MICROBIOLOGY SUMMARY

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Overview:

Microbiology is the branch of biology that studies microorganisms. These are multicellular, unicellular, and a-cellular organisms that cannot be observed by the naked eye, i.e. Bacteria, Algae, Fungi, Protozoa. Viruses are also studied but differ from microorganisms in that they are non-cellular organizations, lacking a metabolism, and their survival depends on acting as intercellular parasites. It's hard to define viruses as living or not living.

The scope of studies is between 1um(micrometer) and about 10nm(nanometer), including large bacterium all the way to structures such as flagellum. Under 10nm is not currently considered forms of life. This range encompasses structures able to be observed by a light microcore, and an electron microscope, but not a human eye or special microscopes. For this reason, microbiology as a discipline began with the invention of the microscope (1600's). Studying microbiology is in three main areas, watching and observing through a microscope, culturing in vitro by observing their spread in a substrate, either solid or liquid, and molecular approach i.e. proteins and nucleic acids, mostly theory.

Bacterial cells are highly diverse, and the great majority of them have yet to be cataloged or studied, about 99%. From this diversity, we see bacterial cells have colonized all ecosystems on earth, even those unhospitable for animals and plants such as hot springs, hypersaline lakes, icebergs. There are 21 orders of magnitude more bacteria than humans.

Phylogenetic tree of life*: LUCA* is a hypothetical organism that is hinted to by the conservation of certain elements in all forms of life. Each fork is called a *speciation point* or event. Archaea are ancient bacteria.

Major cell morphologies are broken into three categories, but they are generalizations and we can see it as more of a spectrum then defined categories.

- *Coccus*: Roughly circular, can form dimers or more, even forming perfect cubes
- *Rod*: long and tubular
- *Spirillum/Spirochete*: Twisted spiral shapes

Spirillum can swim through median by their curved shape, and differ from spirochetes because they have a flagellum, but it is hidden in spirochete.

Prokaryotic cells and eukaryotic cells are the two distinct patterns. Eukaryotes have a nucleus, and therefor transcription and translation occur in two distinct areas (nucleus and cytoplasm), eukaryotic microorganisms include algae and protozoa, broadly called protists, as well as fungi and slime molds. Eukaryotes also contain membrane bound organelles. Prokaryotes have a special cell wall that is different from eukaryotes called the plastmic wall that protects the cell.

Prokaryotes instead can couple transcription and translation, because they both occur in the cytoplasm. Prokaryotes have small compact genomes, consisting of circular DNA. Prokaryotes use their plasma membrane in energy conversion reactions similarly to those found in the mitochondria. In size, prokaryotes are much smaller, 1-5um long while eukaryotes range widely in size.

All cells must have a *catalytic function* in order to synthesize new macromolecules. These energy spending processes include *genetic functions*, and *catalytic functions (Machine Function)*, which converge to ultimately synthesize proteins and other macromolecules needed for the cell to reproduce. Catabolism + anabolism = Metabolism. *Catabolism* is the process of breaking down molecules, releasing energy, *anabolism* is the process of synthesizing complex molecules from simpler subunits, spending energy.

Microbial catabolism has two main energy sources:

- Chemicals: called *chemotrophy*, can rely on organic molecules (Glucose) or Inorganic(H2S)
- Light: Relies on light energy, called phototrophy, and converts to chemical energy

Chemotrophs relying on organic chemicals are called *chemoorganotrophs*, and are *heterotrophs*, meaning they use organic compounds as their carbon source. *Autotrophs* by contrast, rely on CO_2 for their carbon. *Chemotrophs* who use inorganic chemicals (called *chemolithotrophs*) and *phototrophs* are both autotrophs. All microbes eventually store their energy in the form of a high energy phosphate bond, from *ATP*.

Bacteria contain a signal circular chromosome called a *nucleoid*, with two notable exceptions:

- *Vibrio Cholerae* contain two circular nucleoids
- *Borrelia burgdorferi and Streptomyces coelicolor* have linear nucleoids this is somewhat common for larger genomes even in microorganisms.

