surface. [L15 p38]

In **Flavobacterium**, **H+** flows through proteins in the cytoplasmic membrane. [L15 p38]

In **Flavobacterium**, motility proteins in the membrane are thought to act like **feet**. [L15 p37, L15 p38]

In Flavobacterium, no slime is involved. [L15 p37, L15 p38]

In **Flavobacterium**, outer membrane proteins move along the surface. [L15 p38]

In **Flavobacterium**, the movement of the cell is in the opposite direction from the movement of the outer membrane proteins. [L15 p38]

Sliding

Sliding *(passive spreading)* across a surface is powered by the pushing force of dividing cells and requires synthesis of additional **surface factors**: **surfactants** *(soap-like molecules)* or **exopolysaccharide** *(sugar chains outside the cell)* or a protein slime layer *(loose sticky coat outside)*. [L15 p39]

▸ Twitching motility

Book: 3.6 Specialized Structures, Pili and Stalks (chapter 3 PDF, p. 123).

⚠ Do not mix up: Twitching *(jerky crawl using pili)* uses type IV pili *(thin hairs on the cell)*, NOT flagella *(whip tails that spin)*.

What twitching is

Twitching:

- Movement across a surface.

Is mediated by a type of pili (type IV).
Is observed in several **Gram-negative** *(thin wall plus outer membrane)* bacteria species. [L15 p35]
Is not flagella mediated.

Twitching example: Pseudomonas aeruginosa moves across a surface using its type IV pili. [L15 p35]

Type IV pilus cycle

Type IV pili are thought to extend, attach and then shorten to pull the cell forward. [L15 p36]

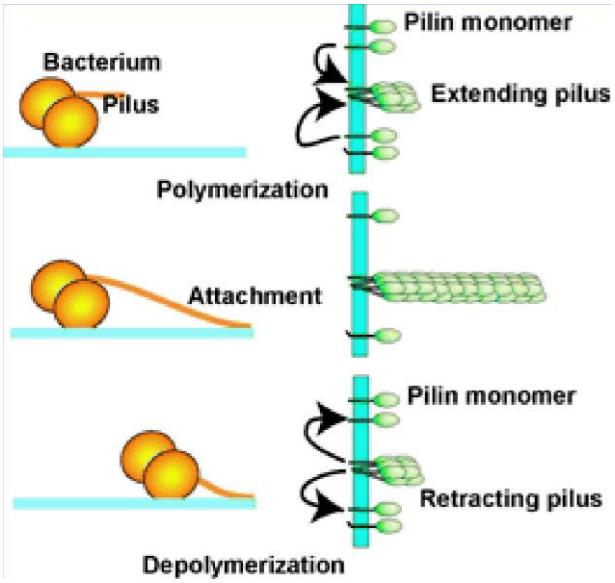
In twitching, the pili act as limbs that walk the bacterium across a substrate (textbook).

Type IV pilus *(thin hair for sticking or DNA swap)* **extension**: **polymerization** adds pilin *(protein that builds a hair)* monomers at the **base** of the pilus and extends it. [L15 p36]

Type IV pilus attachment: the pilus attaches. [L15 p36]

Type IV pilus retraction: **depolymerization** retracts *(pull back in)* the pilus. [L15 p36]

Retracting pilus: **pilin monomers** *(protein subunits)* are removed from the **base** of the pilus, so it shortens. [L15 p36]



Type IV pilus extends, attaches and retracts to pull the cell [L15 p36]

On the figure: pilin monomers · polymerization · depolymerization · attachment · Retracting pilus

What pili do

Pili:

Mediate attachment to surfaces.
Mediate transfer of DNA *(cell genetic instruction molecule)* between cells.

Sex pili of **Gram-negative** enteric bacteria attach a donor cell to a recipient cell for DNA transfer, called conjugation (textbook).

Pili mediate a form of motility *(ability to move)*.

Chemotaxis: what it

Book: 3.6 Specialized Structures, Rotary Flagella (chapter 3 PDF, p. 126).

⚠ **Do not mix up: Cells can do chemotaxis *(moving toward or away from chemicals)*** toward food but cannot move in a straight line to it.

Taxis and stimuli

Taxis *(moving toward or away from something)*:

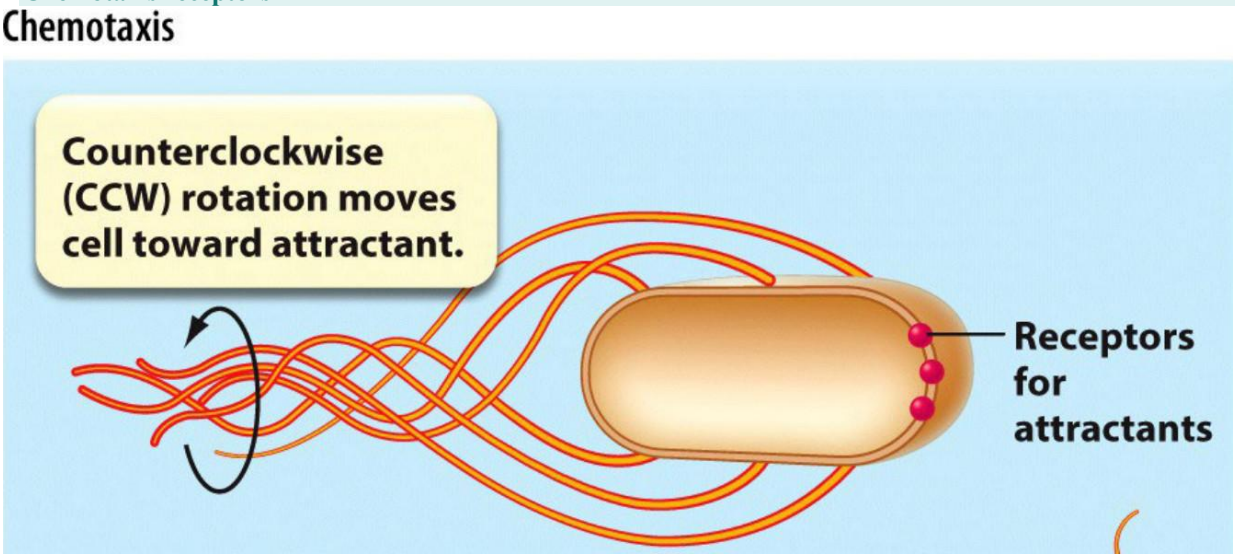
Movement in response to an external stimulus or effector *(outside signal that triggers a response)*.
Can be movement toward or away. [L15 p22, L15 p23]

A **wide variety** of substances and conditions affect motility *(ability to move)* and taxis. [L15 p23]

Stimuli that affect motility and taxis: chemicals (some nutrients), metals (many are toxic). [L15 p22, L15 p23]

More stimuli: light, temperature, pH, oxygen level. [L15 p22, L15 p23]

Chemotaxis receptors



Receptors for attractants at one pole; CCW rotation moves the cell toward the attractant [L15 p24]

On the figure: receptors for attractants · CCW rotation · chemotaxis

Chemotaxis:

> *(moving toward or away from chemicals)*: cells have receptors *(proteins a signal or virus binds to)* for attractants *(chemical that pulls cells in)* and repellents *(chemical that pushes cells away)*.
> The receptors send a signal that affects whether the flagella *(whip tails that spin)* turn. [L15 p24]

Chemoreceptors act like a nose, telling the bacterium when it is swimming toward a source of attractant (textbook).

Chemotaxis: CCW rotation moves the cell toward an attractant. [L15 p24]

Receptors for attractants sit at one pole of the cell, the front end as it swims toward the attractant. [L15 p24]

Arrays of chemoreceptors near a cell pole detect the attractant and repellent molecules (textbook).

The **CCW** flagella trail behind as one bundle. [L15 p24]

Runs and tumbles

No attractant or repellent: a series of runs (R) and tumbles (T). [L15 p26]

Path shape: runs (R) are the straight segments and tumbles (T) are the turns. [L15 p26]

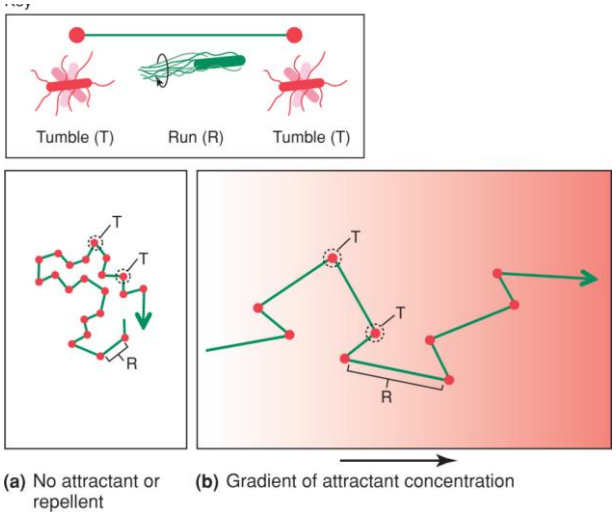
No attractant or repellent: the tumbles reorient the cell randomly. [L15 p26]

Gradient of attractant

Gradient *(gradual change in amount)* **of attractant**: the path ends up where the attractant is most concentrated, the highest concentration. [L15 p26]

In a gradient of attractant there are fewer tumbles and longer runs than with no attractant. [L15 p26]

Runs up the gradient last longer than runs down it. [L15 p26]



Runs and tumbles with no attractant and in an attractant gradient [L15 p26]

On the figure: No attractant or repellent · Gradient of attractant · tumbles · runs

Reaching food

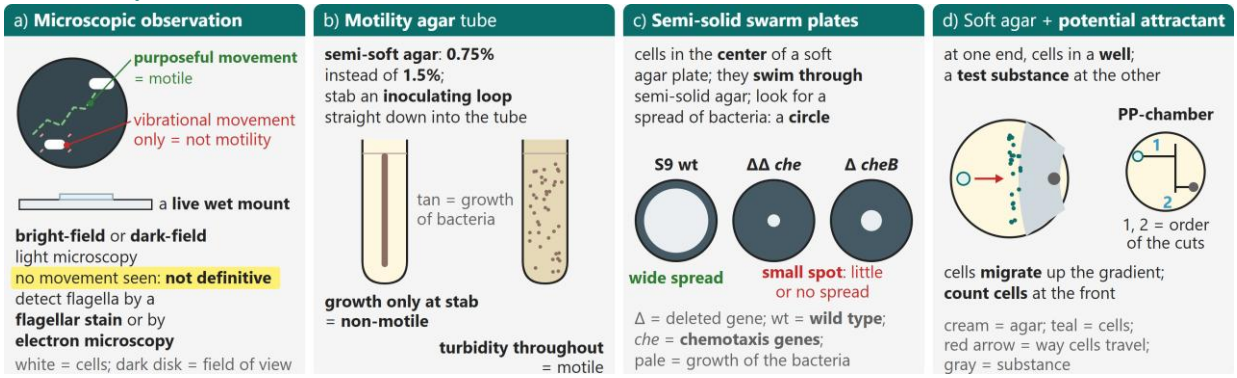
Chemotaxis: bacteria can move toward a desired food source.

Straight line: they cannot move continuously in a straight line to food.

How one could determine if a bacterium is motile and if it has flagella

⚠ **Do not mix up: Motility *(ability to move)* agar: growth only at the stab means non-motile; turbidity *(cloudiness of liquid)* throughout means motile.** [L15 p41]

Four assays



Four motility assays: microscope, agar tube, swarm plate, soft agar with attractant [L15 p41]

On the figure: microscopic observation · Motility agar · semi-solid swarm plates · soft agar · potential attractant · PP-chamber · turbidity · stab

Four assays:

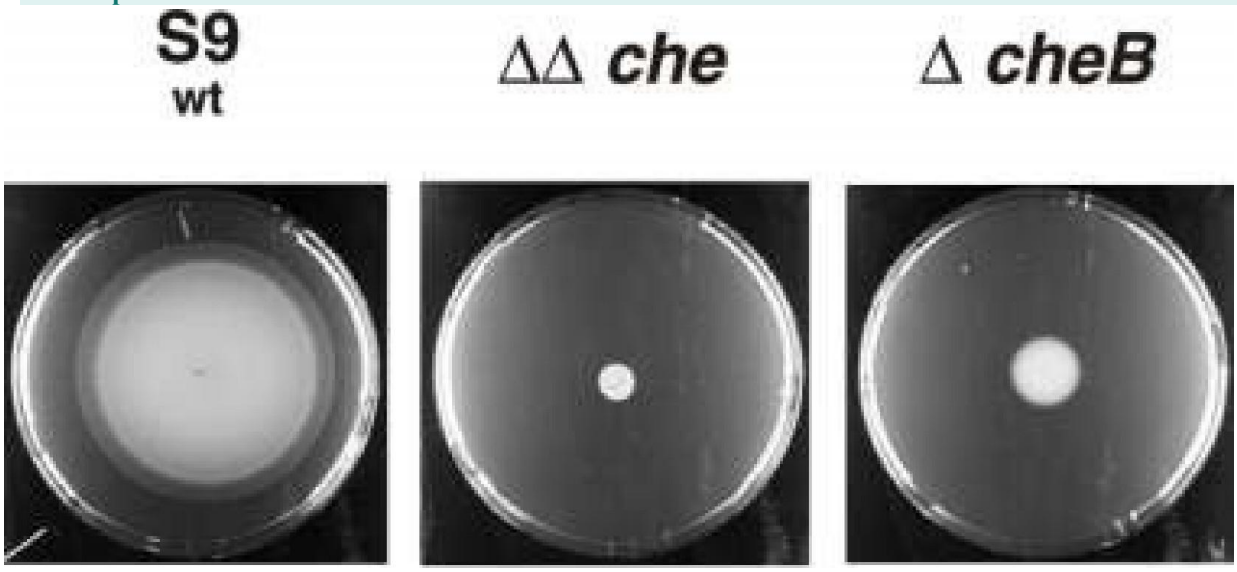
(a) microscopic observation, (b) motility agar.

(c) semi-solid swarm plates, (d) agar plates with soft agar and a potential attractant *(chemical that pulls cells in)*. [L15 p40, L15 p41, L15 p42, L15 p43]

Motility agar tubes

Motility agar tubes: growth **only** at the stab means non-motile; turbidity throughout means motile. [L15 p41]

Swarm plate results



Swarm plates: wild type spreads wide, che mutants stay a small spot [L15 p42]

On the figure: S9 wt · ΔΔche · ΔcheB

Swarm plate:

The wild type (S9 wt) spreads out in a large circle.

The che mutants (ΔΔche and ΔcheB) stay as a small spot. [L15 p42]

Agar plate techniques

PP-chamber version: the agar has cuts made in a sequence (**1, 2**) between the cells and the test substance. [L15 p43]

Agar-plate techniques: cells are counted at the test substance end. [L15 p43]

Soft agar plate with an attractant: cells are put in a well at one end and the test substance at the other; if it attracts them, the cells migrate toward the substance. [L15 p43]

Assay	How	Result and caution
Microscopic observation	bright field or dark-field light microscopy of live wet mounts	make sure the movement is purposeful and not just vibrational; lack of observed movement is not definitive
Seeing flagella	flagellar stain or electron microscopy	part of microscopic observation; shows whether flagella are present
Motility agar	semi-soft agar (**0.75%** instead of **1.5%**); stab an inoculating loop into a tube of media	growth **only** at the stab means non-motile; turbidity throughout means motile; not sure it detects all forms of motility
Semi-solid swarm plates	put cells in the center of a soft agar plate (bacteria can swim through semi-solid agar)	look for spread of bacteria as a circle; a wild type strain spreads widely, while che mutants stay as a small spot at the center
Soft agar with potential attractant	put cells in a well at one end and the test substance at the other end	count cells moving toward the test substance

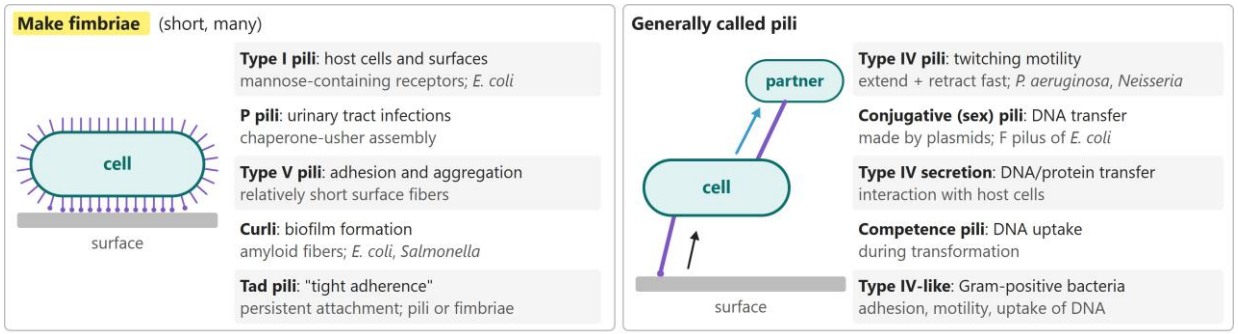
Table source: [L15 p40, L15 p41, L15 p42, L15 p43]

2. Pili and Fimbriae (3.6 and 25.2 6e)

Book: 3.6 Specialized Structures, Pili and Stalks (chapter 3 PDF, p. 123); 25.2 Microbial Attachment: First Contact (chapter 25 PDF, p. 36).

⚠ **Do not mix up: Fimbriae *(short sticky hairs)* are thinner than flagella *(whip tails that spin)***, not thicker or longer.

⚠ **Do not mix up: Fimbriae are the common pili (Type I, P, Type V, curli); Type IV and sex pili are generally called pili, NOT fimbriae.** [L16 p05, L16 p06]



Key: teal = cell; purple line = pilus; blue arrow = DNA moving; gray band = surface; gray text = features and examples

TRAP: sex pili are NOT fimbriae (not generally called fimbriae)

Types that make fimbriae and types generally called pili [L16 p08]

On the figure: Fimbriae · Type I · Type V · curli · Tad · Type IV · conjugative

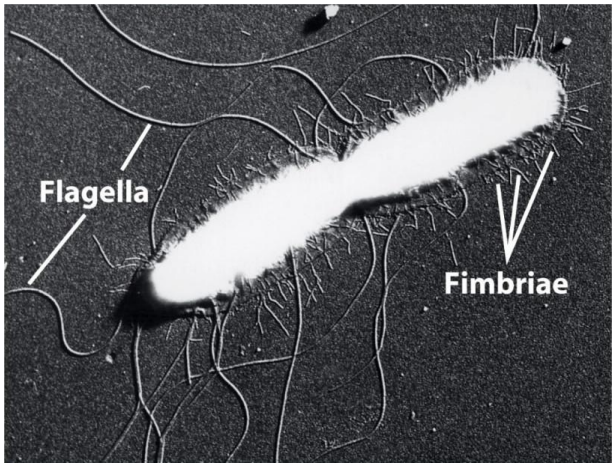
What they are

Pili and fimbriae are protein filament tubes that extend from the cell. [L16 p04]

They are thinner, shorter and more numerous than **flagella**. [L16 p04]

Several different types exist: **Type I**, P, **Type IV**, **Type V**, **curli**, **conjugative** (sex), Type IV secretion, competence *(ability to take up DNA)*, **Tad** *(tight adherence pili)*, and Type IV-like pili in **Gram positive** *(thick wall, stains purple)* bacteria. [L16 p04]

They are more commonly observed in **Gram negative** *(thin wall plus outer membrane)* bacteria. [L16 p04]



Flagella are long; fimbriae are short bristles along the cell [L16 p04]

On the figure: flagella · Fimbriae

Three types of pili

Pili form **3** types of structures, each with a different role: [L16 p04, L16 p05, L16 p06, L16 p09, L16 p10]

1) Common pili or fimbriae: adherence. [L16 p04, L16 p05, L16 p06, L16 p09, L16 p10]

2) Type IV pili: twitching *(jerky crawl using pili)* motility *(ability to move)*. [L16 p04, L16 p05, L16 p06, L16 p09, L16 p10]

3) Conjugative, sex or F pili: DNA *(cell genetic instruction molecule)* transfer. [L16 p04, L16 p05, L16 p06, L16 p09, L16 p10]

Roles and comparison with flagella

Flagella, capsule *(firm sugar coat outside the cell)* and S-layer *(crystal like protein coat outside)* do not mediate all three: flagella give motility, while capsule and S-layer roles are attachment and protection.

Pili vs fimbriae

	Fimbriae (common pili)	Other pili (Type IV, sex pili and more)
What it is	Fine, hairlike protein filament tubes from the cell surface	Protein filament tube; the DNA transfer pilus is an elongate, hollow appendage
Main role	Adherence to other cells and surfaces; biofilm	Type IV: twitching motility, DNA uptake. Sex (F) pili: DNA transfer by conjugation
Width	**3-10 nm**	Sex pili: about **7 nm** (flagellum **14-20 nm**)
How many per cell	Numerous: **100**s per cell	Sex pili: **only** a few (<**10**)
Key part or action	Adhesin protein at the **tip** binds specific molecules (usually sugars)	Type IV: extend, attach, then shorten (retract). Sex pilus: shortens to bring cells close
Types with this name	Type I, P, Type V, curli (Tad: can be called pili or fimbriae)	Type IV, conjugative (sex), Type IV secretion, competence, Type IV-like in **Gram positive**

Table source: [L15 p04, L16 p04, L16 p05, L16 p06, L16 p08, L16 p09, L16 p10, L16 p11]

a. Structure, arrangement in cells and assembly

Book: 3.6 Specialized Structures, Pili and Stalks (chapter 3 PDF, p. 123); 25.2 Microbial Attachment: First Contact (chapter 25 PDF, p. 36).

⚠ **Do not mix up: Pili *(thin hairs on the cell)* add subunits *(building block piece)* at the base; flagella *(whip tails that spin)* add flagellin *(protein that builds the whip)*** at the tip. Do not flip them.

⚠ **Do not mix up: The pilus *(thin hair for sticking or DNA swap)* tip is NOT made of the filament protein: it is 1-2 proteins different from the filament.** [L16 p13, L16 p14]

Basic structure and size

Basic structure: protein filament tube extending from the cell. [L16 p04, L16 p06]

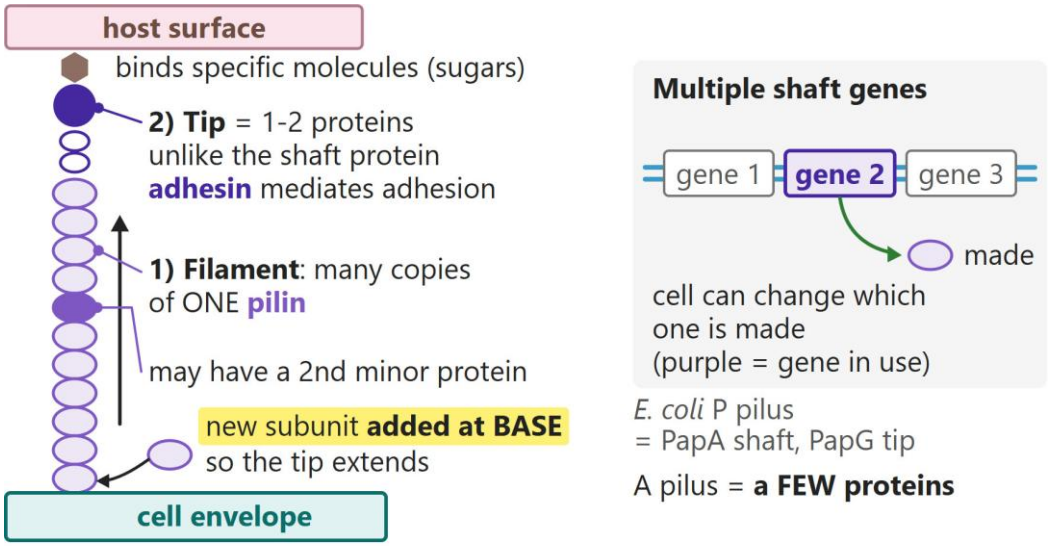
Common pili are thinner and shorter than flagella. [L16 p04, L16 p06]

Diameter: **3-10 nm** for a common pilus vs **14-20 nm** for a flagellum *(whip tail that spins)*. [L16 p04, L16 p06]

Number per cell

Number per cell: common pili are numerous (**100**s per cell). [L16 p06, L16 p10, L16 p09]

Sex pili are few (<**10**). [L16 p06, L16 p10, L16 p09]



Pilus filament of pilin, adhesin at the tip, new subunits added at the base [L16 p10]

On the figure: Pilin · adhesin · tip · base · PapA · PapG

Pilin and tip protein

Composition, filament: mostly many copies of a single protein. [L16 p13]

It is called a **pilin** *(the protein subunit that makes the pilus)*. [L16 p13]

It may have a second minor protein. [L16 p13]

Composition, tip: **1-2 proteins** that are different from the filament. [L16 p13, L16 p14]

The **tip** (**adhesin**) protein mediates adhesion *(sticking to a surface)*. [L16 p13, L16 p14]

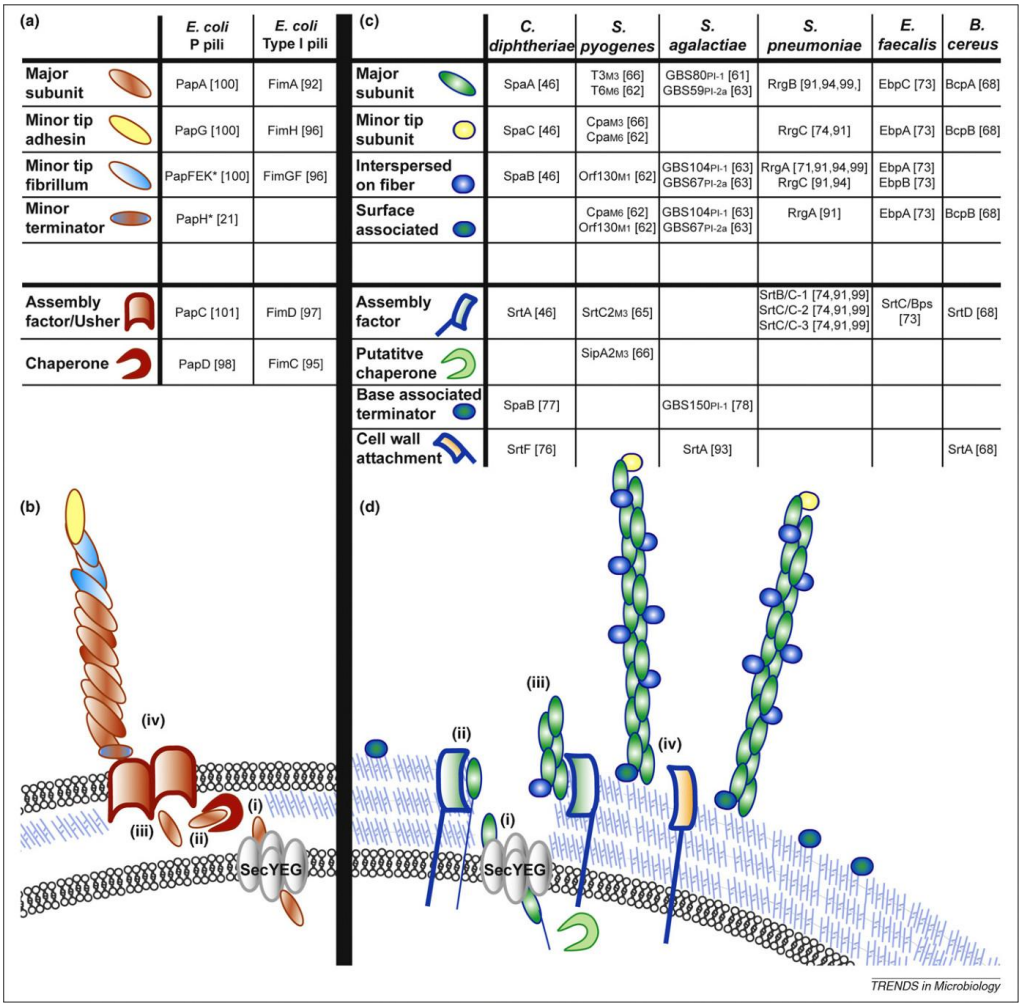
It binds specific molecules (sugars). [L16 p13, L16 p14]

Proteins that form a pilus

How many different proteins form a pilus? [L16 p12]

Answer: more than one protein: one major subunit plus a few minor subunits (**tip** adhesin and **tip** fibrillum at the **tip**, a terminator at the **base**). [L16 p12]

One major subunit is repeated many times. [L16 p12]



Pilus components of E. coli P and Type I pili and Gram positive pili [L16 p12]

On the figure: PapA · PapG · PapFEK · PapH · PapC · PapD · FimA · FimH

E. coli P pili:

Major subunit **PapA**; minor **tip** adhesin *(sticky protein for attaching)* **PapG**.

Also list minor **tip** fibrillum *(thin tip fiber)* **PapFEK** and minor terminator **PapH**.

Also have assembly factor/usher *(channel that assembles pili)* **PapC** and chaperone *(helper protein that guides others)* **PapD**. [L16 p12]

E. coli Type I pili:

FimA (major), **FimH** (**tip** adhesin), FimGF (**tip** fibrillum).

Also have usher FimD and chaperone FimC. [L16 p12]

Gram positive *(thick wall, stains purple)* pili also have a cell wall *(stiff layer around the cell membrane)* attachment factor. [L16 p12]

Gram positive sortase enzymes form a peptide *(short chain of amino acids)* bond from a cell wall cross-bridge to a protein extending from the cell (textbook).

Among the six **Gram positive** pili listed (C. diphtheriae, S. pyogenes, S. agalactiae, S. pneumoniae, E. faecalis, B. cereus), the **only putative chaperone** *(helper protein that guides others)* is **SipA2**, from S. pyogenes. [L16 p12]

Changing which pilin is made

Cells may have multiple different pilin genes. [L16 p14]

They can change which pilin is made. [L16 p14]

Assembly compared with flagellum

Assembly in general: assembly varies by type. [L16 p14, L16 p15, L16 p19]

Feature	Common pili / fimbriae	Sex (F) pili	Flagellum
Diameter	**3-10 nm**	**~7 nm**	**14-20 nm**
Number per cell	**100**s	**only** a few (<**10**)	not given
Length	thinner and shorter than flagella	longer than common pili and described as wider, though **~7 nm** falls inside the **3-10 nm** common pili range	longer than common pili

Table source: [L16 p06, L16 p10]

Gram positive pili	C. diphtheriae	S. pyogenes	S. agalactiae	S. pneumoniae	E. faecalis	B. cereus
Major subunit	SpaA	T3 and T6	GBS80 and GBS59	RrgB	EbpC	BcpA
Minor tip subunit	SpaC	Cpa	none listed	RrgC	EbpA	BcpB
Interspersed on fiber	SpaB	Orf130	GBS104 and GBS67	RrgA and RrgC	EbpA and EbpB	none listed
Surface associated	none listed	Cpa and Orf130	GBS104 and GBS67	RrgA	EbpA	BcpB
Base associated terminator	SpaB	none listed	GBS150	none listed	none listed	none listed
Assembly factor	SrtA	SrtC2	none listed	SrtB/C-**1**, SrtC/C-**2**, SrtC/C-**3**	SrtC/Bps	SrtD
Putative chaperone	none listed	SipA2	none listed	none listed	none listed	none listed
Cell wall attachment	SrtF	none listed	SrtA	none listed	none listed	SrtA

Table source: [L16 p12]

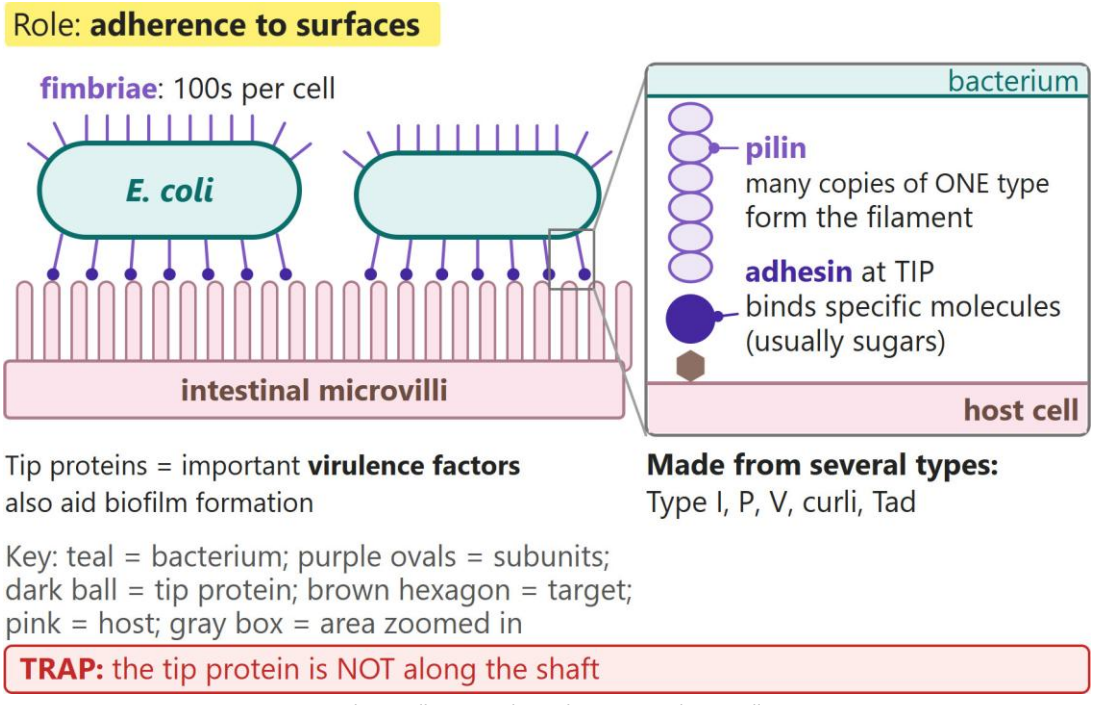
b. Roles of Common pili, Sex pili and Type IV pili

Book: 3.6 Specialized Structures, Pili and Stalks (chapter 3 PDF, p. 123).

⚠ **Do not mix up: Type IV pili *(thin hairs on the cell)* do twitching *(jerky crawl using pili)* motility *(ability to move)*; F (sex) pili do DNA *(cell genetic instruction molecule)* transfer by conjugation *(cells passing DNA by contact)*.** [L16 p09, L16 p10]

⚠ **Do not mix up: The adhesin *(sticky protein for attaching)* protein is at the TIP of the filament.** [L16 p08]

Common pili or fimbriae



Common pili: tip adhesins stick E. coli to intestinal microvilli [L16 p08]

On the figure: adherence · adhesin · tip · intestinal microvilli · virulence · biofilm · E. coli

Common pili or fimbriae *(short sticky hairs)*: role is **adherence** to surfaces. [L16 p07, L16 p08]

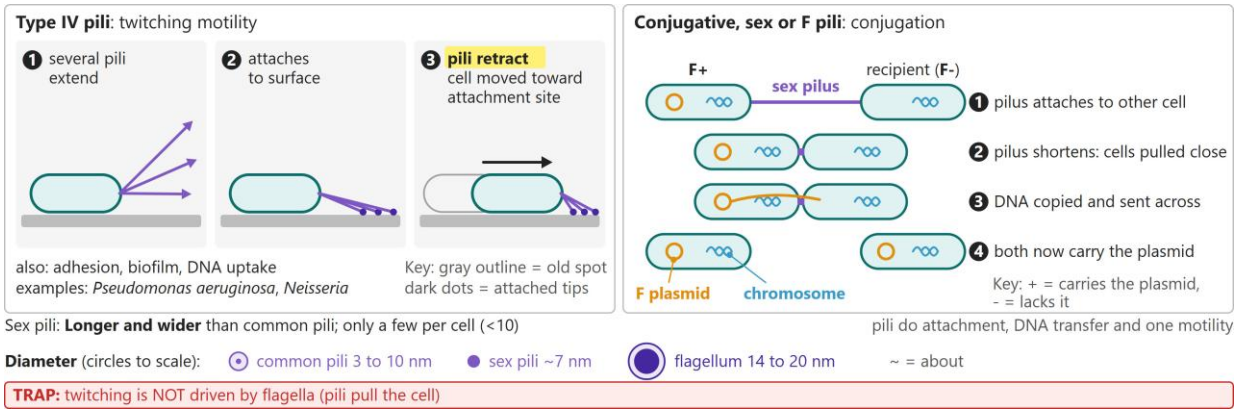
The **adhesin** protein at the **tip** binds specific molecules (usually sugars). [L16 p07, L16 p08]

Adhesins are important **virulence** *(ability to cause disease)* factors *(help germs cause disease)*. [L16 p07, L16 p08]

Common pili can also be important for formation of **biofilm** *(slimy community on a surface)*. [L16 p07, L16 p08]

E. coli cells stick to the **intestinal microvilli** *(finger-like folds of the gut lining)* of the host. [L16 p08]

Sex pili



Type IV pili pull the cell by twitching; sex pili pass the F plasmid across [L16 p09]

On the figure: Type IV · twitching · Conjugative · sex pilus · F plasmid · chromosome · Diameter · conjugation

Conjugative, sex or F pili: longer and wider than common pili. [L16 p10]

Diameter: sex pili **~7 nm**, common pili **3-10 nm**, flagellum *(whip tail that spins)* **14-20 nm**. [L16 p10]

Watch out: Sex pili are called wider than common pili, yet **~7 nm** falls inside the **3-10 nm** range. [L16 p10]

Number: **only** a few per cell (**<10**). [L16 p10]

Their role is DNA transfer by **conjugation** *(cell to cell DNA transfer)*. [L16 p10]

In **Gram negative** *(thin wall plus outer membrane)* enteric bacteria, sex pili attach a donor cell to a recipient cell for transfer of DNA (textbook).

Sex pilus steps and F plasmid

Sex pilus *(thin hair for sticking or DNA swap)* **steps**: **1.** the pilus attaches to another bacterial cell. [L16 p11]

Sex pilus step 2: the pilus shortens and brings the cells close together. [L16 p11]

Sex pilus step 3: DNA is transferred to the recipient cell. [L16 p11]

The **F plasmid** *(small extra DNA circle)* replicates. [L16 p11]

A copy goes from the F+ cell to the F- cell. [L16 p11]

Both cells end with the F plasmid. [L16 p11]

Each cell's **chromosome** *(the main DNA)* stays in its own cell. [L16 p11]

Type IV pili and twitching

Type IV pili: mediate **twitching motility** *(jerky crawling on a surface)*. [L16 p09]

Several pili extend, attach to a surface, and then shorten. [L16 p09]

This moves the cell in the direction of the attachment site. [L16 p09]

In twitching, the pili act as limbs that walk the bacterium across a substrate (textbook).

PilB/PilT sit at the **base** of the Type IV pilus, in the **cytosol** *(jelly inside the cell membrane)*. [L16 p09]

Watch out: another Type IV pilus figure names the **base** proteins PilF and PilT instead of PilB and PilT. [L16 p09, L16 p17]

Twitching motility is not mediated by flagella *(whip tails that spin)*.

Other pilus types

Other pilus types: Type V (adhesion and aggregation, short surface fibers). [L16 p05]

Curli: amyloid *(stacked protein fibers)* fibers, surface attachment, aggregation *(clumping together)*, biofilm; E. coli and Salmonella. [L16 p05]

Type IV secretion pili: DNA/protein transfer, interaction with host cells. [L16 p05]

Competence *(ability to take up DNA)* **pili**: DNA uptake during transformation *(cell taking up free DNA)*. [L16 p05]

Tad *(tight adherence pili)* **pili** ("tight adherence"): strong attachment and colonization. [L16 p05]

Type IV-like pili in Gram positive *(thick wall, stains purple)* **bacteria**: adhesion *(sticking to a surface)*, motility, DNA uptake. [L16 p05]

Which types are called fimbriae

Types of pilin *(protein that builds a hair)* **that form fimbriae**: Type I, P, V, curli, Tad. [L16 p07, L16 p08]

Terminology: Type I, P, V and curli form fimbriae. [L16 p05, L16 p07]

Tad "can be called pili or fimbriae". [L16 p05, L16 p07]

Type IV, Type IV secretion and competence pili are "generally called pili rather than fimbriae"; **Type IV-like pili in Gram positive bacteria** are "generally called pili". [L16 p05, L16 p07]

Conjugative pili are not generally called fimbriae. [L16 p05, L16 p07]

Pilus type	Major roles	Features / examples
Common pili (Type I, P, Type V, curli and Tad form fimbriae; Tad can be called pili or fimbriae)	Adherence to surfaces and host cells, biofilm	Adhesin at **tip** binds specific molecules (usually sugars); adhesins are important virulence factors
Type IV pili	Twitching motility, adhesion, biofilm, DNA uptake	Rapidly extend and retract; Pseudomonas aeruginosa and Neisseria
Conjugative (sex, F) pili	DNA transfer between cells by conjugation	Longer and wider than common pili; **only** a few per cell (<**10**); F pilus of E. coli

Table source: [L16 p05, L16 p07, L16 p08, L16 p09, L16 p10]

Pilus type	Major function	Feature
Type I pili	Adhesion to host cells and surfaces; biofilms	Forms fimbriae; E. coli type **1** fimbriae bind mannose-containing receptors
P pili	Adhesion, especially in urinary tract infections	Forms fimbriae; E. coli P fimbriae bind receptors on urinary tract cells
Tad pili	Strong surface attachment and colonization	"Tight adherence" structures important for persistent attachment
Conjugative (sex) pili	DNA transfer between bacterial cells	Produced by conjugative plasmids; the F pilus of E. coli is the classic example

Table source: [L16 p05]

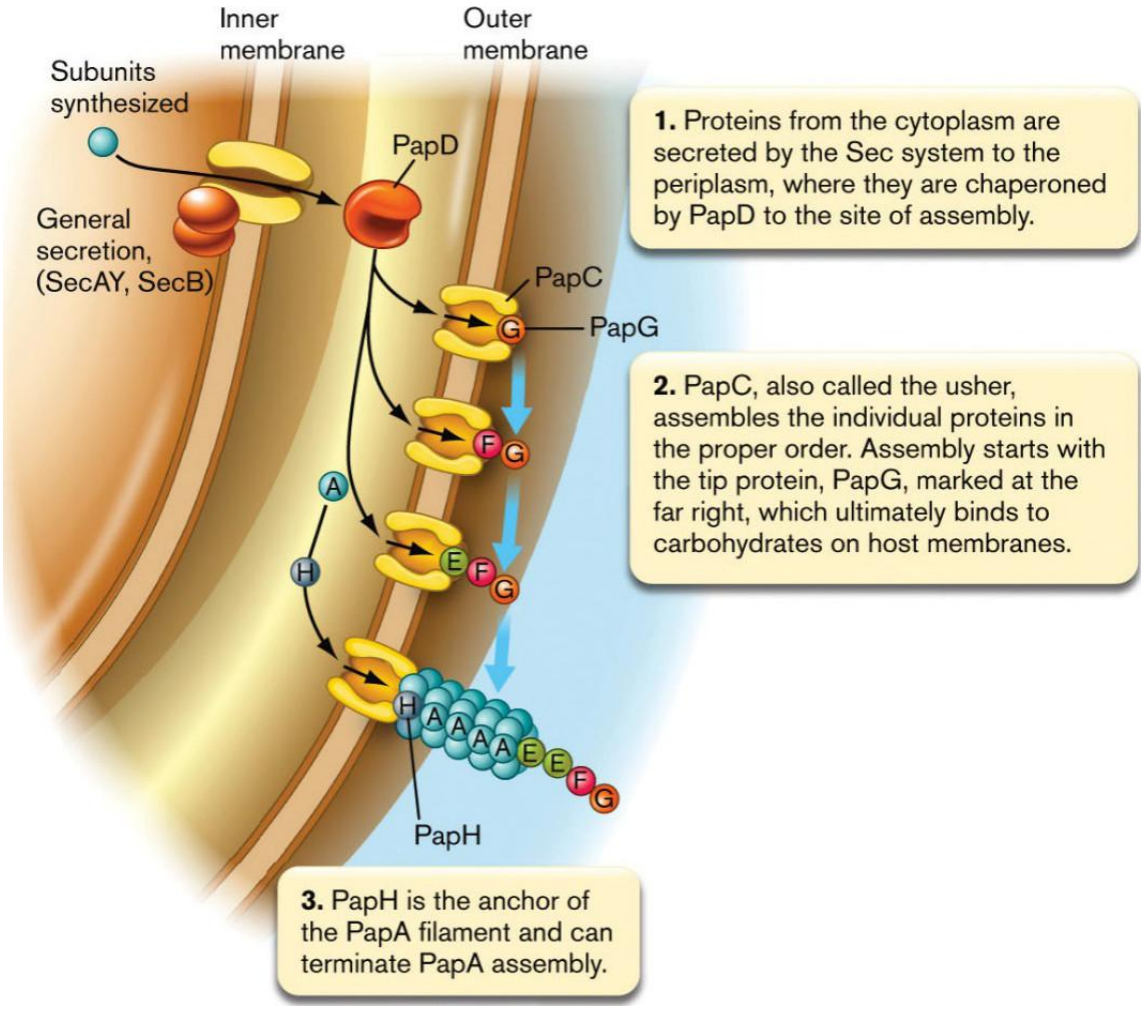
c. Assembly of Type I and Type IV, how it differs from flagellar

Book: 25.2 Microbial Attachment: First Contact (chapter 25 PDF, p. 36); 3.6 Specialized Structures, Pili and Stalks (chapter 3 PDF, p. 123).

⚠ **Do not mix up: Pili** ***(thin hairs on the cell)*** **subunits** ***(building block piece)*** **go on at the BASE; flagellin** ***(protein that builds the whip)*** **goes on at the TIP.** [L16 p19]

Type I pili assembly

Type I pili assembly has **4** steps. [L16 p16]



Type I pilus assembly: Sec system, chaperone PapD, usher PapC, tip PapG first [L16 p16]

On the figure: PapD · PapC · PapG · SecAY · SecB · inner membrane · outer membrane

Type I pili, step 1: proteins are secreted into the periplasm *(space between the two membranes)* by the Sec system *(protein export machine)*. [L16 p16]

The general secretion system includes **SecAY**, **SecB**. [L16 p16]

Type I pili, step 2:

- Proteins are bound by the chaperone (**PapD**).
- They then enter the usher *(channel that assembles pili)* channel (**PapC**). [L16 p16]

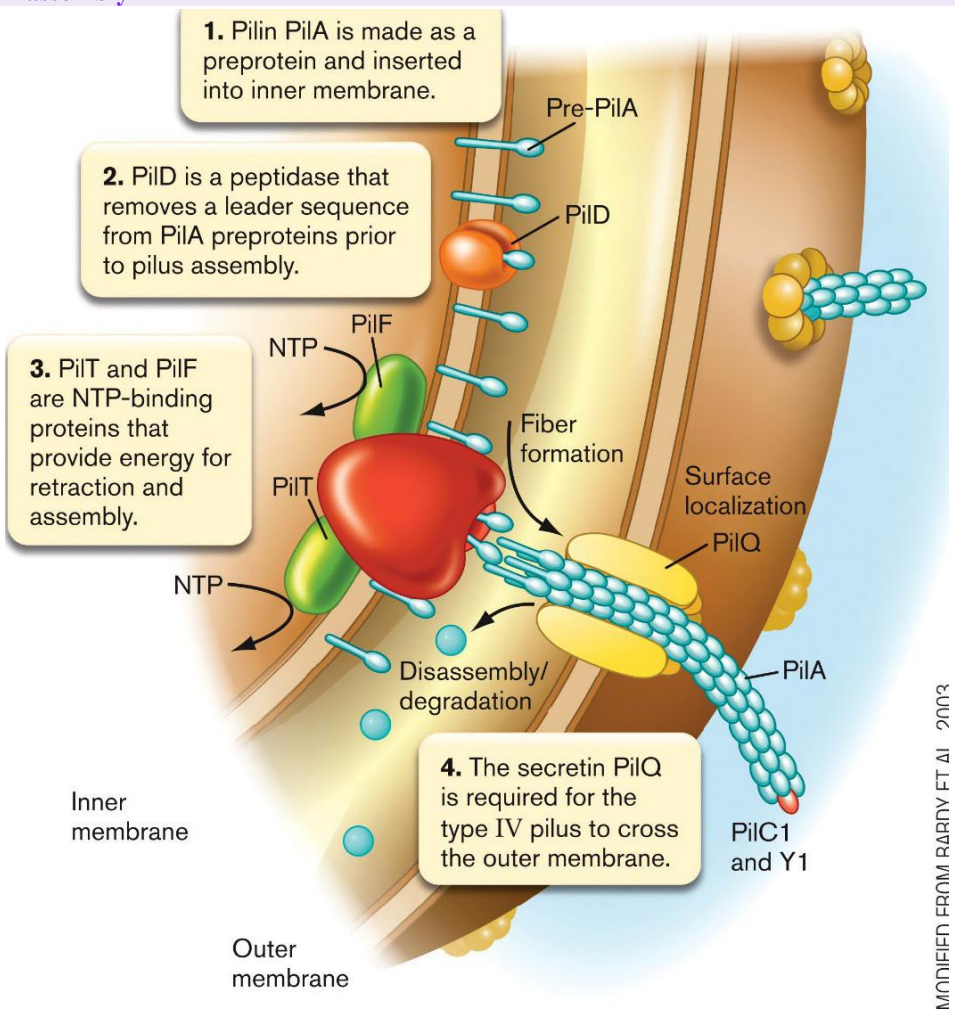
Type I pili, step 3: the pilus *(thin hair for sticking or DNA swap)* is assembled on the **outer membrane** *(extra membrane outside the wall)*. [L16 p16]

Type I pili, step 4: it starts with the adhesin *(sticky protein for attaching)* on the **tip**. [L16 p16]

The **tip** protein **PapG** binds **carbohydrates** *(sugars)* on host membranes *(thin lipid layer)*. [L16 p16]

Pilin *(protein that builds a hair)* proteins are then added to the **base** of the shaft to extend the filament. [L16 p16]

Type IV pili assembly



Type IV pilus assembly: PilA in inner membrane, PilT and PilF with NTP, PilQ channel [L16 p17]

On the figure: PilA · PilC1 · PilT · PilF · inner membrane · outer membrane

Type IV pili assembly has **3** steps. [L16 p17]

Type IV pili, step 1: pilin protein (**PilA**) forms a filament in the **inner membrane** *(the skin around the cytoplasm)*. [L16 p17]

PilC1 and Y1 sit at the **tip** of the Type IV pilus. [L16 p17]

Type IV pili, step 2: the assembled pilus is pushed through an **outer membrane** channel. [L16 p17]

Type IV pili, step 3: it shortens by disassembling pilin proteins from the **base**. [L16 p17]

PilT and PilF are NTP-binding proteins *(proteins that bind an energy molecule)* that provide energy for retraction and assembly. [L16 p17]

Pili versus flagellum filament

How the filament differs from a flagellum *(whip tail that spins)*: pili subunits are added to the **BASE**. [L16 p19, L16 p14, L16 p17, L16 p18]

This causes the filament to extend. [L16 p19, L16 p14, L16 p17, L16 p18]

In a pilus the **tip** is made first and goes out first; new pilin joins at the **base** and **pushes the tip out** *(the tip extends outward)*. [L16 p14, L16 p16, L16 p19]

Flagellum subunits (flagellin) are added to the **TIP**. [L16 p19, L16 p14, L16 p17, L16 p18]

They pass through the basal body *(anchor of the whip in the envelope)* and the hollow filament. [L16 p19, L16 p14, L16 p17, L16 p18]

Type IV pili can also retract *(pull back in)* by disassembly from the **base**. [L16 p19, L16 p14, L16 p17, L16 p18]

Flagellum assembly order

Flagellum assembly order: two ring structures form in the cell membrane *(thin skin around the cell)*.

The rod is added.

The rod is **capped** after it is added (step **3**). [L16 p18]

Then two more rings are added in the cell wall *(stiff layer around the cell membrane)* layer or outer membrane.

The **P ring** (step **4**) sits in the peptidoglycan; the **L ring** (step **5**) sits at the outer membrane. [L16 p18, L15 p14]

The hook is added.

The filament is formed.

The last step (**9**) adds the **Mot proteins** *(motility proteins, the motor parts)* in the cell membrane. [L16 p18]

Step / feature	Type I pilus (chaperone-usher)	Type IV pilus	Flagellum
Starts	Proteins secreted into the periplasm by the Sec system	Pilin PilA made as a preprotein (pre-PilA) and inserted into the inner membrane; PilD peptidase removes the leader sequence	Rings of the basal body inserted into the cell membrane (steps **1** and **2**)
Middle	Bound by chaperone (PapD); the proteins enter the usher channel (PapC)	Pilin assembled into a filament at the inner membrane; PilF and PilT NTP-binding proteins give energy for assembly and retraction	Rod added and capped (**3**), two outer rings added (**4** and **5**), hook added and finished (**6** and **7**)
Later steps	On the outer membrane at the usher (PapC)	Pushed through a channel (secretin PilQ) in the outer membrane	Filament added (**8**), then motility proteins added in the cell membrane (**9**)
How it grows	Adhesin **tip** protein (PapG) goes first, then pilins are added at the **BASE** of the shaft; PapH anchors and can end assembly	New pilin added at the **base**	Flagellin subunits pass through the hollow core and are added at the **TIP**
Retraction	Not stated	Yes, shortens by disassembling pilin from the **base**	Not stated

Table source: [L16 p16, L16 p17, L16 p18, L16 p19]

3. Other external structures

Book: 3.6 Specialized Structures, Pili and Stalks (chapter 3 PDF, p. 123).

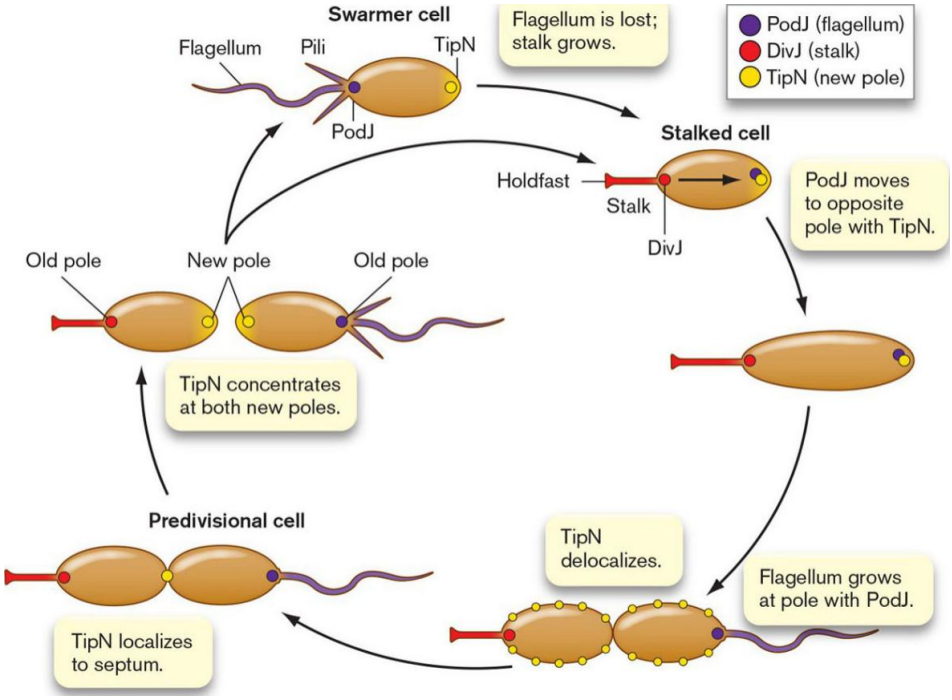
⚠ **Do not mix up: Stalks *(stem that anchors the cell)* are for adhesion *(sticking to a surface)*; nanotubes *(tiny tube joining two cells)* connect the cytoplasm *(jelly inside the cell membrane)* or periplasm *(space between the two membranes)* of two cells.** [L16 p20, L16 p21]

Others as an external structure

Stalks and nanotubes are "Others", the third kind of external structure. [L16 p03]

Stalks and Caulobacter cycle

Stalks (Caulobacter and other bacteria): used for adhesion. [L16 p20]



Caulobacter cycle: swarmer cell, stalk and holdfast, TipN and PodJ at the poles [L16 p20]

On the figure: swarmer · holdfast · PodJ · DivJ · TipN · predivisional cell · septum · new poles

Caulobacter life cycle: the **swarmer** *(swimming daughter cell)* cell has a flagellum *(whip tail that spins)* and pili *(thin hairs on the cell)*. [L16 p20]

Then the flagellum is lost and the stalk grows. [L16 p20]

The stalked cell has a **holdfast** *(sticky foot at the stalk tip)* at the stalk **tip**. The protein **DivJ** marks the stalk end. [L16 p20]

The **tip** of the stalk secretes adhesion factors that form the holdfast, which firmly attaches the bacterium (textbook).

Before division, the flagellum grows at the pole opposite the stalk (the pole with **PodJ**, the flagellum marker), and the stalked cell becomes a **predivisional cell**. [L16 p20]

The cell then divides; the end with the flagellum becomes the old pole of the new swarmer cell. [L16 p20]

The cycle gives a stalked cell and a **new swarmer** cell *(swimming daughter cell)*. [L16 p20]

TipN protein

TipN marks the new pole *(end of the cell made at division)*. [L16 p20]

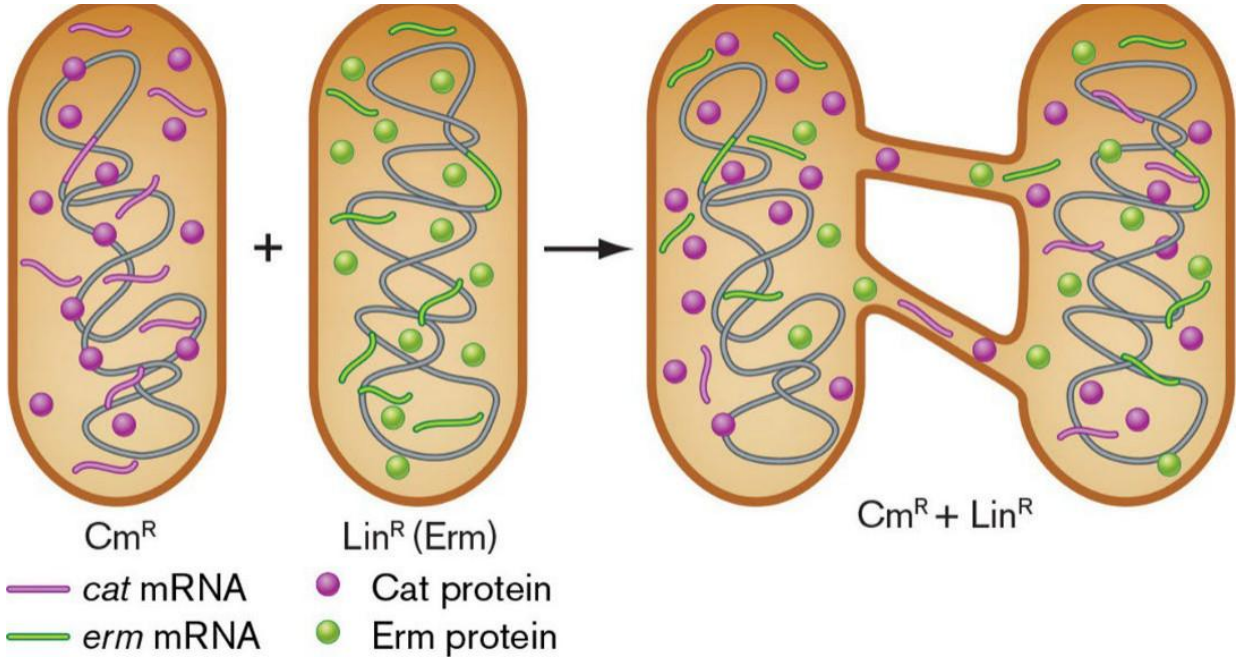
In the predivisional cell, TipN localizes to the **septum** *(dividing wall in the middle of the cell)*, then sits at both **new poles**. [L16 p20]

Nanotubes

Nanotubes: extension of the cytoplasm or periplasm between two different cells. [L16 p21, L16 p22]

They can be between different species. [L16 p21, L16 p22]

Components shared through nanotubes: protein and nucleic acids. [L16 p21, L16 p22]



Nanotube joins a Cm resistant and a Lin resistant cell, sharing mRNA and protein [L16 p22]

On the figure: Cm · Lin · cat mRNA · Cat protein · erm mRNA · Erm protein

> **Nanotube example**: a **Cm** resistant (CmR) cell and a **Lin** resistant (LinR, Erm) cell. [L16 p21, L16 p22]
> They join by a tube. [L16 p21, L16 p22]
> Each ends with both **cat mRNA** *(message copy of a gene)*/**Cat protein** and **erm mRNA**/**Erm protein**. [L16 p21, L16 p22]

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).

⚠ **Do not mix up: Mycobacteria *(waxy walled bacteria)* are Gram positive but have NO outer membrane *(extra membrane outside the wall)* with LPS *(outer membrane sugar and fat coat)***.

⚠ **Do not mix up: Lysozyme *(enzyme that cuts wall sugars)*** cuts the link between the sugars; penicillin forms a D-Ala-D-Ala like structure.

Cell envelope parts

Cell envelope *(layers wrapped around the cell)*: includes the **cell membrane** *(thin skin around the cell)*, **cell wall** *(stiff layer around the cell membrane)*, outer membrane and **glycocalyx** *(sugary coat outside the cell)*/capsule *(firm sugar coat outside the cell)*. [L16 p25]

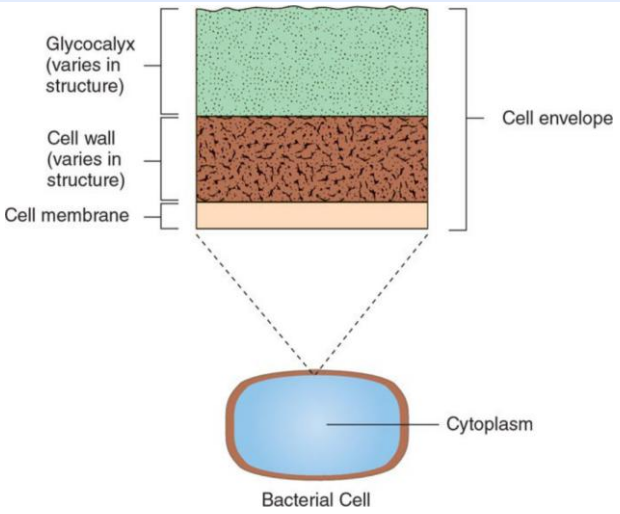
Different envelope styles are seen: **Gram positive**, **Gram negative** *(thin wall plus outer membrane)*, mycobacteria, Mycoplasma (mollicutes) and archaea *(single cell microbes without a nucleus, their own domain (also called archaeal bacteria))*. [L16 p25, L21 p28]

Each style has different layers and components. [L16 p25]

The envelope topic has **three parts**: the major **styles**, the **layers and components** of each, and **antibacterial chemicals and resistance**. [L16 p27]

Envelope style is a major way to group bacterial species. [L16 p25]

It is detectable by staining techniques and microscopy (Gram stain and acid fast). [L16 p25]

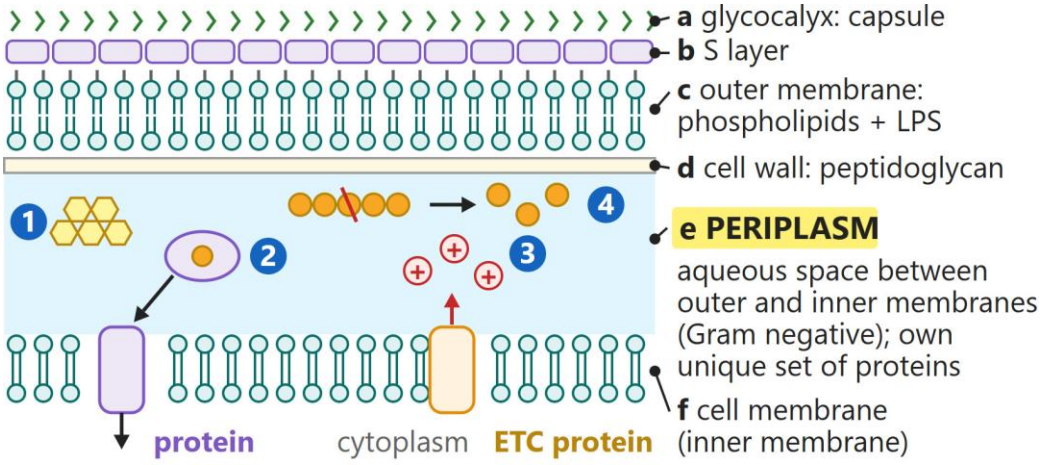


Cell envelope made of glycocalyx, cell wall and cell membrane around the cytoplasm [L16 p37]

On the figure: glycocalyx · cell wall · cell membrane · Cell envelope

Five envelope styles

Five envelope styles: **Gram positive**, Mycobacterial (**Gram positive** actinomycete, acid fast), **Gram negative**, **Mollicutes** (Mycoplasma, no cell wall), **Archaeal** *(of archaea)*. The envelope topic has three parts: styles, layers and components, and antibacterial chemicals and resistance. [L16 p27]



Main Functions

1 osmotic protection: holds oligosaccharides
2 transport: binding proteins bring solutes (food) to membrane transporters
3 oxidation-reduction reactions of the electron transport chain; stores H+ ions
4 hydrolysis: cuts nutrients (red slash) into subunits small enough to import

TRAP: the periplasm is NOT outside the cell; it lies inside the envelope

Gram negative layers outside to inside, with the periplasm between the membranes [L16 p36]

On the figure: glycocalyx · outer membrane · peptidoglycan · Periplasm · cell membrane · phospholipids · LPS

Gram negative layers

Gram negative envelope layers: **glycocalyx** or capsule (sugars or proteins).

Watch out: The **Gram negative** envelope list says capsule in some, made of sugar.

Outer membrane: lipopolysaccharide, proteins, **phospholipids** *(membrane fat with a charged head)*.

The outer membrane is porous to most ions *(charged particle)* and many small organic molecules but prevents the passage of proteins and other macromolecules (textbook).

Cell wall: **peptidoglycan** *(sugar and peptide mesh of the wall)*.

Periplasm *(space between the two membranes)*: part of the **Gram negative** envelope.

The periplasm lies between the outer and inner membranes *(the skin around the cytoplasm)*, includes the cell wall, and holds enzymes and nutrient transporters *(protein that carries molecules across)* not found within the cytoplasm (textbook).

Cell membrane: phospholipids and proteins.

