Exam 2 Study Guide (text version)

BIOL 4640 Microbiology, Dr. Rowen, Exam 2 on Wednesday 10/28/26. This is the essay version of the study guide as plain text, so it is quick to read and search. Each section links its textbook chapter (Slonczewski, *Microbiology: An Evolving Science* 6e). The PDF versions are on the Study Guides page.

A. Size and shapes of bacteria B. External Structures of Bacteria

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E Cell division and Differentiation F. Viruses G. Atypical Bacteria and Viruses (notes)

A. Size and shapes of bacteria

Textbook: Ch 3 Cell Structure and Function

Bacteria can be described by their size and by their shape. In terms of **size**, eukaryotic cells are bigger than bacteria, and bacteria are bigger than viruses. This means the order from largest to smallest is eukaryotic cells, then bacteria, and then viruses.

Bacteria also come in a few basic **shapes**. Cocci are spherical, whereas bacilli are rods. In addition, coccobacilli are short, vibrios are curved rods, and spirals are helical.

B. External Structures of Bacteria

Textbook: Ch 3 (3.6 Specialized Structures) · Ch 25 (25.2 Microbial Attachment)

The external structures of bacteria are the parts found on the outside of the cell. **Flagella** are used to move, while **pili** and fimbriae are used for adhesion, which means sticking. Stalks are also used for adhesion, and **nanotubes** are extensions between 2 different cells.

1. Flagella and motility

Flagella are closely tied to motility, and each flagellum has four parts, which are the basal body, the hook, the filament, and the motor. The **motor** turns either CCW or CW, and this turning is used to drive swimming, periplasmic, and swarming motility, as well as chemotaxis.

In contrast, **non-flagellar motility** uses no flagella at all, and it happens only on a surface. Examples of this kind of movement are twitching, gliding, and sliding.

ARRANGEMENTS OBSERVED

Bacteria show several different arrangements of flagella. A cell could have 0 flagella or many flagella, and the flagella can be seen by a **flagellar stain** or by electron microscopy. Flagella can be found in 3 locations, which are at one end of the cell, at both ends of the cell, or all over the cell, and this all-over arrangement is called **peritrichous**. **Spirochetes** are special because their flagella are in the periplasm, where they extend from each pole and then wrap around the cell. Cells can also lose or regain their flagella, and the number and location of the flagella can be used to describe species and tell them apart.

PARTS AND FEATURES OF FLAGELLA

Flagella are built from several parts, and each part has its own features. A flagellum is made of 40 proteins, and its parts are both in and out of the membrane and the cell.

The **basal body** has 15 proteins and spans the membrane. The L ring sits in the outer membrane and the P ring sits in the peptidoglycan, while the S and M rings sit at the cell membrane, and a central rod finishes the basal body. Next comes the **hook**, which is made of a hook protein and 2 HAPs.

The **filament** is usually made of just one protein, called **flagellin**. Its subunits self-polymerize in a helix to form a hollow tube that is 14-20 nm wide and up to 5-10 μm long, and a different protein (the cap) sits on the tip. Cells can also have several flagellin genes and change which one is used.

Finally, the **motor** is made up of a rotor and Mot proteins, which help with the movement of the flagellum. The rotor is the C ring (switch) that determines whether the flagellum turns CW or CCW, while the Mot proteins are what H+ flows through.

Order of assembly of flagellum.

The flagellum is assembled in a set order, and it starts with the **basal body** in steps 1-5. First, the M ring is added in step 1 and the S ring is added in step 2, and both of these rings go in or at the cell membrane. Next, in step 3, the rod is added and capped, and it runs from the cell membrane to the peptidoglycan. Then the P ring is added in the peptidoglycan in step 4, and finally the L ring is added in the outer membrane in step 5.

After the basal body, the **hook** is built in steps 6 and 7, which happen outside the cell. In step 6 the hook is added, and then in step 7 the hook is finished.

Next, the **filament** is added in step 8, and it sticks out from the cell. The filament grows at the tip as flagellin subunits are pushed out through the hollow core of the flagellum and added to the end.

Finally, the **motor** comes last, in step 9. In this step the motility (Mot) proteins are added in the cell membrane, which enables movement.

TYPES OF MOTILITY OBSERVED IN BACTERIA: KNOW HOW EACH MEDIATED

Bacteria show several types of **motility**, which means purposeful movement in liquid or across a surface. Some of these types use flagella, and the standard one is **swimming**, which happens in liquid and is made up of runs and tumbles. During a run the flagella are on and the cell moves forward, while during a tumble the cell stops and reorients, so the next run goes in a random direction. In a cell with many flagella (**peritrichous**), the turning happens for all of the flagella together. In this kind of cell, CCW rotation bundles the flagella into a run that is slower than the run of a cell with a single flagellum, and CW rotation pushes all the flagella apart so the cell tumbles. In contrast, a cell with a single flagellum runs by going either CW or CCW, and it tumbles by stopping or by reversing the direction it was originally running.

Cells also use runs and tumbles for **taxis**, which is movement in response to a stimulus, either toward an **attractant**, such as nutrients, or away from a **repellent**, such as waste products. Cells have receptors for attractants and repellents, so they can sense a change in concentration and control how long their runs last. While heading toward an attractant, the cell keeps its flagella on for longer runs, or it tumbles when it stops. If there is no attractant or repellent, the path is a short random zigzag, because the cell keeps on running (moving forward) and then tumbling (stopping and then changing its direction or angle). Even during taxis, however, tumbles never stop completely. The cell still drifts toward the attractant overall because the CCW runs heading toward it last longer, while tumbles (stopping and reorienting) still happen between the runs.

Another type of motility that uses flagella is **periplasmic motility**, which happens in liquid in spirochetes. In the periplasm of spirochetes, the flagella extend from each pole of the cell and wrap around the cell. The direction of rotation at each pole decides

which way the cell moves. This turning makes a wiggling or corkscrew motion, which is good for burrowing or for going inside thick mucus.

Swarming also uses flagella, but it happens on a surface. In swarming, the bacteria move across a surface or semi-solid media together as a group (raft), and this requires a slime layer. The flagella may also change position to the sides (lateral). Swarming is seen in some species, like E. coli or Proteus.

Other types of motility use no flagella at all. **Twitching** is motility across a surface that uses type IV pili, and it is seen in several Gm neg species, where the pili are thought to extend, attach, and shorten, which then pulls the cell forward. **Gliding** is also motility across a solid surface that is not done by flagella, and it is seen in over 12 genera, for example Myxococcus. Its mechanism is not understood in all genera, but in Bacteroidetes, for example Flavobacterium, it has been proposed that motility proteins in the membrane act like feet, with no slime. Finally, **sliding** is passive spreading that is pushed by dividing cells, and it needs extra surface factors such as surfactants or an exopolysaccharide or protein slime layer.

CHEMOTAXIS: WHAT IS IT

To understand **chemotaxis**, it helps to start with **taxis**, which is the general term for movement in response to an external stimulus or effector, either toward it or away from it. These stimuli include nutrients and other chemicals, metals (many of them toxic), light, temperature, pH, and oxygen level. These stimuli can affect both motility and taxis.

Chemotaxis is one kind of taxis, and it is the kind that involves chemicals. It is movement toward **attractants** or away from **repellents**, such as waste products. The cell has receptors for them, which are proteins in the cell membrane, and these receptors send a signal that affects whether the flagella turn. As a result, the cell either goes forward (run) or stops and reorients (tumble). CCW rotation moves the cell toward an attractant, and as the cell moves up an attractant gradient, the runs get longer between each tumble.

HOW ONE COULD DETERMINE IF A BACTERIUM IS MOTILE AND IF HAS FLAGELLA

There are several ways to determine if a bacterium is motile and if it has flagella. The first is **microscopy**, where live wet mounts are watched under bright-field or dark-field. The movement must be purposeful and not just vibrational. However, a lack of observed movement is not definitive, which means it does not prove that the bacterium is non-motile.

Another way is to use a **flagellar stain** or EM, which detects the flagella themselves. The stain makes the flagella on the cells visible under the light microscope, while EM images show the flagella.

A third method is **motility agar**, where the cells are stabbed into semi-soft agar (0.75% instead of 1.5%). If there is turbidity throughout the agar, the bacterium is motile, but if there is growth only at the stab, it is non-motile. However, this method may not detect all forms of motility.

A **swarm plate** can also be used. For this test, cells are put in the center of a soft agar plate, since bacteria can swim through semi-solid agar, and then you look for the bacteria to spread out in a circle. The wild type (the normal strain, with working chemotaxis) makes a large, wide zone, while che (chemotaxis) mutants, which have che genes deleted, for example cheB, stay as a small spot at the center. Swarming shows up as a ring pattern (concentric rings of growth) or a dendritic pattern (branching arms of growth).

Finally, for a **chemotaxis plate**, cells are put in a well at one end of a soft agar plate and the test substance (a potential attractant) is put at the other end, and then the cells are counted. If more cells move toward the substance, then it is an attractant. This plate relies on taxis, which is movement toward or away from a stimulus (attractants and repellents).

2. Pili and Fimbriae

Pili are protein tubes that are thinner, shorter, and more numerous than flagella, and they are found mostly in Gm neg bacteria. There are 3 main types of pili structures, which are common pili (**fimbriae**), type IV pili, and sex pili. Together, they are used for adherence, twitching, and DNA transfer.

A. STRUCTURE, ARRANGEMENT IN CELLS AND ASSEMBLY

Structurally, a pilus is made of one **pilin** and its many copies, and it has an **adhesin** tip (made of 1-2 different proteins) that can bind sugars. New subunits are added at the base of the pilus. Cells can also switch which pilin they make, since they may have multiple pilin genes. **Sex pili** are longer and wider than common pili, but they are less numerous, with under 10 per cell. Finally, fimbriae are formed by Type I, P, V, curli, and Tad pili.

B. ROLES OF COMMON PILI, SEX PILI AND TYPE IV PILI

Common pili, sex pili, and Type IV pili each have their own roles. **Common pili** (Type I, P, V, curli, and Tad) are fimbriae that attach to surfaces with tip adhesins that bind sugars, and these adhesins are virulence factors. Common pili also build communities of bacteria that stick to a surface and to each other and are held together in a slime, which is called a **biofilm**.