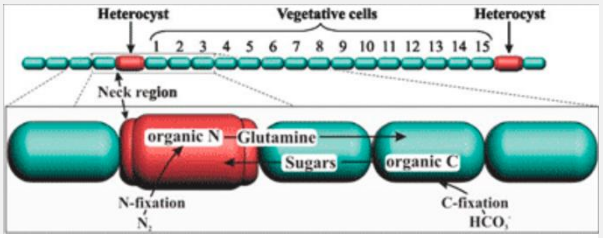
Bacteria also contain *plasmids*, smaller circular DNA distinct from the chromosomes that do not contain core genes, but instead confer special properties (unique metabolism, shape) to the cell. Bacterial genomes usually have one copy of each gene and are therefore *haploid cells*.

Bacterial cells undergo *binary fusion*, the duplication of DNA, followed by *elongation* of the cell and finally segregation of the DNA at the *septum* to form two daughter cells. They therefor undergo mutations during DNA replications, called *Vertical Evolution* (hereditary transmission) and is found in only one of the daughters. *Horizontal Evolution* by contrast refers to the involves the disruption or injection of foreign coding sequences (DNA or RNA) into the cell through viruses, plasmid transfer, or partial chromosome donation.

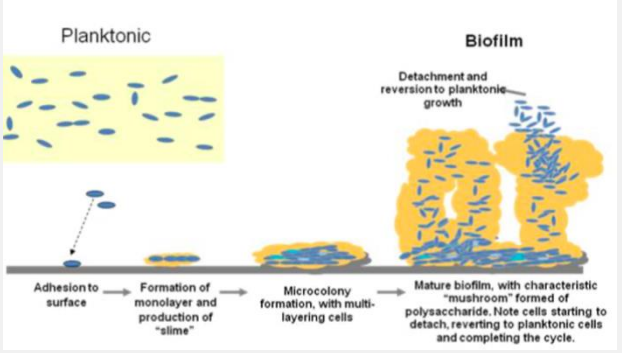
In *transformation*, donor DNA enters receipt cell, can be positive or negative. In *transduction*, viruses enter the receipt cell, mediating the transfer of DNA from the host to the donor. *Conjugation* instead involves the fusion of the plasma membranes through a *cytoplasmic bridge*, either a plasmid or a partial or full chromosome. Horizontal gene transfer turned the tree of life into a web of intermeshed pieces of genes and nucleic acids. In this theory, called the panspermia theory, LUCA would not exist, and instead earth was inoculated by many ancestors from the cosmos. Bacteria reproduce fast due to their short life span, and their haploid genome, because all the genes are dominant.

Cells can be specialized to drive the cell cycle. *Sporulation* refers to the process in which a specialized *endospore* is formed, separating from the *vegetative cell*. Sporulation allows the bacterium to leave its immediate surrounding more readily, and is induced by environmental stimulus such as: shortage of materials, high cell density, etc. Endospores are more resistant to heat, radiation, and chemicals than vegetative cells, but have a decreased *enzymatic activity, respiratory rate,* and the absence of *macromolecule synthesis.*

Differentiated cells can also form specialized cells, such as the *heterocyst cells,* which can perform *nitrogen fixation* by forming *Glutamine* from atmospheric nitrogen. Heterocyst cells cannot divide, only vegetative cells can.



These *Microbial communities* form structures called *biofilms*. Biofilms serve as a protective community for the cells in the presence of antibiotics, bacteriophage, and dehydration. Cell activity and growth is stratified, with the outer cells being highly active while the inner cells are dormant. *Planktonic* bacteria are one phase of a bacteria's lifecycle. Biofilms cause problems when they grow inside the valves of the heart.



Quorum sensing is the minimum accumulation of a certain signal molecule to signal a specific cellular response and can come from nearby bacterial cells. In the case of *AHL* the receipt of the extracellular AHL activates proteins that will increase gene transcription of more AHL, leading to a *signaling cascade*. The AHL signal cascade only works in high density of the same bacteria, signaling the need sporulation These communications can be both *intra- and inter- species*. Functions such as bioluminescence, horizontal gene transfer, antibiotic production, biofilm formation, and virulence are all triggered by quorum sensing in different bacteria.

Bacteria use one or more flagellum to swim through the extracellular environment(swimming) or the flagellum pushes the bacterium across a surface (squirming). The rotation of the flagellum is powered by the H+ gradient, similarly to the ATP-synthase in mitochondria. Bacteria can also twitch, glide or slide using various other specialized proteins.