Archaea and S-layer

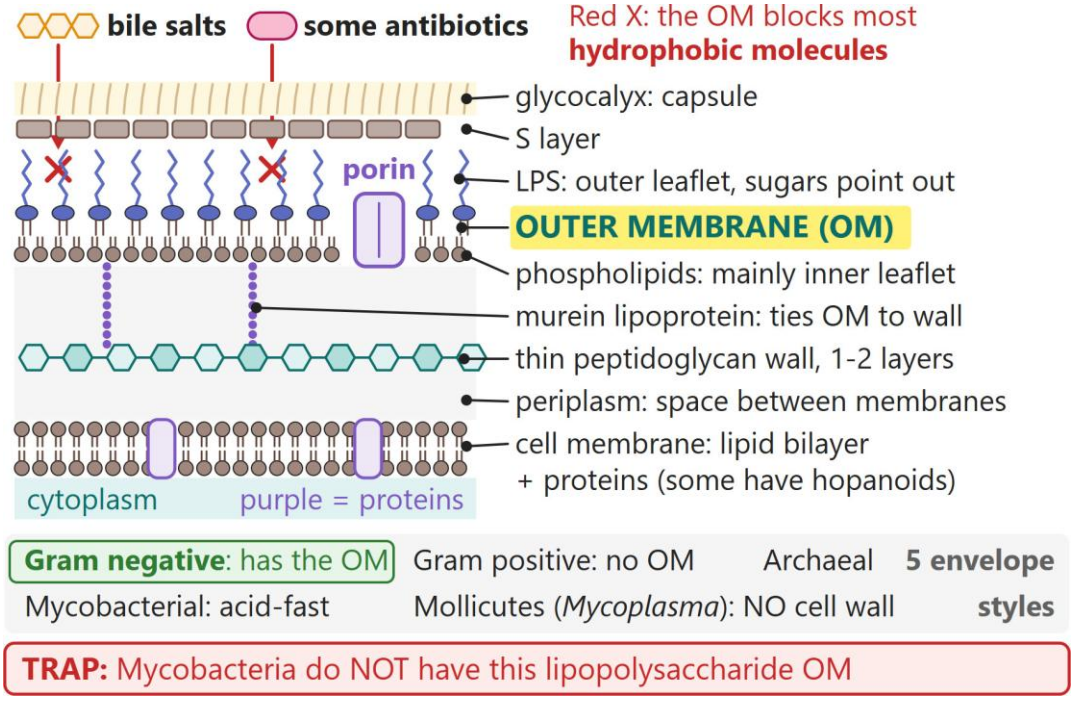
Archaea: no mycolic acid *(waxy fatty acid of mycobacteria)* in the envelope.

Pseudopeptidoglycan *(archaea version of wall mesh)* is in the cell wall of some archaea.

S-layer *(crystal like protein coat outside)* is a protein coat.

Layers outside to inside

Envelope layers, outside to inside: [L17 p04, L17 p30]



Envelope layers outside to inside, with the outer membrane blocking hydrophobic molecules [L17 p04]

On the figure: Glycocalyx · S layer · LPS · Outer membrane · phospholipids · Periplasm · Cell membrane

Glycocalyx (capsule). [L17 p04, L17 p30]

S layer. [L17 p04, L17 p30]

Outer membrane (**LPS** and **phospholipids**). [L17 p04, L17 p30]

Cell wall (peptidoglycan, pseudopeptidoglycan, teichoic acid, mycolic acids, arabinogalactan). [L17 p04, L17 p30]

Periplasm. [L17 p04, L17 p30]

Cell membrane (phospholipids, isoprenoids and hopanoids). [L17 p04, L17 p30]

Peptidoglycan, lysozyme and penicillin

Peptidoglycan: repeats of N-acetyl-glucosamine (NAG) and N-acetyl-muramic acid (NAM).

Lysozyme: a defensive enzyme of our body that cleaves the linkage between the sugars in peptidoglycan.

Penicillin: **only** works on growing cells making new wall.

S layer and archaeal envelope

S layer: a protein (or glycoprotein) coating on the outside of the cell wall or membrane, seen in some bacteria and archaea.

Pseudopeptidoglycan: found in the cell wall of some archaeal bacteria.

Archaeal envelope: isoprenoid *(branched five carbon repeat fat)* membrane, S layer often present, pseudopeptidoglycan in some, no mycolic acid.

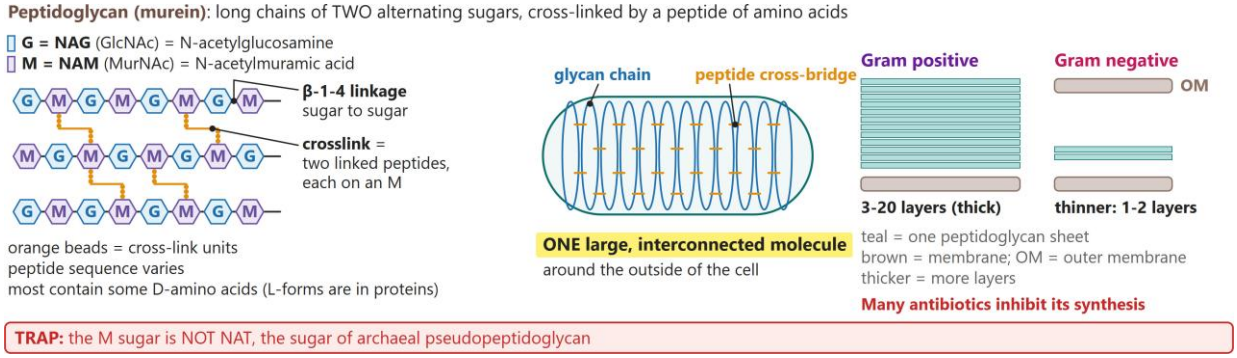
Envelope differences

Acid-fast *(holds dye despite acid wash)* **envelope**: no outer membrane with phospholipids and LPS; it has a mycomembrane of mycolic acids (the mycobacterial outer membrane) instead.

Staph vs Gram negative peptidoglycan: cross-link amino acids and number of layers differ.

Envelope overview

Cell envelope: layers and components; peptidoglycan synthesis and inhibition; antibacterial chemicals and resistance. [L18 p03]



Peptidoglycan: sugar chains of NAG and NAM joined by peptide cross-bridges [L18 p27]

On the figure: peptidoglycan · NAG · NAM

Peptidoglycan *(sugar and peptide mesh of the wall)* **synthesis** and inhibition. [L18 p03]

Antibiotics interfere with or inhibit peptidoglycan synthesis; **lysozyme** and autolysin *(wall-cutting enzyme made by the bacterium itself)* cleave peptidoglycan. [L18 p03]

Envelope layers

Envelope layers:

> Glycocalyx (capsule), S layer, outer membrane (LPS and phospholipids).
>
> Continued: cell wall (peptidoglycan, pseudopeptidoglycan, teichoic acid, mycolic acids, arabinogalactan). [L18 p04]

Mycobacterial envelope style (**Gram positive** actinomycete): **acid-fast** *(keeps its stain after an acid wash)*. [L18 p03]

Envelope layers continued: periplasm, cell membrane (phospholipids, isoprenoids and hopanoids). [L18 p04]

Peptidoglycan sugars and carrier

Peptidoglycan sugars: both sugar precursors (**NAM** and **NAG**) are made from fructose-**6**-P in the cytosol *(liquid part of the cytoplasm)*; then NAG *(one wall sugar)* is linked to NAM *(the other wall sugar)*.

Bactoprenol (C55): NAM-pentapeptide is transferred to it in the cell membrane; it helps to carry peptidoglycan units through the cell membrane.

Antibiotics and lysozyme

Lysozyme cleaves the linkage between the sugars in peptidoglycan (the beta-**1,4** bond between NAM and NAG).

Penicillin and cephalosporin both form a structure similar to D-Ala-D-Ala.

Confirming a Gram positive *(thick wall, stains purple)* **envelope**: test for sensitivity to a penicillin that is more effective against **Gram positive** but not **Gram negative**.

Transport and cell division

Active transport: ion coupled (an ion moving with its concentration gradient powers transport) or ATP *(cell energy molecule)* driven (cleavage of the high energy phosphate bond).

Envelope type	Envelope contents
Gram negative	outer membrane with LPS, thin peptidoglycan (**1-2 layers**), periplasm, cell membrane of phospholipids
Gram positive	thick peptidoglycan (**3-20 layers**), teichoic acids (most **Gram positive**), cell membrane; no outer membrane
Mycobacteria (acid-fast)	peptidoglycan, arabinogalactan, mycolic acids (mycomembrane), cell membrane
Mollicutes (Mycoplasma)	no cell wall
Archaea	pseudopeptidoglycan in some, isoprenoid lipids in the membrane

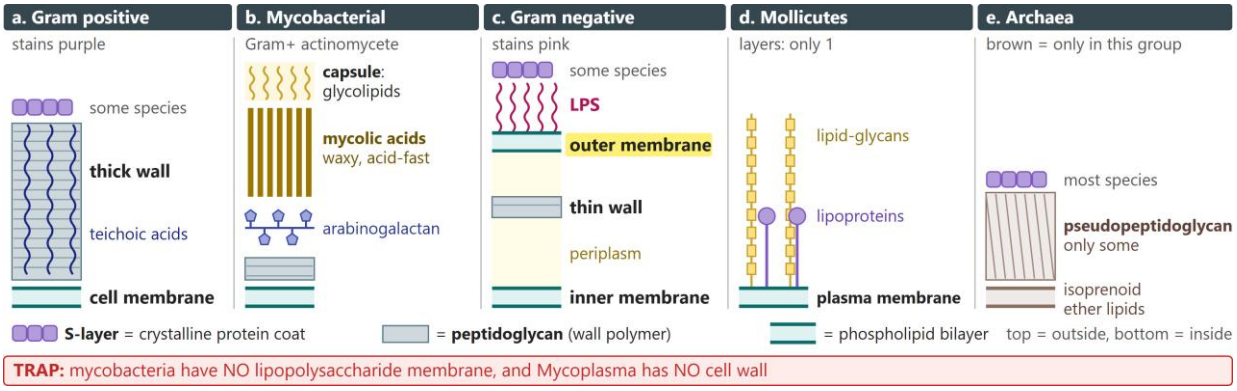
Table source: [L17 p03, L17 p05, L17 p15, L17 p24, L17 p26, L17 p28, L17 p29, L17 p39]

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).

⚠ **Do not mix up: Only Gram negative *(thin wall plus outer membrane)* has an outer membrane *(extra membrane outside the wall)* with LPS *(outer membrane sugar and fat coat)*; Gram positive and mycobacteria *(waxy walled bacteria)* have none.** [L16 p30, L16 p31, L16 p32]

Five envelope styles



Five envelope styles side by side, outside at top [L16 p27]

On the figure: Gram positive · Gram negative · Mollicutes · S-layer · LPS · teichoic acids · outer membrane · peptidoglycan

Five envelope *(layers wrapped around the cell)* **styles**: Gram positive, mycobacterial *(about waxy walled bacteria)*, Gram negative, Mollicutes *(bacteria with no cell wall)*/Mycoplasma, archaeal *(of archaea)*. [L16 p29]

What the styles share

Similar: **Gram positive**, **Gram negative**, mycobacteria, Mycoplasma and archaea *(single cell microbes without a nucleus, their own domain (also called archaeal bacteria))* all have a cell membrane *(thin skin around the cell)*. [L16 p30, L16 p31, L16 p32, L16 p34, L16 p43, L21 p29, L21 p30, L21 p31, L21 p32, L21 p35]

Gram positive, mycobacteria and **Gram negative** all have peptidoglycan *(sugar and peptide mesh of the wall)*. [L16 p30, L16 p31, L16 p32, L16 p34, L16 p43]

An S-layer *(crystal like protein coat outside)* can be found on **Gram positive** *(thick wall, stains purple)*, **Gram negative** and archaea. [L16 p30, L16 p31, L16 p32, L16 p34, L16 p43]

The **S-layer** is **NOT** on all bacteria. [L16 p30, L16 p31, L16 p32, L16 p34, L16 p43]

How they differ

Different: **only Gram negative** has an outer membrane with LPS. [L16 p30, L16 p31, L16 p32, L16 p33, L16 p34]

Only **Gram negative** has a periplasm *(space between the two membranes)* holding a thin peptidoglycan. [L16 p30, L16 p31, L16 p32, L16 p33, L16 p34]

Gram positive has a thick peptidoglycan with teichoic acids *(charged chains in Gram positive wall)*. [L16 p30, L16 p31, L16 p32, L16 p33, L16 p34]

Gram positive has no outer membrane. [L16 p30, L16 p31, L16 p32, L16 p33, L16 p34]

Mycobacteria add arabinogalactan *(chain of arabinose and galactose sugars in the wall)* and mycolic acids *(waxy fatty acids of mycobacteria)* to the wall. [L16 p30, L16 p31, L16 p32, L16 p33, L16 p34]

Mollicutes have no cell wall *(stiff layer around the cell membrane)*. [L16 p30, L16 p31, L16 p32, L16 p33, L16 p34]

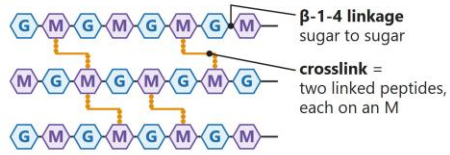
Archaea have no peptidoglycan (pseudopeptidoglycan or S-layer). [L16 p30, L16 p31, L16 p32, L16 p33, L16 p34]

Archaea have isoprenoid *(branched five carbon repeat fat)* membrane lipids. [L16 p30, L16 p31, L16 p32, L16 p33, L16 p34]

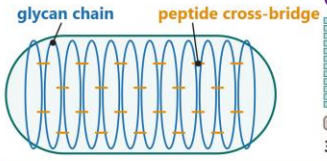
Peptidoglycan differences

Peptidoglycan (murein): long chains of TWO alternating sugars, cross-linked by a peptide of amino acids

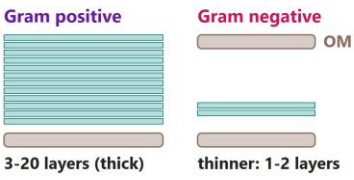
G = NAG (GlcNAc) = N-acetylglucosamine
M = NAM (MurNAc) = N-acetylmuramic acid



orange beads = cross-link units
peptide sequence varies
most contain some D-amino acids (L-forms are in proteins)



ONE large, interconnected molecule
around the outside of the cell



teal = one peptidoglycan sheet
brown = membrane; OM = outer membrane
thicker = more layers
Many antibiotics inhibit its synthesis

TRAP: the M sugar is NOT NAT, the sugar of archaeal pseudopeptidoglycan

Peptidoglycan chains cross-linked by peptides; thick in Gram positive, thin in Gram negative [L16 p27]

On the figure: peptidoglycan · glycan chains · 3-20 layers · 1-2 layers · Gram positive · Gram negative

Peptidoglycan thickness differs: **Gram positive** has **3-20 layers**, **Gram negative** is thinner with **1-2 layers**.

Peptidoglycan is made of parallel glycan *(sugar chain)* chains cross-linked by peptides *(short chain of amino acids)* of four to six amino acids (textbook).

Electron microscope appearance

The **Gram positive** envelope appears as a single dark line. [L16 p28]

A diffuse gray zone lies outside it (one thick wall layer). [L16 p28]

The **Gram negative** envelope appears as separate dark bands. [L16 p28]

Light spaces lie between the bands (outer membrane, thin wall and inner membrane). [L16 p28]

Envelope types: **Gram positive**, mycobacterial, **Gram negative**, Mollicutes (Mycoplasma), archaeal. [L21 p28]

Style	Layers, outside to inside	Cell wall	Outer membrane
Gram positive	glycosyl chains, S-layer, peptidoglycan with teichoic acids, cell membrane with membrane proteins	thick peptidoglycan (**3-20 layers**)	none
Mycobacteria (acid fast, Gram positive actinomycete)	capsule (glycolipids, sugar mycolates), mycolic acids on arabinan, arabinan, galactan, peptidoglycan, cell membrane	peptidoglycan plus arabinogalactan plus mycolic acid	none with LPS; Watch out: one envelope diagram calls the mycolic acid layer a mycomembrane (mycobacterial outer membrane)
Gram negative	S-layer (some), LPS, outer membrane with porins, lipoproteins, periplasm with thin peptidoglycan, inner membrane	thin peptidoglycan (**1-2 layers**) in the periplasm	yes, with LPS
Mollicutes (Mycoplasma)	plasma membrane **only**, with lipid-glycans and lipoproteins on its surface	none	none
Archaea	S-layer (and pseudopeptidoglycan in some), cell membrane of isoprenoids	pseudopeptidoglycan in some, S-layer **only** in others (Sulfolobus)	none

Table source: [L16 p27, L16 p30, L16 p31, L16 p32, L16 p33, L16 p34, L16 p36, L17 p15]

a. Gram positive bacteria

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

⚠ **Do not mix up: Gram positive *(thick wall, stains purple)* wall is thick (3-20 layers) and has NO outer membrane *(extra membrane outside the wall)*.** [L17 p15, L16 p30]

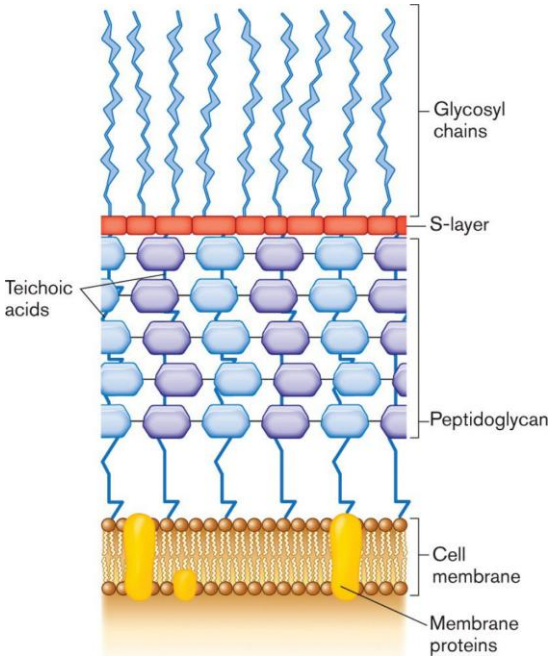
Layers outside to inside

Gram positive envelope *(layers wrapped around the cell)*: from outside to inside, glycosyl *(sugar group)* chains, **S-layer** *(crystal like protein coat outside)*. [L16 p30]

Next: **peptidoglycan** *(sugar and peptide mesh of the wall)* with **teichoic acids** *(charged chains in Gram positive wall)* running through it. [L16 p30]

Teichoic acid cross-threads and the thick wall help the **Gram positive** cell retain the Gram stain (textbook).

Last: **cell membrane** *(thin skin around the cell)* with **membrane proteins**. [L16 p30]



Gram positive envelope: glycosyl chains, S-layer, thick peptidoglycan with teichoic acids, membrane [L16 p30]

On the figure: glycosyl chains · S-layer · peptidoglycan · teichoic acids · cell membrane · membrane proteins

Thick wall, no outer membrane

Peptidoglycan is thick: **3-20 layers** in **Gram positive**. [L17 p15]

No outer membrane: a TEM of a **Gram positive** envelope shows this. [L16 p28, L16 p30]

It also shows a thick peptidoglycan layer. [L16 p30]

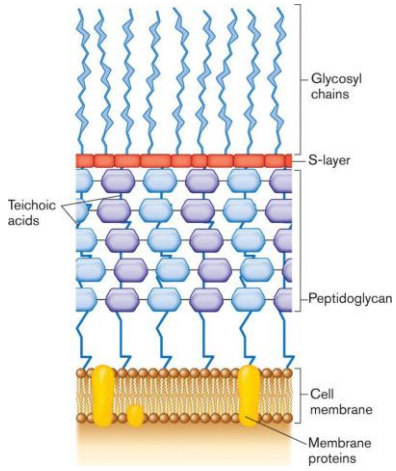
Gram positive has **only** one membrane (the cell membrane), under the peptidoglycan. [L16 p30]

Molecules to know

Cell wall *(stiff layer around the cell membrane)*: teichoic acids and peptidoglycan. [L16 p30, L16 p36]

Cell membrane: phospholipids *(membrane fat with a charged head)* and proteins. [L16 p30, L16 p36]

Gram positive capsule (some): sugar or proteins. [L21 p29]



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

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

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, 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).

⚠ **Do not mix up: Gram negative *(thin wall plus outer membrane)* peptidoglycan *(sugar and peptide mesh of the wall)* is thin (1-2 layers) and sits in the periplasm *(space between the two membranes)*.** [L17 p15, L16 p32]

Layers outside to inside

Gram negative envelop (C. crescentus): from outside to inside, **S-layer** *(crystal like protein coat outside)*. [L16 p32]

Next: **LPS** (with **Mg2+** ions between the chains). [L16 p32]

Magnesium ions *(charged particle)* form ionic cross-links that bridge the negatively charged LPS chains and give structural support (textbook).

Next: **outer membrane** *(extra membrane outside the wall)* with porin *(water filled channel in outer membrane)*. [L16 p32]

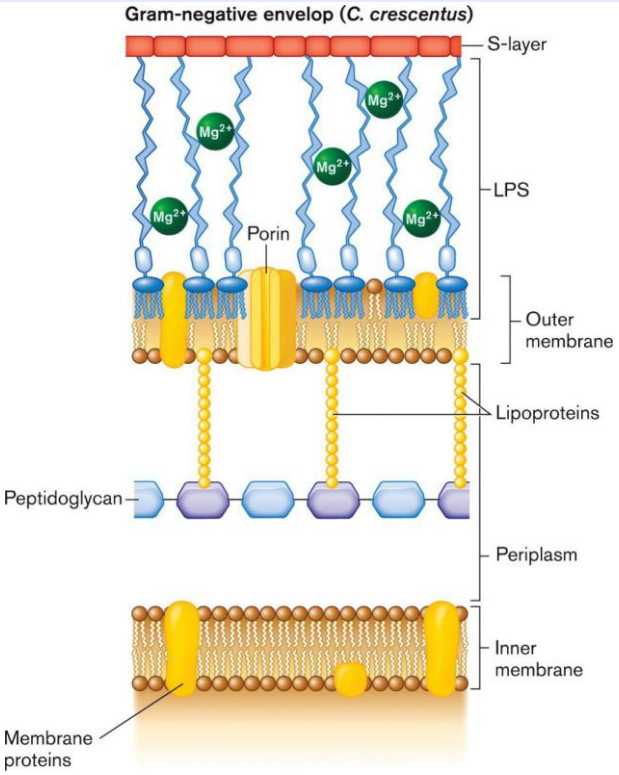
Porins *(channels in the outer membrane)* are outer membrane beta barrel channels that permit entry of nutrients such as sugars and peptides (textbook).

Next: **lipoproteins** *(proteins with fat tails)* linking the outer membrane to the **peptidoglycan**. [L16 p32]

Cells that fail to make murein lipoprotein *(protein anchoring outer membrane to wall)* cannot attach the outer membrane to the cell wall *(stiff layer around the cell membrane)*, so it balloons out where daughter cells separate (textbook).

Next: **periplasm** containing a thin peptidoglycan. [L16 p32]

Last: inner (cell) membrane *(thin lipid layer)* with membrane proteins. [L16 p32]



Gram negative envelope: S-layer, LPS, outer membrane, lipoproteins, periplasm, inner membrane [L16 p32]

On the figure: S-layer · LPS · Mg2+ · outer membrane · Porins · lipoproteins · periplasm · peptidoglycan

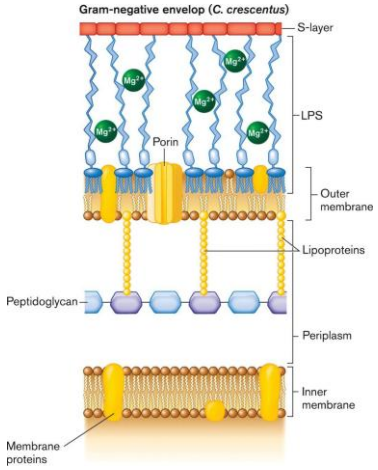
Peptidoglycan thickness

Peptidoglycan is thinner: **1-2 layers** in **Gram negative**. [L17 p15]

Gram positive *(thick wall, stains purple)* has **3-20 layers**. [L17 p15]

Outer membrane

Outer membrane: LPS and phospholipids *(membrane fat with a charged head)*. [L16 p36]



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 S layer (some): protein. [L21 p30]

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

Outer membrane (all): **LPS**, proteins, phospholipids. [L21 p30]

Gram negative cell wall (all): thin layer of **peptidoglycan**. [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).

⚠ **Do not mix up: Mycolic acid *(waxy fatty acid of mycobacteria)* is in the acid fast wall; an LPS *(outer membrane sugar and fat coat)* outer membrane *(extra membrane outside the wall)*** is NOT.

What mycobacteria are

Mycobacterial *(about waxy walled bacteria)* **envelope** *(layers wrapped around the cell)*: a **Gram positive** actinomycete *(branching thread bacterium)*. [L16 p27, L16 p31]

It is **acid fast** (detected by the acid fast stain). [L16 p27, L16 p31]

Mycolic acids *(waxy fatty acids of mycobacteria)* provide the basis for acid fast staining: the cells retain the dye carbolfuchsin (textbook).

Layers outside to inside

Mycobacterial layers, from outside to inside: **capsule** (glycolipids and sugar mycolates). [L16 p27, L16 p31]

The capsule also has phenolic glycolipid and phospholipid *(membrane fat with a charged head)*. [L16 p27, L16 p31]

Next: **mycolic acids**, attached to **arabinan** *(five carbon sugar chain)*. [L16 p27, L16 p31]

Next: arabinan, then **galactan** (mycolic acids, arabinan and galactan together are the cell wall core), then **peptidoglycan** *(sugar and peptide mesh of the wall)*. [L16 p27, L16 p31]

Last: the cell membrane *(thin skin around the cell)*. [L16 p27, L16 p31]

Wall sugars and mycolic acid

Arabinogalactan *(chain of arabinose and galactose sugars in the wall)*: sugar chains made of arabinan and galactan. [L16 p31, L16 p36]

Mycolic acid is in the cell wall *(stiff layer around the cell membrane)*. [L16 p31, L16 p36]

Other parts

Other parts: a **porin** (OmpA Tb) channel crosses the mycolic acid layer. [L16 p31]

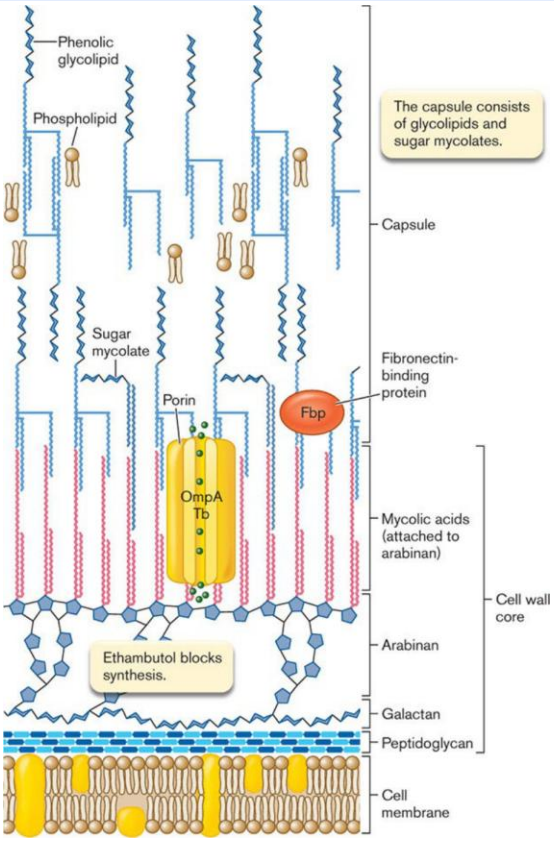
A fibronectin binding protein (**Fbp**) sits in the capsule. [L16 p31]

The drug **ethambutol** blocks arabinan synthesis. [L16 p31]

Acid fast envelope

Acid fast envelope: peptidoglycan, arabinan-galactan sugars, mycolic acid.

They also have a phospholipid bilayer *(two layers)* membrane.



Mycobacterial envelope: capsule, mycolic acids, arabinan, galactan, peptidoglycan, membrane [L16 p31]

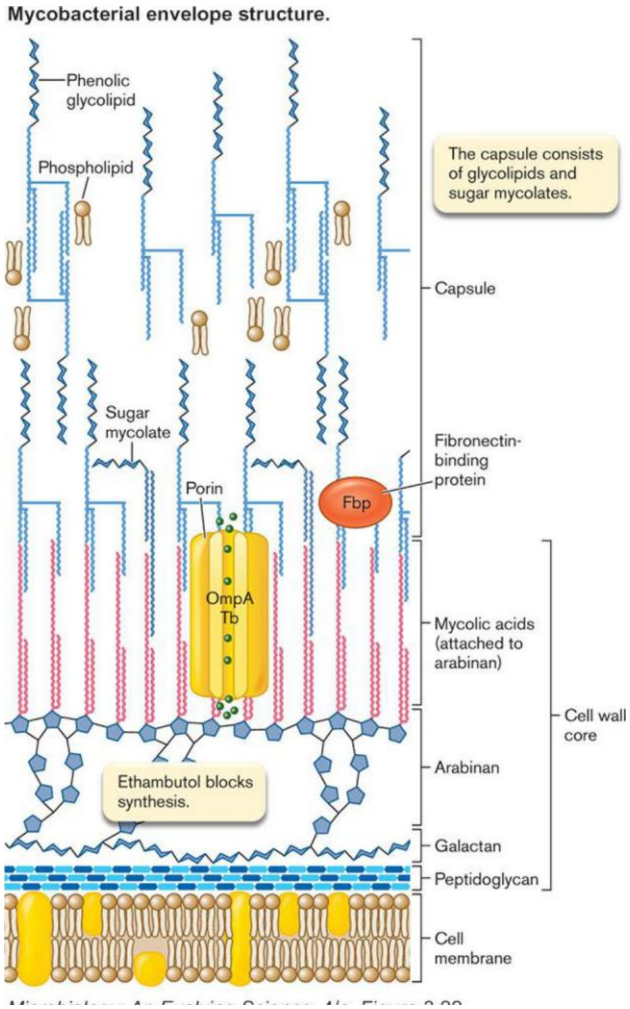
On the figure: capsule · Mycolic acids · arabinan · galactan · peptidoglycan · Fbp · porin · ethambutol

They do **NOT** have an outer membrane with phospholipids and LPS.

Watch out: One envelope diagram calls the mycolic acid layer a mycomembrane (mycobacterial outer membrane).

The thick waxy envelope excludes many antibiotics and retards nutrient uptake, so mycobacteria *(waxy walled bacteria)* grow extremely slowly (textbook).

Mycobacteria: **Gram positive** actinomycete. [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 capsule (all?): sugars and glycolipids. [L21 p31]

Mycobacterial cell wall (all): **mycolic acids**, arabinan-galactan (sugars), **peptidoglycan**. [L21 p31]

Mycobacterial cell membrane (all): phospholipids and proteins. [L21 p31]

Ethambutol blocks **arabinan** synthesis. [L21 p31]

d. Archaea (19.1 6e)

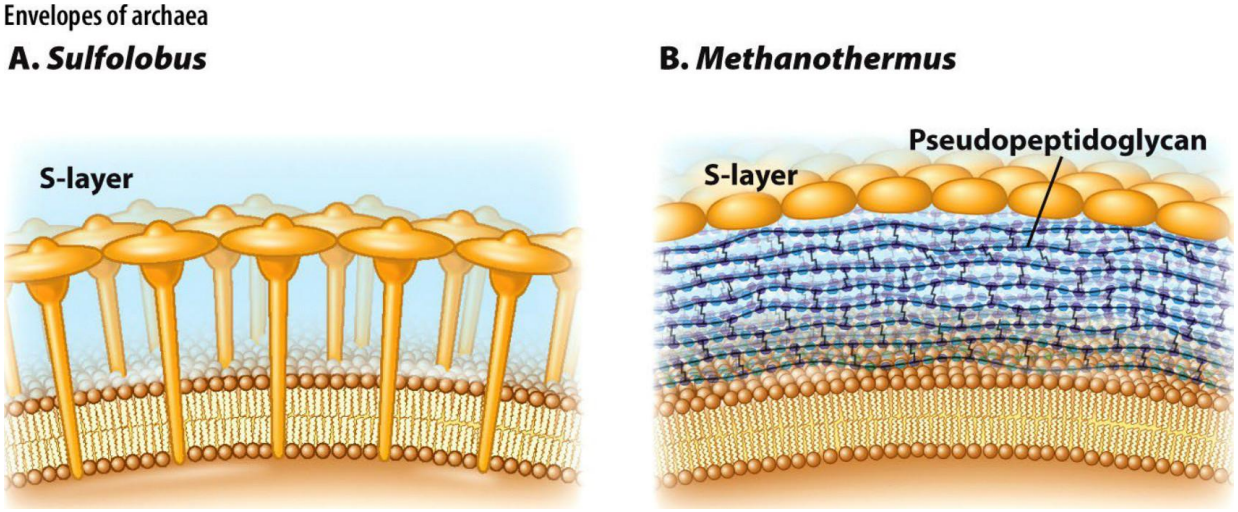
Book: 19.1 Archaeal Diversity at a Glance (chapter 19 PDF, p. 5).

⚠ **Do not mix up: Some archaea *(single cell microbes without a nucleus, their own domain (also called archaeal bacteria))* have pseudopeptidoglycan (others have only an S-layer); all archaea have isoprenoid *(branched five carbon repeat fat)* membranes *(thin lipid layer)*; none have mycolic acid *(waxy fatty acid of mycobacteria)*.**

S-layer in archaea

Most archaea have a crystalline *(neat repeating pattern)* S protein layer.

Archaea with no cell wall *(stiff layer around the cell membrane)* have just the S-layer *(crystal like protein coat outside)*. [L16 p36, L16 p43]



Archaeal envelopes: Sulfolobus with only an S-layer, Methanothermus with pseudopeptidoglycan [L16 p34]

On the figure: Sulfolobus · S-layer · Methanothermus · pseudopeptidoglycan

Archaeal *(of archaea)* **envelope** *(layers wrapped around the cell)*: **Sulfolobus** *(an archaeon)* has an **S-layer** held above the cell membrane *(thin skin around the cell)*. [L16 p34, L16 p43]

Sulfolobus has no cell wall. [L16 p34, L16 p43]

Methanothermus has an S-layer over a **pseudopeptidoglycan** layer over the cell membrane. [L16 p34, L16 p43]

Cell wall

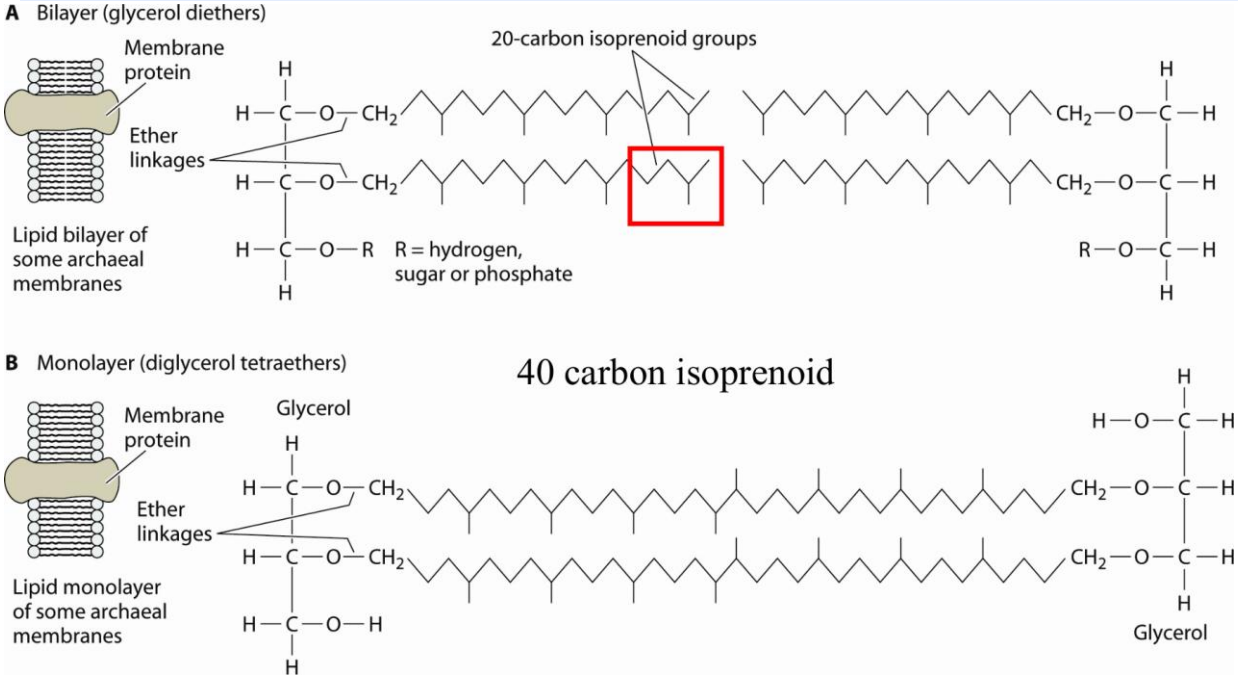
Only some archaea have a cell wall with chains of sugar.

Archaeal wall (in archaea that have one): pseudopeptidoglycan instead of peptidoglycan *(sugar and peptide mesh of the wall)*.

Pseudopeptidoglycan is found in some archaeal bacteria. [L17 p24]

Mycolic acid is **NOT** in the archaeal wall.

Membrane lipids



Archaeal membrane lipids: ether linkages, isoprenoid groups, bilayer and monolayer [L17 p39]

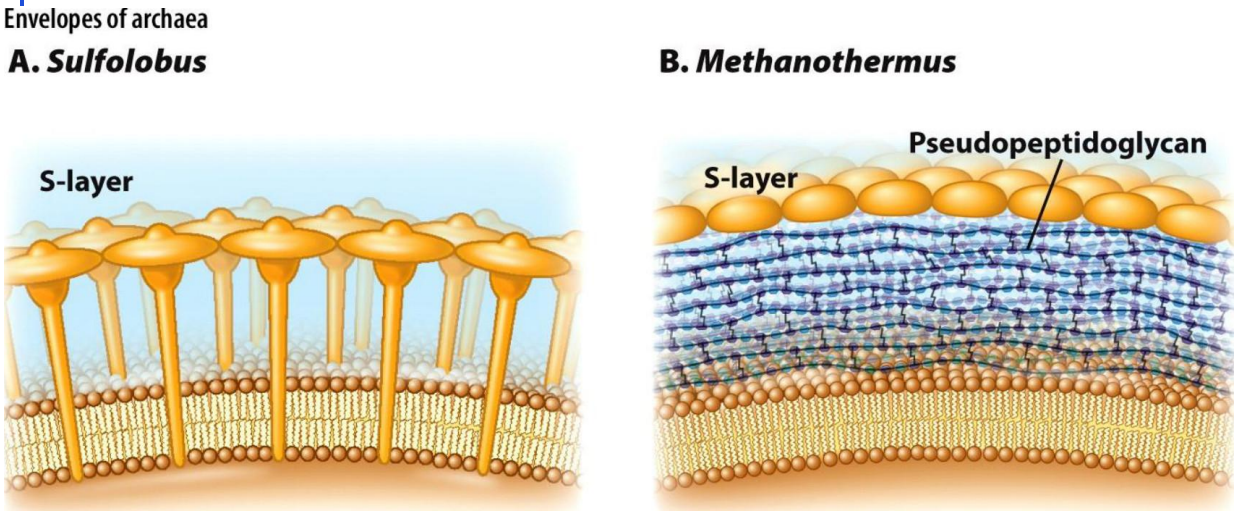
On the figure: isoprenoids · ether linkages

Archaeal membranes are **isoprenoids** *(branched five carbon repeat fats)* instead of phospholipids *(membrane fat with a charged head)*.

Membrane: isoprenoids (not phospholipids). [L17 p39]

Isoprenoids are joined to glycerol *(three carbon fat backbone)* by **ether** *(C O C bond)* **linkages** *(the bond type holding the chains to glycerol)*. [L16 p34, L17 p39]

The ether linkage resists hydrolysis *(breaking with water)*, and branched isoprenoid chains increase membrane stability by hooking each other in place (textbook).



Archaeal envelopes: S layer on stalks and over a pseudopeptidoglycan wall [L21 p35]

On the figure: S layer · Sulfolobus · Methanothermus · pseudopeptidoglycan

Archaeal S layer (some to all): protein. [L21 p35]

Archaeal cell wall (some): **pseudopeptidoglycan**. [L21 p35]

Archaeal cell membrane: isoprenoids (no phospholipids) and proteins. [L21 p35]

Sulfolobus: S-layer 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]

No cell wall

Mollicutes (Mycoplasma): no cell wall. [L16 p27, L16 p33]

The **only** membrane *(thin lipid layer)* is the plasma membrane (**PM**); a sugar layer of lipid-glycans on its surface may be a capsule *(firm sugar coat outside the cell)*. [L16 p27, L16 p33]

Mycoplasmas need no cell wall because they are parasites living in host environments, where they are protected from osmotic *(about water moving across membranes)* shock (textbook).

Membrane proteins

The PM has membrane proteins. [L16 p27, L16 p33]

IMP is intermembrane protein. [L16 p27, L16 p33]

PMP is peripheral membrane protein. [L16 p27, L16 p33]

Membrane surface

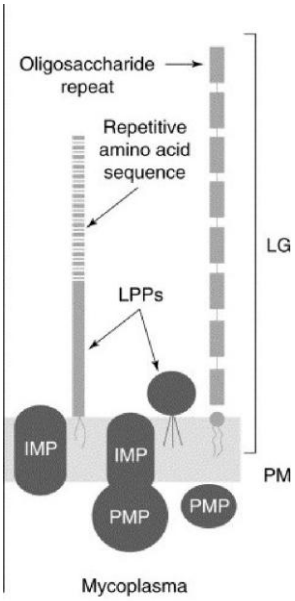
Surface of the membrane: lipoproteins (**LPPs**) with a **repetitive amino acid sequence**. [L16 p33]

Lipid-glycans (**LG**) have an **oligosaccharide repeat**. [L16 p33]

Mycoplasma sugar layer (all have one): it may be a capsule. [L21 p32]

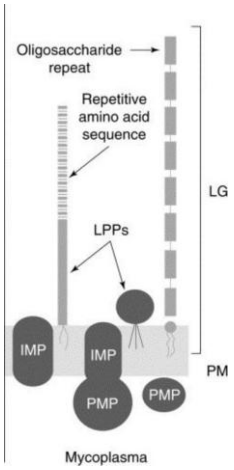
Mycoplasma cell membrane (all): proteins, phospholipids *(membrane fat with a charged head)*, sterols *(stiff ring shaped fat)*. [L21 p32]

Sterols in the membrane help stabilize it. [L21 p34]



Mycoplasma plasma membrane with IMP, PMP, lipoproteins and lipid-glycans [L16 p33]

On the figure: **PM** · **IMP** · **PMP** · **LPPs** · **LG** · **oligosaccharide repeat** · **repetitive amino acid sequence** · **Mycoplasmas**



Mycoplasma surface: lipoglycans and lipoproteins anchored in the plasma membrane [L21 p32]

On the figure: **LG** · **LPPs** · **IMP** · **PMP** · **PM**

Mycoplasma: no cell wall. [L21 p32]

Abbreviations: **LG** = lipid-glycans, **LPPs** = lipoproteins, **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]

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: A Gram stain *(stain that sorts bacteria purple or pink)* color does not always match the actual envelope *(layers wrapped around the cell)* style.** [L16 p35]

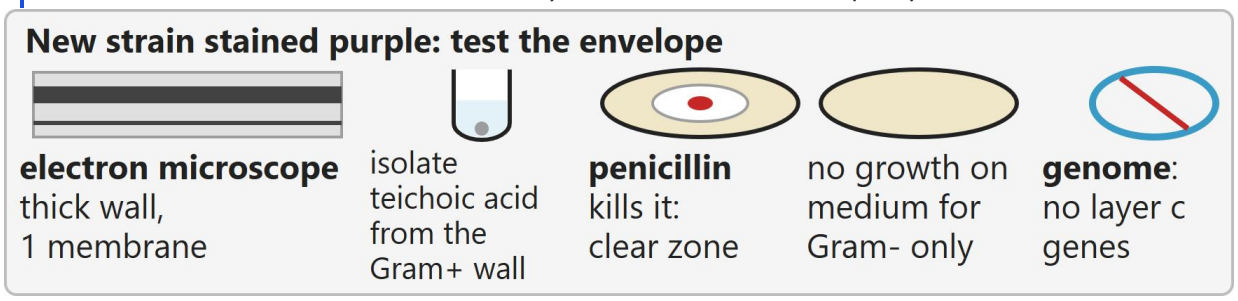
⚠ **Do not mix up: Stains are not the only way: media, electron microscopy, biochemistry and genes also predict the envelope.** [L21 p36]

Staining techniques

Staining techniques: the Gram stain and the acid fast stain detect differences in the envelope. [L16 p25, L16 p26]

They are basic tools for grouping bacteria by envelope. [L16 p25, L16 p26]

Limit of the Gram stain: color does not always match the actual envelope style. [L16 p35]



Tests for a purple strain: electron microscope, teichoic acid, penicillin, selective medium, genome [L16 p35]

On the figure: teichoic acid · penicillins · genome

Other methods can determine or predict the envelope of a newly isolated bacterium: electron microscopy, **teichoic acid** *(charged chains in Gram positive wall)* isolation, penicillin sensitivity, selective media and **genome** genes. [L16 p35]

Electron microscopy

Microscopy: electron microscope images (**50 nm** bar) show a single dark line for **Gram positive** *(thick wall, stains purple)*. [L16 p28]

They show separate dark bands for the **Gram negative** *(thin wall plus outer membrane)* envelope. [L16 p28]

Confirming a Gram positive envelope

Purple cells from an insect gut: how could you confirm a **Gram positive** envelope?

Method 1: transmission electron microscopy.

Method **1** shows a thick peptidoglycan *(sugar and peptide mesh of the wall)* layer and no outer membrane *(extra membrane outside the wall)*.

Method 2: isolate teichoic acid from the cell wall *(stiff layer around the cell membrane)*.

Method 3: test sensitivity to a penicillin.

Method **3**: some **penicillins** are more effective against **Gram positive** than **Gram negative** bacteria, because some do not pass through the **Gram negative** outer membrane.

Penicillin disables cross-bridge formation: the transpeptidase *(enzyme making wall cross bridges)* that cross-links the wall peptides *(short chain of amino acids)* is its target (textbook).

Method 4: no growth on selective media for **Gram negative**.

Method 5: look at genome predicted genes.

Method **5**: no genes for LPS *(outer membrane sugar and fat coat)* synthesis, outer membrane and periplasmic *(in the space between the two membranes)* proteins.

Method **5**: genes for teichoic acid synthesis are found.

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:

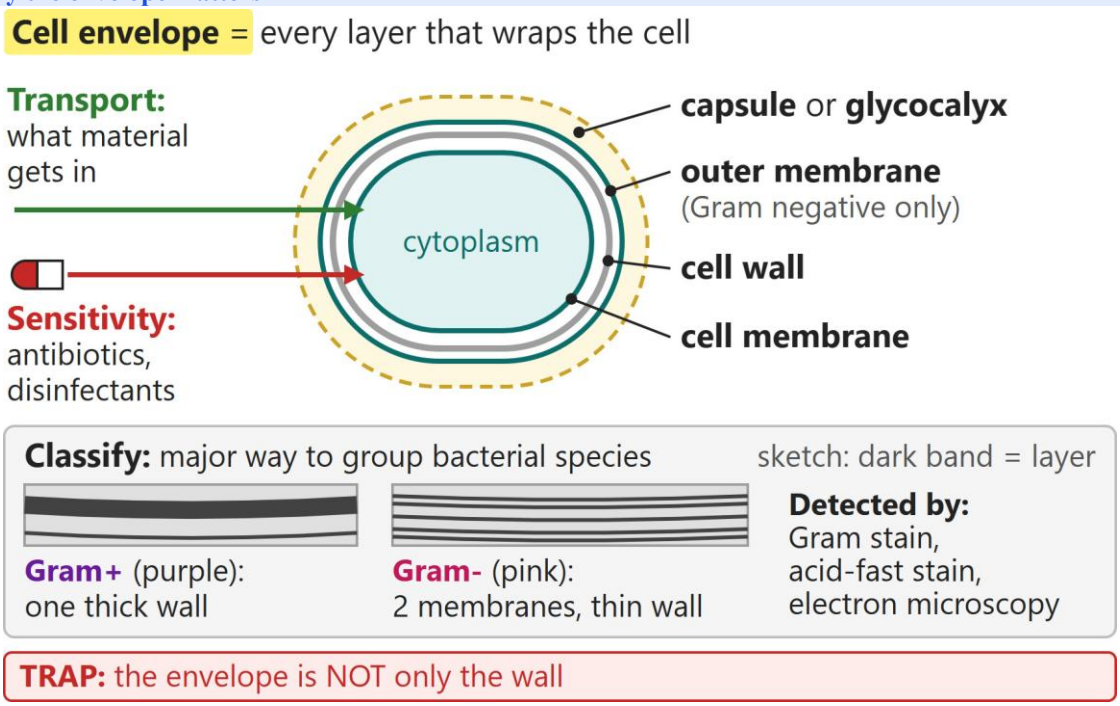
> Outer membrane proteins and LPS for **Gram negative**; teichoic acids for **Gram positive**.
>
> Mycolic acids *(waxy fatty acids of mycobacteria)* for Mycobacterium; pseudopeptidoglycan *(archaea version of wall mesh)* for Archaea *(single cell microbes without a nucleus, their own domain (also called archaeal bacteria))*. [L21 p36]

How the nature of the envelope affects bacterial cells?

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

⚠ **Do not mix up: Mollicutes *(bacteria with no cell wall)* have no cell wall *(stiff layer around the cell membrane)*; the plasma membrane *(thin lipid layer)* is their only membrane, with a sugar layer on its surface that may be a capsule *(firm sugar coat outside the cell)*.** [L16 p27, L16 p33]

Why the envelope matters



Cell envelope layers; what gets in, antibiotic sensitivity, and classifying species [L16 p26]

On the figure: classify · antibiotics · disinfectants · Glycocalyx

Why the envelope *(layers wrapped around the cell)* matters: reason **1,** major feature used to **classify** bacteria. [L16 p26]

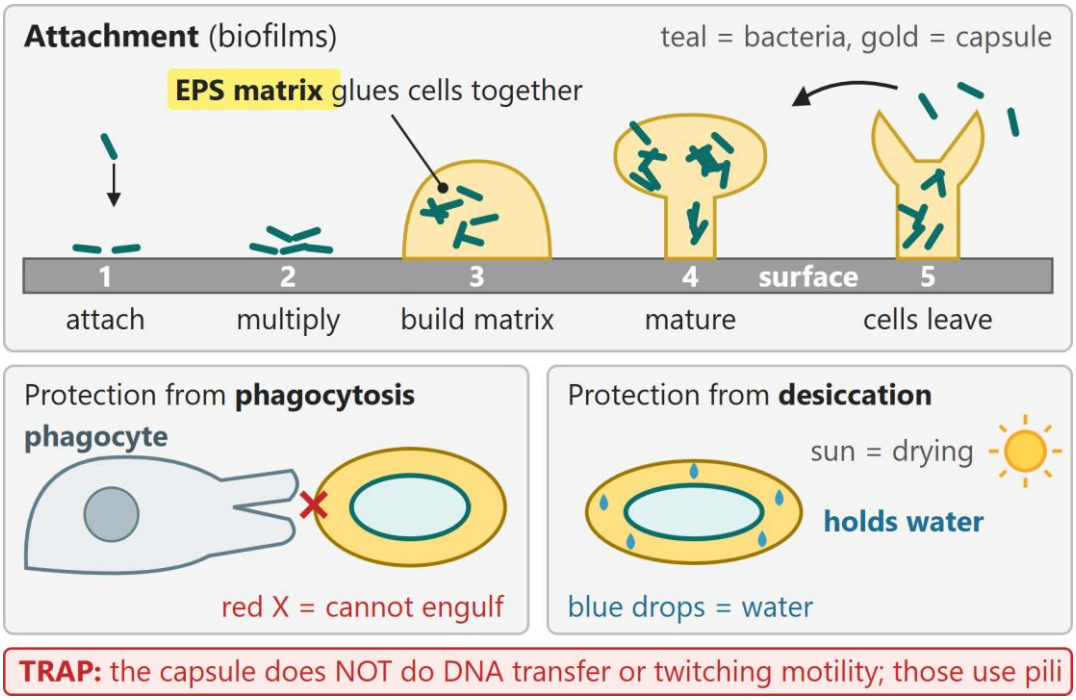
Staining techniques detect differences. [L16 p26]

Reason 2: affects transport of material into the cell. [L16 p26]

Reason 3: affects sensitivity of the bacterium to **antibiotics** and **disinfectants**. [L16 p26]

Drugs and chemicals have to pass through it to enter the cell. [L16 p26]

Glycocalyx and S-layer effects



Glycocalyx roles: biofilm attachment, protection from phagocytosis and desiccation [L16 p41]

On the figure: phagocytosis · desiccation · biofilms · attachment

> Glycocalyx *(sugary coat outside the cell)* **effects**: protection from phagocytosis *(immune cells eating germs)* and from desiccation *(drying out)*. [L16 p41, L16 p44]
>
> Glycocalyx also helps attachment to surfaces and biofilms *(slimy community on a surface)*. [L16 p41, L16 p44]
>
> **S-layer** *(crystal like protein coat outside)* **hypothesized effects**: protection from osmotic *(about water moving across membranes)* stress, protection from viruses. [L16 p41, L16 p44]
>
> S-layer may also be a barrier to large molecules. [L16 p41, L16 p44]

Penicillin in stream water

> **Penicillin treatment of stream water**: why might penicillin not work?
>
> The bacteria may not be growing.
>
> It may not be effective against the type of bacteria (**Gram negative**).
>
> The bacteria may be resistant or the concentration too low.

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

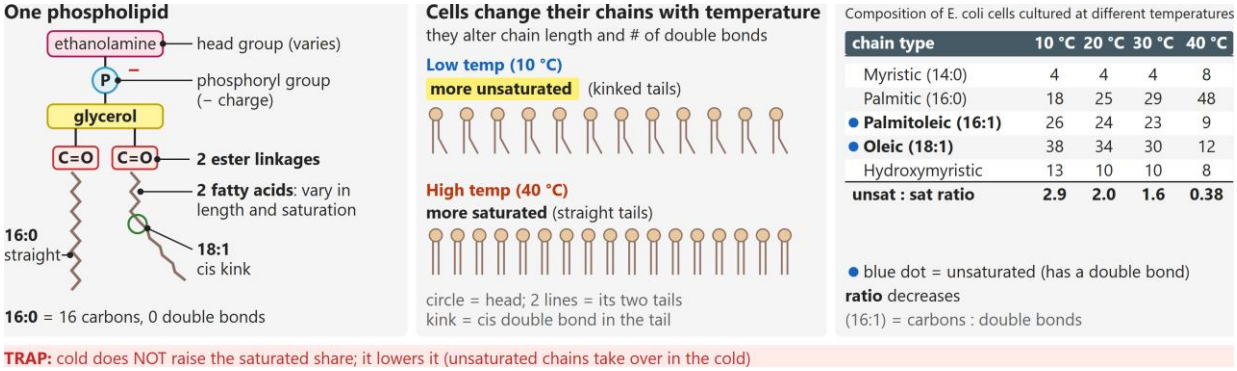
Book: 3.3 The Envelope and Cytoskeleton, Cell Envelope of Bacteria (chapter 3 PDF, p. 49); 3.3 The Envelope and Cytoskeleton, Proteobacterial Cell Envelope: Gram-Negative (chapter 3 PDF, p. 56).

⚠ **Do not mix up: Mycolic acid *(waxy fatty acid of mycobacteria)* is NOT an archaeal *(of archaea)* feature; archaea *(single cell microbes without a nucleus, their own domain (also called archaeal bacteria))* have isoprenoid *(branched five carbon repeat fat)* membranes *(thin lipid layer)*** and often an S layer.

⚠ **Do not mix up: Lysozyme *(enzyme that cuts wall sugars)* is a defensive enzyme of our body; autolysin *(wall-cutting enzyme made by the bacterium itself)* is produced by bacteria.** [L18 p27, L18 p39]

Membrane lipids

> **Membrane components differ**: archaea have isoprenoid lipids while bacteria have phospholipids *(membrane fat with a charged head)*. [L17 p39]
>
> Some bacteria have hopanoids *(five ring cholesterol like fat)*, molecules similar to sterols *(stiff ring shaped fat)*. [L17 p40]

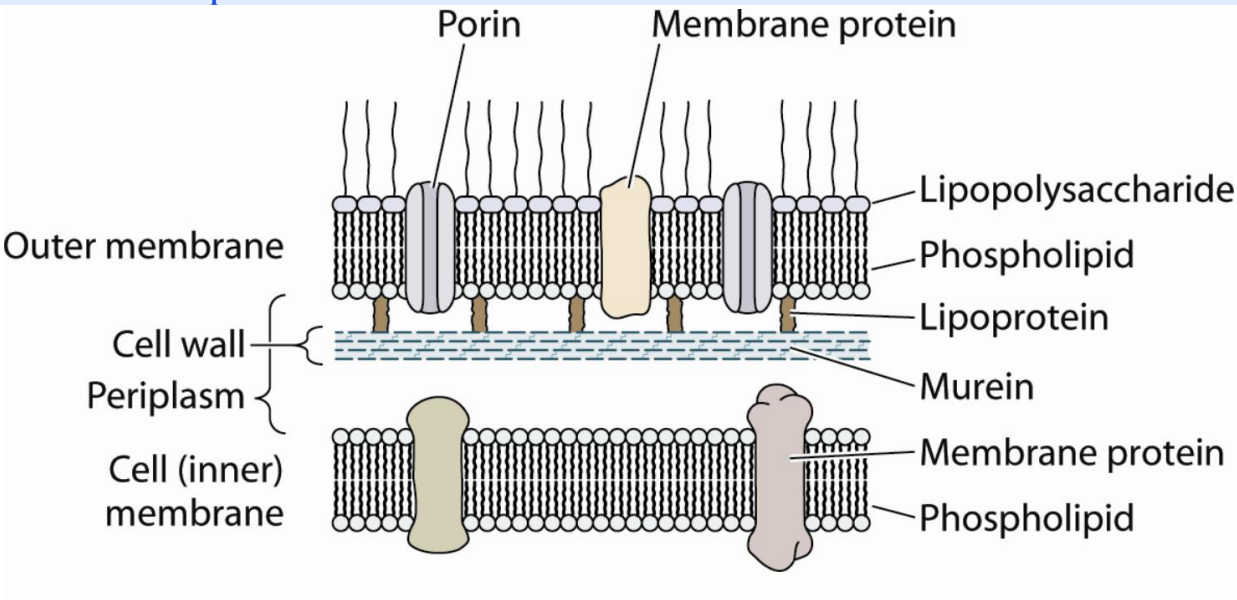


Phospholipid head group and chains; more unsaturated chains in the cold [L16 p40]

On the figure: head groups · unsaturated · 2.9 · 0.38 · 10 °C · 40 °C

> Fatty acid chains vary in length and saturation (number of double bonds) with temperature; at low temperature E. coli has a higher ratio of **unsaturated** to saturated fatty acids (**2.9** at **10 °C** versus **0.38** at **40 °C**). [L17 p37]
>
> Phospholipid **head groups** vary (for example phosphatidylethanolamine and phosphatidylglycerol). [L17 p36]

LPS and membrane proteins



Gram negative outer membrane with lipopolysaccharide, phospholipid, porin and membrane proteins [L17 p31]

On the figure: phospholipids · membrane proteins

> **Gram negative** *(thin wall plus outer membrane)* has LPS *(outer membrane sugar and fat coat)* in its outer membrane *(extra membrane outside the wall)*. [L16 p36]
>
> LPS act as endotoxins *(toxin built into the cell)*: harmless while the pathogen is intact, but released by a lysed *(broken open)* cell they overstimulate host defenses (textbook).
>
> **Membrane proteins** differ between bacterial species and in bacteria living in different conditions.

Layers overview

Outer membrane and **periplasm** *(space between the two membranes)* are **Gram negative** layers. [L17 p04]

Gram negative layers in order

Gram negative envelope *(layers wrapped around the cell)* **layers**: outside to inside.

Layer 1: glycocalyx *(sugary coat outside the cell)* or capsule (sugars, proteins or DNA).

Layer 2: outer membrane (lipopolysaccharide, proteins, **phospholipids**).

Layer 3: periplasm (aqueous compartment between the outer membrane and the **inner membrane**), which holds the thin cell wall *(stiff layer around the cell membrane)*.

Inside layer 3: cell wall (**peptidoglycan**), thin, **1-2 layers**; it sits in the periplasm.

Layer 4: cell membrane (phospholipids and proteins).

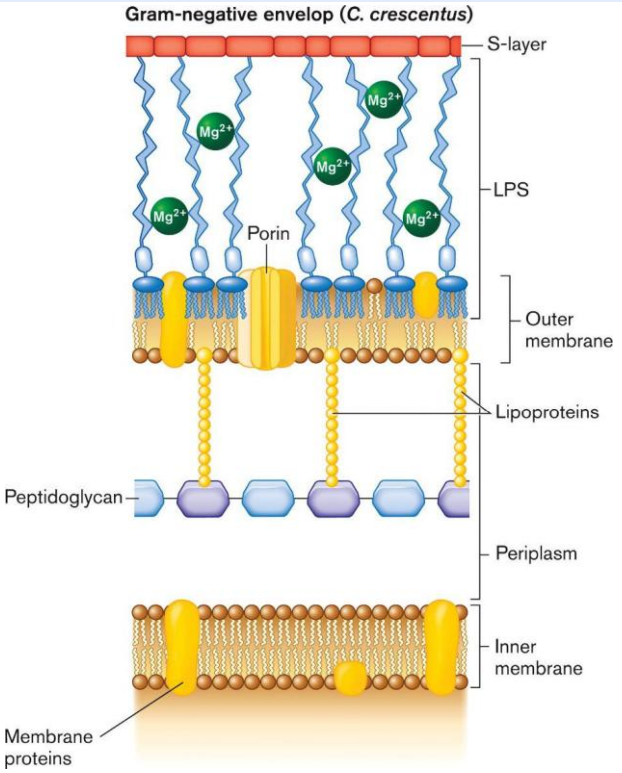
Penicillin and the outer membrane

Penicillin: some penicillins are more effective against **Gram positive** *(thick wall, stains purple)* than **Gram negative** bacteria, because some do not pass through the **Gram negative** outer membrane.

Cell membrane and transport

Cell membrane: components, function and transport. [L18 p05, L18 p06]

Membrane barrier: the cell membrane acts as a barrier to keep water-soluble proteins and other cell components within the cytoplasm (textbook). [L18 p05, L18 p06]



Gram negative envelope: LPS, outer membrane, lipoproteins, periplasm, inner membrane [L17 p05]

On the figure: **Outer membrane** · **periplasm** · **peptidoglycan** · **inner membrane**

Membrane: cells try to maintain concentration of certain ions *(charged particle)* and solutes inside the cell that differ from the outside environment. [L18 p05, L18 p06]

Peptidoglycan in the wall

Cell wall (peptidoglycan): peptidoglycan *(sugar and peptide mesh of the wall)* synthesis is unique to bacteria. [L18 p27, L18 p28]

Many antibacterial drugs block peptidoglycan synthesis. [L18 p27, L18 p28]

New peptidoglycan is needed for both cell growth/division and repair/replacement. [L18 p27, L18 p28]

Peptidoglycan sugars: made from fructose-**6**-P in the cytosol *(liquid part of the cytoplasm)*; NAM-pentapeptide is transferred to bactoprenol *(fat carrier moving wall pieces)* in the cell membrane.

Keeping the layer intact

Layer integrity: the layer must stay intact while links between existing and new peptidoglycan are cut. [L18 p27, L18 p28]

Enzymes that cut peptidoglycan

Enzymes that cleave peptidoglycan: lysozyme (a defensive enzyme of our body) and autolysin (produced by bacteria). [L18 p27]

Lysozyme:

> Found in tears, saliva and mucus (textbook fill).
> Cleaves cell wall peptidoglycan (textbook fill). [L18 p39]

Layer	Components	Where it is found
Glycocalyx (capsule or slime layer)	polysaccharides, peptides, DNA	beyond the cell wall; capsule in some **Gram positive** and some **Gram negative** cells and in mycobacteria
S-layer	proteins or glycoproteins	outside the cell wall or membrane: **Gram positive** (possibly all, not certain), **Gram negative** (some), archaea (some to all); not found on all bacteria, and usually lost in lab cultured strains
Outer membrane	LPS and phospholipids	**Gram negative**
Cell wall	peptidoglycan, pseudopeptidoglycan, teichoic acid, mycolic acids, arabinogalactan	**Gram positive**: thick peptidoglycan, teichoic acid in some (another list says most **Gram positive** bacteria, so the sources differ); **Gram negative**: thin peptidoglycan; mycobacteria: mycolic acids, arabinogalactan, peptidoglycan; archaea (some): pseudopeptidoglycan; Mycoplasma: none
Periplasm	thin peptidoglycan, oligosaccharides (osmotic protection), its own unique set of proteins (proteins that bind solutes)	**Gram negative**
Cell membrane	phospholipids and proteins (bacteria; some also hopanoids); isoprenoids (archaea); sterols (Mycoplasma)	all five: **Gram positive**, **Gram negative**, mycobacteria, archaea, Mycoplasma

Table source: [L16 p32, L16 p36, L16 p37, L16 p40, L16 p42, L17 p31, L17 p32, L21 p29, L21 p30, L21 p31, L21 p32, L21 p35]

Layer	Role	Feature	Composition
Outer membrane	LPS sugars block diffusion of hydrophobic molecules (bile salts, antibiotics, dyes); the membrane blocks hydrophilic molecules except through porins; lipid A induces an inflammatory response	a second membrane beyond the cell membrane, **Gram negative**	LPS (outer leaflet), phospholipids (mainly inner leaflet), porins, murein lipoprotein
Cell wall	structural support and rigidity; prevents lysis in hypotonic conditions; helps determine cell shape	semi-rigid, porous layer beyond the cell membrane; synthesis blocked by some antibiotics	peptidoglycan, pseudopeptidoglycan, teichoic acid, mycolic acid, arabinogalactan
Periplasm	osmotic protection, nutrient transport, redox reactions of the electron transport chain, hydrolysis of nutrients	aqueous compartment between outer and inner membrane, **Gram negative**, own unique proteins	oligosaccharides and a unique set of proteins
Cell membrane	boundary and barrier, regulates what is inside, energy reactions and storage, sensing, lipid synthesis	lipids and proteins, proteins a major component by weight (E. coli: **75%** protein, **25%** phospholipid)	phospholipids, isoprenoids, hopanoids, cardiolipin, proteins

Table source: [L17 p05, L17 p08, L17 p12, L17 p14, L17 p31, L17 p32, L17 p33, L17 p35, L17 p43, L17 p44, L17 p45, L17 p46]

a. Glycocalyx (capsule and slime layer): role and composition

Book: 3.3 The Envelope and Cytoskeleton, Firmicute Cell Envelope: Gram-Positive (chapter 3 PDF, p. 52); 3.3 The Envelope and Cytoskeleton, Proteobacterial Cell Envelope: Gram-Negative (chapter 3 PDF, p. 56).