Within the common pili, each type has a more specific job. Type I pili bind mannose and are used for adhesion and biofilms, while P pili bind urinary tract cells and are used for adhesion in UTIs. Type V pili are short adhesion fibers that are used for adhesion and aggregation. Curli are amyloid fibers of E. coli and Salmonella, and they are used for attachment, aggregation, and biofilms. Finally, Tad stands for "tight adherence," and it is used for strong surface attachment.

Besides the common pili, **Type IV pili**, found in Pseudomonas and Neisseria, twitch by extending, attaching, and shortening. They also adhere, build biofilms, and take up DNA. In contrast, **sex (F) pili** are made by conjugative plasmids. They attach to another cell and shorten to pull the two cells together, and then the DNA is transferred by conjugation.

Less important details: Type IV secretion pili move DNA or protein, and competence pili take up DNA in transformation (DNA uptake). Type IV-like pili are also found in Gm pos bacteria.

C. ASSEMBLY OF TYPE I AND TYPE IV, HOW DIFFERS FROM FLAGELLAR

The assembly of Type I and Type IV pili differs from the assembly of flagella. Pili subunits are added to the base of the filament, which causes the tip to extend. In contrast, flagella grow from the tip, because the flagellin subunits pass through the hollow core and are added at the tip.

Type I pili are chaperone-usher assembled, which means they are built with the help of a chaperone protein that carries each subunit and an usher channel that puts it in place. P pili are built the same way, and the example proteins **PapD** and **PapC** are from P pili. First, the proteins are secreted into the periplasm by the Sec system (the general secretion system), and then they are bound by the chaperone PapD. Next, they enter the usher channel PapC in the outer membrane, where a single pilus is assembled on the outer membrane. The pilus starts with the adhesin (the sugar-binding tip protein) on the tip, and then pilin proteins are added on the base of the shaft of the filament.

Type IV pili are built in a different way. In **Type IV** pili, the pilin called **PilA** is assembled into a filament in the inner membrane, and then it is pushed out through a channel in the outer membrane using the channel protein **PilQ**. The pilus is shortened by disassembling pilin from the base, and this is the twitching shortening that pulls the cell forward.

3. Other external structures

Besides flagella and pili, bacteria have other external structures, such as stalks and nanotubes. **Stalks** are used for adhesion. **Nanotubes** are extensions of the cytoplasm or periplasm between 2 cells, and the 2 cells can be different species. Nanotubes help the cells share proteins and nucleic acids (mRNA).

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

Textbook: Ch 3 (3.3 The Envelope) · Ch 19 (19.1 Archaea) · Ch 27 (27.1-27.3 Antibiotics and Resistance)

The **cell envelope** has a cell membrane, cell wall, outer membrane, glycocalyx or capsule, S layer and periplasm. Different styles of envelope have different layers and components, and these affect the transport of material into the cell and the sensitivity to antibiotics and disinfectants, because drugs and chemicals have to pass through the envelope to enter the cell. These differences can also be used to group or identify bacteria.

These differences change how sensitive bacteria are. For example, Gm + bacteria are more sensitive to some penicillins (susceptible, so the drug is more effective), while Gm - bacteria are less sensitive (the drug is less effective), because the drug does not pass through the outer membrane and so does not reach peptidoglycan synthesis in the periplasmic space. With hydrophobic molecules such as bile salts, dyes or some antibiotics, Gm - is less sensitive (resistant, so they are less effective) because the LPS sugars of the outer membrane block diffusion, while Gm + have no outer membrane to block them. The Gm - outer membrane blocks hydrophilic molecules as well (so they are less effective), except small ones (under 700 daltons) that pass through porins. In the same way, Gm + are more sensitive to disinfectants, which are more effective on them since the chemicals reach the wall and membrane, whereas Gm - are less sensitive and disinfectants are less effective since the outer membrane keeps them out.

Other envelope types have their own sensitivities. **Mycobacteria** are LESS sensitive (resistant, so these are less effective) to acids, bases, antibiotics and dehydration because of their mycolic acids. Mycoplasma and Archaea, on the other hand, are NOT sensitive (they are resistant, and penicillin is not effective) because they have no peptidoglycan. **Lysozyme**, in contrast, works on both Gm + and Gm - (it is effective on both), and Gm + is left as a protoplast while Gm - is left as a spheroplast in isotonic solution.

The envelope can also be used to identify bacteria, and the first way is the **Gram stain**, where Gm + is purple because the thick peptidoglycan wall holds the stain, while Gm - is pink and has a thin wall. However, the color does not always match the actual envelope, and some bacteria stain poorly or not at all, such as Rickettsia or Chlamydia, which have Gram negative morphology but stain poorly. Next, the **acid-fast stain** picks out mycobacteria (they turn red), since it detects the mycobacterial envelope with mycolic acids. With EM (TEM), you can see the layers directly, so Gm + shows one thick dark layer of peptidoglycan and no outer membrane, while Gm - has 2 dark bands separated by a lighter space.

Other tests also help. Selective media such as MacConkey agar are useful, since no growth on a selective medium for Gm - supports Gm +. Penicillin sensitivity is another clue, because some penicillins are more effective against Gm + as they cannot pass the Gm - outer membrane to reach the peptidoglycan in the periplasm, and they have no effect on Mycoplasma or Archaea as these have no peptidoglycan. Finally, biochemical isolation finds unique components in a specific type of envelope, such as teichoic acid from the wall of Gm +, and you can also look for the genes encoding the components or the enzymes that make them, such as outer membrane proteins and LPS (Gm -), teichoic acids (Gm +), mycolic acids (Mycobacterium) or pseudopeptidoglycan (Archaea).

In general, cell envelopes have **six layers**, which from outside to inside are the **glycocalyx or capsule**, then the **S layer**, then **the outer membrane**, then the **cell wall** (peptidoglycan), then the **periplasm**, and finally the **cell membrane**. Of the two membranes, the outer membrane is made up of lipopolysaccharides, phospholipids and proteins, while the cell membrane is made up of phospholipids and proteins. While all types of bacteria have the cell membrane as the innermost layer, each type has a different order from outside to inside.

In **Gm +**, the outermost layer is a **capsule** in some, made of sugars (glycosyl sugar chains) or proteins, with peptide capsules more common in Gm +, then an **S layer** of protein in some, and then the **cell wall** of thick peptidoglycan (3-20 layers) with teichoic acid in most, which runs through the peptidoglycan and is linked to it or to membrane lipids (lipoteichoic acid). Under the wall is the **cell membrane** (proteins, phospholipids and other lipids), but there is no outer membrane, and in EM Gm + shows one thick dark layer.

In **Gm -**, the order is an **S layer** of protein in some, then a **capsule** of sugar in some, and then the **outer membrane** (LPS with Mg2+, proteins such as porins, and phospholipids), which is linked by lipoproteins to the thin peptidoglycan **cell wall** in the **periplasm**. After that come the periplasm with the thin peptidoglycan and then **the inner (cell) membrane** made up of proteins and phospholipids, and in EM Gm - shows two dark bands separated by a lighter space.

Mycobacteria (detected using acid-fast) have their own order, starting with a **capsule** (sugars and glycolipids) on the outside and then a **cell wall** that goes from mycolic acids (a mycomembrane attached to arabinan) to arabinogalactan (chains of arabinose and galactose sugars, linked to peptidoglycan and mycolic acid) and then to peptidoglycan. The arabinan, galactan and peptidoglycan form the cell wall core, and under it is the **cell membrane** (phospholipids and proteins). Some antibiotics used against mycobacteria, such as ethambutol, block arabinogalactan synthesis. **Archaea** have an **S layer** in most (and it is sometimes the only wall), then **pseudopeptidoglycan** in some, and then an **isoprenoid cell membrane** with no phospholipids. Finally, **Mollicutes** have a sugar layer (lipoglycans) and then a **cell membrane** with sterols (that help stabilize the membrane), but they have **no cell wall**. This means penicillin won't work on them, as there is no peptidoglycan.

2. Know the role, feature and basic composition of each of these

The cell envelope is made of **six layers**, which are the glycocalyx (capsule), the S layer, the outer membrane, the cell wall, the periplasm and the cell membrane. Each one of these layers has its own role, features and basic composition.

Looking at the main components, the **outer membrane** is made up of LPS (lipopolysaccharide) and phospholipids. The **cell wall** components are peptidoglycan, pseudopeptidoglycan, teichoic acid, mycolic acids and arabinogalactan, and finally the **cell membrane** is made up of phospholipids, isoprenoids and hopanoids.

A GLYCOCALYX (CAPSULE AND SLIME LAYER)

The **glycocalyx** is the extracellular (outside the cell) material beyond the cell wall, and its composition varies between species. Under darkfield, it shows up as halos around the cells. It comes in two types, which are a slime layer that is loosely attached or a capsule that is more firmly attached.

In terms of its composition, the glycocalyx is usually **polysaccharides**, which are short chains of a homopolymer or heteropolymer of sugars (the same sugar repeated or different sugars mixed) that are called extracellular polysaccharide (EPS). It may also be peptides (short amino acid chains), which are more common in Gm +, for example the polyglutamic acid of Bacillus anthracis. In addition, it may contain DNA. This means the 3 molecule types to list are sugars, peptides (proteins) and DNA, while glycolipids are not on this list because they belong to the mycobacterial capsule.

The composition varies not only between species but also between strains of the same species, and even during the life of an organism, like how E. coli has about 80 capsular polysaccharides (the K antigen). As for its **role**, the glycocalyx is used for attachment (biofilms), protection from phagocytosis (getting engulfed or eaten by a cell), and protection from desiccation (drying out).

B. DAM: COMPOSITION AND WHERE LOCATED

The **S layer** (surface layer) is a layer of proteins or glycoproteins (proteins with sugars attached), made of a complex of a few proteins, that makes a crystalline (regular repeating pattern), porous matrix. It is found on most (?) bacteria, especially Archaea, but not on all of them, and it is usually lost in strains that are cultured in the lab.