Bacteria are absolutely essential for the existence of higher life on earth, in face they made the planet hospitable by producing the oxygen we breathe. They also have a mutualistic relationship with plants, specifically through decomposers such as bacteria and fungi, and nitrogen fixation cycle mediated by many bacteria found in the soil. Humans also rely on bacteria in the gut, to maintain our metabolism and protect us, in exchange for a safe harbor. Bacteria also play a structural

role in humans (Intestines and crypt formation), a metabolic role (mitochondria, as well as the breakdown of non-digestible substances through fermentation), and a protective (resistance to colonization due to diversity).

Koch's Postulates: Guidelines to determine if a bacteria is the cause of a disease:

1. Suspected causing agent must be absent in healthy organisms
2. The agent must be isolated from the diseased organism
3. The agent must produce the same effect when injected into a healthy host

Bacteria also allow important advances in technology, including yogurt, cheese, as well as bioremediation and bioengineering. Bioremediation is a promising solution to waste removal, especially oil in water.

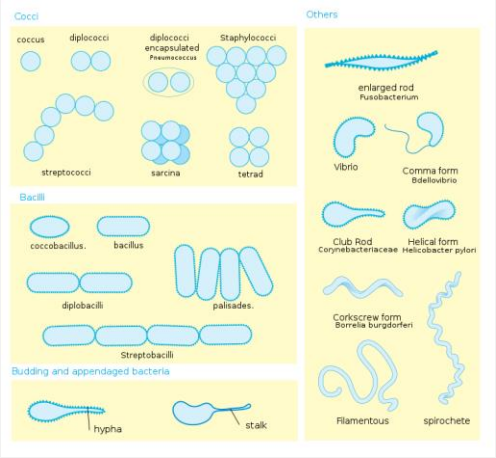
Cell structure and Cytoplasmic Membrane:

Cell size

Prokaryotic cells differ from eukaryotic cells in the reasons mentioned earlier in the summary, including the absence of intercellular compartments, and chromatin structure. Prokaryotes are also, however much smaller than Eukaryotic cells ranging from 0.2 r to 750 micrometer. In larger bacterial, *vacuoles* or *stored hydrogen nitrate* are used to increase their size.

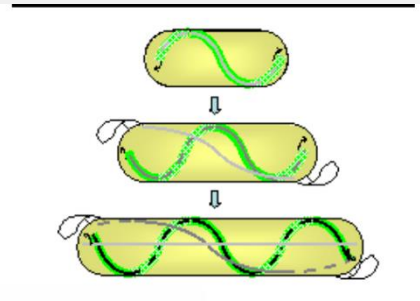
Cell size is limited on both the upper bound and lower bound. As cell size increases, it takes longer for materials to diffuse from the cell membrane to its necessary area within the cell. This means larger cells are less efficient at up taking materials because once the materials enter the cell, they have to travel further. This phenomenon is explained because as a cell grows, the volume of the cell increases 10x faster than the surface area. On the small bound of the spectrum, we see that 0.2 micrometer is the smallest a cell can be while still housing the necessary proteins, nucleic acids and ribosomes to maintain and grow. This upper limit is overcome by eukaryotic cells through intercellular compartments.

Morphology:



Bacterial cells fall into a few major categories, *coccus* is spherical, *rods* are cylindrical, and *spirillum* have one curve, *spirochete* have many curves. Bacterial cells from these categories can also aggregate to form dimers, tetramers or even cube shapes. Some interesting but rare morphologies include *square-like* (Haloquadratum), *star-like* (stella humosa), and *pleomorphic bacteria*, meaning different morphology (H. Pylori). Division and organization of cocci depends on the *plane of division*.

Morphology of the cell depends on *MreB*, and *actin like filament* protein. MreB is not found is naturally *Coccoid cells*, indication that coccoid is the 'default' shape. MreB forms a helical structure and spans the interior of the cell and is thought to recruit other proteins to extend the cell wall in specific locations, forming the rod shape. Like actin, the triphosphate bound monomers attach to the plus end, and after ATP is hydrolyses to ADP, they disassociate, identically to the *treadmilling effect* of actin filaments. MreB filaments are formed by two strands of MreB monomers.



Crescentin (CreS) proteins are an example of an *intermediate filament* in bacterial cells and are thought to play a role in the curved shape of some cells. Caulobacter, an aqueous bacteria exhibits these filaments locally in their curved regions. MreB, CreS and FtsZ (introduced later) form the prokaryotic cytoskeleton.