⚠ **Do not mix up: Slime layer *(loose sticky coat outside)* is loosely attached; capsule *(firm sugar coat outside the cell)* is more firmly attached. Both are glycocalyx *(sugary coat outside the cell)*.** [L16 p37, L16 p38]

What the glycocalyx is

Glycocalyx: extracellular material beyond the cell wall *(stiff layer around the cell membrane)*. [L16 p37, L16 p38]

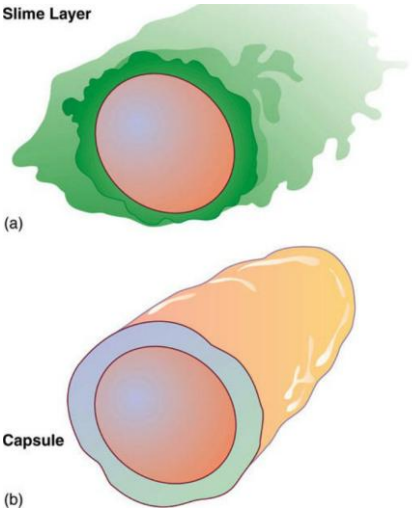
It has two types. [L16 p37, L16 p38]

Slime layer is loosely attached. [L16 p37, L16 p38]

Capsule is more firmly attached. [L16 p37, L16 p38]

A **darkfield microscopy** *(view with a dark background where objects glow bright)* image shows the coat as a bright halo around clusters of cells. [L16 p37]

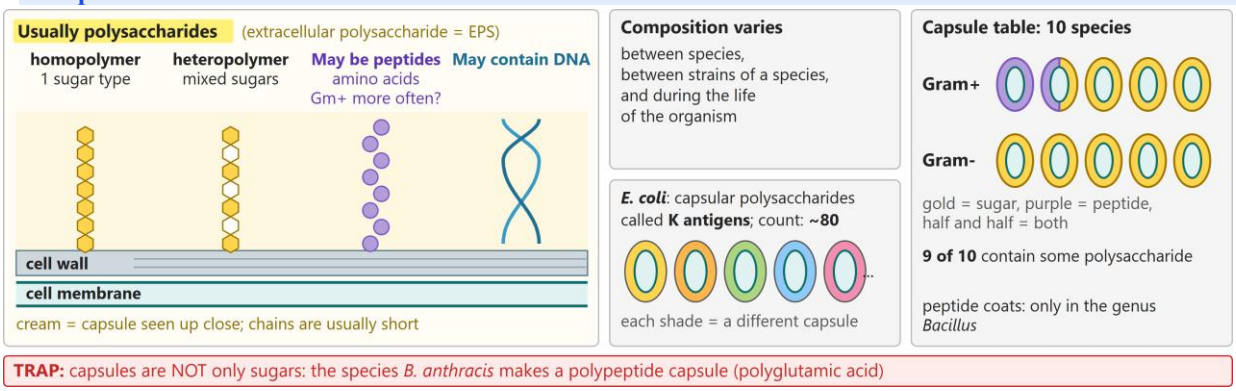
Layers from inside out: inner (cell) membrane *(thin lipid layer)*, **murein** *(another name for peptidoglycan)*, outer membrane *(extra membrane outside the wall)*, capsule. [L16 p38]



Slime layer loosely attached and capsule firmly attached around a cell [L16 p38]

On the figure: Slime layer · capsules

Composition



Glycocalyx composition: polysaccharides, peptides, DNA; 10 capsule examples [L16 p37]

On the figure: polysaccharides · EPS · homopolymer · heteropolymer · peptides · DNA · K antigen · Bacillus

Glycocalyx molecules: sugars, **peptides** (proteins) and **DNA** *(cell genetic instruction molecule)*.

Composition: usually **polysaccharides** *(long chain of sugars)*. [L16 p40]

May be peptides (more common in **Gram positive**, not certain). [L16 p40]

Peptides are short chains of amino acids. [L16 p40]

May also contain DNA. [L16 p40]

It varies between species, strains of a species, and during the life of an organism. [L16 p40]

Sugar chains and EPS

Usually short chains of a **homopolymer** or **heteropolymer** *(one repeated sugar or mixed sugars)* of sugars. [L16 p40]

These are called extracellular polysaccharide (**EPS**). [L16 p40]

E. coli K antigen: ~**80** different capsular polysaccharides have been observed. [L16 p40]

Capsule examples and conclusion

Conclusion: **8** of the **10 capsules** listed are polysaccharide **only**. [L16 p39, L16 p40]

The **2** that contain a peptide are both **Gram positive** *(thick wall, stains purple)* **Bacillus** species. [L16 p39, L16 p40]

B. anthracis has polyglutamic acid; B. megaterium has both. [L16 p39, L16 p40]

This fits "peptides more common in **Gram positive**". [L16 p39, L16 p40]

All **5 Gram negative** *(thin wall plus outer membrane)* examples are polysaccharides. [L16 p39, L16 p40]

Roles

Roles: attachment (biofilms). [L16 p41]

Biofilms *(slimy community on a surface)*: the glycocalyx is a matrix for their formation.

Protection from phagocytosis *(being eaten by immune cells)*. [L16 p41]

The capsule can prevent phagocytosis by a **phagocyte** *(white blood cell that swallows microbes)*. [L16 p41]

Protection from **desiccation** *(drying out)*. [L16 p41]

Bacterium	Capsule composition	Subunits
Bacillus anthracis (Gram positive)	polypeptide (polyglutamic acid)	D-glutamic acid
Bacillus megaterium (Gram positive)	polypeptide and polysaccharide	D-glutamic acid, amino sugars, sugars
Streptococcus mutans (Gram positive)	polysaccharide (dextran)	glucose
Streptococcus pneumoniae (Gram positive)	polysaccharides	sugars, amino sugars, uronic acids
Streptococcus pyogenes (Gram positive)	polysaccharide (hyaluronic acid)	N-acetyl-glucosamine and glucuronic acid
Acetobacter xylinum (Gram negative)	polysaccharide (cellulose)	glucose
Escherichia coli (Gram negative)	polysaccharide (colonic acid)	glucose, galactose, fucose, glucuronic acid
Pseudomonas aeruginosa (Gram negative)	polysaccharide	mannuronic acid
Azotobacter vinelandii (Gram negative)	polysaccharide	glucuronic acid
Agrobacterium tumefaciens (Gram negative)	polysaccharide (glucan)	glucose

Table source: [L16 p39]

Biofilm step	What happens (textbook)
1	Cells attach to a surface
2	Microcolonies form
3	The cells form a thick extracellular matrix *(sticky sugar web, the EPS)* of polysaccharide polymers
4	The biofilm matures
5	Dispersal: some cells leave to start new biofilms

Table source: [L16 p41]

b. S-layer: composition and where located

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

⚠ **Do not mix up: S-layer *(crystal like protein coat outside)* is protein, found especially on archaea *(single cell microbes without a nucleus, their own domain (also called archaeal bacteria))*, and is NOT on all bacteria.** [L16 p42, L16 p43]

What the S-layer is

S-layer: a protein coating beyond the cell wall *(stiff layer around the cell membrane)*.

It is observed in some bacteria and archaea.

S-layer (surface layer) features: found on most bacteria (not certain), especially archaea. [L16 p42]

Watch out: It is also described as observed in some bacteria and archaea, and as not found on all bacteria. [L16 p42]

Usually lost in lab cultured strains. [L16 p42]

A layer of proteins or glycoproteins, a complex of a few proteins. [L16 p42]

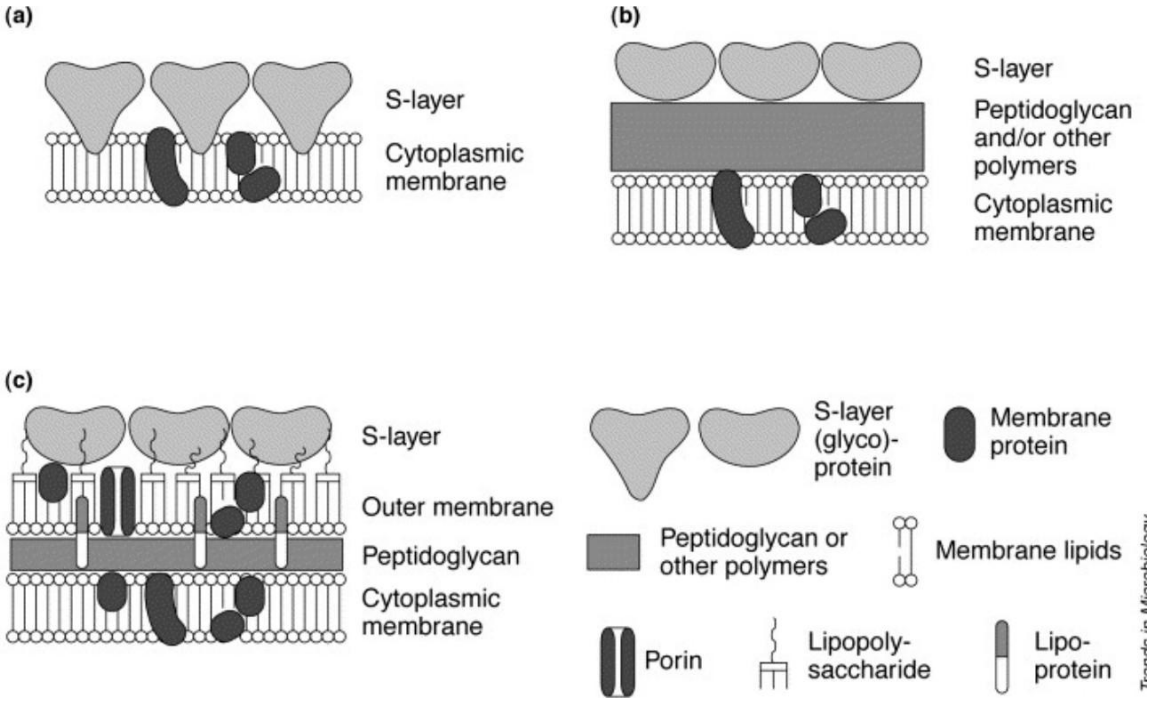
It makes a crystalline *(neat repeating pattern)*, porous matrix. [L16 p42]

The S-layer is built of protein subunits *(building block piece)* that fit together like tiles, and it is rigid but flexes (textbook).

Where it is located

On the outside of the cell wall or membrane *(thin lipid layer)*. [L16 p42]

Where located: the S-layer sits in three places (Sleytr and Beveridge). [L16 p43]



S-layer on archaea, Gram positive and Gram negative envelopes [L16 p43]

On the figure: S-layer · peptidoglycan · outer membrane

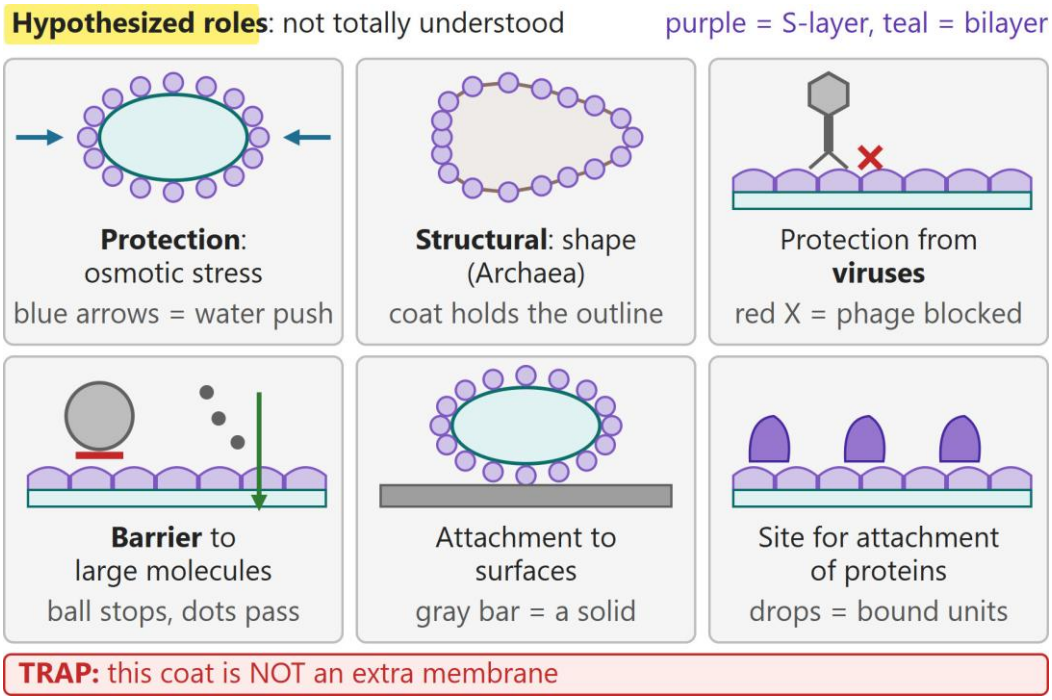
Archaea: no cell wall, just **S-layer** on the membrane. [L16 p43]

Gram positive *(thick wall, stains purple)*: S-layer outside the **peptidoglycan** *(sugar and peptide mesh of the wall)*. [L16 p43]

Gram negative *(thin wall plus outer membrane)*: S-layer outside the **outer membrane** *(extra membrane outside the wall)*. [L16 p43]

S-layer is **NOT** found on all bacteria. [L16 p43]

Hypothesized roles



S-layer hypothesized roles: osmotic stress, viruses, large molecules, attachment [L16 p44]

On the figure: osmotic stress · viruses · large molecules · Attachment to surfaces · Site for attachment of proteins · Structural

> **Hypothesized roles** (not totally understood): protection from osmotic *(about water moving across membranes)* stress. [L16 p44]
>
> **Structural** (shape, in archaea). [L16 p44]
>
> Protection from **viruses**. [L16 p44]
>
> Barrier to **large molecules**. [L16 p44]
>
> **Attachment to surfaces**. [L16 p44]
>
> **Site for attachment of proteins**. [L16 p44]

c. Outer membrane

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

⚠ **Do not mix up: Murein lipoprotein *(protein anchoring outer membrane to wall)* links outer membrane *(extra membrane outside the wall)* to peptidoglycan *(sugar and peptide mesh of the wall)*; porins *(channels in the outer membrane)* pass only molecules up to 700 daltons *(unit of molecule weight)*.** [L17 p11]

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

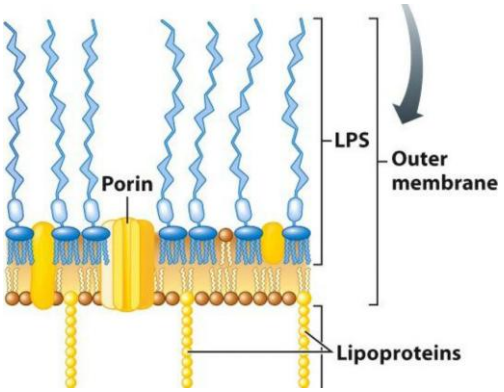
What the outer membrane is

Outer membrane: a second membrane *(thin lipid layer)* beyond the cell membrane *(thin skin around the cell)*. [L17 p05]

Where: found in **Gram negative** *(thin wall plus outer membrane)* bacteria. [L17 p05]

Outer membrane composition: lipopolysaccharide, proteins, and phospholipids *(membrane fat with a charged head)*.

Function: inhibits diffusion *(drifting from high to low amount)* of most hydrophobic molecules (bile salts, some antibiotics); the **LPS** sugars block the hydrophobic molecules, and the membrane itself blocks hydrophilic molecules, which get through **only** by **porins** (under **700 daltons**). [L17 p05]

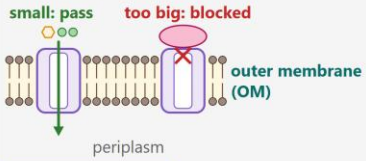


Outer membrane with LPS, porin and lipoproteins [L17 p09]

On the figure: outer membrane · LPS · porins · lipoprotein

Porins: protein channels

outside the cell



MAX 700 daltons

short chains of sugars and amino acids

a few different types

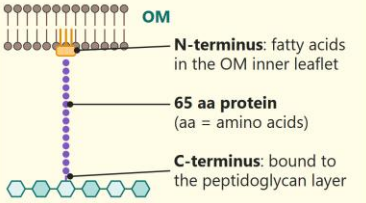
selective for type or size of molecule

E. coli

Protein	Function	Receptor for
OmpF	nonspecific: small molecules	phage T2
OmpC		phage T4
OmpA	specific for peptides	mating pili
LamB	maltose, maltodextrin	phage λ
PhoE	anion-selective channel	none listed

receptor = docking site a phage or pilus binds
phage = virus that infects bacteria
Omp = OM protein; λ = lambda

Murein lipoprotein



Job: LINKS OM TO PEPTIDOGLYCAN

orange = lipid tails; purple = protein

TRAP: these channels do NOT let in molecules of any size; big ones stay out

Porins pass small molecules; murein lipoprotein links outer membrane to peptidoglycan [L17 p10]

On the figure: porins · OmpF · OmpC · OmpA · LamB · PhoE · Murein lipoprotein · 700 daltons

Porins

Porins *(protein channels through the outer membrane)*: a few different types; E. coli has **OmpF**, **OmpC**, **OmpA**, **LamB** and **PhoE**. [L17 p09]

Porin *(water filled channel in outer membrane)* **selectivity**: selective for type or size of molecule. [L17 p09]

Porin size limit: maximum **700 daltons** (short chains of sugars and amino acids). [L17 p09]

In a rich environment, cells down-regulate large porins and express smaller-pore porins, selecting **only** smaller nutrients and avoiding toxins (textbook).

Receptors and phage

Receptor *(docking site on the cell surface)*: a phage *(virus that infects bacteria)* or a mating pilus *(thin hair for sticking or DNA swap)* attaches to its porin receptor. [L17 p10]

Phage *(virus that infects bacteria)*: OmpF, OmpC and LamB are phage receptors *(proteins a signal or virus binds to)*. [L17 p10]

Murein lipoprotein

Murein lipoprotein: a **65** amino acid protein. [L17 p11]

Murein lipoprotein N-terminus: linked to fatty acids in the inner leaflet of the **outer membrane**. [L17 p11]

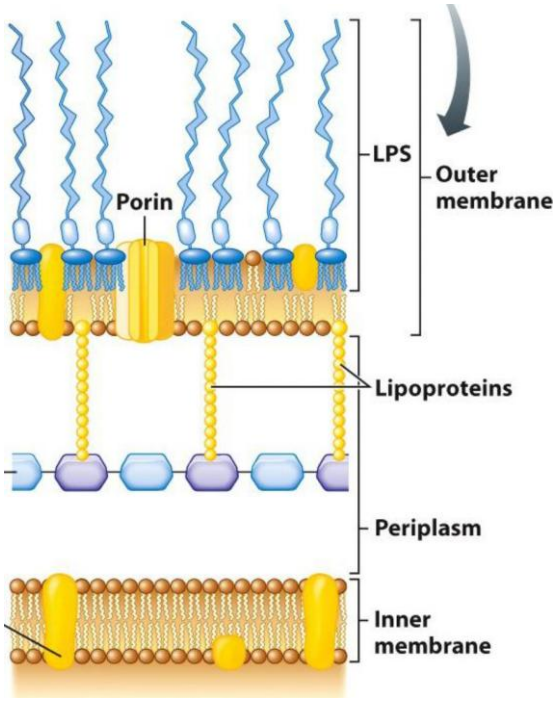
Murein lipoprotein C-terminus: linked to the peptidoglycan layer. [L17 p11]

Murein lipoprotein job: links the outer membrane to peptidoglycan. [L17 p11]

A mutant without murein lipoprotein cannot attach its outer membrane to the growing wall, so the outer membrane balloons out where daughter cells separate (textbook).

Gram negative outer membrane: the membrane itself blocks diffusion of hydrophilic *(water-loving)* molecules. [L21 p51]

Porin: hydrophilic molecules under **700 daltons** can go through. [L21 p51]



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

On the figure: outer membrane · LPS · Porins

Component of the outer membrane	Where and what
1) Lipopolysaccharide (LPS)	outer leaflet, sugar chain pointing out
2) Phospholipids	mainly in the inner leaflet
3) Protein channels (porins)	selective for type or size of molecule, maximum size **700 daltons**
4) Murein lipoprotein	**65** amino acid protein linking the outer membrane to peptidoglycan

Table source: [L17 p06, L17 p09, L17 p11]

E. coli porin	Function	Also
OmpF	nonspecific, small molecules	receptor for phage T2
OmpC	nonspecific, small molecules	receptor for phage T4
OmpA	specific for peptides	receptor for mating pili
LamB	porin for maltose, maltodextrin	receptor for phage lambda
PhoE	anion-selective channel	none listed

Table source: [L17 p10]

▸ LPS

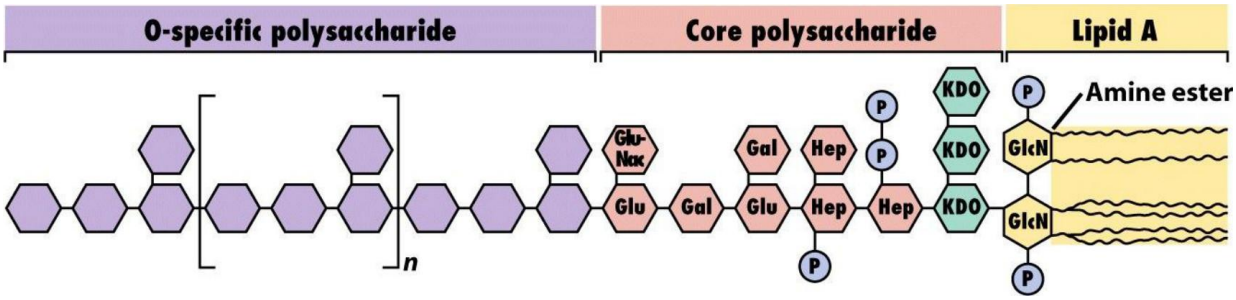
Book: 3.3 The Envelope and Cytoskeleton, Proteobacterial Cell Envelope: Gram-Negative (chapter 3 PDF, p. 56).

⚠ **Do not mix up: LPS *(outer membrane sugar and fat coat)* polysaccharides *(long chain of sugars)* block diffusion *(drifting from high to low amount)*; lipid A *(toxic fatty part of LPS)* is the part that induces inflammation.** [L17 p08]

What LPS is

LPS (lipopolysaccharide):

> Sits in the outer leaflet of the outer membrane *(extra membrane outside the wall)*. [L17 p06, L17 p07]
>
> Found in **Gram negative** *(thin wall plus outer membrane)* bacteria, in the outer membrane. [L17 p07, L17 p08]



LPS in three parts: O-specific polysaccharide, core polysaccharide, lipid A [L17 p07]

On the figure: O-specific polysaccharide · core polysaccharide · lipid A · KDO · Hep · Gal · Glu

LPS structure: three parts, **lipid A**, **core polysaccharide** and **O-specific polysaccharide**. [L17 p07]

LPS sugar chain: points out. [L17 p06, L17 p07]

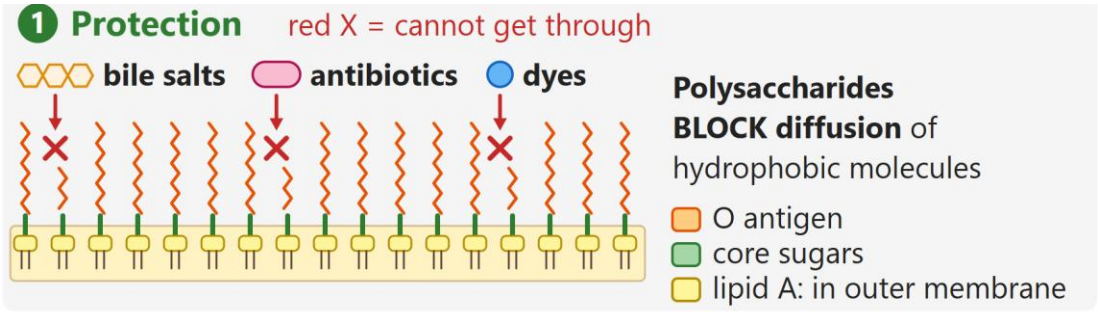
The LPS sugar chains carry negative charges that bind magnesium ions *(charged particle)*, and the ions form ionic cross-links that bridge LPS chains (textbook).

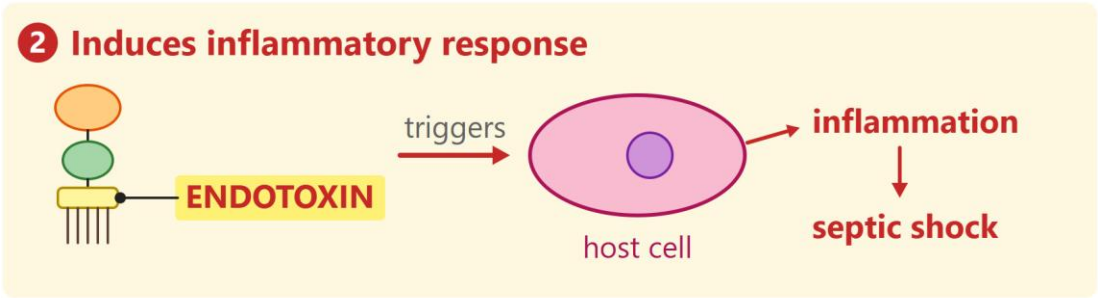
LPS parts

Core polysaccharide sugars: **KDO** *(a core sugar)*, **Hep**, **Gal** and **Glu** *(glucose, not glutamic acid)*. [L17 p07]

O antigen *(the outermost sugar repeat of LPS)*: another name for the **O-specific polysaccharide**. [L17 p08]

Jobs of LPS





TRAP: the sugar chains do NOT trigger this response

Two jobs of LPS: block bile salts, antibiotics and dyes; lipid A triggers inflammation [L17 p08]

On the figure: bile salts · antibiotics · dyes · lipid A · O antigen · Endotoxin

Function of LPS: two jobs. [L17 p08]

LPS job 1, protection: the polysaccharides block diffusion of hydrophobic *(water hating)* molecules (**bile salts**, **antibiotics**, **dyes**). [L17 p08]

LPS job 2: **lipid A** induces an inflammatory response. [L17 p08]

Endotoxin *(toxin built into the cell)* is harmless while the pathogen is intact, but released by a lysed *(broken open)* cell it overstimulates host defenses and can cause potentially lethal shock (textbook).

LPS is tightly packed (**only** saturated fatty acids). [L21 p51]

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

Part	What it is
Lipid A	**4-6** fatty acids attached to a disaccharide of N-acetylglucosamine phosphate; the **endotoxin** *(toxin that causes septic shock)*
Core polysaccharide	branched chain of sugars, same for a species
O-specific polysaccharide	usually a **4-5** sugar repeat; length and composition vary; also contributes to the glycocalyx; sticks out from the outer membrane

Table source: [L17 p07]

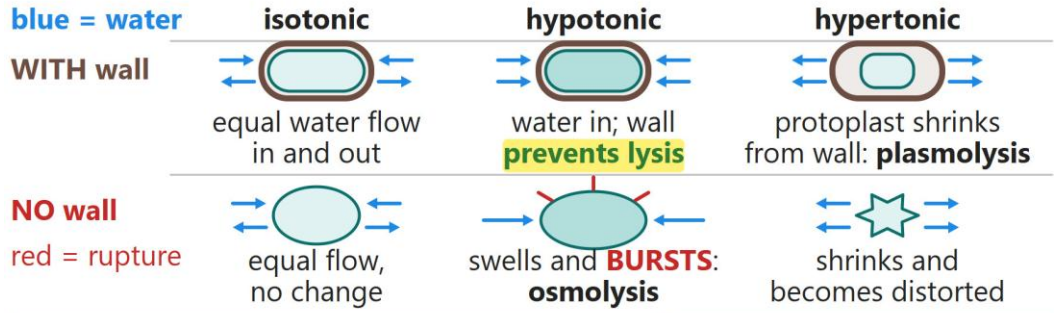
d. Cell Wall: Role

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

⚠ **Do not mix up: Penicillin only kills growing bacteria making new wall; it may fail on Gram negative *(thin wall plus outer membrane)*.**

Cell wall roles

Cell wall: semi-rigid, porous layer beyond the cell membrane
Role: structural support, rigidity; helps determine cell shape
Its synthesis is blocked by some antibiotics



Bacteria: wall components

a) peptidoglycan (murein)
b) pseudopeptidoglycan
c) teichoic acid
d) mycolic acid
e) arabinogalactan

most eubacteria: ONE principal component

Plants and fungi

Plants: **cellulose**, chains of glucose
Fungi: **chitin**, chains of a modified glucose

TRAP: a walled cell losing water does NOT rupture; it shrinks away from the wall

Cell wall in isotonic, hypotonic and hypertonic water, and wall components [L17 p13]

On the figure: cell walls · hypotonic · peptidoglycan · pseudopeptidoglycan · teichoic acid · mycolic acid · arabinogalactan · cellulose

Cell wall *(stiff layer around the cell membrane)*: a semi-rigid, porous layer beyond the cell membrane *(thin skin around the cell)*. [L17 p12]

Role 1: provides structural support and rigidity. [L17 p12]

Role 2: prevents cells from lysing in **hypotonic** *(low solute outside)* conditions. [L17 p12]

Without a countering wall, osmotic *(about water moving across membranes)* pressure makes a cell burst, which is how penicillin kills bacteria by disrupting wall synthesis. A growing cell keeps opening its wall (autolysins) to insert new units, so when new units cannot be made or cross-linked the wall weakens and the cell bursts (textbook).

Role 3: helps determine cell shape. [L17 p12]

Wall composition

Composition varies, but most eubacteria have one principal component. [L17 p12]

Antibiotics: synthesis of the cell wall is blocked by some antibiotics, such as penicillin and vancomycin *(drug blocking wall building)*, which block cross-bridge formation. [L17 p12]

Components found in bacterial cell walls: **peptidoglycan** (murein), **pseudopeptidoglycan** *(archaea version of wall mesh)*, **teichoic acid** *(charged chains in Gram positive wall)*, **mycolic acid** *(waxy fatty acid of mycobacteria)*, **arabinogalactan** *(chain of arabinose and galactose sugars in the wall)*. [L17 p14]

For comparison, plants use **cellulose** (chains of glucose). [L17 p14]

For comparison, fungi use chitin (chains of a modified glucose). [L17 p14]

Why penicillin may fail

Why penicillin may fail: four reasons treating water with penicillin may not make it safe to drink.

Reason 1:

The bacteria may not be growing.

Penicillin **only** kills bacteria that are growing.

Growing bacteria synthesize new cell wall material.

Reason 2:

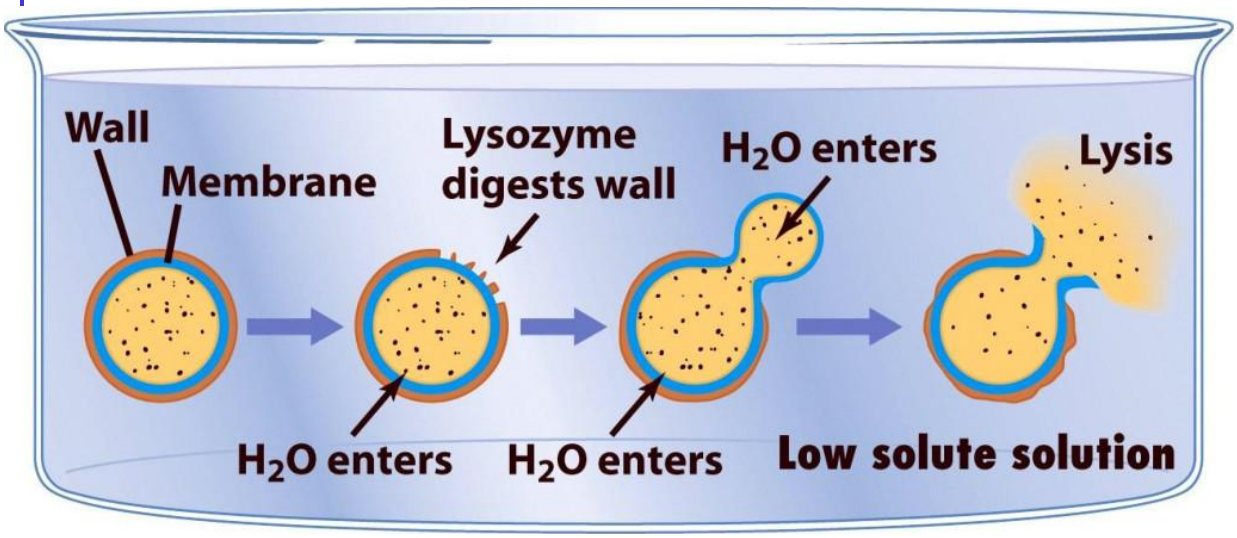
Penicillin may not work against the bacteria causing the illness.

That type is **Gram negative**.

Reason 3: the bacteria may be resistant.

Beta-lactamase *(enzyme that destroys beta lactam drugs)* cleaves the lactam ring of penicillin, so penicillin can no longer inhibit the transpeptidase (textbook).

Reason 4: you may not reach a high enough concentration.



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

On the figure: wall · membrane · lyse

Cell wall role: keeps the **membrane** 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]

Condition	Cell with a cell wall	Cell lacking a cell wall
Isotonic	water concentration equal inside and outside, equal diffusion in both directions	rates of diffusion equal in both directions
Hypotonic	net diffusion of water into the cell; swells the protoplast and pushes it tightly against the wall; the wall usually prevents the cell from bursting	water diffuses in, the cell swells and may burst (osmolysis) if no mechanism exists to remove the water
Hypertonic	water diffuses out and the protoplast shrinks away from the wall (plasmolysis)	water diffuses out, the cell shrinks and becomes distorted

Table source: [L17 p13]

▸ 1) Peptidoglycan: structure, sugars

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

⚠ Do not mix up: Gram negative *(thin wall plus outer membrane)* cross-link joins DAP (position 3) directly to D-Ala (position 4); staphylococci use L-Lys and a (Gly)5 bridge instead. [L17 p19]

What peptidoglycan is

Peptidoglycan (murein): long glycan *(sugar chain)* chains of two alternating sugars. [L17 p15, L17 p16]

Peptidoglycan sugars: repeats of N-acetyl-glucosamine and N-acetyl-muramic acid.

Lysozyme *(enzyme that cuts wall sugars)*: a defensive enzyme of our body that cleaves the linkage between the sugars in peptidoglycan.

Size: one large, interconnected molecule around the outside of the cell. [L17 p15, L17 p16]

Peptide positions in Gram negative

Peptide *(short chain of amino acids)* **of a Gram negative peptidoglycan**: positions counted from the NAM *(the other wall sugar)*. [L17 p19]

Each peptide hangs from an **NAM** (M) sugar of the glycan chain; the cross-link then joins two peptides. [L17 p19]

Position 1: **L-Ala**. [L17 p19]

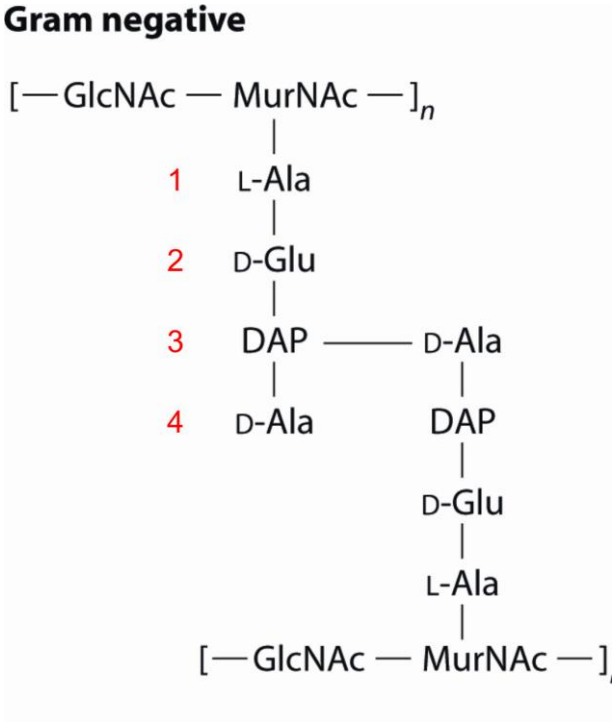
Position 2: **D-Glu** *(D form of glutamic acid)*. [L17 p19]

Position 3: **DAP** *(diaminopimelic acid, a modified lysine)*. [L17 p19]

Position 4: **D-Ala** *(D form of alanine amino acid)*. [L17 p19]

D amino acids sit in positions **2** and **4.** [L17 p19]

D and L *(mirror-image forms of the same amino acid)*: proteins use L-forms, wall peptides have some D-forms. [L17 p17]



Gram negative peptide: L-Ala, D-Glu, DAP and D-Ala with a direct cross-link [L17 p19]

On the figure: L-Ala · D-Glu · DAP · D-Ala

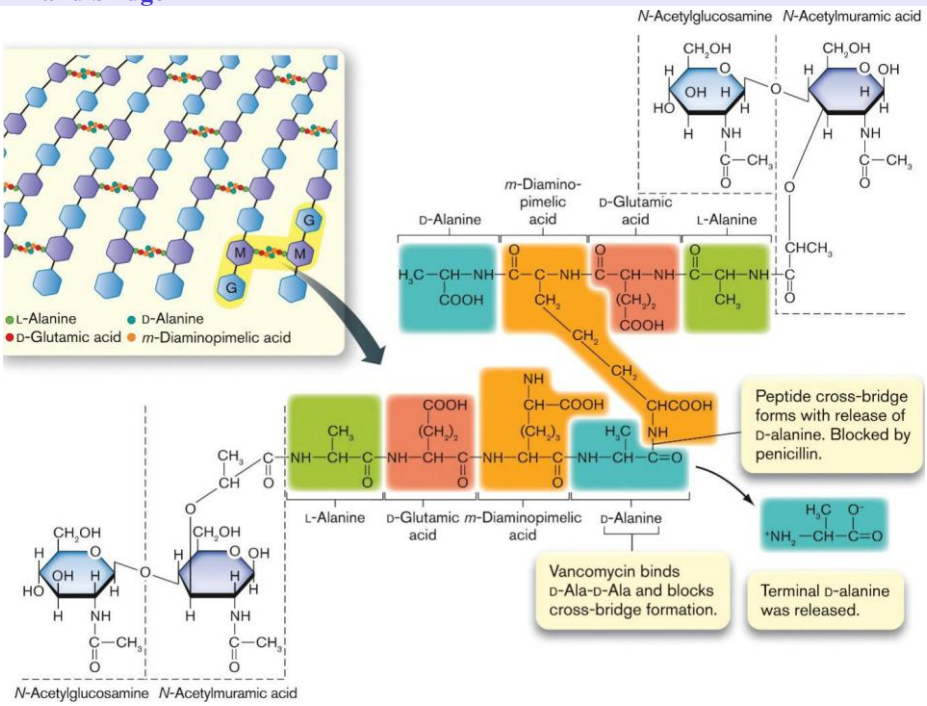
Position 3 varies

Position 3 varies between species. [L17 p21]

Position 3 options: L-alanine, L-homoserine, L-diaminobutyric acid, L-glutamic acid, L-ornithine, L-lysine, or LL-DAP and meso-DAP. [L17 p21]

Other positions: L-alanine at position **1,** D-glutamate at **2,** D-alanine at **4.** [L17 p21]

Cross-link and bridge



Cross-bridge forms with release of D-alanine; blocked by penicillin and vancomycin [L17 p22]

On the figure: Cross-bridge · penicillin · Vancomycin · D-Ala-D-Ala

Cross-link: a peptide of amino acids joins the chains. [L17 p15, L17 p16]

Gram negative cross-link: joins DAP (position **3**) to D-Ala (position **4**) of the neighboring peptide. [L17 p19]

Peptide at cross-linking: starts out with five amino acids. [L17 p22]

5th amino acid: cleaved off when the cross-link is made. [L17 p22]

Cut site: between the two D-alanines. [L17 p22]

Cross-bridge formation releases D-alanine. [L17 p22]

Antibiotics

Antibiotics: many inhibit peptidoglycan synthesis. [L17 p15, L17 p16]

Peptidoglycan is unique to bacteria, and many of its synthesis enzymes bind **penicillin**, so they are called penicillin-binding proteins (textbook).

Penicillin blocks the cross-bridge. [L17 p22]

The transpeptidase *(enzyme making wall cross bridges)* that cross-links the peptides is the target of penicillin (textbook).

Vancomycin *(drug blocking wall building)* binds **D-Ala-D-Ala** and blocks cross-bridge formation. [L17 p22]

Vancomycin binding the terminal D-Ala-D-Ala prevents release of the terminal D-alanine, so the cross-bridge cannot form (textbook).

Feature	Content
Sugar 1	N-acetyl-glucosamine (GlcNAc, NAG or G)
Sugar 2	N-acetylmuramic acid (MurNAc, NAM or M)
Sugar linkage	beta-**1**-**4** between the alternating sugars
Cross-link	by a peptide of amino acids (two linked peptides); sequence varies
Amino acids	most contain some D-amino acids (proteins use L-forms)

Table source: [L17 p17, L17 p18]

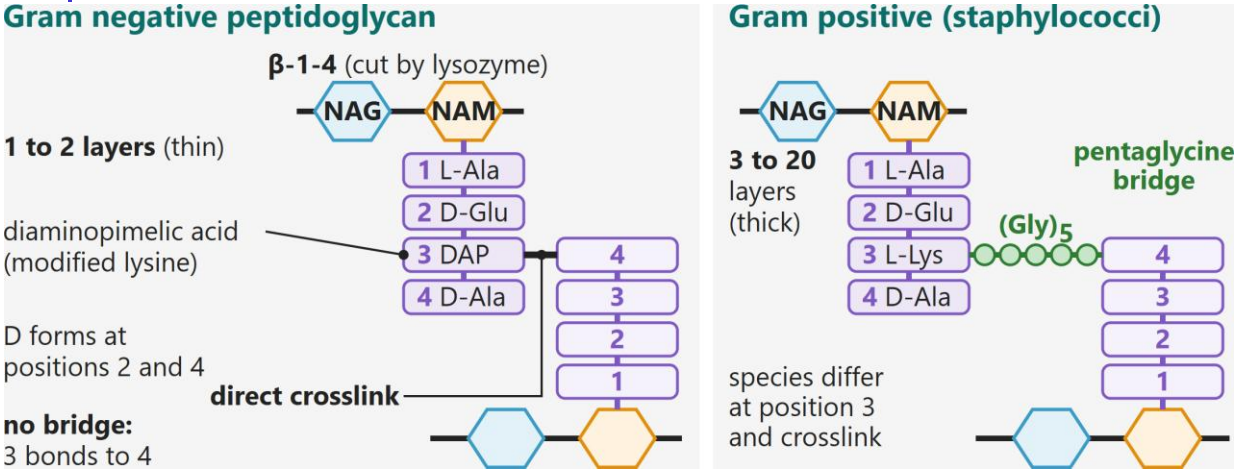
- **Know how it differs in Gram negative vs Gram positive**

  Book: 3.3 The Envelope and Cytoskeleton, Cell Envelope of Bacteria (chapter 3 PDF, p. 49); 3.3 The Envelope and Cytoskeleton, Firmicute Cell Envelope: Gram-Positive (chapter 3 PDF, p. 52).

  ⚠ **Do not mix up: Staph vs Gram negative *(thin wall plus outer membrane)***: cross-link amino acids and layers differ, sugars do not.

Side by side comparison

Gram positive *(thick wall, stains purple)* **example** is the staphylococci. [L17 p15, L17 p19, L17 p20]



Gram negative, staphylococcal and archaeal peptidoglycan: layers, position 3 and cross-link [L17 p20]

On the figure: DAP · L-Lys · (Gly)5 · pentaglycine

Gram positive wall: thicker, with more layers.

Layers differ: **3-20** in **Gram positive** versus **1-2** in **Gram negative**. [L17 p15, L17 p23]

Staph vs Gram negative: the amino acids in the peptide *(short chain of amino acids)* cross-link differ.

Position 3 differs: **L-Lys** in **Gram positive** versus **DAP** *(special amino acid in wall peptide)* in **Gram negative**. [L17 p19, L17 p20, L17 p23]

Cross-link differs: **(Gly)5** bridge versus direct DAP to D-Ala *(D form of alanine amino acid)*. [L17 p19, L17 p20, L17 p23]

(Gly)5 is **pentaglycine** *(five glycines in a row)*. [L17 p20]

What stays the same

Sugars and sugar linkage: the same in staph and **Gram negative**.

Evidence for a Gram positive envelope

Evidence for a Gram positive envelope *(layers wrapped around the cell)*: techniques or experiments that support it.

Evidence:

TEM showing a thick peptidoglycan *(sugar and peptide mesh of the wall)* layer and no outer membrane *(extra membrane outside the wall)*.

Isolate teichoic acid *(charged chains in Gram positive wall)* from the wall.

Sensitivity to a penicillin more effective on **Gram positive**.

No growth on selective media for **Gram negative**.

Genome lacking genes for LPS *(outer membrane sugar and fat coat)*, outer membrane and periplasmic *(in the space between the two membranes)* proteins.

Genome having genes for teichoic acid synthesis.

Feature	Gram negative	Gram positive (staphylococci)
Thickness	thinner, **1-2 layers**	**3-20 layers**
Position 1 and 2	L-Ala, D-Glu	L-Ala, D-Glu
Position 3 of the peptide	DAP	L-Lys
Position 4	D-Ala	D-Ala
Cross-link	direct: DAP (position **3**) to D-Ala (position **4**)	bridge of five glycines: L-Lys to (Gly)**5** to D-Ala
Sugars and linkage	NAG and NAM, beta-**1-4**	NAG and NAM, beta-**1-4** (same)
Other wall layers	outer membrane outside the thin wall; the wall sits in the periplasm	teichoic acids found in most **Gram positive** bacteria

Table source: [L17 p05, L17 p15, L17 p19, L17 p20, L17 p26]

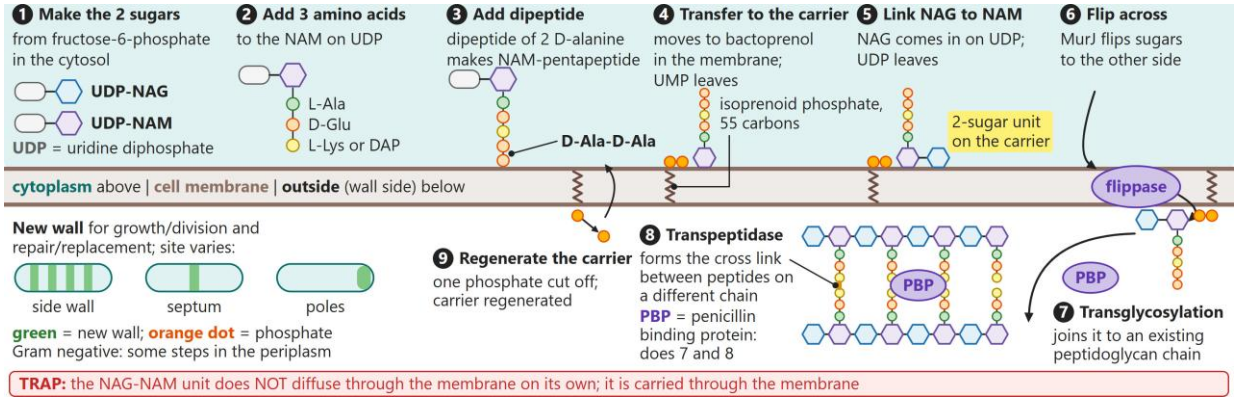
Basic order of events in synthesis

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

⚠ **Do not mix up: Step 7 transglycosylation *(joining sugar chains longer)* links sugars; step 8 transpeptidation *(making peptide bridges between chains)* cross links peptides *(short chain of amino acids)*.** [L18 p34]

⚠ **Do not mix up: Transglycosylation links SUGARS (two sugar units); transpeptidation cross links PEPTIDES.** [L21 p39, L21 p40]

Overview of synthesis



The nine steps of peptidoglycan synthesis in order [L18 p34]

On the figure: UDP · NAG · NAM · Bactoprenol · MurJ · transglycosylation · transpeptidases

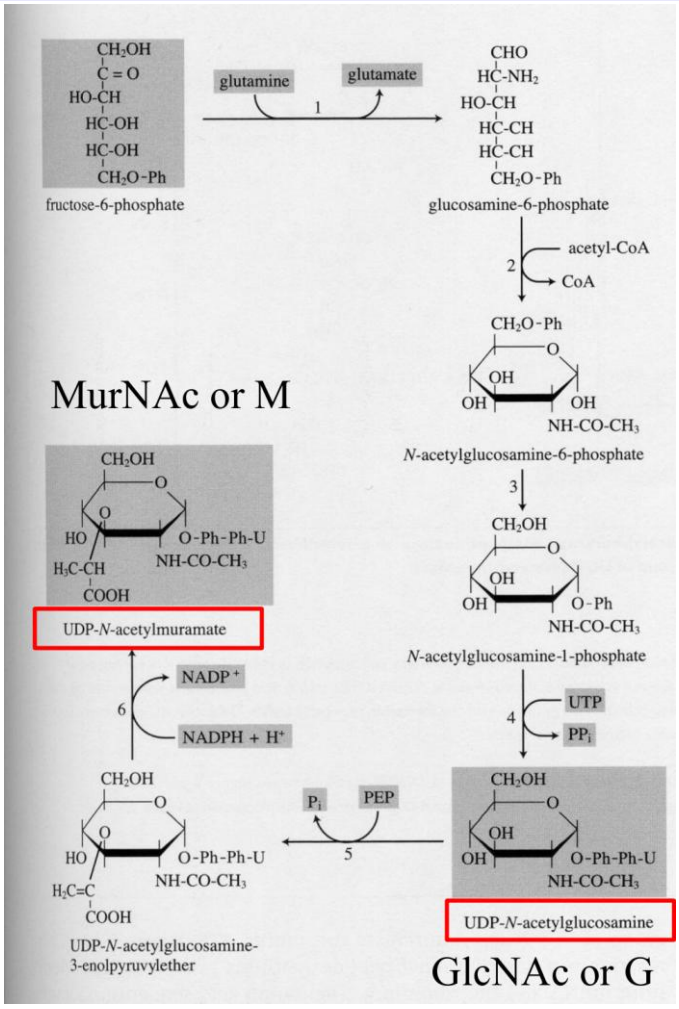
Overview step 1:

> Synthesize two sugar units, **NAG** *(one wall sugar)* and **NAM** *(the other wall sugar)*.
> Also makes a peptide off NAM. [L18 p30]

Overview step 2: add the **2** sugar unit to an existing chain. [L18 p30]

Overview step 3: then form a crosslink to a peptide in a different chain. [L18 p30]

Making NAG and NAM



Step 1 pathway from fructose-6-phosphate to UDP-NAG and UDP-NAM [L18 p32]

On the figure: glutamine · acetyl-CoA · UTP · PEP · NADPH · UDP-N-acetylglucosamine · UDP-N-acetylmuramate

Step 1 pathway: fructose-**6**-P plus **glutamine** gives glucosamine-**6**-P. [L18 p32]

Step 1 continues:

(**acetyl-CoA**) N-acetylglucosamine-**6**-P, then N-acetylglucosamine-**1**-P.

(**UTP**) **UDP-N-acetylglucosamine** (UDP-GlcNAc, G). [L18 p32]

UDP *(uridine diphosphate)*: the sugars are initially attached to UDP. [L18 p32]

Step 1 continues: (**PEP**) UDP-NAG-**3**-enolpyruvylether, then (**NADPH**) **UDP-N-acetylmuramate** (UDP-MurNAc, M). [L18 p32]

NAM precursor is made from the UDP-NAG one. [L18 p32]

Bactoprenol carrier

Bactoprenol (undecaprenyl):

A large isoprenoid *(branched five carbon repeat fat)* phosphate *(long fat-like carrier with a phosphate)* with **55 carbons** (C55).

Carries peptidoglycan *(sugar and peptide mesh of the wall)* units, LPS *(outer membrane sugar and fat coat)* and teichoic acid *(charged chains in Gram positive wall)* through the cell membrane *(thin skin around the cell)*.

The sugar is attached via the phosphate. [L18 p35]

Lipid I, lipid II and MurJ

Lipid I:

Is NAM-pentapeptide on bactoprenol-PP.

Gets NAG from UDP-NAG to become lipid II. [L18 p35, L18 p36]

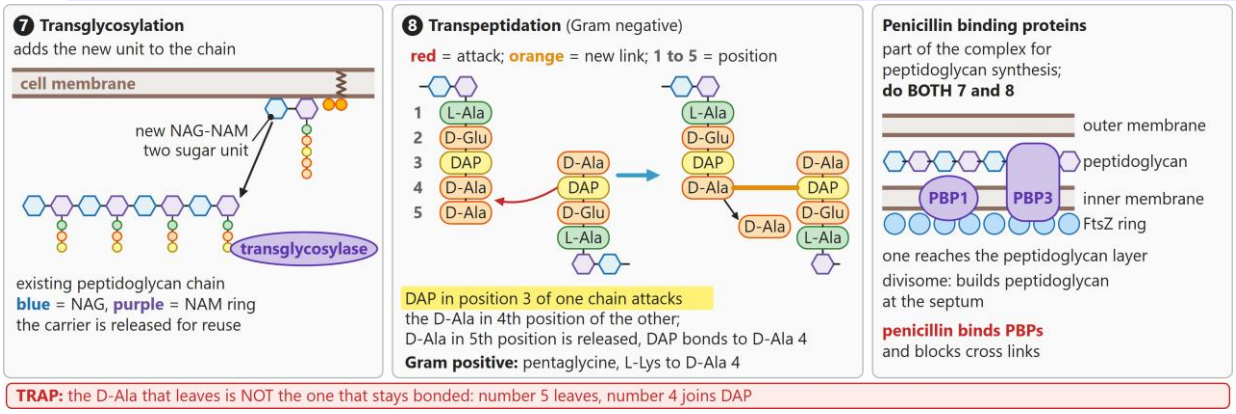
Step 5: UDP-NAG supplies the NAG to NAM and **UDP leaves**. [L18 p33]

MurJ *(flipper protein for wall units)*:

Transmembrane protein (flippase).

Flips sugars to the other side (the lipid-linked NAM-NAG unit, lipid II). [L18 p35, L18 p36]

Step 7 linking sugars



Transglycosylation, transpeptidation and the penicillin binding proteins [L18 p36]

On the figure: transglycosylation · transpeptidation · DAP · pentaglycine · PBPs

Step 7, transglycosylation: link sugars to a glycan *(sugar chain)* chain. [L18 p34]

Step 7:

The transglycosylase attaches the new disaccharide unit to the existing chain.

Linking newly synthesized two sugar units to the existing peptidoglycan chain. [L18 p36]

Step 8 crosslinking

Step 8, transpeptidation: form the cross link. [L18 p34]

Step 8: crosslinking of peptides. [L18 p37]

Transpeptidase *(enzyme making wall cross bridges)* catalyzes the cross-link between side chains of adjacent strands, and cross-linking strengthens the cell wall (textbook).

Gram negative cross link

Gram negative *(thin wall plus outer membrane)*:

DAP *(special amino acid in wall peptide)* in the 3rd position attacks D-Ala *(D form of alanine amino acid)* in the 4th position.

This displaces the D-Ala in the 5th position. [L18 p30, L18 p37]

Gram negative cross link:

DAP of one chain attacks the D-Ala-D-Ala bond of the other chain.

The terminal D-Ala is released and DAP joins the 4th D-Ala. [L18 p30]

Gram positive cross link

Gram-positive *(thick wall, stains purple)* **cross link**:

A **pentaglycine** bridge links L-Lys on one chain

The bridge joins the penultimate D-Ala on the other chain. [L18 p36]

PBPs

PBPs:

Penicillin binding proteins *(enzymes that build the wall)* do the transglycosylation (step **7**) reaction.

Also do the transpeptidation (step **8**) reaction. [L18 p34, L18 p38]

On the outer side of the membrane, **transpeptidases** and transglycosylases bind the D-Ala-D-Ala part of the pentapeptide (textbook).

PBPs: part of the complex involved in synthesis of peptidoglycan. [L18 p38]

Gram positive and Gram negative

Gram positive vs Gram negative: the synthesis pathway above is the **Gram-positive** version. [L18 p33]

Gram negative is similar, but some steps occur in the periplasm *(space between the two membranes)* between the membranes. [L18 p33]

Watch outs and step numbers

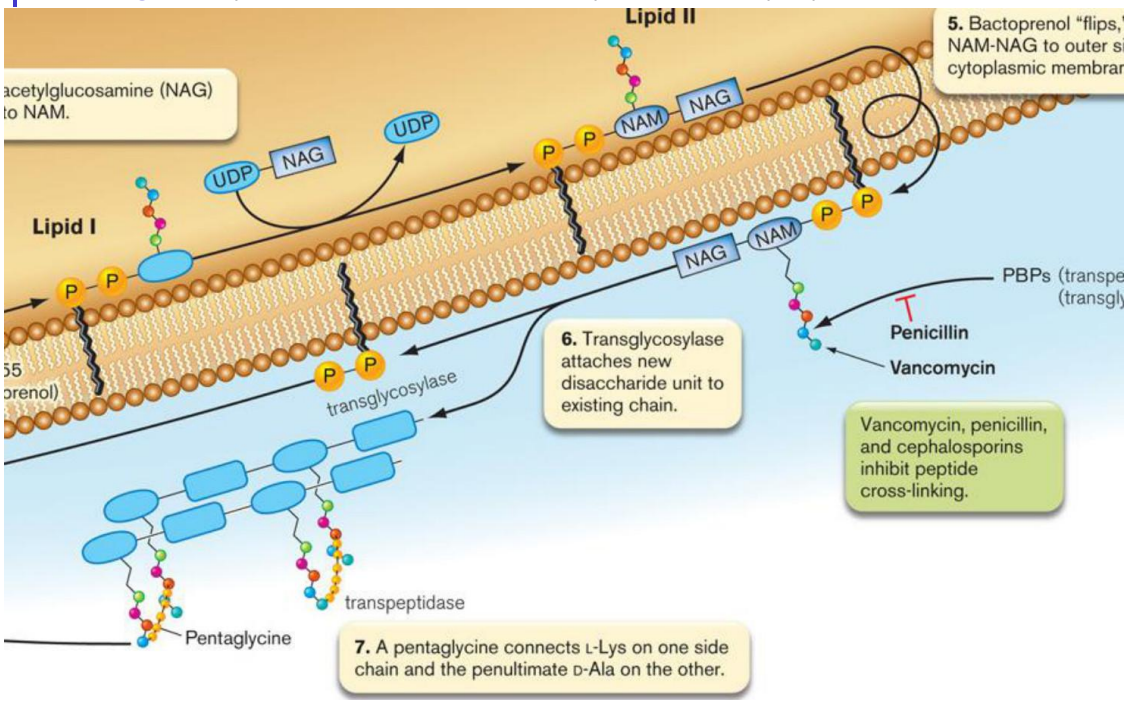
Watch out: in overview step **2** the **2** sugar unit is added to an existing chain; **bactoprenol** helps to carry the units. [L18 p30, L18 p35]

Numbering rule: learn the step numbers **1** to **9** from the numbered step list (step **7** transglycosylation, step **8** transpeptidase). [L18 p34]

Watch out: the synthesis diagram with the drug block points uses its own numbers: flip is **5,** transglycosylase **6,** transpeptidase **7,** bactoprenol return **8.** [L18 p33, L18 p36]

Do not mix the pathway numbers with the step numbers **1** to **9.** [L18 p33, L18 p34, L18 p36]

Gram negative: synthesis is similar, but some steps occur in the periplasm between membranes. [L21 p38]



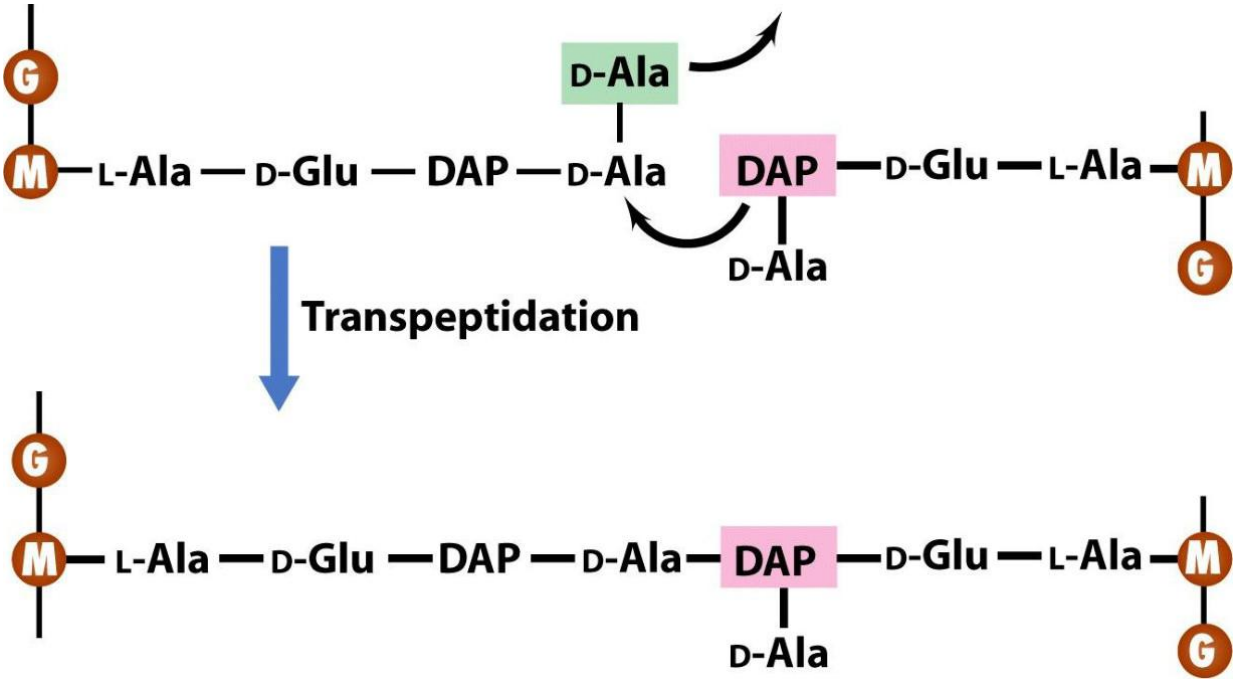
Transglycosylase and transpeptidase steps with the pentaglycine bridge [L21 p39]

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

Transglycosylation: linking newly synthesized two sugar units to the existing peptidoglycan chain. [L21 p39]

Gram positive cross link: **pentaglycine** connects **L-Lys** on one side chain to the penultimate **D-Ala**. [L21 p39]

Transpeptidation: crosslinking of peptides, done by penicillin binding proteins. [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 in the 3rd position attacks D-Ala in the 4th position. [L21 p40]
This displaces the D-Ala in the 5th position. [L21 p40]

Step	Event	Detail	Blocked by (textbook fill marked)
1	Make the sugar precursors NAM and NAG	Both made from fructose-6-P in the cytosol; sugars first attached to UDP (UDP-GlcNAc = G, UDP-MurNAc = M)	none
2	Add 3 amino acids to UDP-NAM to begin the peptide	L-Ala, D-Glu, L-Lys (DAP in **Gram negative**)	none
3	Add the dipeptide of 2 D-alanine to the peptide	Gives the pentapeptide (D-Ala-D-Ala is added to the peptide)	D-cycloserine (cycloserine inhibits formation of the dipeptide: it blocks L-Ala to D-Ala by the Ala racemase and the joining of D-Ala-D-Ala by the ligase)
4	Transfer NAM-pentapeptide to bactoprenol (C55) in the cell membrane	UMP released	none
5	Link NAG to NAM	UDP-NAG supplies the NAG	none
6	Flip the 2 sugar unit to the other side of the bilayer	MurJ flippase	none
7	Transglycosylation: link sugars to a glycan chain	Link sugars to a glycan chain; the transglycosylase adds the new disaccharide unit to the chain; done by PBPs	penicillin and cephalosporins (they bind the PBPs that do transglycosylation); textbook fill: vancomycin
8	Transpeptidase: form the cross link	Cross link between peptides of adjacent chains; done by PBPs	penicillin, cephalosporins, vancomycin
9	Regenerate bactoprenol-P by cleaving off a phosphate group	Carrier is recycled (bactoprenol-PP loses a P and returns)	bacitracin

Table source: [L18 p32, L18 p33, L18 p34, L18 p35, L18 p36, L18 p39]

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]

2) Pseudopeptidoglycan: how it differs from peptidoglycan

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

⚠ **Do not mix up: Pseudopeptidoglycan *(archaea version of wall mesh)* has NAT *(archaeal wall sugar)*, beta-1-3 links, all L-amino acids; Gram negative *(thin wall plus outer membrane)* has NAM *(the other wall sugar)*, beta-1-4.** [L17 p24]

Where it is found

Pseudopeptidoglycan:

> Found in some archaeal *(of archaea)* bacteria. [L17 p24]
>
> Is not in **Gram positive**, **Gram negative** or mycobacteria *(waxy walled bacteria)* walls.