The S layer is located on the outside of the cell wall or membrane, so where it sits depends on the type of cell. In Gm +, it is above the peptidoglycan, while in Gm - it is above the outer membrane (LPS). In Archaea, it sits right on the cell membrane, and in some of them it is the only wall.

The roles of the S layer are only **hypothesized**, because they are not totally understood. These hypothesized roles are protection from osmotic stress and viruses, a barrier to large molecules, shape (in Archaea), attachment to surfaces, and a site for attaching

proteins.

C. OUTER MEMBRANE

The **outer membrane** is the second membrane of Gm - bacteria. It has LPS (lipopolysaccharide) in the outer leaflet (the outer half of the membrane), and it also has porins (protein channels) that let hydrophilic (water-loving) molecules under 700 daltons pass through. Finally, it has murein lipoprotein, which links the outer membrane to the peptidoglycan.

The outer membrane also acts as a **barrier**. For hydrophobic (water-fearing) molecules like dyes, bile salts and some antibiotics, the LPS is tightly packed, so it inhibits the diffusion of most of them. Hydrophilic molecules, on the other hand, are blocked by the membrane itself. As for the porins, they only let small molecules get through, and they are selective for type and size.

LPS

LPS (lipopolysaccharide) is found only in the outer leaflet of the Gm - outer membrane, where it is held in place with Mg2+ and its sugar chains point out. It is tightly packed because it only has saturated fatty acids, and bactoprenol is what carries it across the cell membrane.

LPS has 3 parts. The first is **lipid A**, which has 4-6 fatty acids attached to a disaccharide (2 sugars) of N-acetylglucosamine phosphate. Lipid A is an endotoxin (a toxin that is part of the cell itself) that can cause septic shock, and it induces an inflammatory response. The second part is the **core polysaccharide**, which is a branched chain of sugars that is the same for a species. The third part is the **O-specific polysaccharide** (O antigen), which is usually a 4-5 sugar repeat whose length and composition vary, and it sticks out from the outer membrane and also contributes to the glycocalyx.

The role of LPS is protection. This works because its sugars block the diffusion of hydrophobic molecules like bile salts, antibiotics and dyes.

D. CELL WALL - ROLE

The **cell wall** is a semi-rigid, porous layer beyond the cell membrane. Its role is to give structural support and rigidity, help determine cell shape, and prevent cells from lysing (bursting) in hypotonic conditions (less solute outside than inside, so water comes in). Its composition varies, but most eubacteria have one principal (main) component. The bacterial wall components are peptidoglycan (murein), pseudopeptidoglycan, teichoic acid, mycolic acid and arabinogalactan.

The wall also affects **water movement**. In cells with a wall, the protoplast (the cell inside the wall) shows plasmolysis (shrinks away from the wall) in hypertonic solution. In contrast, cells lacking a wall swell in hypotonic solution until it ends in osmolysis (bursting), and they shrink in hypertonic solution.

Some **antibiotics** block cell wall synthesis. Most antibiotics are only **bacteriostatic** (stop growth), but the ones that block peptidoglycan synthesis, like penicillin, are **bactericidal** (kill) in hypotonic conditions such as the human body or the lab. They kill because, in growing cells, holes form in the peptidoglycan, so the membrane expands out until it lyses as water comes in by osmosis. The drug may also activate autolysin, which is a bacterial enzyme that cleaves (cuts) peptidoglycan.

Less important details: other organisms have other kinds of walls. For example, plants have cellulose (chains of glucose), and fungi have chitin (chains of a modified glucose).

1) Peptidoglycan: structure: sugars and

Peptidoglycan (murein) is the main component of most bacterial cell walls, and it forms one large, interconnected molecule around the outside of the cell. Its sugars are long glycan (sugar) chains of two alternating sugars, N-acetylglucosamine (GlcNAc, NAG or G) and N-acetylmuramic acid (MurNAc, NAM or M), which are linked by a β-1-4 linkage. These chains are cross-linked by

peptides of amino acids that come off NAM, and while the sequence varies, most contain some D-amino acids (the L-forms are the ones in proteins). For the **cross-link**, the peptide starts with five amino acids, and the 5th one (D-Ala) is cut off between the two D-alanines when the cross-link is made. Penicillin blocks this step, and vancomycin binds the D-Ala-D-Ala. The thickness of peptidoglycan also varies, and many antibiotics inhibit its synthesis.

Peptidoglycan is different in Gm - and Gm +. In **Gm -**, it is thinner, with 1-2 layers (in the periplasm), and the peptide is L-Ala, then D-Glu, then DAP (diaminopimelic acid, a modified lysine), then D-Ala, so the D amino acids are in the 2nd and 4th positions. The DAP (3rd) links directly to the D-Ala (4th) of the next peptide. In **Gm +**, for example Staphylococcus, there are 3-20 layers, and the peptide is L-Ala, then D-Glu, then L-Lys, then D-Ala. Here the L-Lys (3rd) links to the D-Ala of the next peptide through a pentaglycine (Gly)5 bridge (a bridge of 5 glycines). So the main differences are that Gm - has fewer layers, DAP as the 3rd amino acid and a direct link, while Gm + has more layers, L-Lys as the 3rd and a (Gly)5 bridge.

Peptidoglycan synthesis follows a basic order of events. First, the cell **makes UDP-NAM and NAG**, then it **adds** the **pentapeptide** (the 5 amino acid peptide), and next the unit is **attached** to **bactoprenol**. After that, **NAG** is **added**, and then the **unit is flipped out** to the other side of the membrane (**MurJ**). Finally, **PBPs** (penicillin binding proteins) **link** the **sugars** (transglycosylation) and then **cross-link** the **peptides** (transpeptidation). **Bactoprenol** is used because it carries the peptidoglycan units (and also LPS and teichoic acid) across the cell membrane, and then it must be recycled back to bactoprenol-P, which is the step bacitracin blocks.

2) Pseudopeptidoglycan: how differs from peptidoglycan

Pseudopeptidoglycan is found in the cell wall of some archaeal bacteria. For example, in Methanothermus it is a thick layer under the S layer.

Pseudopeptidoglycan differs from peptidoglycan in a few ways. First, for its sugars, it keeps NAG (N-acetylglucosamine) but has NAT (N-acetyltalosaminuronic acid) instead of NAM. Next, the sugars are joined by a β-1-3 linkage instead of β-1-4, and this link is lysozyme-insensitive (lysozyme can't break it). Then, it is still cross-linked by peptides, but they are all L-amino acids (Glu, Ala, Lys), while peptidoglycan peptides contain some D-amino acids. Finally, it can have many layers, while Gm - peptidoglycan has 1-2.

3) Teichoic acids

Teichoic acids are polyanionic (many negative charges) polymers of glycerol phosphate or ribitol phosphate, modified with amino acids (D-Ala, D-Lys) or sugars, and they are found in most Gm +. They run through the peptidoglycan and are linked to it (teichoic acid) or to membrane lipids (**lipoteichoic acid**), and bactoprenol carries them across the cell membrane. Their composition varies, so they can be used for identification. As for their role, they regulate cell shape and cell division, keep the cell wall intact, and affect antibiotic resistance.

4) Mycolic acids

Mycolic acids are complex hydroxylated (have OH groups), branched-chain fatty acids with 60-90 carbons, and they are very hydrophobic (water-fearing). They are found in Mycobacteria (acid-fast), where they are linked to arabinogalactan sugars, making a mycomembrane in the cell wall. There, mycolic acids block the diffusion of most molecules, including nutrients. They also make the cells resistant to acids, bases, antibiotics and dehydration (drying out).

E. PERIPLASM

The **periplasm** is the aqueous (watery) compartment between the 2 Gm - membranes, which are the outer membrane and the inner membrane. This compartment carries out several functions.

The first function is **osmotic protection**, which comes from the oligosaccharides (short sugar chains) it contains. It also helps with transport, because proteins bind solutes and hand them off to the transporters in the inner membrane. In addition, the periplasm carries out the hydrolysis (breaking down) of nutrients into smaller subunits that can be transported into the cytoplasm. Finally, it has a role in electron transport reactions that store H+ ions.

This leads to the **proton motive force**, because the stored H+ ARE the proton gradient across the inner membrane. So when H+ flows back in, it powers ATP synthesis (ATP synthase makes ATP) and the ion-coupled transport of molecules against their gradient.

F. CELL MEMBRANE

The **cell membrane** is the lipid and protein boundary, and its jobs are being a permeability barrier (controlling what gets through), transport, ion gradients, sensing and lipid synthesis. For energy, the electron transport chain pumps H+ out, which creates a proton gradient (chemiosmotic force or proton motive force), and then H+ flowing back in through ATP synthase makes ATP. This happens across the inner membrane of Gm - bacteria and mitochondria. In bacteria, the **proton motive force** powers 3 things, which are ATP synthesis, the transport of solutes (ion-coupled transport and efflux pumps), and flagellar rotation.

Hopanoids and isoprenoids each have their own role and function. **Hopanoids** are 5-ring hydrocarbons similar to sterols (like cholesterol), found in the cell membrane of some bacteria, where they sit among the phospholipid tails and have hydroxyl (OH) groups. Their role comes from being sterol-like, since sterols help stabilize the membrane (as in Mycoplasma). **Isoprenoids**, on the other hand, are the ether-linked 20 or 40 carbon lipids of the Archaea cell membrane (covered in the Archaea part below), and they are not in the outer membrane, which has LPS and phospholipids. **Bactoprenol** (undecaprenyl) is a large isoprenoid phosphate (C55) in the bacterial cell membrane, and its role is to carry peptidoglycan units, LPS and teichoic acid through the cell membrane.

In bacteria, the membrane is made of lipids and proteins, and there are several types of lipids. **Phospholipids** are ester-linked (glycerol, 2 fatty acids and a phosphoryl head group) and are found in most bacteria, and their head group, chain length and double bonds vary. Hopanoids (5-ring, sterol-like) are also found in some Gm - and a few Gm +. Next, **cardiolipin** (2 phospholipids linked by a glycerol) is made in stationary phase and gathers at the bent poles. Finally, **membrane proteins** are the largest part by weight (75% in E. coli), and many of them are for transport.

The **Archaea cell membrane** is different, because it is made of isoprenoids and proteins, with no phospholipids. These isoprenoids are 20 or 40 carbon terpenoids made of branched 5 carbon isoprenoid repeats. What makes them different is that they are linked to glycerol by ether linkages, instead of the ester-linked fatty acids that bacteria have. A bilayer (glycerol diethers) has 20 carbon isoprenoids (the R group is hydrogen, sugar or phosphate), while a monolayer (diglycerol tetraethers) has 40 carbon isoprenoids.

Phospholipids also alter (change) their fatty acid chain length and saturation (number of double bonds) in response to **temperature**. A low temperature leads to more unsaturated fatty acids. For example, in E. coli the ratio of unsaturated to saturated is 2.9 at 10 °C, 2.0 at 20 °C, 1.6 at 30 °C and 0.38 at 40 °C, so the colder it is, the higher the ratio. To compare the two kinds, palmitic acid 16:0 is saturated (16 carbons and 0 double bonds), while oleic acid 18:1 has one double bond, so it is unsaturated (cis or trans).

3. Movement of Material across Cell membrane

To understand movement of material across the cell membrane, the first thing to know is that cells keep certain ions and solutes inside at levels that differ from the outside. For example, K+ is high inside, while Na+ and Cl- are high outside, and the **membrane potential** is net negative (about -60 mV, with the cytoplasm negative). For energy, the electron transport chain pumps H+ out, which creates an electrochemical gradient with + outside and the cytoplasm negative. This gradient is called the **proton gradient** (the chemiosmotic force or proton motive force), and it is found across the inner membrane of Gm - bacteria and mitochondria. When H+ flows back in through ATP synthase, it makes ATP, and in bacteria this flow also powers transport.

There are different types of transport across the membrane, and the first is **passive transport**, which moves solutes down their concentration gradient, their charge gradient, or both (from high to low), so no energy is needed. In **simple diffusion**, molecules go straight through the bilayer. The molecules that can do this are small uncharged molecules (O2, H2O), hydrophobic molecules, and the uncharged form of weak acids and bases, which include many drugs like aspirin, Prozac, penicillin, and tetracycline. Once inside, these weak acids and bases deprotonate (lose an H+) and become water soluble. In **facilitated diffusion**, the molecule goes through a membrane protein instead, and this also needs no energy. The protein is either a channel protein (the outer membrane porins) or a carrier in the cell membrane, which is rare (for example, the glycerol transporter).

In contrast, **active transport** moves a molecule against its concentration gradient, so energy is required, either from an ion gradient or from a high energy phosphate bond. In **ion-coupled** transport, an ion moving with its gradient powers the transport of a molecule against its gradient. A symport moves them in the same direction (for example, lactose permease with H+), whereas an antiport moves them in opposite directions (for example, Na+ and H+). The energy for this comes from the H+ gradient in most cases, with H+ going in, or from the Na+ gradient in some cases.

Another kind of active transport uses **ABC transporters**, which are a transmembrane domain plus an ATP binding domain. Each one is made of 2 or 4 proteins, and E. coli has 78 of them. They may use a solute-binding protein in the periplasm to bring the solute, and they either bring solutes in (galactose, GAGs, and siderophore-Fe3+) or pump chemicals out (the multidrug exporters). Their energy is ATP, through cleavage of a high energy phosphate bond.

Finally, **PTS group translocation** modifies the sugar during transport. A phosphate from PEP is passed from Enzyme I to HPr and then to Enzyme II, and it is added to the sugar as the sugar enters, so glucose becomes glucose-6-phosphate. This type of transport is found only in bacteria and some Archaea, and its energy comes from PEP (a high energy phosphate bond).

TYPE OF TRANSPORT PROTEINS OBSERVED IN BACTERIA

- The transport proteins observed in bacteria include **channels**, also called **porins**, which are protein channels that form a barrel in the Gm - outer membrane. They work by facilitated diffusion, so they are passive and use no energy, and there are a few different types that are selective for the type or size of molecule, with a max of 700 daltons (short chains of sugars and amino acids). However, porins only get molecules from the outside into the periplasm, and then an inner membrane transporter has to move them into the cytoplasm.
- Several specific porins are good to know, starting with OmpF, which is a nonspecific channel for small molecules and the receptor for phage T2. OmpC is also a nonspecific channel for small molecules, but it is the receptor for phage T4 instead. In contrast, OmpA is specific for peptides and is the receptor for mating pili. LamB lets in maltose and maltodextrin and is the receptor for phage λ, and finally PhoE is an anion-selective channel, which means it selects for negative ions.
- Another type of transport protein is the **carrier**, which binds a specific molecule and changes shape to carry it across, but carriers are uncommon. One example is **GlpF**, the glycerol transporter, which brings glycerol in because glycerol stays low inside as it gets used up.
- Bacteria also have **ion-coupled** transporters. In a symporter such as lactose permease, lactose and H+ come in together, whereas in an antiporter, Na+ goes out while H+ comes in.
- Next, **ABC transporters** have a transmembrane domain plus an ATP-binding domain, and ATP powers the move. For example, the galactose-binding protein is a solute-binding protein in the periplasm that hands galactose to the transporter, and the GAG transporter in Streptobacillus brings in glycosaminoglycan (GAG) disaccharides.
- Another example is **MacB, MacA, and TolC**, which make up an ABC multidrug exporter (efflux pump) that transports chemicals out, such as erythromycin. In this pump, MacB in the inner membrane uses ATP, then MacA links it across the periplasm, and TolC is the outer membrane channel, so together they form one tube through the Gm - envelope.
- Another set, **FepA through FepG**, moves Fe3+ that is bound to a siderophore (an iron-binding molecule) into the cell. First, the siderophore-Fe3+ complex goes through FepA, which crosses the outer membrane, and then FepB escorts the complex.

Next, FepG, FepD, and FepC are the ABC pump, with FepC as the ATPase, and finally, inside the cell, Fe3+ is released and reduced to Fe2+.

• Finally, the **PTS enzymes** pass a phosphate from PEP to the sugar as it enters, so glucose becomes glucose-6-P, and they are found only in bacteria and some archaea. First, Enzyme I takes the phosphate from PEP, and then HPr passes the phosphate on to Enzyme II. **Enzyme II** includes IIA, IIB, and IIC, and IIC is the membrane channel that also phosphorylates (adds the phosphate to) the sugar as it comes in. Because the sugar is changed, free glucose stays low inside, and so glucose keeps moving in.

DIFFERENCE IN TRANSPORT OF IONS VERSE SUGARS

To understand the difference in transport of ions versus sugars, start with **ions**, which move with their own gradient (concentration and charge) and power other transport. Bacteria mostly use H+ going in, which is the proton motive force, while some use Na+, and haloalkaliphiles (bacteria that like salty, alkaline places) in Nebraska alkaline and brine lakes use both. The **ion carriers** are the antiporter for Na+ and H+, the K+ (potassium) uniporter, and the phosphate (HPO4 2-) and sulfate (HSO4-) symporters, which move them together with H+. These same gradients also drive ATP synthase, the flagellar motor, and drug efflux pumps.

Sugars, in contrast, are moved against their gradient, so they need energy. This energy can come from an ion gradient (for example, lactose moving with H+ on lac permease), from ATP through ABC transporters (for example, for galactose), or from PEP through PTS group translocation (for example, glucose becomes glucose-6-phosphate). So the main difference is that ions go with their own gradient and power other transport, while sugars get moved against their gradient using energy from an ion gradient, ATP, or PEP.

4. How do different antibiotics interfere with peptidoglycan synthesis?

Different antibiotics interfere with peptidoglycan synthesis by acting as **peptidoglycan blockers**, where each drug blocks one step of the synthesis, so growing cells get holes in the wall and burst. Most antibiotics only stop growth, but wall blockers like **penicillin** kill in hypotonic conditions, where water rushes in. These wall blockers may also activate **autolysin**, which is the bacteria's own enzyme that cuts peptidoglycan.

When we look at the targets of antibiotics more broadly, most antibiotics target **unique features** of bacteria, and most of them inhibit an enzyme involved in biosynthesis, which blocks the synthesis of a new cell component. These drugs may be cidal (they kill) or static (they inhibit growth). The first target is **cell wall synthesis**, since peptidoglycan synthesis is unique to bacteria, and the drugs here are penicillins, cephalosporins, monobactams, bacitracin, vancomycin, and D-cycloserine. Also, in mycobacteria, ethambutol blocks arabinogalactan synthesis.

Another target is **protein synthesis**, and this works because 70S ribosomes differ in size and components from eukaryotic ones, so they are selectively inhibited by drugs for the 50S and 30S subunits. These drugs are chloramphenicol, tetracyclines, aminoglycosides, macrolides, and lincosamides. Antibiotics can also target **cell membrane integrity**, and the drugs that do this are polymyxin, daptomycin, gramicidin, amphotericin, and imidazoles. Next, the drugs that target **nucleic acid function** (DNA or RNA) are quinolones, which act on DNA replication, rifampin and pyronins, which act on RNA polymerase, and also nitroimidazoles and nitrofurans. Finally, sulfonamides and trimethoprim target intermediary metabolism (metabolites).

KNOW SPECIFIC MECHANISM OF ACTION OF EACH OF THE FOLLOWING

Each drug and enzyme here has its own specific mechanism of action, starting with **penicillin** (β-lactam family), which has a structure similar to D-Ala-D-Ala, the bond that is cleaved in the transpeptidase reaction. Penicillin binds penicillin binding proteins (PBPs) and blocks transpeptidation (peptide cross-linking) and transglycosylation. It works only on actively growing cells, because growing cells must keep making new peptidoglycan for growth, division, and repair by cutting links in the old wall, so blocking cross-linking leaves holes and the cells lyse (burst). In contrast, dormant cells make little new peptidoglycan, so their existing wall stays intact and they survive.