Its features

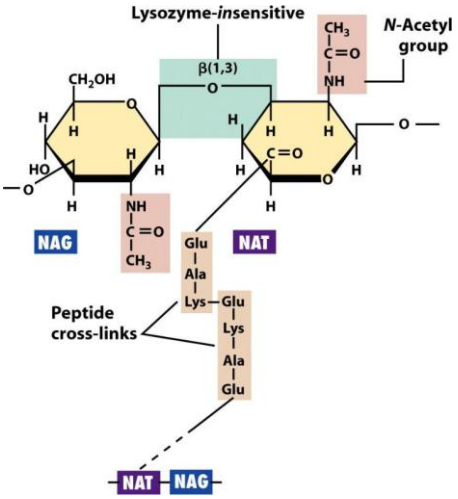
Pseudopeptidoglycan sugars: N-acetyl-glucosamine (**NAG**) and N-acetyltalosaminuronic acid (**NAT**). [L17 p24]

Layers: can have many. [L17 p24]

Sugar linkage: beta-**1-3.** [L17 p24]

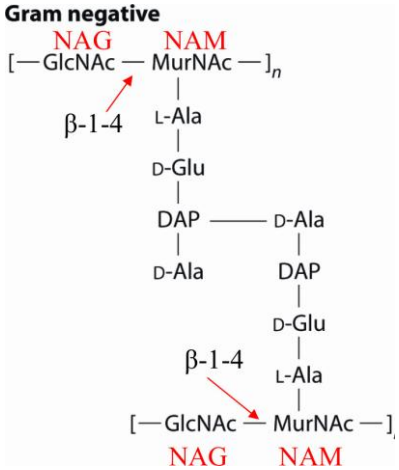
Cross-link: peptide *(short chain of amino acids)* made of all L-amino acids (Glu, Ala and Lys). [L17 p24]

Pseudopeptidoglycan is **lysozyme-insensitive** *(not cut by wall cutting enzyme)*. [L17 p24]



Pseudopeptidoglycan: NAG and NAT, beta 1,3 link, lysozyme insensitive, peptide cross-links [L17 p24]

On the figure: NAG · NAT · lysozyme-insensitive



Gram negative peptidoglycan: NAG and NAM with beta-1-4 links, for comparison [L17 p25]

On the figure: NAG · NAM · Gram negative

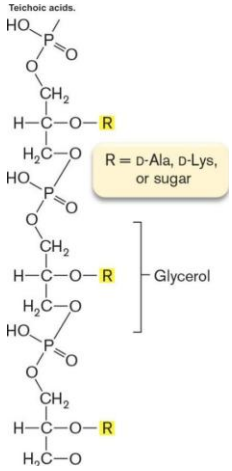
Feature	Gram negative peptidoglycan	Pseudopeptidoglycan
Sugars	NAG and NAM	NAG and NAT (N-acetyltalosaminuronic acid)
Sugar linkage	beta-**1**-**4**	beta-**1**-**3**
Peptide	D-amino acids (D-Glu, D-Ala) and DAP	all L-amino acids
Layers	**1**-**2**	can have many layers
Lysozyme	linkage between the sugars is cut by lysozyme	lysozyme-insensitive

Table source: [L17 p15, L17 p19, L17 p24, L17 p25]

3) Teichoic acids

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

⚠ **Do not mix up: Teichoic acids *(charged chains in Gram positive wall)* link to peptidoglycan *(sugar and peptide mesh of the wall)*; lipoteichoic *(teichoic acid anchored in membrane)* acid links to membrane *(thin lipid layer)* lipids.** [L17 p27]



Teichoic acid: glycerol phosphate chain with R group of D-Ala, D-Lys or sugar [L17 p26]

On the figure: Teichoic acids · glycerol · R

What teichoic acids are

Teichoic acids:

> Polyanionic *(many negative charges)* polymers *(long chains of repeating units)* of **glycerol** *(three carbon fat backbone)* phosphate *(PO4 group)* or ribitol *(five carbon sugar alcohol)* phosphate; each phosphate group carries a negative charge.
> Are modified with amino acids (**R**): R is D-Ala *(D form of alanine amino acid)*, D-Lys or a sugar. [L17 p26]

Where seen: most **Gram positive** *(thick wall, stains purple)* bacteria. [L17 p26]

Watch out: The envelope *(layers wrapped around the cell)* summary list says teichoic acid is in some **Gram positive** walls (most versus some). [L17 p26]

Composition varies, so they can be used for identification. [L17 p26]

Placement

Placement: run through the peptidoglycan. [L17 p27]

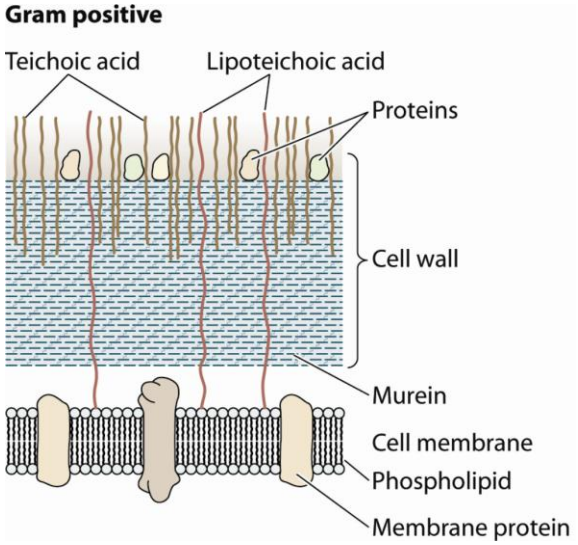
Lipoteichoic acid: linked to lipids in the membrane. [L17 p27]

Teichoic acid can also be linked to peptidoglycan. [L17 p27]

Teichoic acids run through the peptidoglycan. [L17 p27]

Roles

Role 1: regulation of cell shape and cell division. [L17 p27]



Gram positive wall with teichoic acid and lipoteichoic acid through the murein [L17 p27]

On the figure: Teichoic acids · lipoteichoic acid · cell wall

Role 2: affects antibiotic resistance. [L17 p27]

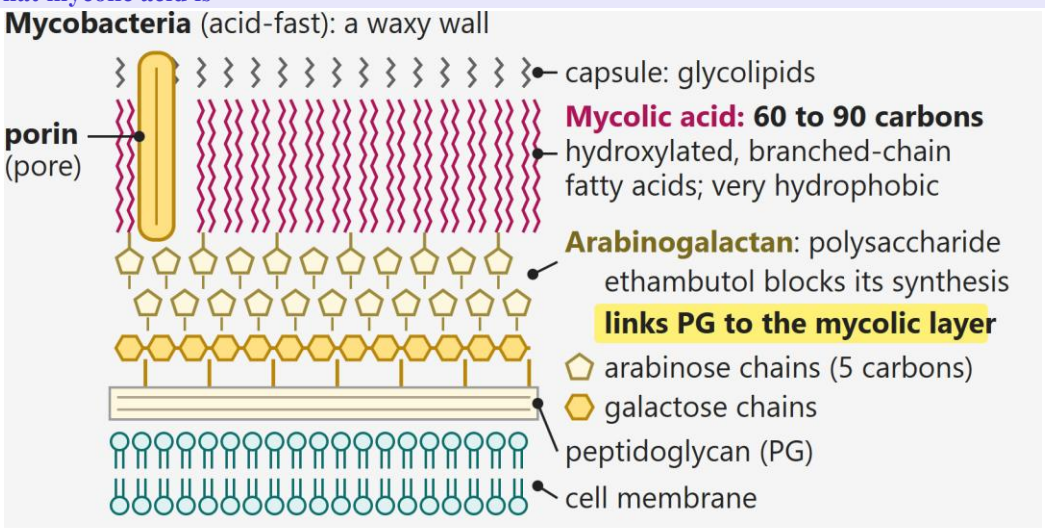
Role 3: integrity of the **cell wall** *(stiff layer around the cell membrane)*. [L17 p27]

▸ 4) Mycolic acids

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

⚠ **Do not mix up: Acid-fast *(holds dye despite acid wash)* envelope *(layers wrapped around the cell)* has a mycolic acid *(waxy fatty acid of mycobacteria)* layer but NOT an outer membrane *(extra membrane outside the wall)* with LPS *(outer membrane sugar and fat coat)*.**

What mycolic acid is



Mycolic acid, arabinogalactan and thin peptidoglycan in the acid fast wall [L17 p28]

On the figure: Mycolic acids · arabinogalactan · Ethambutol · porins · hydrophobic · galactose · arabinose

Mycolic acid: complex hydroxylated *(with OH groups added)*, branched-chain fatty acids. [L17 p28]

Mycolic acid carbons: elevated carbon numbers, **60-90 carbons**. [L17 p28]

Mycolic acid is very **hydrophobic** *(water hating)*. [L17 p28]

Found in Mycobacteria *(waxy walled bacteria)*. [L17 p28]

Linked to **arabinogalactan** *(chain of arabinose and galactose sugars in the wall)* sugars. [L17 p28]

Role of mycolic acid

Role:

Blocks diffusion *(drifting from high to low amount)* of most molecules including nutrients.

Makes cells resistant to acids, bases, antibiotics and dehydration. [L17 p28]

Arabinogalactan

Arabinogalactan:

Polysaccharide *(long chain of sugars)* in the Mycobacteria cell wall *(stiff layer around the cell membrane)*.

Has chains of **arabinose** (**5 carbons**) and chains of **galactose**.

Is linked to peptidoglycan *(sugar and peptide mesh of the wall)* and mycolic acid. [L17 p29]

Arabinogalactan synthesis: inhibited by some antibiotics used against mycobacteria. [L17 p29]

Ethambutol: blocks arabinan *(five carbon sugar chain)* synthesis (the arabinose chains of arabinogalactan). [L17 p29]

Mycobacteria and the acid-fast envelope

Mycobacteria are **Gram positive** **actinomycetes** *(a group of Gram positive bacteria)* with an acid-fast envelope. [L17 p03]

Mycolic acids *(waxy fatty acids of mycobacteria)* provide the basis for acid-fast staining, in which cells retain the dye carbolfuchsin (textbook).

Acid-fast envelope:

Has peptidoglycan.

Also has arabinan-galactan sugars and mycolic acid.

Also has a phospholipid *(membrane fat with a charged head)* bilayer *(two layers)* membrane *(thin lipid layer)*.

Has NO outer membrane containing phospholipids and LPS; its outer layer is the mycomembrane of mycolic acids, called the mycobacterial *(about waxy walled bacteria)* outer membrane.

Wall layers inside to outside

Mycobacterial envelope layers, inside to outside (**peptidoglycan** through the **mycomembrane** form the cell wall core): [L17 p29]

Layer 1: cell membrane *(thin skin around the cell)*. [L17 p29]

Wall layer 2: peptidoglycan. [L17 p29]

Wall layer 3: **galactan** *(chain of galactose sugars)*. [L17 p29]

Wall layer 4: **arabinan**. [L17 p29]

Wall layer 5: mycomembrane of mycolic acids. [L17 p29]

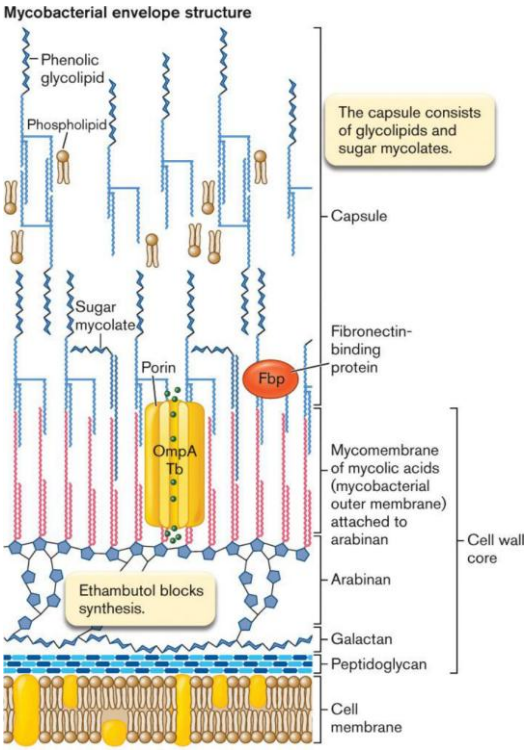
Mycomembrane of mycolic acids (mycobacterial outer membrane), attached to arabinan, with a porin *(water filled channel in outer membrane)*. [L17 p29]

Layer 6: **capsule** *(firm sugar coat outside the cell)* of glycolipids and **sugar mycolates**. [L17 p29]

Capsule components: **phenolic glycolipid**, phospholipid, sugar mycolate and **fibronectin-binding protein** (**Fbp**). [L17 p29]

Wall layers outside to inside

Mycobacterial wall layers, outside to inside: **porins** *(channels in the outer membrane)* and surface proteins, **acyl lipids**, mycolic acid, arabinogalactan, **lipoarabinomannan**, peptidoglycan, **PIM**, cytoplasmic membrane. [L17 p28]



Mycobacterial envelope: capsule, mycomembrane, arabinan, galactan, peptidoglycan, membrane [L17 p29]

On the figure: **capsule** · **phenolic glycolipid** · **sugar mycolates** · **Fbp** · **arabinan** · **galactan** · **peptidoglycan** · **mycomembrane**

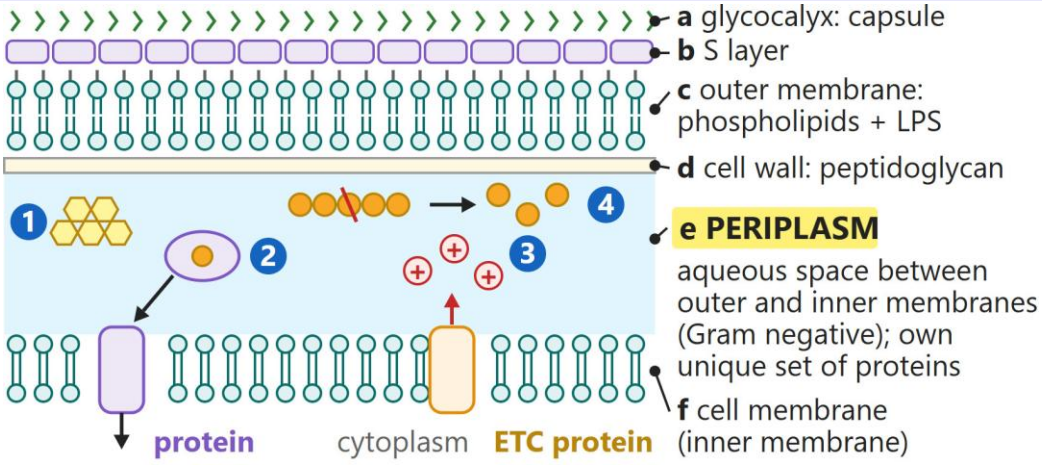
Mycolic acid is not archaeal

Mycolic acid: not part of the archaeal *(of archaea)* envelope.

e. Periplasm

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

What the periplasm is



Main Functions

1 osmotic protection: holds oligosaccharides
2 transport: binding proteins bring solutes (food) to membrane transporters
3 oxidation-reduction reactions of the electron transport chain; stores H+ ions
4 hydrolysis: cuts nutrients (red slash) into subunits small enough to import

TRAP: the periplasm is NOT outside the cell; it lies inside the envelope

Periplasm between the outer and inner membranes, with its four functions [L17 p31]

On the figure: periplasm · outer membrane · inner membrane · Peptidoglycan · unique set of proteins

Periplasm *(space between the two membranes)*: the aqueous compartment between the **outer membrane** *(extra membrane outside the wall)* and the **inner membrane** *(the skin around the cytoplasm)*. [L17 p31]

Where: **Gram negative** *(thin wall plus outer membrane)* bacteria. [L17 p31]

Periplasm contains its own **unique set of proteins**. [L17 p31]

The outer membrane prevents passage of proteins and other macromolecules, so the periplasm is a separate membrane-enclosed compartment (textbook).

Peptidoglycan (murein) layer: sits inside the periplasm. [L17 p31]

Gram positive contrast

Gram positive *(thick wall, stains purple)* **strain**: lacks genes for periplasmic *(in the space between the two membranes)* proteins.

#	Main function of the periplasm
1	osmotic protection: contains oligosaccharides
2	transport of nutrients: proteins bind solutes (food) and carry them to inner membrane transporter proteins
3	oxidation-reduction reactions of the electron transport chain; stores H+ ions
4	hydrolysis of nutrients into smaller subunits that can be transported into the cytoplasm

Table source: [L17 p32]

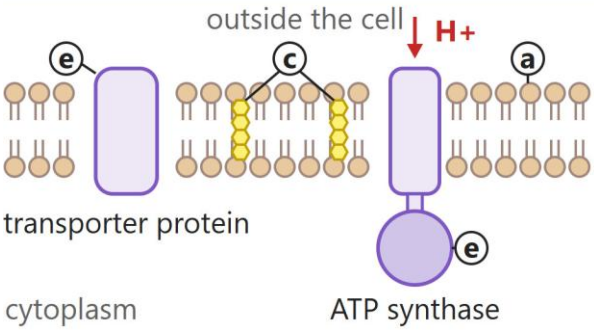
f. Cell membrane (3.2 6e)

Book: 3.2 Membrane Molecules and Transport (chapter 3 PDF, p. 24).

⚠ **Do not mix up: E. coli membrane** ***(thin lipid layer)*** **is 75% protein, 25% phospholipid** ***(membrane fat with a charged head)*** **by weight, with no sterol** ***(stiff ring shaped fat)*****.** [L17 p33]

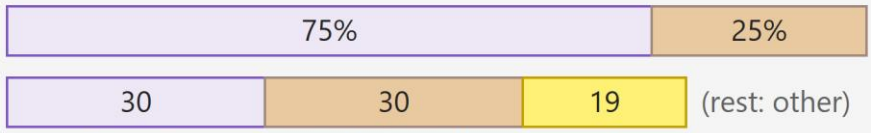
What the cell membrane is

Cell membrane = lipids + proteins; it is the boundary and barrier



Components:
a Phospholipids: most bacteria
b Isoprenoids: archaea
c Hopanoids: similar to sterols
d Cardiolipin
e Proteins:
largest component by weight

% by weight (E. coli membrane)



Bars: purple = protein, tan = phospholipid, yellow = sterol;
E. coli has no sterol; for comparison

TRAP: lipid is NOT the main component by weight in bacteria

Cell membrane of lipids and proteins; protein is 75% of E. coli membrane [L17 p33]

On the figure: lipids · proteins · E. coli · myelin · sterol · 75% · 25%

Cell membrane *(thin skin around the cell)*: composed of **lipids** and **proteins**. [L17 p33]

Cell membrane proteins: a major component by weight. [L17 p33]

Cell membrane is a boundary and a barrier. [L17 p33]

Membrane makeup compared

E. coli membrane: **75%** protein and **25%** phospholipid by weight. [L17 p33]

Human myelin sheath *(fatty covering of nerves)*: **30%** protein, **30%** phospholipid and **19%** sterol (cholesterol). [L17 p33]

E. coli membrane: no sterol. [L17 p33]

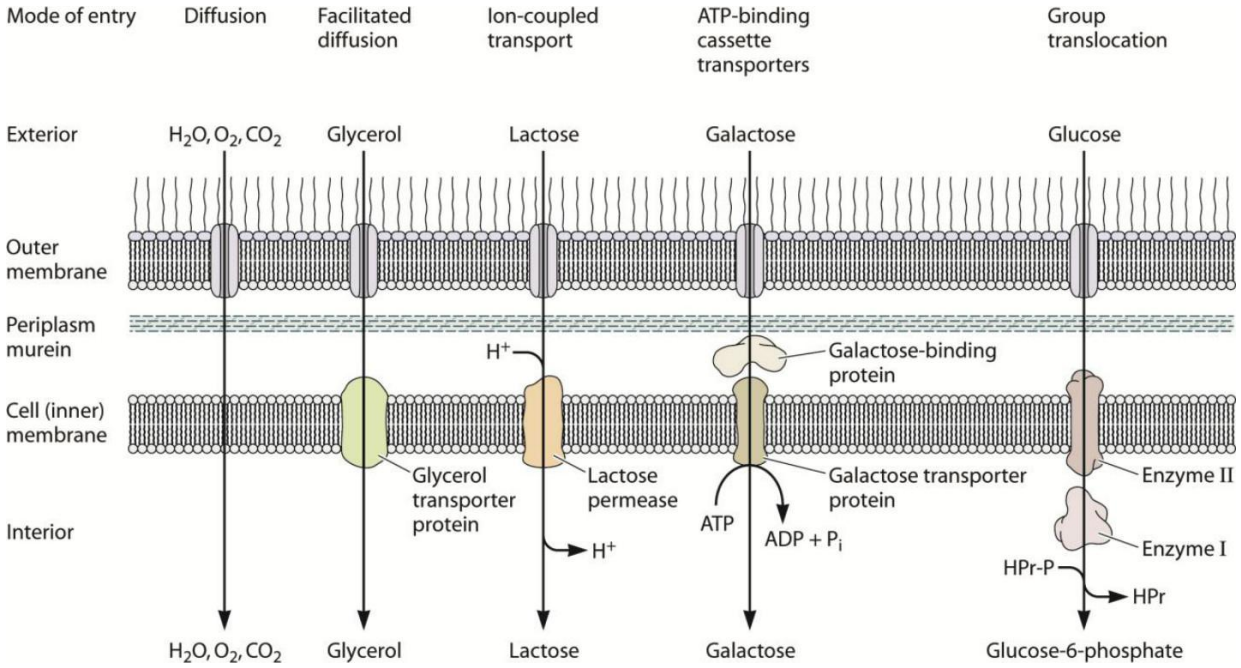
Modes of entry

Modes of entry across a **Gram negative** *(thin wall plus outer membrane)* envelope *(layers wrapped around the cell)*: five in all. [L17 p34]

Mode 1: diffusion (H_2O, O_2, CO_2). [L17 p34]

Mode 2: facilitated diffusion (glycerol). [L17 p34]

Mode 3: ion-coupled transport (lactose enters with H+). [L17 p34]



Five modes of entry across the Gram negative envelope [L17 p34]

On the figure: diffusion · facilitated diffusion · ion-coupled transport · ATP-binding cassette transporters · group translocation

Energy released as an ion *(charged particle)* moves down its concentration gradient *(gradual change in amount)* drives a second molecule against its gradient (textbook).

Mode 4: ATP-binding cassette *(protein part)* transporters (galactose). [L17 p34]

A substrate-binding protein delivers the solute to the channel, then ATP *(cell energy molecule)* hydrolysis *(breaking with water)* signals the channel to open to the cytoplasm (textbook).

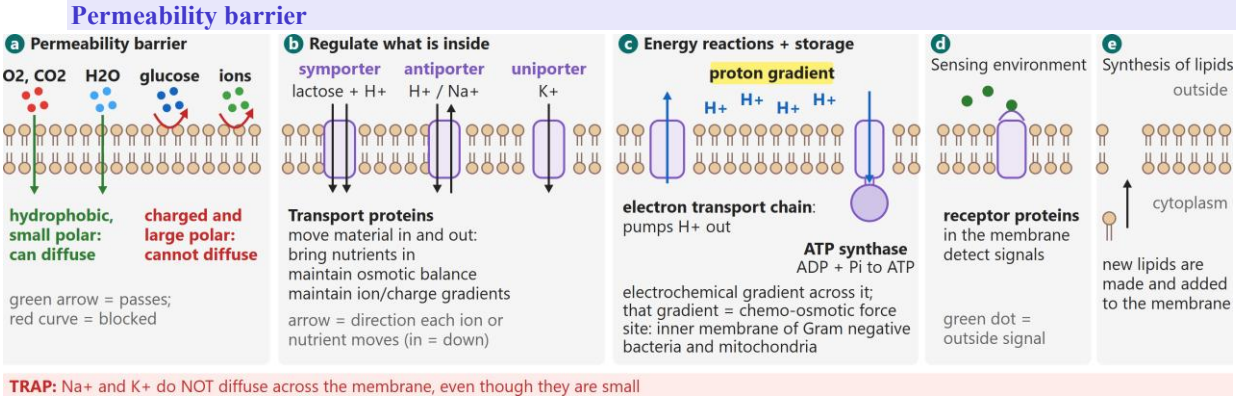
Mode 5: **group translocation** (glucose enters as glucose-**6**-phosphate). [L17 p34]

Group translocation attaches a group such as phosphate *(PO4 group)* to the nutrient during transport, so the unmodified solute always moves down its gradient (textbook).

▸ Role, function

Book: 3.2 Membrane Molecules and Transport, Molecules Cross the Cell Membrane (chapter 3 PDF, p. 35).

⚠ Do not mix up: Hydrophobic *(water hating)* and small polar molecules diffuse; charged and large polar molecules cannot. [L17 p43]



Five membrane functions: barrier, regulate, energy, sensing, lipid synthesis [L17 p43]

On the figure: Permeability barrier · Hydrophobic · proton gradient · chemo-osmotic force · ATP synthase · receptor proteins · synthesis of lipids

Function a, permeability barrier: **hydrophobic** and small polar molecules can diffuse through membranes *(thin lipid layer)*. [L17 p43]

Cannot diffuse: charged and large polar molecules. [L17 p43]

Ions *(charged particle)* such as Na+ and K+ are charged and cannot diffuse through the membrane (impermeable). [L17 p43]

Regulating the inside

Function b, regulate what is inside the cell: transport nutrients in. [L17 p44]

Function b:

- Maintain osmotic *(about water moving across membranes)* balance.
- Maintain ion and charge gradients *(gradual change in amount)*. [L17 p44]

Energy reactions

Function c, energy reactions and storage: an electrochemical gradient is created across the membrane. [L17 p45]

Function c: electron transport chain. [L17 p45]

Electron transport: the **reductant** is the electron **donor** and the **oxidant** is the electron **acceptor**. [L17 p45]

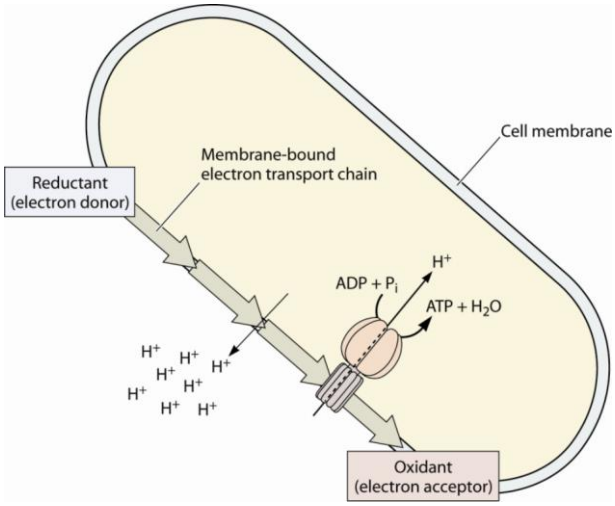
Electron transport chain *(chain of carriers in the membrane)* **pumps H+ out** across the membrane, which builds the **proton gradient**. [L17 p45]

Function c:

- Proton gradient (**chemo-osmotic force**).
- Synthesis of ATP *(cell energy molecule)*. [L17 p45]

ATP synthase *(enzyme that makes ATP)*: **ADP** *(used up energy molecule)* **+ Pi** to ATP (+ H2O); Pi is inorganic phosphate *(PO4 group)*. [L17 p45]

Function c happens across the inner membrane *(the skin around the cytoplasm)* in **Gram negative** *(thin wall plus outer membrane)* bacteria and mitochondria. [L17 p45]



Electron transport chain pumps H+ out; ATP synthase makes ATP from ADP + Pi [L17 p45]

On the figure: reductant · oxidant · electron transport chain · ADP + Pi · H+

Sensing the environment

Function d: sensing the environment (**receptor proteins** in the membrane). [L17 p46]

Receptors *(proteins a signal or virus binds to)* for attractants *(chemical that pulls cells in)* sit in the cell membrane *(thin skin around the cell)*. [L17 p46]

Chemotaxis *(moving toward or away from chemicals)*: **counterclockwise** (**CCW**) rotation of the flagella *(whip tails that spin)* moves the cell toward an attractant. [L17 p46]

Lipid synthesis

Function e: **synthesis of lipids**. [L17 p46]

Molecule type	Examples	Permeability
Hydrophobic molecules	O2, CO2, N2	freely permeable
Small, uncharged polar molecules	H2O, glycerol	slightly permeable
Large, uncharged polar molecules	glucose, sucrose	impermeable
Ions	H+, Na+, HCO3-, Ca2+, Cl-, Mg2+, K+	impermeable

Table source: [L17 p43]

Carrier type	What it does	Example
Symporter	two solutes in the same direction; Lac permease moves H+ together with lactose	Lac permease (lactose and H+ together); phosphate symporter; sulfate symporter
Antiporter	two like charged ions, opposite directions	sodium-proton antiporter
Uniporter	one solute, one direction	potassium uniporter (K+)

Table source: [L17 p44]

Components (types of lipids, etc) observed in bacteria

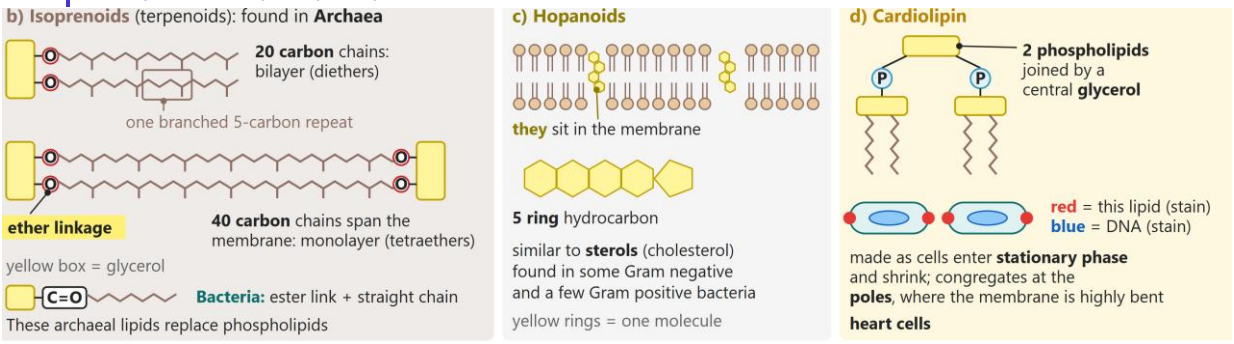
Book: 3.2 Membrane Molecules and Transport, Membrane Lipids (chapter 3 PDF, p. 24).

⚠ **Do not mix up: Archaea *(single cell microbes without a nucleus, their own domain (also called archaeal bacteria))* use isoprenoid *(branched five carbon repeat fat)* lipids; bacteria use phospholipids *(membrane fat with a charged head)*; hopanoids *(five ring cholesterol like fat)*** are 5 ring hydrocarbons similar to sterols (cholesterol).

Five components

Components of the cell membrane *(thin skin around the cell)*: five, a to e. [L17 p35]

Component a: phospholipids, in most bacterial cells. [L17 p35]



TRAP: archaeal membrane lipids are NOT ester-linked to glycerol (they are ether-linked)

Isoprenoids in archaea, hopanoids in the membrane, cardiolipin at the poles [L17 p36]

On the figure: isoprenoids · Archaea · hopanoids · sterols · Cardiolipins · poles · stationary phase

Component b: isoprenoids (found in Archaea). [L17 p35]

Component c: hopanoids. [L17 p35]

Component d: cardiolipin *(double phospholipid joined by glycerol)*. [L17 p35]

Component e: proteins. [L17 p35]

Lipid differences

Ways membrane components differ:

Lipids: archaea have isoprenoid lipids while bacteria have phospholipids.

Fatty acid chains: more or less saturated, changing with temperature.

Hopanoids and cardiolipin

Hopanoids (sterol-like molecules): some bacteria have them; like sterols in eukaryotes, they fit between fatty acid chains and stabilize the membrane (textbook).

Cardiolipins: other bacteria have them; made in bacterial cells entering stationary phase, when cell size decreases.

Cardiolipin (diphosphatidylglycerol) gathers at the cell poles. [L17 p41]

Cardiolipin has a narrow head group and wide fatty acid group, thought to form concave domains that stabilize the curve of the polar membrane (textbook).

DAPI *(dye that stains DNA)* stains the **DNA** *(cell genetic instruction molecule)*; in the same cells, cardiolipin shows at the poles. [L17 p41]

LPS and proteins

LPS *(outer membrane sugar and fat coat)*: **Gram negative** *(thin wall plus outer membrane)* bacteria can have it in the outer membrane *(extra membrane outside the wall)*.

Membrane proteins differ between species and conditions.

Phospholipid types

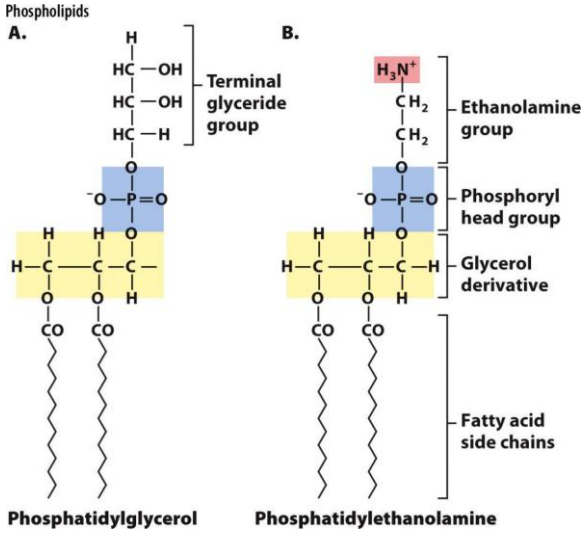
Phospholipid types vary: the head group varies, and the fatty acid chains vary in length and number of double bonds.

Phosphatidylethanolamine:

> A phospholipid; the polar head group is **ethanolamine**.
>
> Is joined through the phosphoryl group to glycerol *(three carbon fat backbone)*, with two fatty acid tails. [L17 p36]

Phosphatidylglycerol:

> Its polar head is a **terminal glyceride group** on the **phosphoryl head group**.
>
> Also has a **glycerol derivative** and two **fatty acid side chains**. [L17 p36]



Phosphatidylglycerol and phosphatidylethanolamine: head groups, glycerol derivative, fatty acid side chains [L17 p36]

On the figure: **Phosphatidylethanolamine** · **Phosphatidylglycerol** · **ethanolamine** · **terminal glyceride group** · **phosphoryl head group** · **glycerol derivative** · **fatty acid side chains**

Component	Description	Where
Phospholipids	glycerol with ester linkages to two fatty acids and a phosphoryl head group; head group varies; fatty acid chains vary in length and number of double bonds	most bacterial cells
Hopanoids	**5** ring hydrocarbon similar to sterols (cholesterol)	membrane of some **Gram negative** and a few **Gram positive** bacteria
Cardiolipin	**2** phospholipids linked by a glycerol; made in cells entering stationary phase when cell size decreases; congregates at the poles where the membrane is highly bent	bacteria; also some other organisms (heart cells)
Membrane proteins	largest component by weight; many transport material in and out of the cell	in the cell membrane

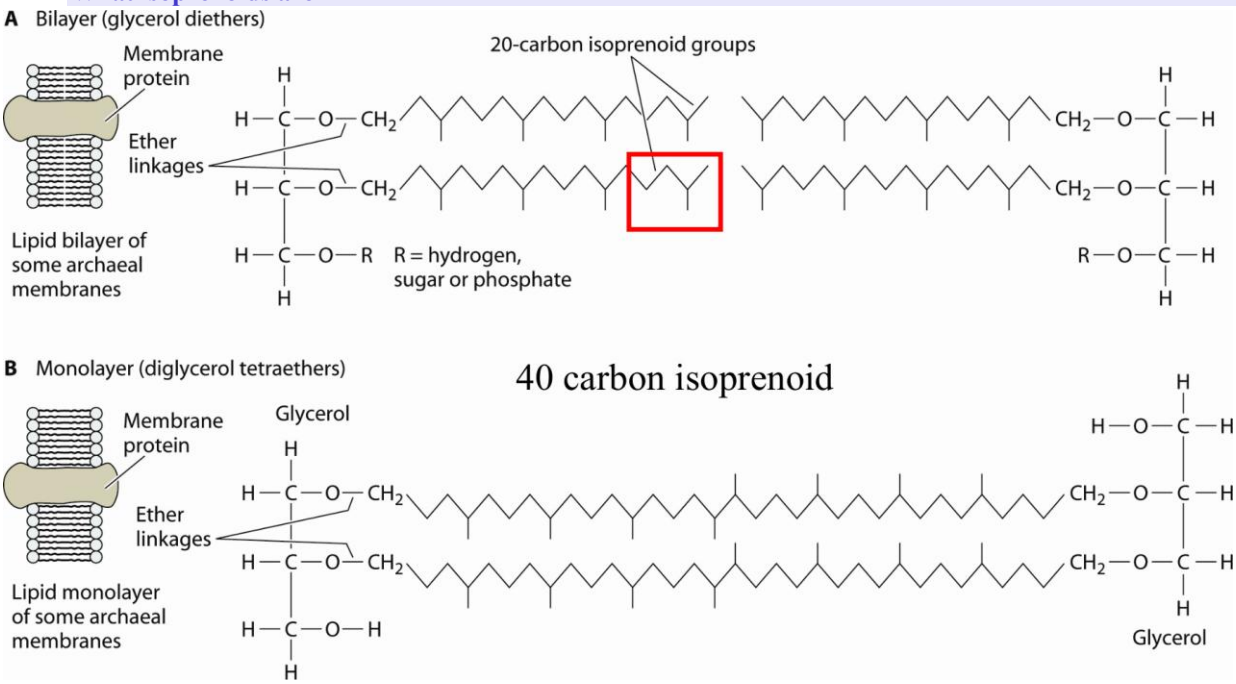
Table source: [L17 p35, L17 p36, L17 p40, L17 p41, L17 p42]

▸ **Components observed in Archaea and how different? Isoprenoids**

Book: 3.2 Membrane Molecules and Transport, Membrane Lipids (chapter 3 PDF, p. 24).

⚠ **Do not mix up: Isoprenoids *(branched five carbon repeat fats)* link to glycerol *(three carbon fat backbone)* by ether *(C O C bond)* bonds (Archaea); phospholipids *(membrane fat with a charged head)* use ester *(bond of acid and alcohol)* bonds.** [L17 p39]

What isoprenoids are



Archaeal lipids: ether linked isoprenoids as a bilayer or a monolayer [L17 p39]

On the figure: ether linkage · glycerol diethers · diglycerol tetraethers · bilayer · monolayer · glycerol

Isoprenoids: **20** or **40 carbon** terpenoids. [L17 p39, L17 p36]

Isoprenoid *(branched five carbon repeat fat)* **units**: branched **5 carbon** repeats. [L17 p39, L17 p36]

Isoprenoids link to **glycerol** via **ether linkage** *(a C-O-C bond instead of the ester bond of phospholipids)*. [L17 p39, L17 p36]

Found in Archaea *(single cell microbes without a nucleus, their own domain (also called archaeal bacteria))*. [L17 p39, L17 p36]

Ether bonds are much more stable than ester bonds, which hydrolyze easily in water, so some archaea grow at higher temperatures (textbook).

Archaeal membranes

Archaeal *(of archaea)* **cell membrane** *(thin skin around the cell)*: made of isoprenoids instead of phospholipids.

Archaeal isoprenoid lipids: **20 carbon glycerol diethers** form a **bilayer** *(two layers)*; **40 carbon diglycerol tetraethers** form a **monolayer** *(one layer)*; the head group R can be hydrogen, sugar or phosphate *(PO4 group)*. [L17 p36, L17 p39]

Feature	Bacterial phospholipid	Archaeal isoprenoid lipid
Hydrocarbon	fatty acid chains	**20** or **40 carbon** isoprenoid chains with branched **5 carbon** repeats
Link to glycerol	ester linkage	ether linkage

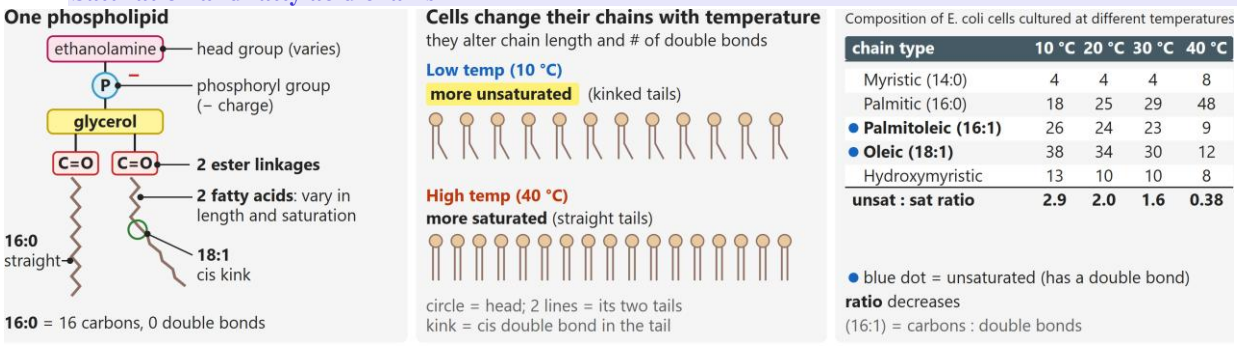
Table source: [L17 p36, L17 p39]

▸ **How types of fatty acids present change in response to temperature?**

Book: 3.2 Membrane Molecules and Transport, Membrane Lipids (chapter 3 PDF, p. 24).

⚠ **Do not mix up: Cold: more unsaturated fatty acids (ratio 2.9 at 10 C); warm: more saturated palmitic acid.** [L17 p37]

Saturation and fatty acid chains



Composition of E. coli cells cultured at different temperatures

chain type	10 °C	20 °C	30 °C	40 °C
Myristic (14:0)	4	4	4	8
Palmitic (16:0)	18	25	29	48
● **Palmitoleic (16:1)**	26	24	23	9
● **Oleic (18:1)**	38	34	30	12
Hydroxymyristic	13	10	10	8
unsat : sat ratio	**2.9**	**2.0**	**1.6**	**0.38**

● blue dot = unsaturated (has a double bond)
ratio decreases
(16:1) = carbons : double bonds

TRAP: cold does NOT raise the saturated share; it lowers it (unsaturated chains take over in the cold)

Straight saturated and kinked unsaturated tails; E. coli fatty acids at four temperatures [L17 p37]

On the figure: cis · unsaturated · saturated · 16:0 · 18:1 · 2.9 · 0.38 · palmitic

Saturation is the number of double bonds: a **saturated** chain has none, and each double bond makes it more **unsaturated**. [L17 p37]

Unsaturated tail: has a **cis double bond** *(C=C with both chain parts on the same side)* that bends (kinks) the tail; a saturated tail has no double bond and stays straight. [L17 p37]

Response to temperature

Temperature response: bacteria alter the chain length and saturation of phospholipid *(membrane fat with a charged head)* fatty acids. [L17 p37]

Kinked unsaturated chains pack less closely than straight chains, so the membrane *(thin lipid layer)* is more fluid and works better at low temperature (textbook).

E. coli results

E. coli: fatty acid composition changes with growth temperature. [L17 p37]

Cold: unsaturated fatty acids (**16:1** and **18:1**, with double bonds) are more abundant. [L17 p37, L17 p38]

Warm: saturated **palmitic** acid (**16:0**) rises as temperature rises. [L17 p37, L17 p38]

Unsaturated to saturated ratio: **2.9** at **10** C and **0.38** at **40** C. [L17 p37, L17 p38]

Fatty acid (percent of total)	10 C	20 C	30 C	40 C
Myristic 14:0	4	4	4	8
Palmitic 16:0 (saturated)	18	25	29	48
Palmitoleic 16:1	26	24	23	9
Oleic 18:1	38	34	30	12
Hydroxymyristic	13	10	10	8
Ratio unsaturated to saturated	2.9	2.0	1.6	0.38

Table source: [L17 p37, L17 p38]

3. Movement of Material across Cell membrane (3.2 and 4.2 6e)

Book: 4.2 Nutrient Uptake (chapter 4 PDF, p. 23).

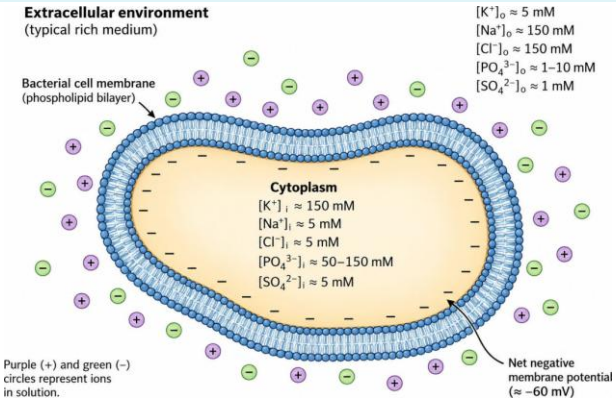
⚠ **Do not mix up: Passive *(needs no energy)* transport follows the concentration gradient *(gradual change in amount)*; active transport requires energy.** [L18 p07]

Why membranes matter

Why membranes *(thin lipid layer)* **matter**:

> Cells try to maintain concentrations of certain ions *(charged particle)* and solutes inside the cell.
>
> These inside levels differ from the outside environment. [L18 p06]

Membrane potential: net negative, about -**60 mV**. [L18 p06]



Ion levels inside and outside a bacterial cell and the membrane potential [L18 p06]

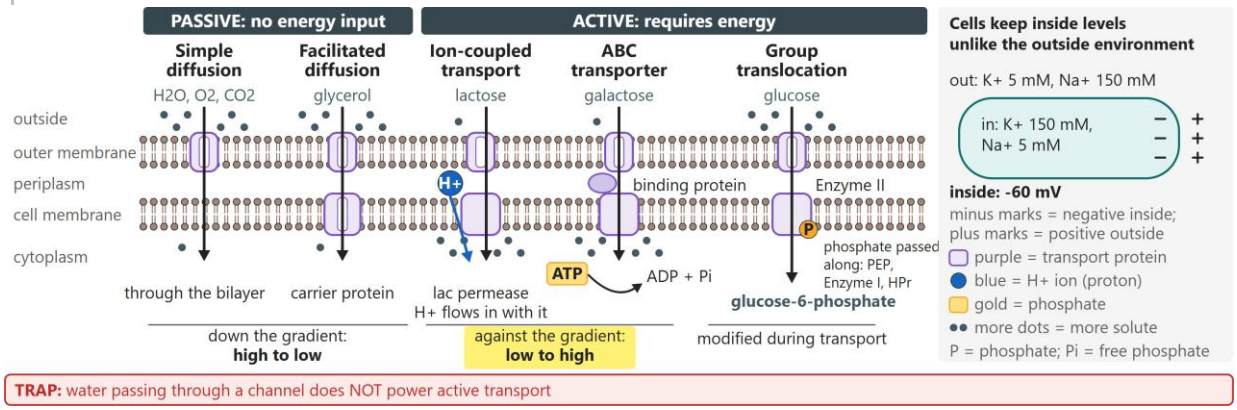
On the figure: K+ · Na+ · Cl- · Membrane potential

Ion levels in mM

Watch out, inside levels differ: the bacterial cell figure gives Cl- about **5 mM** and Na+ about **5 mM** inside; the ion table gives E. coli Cl- **10**-**200** (media dependent) and Na+ **10.** [L18 p06]

Ion table (mM):

> E. coli: K+ **30**-**300,** Na+ **10.**
>
> E. coli: Mg2+ **30**-**100** bound and **0.01**-**1** free.
>
> E. coli: Ca2+ **3** bound and **100** nM free.
>
> E. coli: Cl- **10**-**200** (media dependent).
>
> Sea water: K+ about **10,** Na+ about **500,** Cl- about **500.**
>
> Blood plasma: K+ **4,** Na+ **100**-**200,** Cl- **100. Ion table (mM)**, S. cerevisiae: K+ **300,** Na+ **30,** Mg2+ **50,** Ca2+ **2** bound. **Ion table (mM)**, mammalian cell (heart or RBC): K+ **100,** Na+ **10,** Mg2+ **10** bound and **0.5** free, Ca2+ **10**-**100** nM free, Cl- **5**-**100.** [L18 p06]



Five ways into the cell, passive or active [L18 p07]

On the figure: simple diffusion · facilitated diffusion · ion-coupled transport · ABC · group translocation · Passive · active

Transport mechanisms

Several mechanisms are used: **simple diffusion** *(passing straight through membrane)*, **facilitated diffusion** *(passing through helper protein)*, **ion-coupled transport**, **ABC** (ATP-binding cassette) transporters *(protein that carries molecules across)* and **group translocation** *(carry in while adding a phosphate)*. [L18 p07]

Passive mechanisms depend on the concentration gradient. [L18 p07]

Active mechanisms require energy. [L18 p07]

Ion	Inside the cell (approx.)	Outside, typical rich medium (approx.)
K+	150 mM	5 mM
Na+	5 mM	150 mM
Cl-	5 mM	150 mM
PO4 3-	50-150 mM	1-10 mM
SO4 2-	5 mM	1 mM

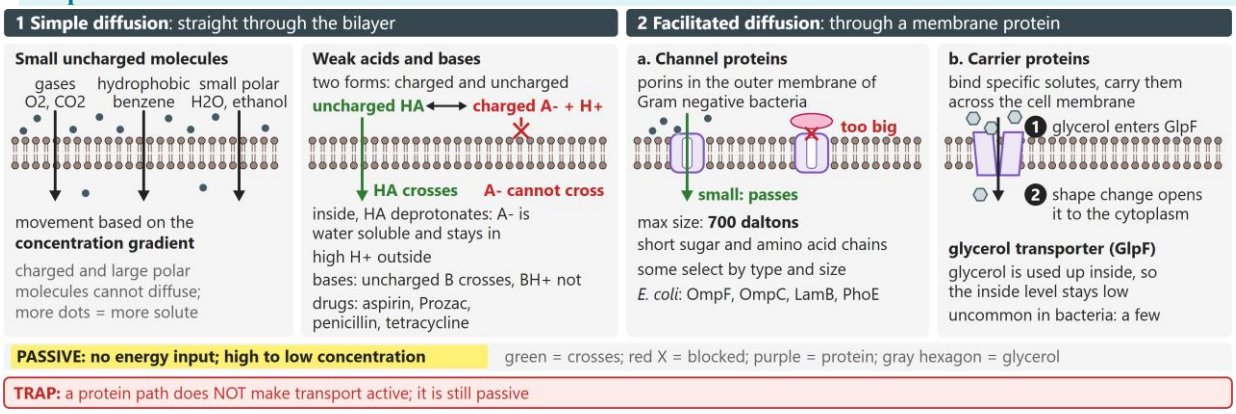
Table source: [L18 p06]

Different types of transport

Book: 4.2 Nutrient Uptake, Facilitated Diffusion (chapter 4 PDF, p. 23); 4.2 Nutrient Uptake, Active Transport Requires Energy (chapter 4 PDF, p. 26).

⚠ **Do not mix up: Antiport *(two molecules, opposite directions)* moves ions *(charged particle)* in opposite directions; symport *(two molecules, same direction)* moves them the same direction.** [L18 p15]

Simple diffusion



Passive transport: simple diffusion, weak acids, channels and carriers [L18 p09]

On the figure: Simple diffusion · Weak acids · Facilitated diffusion · porins · glycerol · benzene · aspirin · Prozac

- **Simple diffusion** *(passing straight through membrane)*:
  - Through the bilayer *(two layers)*.
  - Moves small uncharged molecules (O2, H2O) and hydrophobic *(water hating)* molecules. [L18 p08]
- **Gases** (CO2, O2), hydrophobic molecules (**benzene**) and small polar molecules (H2O, ethanol) pass through the bilayer. [L18 p08]
- **Simple diffusion** movement is based on the concentration gradient *(gradual change in amount)*. [L18 p08]

Weak acids in two forms

- **Weak acids and bases** can exist in two forms. [L18 p09]
- **Weak acid forms**: uncharged acid (HA) or charged (A-). [L18 p09]
- **Uncharged HA** may be able to cross the membrane *(thin lipid layer)*. [L18 p09]
- **Once inside**, HA will deprotonate *(lose its H+)* and be water soluble. [L18 p09]
- **High H+ outside** can drive weak acids into the cell. [L18 p09]
- **H+** protonates weak acids to the uncharged HA form. [L18 p09]

Drugs that are weak acids or bases

- **Many drugs** are weak acids and bases: **aspirin**, **Prozac**, penicillin, tetracycline. [L18 p09]
- **Weak acids** (aspirin, penicillin):
  - Protonated HA is membrane soluble and crosses.
  - Deprotonated A- is water soluble and does not cross. [L18 p09]
- **Weak bases** (Prozac, tetracycline):
  - Uncharged B crosses.
  - Protonated BH+ does not cross. [L18 p09]

Facilitated diffusion

- **Facilitated diffusion** *(passing through helper protein)*:
  - Goes through a membrane protein.
  - Is passive *(needs no energy)*, with no energy input.
  - Flow is determined by the concentration and/or charge gradient.
  - Direction: from high to low. [L18 p10]
- **Examples in bacteria**:
  - Channel proteins (outer membrane **porins**).
  - Carriers in the inner (cell) membrane (rare, **only** a few; one example is the **glycerol** transporter). [L18 p10]

Active transport kinds

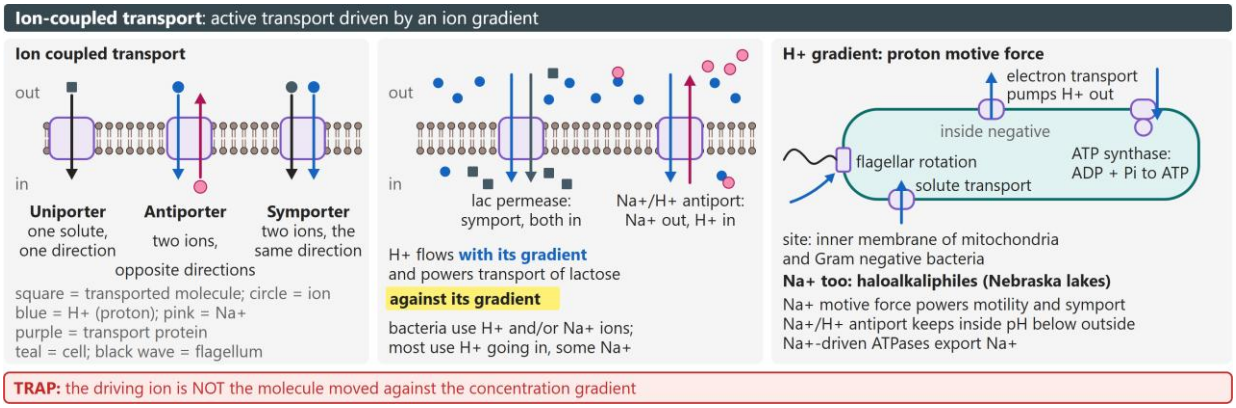
Active transport: against the concentration gradient and requires energy. [L18 p14]

Two kinds of active transport: ion coupled and ATP *(cell energy molecule)* driven. [L18 p14]

Ion coupled transport uses an ion gradient to drive transport. [L18 p14]

ATP driven transport uses energy from cleavage of the high energy phosphate *(PO4 group)* bond. [L18 p14]

Ion coupled transport



Ion coupled transport, proton motive force and the sodium gradient [L18 p15]

On the figure: Uniporter · Antiport · symport · lac permease · Proton motive force · ATP synthase · flagellar rotation · Electron transport

Ion coupled transport:

- An ion moves with its concentration gradient.
- Powers a molecule moving against its concentration gradient. [L18 p15]

Antiport: two like charged ions in opposite directions. [L18 p15]

Symport: two ions in the same direction. [L18 p15]

Watch out: The **lac permease** symporter *(carries two same direction)* example carries lactose (not an ion) in with H+. [L18 p15]

Uniporter *(carries one molecule)*: one solute, one direction. [L18 p15]

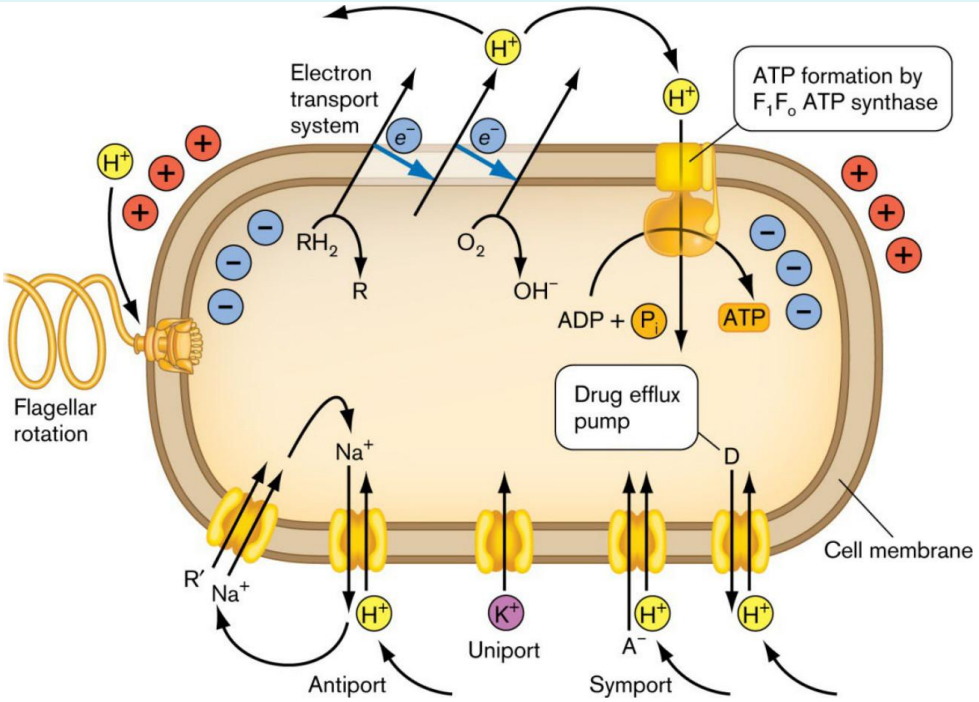
Which ions power transport

Which ions power it: bacteria use H+ and/or Na+ ions to power transport. [L18 p16]

Most bacteria use H+ going in (proton gradient). [L18 p16]

Some bacteria use Na+. [L18 p16]

Proton motive force



Proton motive force: electron transport, ATP synthase, efflux pump and flagellar rotation [L18 p17]

On the figure: Electron transport system · ATP synthase · flagellar rotation · drug efflux pump · Antiport · Symport

Proton motive force *(stored energy from H+ imbalance)*:

> The best known electrochemical gradient is with H+ ions (protons).
>
> Is across the inner membrane *(the skin around the cytoplasm)* of mitochondria and **Gram negative** *(thin wall plus outer membrane)* bacteria.
>
> In bacteria is used for transport in addition to ATP synthesis. [L18 p17]

Electron transport system *(respiratory chain)*: pumps H+ out of the cell to build the proton gradient. [L18 p17, L18 p18]

Proton motive force also drives flagellar rotation and a drug efflux *(pumping the drug out)* pump. [L18 p17]

ATP synthase: H+ flowing back in drives **ADP** *(used up energy molecule)* **+ Pi to ATP**. [L18 p17, L18 p18]

Na+ motive force

Na+ motive force powers flagellar motility *(ability to move)* and the symport of some solutes. [L18 p18]

Na+/H+ antiporters *(carrier moving two ions in opposite directions)* send Na+ out and bring H+ in, which keeps the internal pH below the external pH. [L18 p18]

Sodium channel activated by high pH: replenishes the Na+ needed for **pH homeostasis** *(keeping the inside pH steady)*. [L18 p18]

Na+ driven ATPases export Na+. [L18 p18]

Haloalkaliphilic *(salt and high pH loving)* bacteria use both Na+ and H+ gradients. [L18 p18]

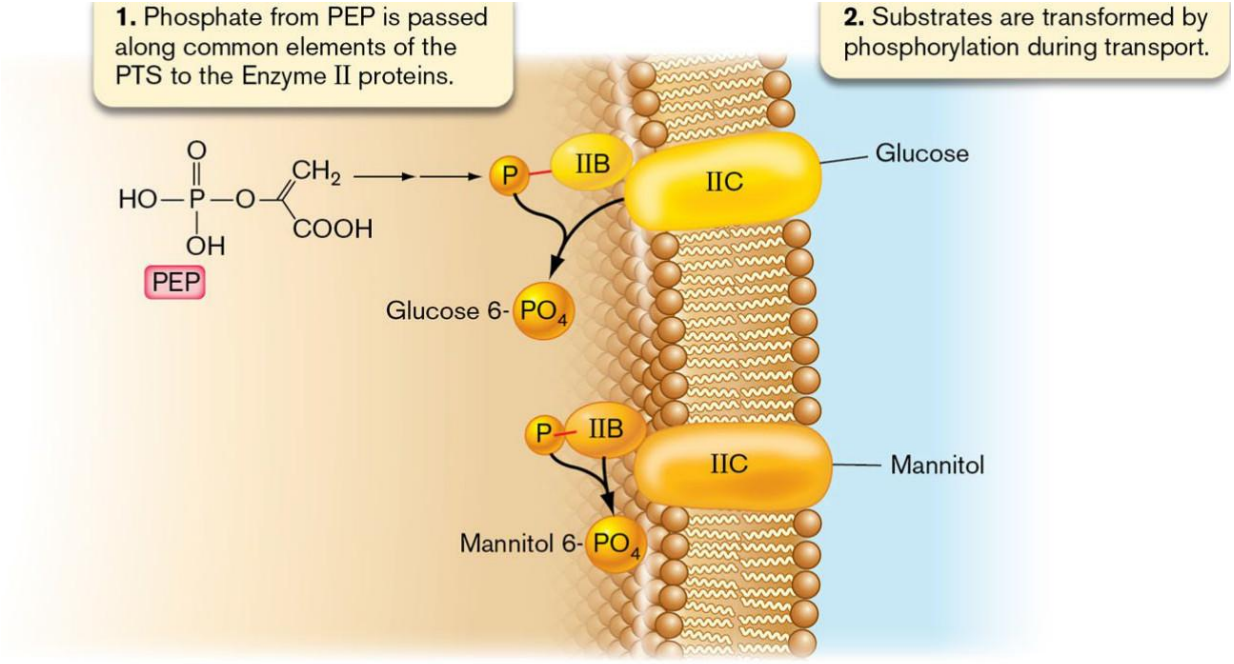
ATP driven transport

ATP driven transport has two forms: a) ABC transporters *(ATP powered pump with binding protein)* and b) group translocation (PTS). [L18 p21, L18 p26]

Watch out: The PTS phosphate comes from PEP *(high energy phosphate donor)*, not ATP. [L18 p21, L18 p26]

ABC transporters are one form of ATP driven transport. [L18 p21, L18 p26]

Group translocation (PTS)



Group translocation: PEP phosphate passed to glucose and mannitol during transport [L18 p26]

On the figure: PEP · PTS · phosphate · glucose

Group translocation (phosphotransfer system, **PTS**): the molecule is modified during transport. [L18 p21, L18 p26]

PTS stands for phosphotransfer system; the textbook name is **phosphotransferase system**, the same thing. [L18 p26]

Group translocation:

- Used for transport of some sugars into cells.
- A **phosphate** from **PEP** is added to the sugar as it is transported in.
- Is **only** found in bacteria and some archaea *(single cell microbes without a nucleus, their own domain (also called archaeal bacteria))*. [L18 p26]

PTS and glucose

PTS and glucose (textbook):

- The PEP phosphate is added as **glucose** crosses.
- Glucose-**6**-phosphate (a different molecule) is inside.
- Glucose itself never accumulates in the cytoplasm (textbook).
- So glucose always moves down its own gradient. [L18 p26]

Western Sandhill lakes

Nebraska has alkaline and brine lakes and marshes. [L18 p18]

Nebraska examples: Western Sandhill alkaline lakes (Garden and Sheridan Counties). [L18 p19, L18 p20]

Western Sandhill lakes:

- Many lakes reach pH above **10.**
- Carbonate-rich water, unusually high potassium. [L18 p19, L18 p20]
- White salt **crusts** can form along shorelines. [L18 p19]
- Evaporation concentrates K+ and carbonate salts. [L18 p19, L18 p20]
- Select for haloalkaliphiles *(salt and high pH loving microbes)* and other **polyextremophiles** *(microbes that handle several extremes at once)*.
- Some microbial communities include **chemolithoautotrophs** *(microbes that live on inorganic chemicals and make their own carbon food)*. [L18 p19]

Eastern saline wetlands

Eastern saline wetlands:

Salt Creek floodplain in Lancaster and southern Saunders Counties.

Saline groundwater from ancient marine salt-bearing formations. [L18 p19, L18 p20]

Summer **drying** can leave visible salt crusts.

Salt-tolerant plants and microbial communities adapted to **osmotic** *(salt and water balance)* stress. [L18 p20]

Type	Passive or active	Energy and direction	Example
Simple diffusion	Passive	No energy; through the bilayer, down the concentration gradient	O2, H2O, CO2, hydrophobic molecules (benzene), small polar molecules (H2O, ethanol)
Facilitated diffusion, channel proteins	Passive	No energy input; through a membrane protein, high to low	Outer membrane porins in **Gram negative**
Facilitated diffusion, carrier proteins	Passive	No energy input; carrier binds a specific molecule; rare in bacteria	Glycerol transporter
Ion-coupled transport	Active	An ion moving down its gradient powers a molecule moving against its gradient	Lactose permease with H+
ATP driven: ABC transporters	Active	Energy from cleavage of the high energy phosphate bond of ATP	Galactose with galactose-binding protein; siderophore-iron
ATP driven: group translocation (PTS)	Active	Phosphate from PEP is added to the molecule as it enters	Glucose enters as glucose-**6**-phosphate

Table source: [L18 p07, L18 p08, L18 p10, L18 p13, L18 p14, L18 p15, L18 p21, L18 p26]

Type of transport proteins observed in bacteria (section 4.2)

Book: 4.2 Nutrient Uptake, ABC Transporters Are Powered by ATP (chapter 4 PDF, p. 30); 4.2 Nutrient Uptake, Group Translocation Avoids "Uphill" Battles (chapter 4 PDF, p. 35).

⚠ **Do not mix up: ABC transport uses ATP *(cell energy molecule)*; the PTS adds a phosphate *(PO4 group)* from PEP *(high energy phosphate donor)* to the sugar.** [L18 p26]

Glycerol transporter

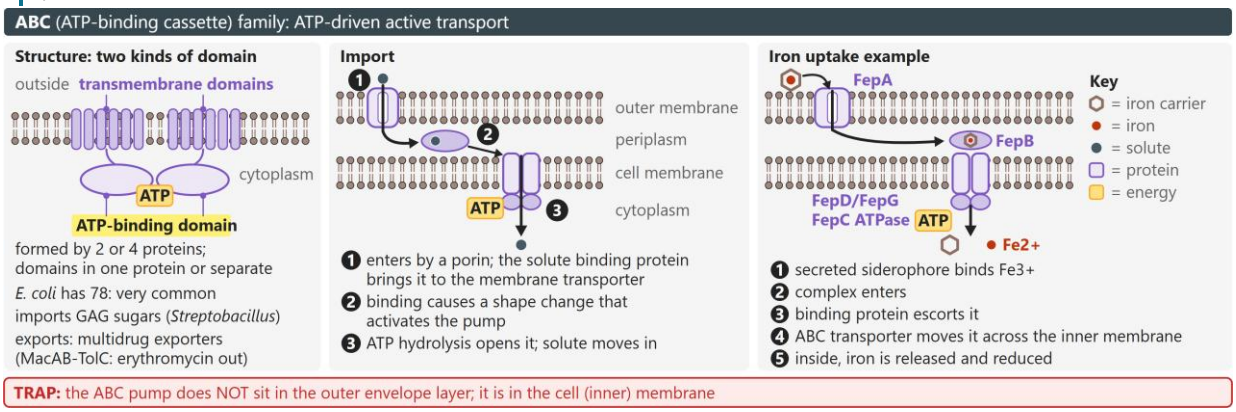
Carrier example, glycerol *(three carbon fat backbone)* **transporter** *(protein that carries molecules across)*: usually transports glycerol in. [L18 p13]

Glycerol transporter: the glycerol concentration inside the cell is very low because it is used up. [L18 p13]

Glycerol channel step 1: glycerol enters the GlpF channel from the extracellular fluid. [L18 p13]

Glycerol channel step 2: a conformational change in GlpF opens the channel to the cytoplasm *(jelly inside the cell membrane)*, and glycerol enters the cell. [L18 p13]

Watch out: the text lists the glycerol transporter as a carrier protein, while the diagram calls GlpF a channel. [L18 p13]



ABC transporter structure, solute import and iron uptake with siderophores [L18 p21]

On the figure: ABC · transmembrane · periplasm · siderophores · FepA · FepB · GAG · TolC

ABC family basics

ABC (ATP-binding cassette) family: common in bacteria (E. coli has **78**). [L18 p21]

ABC family:

Formed by **2** or **4 proteins**.

Has two domains: **transmembrane** and ATP binding. [L18 p21]

ABC transporter *(ATP powered pump with binding protein)*: hinge regions link the transmembrane domains to the ATP site. [L18 p21]

ABC domains can be in the same protein or separate. [L18 p21]

ABC with a solute binding protein *(protein that grabs one solute)*: may also have a solute binding protein in the **periplasm** *(space between the two membranes)*. [L18 p24]

Solute binding protein brings the solute to the transporter. [L18 p24]

Substrate-binding proteins have a high affinity for their matched solutes, which makes transport efficient when solute concentrations are low (textbook).

ABC import steps

ABC import step 1:

After entering through a porin *(water filled channel in outer membrane)*, the solute binds its periplasmic *(in the space between the two membranes)* binding protein.

The complex then binds the membrane *(thin lipid layer)* transporter. [L18 p24]

ABC import step 2: the solute binds the transporter and a conformational change activates it. [L18 p24]

Conformational change *(shape change)* activates the transporter. [L18 p24]

ABC import step 3:

After ATP hydrolysis (**2** ATP to **2** ADP + phosphate)

The dimer opens and releases the solute into the cytoplasm. [L18 p24]

ABC example, GAG transporter

ABC example: a transporter of glycosaminoglycan (**GAG**) disaccharides in Streptobacillus (sulfated chondroitin); Streptococcus takes up its GAG disaccharide (hyaluronan) by a PTS instead. [L18 p22]

GAG transporter has a periplasmic binding protein and ATPase. [L18 p22]

GAG transport: a **polysaccharide** *(long chain of sugars)* **lyase** *(enzyme that cuts a sugar chain)* cuts the polymer into disaccharides before import. [L18 p22]

Siderophore mediated iron uptake

Siderophore *(iron grabbing molecule)* **mediated transport**:

Bacteria secrete **siderophores** which bind an iron (Fe3+) ion *(charged particle)*.

An ABC type transporter moves the complex across the cell membrane *(thin skin around the cell)*.