The functional groups off the ring vary, which changes the spectrum of activity, the properties (acid stable or resistant), and the sensitivity to β-lactamases. Penicillinases attack the β-lactam ring, although methicillin is penicillinase-resistant. Also, **ampicillin** has an effect similar to lysozyme's, where holes in the wall let the membrane bulge out and the cells swell round at higher doses.

Cephalosporins have the same mechanism as penicillin, since they also have a structure similar to D-Ala-D-Ala (the peptide cleaved in the transpeptidase reaction), and they inhibit transpeptidase and transglycosylation. **Bacitracin**, on the other hand, blocks the recycling of bactoprenol, the lipid carrier. It does this by inhibiting the hydrolysis of the phosphodiester on bactoprenol-PP, so bactoprenol-P is not regenerated. With no bactoprenol-P, no new NAM-NAG units get carried out across the membrane.

Next, **vancomycin** is a large glycopeptide that binds the D-Ala-D-Ala end of the pentapeptide (L-Lys-D-Ala-D-Ala), which prevents cleavage and the transpeptidase cross-link, so it targets transpeptidation. Resistance to vancomycin has been seen in VRE (vancomycin-resistant Enterococcus faecium, 1987) and VRSA (S. aureus fully resistant to vancomycin, 2002). **D-cycloserine**, in turn, has a structure similar to ligated (joined) D-Ala-D-Ala. It is a competitive inhibitor that blocks Ala racemase, which turns L-Ala into D-Ala, and ligase, which makes D-Ala-D-Ala, by competing with their normal molecules. As a result, D-alanine and the D-Ala-D-Ala dipeptide are not made. Putting these together, penicillins, cephalosporins, and D-cycloserine all have a structure similar to D-Ala-D-Ala, but vancomycin does not, because it binds D-Ala-D-Ala instead.

Unlike these drugs, **lysozyme** is a defensive enzyme of our body that cleaves peptidoglycan by breaking the glycosidic bond between the sugars NAM and NAG, so a hole appears in the wall. In a low solute solution, water enters, and the cell bulges and lyses. In an isotonic solution, however, the wall peels off, leaving a round protoplast (Gm +) or spheroplast (Gm -). **Autolysin** also cleaves peptidoglycan, but it is the bacteria's own enzyme, and it cuts old peptidoglycan (the β1-4 linkage) at cell division.

Less important details: the penicillin variants, which have different functional groups off the ring, are penicillin G, penicillin V, ampicillin, carbenicillin, methicillin, and ticarcillin. The cephalosporin examples by generation are cephalothin for the first generation, cefoxitin for the second, and cefoperazone and ceftriaxone for the third.

HOW DO BACTERIA RESIST THE EFFECTS OF ANTIBIOTICS?

Bacteria resist the effects of antibiotics by destroying the drug (β-lactamase), pumping it out, keeping it out, changing the target (an altered PBP, which is a penicillin binding protein), or protecting the ribosome. These ways fall into three groups. First, they can **prevent entry**, which lowers drug levels, by destroying the drug (β-lactamase), pumping it out with a multidrug exporter or efflux pump, or reducing uptake by eliminating the transport protein or porin. Then, they can **prevent binding** by changing the target so the drug no longer binds (an altered PBP), or by modifying or cleaving the drug. Finally, they can **reverse binding** by protecting the ribosome.

For **penicillin** specifically, resistance happens when β-lactamase cleaves the β-lactam ring, or when an altered PBP (from a mutation or an acquired gene) no longer binds it. There are also several reasons why penicillin may fail, for example when treating stream water. It may fail because the cells are not growing, because there is no peptidoglycan (as in Mycoplasma and Archaea), or because the Gm - outer membrane blocks it. Penicillin can also fail because of resistance (β-lactamase or an altered PBP), and because it does not affect viruses.

D. Bacterial Internal Structures

Textbook: Ch 3 (3.3, 3.5, 3.6)

Bacteria have **seven structures inside the cell**. First come the cytoplasm and cytosol, then the ribosomes, then the cytoskeleton, and then the nucleoid. Next are the membrane compartments and the inclusions and granules, and finally there are the endospores. Several other things can also be seen in the cytoplasm of some bacteria. These include membrane bound compartments and, in only one type of bacterium, DNA inside an intracellular membrane. They also include all three types of cytoskeleton, aggregates (clumps) of metabolites or glycogen, and iron (magnetite crystals) and sulfur granules.

Looking at the whole cell, the **types of structures seen in bacteria** are grouped by where they are, going from the outside, to the envelope, and then to the inside. On the outside are the appendages, which are the flagella, pili and fimbriae. Next is the envelope, which is made up of the glycocalyx (capsule or slime layer), the outer membrane (in Gm -), the cell wall and the cell (cytoplasmic) membrane. Finally, the inside holds the cytoplasm, ribosomes, nucleoid (chromosome), plasmids, inclusions (granules), compartments, cytoskeleton and endospores.

1. Cytoplasm and cytosol

The **cytoplasm** is everything inside the cell membrane, which includes the cytosol, the nucleoid, inclusions (in most bacteria) and membrane compartments (only in a few). The **cytosol**, in contrast, is just the liquid portion of the cytoplasm. It is water with minerals, salts, proteins and sugars dissolved in it.

The cytoplasm is also **crowded** with macromolecules (big molecules), especially ribosomes, with about 15,000 per cell. It is viscous (thick) as well, so diffusion is 10 times slower. As for what it is made of, it is mostly water, which is about 70% of the weight. Protein is the next biggest part, then RNA, and there is only a little lipid and DNA.

It also helps to compare **percent of total weight** with **number of molecules**. Percent of total weight is a component's share of the cell's mass, while number of molecules is how many separate molecules there are, so it depends on size. Big molecules weigh a lot but are few; for example, DNA is only 2 molecules and peptidoglycan is 1 giant molecule. Small molecules, however, are many but light, and inorganic ions are the most numerous after water even though they have almost no weight.

2. Ribosomes

Ribosomes in bacteria are 70S, while eukaryotes have 80S ribosomes, so the two are different in both size and components. The bacterial **70S** ribosome is made of a 50S subunit (23S and 5S rRNAs and 34 proteins) plus a 30S subunit (16S rRNA and 21 proteins), and rRNAs make up 65% of ribosomes. In contrast, the eukaryotic **80S** ribosome is made of a 60S subunit (28S, 5.8S and 5S rRNAs and about 45 proteins) plus a 40S subunit (18S rRNA and about 30 proteins).

This difference matters, because it lets some antibiotics inhibit (block) bacterial ribosomes selectively, meaning they block only the bacteria's ribosomes. These drugs act on the 50S or 30S subunit, and they are listed under Target of antibacterial drugs. More generally, **translation inhibitors** block making proteins. Some of them are antibiotic drugs used for bacterial infections, some are toxins produced by organisms, and some are used for research.

Several kinds of cells and organelles also have their **own ribosomes**. These include atypical small bacteria (Rickettsia and Chlamydia), endosymbiotic bacteria, mitochondria and chloroplasts. Viruses, however, use the host's ribosomes instead.

Less important details: The translation inhibitor list includes chloramphenicol, cycloheximide, erythromycin, fusidic acid, paromomycin, puromycin, streptomycin and tetracycline. It also includes diphtheria toxin, as well as ricin, abrin and α-sarcin.

3. Cytoskeleton: types found and roles

For the **cytoskeleton**, bacteria have homologs (similar versions) of all three types of eukaryotic cytoskeletal proteins. Their roles are cell division, cell polarity, cell shape regulation and plasmid partition (splitting plasmids between the new cells), along with other functions.

The first type is **FtsZ**, which makes the Z ring. FtsZ is similar to tubulin and is used for division, and it forms a ring at mid-cell. It is seen in Staphylococcus, E. coli and Caulobacter. Next, **MreB** is similar to actin and is used for polarity, and it coils along the cell in E. coli and Caulobacter. Finally, **crescentin (CreS)** is similar to intermediate filaments and is used for shape, and it is a line along the curved side of Caulobacter crescentus. Together, all three play a role in cell shape and cell division.

4. Nucleoid: DNA and packing proteins

The **nucleoid** is the region in the cytosol where the chromosomal DNA, which is tightly coiled, is located. It lacks ribosomes, so in EM it shows up as the lighter region among the dark ribosome grains. The DNA here is packed and condensed with no histone proteins, and the mechanism of this packing is not well understood.

The packing is mediated (helped along) in part by **packing proteins**, which are HU, IHF, H-NS and Fis. These are common DNA binding proteins with low sequence specificity, which means they are not picky about the DNA sequence. Of these, HU is similar to a histone, while H-NS bridges DNA. The DNA is also organized into **loops** of about 50 kB, which are negatively supercoiled (twisted on itself) by topoisomerases. Once the cell is lysed (broken open), the DNA becomes less condensed and can make the solution viscous (thick).

The **Planctomycetes** (Gemmata) are a special case. In this one type of bacterium, the chromosome appears to be inside a membrane compartment, which is a double "nuclear" membrane that sits inside an inner cell membrane.

5. Compartments: intracellular membrane structures

Compartments are structures (organelles) surrounded by a lipid membrane, and that membrane is what makes the compartment. They are seen in some bacteria, like methanotrophs (which break down methane), nitrogen fixers, nitrifiers and phototrophs (which use light). Compartments have a specialized role, which is to carry out specific metabolic reactions in and across the membrane, such as photosynthesis.

The compartments can also be looked at through their examples, roles and arrangements. In terms of **arrangement**, compartments are found in many types of phototrophic bacteria, and they can be arranged as stacked parallel layers, concentric rings (rings inside rings) or tightly packed stacks.

In cyanobacteria, the compartments are **thylakoids**, which are the site of light harvesting reactions. Their membranes have full lipid bilayers, and some are similar to the thylakoids in chloroplasts. Green sulfur and nonsulfur bacteria, in contrast, have **chlorosomes**, which are a single phospholipid monolayer (one layer, not a bilayer) linked to the cell membrane, and they contain light harvesting pigments.