Inside the cell the Fe3+ is released. [L18 p25]

Iron uptake: once inside the cell, the iron is released and reduced to **Fe2+**. [L18 p25]

Siderophore example: enterochelin is the siderophore that binds Fe3+; **FepA** is the outer membrane *(extra membrane outside the wall)* protein; **FepB** is the periplasmic binding protein; FepG and FepD form the ABC transporter, with FepC as the ATPase. [L18 p25]

ABC exporters

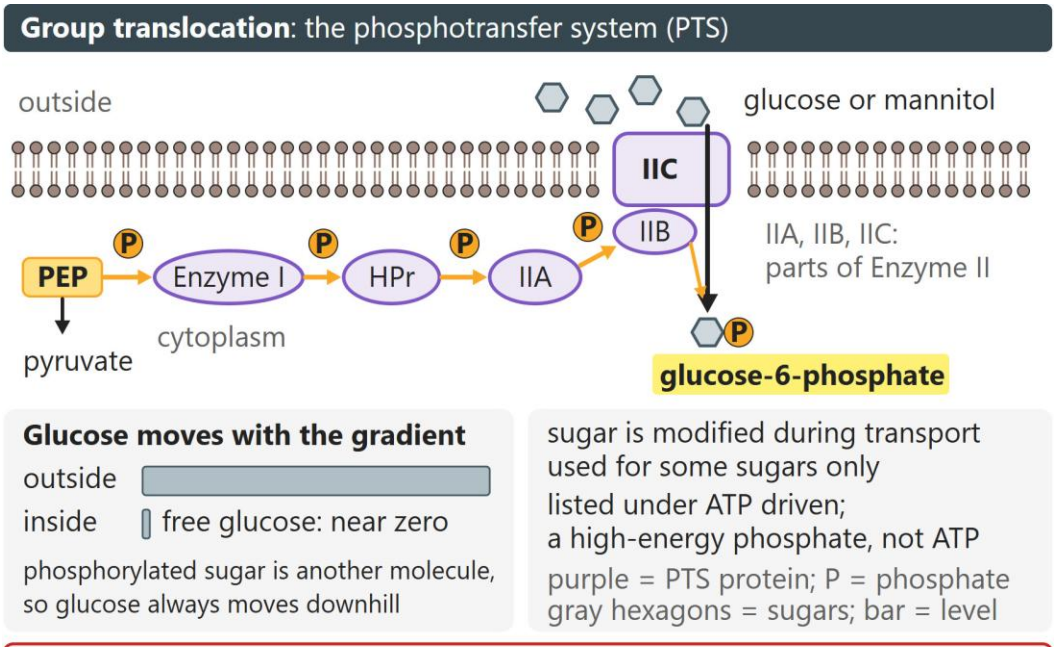
ABC exporters: some ABC transporters move chemicals out, such as some multidrug exporters. [L18 p23]

MacB (ABC protein in the inner membrane):

With MacA and **TolC** spans to the outer membrane.

With MacA and TolC pumps erythromycin out.

With MacA and TolC also secretes **enterotoxin STII** *(a toxin protein made by the bacterium)*. [L18 p23]



Group translocation: phosphate passed from PEP to the sugar as it enters [L18 p26]

On the figure: PEP · Enzyme I · HPr · IIB · IIC · Enzyme II · mannitol · PTS

PTS proteins

PTS:

Phosphate from **PEP** is passed along common **PTS** proteins to the **Enzyme II** proteins.

The Enzyme II proteins shown are **IIB** and **IIC**; the transport overview also shows **Enzyme I** and **HPr** passing the phosphate.

Enzyme II proteins phosphorylate the sugar during transport. [L18 p26]

PTS products: glucose or **mannitol** *(sugar alcohol)* become glucose-**6**-P or mannitol-**6**-P. [L18 p26]

Transport protein	Where	What it does	Example
Porin (channel protein)	Outer membrane of **Gram negative**	Passive channel; some selective for type and size; maximum size **700 daltons** (short chains of sugars and amino acids)	OmpF, OmpC, OmpA, LamB, PhoE
Carrier protein	Inner (cell) membrane	Binds specific molecules and transports them across the membrane; uncommon in bacteria	Glycerol transporter
Ion-coupled transporter (uniporter, antiporter, symporter)	Cell membrane	Uses an H+ or Na+ gradient to move a molecule against its gradient	Lac permease (lactose with H+), Na+/H+ antiporter, phosphate, sulfate symporters, K+ uniporter
ABC transporter	Cell membrane	ATP driven; two transmembrane domains plus ATP binding domains	E. coli has **78**
PTS enzymes (Enzyme I, HPr, Enzyme II)	Cell membrane	Group translocation: phosphate from PEP passed along to Enzyme II, which phosphorylates the sugar as it crosses	Glucose to glucose-**6**-phosphate

Table source: [L18 p05, L18 p11, L18 p13, L18 p16, L18 p21, L18 p26]

Porin (E. coli)	Function
OmpF	Nonspecific channel for small molecules; receptor for phage T2
OmpC	Nonspecific channel for small molecules; receptor for phage T4
OmpA	Specific for peptides; receptor for mating pili
LamB	Porin for maltose and maltodextrin; receptor for phage lambda
PhoE	Anion-selective channel

Table source: [L18 p12]

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).

⚠ **Do not mix up:** Penicillin only kills bacteria that are growing and making new wall material.

Peptidoglycan as drug target

Why peptidoglycan *(sugar and peptide mesh of the wall)* **is a good drug target**: its synthesis is unique to bacteria. [L18 p27, L18 p39]

Antibacterial drugs (antibiotics): many interfere with or inhibit peptidoglycan synthesis. [L18 p27, L18 p39]

Different antibiotics inhibit different steps. [L18 p27, L18 p39]

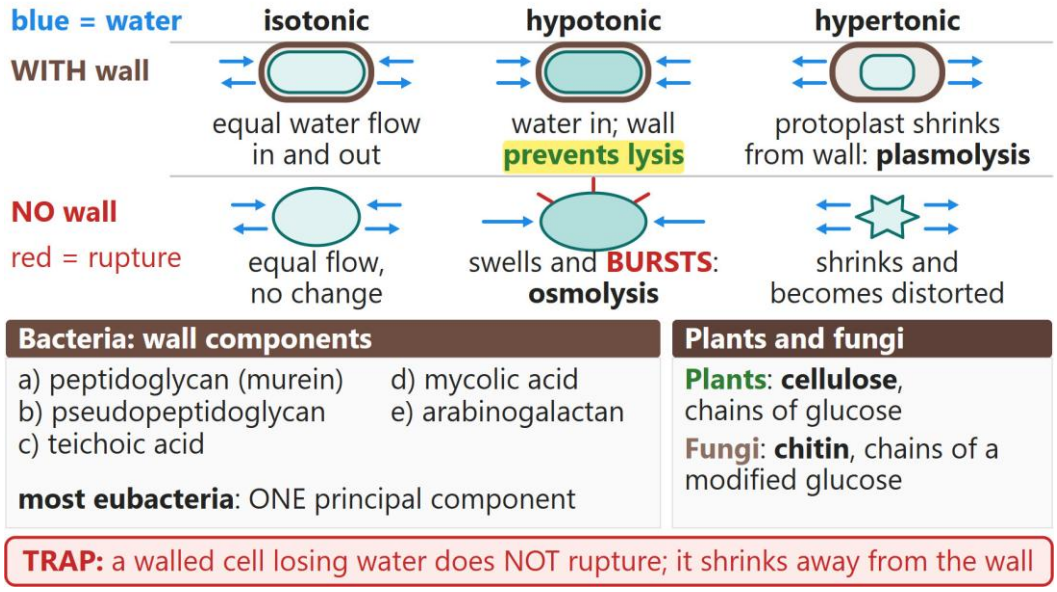
Some antibiotics block wall synthesis. [L17 p12]

The cell wall and its enzymes

Cell wall: semi-rigid, porous layer beyond the cell membrane

Role: structural support, rigidity; helps determine cell shape

Its synthesis is blocked by some antibiotics



The cell wall prevents lysis in hypotonic conditions; without it the cell bursts [L18 p27]

On the figure: cell wall · hypotonic · Water · bursts · Peptidoglycan

Cell wall *(stiff layer around the cell membrane)* prevents cells from lysing *(bursting)* in **hypotonic** *(low solute outside)* conditions. [L17 p12]

Water diffuses into the cell by osmosis, and the resulting turgor pressure **bursts** the cell unless the cell wall counters it (textbook).

Weak wall: autolysins *(wall-cutting enzyme made by the bacterium itself)* keep cutting the existing wall while penicillin blocks the new cross-links, so the wall of a growing cell weakens and the cell bursts (textbook fill). [L18 p39]

Not growing: penicillin blocks the making of new **peptidoglycan** and does not damage the existing layer, so cells that are not growing are barely affected. [L21 p53]

New peptidoglycan is needed for growth/division and repair/replacement. [L18 p28]

Enzymes that cleave peptidoglycan: lysozyme *(enzyme that cuts wall sugars)*, autolysin. [L18 p27, L18 p39]

Penicillin and growing bacteria

Penicillin only kills bacteria that are growing.

Growing bacteria are synthesizing new cell wall material.

Step	Event	What blocks it (textbook fill marked)
3	Add D-Ala-D-Ala dipeptide	D-cycloserine
7	Transglycosylation	Penicillin and cephalosporins (they bind the PBPs); textbook fill: vancomycin
8	Transpeptidation (cross linking)	Penicillin, cephalosporins, vancomycin
9	Regenerate bactoprenol-P	Bacitracin
after synthesis	Cleave the linkage between the sugars in peptidoglycan	Lysozyme (a defensive enzyme of our body that cleaves peptidoglycan; textbook fill: found in tears, saliva and mucus; it cuts, not a synthesis block)

Table source: [L18 p27, L18 p30, L18 p33, L18 p34, L18 p36, L18 p39]

Target of antibacterial drugs / antibiotics

Book: 27.2 Antibiotic Mechanisms of Action (chapter 27 PDF, p. 27).

⚠ **Do not mix up: Conflict: one table puts nitroimidazoles *(drug family, e.g. metronidazole)* under nucleic acid function; another figure puts metronidazole under metabolism.** [L18 p41, L18 p42]

General rules

General rules: most antibacterial drugs (antibiotics) target unique features of bacteria. [L18 p40]

Antibacterial drugs may be cidal *(kill)* or static *(only stop growth)*. [L18 p40]

Most antibiotics inhibit the synthesis of a cell component. [L18 p40, L18 p41]

Most inhibit an enzyme involved in biosynthesis. [L18 p40, L18 p41]

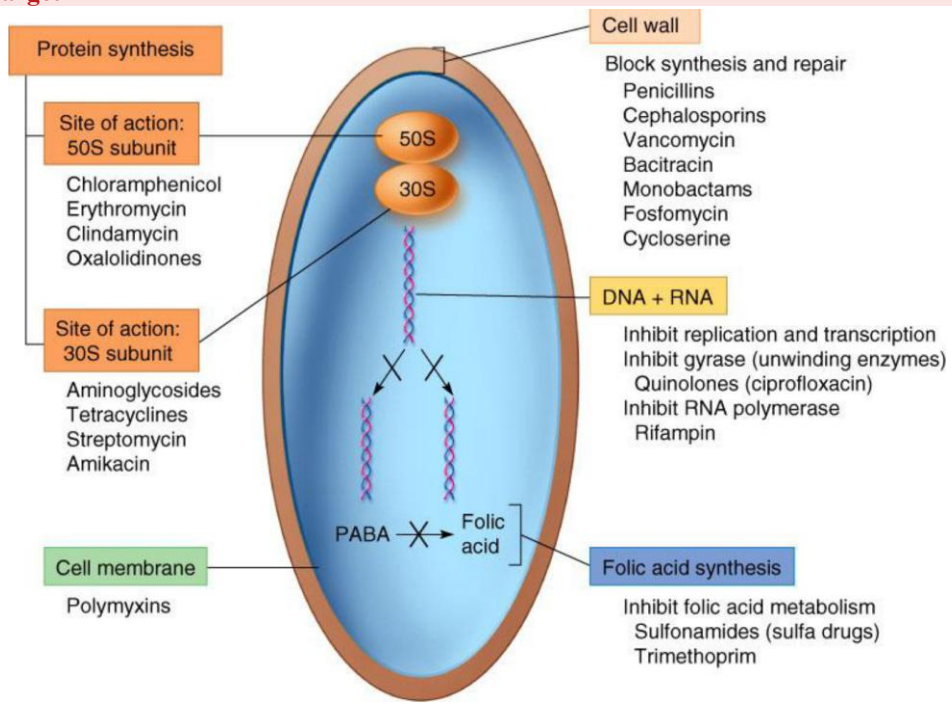
Most block synthesis of new macromolecules (cell components). [L18 p42]

Bacterial pathogens are becoming resistant to commonly used antibiotics. [L18 p40, L18 p41]

Processes blocked

Processes blocked: proteins, cell wall *(stiff layer around the cell membrane)*, nucleic acids (DNA or RNA), metabolites *(small working molecules of the cell)*. [L18 p42]

Drugs by target



Drug targets in the cell and the antibiotics for each target [L18 p43]

On the figure: cell wall · 50S · 30S · Cell membrane · polymyxins · Folic acid · rifampin · quinolones

Cell wall (block synthesis and repair):

> Penicillins, cephalosporins *(penicillin like wall blocking drugs)*, vancomycin *(drug blocking wall building)*, bacitracin *(drug blocking carrier recycling)*.
>
> Also: monobactams *(single ring beta lactam drugs)*, fosfomycin *(drug blocking early wall steps)*, cycloserine *(drug blocking D alanine steps)*. [L18 p43]

50S: chloramphenicol, erythromycin, clindamycin, oxazolidinones *(drug family, e.g. linezolid)*. [L18 p43]

30S: aminoglycosides *(drugs causing ribosome misreading)*, tetracyclines, streptomycin, amikacin. [L18 p43]

Cell membrane *(thin skin around the cell)*: **polymyxins**. [L18 p43]

DNA *(cell genetic instruction molecule)* **and RNA**: inhibit gyrase (**quinolones**, ciprofloxacin) and RNA polymerase (**rifampin**). [L18 p43]

Nucleic acid function: also some antiviral compounds, especially **antimetabolites** *(look-alikes of a normal building block)*. [L18 p41]

Folic acid metabolism: sulfonamides, trimethoprim. [L18 p43]

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
Intermediary metabolism	Sulfonamides, trimethoprim

Table source: [L18 p41]

Site	Drugs
Cell wall (peptidoglycan synthesis)	Penicillins, cephalosporins, vancomycin, bacitracin, monobactams
Cell membrane	Polymyxins, daptomycin, gramicidin
Metabolism	Sulfonamides, trimethoprim, metronidazole
DNA replication	Quinolones
RNA polymerase	Rifampin, pyronins
50S ribosome subunit	Chloramphenicol, macrolides (erythromycin), clindamycin, oxazolidinones (linezolid), streptogramins (Synercid)
30S ribosome subunit	Aminoglycosides (gentamicin), tetracyclines (doxycycline)

Table source: [L18 p42]

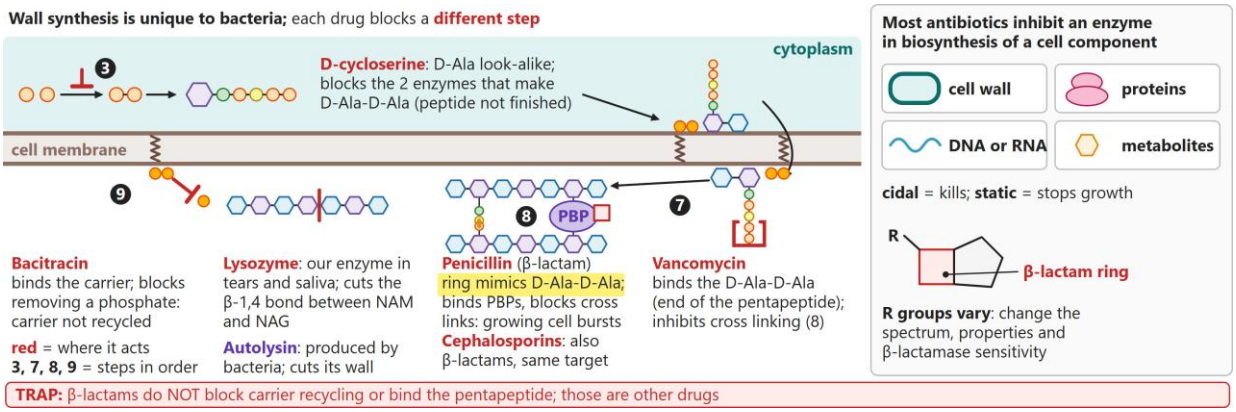
Know specific mechanism of action of each: a. Penicillin, b. Cephalosporin, c. Bacitracin, d. Vancomycin, e. D-cycloserine, f. Lysozyme

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

⚠ **Do not mix up: Penicillin and cephalosporins *(penicillin like wall blocking drugs)* block both transglycosylation (step 7) and transpeptidase cross-linking (step 8); vancomycin blocks the transpeptidase cross-linking (step 8).** [L18 p33, L18 p36, L18 p39]

⚠ **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 and cephalosporin



Where each antibiotic and enzyme acts on peptidoglycan synthesis [L18 p33]

On the figure: Penicillins · vancomycin · Lysozyme · autolysin · cephalosporins · PBPs · D-Ala-D-Ala

Penicillin and cephalosporin are alike: both bind PBPs and inhibit transpeptidase (cross-linking, step **8**) and transglycosylation (step **7**). [L18 p36]

Beta-lactams *(drug family with four member ring)*: both have a ring that mimics D-Ala-D-Ala (textbook fill). [L18 p39]

This mimicry lets beta-lactams target the penicillin-binding proteins that carry out cell wall *(stiff layer around the cell membrane)* synthesis and remodeling (textbook).

Penicillin:

- Blocks the PBPs. [L18 p33]
- Inhibits transpeptidase (peptide cross linking, step **8**) and transglycosylation (step **7**). [L18 p39]

Cephalosporins are modified in generations (textbook fill). [L18 p39]

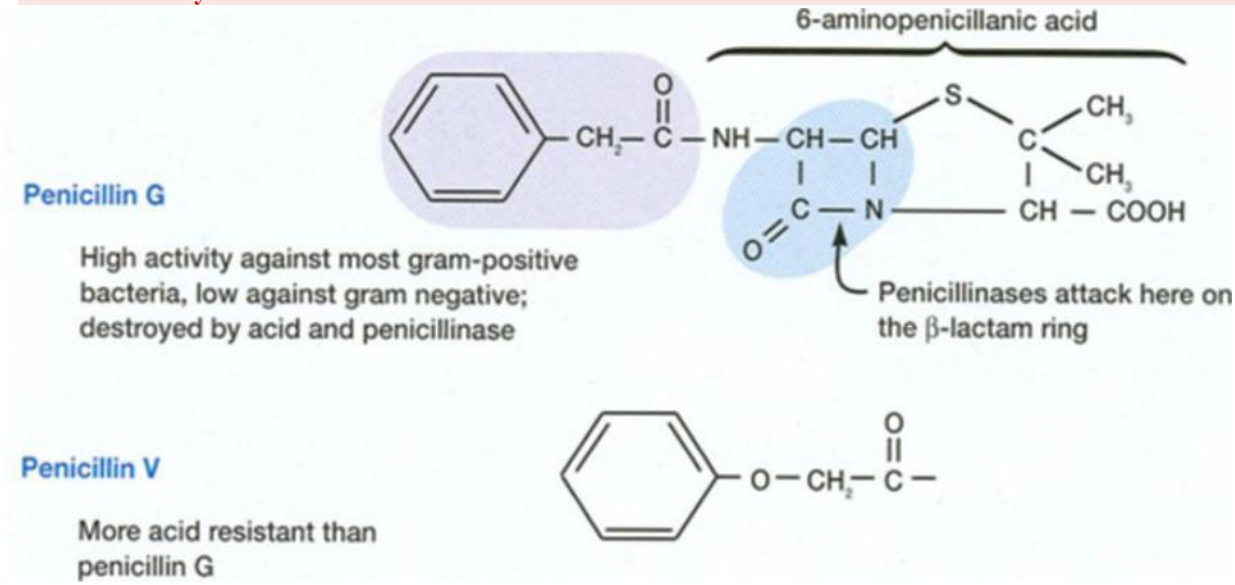
Cephalothin, the first cephalosporin, was introduced in **1964.** [L18 p45]

Vancomycin

Vancomycin *(drug blocking wall building)*:

- Binds D-Ala-D-Ala. [L18 p30]
- Inhibits the transpeptidase (peptide cross-linking, step **8**); textbook fill: it also prevents the action of transglycosylases (step **7**). [L18 p39]

Penicillin family structure



Penicillin G ring core and the site penicillinases attack [L18 p51]

On the figure: 6-aminopenicillanic acid · penicillin G · Penicillinases

Penicillin or beta-lactam family structure: functional groups off the ring vary (different R groups). [L18 p51]

6-aminopenicillanic acid: the ring core of **penicillin G**. [L18 p51]

Penicillins share this core and differ in the group off the ring. [L18 p51]

R groups:

- Affect spectrum of activity.
- Affect properties (acid stability is one example).
- Affect sensitivity to particular beta-lactamases *(enzyme that destroys beta lactam drugs)*. [L18 p51]

Penicillinases *(enzyme that breaks the drug)* attack the beta-lactam ring. [L18 p51]

Lysozyme and autolysin

Lysozyme *(enzyme that cuts wall sugars)* **and autolysin** *(wall-cutting enzyme made by the bacterium itself)*: lysozyme is a defensive enzyme of our body that cleaves peptidoglycan. [L18 p27]

Autolysin: produced by bacteria. [L18 p39]

When penicillin may not work

Penicillin:

- The growing bacteria must be synthesizing new cell wall material.
- May not work on the type of bacteria causing the illness (**Gram negative**).

Penicillin has difficulty passing through the **Gram-negative** *(thin wall plus outer membrane)* outer membrane *(extra membrane outside the wall)*, so it is more effective against **Gram positive** *(thick wall, stains purple)* organisms (textbook).

Penicillin is more effective against **Gram positive**, so sensitivity can serve as a test.

Mycoplasma:

- Mollicutes (Mycoplasma) have no cell wall. [L18 p03]
- Has no wall, so drugs that inhibit peptidoglycan synthesis have no target in it. [L18 p27]

Penicillin family

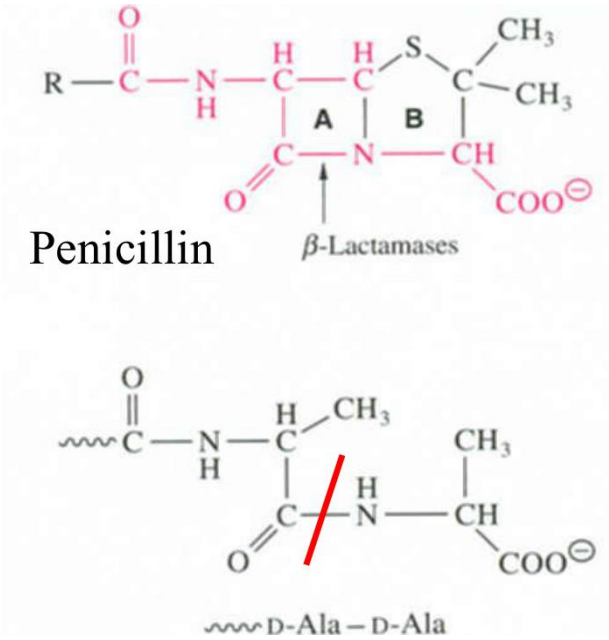
Penicillin (beta-lactam) family: structure similar to **D-Ala-D-Ala**, which is cleaved in the transpeptidase reaction. [L21 p41]

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

Penicillin rings: ring A is the beta-lactam 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]



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

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

Cephalosporins

Cephalosporins: 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]

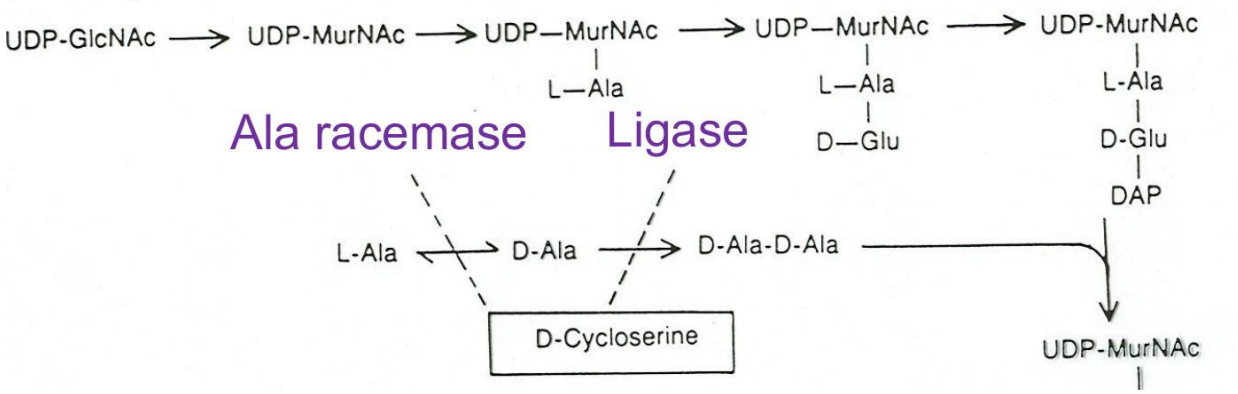
Bacitracin

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:

- 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 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 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** bacteria. [L21 p50]

Reason: some do not pass through the outer membrane 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. [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]

Why wall drugs kill

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

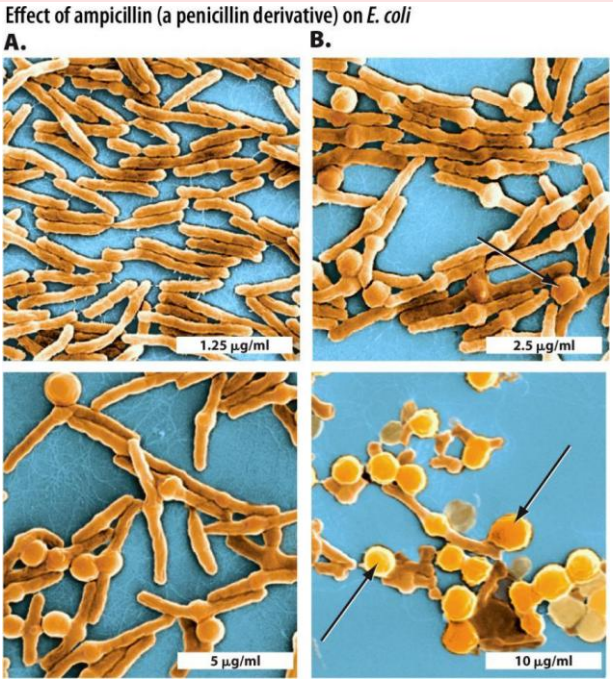
Peptidoglycan synthesis inhibitors such as penicillin are bactericidal *(kill)* in hypotonic *(low solute outside)* conditions. [L21 p55]

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, a bacterial enzyme that cleaves peptidoglycan. [L21 p55]



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

On the figure: Ampicillin · E. coli · Penicillins

Agent	Step	Mechanism of action (textbook fill marked)
a. Penicillin (beta-lactam family)	**7** and **8,** transglycosylation and transpeptidase cross linking	Binds the PBPs that do transpeptidation (peptide cross linking) and transglycosylation, and inhibits both (penicillin blocks the PBPs). Forms a structure similar to D-Ala-D-Ala. Textbook fill: the beta-lactam ring mimics D-Ala-D-Ala, weak wall, growing cell bursts.
b. Cephalosporins	**7** and **8,** transglycosylation and transpeptidase cross linking	Also a beta-lactam: inhibits transpeptidase (peptide cross-linking) and transglycosylation, like penicillin; same D-Ala-D-Ala like structure. Textbook fill: structure modified in a series of generations.
c. Bacitracin	**9,** bactoprenol recycling	Bactoprenol-PP loses a P and returns, and bacitracin blocks this (it inhibits incorporation of disaccharide units). Textbook fill: binds bactoprenol and blocks its dephosphorylation, so the carrier cannot accept a new unit of UDP-NAM.
d. Vancomycin	**8**; **7** and **8** (textbook fill)	Binds D-Ala-D-Ala and blocks cross-bridge formation; vancomycin inhibits the transpeptidase (peptide cross-linking). Textbook fill: binds the D-Ala-D-Ala terminal end of the disaccharide unit and also prevents the action of transglycosylases (step **7**).
e. D-cycloserine	**3,** D-Ala-D-Ala dipeptide	Cycloserine inhibits formation of the D-Ala-D-Ala dipeptide. Textbook fill: D-alanine analog that inhibits the two enzymes that make the D-Ala-D-Ala dipeptide, so the complete pentapeptide cannot be made.

Agent	Step	Mechanism of action (textbook fill marked)
f. Lysozyme	none (cuts finished peptidoglycan)	A defensive enzyme of our body that cleaves peptidoglycan; cleaves the linkage between the sugars in peptidoglycan. Textbook fill: found in tears, saliva and mucus; cleaves cell wall peptidoglycan.

Table source: [L18 p27, L18 p30, L18 p33, L18 p36, L18 p39]

Penicillin type	Note
Penicillin G	High activity against most **Gram positive**, low against **Gram negative**; destroyed by acid and penicillinase
Penicillin V	More acid resistant than penicillin G
Ampicillin	Active against **Gram positive** and **Gram negative**; acid stable
Carbenicillin	Active against **Gram negative** like Pseudomonas and Proteus; acid stable; not well absorbed by small intestine
Methicillin	Penicillinase resistant, but less active than penicillin G; acid labile
Ticarcillin	Similar to carbenicillin, but more active against Pseudomonas

Table source: [L18 p51]

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); 27.1 Fundamentals of Antimicrobial Therapy, Measuring Drug Susceptibility (chapter 27 PDF, p. 13).

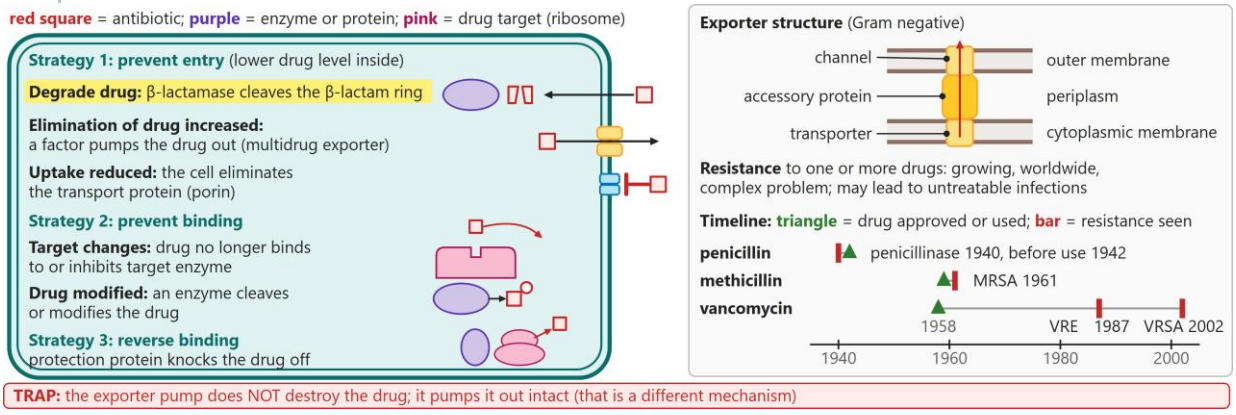
⚠ **Do not mix up: Efflux *(pumping the drug out)* pumps the drug out; beta-lactamase *(enzyme that destroys beta lactam drugs)* cleaves the drug.** [L18 p50]

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

Drug resistance overview

Drug resistance:

Evolution of pathogenic strains resistant to one or more drugs used to treat infections.
A growing, worldwide problem.
A complex problem.
May lead to untreatable infections. [L18 p44]



Three resistance strategies and the multidrug exporter [L18 p49]

On the figure: prevent entry · prevent binding · reverse binding · multidrug exporters · porins · accessory protein · outer membrane · MRSA

Resistance groups: the list gives two, **prevent entry** (lower levels) and **prevent binding**; the strategy diagram adds a third, **reverse binding** (a ribosome protection protein knocks off a bound macrolide). [L18 p50]

Prevent entry

Prevent entry 1) degrade drug (textbook fill: beta-lactamase cleaves the beta-lactam ring). [L18 p50]

Beta-lactamase destroys the drug. [L18 p49, L18 p50]

Beta-lactamase is exported into the surrounding medium (**Gram positive**) or the periplasm (**Gram negative**) and destroys penicillin before it reaches the cell (textbook).

Penicillinases (also called beta-lactamases) attack the beta-lactam *(drug family with four member ring)* ring. [L18 p51]

Prevent entry 2) elimination of drug increased: make a factor that pumps the drug out. [L18 p50]

Multidrug exporter: the factor that pumps the drug out. [L18 p50]

Pumps work because they bail drugs out of the cell faster than the drugs can enter; efflux is usually energized by proton motive force (textbook).

Prevent entry 3) uptake of drug reduced: eliminate the transport protein. [L18 p50]

Efflux or reduced porin *(water filled channel in outer membrane)* **permeability** keep the drug out. [L18 p49, L18 p50]

Gram negative *(thin wall plus outer membrane)* bacteria can express alternative **porins** *(channels in the outer membrane)* with pores too narrow to let the antibiotic pass (textbook).

Watch out, grouping: degrading the drug (beta-lactamase) is listed under Prevent entry (lower levels), and a factor that cleaves or modifies the drug is also listed under Prevent binding (drug modified). [L18 p50]

Multidrug exporter in Gram negative

Gram negative cells have **multidrug exporters**. [L18 p50]

Multidrug exporter in Gram negative: a transporter *(protein that carries molecules across)* in the cytoplasmic membrane *(thin lipid layer)*. [L18 p50]

Multidrug exporter:

- An **accessory protein** across the periplasm.
- An **outer membrane** *(extra membrane outside the wall)* channel.
- The parts form one continuous exit tube. [L18 p50]

Prevent binding

Prevent binding:

- **1**) target of drug changes.
- **1**a) the drug no longer binds to or inhibits the target enzyme. [L18 p50]

Target changes: the drug no longer binds to or inhibits the target enzyme. [L18 p50]

In **MRSA** *(methicillin resistant staph germ)*, the mecA gene encodes an altered penicillin-binding protein with low affinity for beta-lactam antibiotics (textbook).

Prevent binding:

- **2**) drug modified.
- **2**a) makes a factor that cleaves or modifies the drug. [L18 p50]

Gram negative and penicillin

Gram negative peptidoglycan *(sugar and peptide mesh of the wall)*:

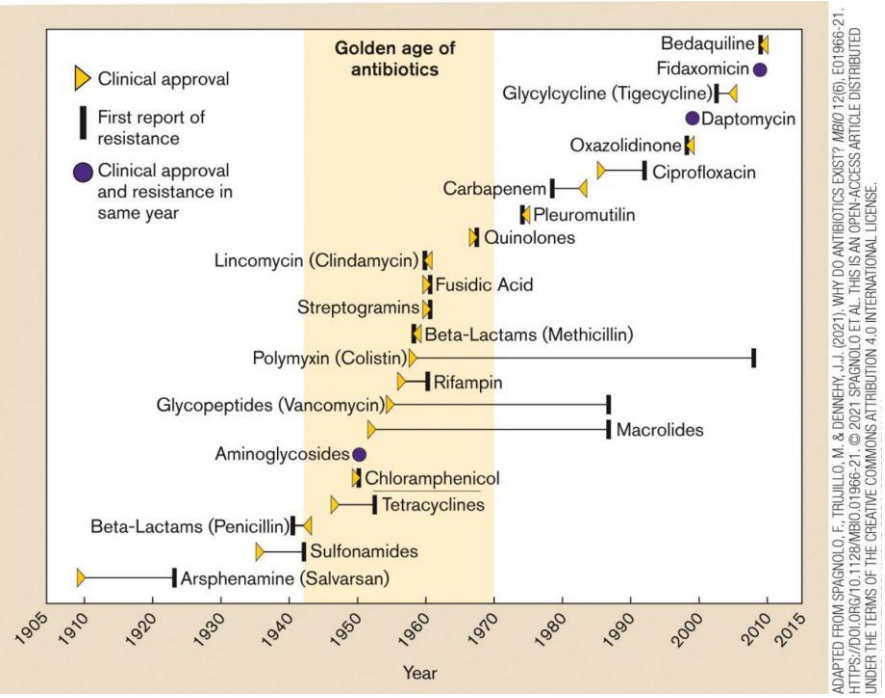
- Is thin (**1-2 layers**). [L17 p15]
- Sits between the outer and inner membranes *(the skin around the cytoplasm)*. [L18 p33, L18 p38]

Porins pass **only** molecules up to **700 daltons** *(unit of molecule weight)*. [L18 p11]

Penicillin G:

- High activity against most **Gram positive** *(thick wall, stains purple)*.
- Low activity against **Gram negative**. [L18 p51]

Approval versus resistance dates



Antibiotic approval and first resistance reports on one timeline, with the golden age [L18 p47]

On the figure: Salvarsan · Ciprofloxacin · Chloramphenicol · daptomycin · fidaxomicin · carbapenem · tigecycline · Golden age of antibiotics

Timeline pattern: resistance usually follows approval within a few years. [L18 p47]

Timeline examples:

> Sulfonamides about **1936** to **1942,** tetracyclines about **1947** to **1953.**
> Rifampin *(drug blocking RNA making)* about **1957** to **1960.** [L18 p47]

Ciprofloxacin: approval about **1986,** resistance about **1992.** [L18 p47]

Chloramphenicol: approval and resistance together at about **1950.** [L18 p47]

Timeline graph, approval and resistance in the same year: aminoglycosides *(drugs causing ribosome misreading)*, daptomycin and fidaxomicin. [L18 p47]

Resistance reported before approval:

> Beta-lactams (resistance about **1940,** approval about **1943**).
> Carbapenem (about **1978** vs **1983**), tigecycline (about **2003** vs **2005**). [L18 p47]

Long gaps:

> Salvarsan about **1910** to **1923,** macrolides *(protein building blocker drugs)* about **1952** to **1987.**
> Vancomycin *(drug blocking wall building)* about **1955** to **1987,** polymyxin/colistin about **1958** to **2008** (longest). [L18 p47]

Rising resistant strains

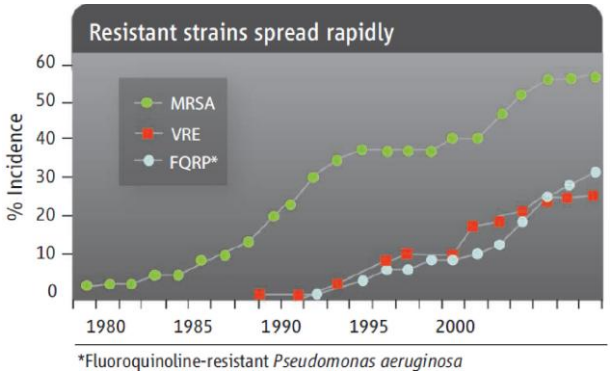
Golden age of antibiotics shaded about **1943** to **1970.** [L18 p47]

Rising incidence of resistant strains (1979 to 2007): MRSA, VRE *(drug resistant gut germ)* and FQRP. [L18 p48]

MRSA: about **2%** in **1979** to about **57%** by about **2007.** [L18 p48]

VRE: first seen about **1989,** up to about **25%.** [L18 p48]

FQRP (fluoroquinolone resistant Pseudomonas aeruginosa): first seen about **1992,** up to about **31%.** [L18 p48]



Rising incidence of MRSA, VRE and FQRP from 1979 to 2007 [L18 p48]

On the figure: MRSA · VRE · FQRP · Pseudomonas aeruginosa

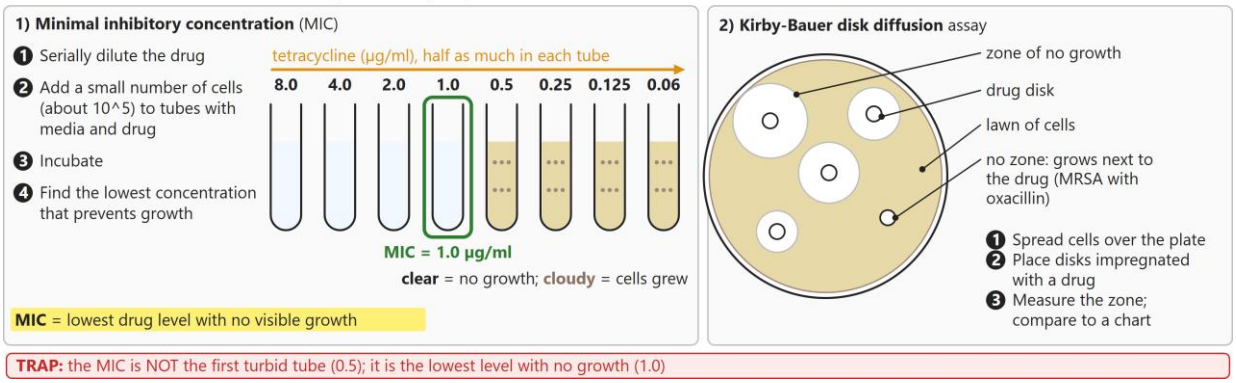
Date conflicts

Date conflict:

> One timeline dates vancomycin introduction to **1958** while another shows about **1955.** [L18 p45, L18 p47]
>
> One timeline gives VRE in **1987** while another gives first seen about **1989.** [L18 p46, L18 p48]

Date conflicts: the timeline graph is approximate; it puts tetracyclines at about **1947** to **1953,** while the dated list gives approval **1952** and resistance **1956.** [L18 p45, L18 p46, L18 p47, L18 p48]

Is an isolate resistant or sensitive to a drug? Two tests, plus genetic methods



MIC tubes and Kirby-Bauer disk plate for antibiotic susceptibility [L18 p53]

On the figure: MIC · tetracyclines · clear · cloudy · Kirby-Bauer · lawn · zone of no growth · oxacillin

MIC tetracycline tubes

MIC tetracycline tubes: drug diluted **8.0, 4.0, 2.0, 1.0, 0.5, 0.25, 0.125, 0.06** ug/ml. [L18 p53]

MIC tubes:

> Each tube has **half as much** drug as the tube before it (**8.0,** then **4.0,** then **2.0**).
>
> The tubes at **8.0** to **1.0** stay clear and the lower ones are cloudy.
>
> A cloudy tube means the cells grew; a clear tube means no visible growth. [L18 p53]

MIC = **1.0** ug/ml for the tetracycline tubes. [L18 p53]

Kirby-Bauer plates

Kirby-Bauer step **1** spreads the cells over the whole plate, an even layer called a **lawn** (textbook word). [L18 p54]

Kirby-Bauer: a disk dispenser places several drug disks at once. [L18 p54]

Kirby-Bauer plates:

> Each disk carries a drug abbreviation, for example OX.
>
> A clear **zone of no growth** around a disk means the cells are sensitive.
>
> One OX (**oxacillin**) disk has no zone around it, meaning those cells are resistant. [L18 p54]

Penicillin in stream water

Stream water: reasons penicillin may not make it safe to drink.

Why penicillin may fail in stream water:

> Reason **1**: the bacteria in the water may not be growing.
>
> > Penicillin **only** kills bacteria that are growing and synthesizing new cell wall *(stiff layer around the cell membrane)* material.

Reason **2**: it may not work on the type of bacteria causing the illness (**Gram negative**).

Why penicillin may fail in stream water:

Reason **3**: the bacteria may be resistant to **penicillin**.

Reason **4**: the dose may not get high enough.

Gram positive envelope tests

Gram positive envelope *(layers wrapped around the cell)* **tests** (to support a purple Gram stain):

Transmission electron microscopy showing a thick peptidoglycan layer and no outer membrane; isolating teichoic acid *(charged chains in Gram positive wall)* from the cell wall.

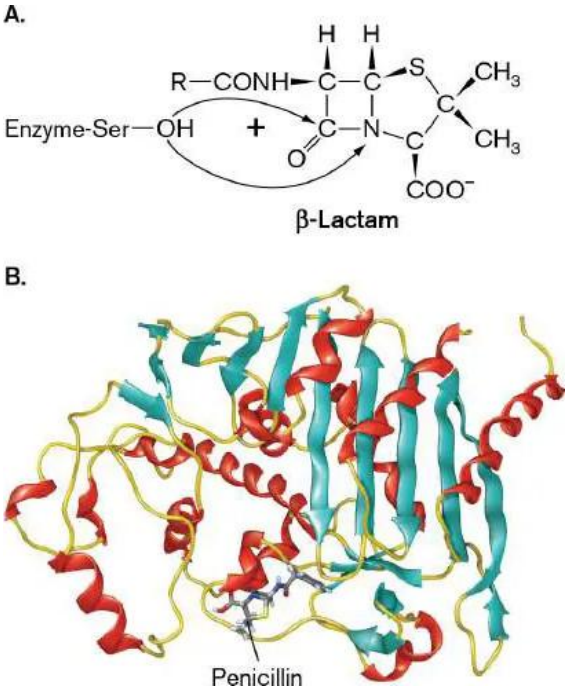
Also: no growth on a selective medium for **Gram negative**; genes for teichoic acid synthesis present and genes for LPS *(outer membrane sugar and fat coat)*, outer membrane and periplasmic *(in the space between the two membranes)* proteins absent.

Use a penicillin more effective against **Gram positive** but not **Gram negative**.

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]



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

On the figure: penicillin

Strategy	Mechanism	Example
1. Prevent entry	Destroy the antibiotic	Beta-lactamase
1. Prevent entry	Pump the antibiotic out	Efflux pump
1. Prevent entry	Reduce membrane permeability	Porin with reduced permeability
2. Prevent binding	Alter the target	Mutated ribosome component
2. Prevent binding	Modify the antibiotic	Modification enzyme
3. Reverse binding	Protect the ribosome	Ribosome protection protein knocks off a bound macrolide

Table source: [L18 p49]

Year	Event (Science 2008 timeline)
1940	Penicillinase (an enzyme that destroys penicillin) identified in bacteria
1942 to **1943**	First therapeutic use of penicillin; mass produced in **1943**
1945	More than **20%** of S. aureus hospital isolates penicillin resistant
1947	Streptomycin approved; resistance observed the same year
1952 to **1956**	Tetracycline approved **1952**; resistance observed **1956**
1958	Vancomycin introduced (rarely used until the mid **1980**s)
1959 to **1961**	Methicillin introduced **1959**; MRSA observed **1961**
1964 to **1966**	Cephalothin (first cephalosporin) **1964**; resistance **1966**
1967 to **1970**	Gentamicin approved **1967**; resistance **1970**
1976	Transferable penicillinase first observed in a gonococcus
1981 to **1983**	Cefotaxime approved **1981**; resistance **1983**; first penicillin resistant Enterococcus **1983**
1987	Vancomycin resistant Enterococcus (VRE); first outbreak of Klebsiella resistant to third generation cephalosporins
1996	S. aureus with intermediate resistance to vancomycin (VISA)
1999	Community acquired MRSA
2000 to **2001**	Linezolid (first oxazolidinone) approved **2000**; resistance **2001**
2002	S. aureus with complete resistance to vancomycin (VRSA)

Table source: [L18 p45, L18 p46]

Test	How it works	Result
MIC (minimal inhibitory concentration)	**1**) Serially dilute the drug, **2**) add a small number of cells (**10^5**) to a tube with media and drug, **3**) incubate, **4**) find the lowest concentration that prevents growth	Lowest drug concentration with no growth (tetracycline tubes: MIC **1.0** ug/ml; microtiter plate: penicillin MIC **0.06** ug/ml, erythromycin MIC **8** ug/ml)
Kirby-Bauer disk diffusion assay	**1**) Spread cells over the surface of a plate, **2**) place one or more disks impregnated with a drug on the plate, **3**) measure the zone of no growth around each disk and compare to a chart	Zone size gives sensitive or resistant
Genetic methods	Listed as the third method	No detail given

Table source: [L18 p52, L18 p53, L18 p54]

D. Bacterial Internal Structures (Chapter 3): know types of structures seen in bacteria

Book: 3.1 The Bacterial Cell: An Overview, Model of a Bacterial Cell (chapter 3 PDF, p. 7); 3.6 Specialized Structures, Thylakoids, Carboxysomes, and Storage Granules (chapter 3 PDF, p. 119).

⚠ **Do not mix up: Bacterial DNA *(cell genetic instruction molecule)* is folded by proteins, but NOT wrapped around an octamer of histones *(eukaryote protein that DNA winds around)*.**

Cell envelopes

Major styles of cell envelope *(layers wrapped around the cell)*: **Gram-positive** *(thick wall, stains purple)*; mycobacterial (Gm+ **actinomycete** *(a type of Gram-positive bacterium)*), **acid-fast** *(holds dye despite acid wash)*; **Gram-negative** *(thin wall plus outer membrane)*; Mollicutes (Mycoplasma) with no cell wall *(stiff layer around the cell membrane)*; archaeal *(of archaea)*. [L19 p03]

Peptidoglycan *(sugar and peptide mesh of the wall)* **synthesis** is **unique** to bacteria; many antibacterial drugs (antibiotics) interfere with or inhibit it, and antibiotics inhibit **different** steps. [L19 p04]

Enzymes that cleave peptidoglycan: lysozyme (a defensive enzyme of our body) and autolysin (produced by bacteria). [L19 p04, L19 p16]

New peptidoglycan is needed for growth and division and for repair and replacement. [L19 p05]

Dividing cell: FtsZ *(protein ring for cell splitting)* ring, wall bands and growth zones (where the new peptidoglycan is). [L19 p05]

Site of new peptidoglycan synthesis varies: it can occur in the middle, at one end or **dispersed**. [L19 p06]

Peptidoglycan synthesis overview

Overview step 1: make the two sugar units (NAG and NAM), with a peptide *(short chain of amino acids)* off NAM *(the other wall sugar)*. [L19 p07]

Overview step 2: add the **2** sugar unit to an **existing** chain. [L19 p07]

Overview step 3: form a **crosslink** to the peptide in a different chain. [L19 p07]

The peptide off NAM in the cross-linked **Gram negative** wall is L-Ala, D-Glu *(D form of glutamic acid)*, m-diaminopimelic acid and D-Ala *(D form of alanine amino acid)*; the terminal (fifth) D-Ala was released. [L19 p07]

The peptide cross-bridge forms with release of **D-alanine**; penicillin blocks this step. [L19 p07]

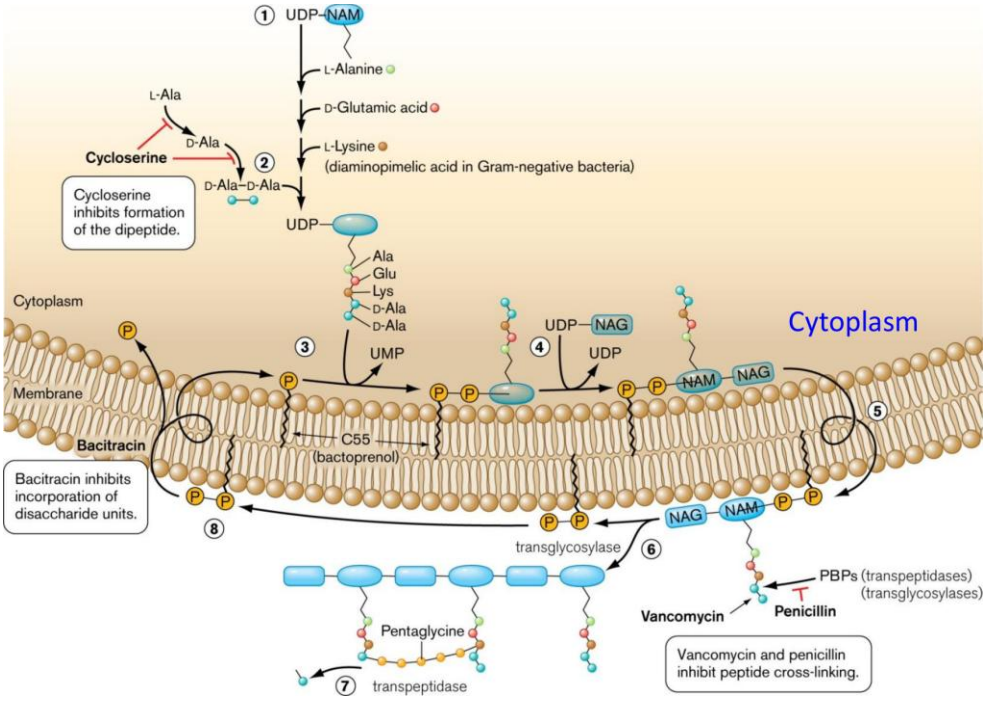
Vancomycin *(drug blocking wall building)* binds D-Ala-D-Ala and blocks cross-bridge formation. [L19 p07]

Vancomycin binds the D-Ala-D-Ala terminal end of the disaccharide unit and prevents transglycosylase and transpeptidase *(enzyme making wall cross bridges)* action (textbook).

Synthesis steps in order

Where it happens: in a **Gram-positive** bacterium. [L19 p10]

Gram-negatives are similar, but some steps occur in the periplasm *(space between the two membranes)* between the membranes *(thin lipid layer)*. [L19 p10]



Peptidoglycan synthesis steps with the points where each antibiotic blocks [L19 p10]

On the figure: UDP · UMP · bactoprenol · Cycloserine · bacitracin · Vancomycin · penicillin · transpeptidase

Step 1: make the sugar precursors (NAM and NAG). Both come from **fructose-6-P** in the cytosol *(liquid part of the cytoplasm)*, and the sugars are first attached to UDP. [L19 p09]

Sugar names: NAG *(one wall sugar)* is also written **GlcNAc** or G, and NAM is written **MurNAc** or M. [L19 p09]

Sugar precursors, step 2: add an acetyl group from **acetyl-CoA** (CoA is released). [L19 p09]

Step 2: add **3** amino acids to UDP-NAM to begin the peptide: L-alanine, D-glutamic acid, then L-lysine (**diaminopimelic acid** *(DAP, the third amino acid in Gram-negatives)* instead of lysine in **Gram-negative** bacteria). [L19 p10, L19 p11]

Step 3: add a dipeptide of **2 D-alanine** amino acids to the peptide. [L19 p11]

Step 4: transfer the NAM-pentapeptide to **bactoprenol** (C55) in the cell membrane *(thin skin around the cell)*; **UMP** is released. [L19 p10, L19 p11]

Step 5: link NAG to NAM (UDP is released). [L19 p10, L19 p11]

Step 6: **flip** the **2** sugar unit to the other side of the bilayer *(two layers)*. [L19 p11]

Step 7: transglycosylation *(joining sugar chains longer)*, linking sugars to a glycan *(sugar chain)* chain. [L19 p11]

Step 8: **transpeptidase**, forming the cross link. [L19 p11]

Step 9: regenerate bactoprenol-P by cleaving off a phosphate *(PO4 group)* group. [L19 p11]

Bactoprenol, flippase and crosslinking

Bactoprenol *(undecaprenyl, a large isoprenoid phosphate with 55 carbons)* helps carry peptidoglycan units, LPS *(outer membrane sugar and fat coat)* and **teichoic acid** *(charged chains in Gram positive wall)* through the cell membrane; the sugar is attached via phosphate. [L19 p12]

MurJ *(flipper protein for wall units)* is a transmembrane protein (flippase) that flips the sugars to the other side. [L19 p12]

Transpeptidation *(making peptide bridges between chains)* **reaction**: in **Gram-negative**s, DAP *(special amino acid in wall peptide)* in the 3rd position attacks D-Ala in the 4th position and displaces D-Ala in the 5th position. [L19 p14]

Drugs that block wall synthesis

Antibiotics that interfere with peptidoglycan synthesis: the **penicillin** (beta-lactam) family, cephalosporins *(penicillin like wall blocking drugs)*, **vancomycin**, **bacitracin** *(drug blocking carrier recycling)* and D-cycloserine *(drug blocking D alanine steps)*. [L19 p16]

Cycloserine inhibits formation of the **dipeptide** (the D-Ala-D-Ala pair). [L19 p10]

Bacitracin inhibits incorporation of disaccharide units. [L19 p10]

Bacitracin binds bactoprenol and blocks its dephosphorylation, so the carrier cannot accept a new unit of UDP-NAM (textbook).

Antibiotics in general

Most antibacterial drugs target unique features of bacteria; most inhibit an **enzyme** involved in biosynthesis, the synthesis of a cell component. [L19 p17, L19 p18]

Cidal *(kills the germs)* drugs kill bacteria; **static** *(only stops germ growth)* drugs inhibit growth. [L19 p17]

Most antibiotics block synthesis of new: proteins, cell wall, **nucleic acids** (DNA or RNA) or metabolites *(small working molecules of the cell)*. [L19 p19]

Folic acid pathway: folic acid is made from **PABA**; sulfonamides and trimethoprim inhibit folic acid metabolism. [L19 p20]

Sulfa drugs bind and inhibit the enzyme that converts PABA to folic acid; humans do not synthesize folic acid (textbook).

Antibiotic resistance

Microbial drug resistance: pathogenic strains evolve resistance to one or more drugs. [L19 p21]

It is a growing, worldwide, complex problem that may lead to untreatable infections. [L19 p21]

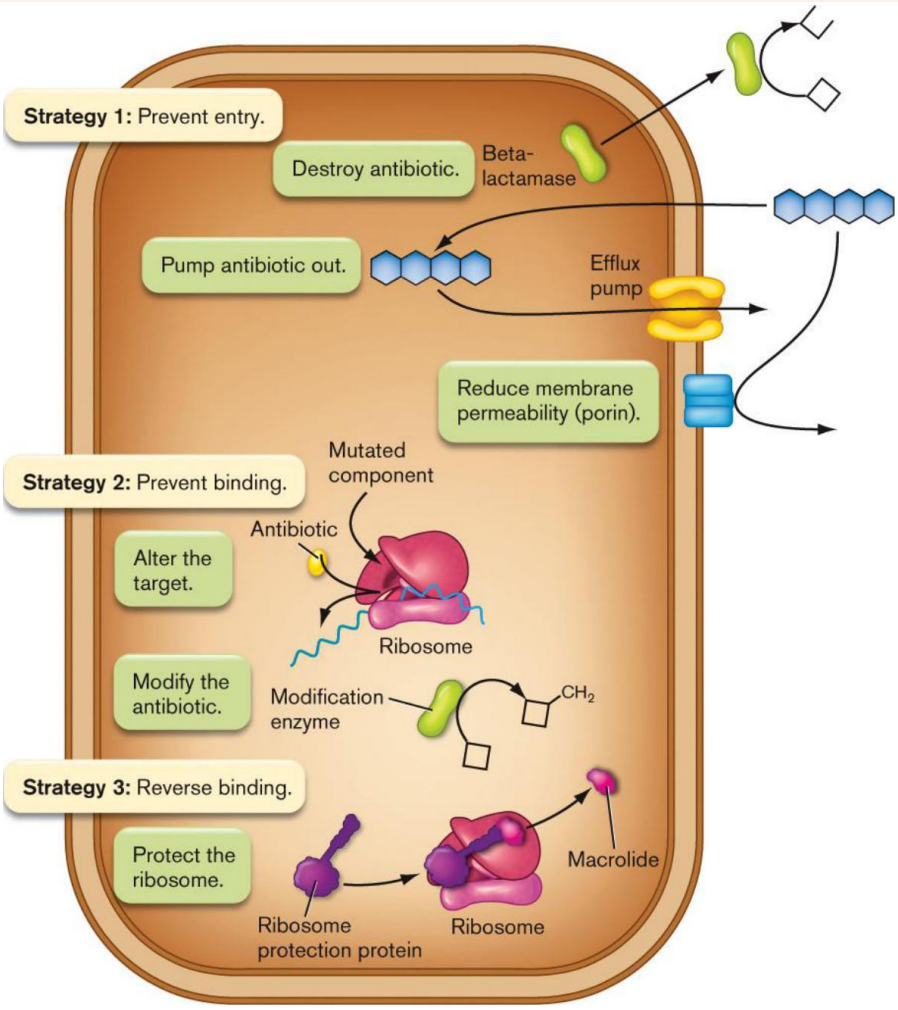
Antibiotic timeline: the earliest drug is **arsphenamine** (Salvarsan), approved about **1910.** [L19 p24]

Golden age of antibiotics is marked on the timeline of antibiotic use and appearance of resistance. [L19 p24]

Incidence of resistant strains: they spread rapidly, and **MRSA** (methicillin-resistant S. aureus) has the highest incidence. [L19 p25]

Resistant strain names: **VRE** *(drug resistant gut germ)* is vancomycin-resistant Enterococcus; **FQRP** is fluoroquinolone-resistant Pseudomonas aeruginosa. [L19 p23, L19 p25]

Resistance mechanisms



Three resistance strategies: prevent entry, prevent binding, reverse binding [L19 p26]

On the figure: Prevent entry · Prevent binding · target

Prevent entry (lower drug levels): **degrade** the drug. [L19 p27]

Increase elimination of the drug: a factor pumps the drug out (the multidrug exporter). [L19 p27]

Pumps bail the drug out faster than it can enter, usually powered by proton motive force (textbook).

Multidrug exporter spans the outer membrane *(extra membrane outside the wall)*, the periplasm and the cytoplasmic membrane. [L19 p27]

Reduce uptake of the drug: eliminate a transport protein. [L19 p27]

Beta-lactams *(drug family with four member ring)*, tetracyclines and fluoroquinolones *(DNA coiling blocker drug)* enter through outer-membrane porins *(channels in the outer membrane)*; **Gram-negative**s can express alternative porins too narrow to admit them (textbook).

Prevent binding: the **target** of the drug changes. [L19 p27]

The drug no longer **binds** to or inhibits the target enzyme. [L19 p27]

Prevent binding, drug modified: a factor cleaves or modifies the drug. [L19 p27]

Watch out: Degrading the drug is also listed under Prevent entry. [L19 p27]

Beta lactam family

Beta-lactam family: the functional groups off the ring **vary**. [L19 p28]

Functional groups off the ring affect spectrum of activity, properties and sensitivity to particular **beta-lactamases** *(enzyme that destroys beta lactam drugs)*. [L19 p28]

Susceptibility tests and MIC

Tests for antibiotic susceptibility: **1**) MIC, **2**) Kirby-Bauer disk diffusion *(drifting from high to low amount)* assay, **3**) genetic methods. [L19 p29]

MIC stands for **minimal inhibitory concentration**: the lowest concentration of drug that prevents growth. [L19 p29, L19 p30]

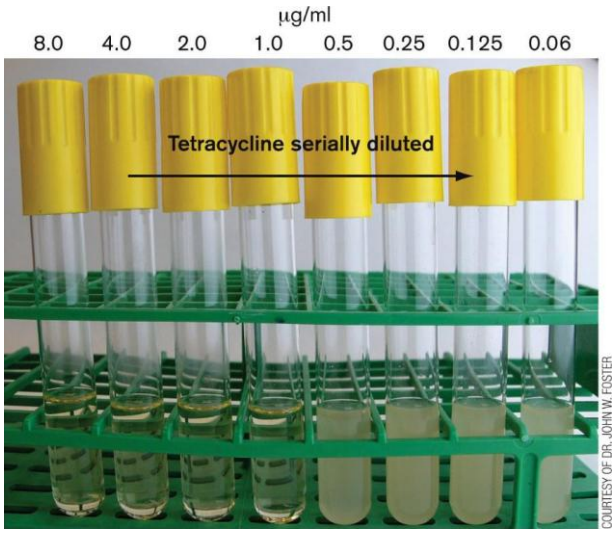
MIC step 1: **serially** dilute the drug. [L19 p30]

MIC step 2: add a small number of cells (**10^5**) to a tube with media and drug. [L19 p30]

MIC step 3: **incubate** the tubes. [L19 p30]

MIC step 4: find the lowest concentration that prevents growth; in the serially diluted **tetracycline** tubes (**8.0** to **0.06** ug/ml) the MIC is **1.0 ug/ml**. [L19 p30]

MIC examples: the MIC is **0.06** ug/ml for penicillin and **8** ug/ml for erythromycin. [L19 p30]



Tetracycline serially diluted in tubes; the MIC is the lowest clear tube [L19 p30]

On the figure: tetracycline · serially · 8.0 · 0.06 · 1.0

Kirby Bauer disk test

Kirby-Bauer is a **disk diffusion assay**: spread cells over the plate, place drug disks, measure the zone of no growth. [L19 p31]

Kirby-Bauer step 1: spread cells over the **surface** of the plate. [L19 p31]

Kirby-Bauer step 2: place one or more disks **impregnated** with a drug on the plate. [L19 p31]

Kirby-Bauer step 3: measure the zone of no growth around the disk and compare it to a **chart**. [L19 p31]

Antibiotic stewardship

Stewardship topic: what a doctor and medical center could do. [L19 p32]

Stewardship goal: reduce the increase of antibiotic resistance in pathogenic bacteria. [L19 p32]

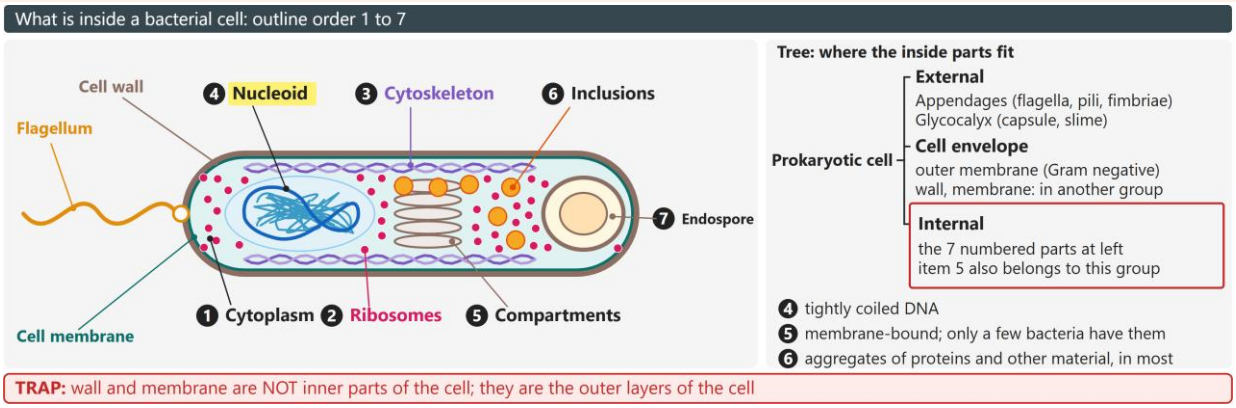
Stewardship:

- No antibiotics for likely viral infections (textbook fill).
- Treat **only** if the patient worsens or does not improve (textbook fill).
- Know which resistant strains are common in the community or hospital (textbook fill).
- Limit how long the drug is given (textbook fill).
- Switch from a broad-spectrum drug to a narrow-spectrum drug (textbook fill).
- Make that switch once the agent and its resistance pattern are known (textbook fill).
- Stop unnecessary antibiotics (textbook fill).
- Confirm a bacterial cause first (textbook fill). [L19 p32]

Sources for internal structures

Internal structures get little space in the textbook; other sources are used. [L19 p34]

Internal structures list



Internal structures of a bacterial cell in outline order [L19 p39]

On the figure: flagellum · cytoplasm · ribosomes · nucleoid · cytoskeleton · compartments · inclusions · endospores

Prokaryotic cell *(bacteria or archaea)*: its structures group into external, cell envelope and internal. [L19 p36, L19 p39]

Structures in the images of a typical bacteria cell: **flagellum** *(whip tail that spins)*, **ribosomes** *(protein building machine)*, **nucleoid** *(DNA region without a membrane)*, **cytoplasm** *(jelly inside the cell membrane)*, chromosome *(one long DNA molecule)*, cell membrane, cell wall. [L19 p36]

Of the structures in that image, ribosomes, nucleoid, cytoplasm and chromosome are internal; the flagellum is external; the cell membrane and cell wall are cell envelope. [L19 p36]

Structures observed inside a bacterial cell: ribosomes; nucleoid (chromosome / DNA); **inclusions** (storage granules, gas vesicles) (textbook fill). [L19 p35]

Internal structures outline: **1.** cytoplasm, **2.** ribosomes, **3.** **cytoskeleton** *(inner scaffold of the cell)*, **4.** nucleoid, **5.** **compartments**, **6.** inclusions and granules, **7.** **endospores** (a few bacteria form spores inside the cell). [L19 p40]

Internal structures (revised list, compartments added next to inclusions): cytoplasm, ribosomes, inclusions, compartments, nucleoid/chromosome, cytoskeleton, endospore. [L19 p39]

Compartments and protein-shell inclusions

Thylakoids *(membrane sacs for photosynthesis)* **and chlorosomes** *(light catching sacs)*: membrane compartments for photosynthesis; chlorosomes of green bacteria are enclosed by a phospholipid *(membrane fat with a charged head)* **monolayer** *(one layer)* linked to the cell membrane.

Carboxysomes *(protein-shelled bodies that fix carbon dioxide)*: site of carbon fixation; a protein shell, not a lipid membrane, so they are inclusions, not membrane compartments.

Gas vesicles *(gas filled floating bubbles)*: store gases inside a protein shell; an inclusion, not a membrane compartment.

A membrane surrounding DNA in one type of bacteria (Gemmata).

Internal structures outline

Internal structure outline: cytoplasm, ribosomes, cytoskeleton, nucleoid, compartments, inclusions and granules, endospores. [L20 p03]

Surface and wall

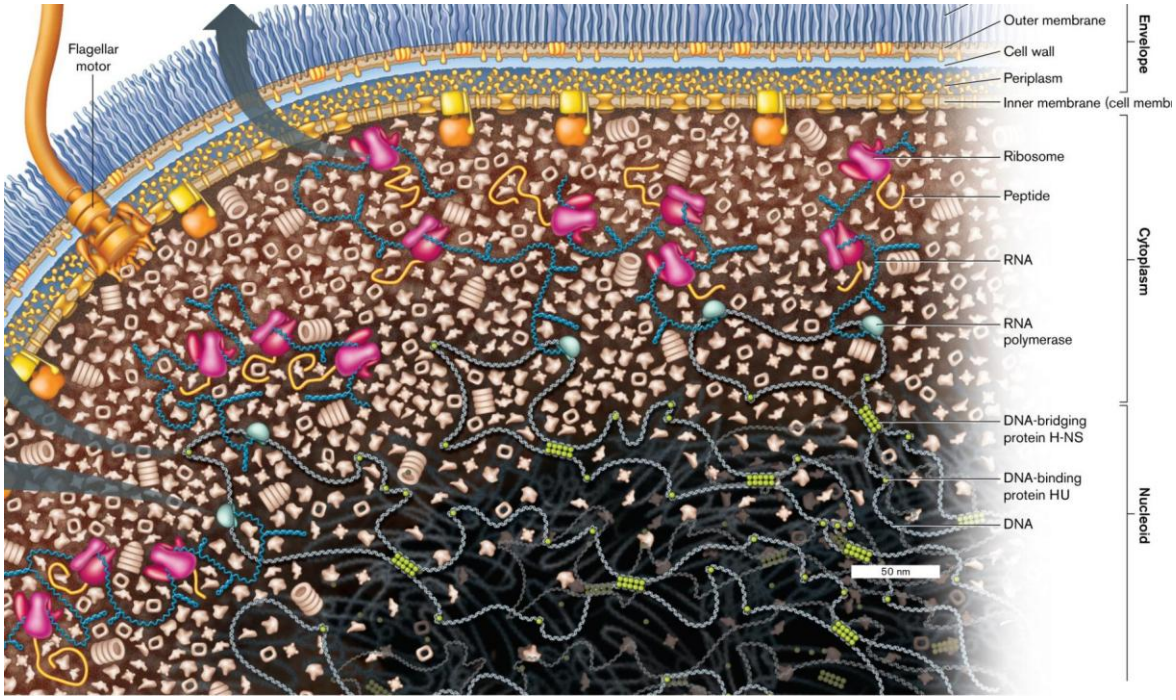
Appendages *(parts that stick out from the surface)* of a **prokaryotic** cell: flagella *(whip tails that spin)*, pili *(thin hairs on the cell)* and **fimbriae** *(short sticky hairs)*. [L20 p04]

Glycocalyx *(sticky coat outside the wall)*: the capsule *(firm sugar coat outside the cell)* and the **slime layer** *(loose sticky coat outside)*. [L20 p04]

Peptidoglycan of an E. coli cell: the murein *(another name for peptidoglycan)* **sacculus** *(bag)*. [L20 p07]

Sacculus: **1 molecule**, **0.8** percent of total weight. [L20 p07]

Cytoplasm contents



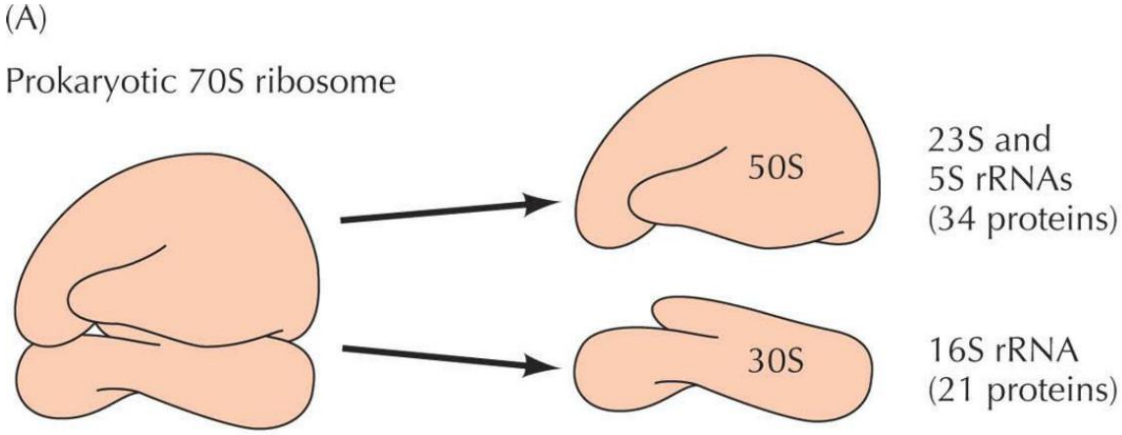
Cytoplasm contents: ribosomes, peptides, RNA and RNA polymerase in the E. coli cell [L20 p06]

On the figure: ribosomes · peptide · RNA · RNA polymerase

Cytoplasm contents: ribosome, **peptide**, **RNA** and **RNA polymerase** *(enzyme copying DNA into RNA)*. [L20 p06]

Polyamines *(small charged helper molecules)* in E. coli: mainly putrescine and **spermidine**. [L20 p07]

Ribosome and inhibitors



Prokaryotic 70S ribosome split into 50S and 30S subunits with their rRNAs [L20 p09]

On the figure: 50S · 23S · 5S · 34 proteins · ribosomes

Large subunit (50S) of the **prokaryotic** ribosome: **23S** and **5S** rRNAs plus **34 proteins**. [L20 p09]

Chloramphenicol inhibits **peptidyl transferase** *(enzyme that joins amino acids)* on the **prokaryotic** large subunit. [L20 p10]

Cycloheximide *(drug blocking eukaryotic peptide bonds)* inhibits peptidyl transferase on the **eukaryotic** *(cells with a nucleus)* large subunit. [L20 p10]

Paromomycin increases the ribosomal *(about protein building machines)* error rate. [L20 p10]

Growth pattern	Bacteria
Dispersed zonal growth	E. coli, Bacillus
Septal zonal growth	Streptococcus, Staphylococcus
Polar growth	Actinomycetes, Rhizobiales

Table source: [L19 p06]

Target of the drug	Drug examples
Cell wall synthesis (peptidoglycan)	penicillins, cephalosporins, vancomycin, bacitracin, monobactams, fosfomycin, cycloserine
Cell membrane integrity	polymyxins, daptomycin, gramicidin; amphotericin and imidazoles (vs. fungi)
Metabolic inhibitors	sulfonamides, trimethoprim, metronidazole (watch out: the other target table lists nitroimidazoles under nucleic acid function)
DNA replication inhibitors	quinolones (ciprofloxacin), which inhibit gyrase *(an unwinding enzyme)*
RNA polymerase inhibitors	rifampin, pyronins
Nucleic acid function	nitroimidazoles, nitrofurans, quinolones, rifampin
50S ribosome subunit	chloramphenicol, macrolides (erythromycin), clindamycin, oxazolidinones (linezolid), streptogramins (Synercid)
30S ribosome subunit	aminoglycosides (gentamicin, streptomycin, amikacin), tetracyclines (doxycycline)

Table source: [L19 p18, L19 p19, L19 p20]

Year	Event
1940	penicillinase, an enzyme that destroys penicillin, identified in bacteria
1942	first therapeutic use of penicillin
1943	penicillin mass-produced
1945	more than **20%** of S. aureus hospital isolates penicillin-resistant
1947	streptomycin approved by FDA; streptomycin resistance observed
1952 and **1956**	tetracycline approved by FDA (**1952**); tetracycline resistance observed (**1956**)
1958	vancomycin introduced, although rarely used until the mid-**1980**s
1959 and **1961**	methicillin introduced (**1959**); methicillin-resistant S. aureus (MRSA) observed (**1961**)
1964 and **1966**	cephalothin, the first antibiotic in the cephalosporin class, introduced (**1964**); cephalothin resistance observed (**1966**)
1967 and **1970**	gentamicin approved by FDA (**1967**); gentamicin resistance observed (**1970**)
1976	transferable penicillinase first observed in a gonococcus
1981 and **1983**	cefotaxime approved by FDA (**1981**); cefotaxime resistance observed (**1983**)
1983	first penicillin-resistant Enterococcus reported
1987	vancomycin-resistant Enterococcus (VRE) observed; first outbreak of Klebsiella pneumoniae resistant to third-generation cephalosporins
1996	S. aureus with intermediate resistance to vancomycin (VISA) reported
1999	community-acquired MRSA reported
2000 and **2001**	linezolid, the first antibiotic in the oxazolidinone class, approved by FDA (**2000**); linezolid-resistant S. aureus and VRE observed (**2001**)
2002	S. aureus with complete resistance to vancomycin (VRSA) observed

Table source: [L19 p22, L19 p23]

Resistance strategy	How the cell does it
Strategy 1: prevent entry	destroy the antibiotic (beta-lactamase); pump the antibiotic out (efflux pump); reduce membrane permeability (porin)
Strategy 2: prevent binding	alter the target (a mutated component, such as the ribosome); modify the antibiotic (modification enzyme)
Strategy 3: reverse binding	protect the ribosome (ribosome protection protein)

Table source: [L19 p26]

Beta-lactam	Properties
Penicillin G	high activity against most **Gram-positive** bacteria, low against **Gram-negative**; destroyed by acid and penicillinase (penicillinases attack the beta-lactam ring)
Penicillin V	more acid resistant than penicillin G
Ampicillin	active against **Gram-positive** and **Gram-negative** bacteria; acid stable
Carbenicillin	active against **Gram-negative** bacteria like Pseudomonas and Proteus; acid stable; not well absorbed by the small intestine
Methicillin	penicillinase-resistant, but less active than penicillin G; acid-labile
Ticarcillin	similar to carbenicillin, but more active against Pseudomonas

Table source: [L19 p28]

External group	Parts	Plain meaning
Appendages	flagella, pili, fimbriae	parts that stick out from the surface
Glycocalyx	capsule, slime layer	sticky coat outside the wall

Table source: [L19 p39]

Structure seen in some bacteria	Observed?	Basis
Membrane bound compartments	Yes (textbook)	Thylakoids, chlorosomes and a membrane around DNA; carboxysomes and gas vesicles have a protein shell and are inclusions, not membrane compartments
DNA within an intracellular membrane	Yes (textbook)	DNA inside a cytoplasmic membrane compartment in one type of bacteria is true
Three types of cytoskeleton	Yes (three types of cytoskeletal filaments: FtsZ, MreB and crescentin)	FtsZ is similar to tubulin, MreB is similar to actin and crescentin is similar to intermediate (filaments)
DNA wrapped around proteins	Yes, for proteins that fold and compact DNA; **NOT** histone octamers (textbook)	Proteins fold and compact bacterial DNA; bacterial DNA is **NOT** wrapped around an octamer of histones. The common DNA binding proteins are HU, IHF, H-NS and Fis
Aggregates of metabolites or glycogen	Yes (textbook)	Storage granules of glycogen come from the textbook; polyhydroxybutyrate (PHB) and poly-**3**-hydroxyalkanoate (PHA) granules are listed as observed in bacterial interiors
Clumps of iron and sulfur	Yes (textbook)	Granules of elemental sulfur and crystals of iron magnetite ARE observed (textbook)
UFOs (unidentified floating objects)	Yes (textbook)	Structures of unknown function are still being found

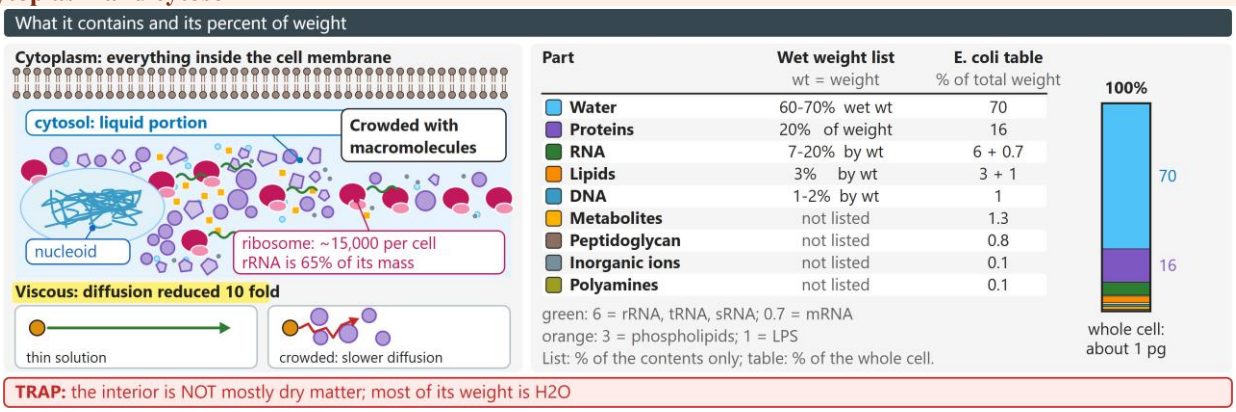
Table source: [L19 p37, L19 p42]

1. Cytoplasm and cytosol

Book: 3.1 The Bacterial Cell: An Overview, Model of a Bacterial Cell (chapter 3 PDF, p. 7); 3.1 The Bacterial Cell: An Overview, Biochemical Composition of Bacteria (chapter 3 PDF, p. 11).

⚠ **Do not mix up: Cytosol *(liquid part of the cytoplasm)* is the liquid part; cytoplasm *(jelly inside the cell membrane)* is everything inside the cell membrane *(thin skin around the cell)*.** [L19 p41]

Cytoplasm and cytosol



Cytosol, contents of the cytoplasm and share of weight [L19 p43]

On the figure: Cytosol · nucleoid · Viscous · Ribosomes · water · proteins · Lipid · DNA

Cytoplasm: everything inside the cell membrane. [L19 p41]

Cytosol: the liquid portion of the cytoplasm. [L19 p41]

Cytoplasm contains: cytosol, dissolved substances, **nucleoid** *(DNA region without a membrane)*, inclusions *(clumps of protein and other material)*, membrane-bound compartments. [L19 p41]

Cytosol:

- The liquid part of the cytoplasm.
- Is a variety of minerals, salts, **proteins**, sugars and other things dissolved in **water**. [L19 p41]

Ribosomes *(protein building machine)* are found in the cytoplasm. [L19 p42]

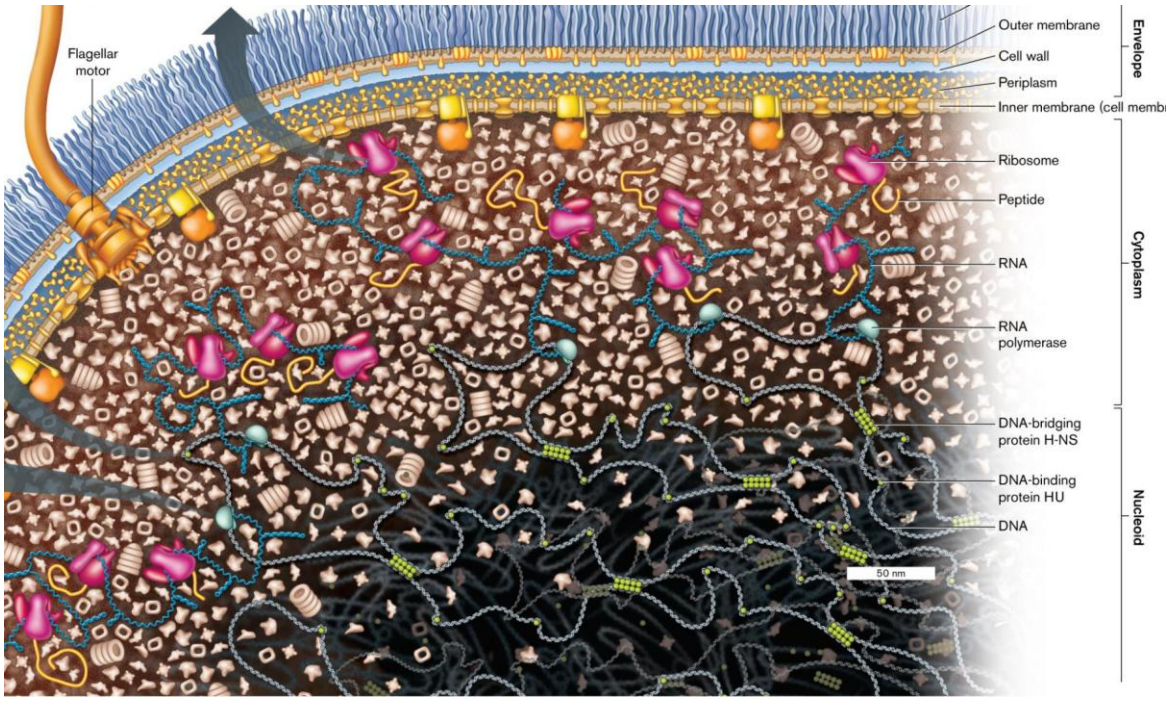
Nucleoid *(the cell's DNA region)*: tightly coiled **DNA** *(cell genetic instruction molecule)*. [L19 p41, L19 p42]

Inclusions:

- Aggregates of proteins and other material.
- Are found in most bacteria. [L19 p41, L19 p42]

Membrane-bound compartments: a few bacteria have them. [L19 p41]

Watch out: most bacteria have inclusions; **only** a few have membrane-bound compartments. [L19 p41, L19 p42]



Cytoplasm contents, nucleoid proteins and envelope layers of a bacterial cell [L19 p42]

On the figure: Ribosomes · RNA polymerase · H-NS · HU · lipopolysaccharide · outer membrane · periplasm · inner membrane

Cytoplasm, nucleoid and envelope contents

Cytoplasm contains ribosome, peptide *(short chain of amino acids)*, RNA and **RNA polymerase** *(enzyme copying DNA into RNA)*. [L19 p42]

Nucleoid contains **H-NS** *(DNA bridging protein)*, **HU** *(DNA binding protein)* and DNA. [L19 p42]

Cell envelope *(layers wrapped around the cell)* holds **lipopolysaccharide** *(outer membrane sugar and fat coat)*, **outer membrane** *(extra membrane outside the wall)*, cell wall *(stiff layer around the cell membrane)*, **periplasm** *(space between the two membranes)* and **inner membrane** (cell membrane); the flagellar motor is also part of the envelope. [L19 p42]

Flagellar motor *(the spinning anchor of the whip tail)*: part of the envelope. [L19 p42]

E. coli composition table

E. coli composition table: here they tried to estimate the number of each component per cell. [L19 p43]

Cell state: balanced exponential growth, a hypothetical average cell. [L19 p43]

Growth medium: glucose minimal salts medium at **37** C. [L19 p43]

Whole cell weight is about **10**^-12 g (**1** pg). [L19 p43]

Two DNA copies: fission lags about one generation behind DNA replication in fast growth. [L19 p43]

Kinds of proteins and mRNA *(message copy of a gene)* are hard to estimate. [L19 p43]

Cytoplasm makeup

Cytoplasm is crowded: packed with macromolecules, particularly **ribosomes** (about **15,000** per cell). [L19 p44]

Viscous *(thick and gel like)*: the rate of diffusion *(drifting from high to low amount)* is reduced **10** fold. [L19 p44]

The weight shares below are for the contents of the cytoplasm. [L19 p44]

Water is **60**-**70%** of the wet weight of the cytoplasm. [L19 p44]

Protein is **20%** of weight. [L19 p44]

RNA is **7**-**20%**. [L19 p44]

rRNAs make up **65%** of ribosomes. [L19 p44]

Lipid is **3%**. [L19 p44]

DNA is **1**-**2%**. [L19 p44]

Check the two lists

Check before you memorize: the cytoplasm percentages do not match the E. coli composition table. [L19 p43, L19 p44]

Protein: **20%** in the cytoplasm list vs **16%** in the composition table. [L19 p43, L19 p44]

DNA: **1**-**2%** in the cytoplasm list vs **1%** in the composition table. [L19 p43, L19 p44]

RNA: **7**-**20%** in the cytoplasm list vs **6.7%** in the composition table. [L19 p43, L19 p44]

The composition table RNA counts rRNA *(RNA building ribosomes)*, tRNA *(adapter that carries amino acids)*, sRNA and mRNA together. [L19 p43, L19 p44]

Lipid: **3%** in the cytoplasm list vs **4%** in the composition table for phospholipids *(membrane fat with a charged head)* plus LPS. [L19 p43, L19 p44]

Learn the cytoplasm list as written; use the composition table for molecule counts. [L19 p43, L19 p44]

Component	% of total weight	Molecules per cell	Different kinds
Water	70	20,000,000,000	1
Proteins	16	2,400,000	4,000
rRNA, tRNA and other small RNA	6	250,000	200
mRNA	0.7	4,000	2,000
Phospholipids (membrane)	3	25,000,000	50
Lipopolysaccharides (outer membrane)	1	1,400,000	1
DNA	1	2	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

Table source: [L19 p43]

2. Ribosomes

Book: 27.2 Antibiotic Mechanisms of Action, Protein Synthesis Inhibitors (chapter 27 PDF, p. 50); 27.2 Antibiotic Mechanisms of Action, Targeting the 50S Subunit (chapter 27 PDF, p. 52).

⚠ **Do not mix up: Penicillin and cephalosporin do NOT inhibit ribosomes *(protein building machine)*; they block peptidoglycan *(sugar and peptide mesh of the wall)* synthesis.** [L19 p16]

⚠ **Do not mix up: 70S is NOT 50S + 30S added up: 50 + 30 would be 80, yet the whole bacterial ribosome is 70S (and 60S + 40S make 80S, not 100S).** [L19 p45]

What ribosomes are

Ribosome *(the protein making machine)*: bacterial and **eukaryotic** *(cells with a nucleus)* ribosomes differ in size and components. [L19 p45, L19 p46]

Size: 70S in bacteria vs 80S in eukaryotes. [L19 p45]

Subunits *(building block piece)*: the **70S** ribosome is made of a 50S and a 30S subunit; the **80S** ribosome is made of a 60S and a 40S subunit. [L19 p45]

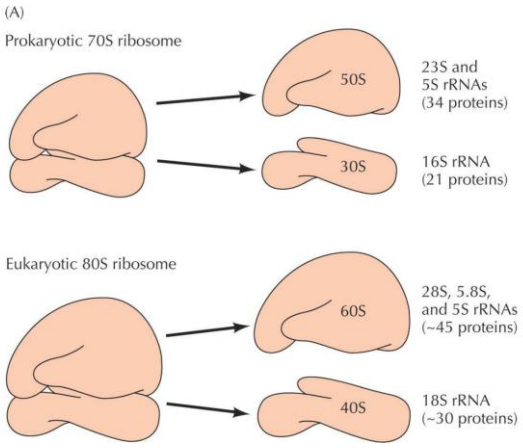
Selective inhibition

Selective inhibition: bacterial ribosomes are selectively inhibited by some antibiotics. [L19 p45, L19 p46]

Selective means the drug hits the bacterial **70S** ribosome and leaves the **eukaryotic 80S** ribosome (ours) working. [L19 p45]

Examples: streptomycin, tetracycline, chloramphenicol, erythromycin. [L19 p45, L19 p46]

Watch out: some antibiotics inhibit ribosomes, not all of them; penicillin and cephalosporin block peptidoglycan synthesis instead. [L19 p45, L19 p46]



Bacterial 70S and eukaryotic 80S ribosomes and their subunits [L19 p45]

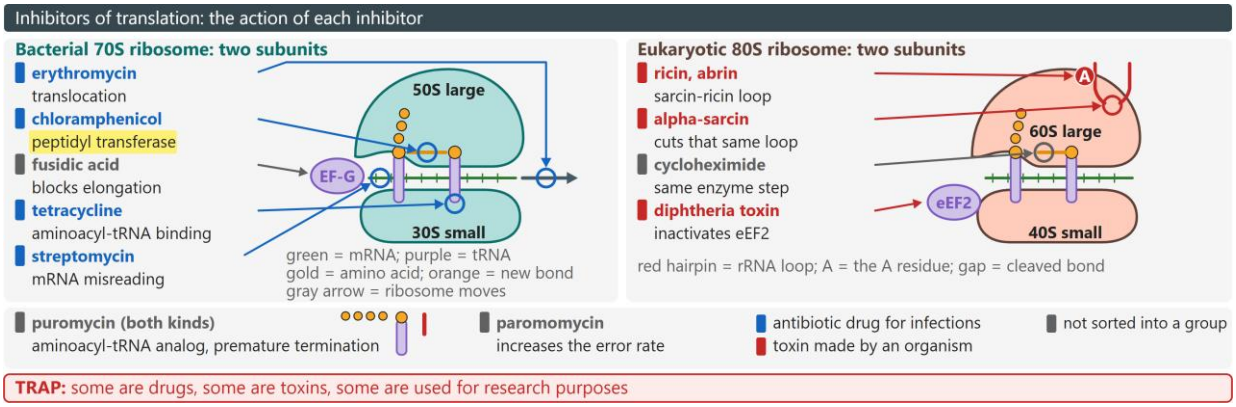
On the figure: 70S · 50S · 30S · 80S · 60S · 40S

Translation inhibitors by target

Translation inhibitors include antibiotic drugs used for bacterial infections. [L19 p46]

Some translation inhibitors:

- Are toxins produced by organisms.
- Are used for research purposes. [L19 p46]



Where each translation inhibitor acts on the ribosome [L19 p46]

On the figure: chloramphenicol · erythromycin · tetracycline · streptomycin · fusidic acid · puromycin · Paromomycin · Diphtheria toxin

Prokaryotic ribosome inhibitors: **chloramphenicol**, **erythromycin**, **tetracycline**, **streptomycin**, **fusidic acid** *(drug trapping EF-G on ribosome)*. [L19 p46]

Eukaryotic ribosome or its elongation factor inhibitors: cycloheximide *(drug blocking eukaryotic peptide bonds)*, ricin *(castor bean poison)*, abrin *(rosary pea poison)*, alpha-sarcin *(fungal toxin that cuts rRNA)*. [L19 p46]

Diphtheria toxin *(toxin shutting down eEF2)* inactivates eEF2 *(eukaryotic elongation helper protein)*. [L19 p46]

Inhibits both kinds of ribosome: **puromycin** *(drug mimicking tRNA, ends protein early)*. [L19 p46]

Paromomycin increases the ribosomal *(about protein building machines)* error rate; no subunit is named for it. [L19 p46]

Penicillin and cephalosporin

Both are **antibiotics that interfere with peptidoglycan synthesis**. [L19 p16]

Penicillin and cephalosporin both form a structure similar to D-Ala-D-Ala.

PBPs *(penicillin binding proteins)* are part of a complex involved in synthesis of peptidoglycan and do the transglycosylation *(joining sugar chains longer)* and transpeptidation *(making peptide bridges between chains)* reactions. [L19 p11, L19 p12]

Penicillin *(a beta-lactam)* blocks the PBPs (transpeptidases and transglycosylases), so peptides *(short chain of amino acids)* are not cross-linked. [L19 p10, L19 p16]

The weakened wall cannot counter intracellular turgor pressure, so the growing cell bursts (textbook).

Cephalosporins *(penicillin like wall blocking drugs)* also inhibit transpeptidase (peptide cross-linking) and transglycosylation, the reactions the PBPs carry out. [L19 p13]

	Prokaryotic 70S ribosome	Eukaryotic 80S ribosome
Large subunit	**50S**: **23S** and **5S** rRNAs, **34 proteins**	**60S**: **28S**, **5.8S** and **5S** rRNAs, about **45 proteins**
Small subunit	**30S**: **16S** rRNA, **21 proteins**	**40S**: **18S** rRNA, about **30 proteins**

Table source: [L19 p45]

Inhibitor	Action	Acts on
Chloramphenicol	Inhibits peptidyl transferase	**Prokaryotic** large subunit
Erythromycin	Inhibits translocation	**Prokaryotic** large subunit
Tetracycline	Inhibits binding of aminoacyl-tRNAs	**Prokaryotic** small subunit
Streptomycin	Causes mRNA misreading and inhibits chain initiation	Prokaryotes
Fusidic acid	Inhibits elongation by binding EF-G-GDP so it cannot dissociate from the large subunit	Prokaryotes
Paromomycin	Increases the ribosomal error rate	Not stated
Puromycin	Aminoacyl-tRNA analog that causes premature chain termination	Prokaryotes and eukaryotes
Cycloheximide	Inhibits peptidyl transferase	**Eukaryotic** large subunit
Diphtheria toxin	Inactivates eEF2 by ADP-ribosylation	eEF2 (no ribosome type named)
Ricin and abrin (plant glycosidases)	Catalytically inactivate the **eukaryotic** large subunit by removing a purine (depurination) from a highly conserved A in the **28S** RNA sarcin-ricin loop, a critical part of the factor binding center	**Eukaryotic** large subunit
Alpha-sarcin (fungal protein)	Cleaves a phosphodiester bond in the sarcin-ricin loop	The sarcin-ricin loop (in the **28S** RNA of the **eukaryotic** large subunit, as for ricin)

Table source: [L19 p46]

3. Cytoskeleton: types found and roles

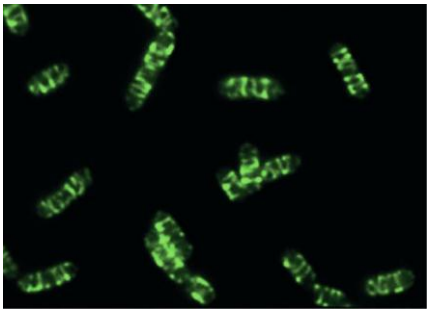
Book: 3.3 The Envelope and Cytoskeleton, Bacterial Cytoskeleton (chapter 3 PDF, p. 67).

⚠ **Do not mix up: Bacteria DO have a cytoskeleton *(inner scaffold of the cell)*: homologs of all three types of eukaryotic *(cells with a nucleus)* cytoskeletal proteins.** [L20 p11]

Cytoskeleton: bacteria have homologs *(related proteins)* of all three types of **eukaryotic** cytoskeletal proteins. [L20 p11]

Roles: cell division, cell polarity *(which end is which)*, cell shape regulation, plasmid *(small extra DNA circle)* partition and other functions. [L20 p11]

Stained actin *(protein of eukaryote thin filaments)* **fibers** show as green bands along rod-shaped cells. [L20 p11]

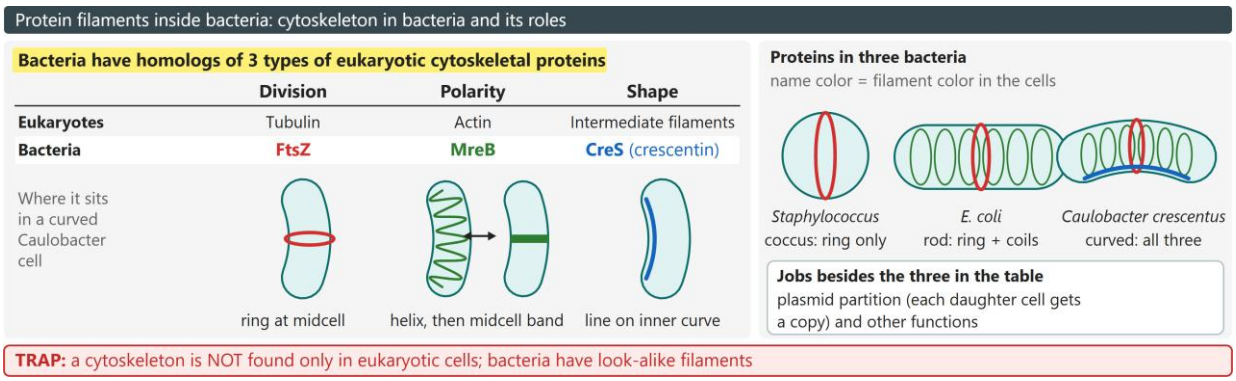


Bacterial cells with green stained actin fiber bands [L20 p11]

FtsZ, MreB and crescentin (CreS): which eukaryotic protein each is like and its role

Book: 3.3 The Envelope and Cytoskeleton, Bacterial Cytoskeleton (chapter 3 PDF, p. 67).

⚠ **Do not mix up: FtsZ *(protein ring for cell splitting)* is like tubulin (division); MreB is like actin (polarity). Do not swap them.** [L20 p11, L20 p12]



FtsZ ring, MreB helix then band, and crescentin line in a curved Caulobacter cell [L20 p11]

On the figure: tubulin · actin · intermediate filaments · FtsZ · MreB · Crescentin · Caulobacter

The three filaments

All 3 types of cytoskeletal filaments play a role in cell shape and cell division. [L20 p12]

FtsZ of the Z ring: similar to **tubulin**. [L20 p12]

The **Z-ring** determines cell diameter and manages growth of the septum *(cross wall)*, the dividing partition (textbook).

MreB:

> Similar to **actin**. [L20 p12]
>
> Travels in a helical arc beneath the cell membrane *(thin skin around the cell)*, guiding peptidoglycan *(sugar and peptide mesh of the wall)* elongation (textbook).
>
> Forms a **helix** *(spiral coil)* along the cell first, then gathers as a band at midcell. [L20 p11]

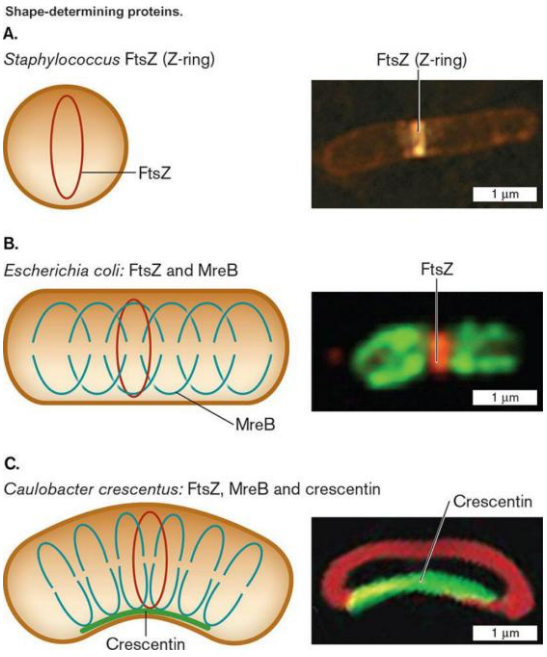
Crescentin *(protein that keeps the cell curved)*: similar to **intermediate filaments** *(rope-like eukaryote filaments)*. [L20 p12]

Shape-determining proteins

Staphylococcus (a coccus): FtsZ forms the Z ring around the middle of the cell. [L20 p12]

E. coli (a rod): an FtsZ ring at midcell plus **MreB** helices along the cell. [L20 p12]

Caulobacter crescentus (curved): FtsZ midcell ring, MreB helices, crescentin along the inner curve. [L20 p12]



FtsZ in a coccus; FtsZ and MreB in E. coli; plus crescentin in Caulobacter [L20 p12]

On the figure: FtsZ · MreB · Crescentin · Staphylococcus · E. coli · Caulobacter crescentus

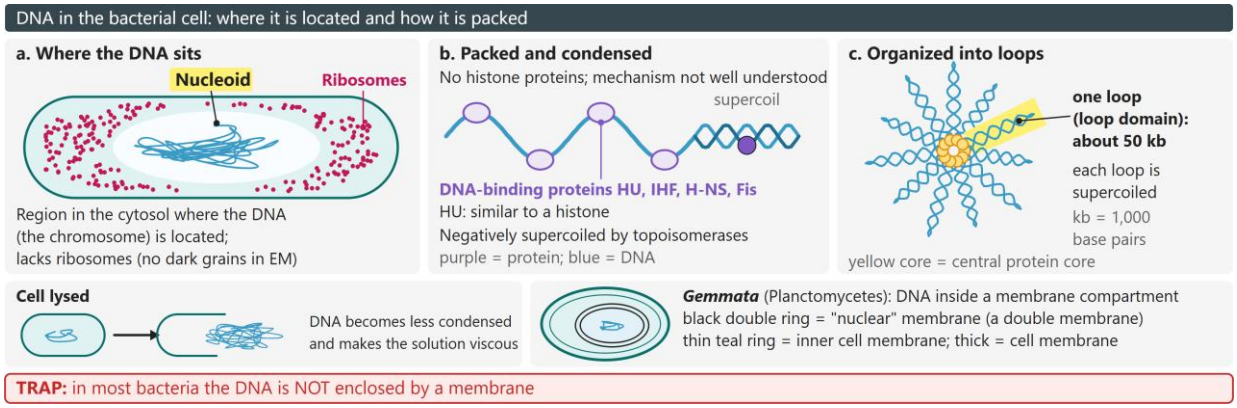
Cytoskeleton proteins: eukaryote vs bacteria

Role	Eukaryotic protein	Bacterial homolog	Caulobacter localization
Division	Tubulin	FtsZ (Z ring)	Ring at midcell
Polarity	Actin	MreB	Helix along the cell, then a band at midcell
Shape	Intermediate filaments	CreS (crescentin)	Line along the inner curve

Table source: [L20 p11, L20 p12]

4. Nucleoid: DNA and packing proteins

Book: 7.2 Genome Organization, Bacterial Chromosomes Are Compacted into a Nucleoid (chapter 7 PDF, p. 21); 3.4 Bacterial Cell Division, DNA Is Organized in the Nucleoid (chapter 3 PDF, p. 81).



Where the DNA sits, how it is packed, loop domain and the Gemmata membranes [L20 p13]

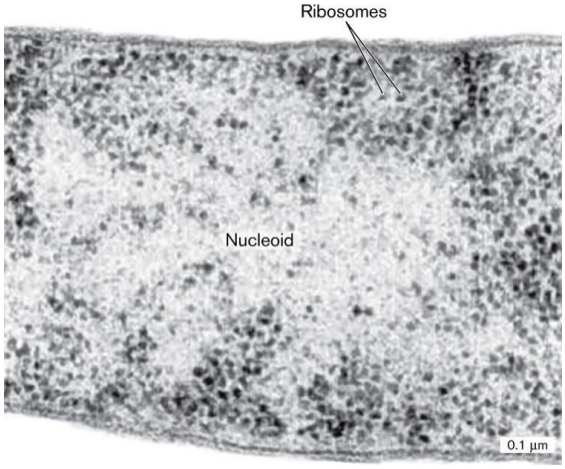
On the figure: Nucleoid · loop domains · Gemmata

> **Nucleoid** *(the cell DNA region)* topics: DNA *(cell genetic instruction molecule)* location, DNA packing, **loop domains**, a membrane *(thin lipid layer)* compartment. [L20 p13, L20 p15, L20 p17, L20 p18]

a. Nucleoid region: where the DNA is located

Book: 3.4 Bacterial Cell Division, DNA Is Organized in the Nucleoid (chapter 3 PDF, p. 81); 7.2 Genome Organization, Bacterial Chromosomes Are Compacted into a Nucleoid (chapter 7 PDF, p. 21).

⚠ **Do not mix up: The nucleoid *(the cell DNA region)* is the area WITHOUT ribosomes *(protein building machine)*; the dark grains around it are ribosomes.** [L20 p13]



Electron micrograph: pale nucleoid region without ribosomes, dark ribosome grains around it [L20 p13]

On the figure: nucleoid · ribosomes

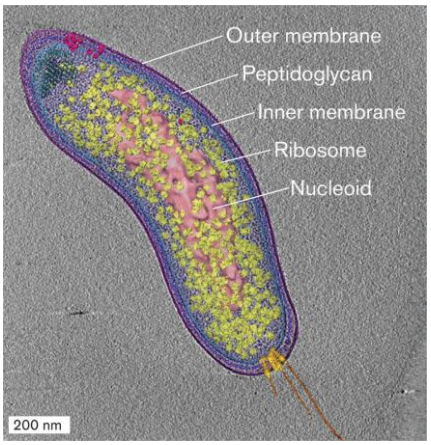
Nucleoid: region in the cytosol *(liquid part of the cytoplasm)* where chromosomal DNA *(cell genetic instruction molecule)* is located. [L20 p13]

Lacks ribosomes: in an electron micrograph, ribosomes are the black dots or grains. [L20 p13]

In an electron micrograph the nucleoid is the lighter area; ribosome-filled cytoplasm *(jelly inside the cell membrane)* is darker. [L20 p13]

Bdellovibrio bacteriovorus: colorized cryo-electron tomograph *(3D electron microscope picture)* of one cell. [L20 p14]

Bdellovibrio tomograph: outer membrane *(extra membrane outside the wall)*, peptidoglycan *(sugar and peptide mesh of the wall)*, inner membrane *(the skin around the cytoplasm)*, ribosome and nucleoid. [L20 p14]



Cryo-electron tomograph of one Bdellovibrio cell with its membranes, ribosomes and nucleoid [L20 p14]

On the figure: outer membrane · peptidoglycan · inner membrane · ribosomes · nucleoid

b. How bacterial DNA is packed: no histones, DNA binding proteins, supercoiling

Book: 7.2 Genome Organization, DNA Supercoiling Compacts the Chromosome (chapter 7 PDF, p. 24); 7.2 Genome Organization, Topoisomerases Supercoil DNA (chapter 7 PDF, p. 33).

⚠ **Do not mix up: Bacteria have NO histones *(eukaryote protein that DNA winds around)*; HU *(DNA binding protein)* is only similar to a histone.** [L20 p15]

Packing basics

Bacterial DNA *(cell genetic instruction molecule)* **is packed and condensed**; the mechanism is not well understood. [L20 p15]

No histone proteins in bacteria. [L20 p15]

DNA binding proteins

Proteins that bind DNA: HU, IHF, H-NS *(DNA bridging protein)* and Fis help pack it, in part. [L20 p15]

These proteins bind DNA but with low sequence specificity *(not tied to one exact DNA sequence)*. [L20 p15]

HU is similar to a histone protein. [L20 p15]

Supercoiling and loops

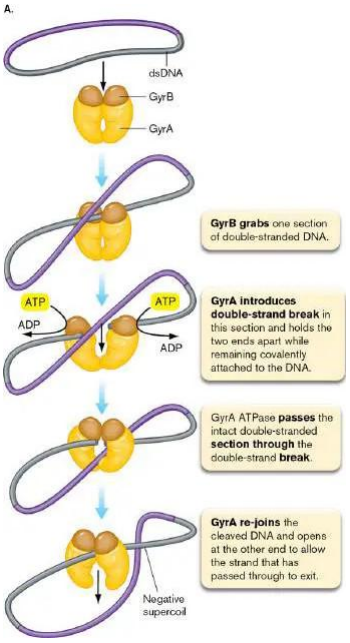
Supercoiling: the DNA is negatively supercoiled *(underwound, then twisted into coils)* by topoisomerases *(enzymes that twist or untwist DNA)*. [L20 p15]

DNA gyrase *(enzyme that coils DNA)*, a type II topoisomerase, introduces negative supercoils and uses ATP (textbook).

In loops: the packed DNA is arranged in loops. [L20 p15]

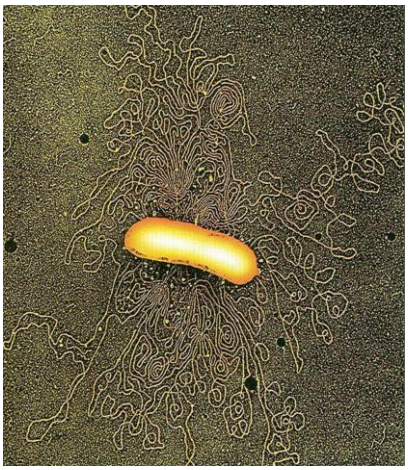
After the cell is lysed

After the cell is lysed *(broken open)* the DNA becomes less condensed. [L20 p16]



DNA gyrase makes a negative supercoil using ATP [textbook 7.10]

On the figure: ATP · negative supercoils



Uncondensed DNA spread around one lysed bacterial cell [L20 p16]

Released DNA can make the solution viscous *(thick)*. [L20 p16]

Uncondensed DNA released from one bacterial cell spreads all around it. [L20 p16]

c. Loop domains of about 50 kB

Book: 7.2 Genome Organization, DNA Supercoiling Compacts the Chromosome (chapter 7 PDF, p. 24).

⚠ **Do not mix up: Not 1,000 nm loops: each loop holds about 50 kB; 1,000 nm is the whole structure.** [L20 p17]

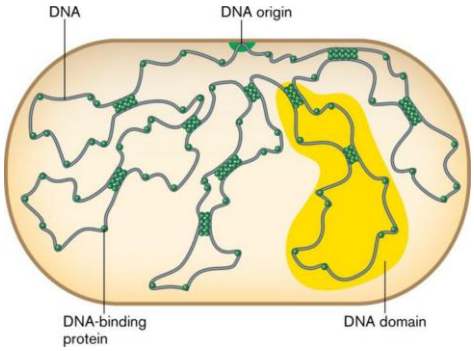
What loop domains are

Loop domains: DNA *(cell genetic instruction molecule)* is organized into loops of about **50** kB and supercoiled *(twisted into coils)*. [L20 p17]

Attachment points: the chromosome *(one long DNA molecule)* attaches to itself about once every **50** kb, forming a loop. [L20 p17]

DNA-binding proteins hold the loops; one loop is a DNA domain. [L20 p17]

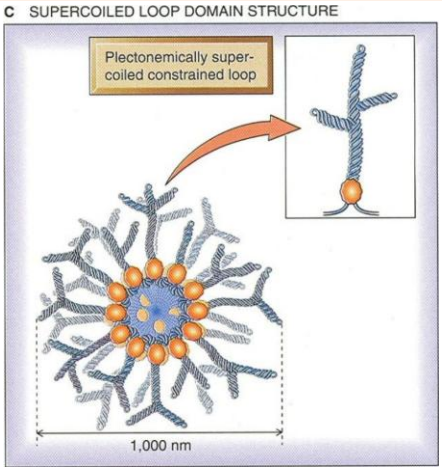
The DNA origin sits at the cell envelope *(layers wrapped around the cell)*. [L20 p17]



Chromosome loops held by DNA-binding proteins; one loop is a DNA domain [L20 p17]

On the figure: DNA-binding proteins · DNA domain · DNA

Supercoiled loops



Supercoiled loop domain structure, about 1,000 nm across, with one constrained loop [L20 p17]

On the figure: supercoiled loop domain structure · 1,000 nm · plectonemically · constrained

Supercoiled: the looped DNA is also supercoiled. [L20 p17]

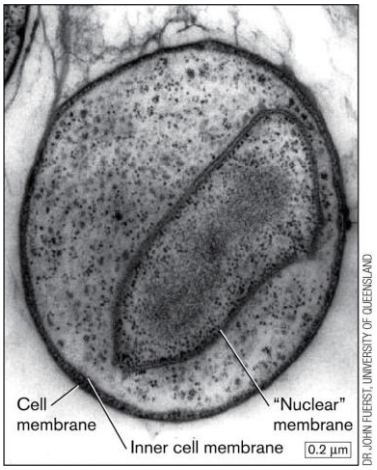
Each loop is a **plectonemically** *(twisted around itself like a coiled phone cord)* supercoiled **constrained** loop. [L20 p17]

Supercoiled loop domain structure: about **1,000 nm** across. [L20 p17]

d. DNA inside a membrane compartment (Gemmata, Planctomycetes)

Book: Not in the assigned textbook sections; see the posted source "Bacterial Intracellular Structures".

⚠ **Do not mix up: The chromosome *(one long DNA molecule)* sits inside TWO membranes *(thin lipid layer)*: the double nuclear membrane and the inner cell membrane *(membrane that lies inside another membrane)*.** [L20 p18]



Gemmata: cell membrane, inner cell membrane and nuclear membrane around the chromosome [L20 p18]

On the figure: cell membrane · inner cell membrane · nuclear membrane

One type of bacterium (Planctomycetes): DNA *(cell genetic instruction molecule)* appears to sit inside a membrane compartment. [L20 p18]

Gemmata is that bacterium. [L20 p18]

Chromosome inside two membranes: the nuclear membrane (a double membrane) and the inner cell membrane. [L20 p18]

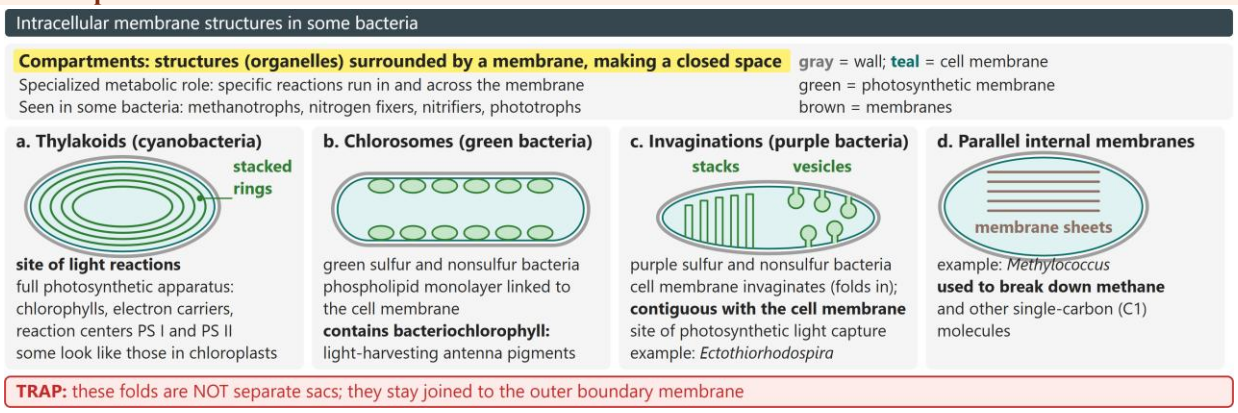
Gemmata membranes: the cell membrane *(thin skin around the cell)*, the inner cell membrane and the nuclear membrane. [L20 p18]

5. Compartments: intracellular membrane structures (know some examples and their role)

Book: 3.6 Specialized Structures, Thylakoids, Carboxysomes, and Storage Granules (chapter 3 PDF, p. 119).

⚠ **Do not mix up: Compartments are surrounded by a lipid membrane *(thin fat skin)*; gas vesicles *(gas filled floating bubbles)* and carboxysomes *(protein-shell bodies that fix carbon dioxide)* have a protein shell.** [L20 p19, L20 p30, L20 p31]

What compartments are



Membrane compartments: thylakoids, chlorosomes, invaginations and methanotroph membranes [L20 p23]

On the figure: Compartments · methanotrophs · nitrogen fixers · nitrifiers · phototrophs · thylakoids

Compartments: structures (organelles) surrounded by a lipid membrane, thus making a compartment. [L20 p19]

Seen in some bacteria: methanotrophs *(bacteria that use methane)*, nitrogen fixers, nitrifiers *(bacteria that turn ammonia into nitrate)*, phototrophs *(organisms that use light for energy)*. [L20 p19]

Specialized physiological role *(a special job in the cell)*: they carry out specific metabolic reactions in and across the membrane. [L20 p19]

Photosynthesis example

Example: photosynthesis. [L20 p19]

Phototroph *(organism that uses light for energy)* **examples**: thylakoids *(membrane sacs for photosynthesis)* and tubular, vesicular and lamellar *(in flat sheets)* internal membranes in phototrophic bacteria. [L20 p20, L20 p21]

Intracellular membrane structures are found in many types of phototrophic bacteria. [L20 p20, L20 p21]

Intracellular membrane structures

Membrane structure	Found in	Built how	Role
Thylakoids	Cyanobacteria	Stacked membranes with full lipid bilayers, some like chloroplast thylakoids	Site of light harvesting reactions
Chlorosomes	Green sulfur and nonsulfur bacteria	Single phospholipid monolayer linked to the cell membrane	Contain light harvesting pigments
Invaginations	Purple sulfur and nonsulfur bacteria	Cell membrane folds inward and stays contiguous with it	Photosynthetic light capture reactions
Membrane compartments	Methanotrophs (Methylococcus)	Stacks of internal membranes	Breakdown of methane and other one carbon molecules

Table source: [L20 p23, L20 p25, L20 p26, L20 p27, L20 p28]

a. Thylakoids in cyanobacteria

Book: 3.6 Specialized Structures, Thylakoids, Carboxysomes, and Storage Granules (chapter 3 PDF, p. 119).

⚠ **Do not mix up: Thylakoids *(membrane sacs for photosynthesis)* = cyanobacteria; chlorosomes *(light catching sacs)* = green bacteria; invaginations *(inward folds)* = purple bacteria.** [L20 p21, L20 p24, L20 p26]

What thylakoids are

Thylakoids *(flat membrane sacs for photosynthesis)*: found in cyanobacteria. [L20 p21, L20 p22]

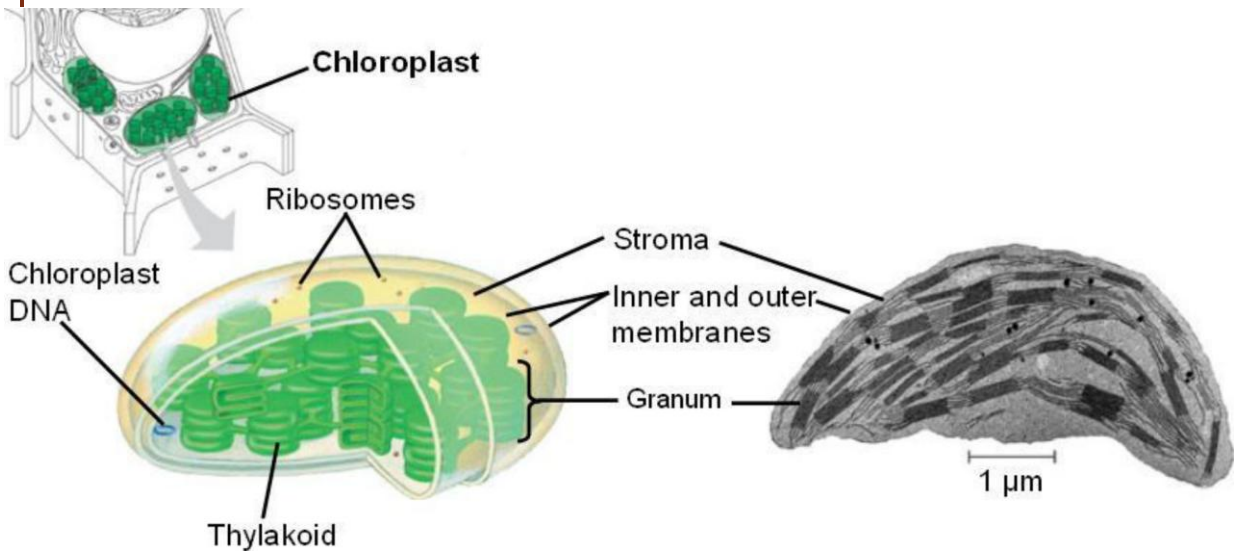
Thylakoids are the site of light harvesting reactions. [L20 p23]

Thylakoid membranes *(thin lipid layer)* have full lipid bilayers *(two-layer membranes, like a normal membrane)*. [L20 p23]

Compared with chloroplasts

Some are similar to the thylakoids in chloroplasts *(plant part that does photosynthesis)*: stacked thylakoid membranes. [L20 p21, L20 p22]

Chloroplast parts: stroma, inner and outer membranes *(extra membrane outside the wall)*, **granum** and thylakoid. [L20 p22]



Chloroplast parts: stroma, inner and outer membranes, granum and thylakoid [L20 p22]

On the figure: chloroplasts · stroma · inner and outer membranes · granum · Thylakoids

Cyanobacteria and examples

Cyanobacteria with oxygen: oxygenic *(gives off oxygen)* phototrophy; chlorophyll *(green light-catching pigment)* a/b in **thylakoids**. [L20 p21]

Cyanobacteria without oxygen (-O2): photolithoautotrophy on reduced sulfur. [L20 p21]

Cyanobacteria reaction centers: PS I and PS II. [L20 p21]

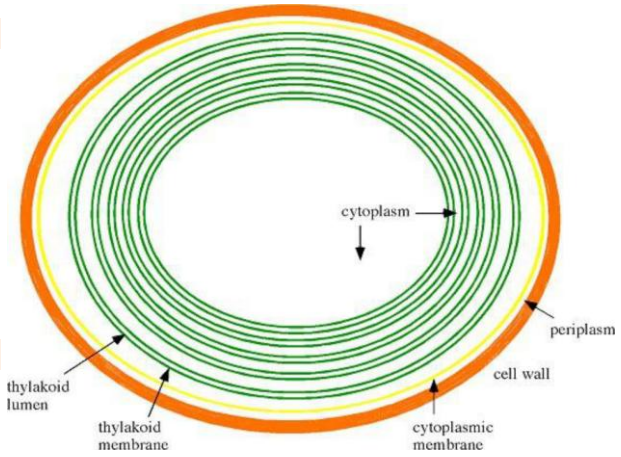
Example: the cyanobacteria example is **Anabaena**. [L20 p21]

Prochlorococcus

Prochlorococcus, a marine phototroph *(organism that uses light for energy)*: concentric thylakoid layers, carboxysomes *(protein-shell bodies that fix carbon dioxide)* in the center. [L20 p23]

Prochlorococcus cell layers: **periplasm** *(space between the two membranes)*, **cell wall** *(stiff layer around the cell membrane)*, **cytoplasmic** membrane, thylakoid membrane and thylakoid **lumen** *(inner space)*. [L20 p23]

The thylakoid membrane surrounds the lumen. [L20 p23]



Prochlorococcus cell layers: periplasm, cell wall, cytoplasmic membrane, thylakoid membrane, lumen [L20 p23]

On the figure: periplasm · cell wall · Thylakoid membranes · lumen

b. Chlorosomes in green bacteria

Book: Not in the assigned textbook sections; see the posted source "Bacterial Intracellular Structures".

⚠ **Do not mix up: A chlorosome has a SINGLE phospholipid *(membrane fat with a charged head)* monolayer *(one layer)*, not a bilayer *(two layers)*, linked to the cell membrane *(thin skin around the cell)*.** [L20 p25]

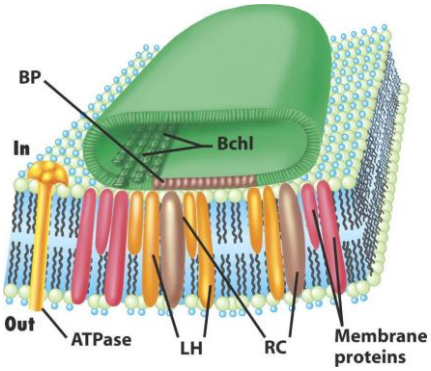
Chlorosome structure

Chlorosomes *(light catching sacs)*: found in green sulfur and green nonsulfur phototrophic bacteria. [L20 p24, L20 p25]

Chlorosome membrane: a single phospholipid monolayer linked to the cell membrane. [L20 p24, L20 p25]

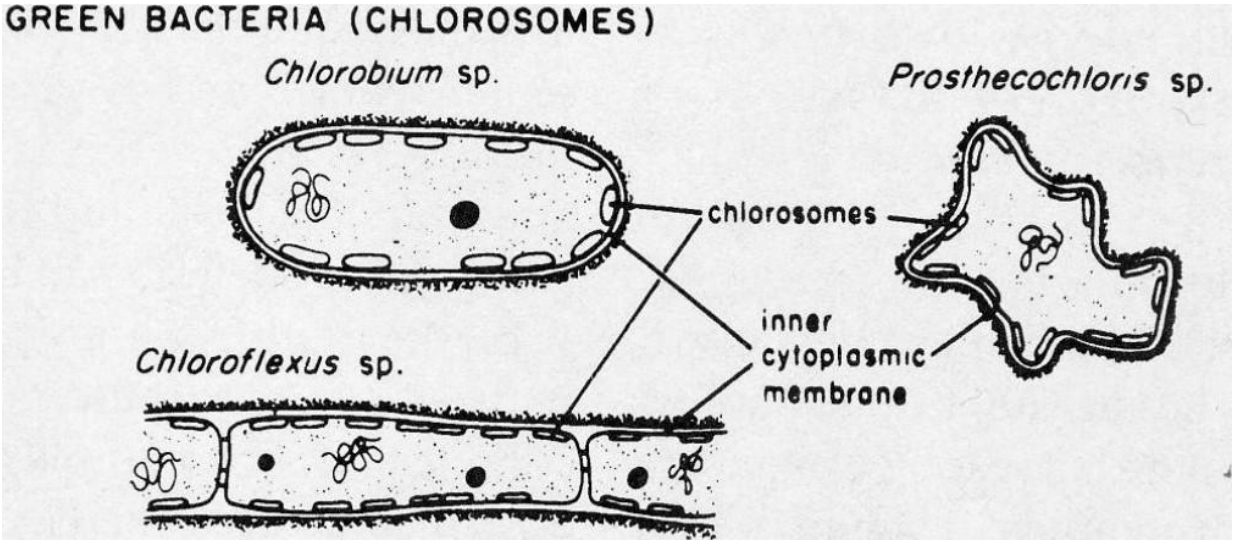
Contains light harvesting pigments *(pigments that catch light)*. [L20 p24, L20 p25]

Chlorosome: it sits on the cell membrane on the baseplate (**BP**); bacteriochlorophyll (**Bchl**) is inside it. [L20 p25]



Chlorosome on the baseplate BP with bacteriochlorophyll inside, sitting on the membrane [L20 p25]

On the figure: BP · Bchl



Chlorosomes along the inner cytoplasmic membrane in Chlorobium, Prosthecochloris and Chloroflexus [L20 p25]

On the figure: Chlorosomes · Chlorobium · Prosthecochloris · Chloroflexus · inner cytoplasmic membrane

> **Chlorosomes** in **Chlorobium**, **Prosthecochloris** and **Chloroflexus** lie along the **inner cytoplasmic membrane**. [L20 p25]

Green nonsulfur bacteria

> **Chloroflexi** (green nonsulfur) have chlorosomes with BChl c/d, reaction center PS II. [L20 p24]
>
> **Chloroflexus** with oxygen uses **heterotrophy** *(food from ready-made organic matter)*; without oxygen it does photoheterotrophy or uses reduced sulfur. [L20 p21, L20 p24]

Green sulfur bacteria

> **Chlorobi** (green sulfur):
>
> > Chlorosomes with BChl c/d/e, reaction center PS I.
> >
> > Live **only** without oxygen (-O2 **only**): photolithoautotrophy on reduced sulfur. [L20 p24]

Example names

> **Chlorobium** is the green sulfur example; **Chloroflexus** is the green nonsulfur example. [L20 p24]

c. Membrane invaginations in purple bacteria

Book: Not in the assigned textbook sections; see the posted source "Bacterial Intracellular Structures".

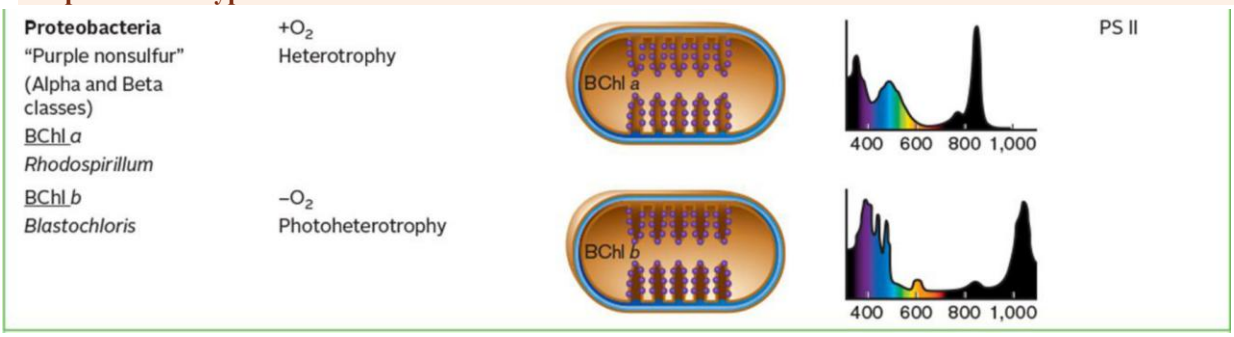
⚠ **Do not mix up: In purple bacteria the cell membrane *(thin skin around the cell)* folds inward; the folds stay CONTIGUOUS *(still joined)* with it.** [L20 p26, L20 p27]

Membrane folds inward

> **Purple sulfur and nonsulfur bacteria**: the cell membrane folds inward (invaginations). [L20 p26, L20 p27]
>
> **Contiguous with the cell membrane**, but the site of photosynthetic reactions. [L20 p26, L20 p27]

Invaginated membrane becomes the site of photosynthetic light capture reactions. [L20 p26, L20 p27]

Purple bacteria types



Purple nonsulfur Proteobacteria: BChl a or b, heterotrophy with oxygen, photoheterotrophy without [L20 p26]

On the figure: BChl a · heterotrophy · photoheterotrophy · PS II

Purple bacteria use **BChl a** or b, reaction center **PS II**. [L20 p26]

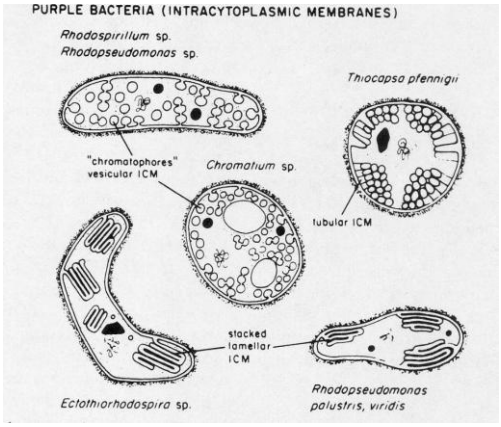
Purple nonsulfur bacteria (Alpha and Beta classes): **heterotrophy** with oxygen, **photoheterotrophy** *(light for energy, ready-made organic food for carbon)* without oxygen. [L20 p26]

Purple sulfur bacteria (Gamma class): the example is **Chromatium**. [L20 p26]

Membrane shapes

Purple bacteria membrane types: **vesicular chromatophores**, **tubular** (Thiocapsa pfennigii) and **stacked lamellar** *(in flat sheets)* membranes. [L20 p27]

Ectothiorhodospira: a curved cell packed with stacked membranes. [L20 p27]



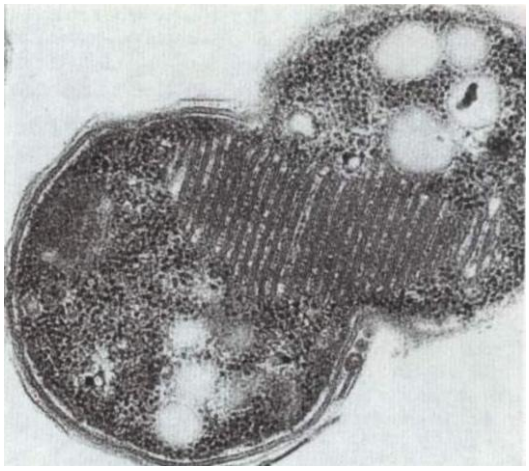
Purple bacteria membranes: vesicular chromatophores, tubular and stacked lamellar [L20 p27]

On the figure: vesicular · tubular · stacked lamellar · Ectothiorhodospira · chromatophores

d. Membrane compartments in methanotrophs

Book: Not in the assigned textbook sections; see the posted source "Bacterial Intracellular Structures".