Purple sulfur and nonsulfur bacteria have **invaginations**, which are when the cell membrane folds in (vesicular, tubular or stacked lamellar) while staying contiguous (connected) with it. This folded membrane becomes the site of photosynthetic light capture reactions, for example in Ectothiorhodospira. Finally, **methanotroph membranes** (Methylococcus) are used for the breakdown of methane and other one carbon molecules.

6. Inclusions and granules (know examples and their role)

Inclusions are granules or clumps of material in the cytoplasm that are visible in the microscope. They are aggregates of proteins and other material, and they are found in most bacteria. Some of them are surrounded by a shell or coat made of protein or a single monolayer of lipids. Their role is to store energy sources, building blocks and metabolites, or to do work.

Examples of inclusions are gas vesicles, carboxysomes, magnetosomes, PHA, glycogen and sulfur granules. **Magnetosomes** are crystals of iron magnetite (Fe_3O_4) lined up in a chain along the cell. These crystals allow bacteria to do magnetotaxis, which is movement in response to a magnetic field.

Two other examples are gas vesicles and carboxysomes. **Gas vesicles** store gases within a protein shell, and they are rod-shaped vesicles packed in bundles. They are found in aquatic bacteria, and they confer buoyancy (help the cell float), so cells can control the depth at which they float. **Carboxysomes**, on the other hand, are polyhedral (many-sided) structures with a protein shell, and they are the site of carbon (CO_2) fixing. They are found in some autotrophic bacteria (which use CO_2), particularly photosynthetic ones, such as the cyanobacterium Prochlorococcus.

Some inclusions are energy and carbon storage polymers or sulfur granules. **PHA** (polyhydroxyalkanoates, such as poly-β-hydroxybutyrate) are energy and carbon storage granules, and they are seen as large white round granules inside the cell. **Glycogen** is a chain of glucose with a length of 4 to 18 units, and it is also an energy and carbon store. Finally, **sulfur granules** contain elemental sulfur and are seen in sulfur oxidizing bacteria, where the cells are packed with small bright granules in dark-field.

Less important details: Sulfur (S) granules also occur in purple sulfur bacteria. In green sulfur bacteria, however, they sit outside the cell.

7. Endospore: What they are

Spores are special reproductive cells for survival or dispersal (spreading out). A few bacteria, such as some Firmicutes (for example Bacillus), form them inside the cell, which is why they are called **endospores**. Endospores are dormant and very hardy, and they can survive boiling, dehydration (drying out) and radiation. They are also wrapped in several coats. Their formation is induced (triggered) by starvation and high cell density, which means lots of cells crowded together.

During **formation**, the cell divides, but there is no septation of the cell wall, which means no wall partition forms between the two cells. Instead, one cell (the forespore) gets engulfed and develops into the hardy spore.

This happens in seven **stages**. First, a septum forms near one pole, with the DNA as an axial filament. Then the forespore and mother cell form, and next comes engulfment. After that, the cortex forms and the mother cell chromosomes disintegrate, then the exosporangium forms, and then the spore coat forms. Finally, the mother cell releases the spore. Germination then returns it to vegetative growth, which is the normal growing cell.

There are also some **variants** of this process. One type forms multiple endospores inside the cell, while another type does "live births" where two offspring emerge from the mother cell.

Less important details: There are other spore types besides endospores. These are akinetes in filamentous cyanobacteria, arthrospores or exospores in actinomycetes, elementary bodies in Chlamydias, and myxospores in Myxobacteria.

E Cell division and Differentiation

Textbook: Ch 3 (3.4 Cell Division, 3.5 Cell Asymmetry)

Cell division in bacteria can happen by binary fission, budding, multiple fission or asymmetrically (differentiation, which produces a different type of cell). Division goes through replication, then cytokinesis, then septation and finally separation, but the mechanism (how each step is done) varies. After binary fission, each cell has a new pole and an old pole. The proteins used in this process are FtsZ, FtsA, ZipA, PBP3 and Min.

As for the **features of cell division**, most bacteria divide by binary fission into two, usually with only a small change in cell size (2 fold, so the cell just doubles in size). In **one generation**, DNA replication happens first, and then cell elongation makes the cell longer. Next comes septum formation, where the wall between the 2 new cells forms. Then the septum is completed with the formation of distinct walls, so each cell gets its own wall, and finally cell separation happens.

The four types of cell division are binary fission (used by most), budding, multiple fission and asymmetrical division, and all four are observed in bacteria. Of these, **budding** produces smaller offspring cells, like in Pedomicrobium, Planctomyces and budding yeast (fungi). **Multiple fission**, on the other hand, makes multiple offspring from multiple fission events, usually in a sac, and these offspring are called baeocytes. For example, Stanieria and Pleurocapsa do this, while Savagella forms its offspring inside the cell and then releases them. Finally, **asymmetrical division** produces a different type of cell (cell differentiation), such as the stalked cells of Caulobacter crescentus and spores.

Less important details: Ancalomicrobium is another example that also does budding. Also, Dermocarpella is another example that does multiple fission.

Bacterial Cell Division Basic Steps in process: Replication, cytokinesis, septation, separation

Bacterial cell division follows a basic order of steps. First is replication, where the chromosomes copy, and then segregation, where the copies segregate (separate) into each end of the cell. Next comes cytokinesis, where the cell membrane divides so there are now 2 cells with 2 membranes. After that is septation, where the cells make a double layer of new cell wall, which is called the septum. Finally, in separation, the cells cut the cell wall between them.

Even though the basic steps are replication, then cytokinesis, then septation and then separation, how they happen changes between E. coli, Bacillus and Staphylococcus. In **E. coli**, which is a Gm - rod with a thin wall, replication happens first. Then cytokinesis, septation and separation all happen at once, as the whole envelope pinches in the middle. This works because the thin wall lets the whole envelope pinch together.

Bacillus, in contrast, is a Gm + rod with a thick wall. It also starts with replication, but then cytokinesis and septation happen together, as the membrane folds in and a wall furrow (groove of new wall) forms. Separation comes later, so the cells often stay in chains. This is because the thick wall has to be built in, while cutting it apart can wait.

Staphylococcus is a Gm + coccus that also has a thick wall. It also starts with replication, but then septation comes first, before cytokinesis, as a wall furrow grows inward from the equator (middle of the cell). This is because a round cell with a thick wall has to build the wall first. Then cytokinesis happens when the furrows meet, and finally separation happens when the wall between the cells is cut. If the Staphylococcus wall isn't cut, the cells stay as pairs or tetrads (groups of 4), and the plane (the direction the septum forms in) sets the shape.

Asymmetrical cell divisions and cell polarity

Asymmetrical division is when a cell divides and produces a different type of cell. One example is **Caulobacter**, where a swarmer cell (with one flagellum) loses its flagellum, grows a stalk and then divides, so the new cell gets a flagellum on the old pole end. The other example is **spores**, such as akinetes, exospores, endospores, myxospores and fungal spores, which are made for survival or dispersal (spreading out).

The second part of this topic is **cell polarity**, which means that the two ends of a rod can differ, such as a flagellum at one end, Caulobacter's stalk or flagellum, or envelope differences at the poles. **TipN** localizes to (goes and sits at) the new pole and marks it, and mutants lacking TipN grow multiple flagella from the stalk end. Also, even in normal symmetrical binary fission, each new cell gets one new pole (labeled 0) and one old pole. The oldest poles have weaker walls, which may explain why some cells in a population die or differ in sensitivity to antimicrobial agents.

Role of FtsZ and Min proteins

To understand the role of **FtsZ**, it helps to know that FtsZ is a homolog (related version) of tubulin, which is the microtubule protein in eukaryotes. FtsZ forms dynamic (always changing) filaments on the inside of the membrane, and it localizes to the cleavage (division) site, where it forms the **Z ring** (the division ring). The Z ring then gets smaller, which causes constriction (pinching in). Other proteins also associate with the Z ring to build the **divisome**, which is the division protein complex. In this way, the FtsZ ring under the inner membrane organizes the divisome, so it anchors the PBPs (PBP1 and PBP3) that build the septum wall.

The proteins on the Z ring include FtsA, ZipA and PBP3. **FtsA** is an actin homolog that binds FtsZ, so it attaches FtsZ to the membrane and bundles the FtsZ filaments together. **ZipA** is a membrane protein with a role similar to FtsA. Finally, **PBP3** makes

new peptidoglycan at the division site, doing both transglycosylation (joining the sugars into chains) and transpeptidation (cross-linking the peptides).

In E. coli, the **Min proteins** make sure that the Z ring and the septum form in the center of the cell. MinD-ATP forms dynamic helical (spiral) filaments on the inside of the membrane, while MinE sits near the center and stimulates ATP breakdown by MinD, which takes the MinD filaments apart. MinC rides with MinD and is an inhibitor of FtsZ polymerization (FtsZ joining into filaments), so it blocks the Z ring from forming. In other words, MinC never builds anything, and it sits at the poles and only blocks FtsZ there. Together, MinC and MinD (called **MinCD**) oscillate (move back and forth) from pole to pole, which keeps FtsZ blocked at the poles, so the ring can only form in the center. In contrast, **Min mutants** make minicells (very small cells), because division happens near one end instead of the middle.

There are also **methods to study division proteins**. One method uses temperature-sensitive mutants that can't divide at high temperature, and these form filaments (long cells that don't split), which is where the name fts comes from (filamentous temperature sensitive). Another method is **fluorescence microscopy**, which shows where the proteins sit, such as FtsZ at mid-cell and TipN-GFP (TipN tagged with a glowing green protein) at the new pole.

F. Viruses

Textbook: Ch 6 Virus Structure and Function

Viruses can be different from each other in several ways. They can differ in size and shape, in their structural features (their components), and in the features used to classify or group them. They can also differ in reproduction, which includes the general features, the steps in the multiplication cycle, and the synthesis stage for the 7 types, and finally they can differ in the outcomes of infection.

A good example to look at is **COVID-19**, which is caused by a coronavirus. This coronavirus is an enveloped, single-stranded, + sense RNA virus, and its parts are the spike glycoprotein (S), the M protein, the E protein, the hemagglutinin-esterase dimer (HE), and the RNA with the N protein. Being **enveloped** means it is covered by a membrane envelope around the capsid, and that envelope is what carries the spikes. Being **single-stranded, + sense RNA** means the genome is one RNA strand that serves as the template for translation (it can be read as mRNA), so it gets translated right away. Then the viral RNA replicase copies it into a (-) strand, and from that (-) strand it makes new (+) genomes, so the order is (+) → (-) → (+).

1. Size and Shape observed

The first way viruses differ is in the size and shape that is observed. When it comes to **size**, most viruses are 0.02 to 0.2 µm, which is 10 to 100 times smaller than bacteria, so you need an electron microscope to see them. Examples go from small ones like yellow fever virus (22 nm) and polio virus (30 nm) up to bigger ones like HIV (110 nm) and herpes simplex virus (150 nm). However, **giant viruses** like Mimivirus (450 nm) and Pandoravirus (1 µm) are similar in size to some bacteria, such as Rickettsia (0.3 µm) and Streptococcus (1 µm).

There is also a large diversity in the **shape** of viruses. They can be an icosahedron (a 20-sided polyhedron), spherical, or complex, which means more complicated, for example an icosahedral head on a long tail. Some enveloped viruses are also elongated or filamentous (long and thread-like), like Ebola virus (1.2 µm).

2. Structural features

The **structural features** of viruses show that they are simpler than cells, so they are considered acellular (not made of cells). When you compare them to cell features, viruses only have nucleic acids, and they have a capsid instead of a cell wall. Virions (virus

particles) may contain just nucleic acid in a protein shell, which is called the capsid. A **naked virus** has no membrane, just the nucleic acid in a capsid, which may carry spikes. In contrast, an **enveloped virus** has the capsid sitting inside a membrane envelope, and the envelope carries the spikes.

The **components of virus particles** can be split into two parts, which are the core and the covering. The core is the nucleic acid, the matrix proteins, and enzymes in some viruses, while the covering is the capsid and, in some viruses, an envelope. In the nucleic acid, genes can overlap, and multiple proteins are made from a single RNA and polypeptide, like in SARS-CoV-2. Enzymes are found only in some viruses, and they are inside the capsid, such as polymerase or reverse transcriptase. For example, HIV, which is a retrovirus, carries reverse transcriptase, integrase and protease. The envelope is also found only in some viruses, and it is a phospholipid bilayer membrane with proteins and glycoproteins (spikes), for example HIV GP-120 and GP-41.

Naked and enveloped viruses also differ in **viability**, which means the virus is still able to cause an infection when it is outside a host cell. Naked (no membrane) viruses are generally tougher, survive longer, and are more resistant to drying, detergents and acids, while enveloped ones are more sensitive and must remain wet. They also differ in how they escape from the infected cell, since naked (nonenveloped) viruses must lyse (burst) the cell, while enveloped ones can escape by budding.

Out of all these parts, the two that are found in all viruses are the **nucleic acid** and the **capsid**, and together they form the nucleocapsid. The nucleic acid can be DNA or RNA but not both (some giant viruses contain both), and it may be single or double stranded, in multiple segments, or in more than 1 copy. Examples are dsDNA in variola virus (smallpox) and herpes simplex 2 (genital herpes), ssDNA in parvovirus, + ssRNA in poliovirus, - ssRNA in influenza virus, dsRNA in rotavirus, and ssRNA with reverse transcriptase in HIV (AIDS).

The capsid is the protein coat or shell, and it is usually icosahedral (20 sided) or helical. Viral capsid proteins form identical capsomeres (the subunits), which in turn form the capsid. In an icosahedral capsid, the capsomeres form facets (flat faces) and vertices (corners), some with fibers, while in a helical capsid, the capsomeres are stacked around the coiled nucleic acid.

Less important details: in avian leukosis virus RNA, the gag, pol and env genes code for the core, enzymes and envelope. This means gag codes for the core, pol codes for the enzymes, and env codes for the envelope.

3. Features used to classify or group viruses

Viruses are classified into **groups** based on the nature of the genetic material (DNA or RNA, and ss or ds) and its means of replication (how it gets copied). The **subgroups** are then based on the outer covering (enveloped or nonenveloped), the shape of the virus particle or capsid, the organisms or cells the virus infects, and whether the genome is segmented (in pieces) or not. However, cell wall chemicals are not a feature used to classify viruses, because viruses have capsids instead of cell walls.

In total, there are **seven groups** in the Baltimore classification, and some call them families. Group I is dsDNA, Group II is ssDNA, Group III is dsRNA, Group IV is (+) sense ssRNA, and Group V is (-) sense ssRNA. Finally, Group VI is the RNA retroviruses, and Group VII is the pararetroviruses, which are DNA reverse-transcribing, meaning they are DNA viruses that use reverse transcription.

Less important details: examples of Group I are phage lambda, fusellovirus, variola (smallpox) and herpes simplex 2. Group II includes geminivirus and parvovirus, Group III includes rotavirus, and Group IV includes phage MS2, rhinovirus 14 and poliovirus. Finally, Group V includes rabies virus and influenza virus, Group VI includes HIV-1, and Group VII includes cauliflower mosaic virus.

4. Reproduction

In terms of **reproduction**, viruses multiply only in host cells through a set cycle, and that cycle differs in the synthesis step across the 7 groups. This is different from bacteria, because typical bacteria are free-living and reproduce by themselves. In contrast, out of the major elements of life (genetics, reproduction, biosynthesis, respiration and others), viruses only have nucleic acids.

One general feature is that viruses are **obligate intracellular parasites**, which means they can only replicate or multiply inside a host cell. This is also seen in some bacteria (like Rickettsia and Chlamydia) and in protozoans. Another general feature is **assembly**, which means viruses are assembled from parts inside the host cell, not by splitting (no binary fission). First, proteins assemble to make the capsomers and sheath, and then the parts of the virus are assembled together. Chlamydia is different, because even though it is also an obligate intracellular parasite, it divides inside the host cell (binary fission of reticulate bodies).

A good example of assembly is the **T4 bacteriophage**. First, capsomers form the head, and then DNA enters to fill the capsid. Next, the tail, sheath and tail fibers are assembled separately, and finally they are joined to the filled head.

B. STEPS IN MULTIPLICATION CYCLE

The **steps in the multiplication cycle** happen in a set order, starting with adsorption, then penetration, then uncoating, then synthesis (see part c), then assembly from parts (see part a), and finally release. **Adsorption** is step 1, and it is when the virus attaches (by its spikes) to receptors on the host cell that will be infected. For example, SARS-CoV-2 binds ACE2, and phages bind E. coli porins, such as OmpF for T2, OmpC for T4, and LamB for λ. This step is the major factor that determines host range, which means the kinds of cells the virus can infect.

Penetration is step 2, and it is how viruses get in (entry into the cell). This happens either by endocytosis into an endosome vesicle, as in SARS-CoV-2, or by fusion of the viral envelope with the cell membrane so the coated genome (nucleocapsid) enters the cytoplasm, as in measles. In the endocytosis route, the virus first attaches to the cell, then it is engulfed into a vesicle, and then the vesicle, envelope and capsid break down (the uncoating step), leaving free DNA. In the fusion route, the virus attaches, its envelope fuses with the membrane, the nucleocapsid enters, and then the nucleic acid is uncoated, leaving free RNA. Next, **uncoating** is step 3, and it is the release of the viral genome. It can happen in the cytoplasm, inside the endosome, or at the nucleus. In hepatitis C, it happens inside the endosome, where a lysosome fuses with and acidifies (lowers the pH of) the endosome, and then the viral envelope fuses with the endosome membrane. In adenovirus, the dsDNA is released at the nucleus, at a nuclear pore.

After synthesis and assembly, **release** is step 6, and it is how the viruses get out, which can happen in three ways. The first way is lysis of the host cell, and naked viruses must lyse it. The second way is budding by enveloped viruses, where the nucleocapsid lines up under the membrane and is pinched off (like filamentous Ebola), and this can give a persistent infection. The third way is cell-to-cell spread to a target cell (along F-actin or across the apical junction), which may lead to cell fusion (syncytia).

A full example of the cycle is **SARS-CoV-2**. First, the spike binds ACE2, and then entry and uncoating happen in the endosome. Next, the genome is translated into a polyprotein, and a protease cleaves (cuts) it, making the RNA replicase (Paxlovid inhibits this step). Then, in a viral factory made from ER, the replicase makes (-) strand RNA (molnupiravir interferes here), and after that it makes viral mRNAs and new (+) genomes. Finally, translation happens, followed by assembly in the Golgi and then release.

For **plant viruses**, the cell wall blocks entry without help, so they get in by an animal vector that damages cells (an insect, like an aphid bite), by contact with damaged tissue with broken cells, or by infecting seeds. Then they move cell to cell through plasmodesma connections (channels between plant cells). As for **bacteriophages**, most inject their DNA into the cell and leave the particle outside. Some can go latent (lysogen, a prophage), and most are released by lysis.

C. SYNTHESIS STAGE FOR 7 TYPES OF VIRUSES

In the **synthesis stage**, every virus must do two jobs, which are copying its genome and making many copies of its proteins (viral mRNA is used to make proteins). To do this, viruses use enzymes from the virus or the host, and all of them use host ribosomes. However, host DNA pol and RNA pol only use DNA as a template, and most host cells have no (or very low) RdRP (RNA-dependent RNA polymerase). This is why RNA viruses bring their own enzymes, since they usually encode their own RdRP or reverse transcriptase.

To read the synthesis paths, you need to know a few symbols and terms. In the paths, ds means double-stranded, ss means single-stranded, and ± means both strands (a double strand). The **+ strand** is the strand that serves as the template for translation (it reads

as mRNA), while the **- strand** is complementary to the + strand, so it must be copied into + before it can be translated. An arrow means "is used as a template to make," so DNA to RNA is transcription, RNA to protein is translation, and RNA to DNA is reverse transcription. The "Genome" path is replicating the genetic material, and the "Protein" path is making mRNA and then proteins. Finally, for the enzymes, RdRP (RNA dependent RNA polymerase) makes RNA from an RNA template, reverse transcriptase makes DNA from an RNA template, and DNA dependent RNA pol makes RNA from DNA (transcription).