⚠ **Do not mix up: Methanotroph *(bacterium that uses methane)* internal membranes *(stacked lipid layers)* break down methane; purple bacteria membranes capture light.** [L20 p28, L20 p27]



Methylococcus: a stack of parallel internal membranes across the cell [L20 p28]

Methanotrophs *(bacteria that use methane)*: have membrane compartments. [L20 p28]

Role: breakdown of methane and other one carbon molecules. [L20 p28]

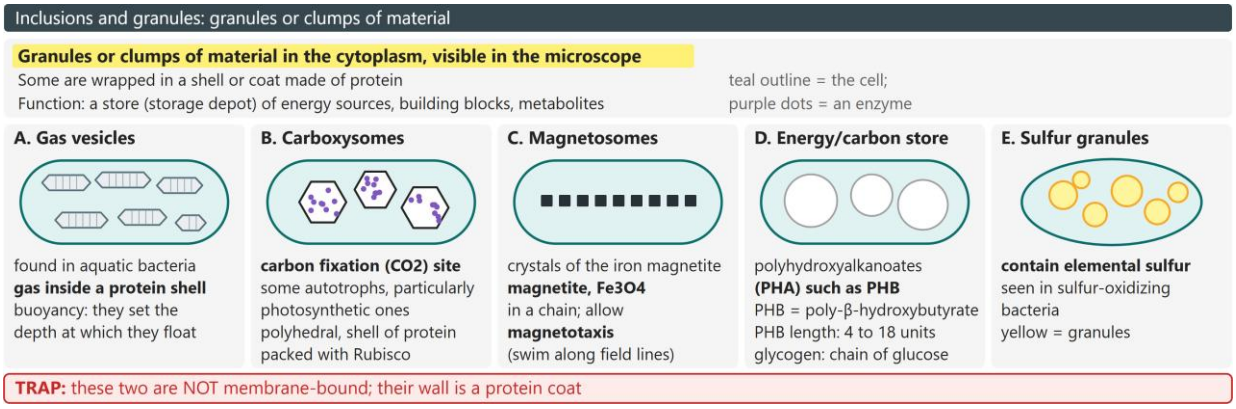
Methylococcus: a large stack of parallel internal membranes across the cell. [L20 p28]

6. Inclusions and granules (gas vesicles, carboxysomes, energy and carbon storage polymers, sulfur granules)

Book: 3.6 Specialized Structures, Thylakoids, Carboxysomes, and Storage Granules (chapter 3 PDF, p. 119).

⚠ **Do not mix up: Inclusions *(clumps of protein and other material)* are granules or clumps in the cytoplasm *(jelly inside the cell membrane)*; compartments are surrounded by a membrane *(thin lipid skin)*.** [L20 p29, L20 p19]

What inclusions are



Inclusions and granules: gas vesicles, carboxysomes, magnetosomes, storage and sulfur granules [L20 p30]

On the figure: Inclusions and granules · gas vesicles · carboxysomes · protein

Inclusions and granules: granules or clumps of material in the cytoplasm. [L20 p29]

Visible in the microscope. [L20 p29]

Some have a shell or coat of **protein** or a single monolayer *(one layer)* of lipids. [L20 p29]

Function: a store of energy sources, building blocks and metabolites *(small working molecules of the cell)*. [L20 p29]

Not membrane compartments

Gas vesicles *(gas filled floating bubbles)* **and carboxysomes** *(protein-shell bodies that fix carbon dioxide)* are classed as inclusions. [L20 p30, L20 p31]

Both have a protein shell, not a membrane. [L20 p19, L20 p30, L20 p31]

Watch out: both enclose a space, but their wall is a protein shell, not a membrane. [L20 p29, L20 p31]

Inclusions and granules

Inclusion	What it is	Role or where found
Gas vesicles	Gas stored inside a protein shell	Aquatic bacteria; buoyancy to control the depth they float at
Carboxysomes	Polyhedral structures with a protein shell	Site of carbon fixing (CO2) in some autotrophs, especially photosynthetic ones
Magnetosomes	Crystal of iron magnetite, Fe3O4	Magnetotaxis
Energy and carbon storage granules	PHA such as poly-beta-hydroxybutyrate; glycogen, a chain of glucose	Store energy and carbon
Sulfur granules	Elemental sulfur	Sulfur oxidizing bacteria

Table source: [L20 p30, L20 p31, L20 p32, L20 p33, L20 p34]

A. Gas vesicles

Book: 3.6 Specialized Structures, Thylakoids, Carboxysomes, and Storage Granules (chapter 3 PDF, p. 119).

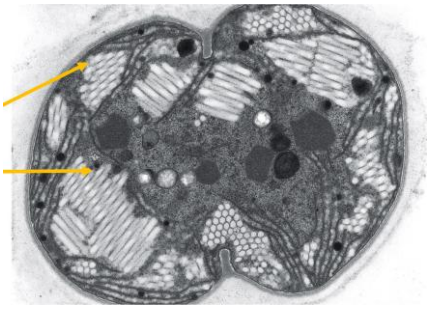
⚠ **Do not mix up: The gas vesicle wall is a protein shell, NOT a lipid membrane *(thin fat layer)*.** [L20 p30]

Gas vesicles *(gas filled floating bubbles)*:

> Store gases within a protein shell.
>
> Are packed in bundles inside the cell. [L20 p30]

Found in aquatic bacteria: they confer buoyancy *(ability to float)*. [L20 p30]

Buoyancy lets the cell control the depth at which it floats. [L20 p30]



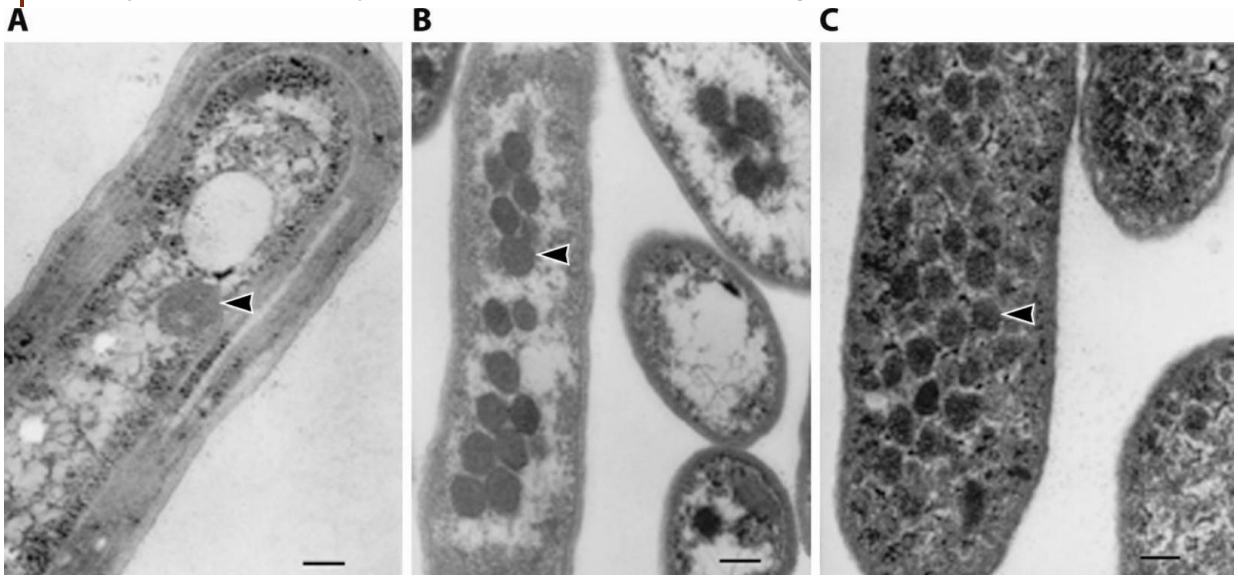
Gas vesicle bundles in a cell; arrows point to them [L20 p30]

B. Carboxysomes

Book: 3.6 Specialized Structures, Thylakoids, Carboxysomes, and Storage Granules (chapter 3 PDF, p. 119).

⚠ **Do not mix up: Carboxysomes *(protein-shell bodies that fix carbon dioxide)* are the site of carbon fixing (CO_2); gas vesicles *(gas filled floating bubbles)* store gases. Both have protein shells.** [L20 p31, L20 p30]

Carboxysomes *(bodies that fix carbon dioxide)*: site of carbon fixing (CO_2). [L20 p31]



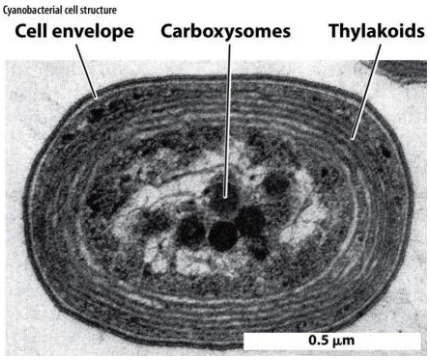
Carboxysomes: dark polyhedral bodies at the arrowheads [L20 p31]

Polyhedral structures with a protein shell. [L20 p31]

Associated with autotrophs *(organisms that make food from CO_2)*. [L20 p31]

Found in some autotrophic bacteria (use CO_2), particularly photosynthetic ones. [L20 p31]

Prochlorococcus has **carboxysomes** in the cell center, inside the **thylakoid** rings. [L20 p23]



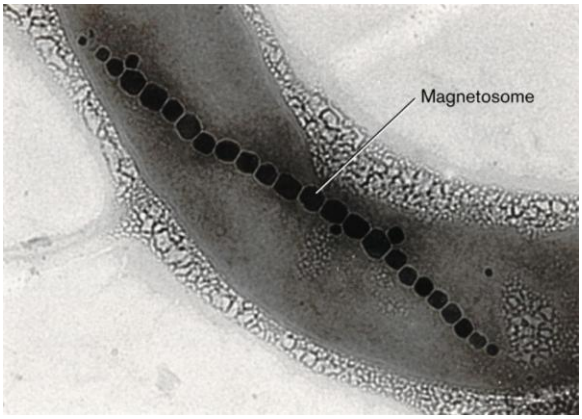
Cyanobacterial cell: carboxysomes in the center inside the thylakoid rings [L20 p23]

On the figure: Carboxysomes · thylakoid

C. Magnetosomes

Book: Not in the assigned textbook sections; see the posted source "Bacterial Intracellular Structures".

⚠ **Do not mix up: Magnetosomes give magnetotaxis *(moving along magnetic field lines)*; gas vesicles *(gas filled floating bubbles)* give buoyancy *(ability to float)*.** [L20 p32, L20 p30]



A chain of dark magnetite crystals along the cell [L20 p32]
On the figure: Magnetosomes

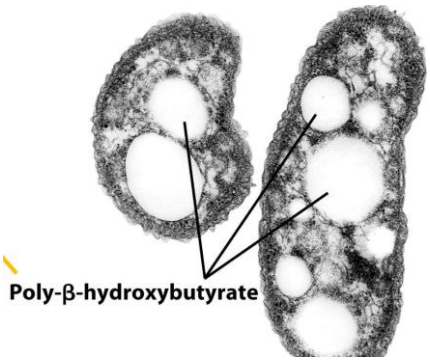
Magnetosomes:
A crystal of iron magnetite *(magnetic iron mineral)*, Fe3O4.
Form a chain of dark magnetite crystals along the cell. [L20 p32]
Magnetite, Fe3O4, is an **iron oxide** *(iron bound to oxygen)*. [L20 p32]
Allow magnetotaxis *(moving along magnetic field lines)*. [L20 p32]

D. Energy and carbon storage granules (PHA, glycogen)

Book: 3.6 Specialized Structures, Thylakoids, Carboxysomes, and Storage Granules (chapter 3 PDF, p. 119).

⚠ **Do not mix up: Glycogen *(stored chain of glucose)* is a chain of glucose; PHA such as poly-beta-hydroxybutyrate is a different polymer.** [L20 p33]

Energy and carbon storage granules: two kinds, PHA and glycogen. [L20 p33]
Polyhydroxyalkanoates (PHA), such as **poly-beta-hydroxybutyrate**. [L20 p33]
Poly-beta-hydroxybutyrate chain length: **4** to **18** units. [L20 p33]
Poly-beta-hydroxybutyrate granules are large and light, filling the cells. [L20 p33]
Glycogen: a chain of glucose. [L20 p33]

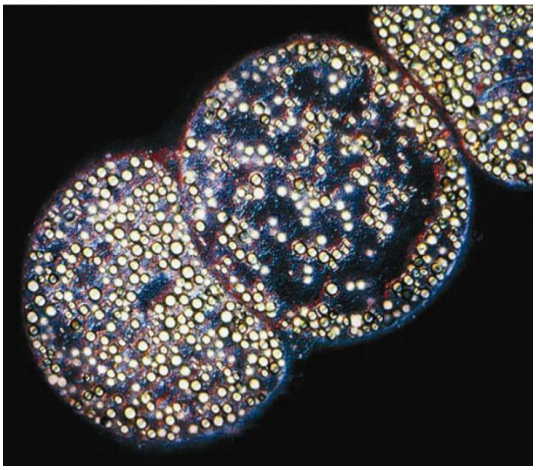


Poly-beta-hydroxybutyrate granules filling the cells [L20 p33]
On the figure: poly-beta-hydroxybutyrate

E. Sulfur granules

Book: 3.6 Specialized Structures, Thylakoids, Carboxysomes, and Storage Granules (chapter 3 PDF, p. 119).

⚠ **Do not mix up: Sulfur granules hold elemental sulfur; magnetosomes hold iron magnetite *(magnetic iron mineral)* crystals.** [L20 p34, L20 p32]



Bright sulfur granules packing large round cells [L20 p34]

> **Sulfur granules**: contain elemental sulfur. [L20 p34]
> **Seen in** sulfur oxidizing bacteria. [L20 p34]
> **Sulfur granules** are bright and pack large round cells. [L20 p34]

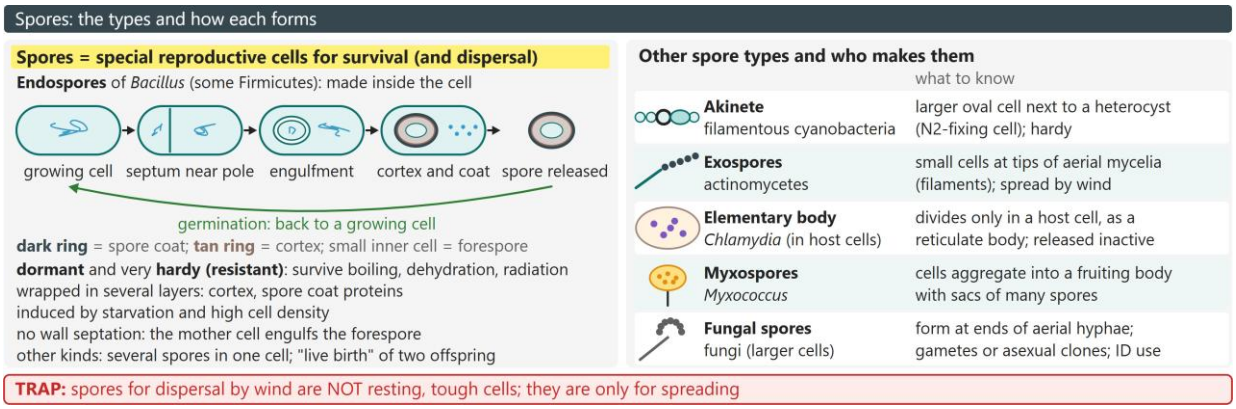
7. Endospore: what they are

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

⚠ **Do not mix up: Endospores *(tough dormant survival form)* form INSIDE the cell; exospores form at the tips of aerial mycelia *(mats of filaments)*.** [L20 p35, L20 p45]

Spores and their types

Spores are special reproductive cells for survival or dispersal. [L20 p35]



Spore types and who makes them, and endospore formation inside the cell [L20 p35]

On the figure: akinete · elementary body · Endospores · myxospore · forespore · mother cell

> **Spore types in bacteria**: akinete *(hardy resting cell)*, arthrospore (exospore), elementary body, endospore, myxospore. [L20 p35]

Endospores

> **Endospores**:
>> A few bacteria form spores inside the cell.
>> Made by Firmicutes *(Gram positive group that includes Bacillus)*. [L20 p35]
>
> **Endospore triggers**: carbon, nitrogen or phosphate *(a mineral nutrient)* starvation, low pH, peptide *(short chain of amino acids)* antibiotics. [L20 p35]
>
> **Endospore formation**: mother cell and forespore *(the part that becomes the spore)*; coat reinforced by keratin and calcium dipicolinate. [L20 p35]
>
> **Endospore properties**: highly heat and desiccation *(drying out)* resistant; viable for centuries, possibly thousands of years. [L20 p35]

Endospores of Bacillus: features and the stages of formation

Book: Not in the assigned sections (endospore stages are in 4.6, not assigned).

⚠ **Do not mix up: Cortex *(thick spore wall layer)* peptidoglycan *(sugar and peptide mesh of the wall)* comes in Stage V; dipicolinic acid *(heat-protecting spore chemical)* and calcium come in Stage VI.** [L20 p36]

Features

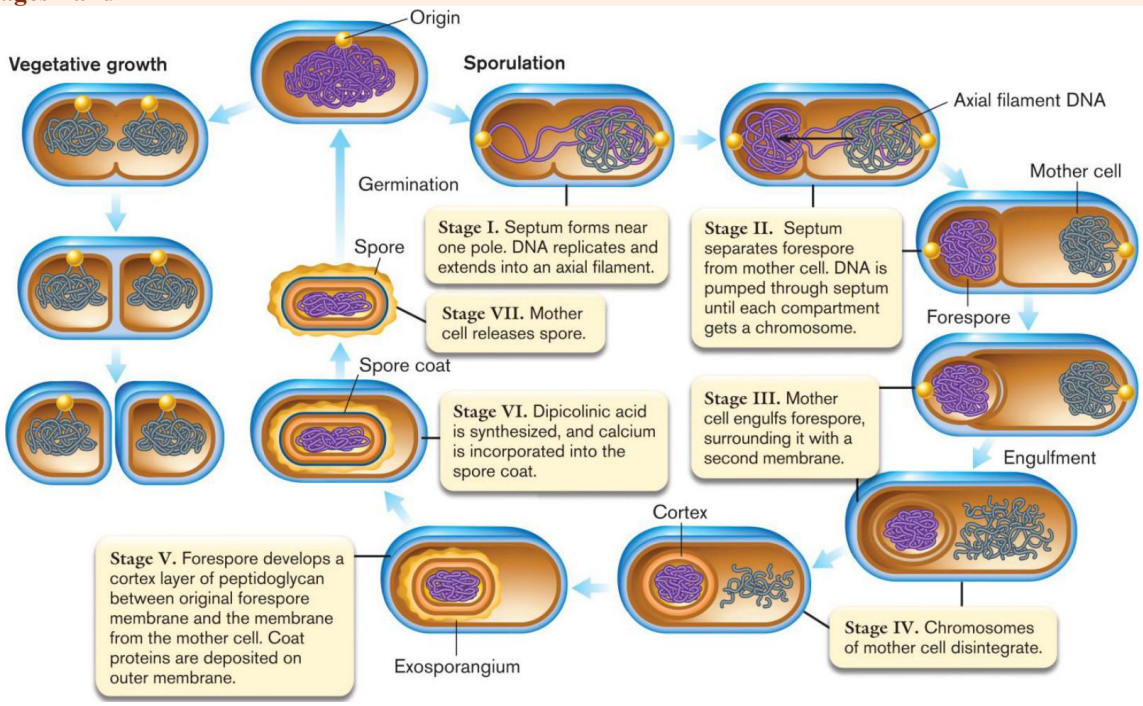
Endospores *(tough dormant survival form)* **of Bacillus** are dormant *(inactive, not growing)* and very hardy. [L20 p36]

They survive boiling, dehydration and radiation. [L20 p36]

Wrapped in several coats. [L20 p36]

Induced by starvation and high cell density. [L20 p36]

Stages I and II



Seven stages of endospore formation with mother cell, forespore, cortex and exosporangium [L20 p36]

On the figure: mother cell · forespore · axial filament · Cortex · Exosporangium · Stage VI

Stage I: septum *(cross wall)* forms near one pole; DNA *(cell genetic instruction molecule)* replicates, extends into an **axial filament** *(stretched-out DNA strand)*. [L20 p36]

Stage II: septum separates **forespore** *(the part that becomes the spore)* from **mother cell**; DNA is pumped through the septum. [L20 p36]

Stage II, also: pumping continues until each compartment gets a chromosome *(one long DNA molecule)*. [L20 p36]

Stages III to V

Stage III: mother cell engulfs forespore; a second membrane (from the mother cell) surrounds it. [L20 p36]

Stage IV: chromosomes of the mother cell disintegrate. [L20 p36]

Stage V: forespore develops a **cortex** of peptidoglycan between its two membranes. [L20 p36]

Stage V, also: coat proteins are deposited on the outer membrane (from the mother cell). [L20 p36]

Exosporangium: named at Stage V, when coat proteins are deposited on the outer membrane. [L20 p36]

Stages VI and VII

Stage VI: dipicolinic acid is made; calcium is incorporated into the spore coat. [L20 p36]

Stage VII: mother cell releases the spore; it can germinate back into a vegetative cell *(normal growing cell)*. [L20 p36]

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).

Cell division:

The FtsZ *(protein ring for cell splitting)* ring.

Where new peptidoglycan *(sugar and peptide mesh of the wall)* is made. [L18 p28, L18 p38]

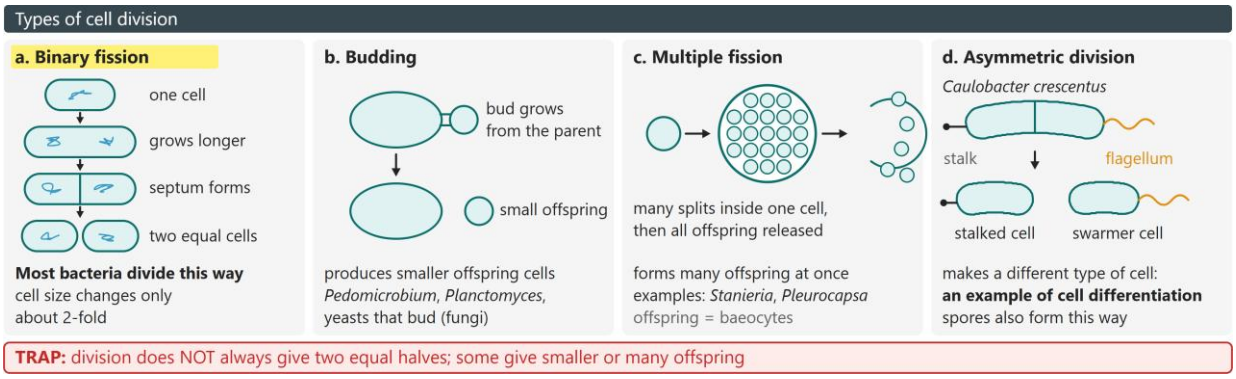
FtsZ forms a ring at the cleavage site during cytokinesis *(splitting of the cell)*.

Cell division topics: types, steps, mechanism, cell polarity *(which end is which)* after division, and division proteins. [L20 p37, L20 p38]

1. Features and Types of Cell division in bacteria

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

⚠ **Do not mix up: Budding gives SMALLER offspring; binary fission gives two equal cells.** [L20 p39, L20 p40]



Binary fission, budding, multiple fission and asymmetric division [L20 p39]

On the figure: binary fission · Budding · multiple fission · Caulobacter crescentus

Four types: binary fission, budding, multiple fission, asymmetric cell division. [L20 p39, L20 p42]

Asymmetric division gives examples of cell differentiation *(becoming a different cell type)*: stalked cells of Caulobacter crescentus, and spores. [L20 p39, L20 p42]

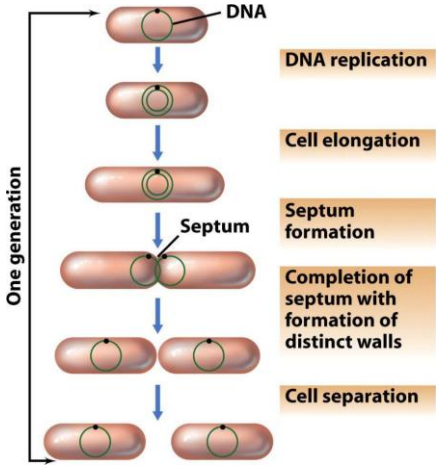
Types of cell division

Type	Offspring	Examples
Binary fission	Two cells; usually **only** a small change in cell size (**2** fold)	Most bacteria
Budding	Smaller offspring cells	Pedomicrobium, Planctomyces, budding yeast (fungi)
Multiple fission	Multiple offspring by multiple fission events, usually in a sac	Stanieria, Pleurocapsa
Asymmetric cell division	A different type of cell	Caulobacter stalked and swarmer cells; spores

Table source: [L20 p39, L20 p40, L20 p41, L20 p42]

a. Binary fission

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



One generation of binary fission: replication, elongation, septum, walls, separation [L20 p39]

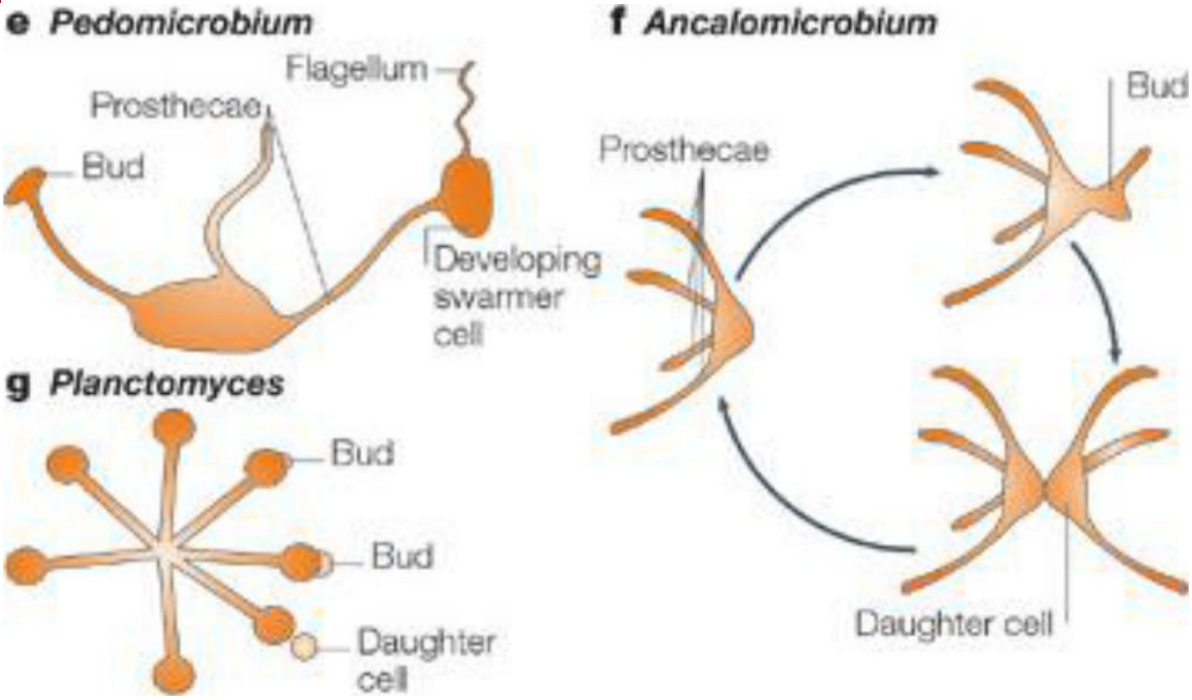
On the figure: DNA replication · cell elongation · septum formation · distinct walls

> **Most bacteria divide by binary fission**: usually **only** a small change in cell size (**2** fold). [L20 p39]
>
> **One generation**: DNA *(cell genetic instruction molecule)* replication, **cell elongation**, septum *(cross wall)* formation. [L20 p39]
>
> **Then** the septum is completed with **distinct walls**, then the cells separate. [L20 p39]

b. Budding

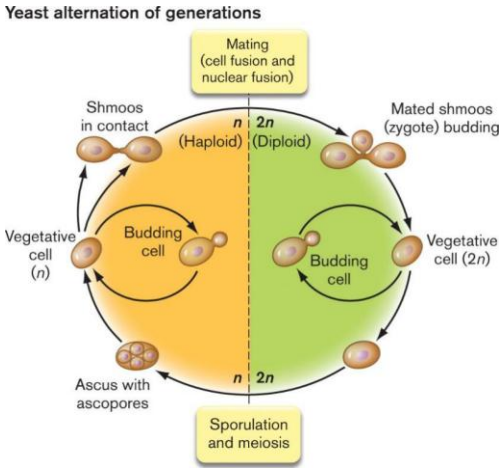
Book: Not in the assigned sections.

> **Budding**:
>
> > Produces smaller offspring cells.
> >
> > Produces a **bud** that becomes a smaller offspring (daughter) cell. [L20 p40]
>
> **Examples**: Pedomicrobium, Planctomyces, budding yeast (fungi). [L20 p40]



Pedomicrobium, Ancalomicrobium and Planctomyces budding [L20 p40]

On the figure: Pedomicrobium · prosthecae · bud · flagellum · developing swarmer cell · Ancalomicrobium · daughter cell



Budding yeast cycle: shmoos, mating, sporulation and meiosis, ascus with ascospores [L20 p40]

Pedomicrobium parts: **prosthecae** *(thin cell extensions)*, **bud**, **flagellum** *(whip tail that spins)*, developing swarmer *(swimming daughter cell)* cell; **Ancalomicrobium**: prosthecae, bud, **daughter cell**. [L20 p40]

Budding yeast cycle	What happens
Haploid cells in contact before mating	shmoos
Mating	Cell fusion and nuclear fusion give a diploid zygote (mated shmoos) that buds
Sporulation and meiosis	Make an ascus with ascospores

Table source: [L20 p40]

c. Multiple fission

Book: 3.6 Specialized Structures, How Do a Hundred Cells Divide at Once? (chapter 3 PDF, p. 139).

⚠ **Do not mix up: Multiple fission gives MULTIPLE offspring, usually in a SAC; budding gives smaller offspring.** [L20 p41, L20 p40]

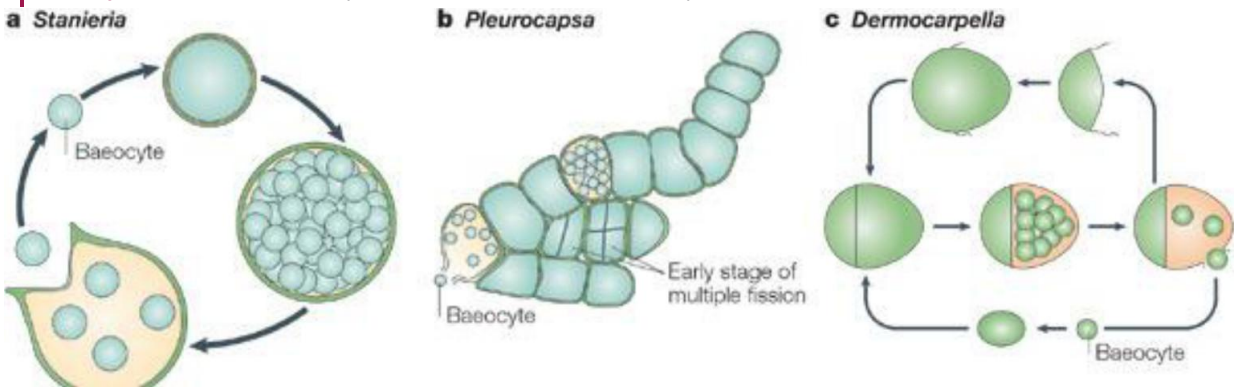
What multiple fission is

Multiple fission: some form multiple offspring by multiple fission events. [L20 p41]

Usually in a sac *(a closed pouch)*. [L20 p41]

Examples

Examples: Stanieria (also spelled Stanleria) and Pleurocapsa. [L20 p41]

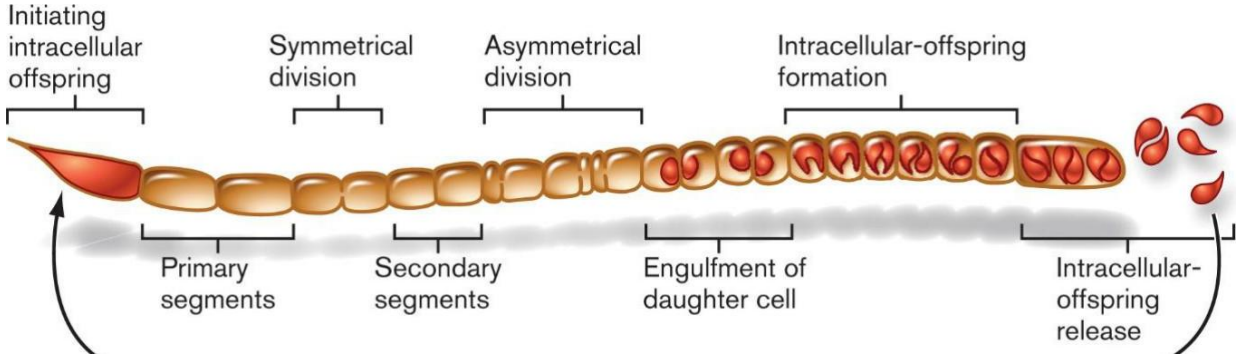


Baeocytes released in Stanieria; early multiple fission in Pleurocapsa [L20 p41]

On the figure: Stanieria · Pleurocapsa · baeocytes · Multiple fission

Stanieria: the cell enlarges, fills with many **baeocytes** *(small offspring cells)*, then releases them. [L20 p41]

Savagella: a filament that forms offspring inside its cells. [L20 p41]



Savagella filament: primary and secondary segments, engulfment of daughter cell, offspring release [L20 p41]

On the figure: primary segments · secondary segments · engulfment of daughter cell

> **Savagella parts**: primary segments, secondary segments, engulfment of daughter cell and intracellular offspring **release**. [L20 p41]

d. Asymmetric cell division and differentiation

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

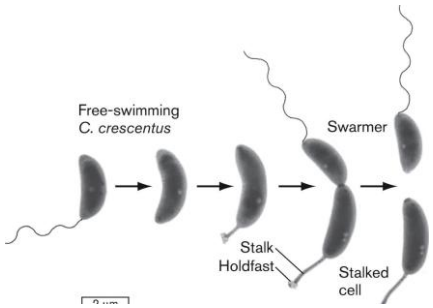
⚠ Do not mix up: Caulobacter: the STALKED cell divides into a swarmer *(swimming daughter cell)* cell and a stalked cell. [L20 p42]

Asymmetrical cell division: produces a different type of cell. [L20 p42]

Examples of cell differentiation *(becoming a different cell type)*: stalked cells of Caulobacter crescentus, and spores. [L20 p42]

Caulobacter: a free-swimming cell loses its flagellum *(whip tail that spins)* and grows a stalk *(stem that anchors the cell)* with a holdfast *(sticky foot at the stalk tip)*. [L20 p42]

The stalked cell divides into a flagellated swarmer cell and a stalked cell. [L20 p42]



Caulobacter crescentus: free-swimming cell, stalk, holdfast, swarmer and stalked cell [L20 p42]

On the figure: free-swimming · stalk · holdfast · swarmer · stalked cells

▸ Spore types: akinetes, exospores, Chlamydia elementary bodies, endospores, myxospores, fungal spores

Book: Not in the assigned sections (akinetes and myxospores are in 4.6, not assigned).

⚠ Do not mix up: Exospores are NOT dormant *(inactive, not growing)* or hardy (wind dispersal); endospores *(tough dormant survival form)* are dormant and very hardy. [L20 p45, L20 p36]

Spores in general

> **Spores**: several types of bacteria form spores, special reproductive cells for survival or dispersal. [L20 p43]
>
> **Some spores** are dormant. [L20 p43]

Akinetes

> **Akinete** *(hardy resting cell)*: a larger, oval cell near a heterocyst *(nitrogen-fixing cell)* in a chain of vegetative cells *(normal growing cell)*. [L20 p44]
>
> **Akinetes form** in response to stress; akinetes are hardy. [L20 p44]
>
> **Akinete triggers**: light limitation, cold, phosphate *(a mineral nutrient)* starvation; viable for decades. [L20 p35, L20 p43]
>
> **Heterocyst cells** fix nitrogen. [L20 p44]
>
> **Anabaena solitaria**: a filament with a heterocyst and an akinete. [L20 p44]

Exospores

> **Exospores**: smaller spore cells at the end of aerial mycelia *(filaments)*, like fungal spores. [L20 p45]
>
> **Exospore triggers**: carbon and phosphate starvation. [L20 p45]
>
> **Tip cells** of an aerial mycelium undergo vegetative division and pinch off as arthrospores. [L20 p45]

Exospores are NOT dormant or hardy: they are just for dispersal by wind. [L20 p45]

Watch out: arthrospores are listed as desiccation *(drying out)* and heat resistant. [L20 p35]

Yet exospores are also called not dormant or hardy, just for dispersal by wind. [L20 p45]

Chlamydia elementary bodies

Chlamydia: obligate intracellular parasite; its life cycle is similar to viruses. [L20 p46]

Elementary bodies *(infectious resting form outside the host cell)* are inactive spores with cross-linked outer membrane *(extra membrane outside the wall)* proteins. [L20 p46]

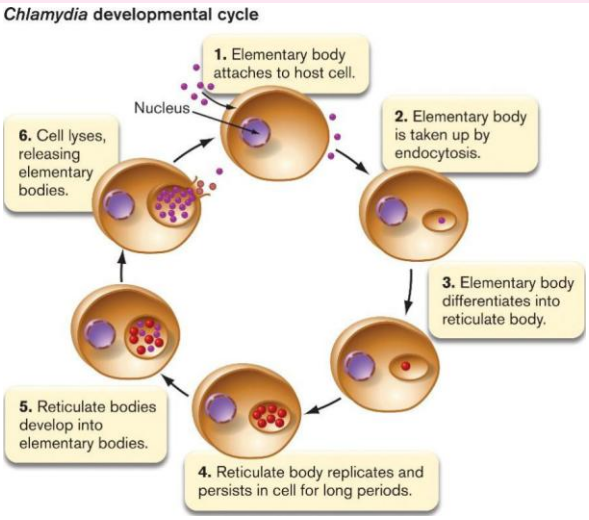
Elementary body formation starts when the intracellular life cycle is complete. [L20 p46]

Chlamydia divides only inside a host cell, as reticulate bodies *(dividing form inside the host)*. [L20 p46]

Chlamydia cycle: elementary body attaches, enters by **endocytosis**, becomes a **reticulate body**. [L20 p46]

Cycle, continued: the reticulate body replicates and persists in the cell for long periods. [L20 p46]

Then reticulate bodies develop into elementary bodies; the cell **lyses** and releases them. [L20 p46]



Chlamydia cycle: elementary body attaches, endocytosis, reticulate body replicates, cell lyses [L20 p46]

On the figure: elementary body · reticulate body · endocytosis · lyses

Endospores

Endospores: some Firmicutes *(Gram positive group that includes Bacillus)* form them. [L20 p47]

Endospore division: the cell divides, but with no septation *(building the cross wall)* of the cell wall *(stiff layer around the cell membrane)*. [L20 p47]

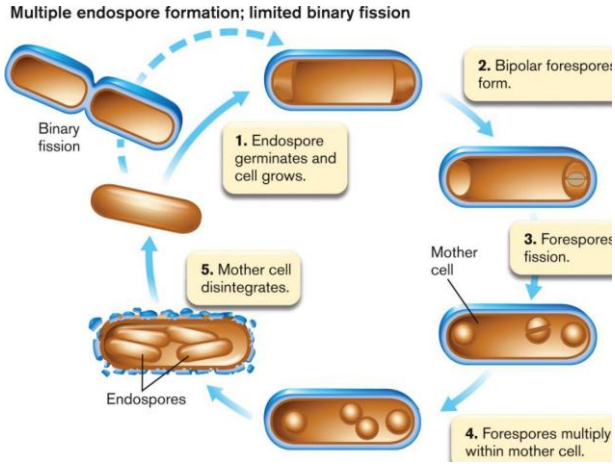
Watch out: in the endospore stages a septum *(cross wall)* near one pole separates forespore *(the part that becomes the spore)* and **mother cell**. [L20 p47]

One cell is engulfed and then develops into a hardy spore. [L20 p47]

Multiple endospores

Multiple endospores:

> One type forms multiple **endospores** inside the cell.
>
> **Bipolar forespores** form, fission, then **multiply** within the mother cell, which **disintegrates**. [L20 p48]



Multiple endospore formation: bipolar forespores form, fission, multiply, mother cell disintegrates [L20 p48]

On the figure: endospores · bipolar · forespores · mother cell · disintegrates

Live birth

Live birth:

> One type does live births; two offspring emerge from the mother cell.
>
> **bipolar** *(at both ends)* division gives two offspring. [L20 p48]

Offspring grow through the length of the mother cell; no binary fission. [L20 p48]

Myxospores

Myxococcus: cells aggregate and form a fruiting body *(raised clump of cells that holds spores)* with sacs of many myxospores. [L20 p49]

Myxospore cycle: free-living cells, starvation, aggregation *(clumping together)*, fruiting body, myxospore dispersal. [L20 p49]

Myxospore triggers: nutrient starvation, heat shock, glycerol *(three carbon fat backbone)* and dimethyl **sulfoxide**. [L20 p35]

Fungal spores

Fungi: most grow as filaments (hyphae). [L20 p50, L20 p51]

Fungal spores form at the end of aerial hyphae *(fungal filaments)*. [L20 p50, L20 p51]

Fungal spores can be gametes or asexual clones. [L20 p50, L20 p51]

Fungal cells are larger than bacteria. [L20 p50, L20 p51]

Spore-forming structures vary in shape and features; they are used for identification. [L20 p50, L20 p51]

Examples: sporangiospores in a sporangium; conidia such as arthrospores, chlamydospores, phialospores. [L20 p50, L20 p51]

Spore types

Spore type	Made by	How it forms	Dormant and hardy?
Akinete	Filamentous cyanobacteria (Anabaena)	Larger oval cell near a heterocyst, in response to stress	Hardy
Exospore (arthrospore)	Actinomycetes	Smaller spore cells at the end of aerial mycelia, like fungal spores	**NOT** dormant or hardy; for dispersal by wind (also listed as desiccation and heat resistant)
Elementary body	Chlamydia	Reticulate bodies divide in a host cell, then become elementary bodies	Inactive spores; survive outside the host; desiccation resistant
Endospore	Some Firmicutes (Bacillus)	Cell division with no wall septation; one cell is engulfed and becomes the spore	Dormant and very hardy
Myxospore	Myxococcus	Cells aggregate into a fruiting body with sacs of many myxospores	Desiccation and UV resistant
Fungal spores	Fungi	At the end of aerial hyphae; gametes or asexual clones	Not stated

Table source: [L20 p43, L20 p44, L20 p45, L20 p46, L20 p47, L20 p49, L20 p50]

Actinomycete life cycle	What happens
Favorable conditions	One or two germ tubes emerge from a spore and make the substrate mycelium
Nutrient depletion	After **48** to **72** hours, bld genes are expressed and make aerial hyphae (aerial mycelia)
Growth stops	The compartment segments of the aerial hyphae (this requires the whi genes) change shape and thicken the wall to become desiccation resistant spores
Glucose drops	Nonbranching exploratory cells develop to find new nutrients and form mycelia

Table source: [L20 p45]

Fungal structure	Name
Root-like structures at the base of the reproductive hyphae	rhizoids
What a germinating fungal spore sends out; it becomes a hypha	germ tube
Inside the sporangium, which sits on the sporangiophore	columella
Between the conidiophore and the phialospores	sterigma
Other conidia besides arthrospores, chlamydospores, blastospores, macroconidia and microconidia	porospore

Table source: [L20 p50, L20 p51]

2. Basic Steps in process: Replication, cytokinesis, septation, separation

Book: 3.4 Bacterial Cell Division, DNA Replication Regulates Cell Division (chapter 3 PDF, p. 85); 3.4 Bacterial Cell Division, Cell Division by Septation (chapter 3 PDF, p. 78).

⚠ **Do not mix up: Cytokinesis *(splitting the cell in two)*: splitting into two cells, each with its own cell membrane *(thin skin around the cell)*. Septation *(building the cross wall)*: envelope *(layers wrapped around the cell)* partition. Separation: detachment.** [L20 p53]

The four steps

Four steps: replication and segregation of chromosomes *(one long DNA molecule)*, cytokinesis, septation, separation of the cell. [L20 p52]

The replicating chromosome has an **origin of replication** and a **terminator sequence** *(the DNA site where copying ends)*. [L20 p52]

Watch out: in Staphylococcus the septum *(cross wall)* furrow *(groove)* grows in first; cytokinesis happens when furrows meet. [L20 p58]

Replication and segregation

Replication: the origin replicates and migrates; **replication continues bidirectionally**. [L20 p52]

After the DNA *(cell genetic instruction molecule)* **origin replicates and migrates**, two **replisomes** *(DNA copying machines)* are present. [L20 p52]

Segregation *(copies pulled apart)*: the two copies of the DNA origin move apart as the cell expands. [L20 p52]

Replication of the DNA termination site triggers proteins to form the divisome (textbook).

The divisome manages assembly of the septum (textbook).

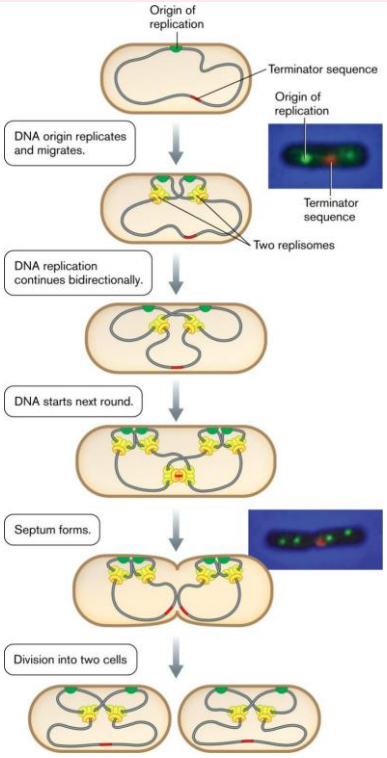
Division follows

Then DNA starts the next round, the **septum forms**, and the cell divides into two. [L20 p52]

FtsN, a divisome part, helps time septum constriction (textbook).

Cells lacking FtsN balloon out at the septum (textbook).

Staphylococcus: septum grows in, two partitions complete, then the cells peel apart. [L20 p53]



Origin replicates and migrates, two replisomes, septum forms, division into two cells [L20 p52]

On the figure: replisomes · DNA origin replicates and migrates · replication continues bidirectionally · septum forms

Steps in cell division

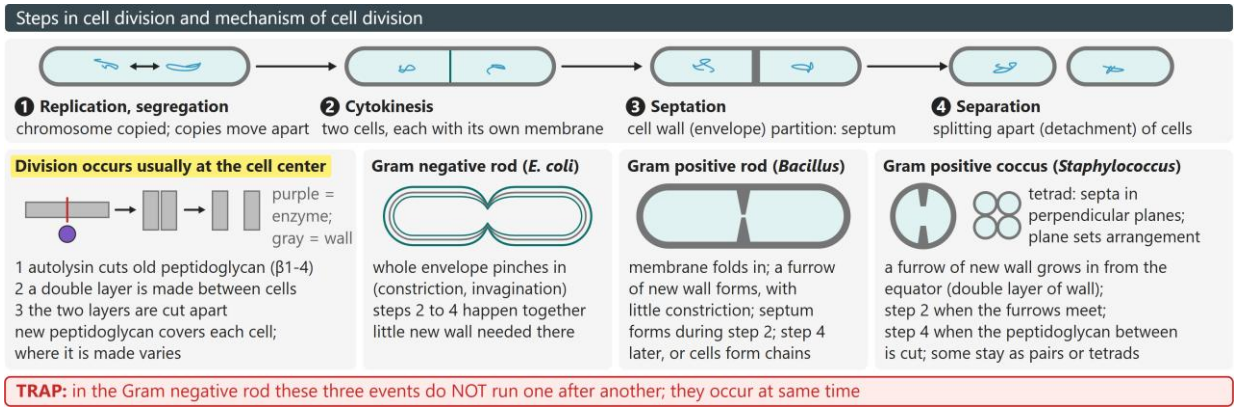
Step	Meaning
Cytokinesis	Splitting of the cell to form two cells, each surrounded by its own cell membrane
Septation	Formation of an envelope partition (septum) between dividing cells
Separation	Detachment of the two cells

Table source: [L20 p53]

3. Mechanism of cell division: how it differs between cell types

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

⚠ Do not mix up: E. coli: all three steps at the same time; Bacillus: separation may come later. [L20 p56, L20 p57]



Division in E. coli, Bacillus and Staphylococcus compared [L20 p56]

On the figure: Gram negative rod · Gram positive rod · E. coli · Bacillus · Staphylococcus

Mechanism varies: cytokinesis *(splitting the cell in two)*, septation *(building the cross wall)* and separation differ between bacteria. [L20 p54]

Location: division usually occurs in the center of most cells. [L20 p54]

Division mechanism in three cell types

	Gram negative rod (E. coli)	Gram positive rod (Bacillus)	Gram positive cocci (Staphylococcus)
What you see	Constriction and inward fold of the entire envelope in the middle	Membrane invaginates in the middle; a furrow of new wall forms, little constriction	A furrow of new wall forms at the equator and grows inward
Timing	Cytokinesis, septation and separation at the same time	Septum forms with cytokinesis; separation may occur later	Cytokinesis when the septa furrows meet; separation when the peptidoglycan between cells is cut
New wall at the division site	Little needed	A furrow of new cell wall	A double layer of cell wall
Afterward	The two cells are already separate (separation happens with cytokinesis)	Often stay attached as a chain or filament	Some stay as pairs or tetrads

Table source: [L20 p56, L20 p57, L20 p58, L20 p59]

Autolysins and new peptidoglycan at the division site

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

⚠ **Do not mix up: Autolysin *(wall-cutting enzyme made by the bacterium itself)* CUTS old peptidoglycan (beta 1-4 linkage); it does not build new wall.** [L20 p54]

Three steps at the septum

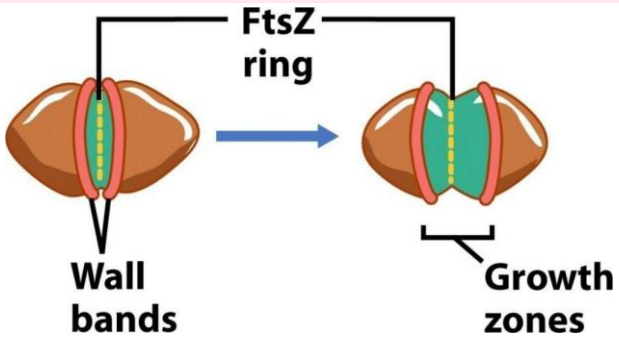
Basic steps, step **1**: autolysin enzyme cuts old peptidoglycan (beta **1-4** linkage). [L20 p54]

Step 2: synthesize a double layer between the cells. [L20 p54]

Step 3: then cut the two layers apart. [L20 p54]

Dividing coccus

Dividing coccus: FtsZ *(protein ring for cell splitting)* ring at the equator, **wall bands**, new wall from **growth zones**. [L20 p54]

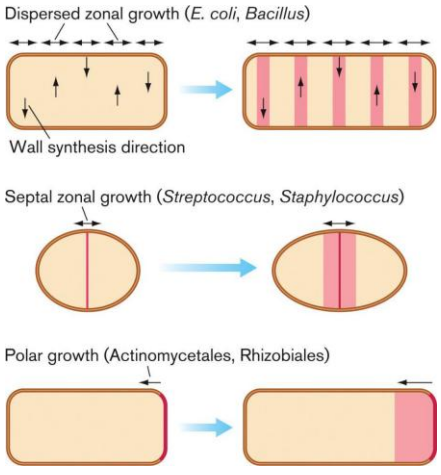


Dividing coccus: FtsZ ring, wall bands and growth zones [L20 p54]

On the figure: FtsZ ring · wall bands · growth zones

New peptidoglycan

New peptidoglycan must be made so each cell is covered when separation occurs. [L20 p55]



Dispersed zonal, septal zonal and polar wall growth patterns [L20 p55]

Location of new peptidoglycan synthesis varies. [L20 p55]

Where new wall is made

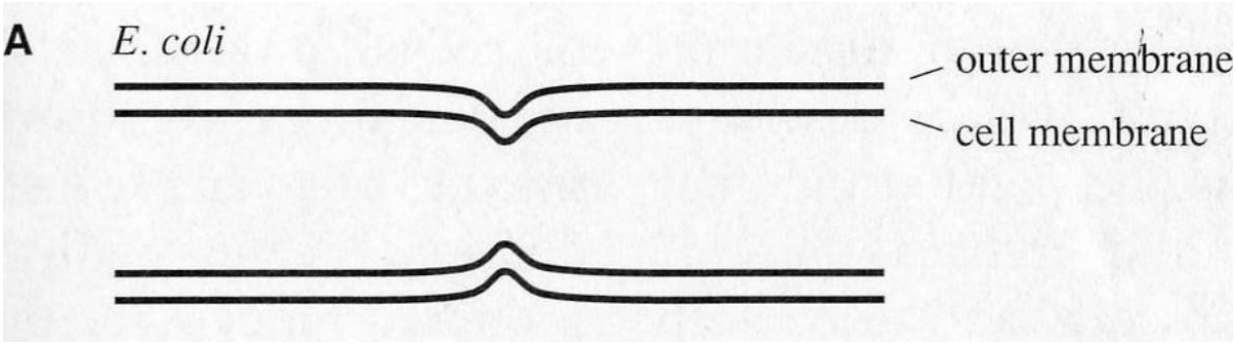
Growth pattern	Where new wall is made	Examples
Dispersed zonal growth	Many bands along the rod	E. coli, Bacillus
Septal zonal growth	At the septum	Streptococcus, Staphylococcus
Polar growth	At one pole	Actinomycetales (actinomycetes), Rhizobiales

Table source: [L20 p55]

a. Gram negative rod (E. coli)

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

⚠ **Do not mix up: E. coli pinches the whole envelope *(layers wrapped around the cell)* inward; little new wall is made at the division site.** [L20 p56]



E. coli: outer membrane and cell membrane fold inward together [L20 p56]

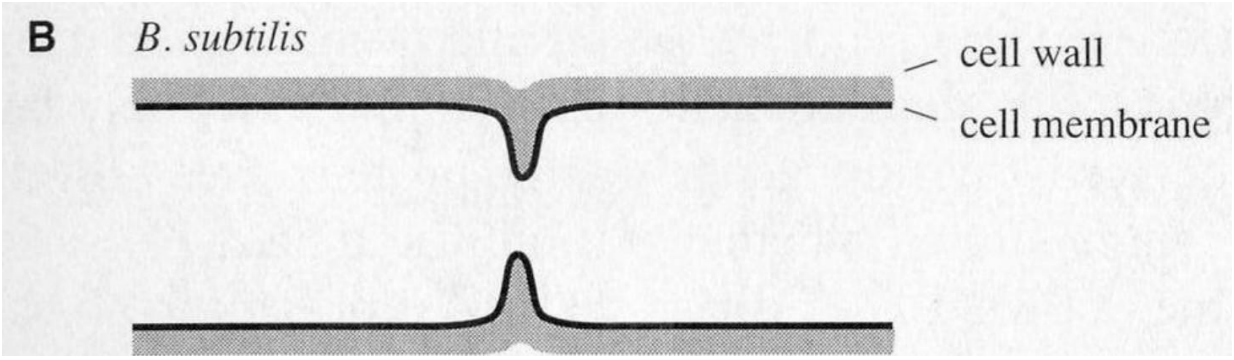
On the figure: E. coli · Outer membrane · cell membrane

> **E. coli (Gram negative rod)**: the entire envelope constricts *(pinches in)* and folds inward (invaginates) in the middle. [L20 p56]
>
> **Outer membrane** *(extra membrane outside the wall)* **and cell membrane** *(thin skin around the cell)* pinch inward together. [L20 p56]
>
> **Cytokinesis** *(splitting the cell in two)*, **septation** *(building the cross wall)* **and separation** occur at the same time. [L20 p56]
>
> **Little new cell wall** *(stiff layer around the cell membrane)* **synthesis** is needed at the division site. [L20 p56]

b. Gram positive rod (Bacillus)

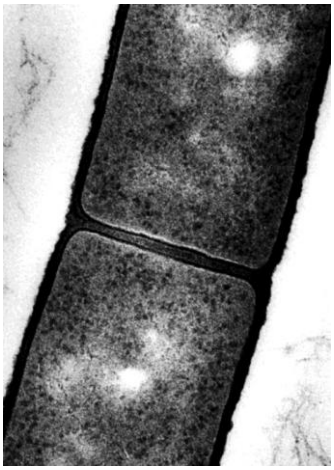
Book: 3.4 Bacterial Cell Division, Cell Division by Septation (chapter 3 PDF, p. 78).

⚠ **Do not mix up: Bacillus *(rod shaped cell)*: little constriction and late separation (chains); E. coli: constriction, all at once.** [L20 p57, L20 p56]



B. subtilis: cell membrane invaginates with a furrow of cell wall [L20 p57]

On the figure: cell wall · membrane



Two Bacillus cells joined by a straight cross wall [L20 p57]

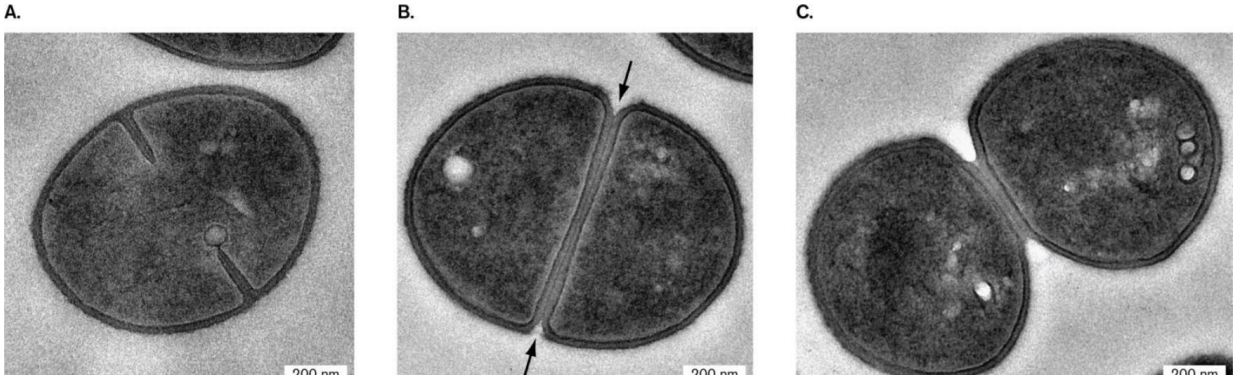
> **Gram positive** *(thick wall, stains purple)* **rod (Bacillus)**: the **membrane** *(thin lipid layer)* invaginates *(folds inward)* in the middle. [L20 p57]
>
> **A furrow** *(groove)* **of new cell wall** *(stiff layer around the cell membrane)* forms there, with little constriction. [L20 p57]
>
> **Septum** *(cross wall)* **forms** between the cells with cytokinesis *(splitting the cell in two)*. [L20 p57]
>
> **Bacillus**: two cells joined by a straight cross wall in a chain. [L20 p57]
>
> **Separation may occur later**; cells often stay attached as a chain or filament. [L20 p57]

c. Gram positive cocci (Staphylococcus): septum, planes and arrangements

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

⚠ **Do not mix up: Pairs or tetrads** ***(two or four cells left stuck together)*** **stay attached; tetrads** ***(groups of four cells)*** **need septation** ***(building the cross wall)*** **in PERPENDICULAR** ***(at right angles)*** **planes.** [L20 p59]

Furrow and septum



Staphylococcus division: septum furrow, completed septum, cells peeling apart [L20 p58]

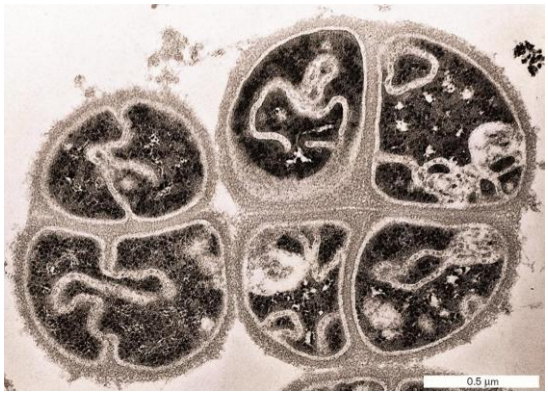
On the figure: furrows · septa

Gram positive *(thick wall, stains purple)* **cocci (Staphylococcus)**: a furrow *(groove)* of new wall forms at the equator and expands inward. [L20 p58]

This forms a double layer of cell wall *(stiff layer around the cell membrane)*. [L20 p58]

When the septa furrows meet, cytokinesis *(splitting the cell in two)* occurs. [L20 p58]

Cell separation occurs when the peptidoglycan *(sugar and peptide mesh of the wall)* between the cells is cut. [L20 p58]



Tetrad of cocci separated by septa in perpendicular planes [L20 p59]

On the figure: septation · Arrangements

Arrangements

Some do not separate: they remain as pairs or tetrads. [L20 p59]

The plane used for septation can determine the arrangement afterward. [L20 p59]

Tetrads: septation uses perpendicular planes. [L20 p59]

FtsZ ring and where new peptidoglycan is made (Lecture 18 slides)

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

⚠ **Do not mix up: FtsZ *(protein ring for cell splitting)* forms the ring; PBPs build peptidoglycan *(sugar and peptide mesh of the wall)*; autolysin *(wall-cutting enzyme made by the bacterium itself)*, produced by bacteria, is one of the enzymes that cleave peptidoglycan.** [L18 p38, L18 p39]

FtsZ ring

FtsZ is involved in formation of a ring at the cleavage site

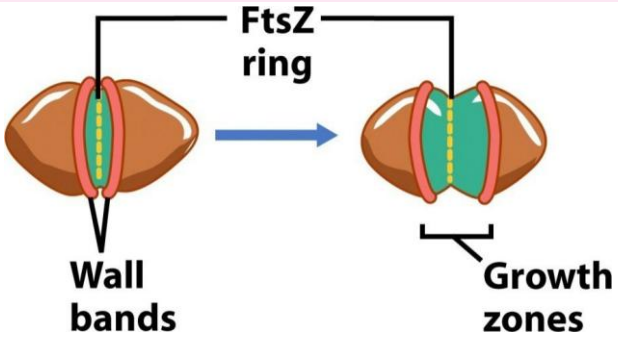
FtsZ ring forms during cytokinesis *(splitting the cell in two)*.

The Z-ring determines the cell diameter and manages the growth of the dividing partition, called the septum (textbook).

FtsZ ring: sits at mid-cell between the **wall bands**. [L18 p28]

Septal *(at the splitting wall)* **ring model**:

> A ring of FtsZ proteins
>
> ZipA connects the ring to the cell membrane *(thin skin around the cell)*. [L18 p38]



FtsZ ring, wall bands and growth zones of a dividing cell [L18 p28]

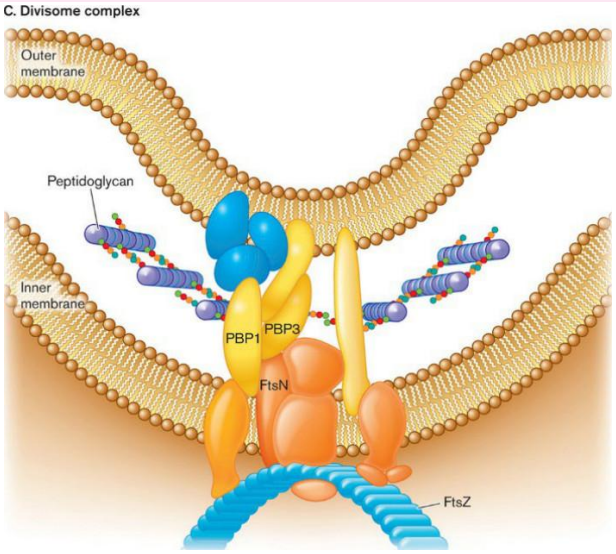
On the figure: FtsZ ring · wall bands · growth zones

Divisome proteins

Divisome *(cell splitting machine)*: the **FtsZ** ring sits under the **inner membrane** *(the skin around the cytoplasm)*. [L18 p38]

Divisome complex: **FtsN**, **PBP1** and **PBP3** sit on the inner membrane in the periplasm *(space between the two membranes)* and reach toward the **peptidoglycan**. [L18 p38]

Other division proteins: FtsA, FtsK and FtsW, FtsL, FtsN and FtsQ. [L18 p38]



Divisome complex: FtsZ ring, PBP1, PBP3 and FtsN at the peptidoglycan [L18 p38]

On the figure: **FtsZ** · **FtsN** · **PBP1** · **PBP3** · **peptidoglycan** · **inner membrane**

PBPs

PBPs in the divisome:

> Penicillin binding proteins *(enzymes that build the wall)* are part of the complex involved in peptidoglycan synthesis.
>
> Do the transglycosylation *(joining sugar chains longer)* and transpeptidation *(making peptide bridges between chains)* reactions. [L18 p38]

PBP3 reaches the peptidoglycan layer. [L18 p38]

Where new peptidoglycan is made

New peptidoglycan fills the **growth zones** at the division site. [L18 p28]

> **Where new peptidoglycan is made varies**: in the middle, at one end or dispersed. [L18 p29]

Keeping the layer intact

> **Layer integrity**:
>
> > The cell has to maintain the integrity of the layer.
> >
> > The cell cuts links between existing peptidoglycan and new peptidoglycan. [L18 p28]

Autolysin

> **Autolysin**, produced by bacteria, cuts old peptidoglycan to open the wall for new units during growth; at division it cuts the layers apart to separate the **daughter cells** *(the two new cells)* (textbook fill). [L18 p39, L20 p54]

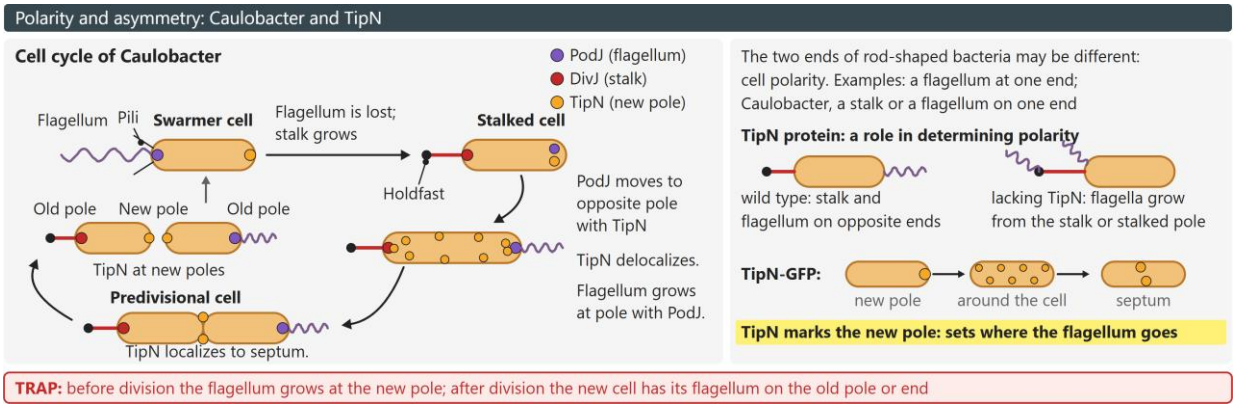
Growth pattern	Where new wall goes	Examples
Dispersed zonal growth	Dispersed along the side wall	E. coli, Bacillus
Septal zonal growth	Middle (septum at mid-cell)	Streptococcus, Staphylococcus
Polar growth	One end	Actinomycetes, Rhizobiales

Table source: [L18 p29]

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]

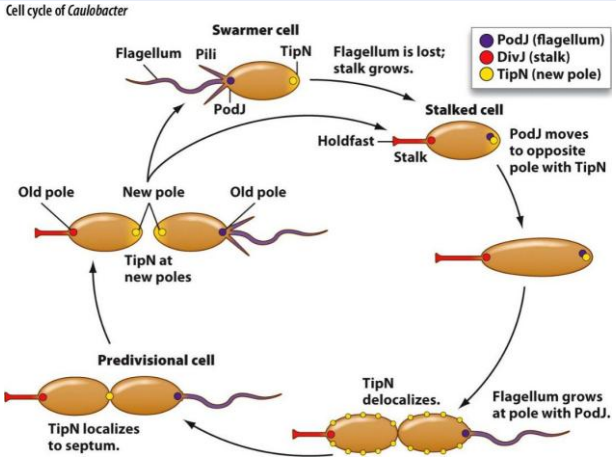
Stalked cell: a stalk with a **holdfast** *(sticky foot)* at its end. [L21 p06]

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]

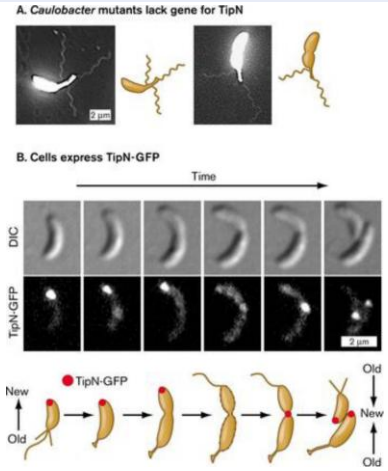


Caulobacter cell cycle with PodJ, TipN, old poles and new poles [L21 p06]

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

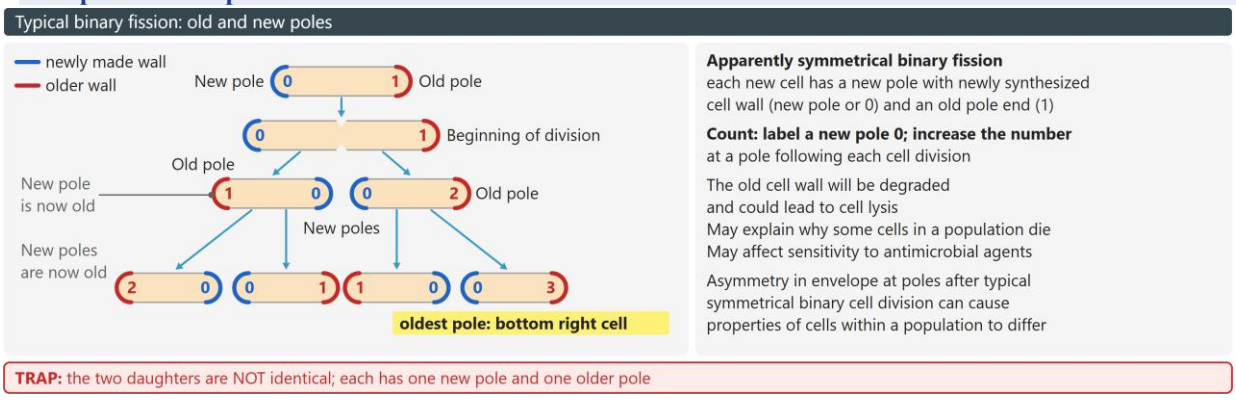
Table source: [L21 p06]

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]

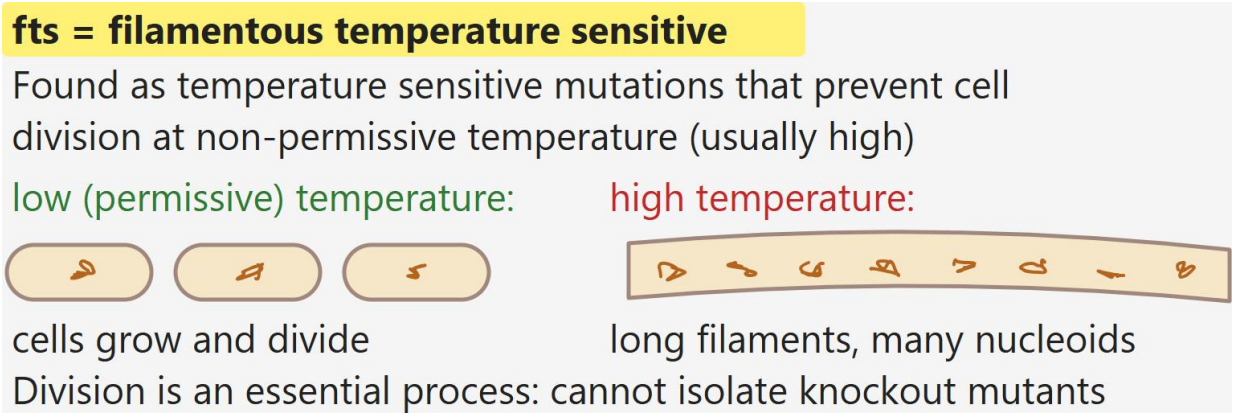
Many division proteins were identified by isolating temperature sensitive mutations. [L21 p11]

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]

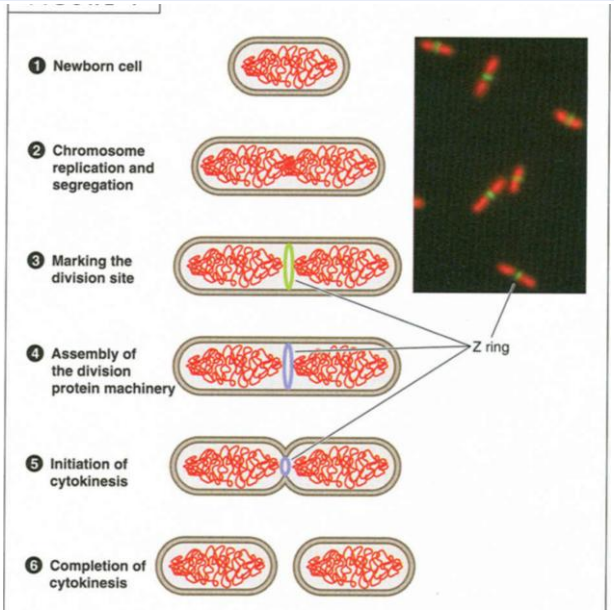
FtsZ in E. coli

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

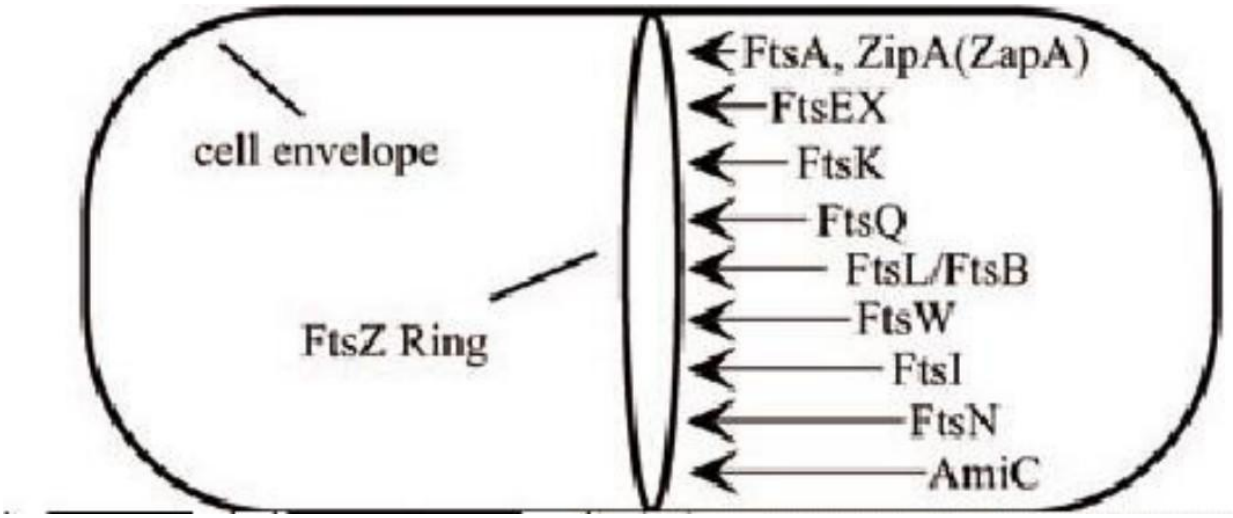
Bacillus (**Gram positive** rod), dividing cells: FtsZ localizes to the middle of the cell. [L21 p15]

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]

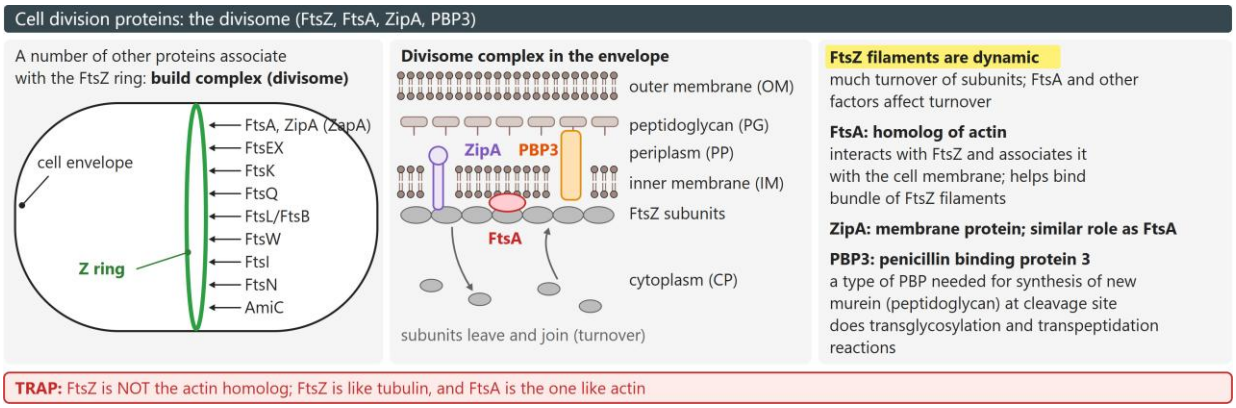
Z ring stages in E. coli

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)*.

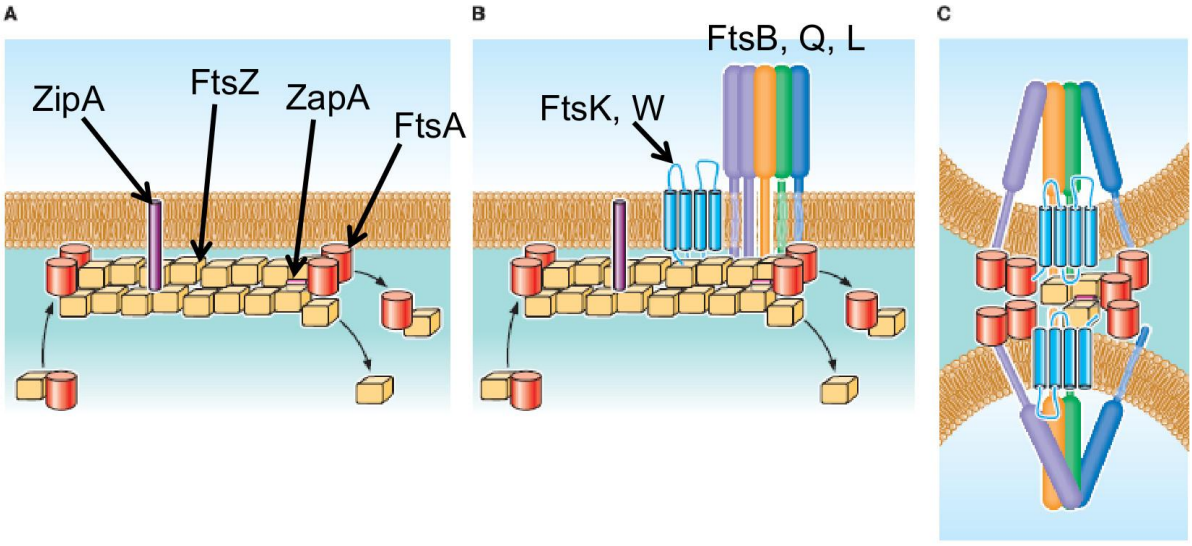
Interacts with **FtsZ** *(protein ring for cell splitting)* and associates with the membrane *(thin lipid layer)*.

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).

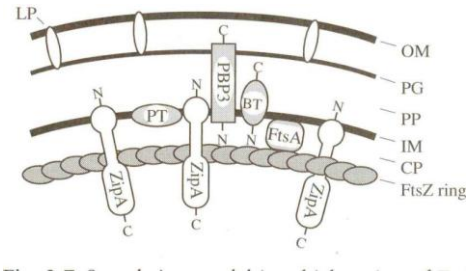
⚠ **Do not mix up: PBP3 does BOTH reactions at the cleavage site: transglycosylation *(joining sugar chains longer)* and transpeptidation *(making peptide bridges between chains)*.** [L21 p19]

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

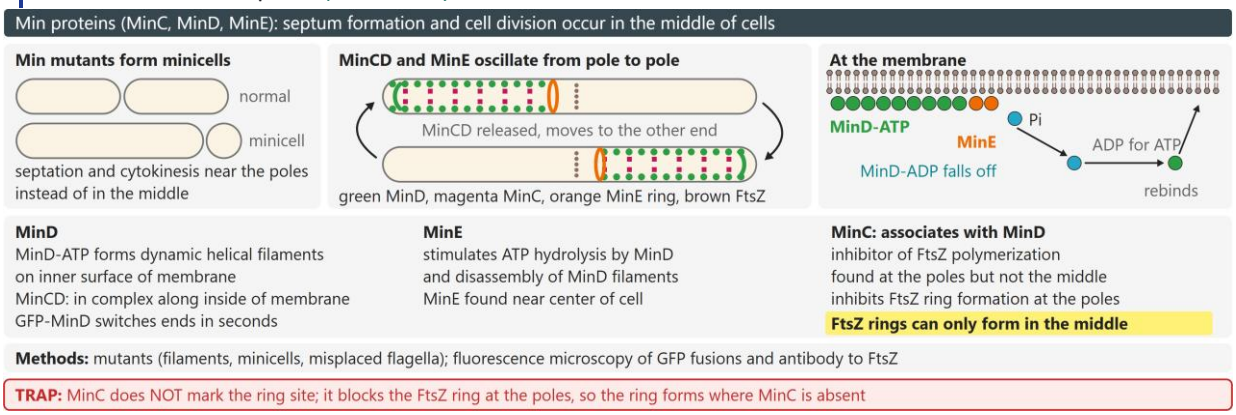
Book: Not in the assigned textbook sections; see the posted review article on cell division proteins.

⚠ **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]

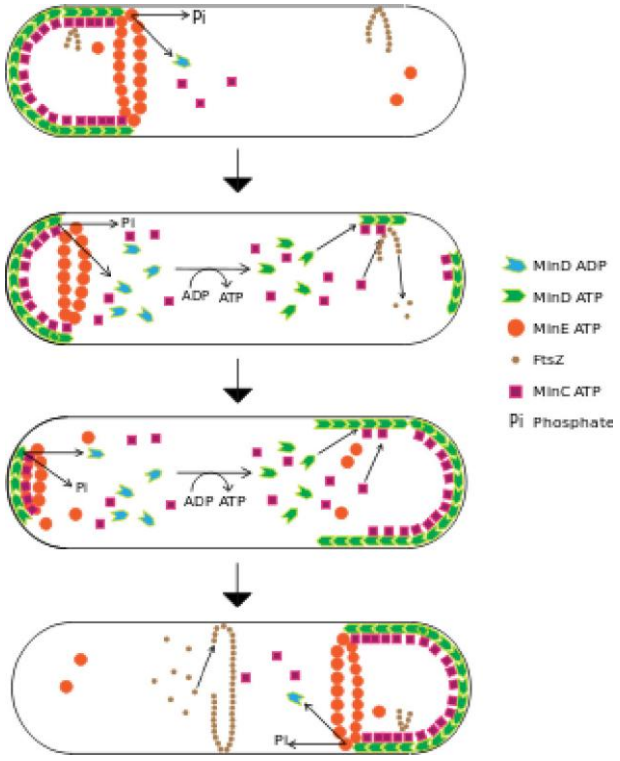
Step 1: **MinD-ATP** with MinC coats the membrane at one pole. [L21 p24]

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]

F. Viruses (Chapter 6)

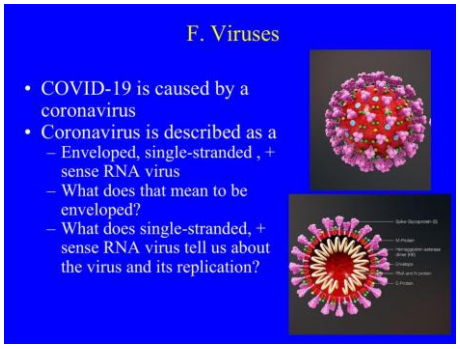
COVID-19 is caused by a coronavirus. [L22 p03]

Coronavirus: an enveloped, single-stranded, + sense RNA virus. [L22 p03]

Coronavirus surface: spike glycoprotein (S), M protein, E protein, hemagglutinin-esterase dimer. [L22 p03]

Inside the envelope *(layers wrapped around the cell)*: the RNA genome with N protein. [L22 p03]

Coronavirus (SARS) is a Group IV, (+) sense ssRNA, enveloped animal virus. [L22 p19]



Coronavirus: spikes on the envelope; S, M, E and hemagglutinin-esterase proteins; RNA with N protein [L22 p03]

1. Size and shape observed

Book: 6.2 Virus Structure (chapter 6 PDF, p. 30); 6.2 Virus Structure, Symmetrical Virions (chapter 6 PDF, p. 31).

⚠ **Do not mix up: Most viruses are 10-100x SMALLER than bacteria; giant viruses are similar in size to some bacteria.** [L22 p05]

Size

Size: most viruses are between **0.02** and **0.2** microns *(one millionth of a meter)*. [L22 p05]

Most are 10-100x smaller than bacteria. [L22 p05]

Need an electron microscope to see them. [L22 p05]

Giant viruses: Pandoravirus and Mimivirus are similar in size to some bacteria. [L22 p05]

Compare: Pandoravirus **1 um**, Mimivirus **450 nm**, E. coli **2 um** long, Rickettsia **0.3 um**. [L22 p05]

Smaller viruses: HIV **110 nm**, influenza **100 nm**, polio **30 nm**, yellow fever **22 nm**. [L22 p05]

Shape

Shape: large diversity. [L22 p06]

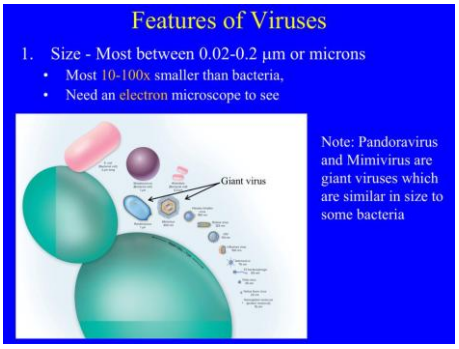
Icosahedron: a **20**-sided polyhedron *(3D shape with flat faces, straight edges, sharp corners)*. [L22 p06]

Other shapes: spherical, and complex *(more complicated)*. [L22 p06]

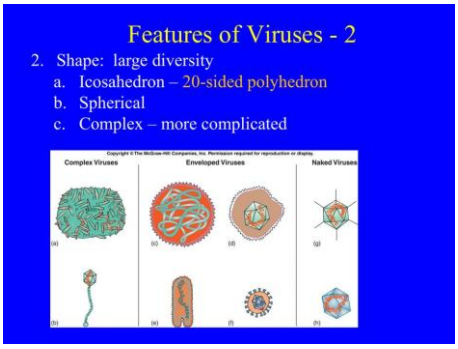
Complex viruses: poxvirus-like oval particles and tailed bacteriophages. [L22 p06]

Enveloped shapes: spherical, envelope *(layers wrapped around the cell)* around an icosahedral capsid, bullet-shaped helical. [L22 p06]

Naked viruses: icosahedrons, some with fibers. [L22 p06]



Size scale from yeast cell down to a hemoglobin molecule; giant viruses near bacteria [L22 p05]



Complex, enveloped and naked virus shapes [L22 p06]

2. Structural features: components of virus particles (core and covering)

Book: 6.2 Virus Structure, Symmetrical Virions (chapter 6 PDF, p. 31).

⚠ **Do not mix up: The CORE is nucleic acid and enzymes; the COVERING is the capsid and envelope *(layers wrapped around the cell)*.** [L22 p08]

Simpler than cells

Viruses are simpler than cells: considered acellular *(not made of cells)*. [L22 p07]

Virus particles (virions) may contain just nucleic acid in a protein shell. [L22 p07]
Naked virus: capsid with spikes at the vertices, nucleic acid inside. [L22 p07]
Enveloped virus: nucleic acid, capsid, an outer envelope, spikes from the envelope. [L22 p07]

Core and covering

Components can be divided into core and covering. [L22 p08]
Core: nucleic acid and enzymes. [L22 p08]
Core also: matrix proteins; enzymes are not found in all viruses. [L22 p08]
Covering: capsid and envelope. [L22 p08]
Envelope: not found in all viruses. [L22 p08]

a. Nucleic acid

Nucleic acid: found in all viruses. [L22 p09]
Can be DNA *(cell genetic instruction molecule)* **or RNA**, but not both. [L22 p09]
May be single or double stranded. [L22 p09]
May be in multiple segments. [L22 p09]
May contain more than 1 copy. [L22 p09]
dsDNA examples: variola (smallpox), herpes simplex **2** (genital herpes). [L22 p09]
ssDNA example: parvovirus (erythema infectiosum). [L22 p09]
RNA examples: poliovirus ss (+), influenza ss (-), rotavirus dsRNA. [L22 p09]
HIV (AIDS): ssRNA with reverse transcriptase. [L22 p09]
Overlapping genes can be seen in viral nucleic acid. [L22 p10]
Multiple proteins can come from a single RNA and polypeptide. [L22 p10]
SARS-CoV-2 genome: about **29,903** nucleotides; ORF1a and ORF1b, then S, E, M, N genes. [L22 p10]
The ORF1a and ORF1b polyprotein is cut into nonstructural proteins NSP1 to NSP16. [L22 p10]
SARS-CoV-2 structural proteins: S, E, M, N; accessory proteins orf3a to orf10. [L22 p10]
Cauliflower mosaic virus: circular DNA genome of **8,000** bp with overlapping genes. [L22 p10]
Avian leukosis virus: RNA genome of **7,200** bp with gag (core), pol (enzymes), env (envelope). [L22 p10]

b. Enzymes

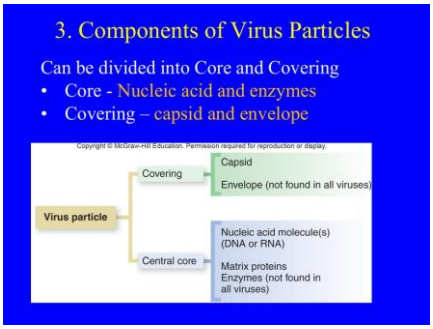
Enzymes: such as polymerase or reverse transcriptase. [L22 p11]
Enzymes sit inside the capsid. [L22 p11]
Enzymes are observed in some viruses. [L22 p11]
HIV (a retrovirus) carries reverse transcriptase, integrase and protease. [L22 p11]
HIV particle: GP-**120** knobs on GP-**41** stems; the capsid holds two RNA strands. [L22 p11]

c. Capsid

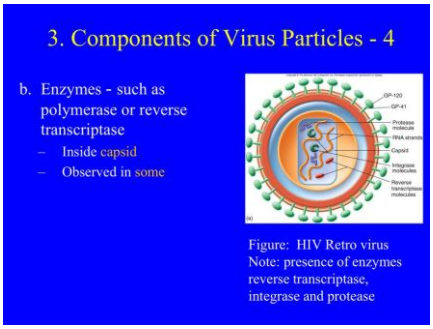
Capsid: protein coat or shell. [L22 p12]
The capsid is found in all viruses. [L22 p12]
Usually icosahedral (**20** sided) or helical in shape. [L22 p12]
Capsid proteins form identical capsomeres, which in turn form the capsid. [L22 p12]
Capsid and nucleic acid together form the nucleocapsid. [L22 p12]
Capsomeres form triangular facets and cluster at each vertex. [L22 p12]
Helical capsid: capsid proteins stacked around a helical nucleic acid. [L22 p12]

d. Envelope

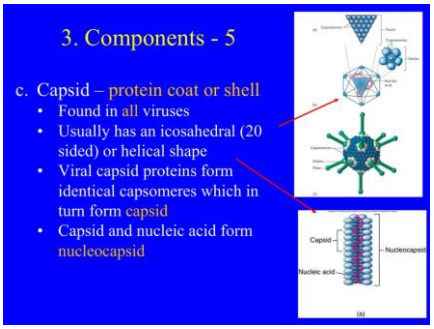
Envelope: phospholipid *(membrane fat with a charged head)* bilayer *(two layers)* membrane *(thin lipid layer)* with proteins and glycoproteins (spikes). [L22 p13]
The envelope is observed in some viruses. [L22 p13]



Virus particle: covering (capsid, envelope) and central core (nucleic acid, matrix proteins, enzymes) [L22 p08]



HIV particle: envelope with GP-120 and GP-41; capsid with RNA and enzymes [L22 p11]



Capsomeres, facets and vertices of an icosahedral capsid; helical nucleocapsid [L22 p12]

Components of virus particles

Component	Core or covering	In all viruses?	What it is
Nucleic acid	Core	Yes	DNA or RNA, but not both
Enzymes	Core, inside the capsid	Some	Such as polymerase or reverse transcriptase
Capsid	Covering	Yes	Protein coat or shell
Envelope	Covering	Some	Phospholipid bilayer membrane with proteins and glycoproteins (spikes)

Table source: [L22 p08, L22 p09, L22 p11, L22 p12, L22 p13]

Which components are found in all viruses?

Book: 6.2 Virus Structure, Symmetrical Virions (chapter 6 PDF, p. 31).

⚠ **Do not mix up: Nucleic acid and capsid are in ALL viruses; enzymes and envelope *(layers wrapped around the cell)* only in SOME.** [L22 p09, L22 p12, L22 p13]

Found in all viruses: nucleic acid and capsid. [L22 p09, L22 p12]

Found in some viruses only: enzymes and envelope. [L22 p08, L22 p11, L22 p13]

Naked vs enveloped viruses

Book: 6.2 Virus Structure, Symmetrical Virions (chapter 6 PDF, p. 31).

⚠ **Do not mix up: Naked viruses are TOUGHER; enveloped viruses are more sensitive and must remain wet.** [L22 p14]

Significance of the outer covering: naked vs enveloped. [L22 p14]

It affects viability *(able to still cause an infection)* outside a host cell. [L22 p14]

Naked (no membrane) viruses: generally tougher, survive longer. [L22 p14]

Naked viruses are more resistant to drying, detergents and acids. [L22 p14]

Enveloped viruses: more sensitive, must remain wet. [L22 p14]

The covering also determines the means of escape from the infected cell. [L22 p14]

Naked (nonenveloped) viruses must lyse the cell. [L22 p14]

Enveloped viruses can escape by budding. [L22 p14]

Naked vs enveloped viruses

Feature	Naked (nonenveloped)	Enveloped
Outer layer	Capsid, no membrane	Envelope over the capsid
Outside a host cell	Tougher, survive longer; resist drying, detergents, acids	More sensitive; must remain wet
Escape from the cell	Must lyse the cell	Can escape by budding

Table source: [L22 p07, L22 p14]

3. Features used to classify or group viruses (groups, subgroups, Baltimore Groups I to VII)

Book: 6.3 Viral Genomes and Classification, The Baltimore Virus Classification (chapter 6 PDF, p. 53).

⚠ **Do not mix up: GROUPS use genetic material and means of replication; covering, shape, host and segments define SUBGROUPS.** [L22 p17]

Groups and subgroups

Groups:

Are based on the nature of genetic material: DNA *(cell genetic instruction molecule)* or RNA, ss vs ds.

Are also based on the means of replication. [L22 p17, L22 p18]

Subgroups, a: outer covering, whether they have an envelope *(layers wrapped around the cell)* or not (nonenveloped). [L22 p17]

Subgroups, b: shape of the virus particle or capsid. [L22 p17]

Subgroups, c: organisms or cells the virus infects. [L22 p17]

Subgroups, d: whether the genome is segmented or not. [L22 p17]

Baltimore classification: seven groups of viruses, Group I to Group VII. [L22 p18, L22 p19]

Some refer to groups as families. [L22 p18, L22 p19]

Subgroup examples

Group I subgroups: nonenveloped head and tail bacteriophages; nonenveloped and enveloped animal viruses. [L22 p18]

Group I also includes archaeal *(of archaea)* viruses. [L22 p18]

Group III subgroups: nonsegmented enveloped bacteriophages; segmented nonenveloped animal and plant viruses. [L22 p18]

Group IV subgroups: nonenveloped phage (MS2); nonenveloped animal and plant (polio); enveloped (SARS). [L22 p19]

Group V subgroups: segmented enveloped (influenza); nonsegmented enveloped (Ebola, measles, rabies). [L22 p19]

Group VII subgroups: nonenveloped plant viruses (cauliflower mosaic); enveloped animal viruses (hepatitis B). [L22 p19]

Baltimore groups of viruses

Group	Genome	Replication	Examples
I	dsDNA	Host or viral DNA polymerase	T4, lambda, adenovirus, herpes, pox, Mimivirus
II	ssDNA, (+) sense	Host DNA polymerase; nonenveloped	M13, phiX174, parvovirus, geminivirus
III	dsRNA	Viral RNA-dependent RNA polymerase, usually packaged	Cystoviridae phages, reovirus (rotavirus)
IV	(+) sense ssRNA	Viral RNA-dependent RNA polymerase makes a (-) template	MS2, polio, rhinovirus, corona (SARS), Zika
V	(-) sense ssRNA	Viral RNA-dependent RNA transcriptase; genome often segmented	Influenza, Ebola, measles, mumps, rabies
VI	Retroviruses, ssRNA	Viral reverse transcriptase copies RNA to DNA for integration into the host chromosome	HIV, SIV
VII	Pararetroviruses, dsDNA	DNA transcribed to RNA, then reverse-transcribed to DNA	Cauliflower mosaic virus, hepatitis B

Table source: [L22 p18, L22 p19]

4. Reproduction

a. General features: obligate intracellular parasites, assembled from parts

Book: 6.1 Viruses in Ecosystems, Viruses Infect Specific Hosts (chapter 6 PDF, p. 12).

⚠ **Do not mix up: Viruses are ASSEMBLED from parts inside the host cell, not made by splitting.** [L22 p16, L22 p37]

Obligate intracellular parasites: **only** replicate or multiply inside a host cell. [L22 p15]

Obligate intracellular parasitism is also observed in some bacteria and protozoans. [L22 p15]

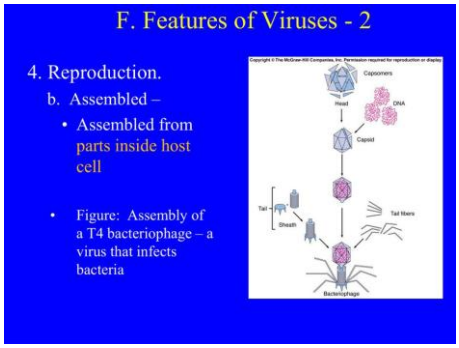
Phage *(virus that infects bacteria)* **cycle**: adsorption, penetration, duplication of phage parts, assembly, maturation, lysis *(bursting open)*, release. [L22 p15]

Lysogenic phase: viral DNA *(cell genetic instruction molecule)* becomes latent as a prophage. [L22 p15]

Assembled from parts inside the host cell. [L22 p16]

T4 bacteriophage *(a virus that infects bacteria)*: capsomers make the head; DNA is packed into the capsid. [L22 p16]

Tail and sheath are built separately; tail fibers are added last. [L22 p16]



Assembly of a T4 bacteriophage: head, DNA packing, tail and sheath, tail fibers [L22 p16]

b. Steps in multiplication cycle

Book: 6.5 Coronavirus SARS-CoV-2 and Other Viral Pathogens, Animal Viruses Bind Host Receptors and Enter the Cell (chapter 6 PDF, p. 86); 6.4 Bacteriophages: The Gut Virome, Bacteriophages Infect a Host Cell (chapter 6 PDF, p. 67).

⚠ **Do not mix up: Penetration is ENTRY into the cell; uncoating is RELEASE of the viral genome.** [L22 p22]

Animal virus cycle

Animal virus cycle: adsorption, penetration, uncoating, synthesis, assembly, release. [L22 p20]

Adsorption: spikes bind cell receptors *(proteins a signal or virus binds to)*. [L22 p20]

Penetration: the virus is engulfed into a vesicle. [L22 p20]

Uncoating frees the viral RNA into the cytoplasm *(jelly inside the cell membrane)*. [L22 p20]

Synthesis: (+) RNA to (-) RNA to new (+) RNA; new capsomers and spikes. [L22 p20]

Assembly: spike proteins go into the cell membrane *(thin skin around the cell)*; RNA and capsomers form the nucleocapsid. [L22 p20]

Release: the enveloped virus buds off the membrane, carrying an envelope *(layers wrapped around the cell)* with spikes. [L22 p20]

a. Adsorption

Adsorption: attachment to receptors on the host cell to be infected. [L22 p21]

Adsorption is the major factor determining host range *(the kinds of cells a virus can infect)*. [L22 p21]

SARS-CoV-2 binds the ACE2 receptor. [L22 p21]

Measles virus: an envelope protein binds a receptor protein, then the membranes fuse. [L22 p21]

b. Penetration and c. Uncoating

Penetration: entry into the cell. [L22 p22]

Uncoating: release of the viral genome. [L22 p22]

Two ways in: endocytosis to an endosome vesicle, or fusion of membranes. [L22 p22]

Endocytosis: attachment, engulfment, virus in a vesicle; vesicle, envelope and capsid break down. [L22 p22]

Fusion:

> Membranes fuse at the receptor-spike complex; the nucleocapsid enters, then uncoats.
> Releases the contents of the viral particle inside the cell. [L22 p22]

Uncoating mechanisms: in the endosome; by fusion and release; or at the nucleus. [L22 p23]

SARS-CoV-2 enters by endocytosis; its genome is uncoated within the endosome. [L22 p23]

Measles: the coated RNA genome enters the cytoplasm. [L22 p23]

Hepatitis C: uncoated in an endosome acidified by lysosome fusion. [L22 p23]

Adenovirus: the genome is uncoated at the nuclear pore complex. [L22 p23]

e. Assembly

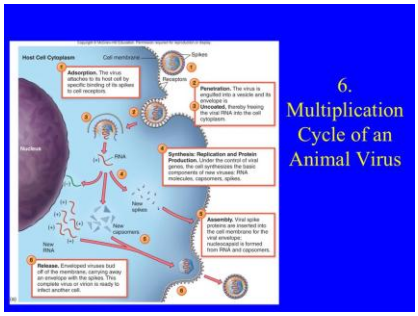
Assembly: assembled from parts, not by splitting. [L22 p37]

Proteins assemble to make capsomers and the sheath. [L22 p37]

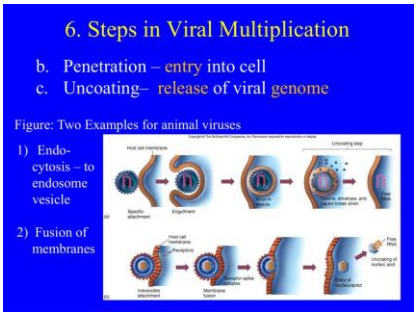
Parts of the virus are then assembled. [L22 p37]

f. Release or escape

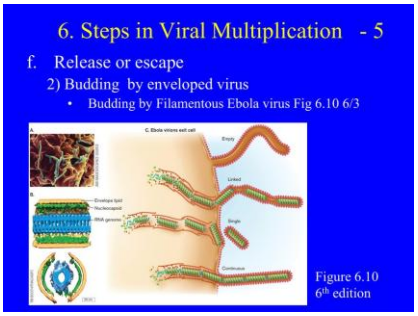
Release, way 1: lysis *(bursting open)* of the host cell. [L22 p38]
Way 2: budding by enveloped viruses. [L22 p38]
Budding can result in a persistent infection. [L22 p38]
Budding: viral spikes and matrix protein go into the host membrane; the nucleocapsid pushes out. [L22 p38]
Filamentous Ebola virus buds out of the cell. [L22 p39]
Ebola virion: lipid envelope, nucleocapsid and RNA genome. [L22 p39]
Ebola virions exit as filaments: empty, linked, single or continuous. [L22 p39]
Tandem arrays of RNA genomes are held together in a flexible lipid envelope. [L22 p39]
Way 3: cell-to-cell spread. [L22 p40]
Cell-to-cell spread may lead to cell fusion: syncytia formation. [L22 p40]
Viral components pass along an F-actin bridge to a target cell. [L22 p40]
In epithelial cells, viral particles pass through the apical junction complex. [L22 p40]



Animal virus cycle: adsorption, penetration, uncoating, synthesis, assembly, release by budding [L22 p20]



Entry by endocytosis vs fusion of membranes, then uncoating [L22 p22]



Ebola virions budding out of a cell as filaments [L22 p39]

Steps in viral multiplication

Step	What happens
a. Adsorption	Attachment to receptors on the host cell
b. Penetration	Entry into the cell, by endocytosis or fusion
c. Uncoating	Release of the viral genome
d. Synthesis	Copies of the genetic material and of the proteins
e. Assembly	From parts, not by splitting
f. Release or escape	Lysis, budding by enveloped viruses, or cell-to-cell spread

Table source: [L22 p20, L22 p21, L22 p22, L22 p24, L22 p37, L22 p38, L22 p40]

c. Synthesis stage for the 7 types of viruses and the source of each enzyme

Book: 6.3 Viral Genomes and Classification, The Baltimore Virus Classification (chapter 6 PDF, p. 53).

⚠ **Do not mix up: Most host cells lack RNA-dependent RNA polymerase *(enzyme copying DNA into RNA)*, so RNA viruses usually encode their own.** [L22 p28, L22 p29]

Two jobs

Synthesis needs two things: replicate the genetic material and make many copies of proteins. [L22 p24]
Replicate: DNA *(cell genetic instruction molecule)* to more DNA, usually ds; ss RNA to more ss RNA. [L22 p24]
Proteins: viral mRNA *(message copy of a gene)* to proteins. [L22 p24]
Done in different ways by different viruses; it depends on the genetic material. [L22 p24]
Needed enzymes can be encoded by the virus or found in the host cell. [L22 p24]
Every group leads to (+) mRNA. [L22 p25]

RNA genomes

RNA genomes: usually single stranded (ss). [L22 p28]
Problem: RNA must be the template to make more RNA (RNA-dependent RNA polymerase, RdRP). [L22 p28]
Main host DNA pol and RNA pol use DNA as the template. [L22 p28]
Most host cells have no RdRP or very low levels. [L22 p28]
Solution: viruses usually encode enzymes that can replicate RNA. [L22 p29]

Mechanism 1: RNA-dependent RNA polymerases: ssRNA to complementary ssRNA to ssRNA. [L22 p29]
Mechanism 2: reverse transcriptase of retroviruses: ssRNA to ssDNA to dsDNA to ssRNA. [L22 p29]
(+) strand: the strand that serves as template for translation. [L22 p30]
(-) strand: the strand complementary to the (+) strand. [L22 p30]

Group I: dsDNA

Group I genome: dsDNA to more dsDNA; DNA polymerase from the host cell or virus. [L22 p26]
Group I proteins: dsDNA to ssRNA to proteins; DNA-dependent RNA pol from host or virus. [L22 p26]
Group I ribosomes *(protein building machine)*: host cell. [L22 p26]
Papillomavirus:

> Genome uncoated at the nuclear pore; host RNA and DNA polymerases do the work.
> Assembles in the nucleus; **only** shedding epithelial cells release virions. [L22 p26]

Group I is the most similar to cellular processes. [L22 p26]

Group II: ssDNA

Group II genome: ssDNA to dsDNA to ssDNA; DNA polymerase from the host. [L22 p27]
Group II proteins: dsDNA to RNA to protein; RNA polymerase from host or virus. [L22 p27]
Group II ribosomes: host. [L22 p27]
Parvovirus infects dividing erythroid precursor cells; its +DNA becomes +/-DNA in the nucleus. [L22 p27]

Group III: dsRNA

Group III genome: dsRNA to (+) ssRNA to dsRNA, using RdRP from the virus. [L22 p31]
Group III proteins: (+) ssRNA to proteins on host ribosomes. [L22 p31]
Reovirus: enters by receptor-mediated endocytosis; uncoating in the endosome releases the core. [L22 p31]
In the core, viral RdRP makes (+) ssRNA; new dsRNA forms inside the capsid. [L22 p31]
Reovirus is released by lysis *(bursting open)* of the host cell. [L22 p31]

Group IV: (+) ssRNA

Group IV genome: (+) ssRNA to (-) ssRNA to (+) ssRNA, using RdRP from the virus. [L22 p32]
Group IV proteins: the (+) ssRNA carried in the particle can be translated immediately. [L22 p32]
Group IV ribosomes: host. [L22 p32]
Picornavirus: the (+) RNA is translated first to make the RdRP. [L22 p32]
Picornavirus (-) RNA is made in a virus-induced vesicle from the ER. [L22 p32]

Group V: (-) ssRNA

Group V genome: (-) ssRNA to (+) ssRNA to (-) ssRNA, using RdRP from the virus. [L22 p33]
Group V proteins: (+) ssRNA to proteins on host ribosomes. [L22 p33]
The virus brings its RdRP in with the (-) RNA genome. [L22 p33]

Group VI: retroviruses

Group VI genome: (+) ssRNA to ssDNA to dsDNA to (+) ssRNA. [L22 p34]
Reverse transcriptase comes from the virus; the last step uses host DNA-dependent RNA pol. [L22 p34]
Group VI proteins: dsDNA to (+) ssRNA to protein; RNA polymerase and ribosomes from host. [L22 p34]
Retrovirus: the dsDNA copy integrates into the host genome; host RNA Pol II transcribes it. [L22 p34]
Retroviruses leave the cell by budding. [L22 p34]

Group VII: DNA reverse-transcribing

Group VII genome: dsDNA to (+) ssRNA to dsDNA. [L22 p35]
Group VII reverse transcriptase comes from the virus or the host cell. [L22 p35]
Group VII proteins: dsDNA to (+) ssRNA to protein on host ribosomes. [L22 p35]
Cauliflower mosaic virus: dsDNA genome passed on through an aphid bite. [L22 p35]
It spreads to uninfected cells through the plasmodesma. [L22 p35]

Enzyme sources compared

Parvovirus and papillomavirus: host DNA pol for the first step. [L22 p36]

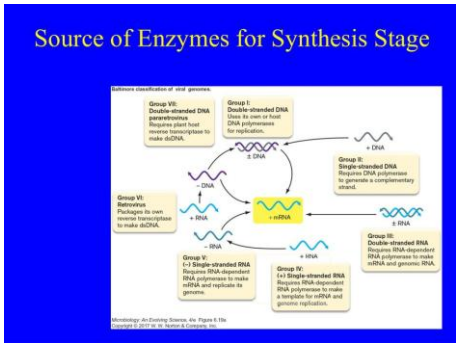
Adenovirus, herpesvirus, poxvirus: virus-encoded DNA-dependent DNA pol for the first step. [L22 p36]

Poxvirus is the exception for transcription: its RNA polymerase is virus encoded. [L22 p36]

RNA retroviruses: virus-encoded RNA-dependent DNA polymerase for the first step. [L22 p36]

(+) and (-) ssRNA viruses: virus-encoded RNA-dependent RNA pol; ribosomes from the host. [L22 p36]

First step: host ribosomes for (+) ssRNA; viral RdRP for (-) ssRNA. [L22 p36]



Every group leads to (+) mRNA; the enzyme each group needs [L22 p25]

Synthesis stage: route and source of each enzyme, Groups I to VII

Group	Route	Replication enzyme	RNA polymerase for transcription	Ribosomes
I dsDNA	dsDNA to dsDNA; dsDNA to ssRNA to protein	DNA polymerase: host or virus	Host or virus (virus in poxvirus)	Host
II ssDNA	ssDNA to dsDNA to ssDNA; dsDNA to RNA to protein	DNA polymerase: host	Host or virus	Host
III dsRNA	dsRNA to (+) ssRNA to dsRNA	RdRP from the virus	RdRP from the virus makes the (+) ssRNA	Host
IV (+) ssRNA	(+) ssRNA to (-) ssRNA to (+) ssRNA	RdRP from the virus	Not needed: the (+) ssRNA is translated immediately	Host
V (-) ssRNA	(-) ssRNA to (+) ssRNA to (-) ssRNA	RdRP from the virus	Not host: viral RdRP makes the (+) ssRNA	Host
VI retrovirus	(+) ssRNA to ssDNA to dsDNA to (+) ssRNA	Reverse transcriptase: virus	Host	Host
VII DNA reverse-transcribing	dsDNA to (+) ssRNA to dsDNA	Reverse transcriptase: virus or host cell	Host	Host

Table source: [L22 p25, L22 p26, L22 p27, L22 p31, L22 p32, L22 p33, L22 p34, L22 p35, L22 p36]

▸ Multiplication cycles of SARS-CoV-2, plant viruses and bacteriophage

Book: 6.5 Coronavirus SARS-CoV-2 and Other Viral Pathogens, Coronavirus (chapter 6 PDF, p. 84); 6.5 Coronavirus SARS-CoV-2 and Other Viral Pathogens, Plant Viruses (chapter 6 PDF, p. 107); 6.4 Bacteriophages: The Gut Virome, Bacteriophages Infect a Host Cell (chapter 6 PDF, p. 67).

⚠ Do not mix up: Plant viruses need help past the cell wall *(stiff layer around the cell membrane)*; most phages *(virus that infects bacteria)* inject DNA *(cell genetic instruction molecule)*, leaving the particle outside. [L22 p42, L22 p43]

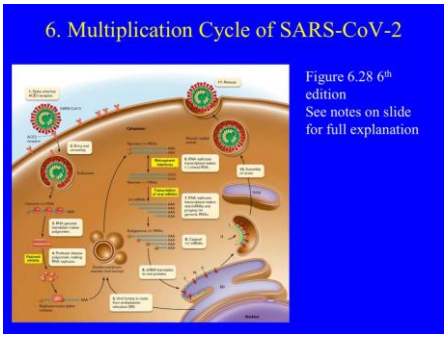
SARS-CoV-2

Step 1: the spike attaches to the ACE2 receptor. [L22 p41]

Step 2: entry by endocytosis and uncoating in the host cytoplasm *(jelly inside the cell membrane)*. [L22 p41]

Step 3: host ribosomes *(protein building machine)* translate the RNA genome into a polyprotein. [L22 p41]

Step 4: a protease cleaves the polyprotein, making RNA replicase (RdRP); Paxlovid inhibits this. [L22 p41]



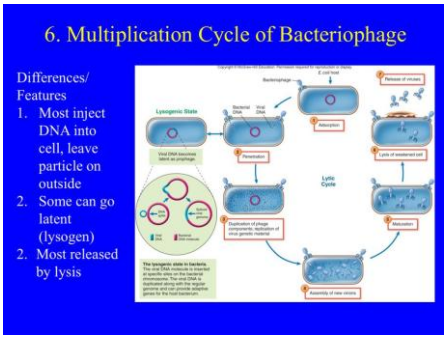
SARS-CoV-2 cycle: ACE2 binding, entry, polyprotein, replicase, ER factories, assembly, release [L22 p41]

Step 5: ER membrane *(thin lipid layer)* becomes viral factories (double-membrane vesicles). [L22 p41]
Step 6: replicase makes (-) strand RNA; molnupiravir interferes. [L22 p41]
Steps 7 and 8: the (-) strand templates viral mRNAs and new (+) genomes. [L22 p41]
Step 9: mRNAs are translated into viral proteins (E, M, S on the ER; N). [L22 p41]
Steps 10 and 11: virions assemble via the Golgi and bud out of the cell. [L22 p41]

Plant viruses

Plant viruses: entry and penetration are different. [L22 p42]
The cell wall blocks entry without help. [L22 p42]
Ways in: an animal vector (insect) damages cells; contact with damaged tissue; infected seeds. [L22 p42]
Then the virus moves cell to cell through plasmodesma connections. [L22 p42]
Cauliflower mosaic virus uses host RNA polymerase *(enzyme copying DNA into RNA)* and host reverse transcriptase. [L22 p42]

Bacteriophage

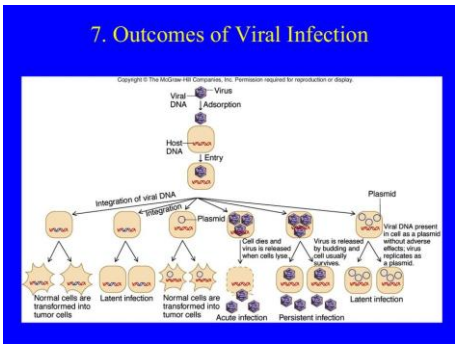


Bacteriophage cycle with the lysogenic prophage state [L22 p43]

Most phages inject DNA into the cell and leave the particle on the outside. [L22 p43]
Some can go latent (lysogen) *(phage DNA kept quietly in the host)*. [L22 p43]
Most are released by lysis *(bursting open)*. [L22 p43]
Prophage: viral DNA inserted at specific sites on the bacterial chromosome *(one long DNA molecule)*, copied with it. [L22 p43]
Prophages can give the host adaptive genes. [L22 p43]

5. Outcomes of viral infections

Possible outcomes: acute infection, persistent infection, latent infection, transformation *(cell taking up free DNA)* into tumor cells. [L22 p44]



Outcomes of viral infection after adsorption and entry [L22 p44]

Different possible outcomes for the host cell

Book: 6.5 Coronavirus SARS-CoV-2 and Other Viral Pathogens, Oncogenic Viruses (chapter 6 PDF, p. 106); 6.1 Viruses in Ecosystems, Integrated Viral Genomes (chapter 6 PDF, p. 15).

⚠ **Do not mix up: Persistent infection is ONLY by enveloped viruses that leave by budding; the cell survives.** [L22 p45]

Acute and persistent

a. Acute infection: death of host cells due to cell lysis *(bursting open)*. [L22 p45]
b. Persistent infection: host cells keep producing virus particles over a long period of time. [L22 p45]
Slow release of virus. [L22 p45]
Persistent infection is only done by enveloped viruses, which leave by budding. [L22 p45]

Latent

c. Chronic, latent infection: the virus goes dormant *(inactive, not growing)* in the host cell. [L22 p46]

It can later reactivate and release virus particles. [L22 p46]

Example: herpes virus goes latent in a nerve cell. [L22 p46]

Herpes will occasionally release virus particles, which cause skin lesions. [L22 p46]

HSV-1: first infection at **3** to **5** years old (cold sores). [L22 p46]

Then it stays latent in a root ganglion neuron; when activated it travels the trigeminal nerve. [L22 p46]

Latent infection can also be viral DNA *(cell genetic instruction molecule)* kept as a plasmid *(small extra DNA circle)* without adverse effects. [L22 p44]

Cytopathic changes

d. Cytopathic changes: infection alters the appearance of the cell. [L22 p47]

Cytopathic changes are sometimes used in diagnosis. [L22 p47]

Transformation and syncytia

e. Transformation *(cell taking up free DNA)*: the cell becomes cancerous. [L22 p48]

Some viruses contain an oncogene, or their integration generates an oncogene. [L22 p48]

The oncogene promotes growth of the host cell. [L22 p48]

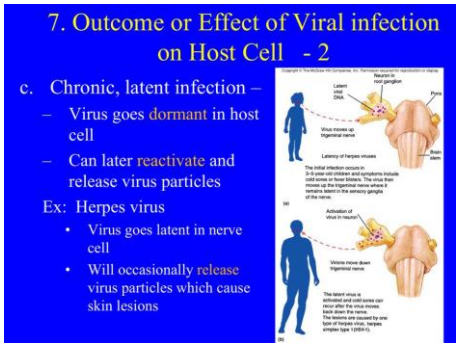
Infected cells round up, clump and detach; normal cells lie flat and spread out. [L22 p48]

f. Syncytia: large, multinucleated cells. [L22 p49]

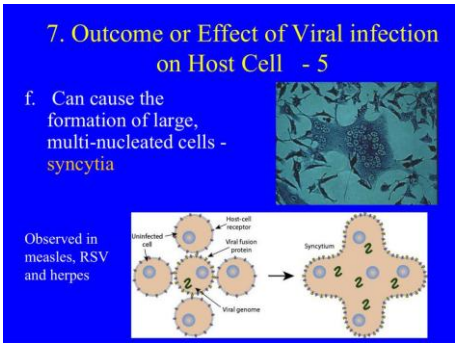
Syncytia are observed in measles, RSV *(respiratory syncytial virus)* and herpes. [L22 p49]

How: a viral fusion protein on an infected cell binds receptors *(proteins a signal or virus binds to)* on uninfected neighbors. [L22 p49]

The cells fuse into one syncytium with several nuclei. [L22 p49]



Herpes latency in a nerve root ganglion and later activation with cold sores [L22 p46]



Syncytium: infected cell fusion protein binds neighbors; one cell with many nuclei [L22 p49]

Outcomes for the host cell

Outcome	What happens to the host cell	Virus release
a. Acute	Host cells die due to cell lysis	Released when the cells lyse
b. Persistent	Cell usually survives	Slow release by budding over a long period
c. Chronic, latent	Virus goes dormant in the host cell	Can later reactivate and release virus particles
e. Transformation	Cell becomes cancerous (tumor cell)	Viral DNA integrated, or kept as a plasmid

Table source: [L22 p44, L22 p45, L22 p46, L22 p48]

Cytopathic changes

Virus	Cytopathic change	Inclusions
Smallpox	Cells round up	Cytoplasmic
Herpes simplex	Cells fuse to form multinucleated syncytia	Nuclear
Adenovirus	Clumping	Nuclear
Poliovirus	Cell lysis	None
Reovirus	Cell enlargement, vacuoles	Cytoplasmic
Influenza	Cells round up	None
Rabies	No change in shape	Cytoplasmic (Negri bodies)
Measles	Syncytia form (multinucleate)	

Table source: [L22 p47]

Effects on populations of microbes

Book: 6.1 Viruses in Ecosystems, Viruses Limit Host Populations and Increase Diversity (chapter 6 PDF, p. 19); 6.1 Viruses in Ecosystems, Viruses Cycle Nutrients and Share Host Genes (chapter 6 PDF, p. 22); 6.4 Bacteriophages: The Gut Virome, Bacteriophages Within the Gut Community (chapter 6 PDF, p. 79).

⚠ **Do not mix up: Phage *(virus that infects bacteria)* lysis *(bursting open)* at the ocean SURFACE releases organic matter that would otherwise sink.** [L22 p51]

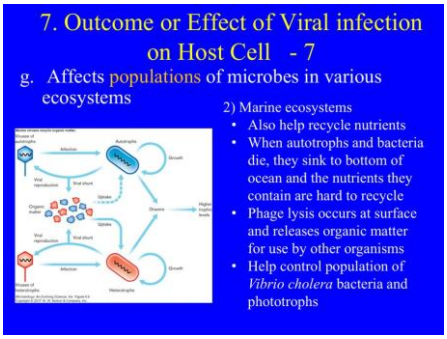
g. Viruses affect populations of microbes in various ecosystems. [L22 p50]

Mammalian gut: lots of bacteriophages that act against bacteria in the intestine. [L22 p50]

Gut phages can transfer host DNA (transduction) and may stimulate the immune system. [L22 p50]

Lysogenized bacteria can have enhanced traits and express virulence *(ability to cause disease)*, such as toxins. [L22 p50]

Marine ecosystems: viruses also help recycle nutrients. [L22 p51]



Marine viruses recycle organic matter through the viral shunt [L22 p51]

Dead autotrophs and bacteria sink to the ocean bottom, where their nutrients are hard to recycle. [L22 p51]

Phage lysis occurs at the surface and releases organic matter for use by other organisms. [L22 p51]

Viral shunt *(organic matter released by lysis)*: taken up by autotrophs and heterotrophs. [L22 p51]

Phages help control populations of Vibrio cholera bacteria and phototrophs *(organisms that use light for energy)*. [L22 p51]

G. Atypical Bacteria and Viruses

Typical bacteria vs typical viruses

Book: Not in Chapter 6; his outline marks this part "(notes)": study the slides.

⚠ **Do not mix up: Typical bacteria reproduce BY THEMSELVES; typical viruses only reproduce INSIDE cells.** [L22 p53]

Typical bacteria are free-living and reproduce by themselves. [L22 p53]

Typical viruses are obligate intracellular parasites: they **only** reproduce inside cells. [L22 p53]

Viruses have nucleic acids but no chromosomes *(one long DNA molecule)*, nucleus, ribosomes *(protein building machine)*, mitochondria or flagella *(whip tails that spin)*. [L22 p52]

Viruses lack mitosis, binary fission and independent biosynthesis. [L22 p52]

No cell wall *(stiff layer around the cell membrane)* in viruses: capsids instead. [L22 p52]

Sizes: viruses under **0.2 um**; bacteria **0.5** to **3 um**; eukaryotes **2** to **100 um**. [L22 p52]

Outside host cells, viruses cannot carry out metabolic or genetic activity. [L22 p52]

Typical bacteria vs typical viruses

Property	Typical bacteria	Typical viruses
Size	**1** to **3 um**	**0.02** to **0.2 um**
Genome	**500** to **10,000** kB; **1,000** to **10,000** genes	**1.8** to **100** kB; **1** to **100 proteins**
Lifestyle	Free-living; reproduces by itself	Obligate intracellular parasite; **only** reproduces inside cells

Table source: [L22 p53]

Atypical small bacteria: how they were found

Book: Not in Chapter 6; his outline marks this part "(notes)": study the slides.

⚠ **Do not mix up: Passing a 1 micron *(one millionth of a meter)* filter did NOT make them viruses: they had ribosomes *(protein building machine)* and metabolic enzymes.** [L22 p54]

Atypical small bacteria: originally identified in searches for the causes of certain diseases. [L22 p54]

First observation: no bacteria seen in a Gram stain *(stain that sorts bacteria purple or pink)*; agent too small or does not stain. [L22 p54]

Not removed by 1 micron filters, so the size is under **1 um**. [L22 p54]

Some material was seen inside some host cells (obligate intracellular parasitism?). [L22 p54]

Some concluded the diseases must be caused by a virus. [L22 p54]

Later observations: the agent contained DNA *(cell genetic instruction molecule)*, RNA and protein. [L22 p54]

The agent also contained ribosomes and metabolic enzymes. [L22 p54]

1. Rickettsia

Book: Not in Chapter 6; his outline marks this part "(notes)": study the slides.

⚠ **Do not mix up: Rickettsia are ENERGY PARASITES: they lack glycolysis and steal ATP *(cell energy molecule)* with a translocase.** [L22 p55]

Rickettsia size: **0.3** x **1 um**. [L22 p55]

Rickettsia genome: about **1,000** kB, about **830** genes. [L22 p55]

Multiplication only inside host cells. [L22 p55]

Gram-negative *(thin wall plus outer membrane)* **morphology**, but they stain poorly. [L22 p55]

Energy parasites: lack full metabolic systems (no glycolysis, no biosynthetic pathways). [L22 p55]

They will steal ATP if available, using a translocase transporter *(protein that carries molecules across)*. [L22 p55]

Gimenez stain of tick hemolymph: host cells packed with small red-stained rods. [L22 p55]

Infection: phagocytosis *(immune cells eating germs)* is induced; the bacterium escapes from the phagosome. [L22 p55]

Exit: lysis (R. prowazekii), filopodium focal lysis (R. rickettsii) or budding (R. tsutsugamushi). [L22 p55]

2. Chlamydia

Book: Not in Chapter 6; his outline marks this part "(notes)": study the slides.

⚠ **Do not mix up: EB is the DORMANT *(inactive, not growing)*, infectious form outside cells; RB is the GROWING form inside.** [L22 p56]

Chlamydia EB form: **only 0.3 um**. [L22 p56]

Chlamydia genome: **1,000** kB, about **900** genes. [L22 p56]

Multiplication only inside host cells: an obligate intracellular parasite. [L22 p56]

Two forms: RB *(reticulate body)* and EB *(elementary body)*. [L22 p56]

RB: the growing form, inside the cell. [L22 p56]

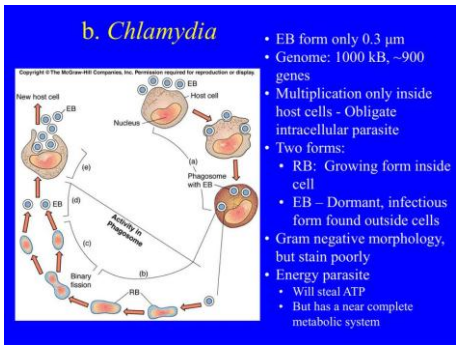
EB: the dormant, infectious form, found outside cells. [L22 p56]

Gram negative *(thin wall plus outer membrane)* **morphology**, but they stain poorly. [L22 p56]

Energy parasite: will steal ATP *(cell energy molecule)*, but has a near complete metabolic system. [L22 p56]

Cycle: EB attach and enter; EB become RB; RB divide by binary fission. [L22 p56]

Then RB turn back into EB, which are released to a new host cell. [L22 p56]



Chlamydia cycle: EB enter, become RB, divide, turn back into EB and leave [L22 p56]

3. Endosymbiotic bacteria

Book: Not in Chapter 6; his outline marks this part "(notes)": study the slides.

⚠ **Do not mix up: Endosymbionts have the SMALLEST bacterial genomes, yet still have their own DNA *(cell genetic instruction molecule)* and ribosomes *(protein building machine)*.** [L22 p57]

Endosymbiotic bacteria live inside cells or a body part of another organism. [L22 p57]

Some are obligate endosymbionts. [L22 p57]

Genome: as small as **150** kB, **182** genes, the smallest bacterial genomes. [L22 p57]

They have their own DNA, ribosomes and some metabolic enzymes. [L22 p57]

They grow independently by dividing. [L22 p57]

Mitochondria and chloroplasts

Book: Not in Chapter 6; his outline marks this part "(notes)": study the slides.

Mitochondria and chloroplasts *(plant part that does photosynthesis)*: obligate intracellular symbionts. [L22 p58]

Mitochondria genome: **6** to **300** kB, **30** to **60** genes. [L22 p58]

Chloroplast genome: **70** to **200** kB, about **120** genes. [L22 p58]

They grow independently by dividing and fusion. [L22 p58]

They synthesize their own DNA *(cell genetic instruction molecule)*, RNA, ribosomes *(protein building machine)*, tRNA *(adapter that carries amino acids)* and some metabolic enzymes. [L22 p58]

They generate ATP *(cell energy molecule)* **for their own use** but get many metabolites *(small working molecules of the cell)* from the host cell. [L22 p58]

Hypothesis: mitochondria came from an ancestor of Rickettsia; chloroplasts from a cyanobacterium. [L22 p58]

4. Giant viruses

Book: 6.3 Viral Genomes and Classification, Viral Genomes: Small or Large (chapter 6 PDF, p. 47).

⚠ **Do not mix up: Typical viruses have DNA *(cell genetic instruction molecule)* or RNA but NOT both; some giant viruses contain both.** [L22 p09, L22 p59]

Giant viruses: size over **0.15 um**. [L22 p59]

Giant virus genome: **100** to **1,000** kB, **100** to **1,100** genes. [L22 p59]

Some contain both DNA and RNA. [L22 p59]

Some produce enzymes involved in translation. [L22 p59]

Some can be infected by viruses. [L22 p59]

Giant viruses have been proposed as a fourth domain of life. [L22 p59]

Genome size: Mimivirus (about **1.2** million bp) is larger than Rickettsia, Chlamydia and Mycoplasma. [L22 p59]

Which would you consider a living organism?

Book: Not in Chapter 6; his outline marks this part "(notes)": study the slides.

Living or not: weigh each on reproducing by itself, ribosomes *(protein building machine)*, metabolism and genome size. [L22 p60]

Viruses have no ribosomes and are assembled from parts, not by splitting. [L22 p37, L22 p52]

Typical and atypical bacteria and viruses compared

Organism	How it reproduces	What it has	Genome
Typical bacteria	By itself; free-living	Ribosomes; independent biosynthesis	**500** to **10,000** kB
Rickettsia	Only inside host cells	DNA, RNA, ribosomes; lacks glycolysis, steals ATP	About **1,000** kB, **830** genes
Chlamydia	Only inside host cells; RB divide by binary fission	Near complete metabolic system; steals ATP	**1,000** kB, about **900** genes
Endosymbiotic bacteria	Grow independently by dividing	Own DNA, ribosomes, some metabolic enzymes	As small as **150** kB, **182** genes
Mitochondria and chloroplasts	Grow by dividing and fusion inside host cells	Own DNA, RNA, ribosomes; many metabolites from the host	**6** to **300** kB; **70** to **200** kB
Giant viruses	Viruses: no activity outside host cells	Some have DNA and RNA; some make translation enzymes	**100** to **1,000** kB
Typical viruses	Only inside cells; assembled from parts	No ribosomes; no metabolism outside host cells	**1.8** to **100** kB, **1** to **100 proteins**

Table source: [L22 p52, L22 p53, L22 p55, L22 p56, L22 p57, L22 p58, L22 p59, L22 p60]