Among the groups, **Group I dsDNA** is the most similar to cellular processes, because it copies DNA to DNA like the cell does. Its DNA pol comes from the host (papillomavirus, parvovirus) or from the virus (adenovirus, herpesvirus, poxvirus), and host RNA pol makes the mRNA, while virus RNA pol is used only in poxvirus. In **Group II ssDNA**, the single strand is first made double (ssDNA → dsDNA), and then host DNA pol copies it back to ssDNA. The dsDNA is also transcribed to mRNA by host or virus RNA pol.

In **Group III dsRNA**, viral RdRP copies the double strand into + strands (dsRNA → (+) ssRNA). Those + strands act as mRNA for proteins, and they are also made double again for new genomes. In **Group IV (+) ssRNA**, the genome already is mRNA, so it is translated immediately as the first step. Then viral RdRP makes a - copy and uses it to make new + genomes ((+) → (-) → (+)). As for the enzymes in Group IV, the virus supplies only the RdRP and the host supplies the ribosomes. No DNA-dependent RNA polymerase is used, because there is no DNA step.

In contrast, in **Group V (-) ssRNA**, the genome cannot be translated, so viral RdRP must first make a + copy as the first step. That + copy is the mRNA for proteins and also the template for new - genomes ((-) → (+) → (-)). In **Group VI RNA retroviruses**, viral reverse transcriptase copies the + RNA into DNA ((+) ssRNA → ssDNA → dsDNA), and the dsDNA integrates into the host genome. Then host RNA pol transcribes it back into + RNA, which is used for new genomes and mRNA. Finally, in **Group VII DNA reverse-transcribing** viruses (pararetroviruses, like cauliflower mosaic virus), the dsDNA is transcribed into + RNA, and then reverse transcriptase (from the virus or host) copies that RNA back into dsDNA (dsDNA → (+) ssRNA → dsDNA).

5. Outcomes of Viral infections

The **outcomes of viral infections** can be acute, persistent or latent. Viral infections can also change the infected cells through cytopathic changes, transformation and syncytia, and they can even change whole microbial populations.

An **acute infection** is when host cells die due to cell lysis (bursting), and the virus is released when the cells lyse. A **persistent infection** is when host cells keep producing virus particles over a long period of time (slow release), and the cell usually survives. This is only done by enveloped viruses, which leave by budding. A **chronic, latent infection** is when the virus goes dormant (inactive) in the host cell, either as integrated viral DNA or as DNA kept as a plasmid without adverse effects (in phages, this is a lysogen). The virus can later reactivate and release virus particles. For example, herpes goes latent in a nerve cell and occasionally releases virus particles that cause skin lesions (cold sores).

Viral infections also have effects on the infected cells, and the first one is **cytopathic changes**, which is when infection alters the appearance of the cell. When this happens, cells can round up, clump, enlarge, fuse or lyse, and there can be inclusions such as Negri bodies in rabies. These changes are sometimes used in diagnosis. Because different viruses cause different changes, cytopathic changes can help identify the virus, so the idea that "every virus causes the same change" is false. Next, **transformation** is when the cell becomes cancerous, which happens because some viruses contain an oncogene, or their integration generates one, and that oncogene promotes growth of the host cell. Finally, **syncytia** are large, multi-nucleated cells formed by cell fusion, which is done by a viral fusion protein, and they are seen in measles, RSV and herpes. Cell-to-cell spread may also lead to syncytia.

Viral infections also affect whole **microbe populations**. In the mammalian gut, lots of phages act against intestinal bacteria. They also transfer DNA by transduction (phages moving DNA between bacteria), and lysogens can express virulence such as toxins. In **marine ecosystems**, phage lysis at the surface releases organic matter for other organisms to use, which matters because nutrients in dead cells that sink are hard to recycle. Phage lysis also helps control Vibrio cholerae and phototrophs.

G. Atypical Bacteria and Viruses (notes)

Textbook: Ch 18 (Rickettsia, Chlamydia) · Ch 6 (giant viruses)

The atypical ones in this section are Rickettsia, Chlamydia, endosymbiotic bacteria, mitochondria and chloroplasts, and giant viruses, and each of them is different in some way from typical bacteria and typical viruses. For comparison, **typical bacteria** are 1 to 3 μm with a 500 to 10,000 kB genome (1000 to 10,000 genes), and they are free-living (live on their own). In contrast, **typical viruses** are 0.02 to 0.2 μm with a 1.8 to 100 kB genome (1 to 100 proteins), and they are obligate intracellular parasites (can only reproduce inside cells). The **atypical small bacteria** were originally found when people were searching for the causative agents of certain diseases (what was causing them).

Looking at the features of each group shows how they differ from each other. Typical bacteria are bigger, have many genes, and reproduce on their own by binary fission (splitting into 2). Typical viruses, on the other hand, are tiny, have few genes and no ribosomes, and only reproduce inside cells, where they are assembled (put together from parts) rather than divided. The **atypical bacteria** (Rickettsia, Chlamydia, endosymbionts) and the organelles (mitochondria and chloroplasts) fall in between, because they are small and live inside host cells like viruses, but they keep DNA, RNA, ribosomes and enzymes and divide by fission like bacteria. At first they were mistaken for viruses, because they didn't show up on a Gram stain, passed through 1 μm filters, and were seen inside host cells. **Giant viruses** come from the other direction, since they reach bacterial size and genome and have some bacterial parts, but they still have no ribosomes and don't divide.

1. RICKETTSIA

- **Rickettsia** is an atypical bacterium that is like bacteria in some ways and like viruses in others. Like bacteria, it is Gram-negative (but stains poorly), has DNA, RNA and ribosomes, and divides by binary fission (splitting into 2). Like viruses, however, it is tiny and only multiplies inside host cells. Inside the host, it escapes the phagosome (the bubble the host cell takes it into), and then it exits by lysis (bursting the cell) or budding (pinching off).
- What makes Rickettsia different is that it is an **energy parasite**, which means it lacks glycolysis and biosynthetic pathways (ways to build its own molecules) and steals the host's ATP using a translocase (a transporter). Rickettsia is also the likely ancestor of mitochondria.

2. CHLAMYDIA

- **Chlamydia** is another atypical bacterium that shares traits with both bacteria and viruses. Like bacteria, it is Gram-negative (but stains poorly), it has DNA, RNA and ribosomes and a near-complete metabolism, and its RB (reticulate body) divides by binary fission (splitting into 2). Like viruses, it is tiny and obligate intracellular (can only multiply inside host cells), and it has a virus-like life cycle.
- In its **life cycle**, the infectious EB (elementary body) first enters the cell, and then it becomes an RB that divides. Next, the RBs turn back into EBs, and finally the cell lyses (bursts).
- What makes Chlamydia different is that it has two forms. The **EB** is dormant, infectious and spore-like and does not divide, while the **RB** is the growing form. When it comes to ATP, Chlamydia steals ATP like Rickettsia does, but it has much more of its own metabolism.

3. ENDOSYMBIOTIC BACTERIA

- **Endosymbiotic bacteria** are like bacteria because they have their own DNA, ribosomes and some metabolic enzymes, and they grow independently (on their own) by dividing. They are also like viruses, since they live inside the cells or a body part of another organism, and some are obligate endosymbionts (can only live inside the host). What makes them different is that they

have the smallest bacterial genomes, as small as 150 kB (182 genes). Finally, in their relationship with the host, they are symbionts (living together with the host), not parasites.

• Less important details: Hodgkinia is the shortest bar on the genome graph. This means it has the smallest genome shown.

4. GIANT VIRUSES

• **Giant viruses** are like bacteria in that they reach bacterial size and genome, some have both DNA and RNA, and some make translation enzymes (enzymes used to make proteins). In fact, some giant virus genomes are BIGGER than some bacterial genomes. For example, the Mimivirus genome is bigger than the Rickettsia genome and the tiny endosymbiont genomes. This means it is false that giant virus genomes are "always smaller than any bacterial genome".

• However, giant viruses are still like viruses, because they have no ribosomes and no binary fission, and they only reproduce inside host cells. What makes them different is that they can be infected by viruses themselves, and they have been proposed as a fourth domain of life.

5. MITOCHONDRIA AND CHLOROPLASTS

• **Mitochondria and chloroplasts** are like bacteria because they synthesize (make) their own DNA, RNA, ribosomes, tRNA and some metabolic enzymes, and they grow independently by dividing and fusion (joining together). They also make ATP for their own use with a proton motive force across the mitochondrial inner membrane, the same way Gm - bacteria do. At the same time, they are like viruses in that they are obligate intracellular symbionts (not parasites) that live only inside host cells, while only SOME endosymbiotic bacteria are obligate.

• Mitochondria and chloroplasts share several things with **endosymbiotic bacteria**, since both have their own DNA, ribosomes and some metabolic enzymes, and both grow by dividing. What makes them different is that they have very few genes (mitochondria 30 to 60, chloroplasts about 120), and they get many metabolites (small molecules they need) from the host. They are descended from bacteria, with mitochondria coming from a Rickettsia ancestor and chloroplasts coming from a cyanobacterium. Also, cyanobacterial thylakoids (membrane sacs) are similar to those in chloroplasts.

• Looking at **all five** atypical ones together, they all live or multiply only inside host cells (for endosymbionts, only some), and they all depend on the host. All of them except giant viruses have ribosomes and binary fission. The big pattern is that going from typical bacteria down to endosymbionts and organelles, each one loses more genes and depends more on the host. In contrast, giant viruses come from the other side and gain bacteria-like parts, but not ribosomes.

How can one predict the structures and properties of the cells of a newly discovered bacterium?

To predict the structures and properties of the cells of a newly discovered bacterium, first look at its **phylogeny** (family tree of relatives), because close relatives likely share the same structures. Next, look in its **genome** for genes for LPS, teichoic acid, mycolic acids, pseudopeptidoglycan and flagella. Then, check these predictions with stains, MacConkey agar, electron microscopy and motility tests.