CPM In Bacteria

Cytoplasmic membrane is the only lipid membrane in bacterial cells, as they do not contain intercellular compartments. This membrane is composed of *phospholipids*, Cations such as Mg2+ and Ca2+ and integral membrane proteins. Phospholipids

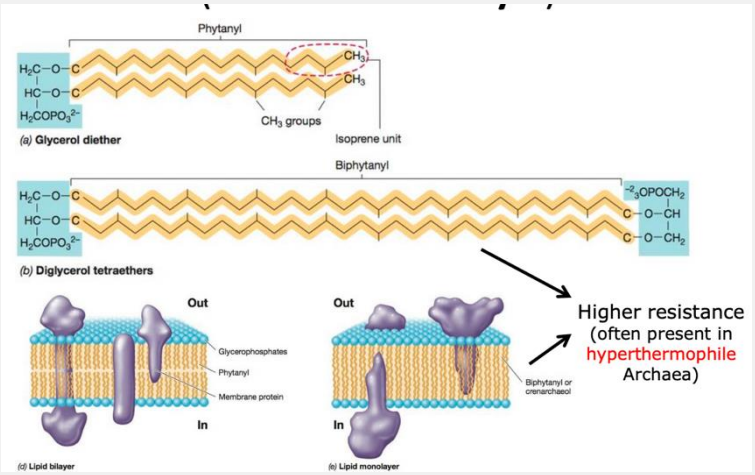
have a hydrophobic head (*glycerophosphate*) and a *hydrophilic tail (fatty acid*) and self-organize into a bilayer in aqueous solutions.

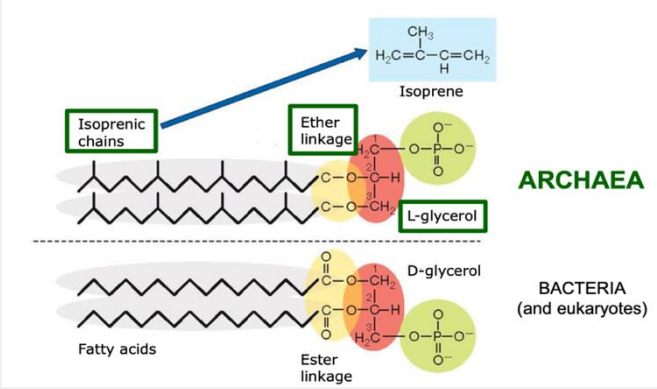
In bacteria the composition of the membrane -that is the amount of saturated vs unsaturated fatty acids- changes in response to environmental temperature, maintaining a constant fluidity. A fall in temperature increases the unsaturated fatty acid content, which as we know contain a double bond, and thus can form cis trans isomers, and branched fatty acids, increasing fluidity. A rise in temperature will decrease the concentration of unsaturated fatty acids, increasing fluidity. In prokaryotes, we find palmitic, palmitoleic (mono-unsaturated) ,steric, and oleic (mono-unsaturated) fatty acids.

Sterols, which increase rigidity of the plasma membrane are not present in prokaryotes, with few exceptions. Instead, bacteria (but NOT archaea) contain opanoids, a cytoplasmic elements structurally and functionally similar to sterols. ADD HOW THEY WORK

In Archaea

Archaea contain a few key differences in their plasma membrane. First, instead of an *ether linkage* between the fatty acid and the *glycerophosphate groups* found in bacteria and eukaryotes, we see an *Ester linkage*. Secondly, archaea lack true fatty acid tails, and instead contain *isoprenic chains*, composted of repeating *hydrophobic hydrocarbon isoprene* units.





Membranes of Archaea can be formed by either *Glycerol diethers and Phytanyl tails*, or *Diglycerol tetraethers and biphytanyl tails*. Because of this, archaea can in fact form a lipid bilayer, or a *lipid monolayer* in the case of diglycerol tetraether, because this molecule contains two hydrophobic *glycerophosphate* groups. A monolayer, in contrast to a bilayer, confers greater resistance to heat, and therefor are found in *hyperthermophile archaea*, prokaryotes that live in extreme temperatures.

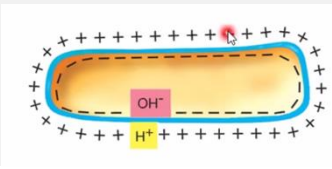
Protein composition of cytoplasmic membrane

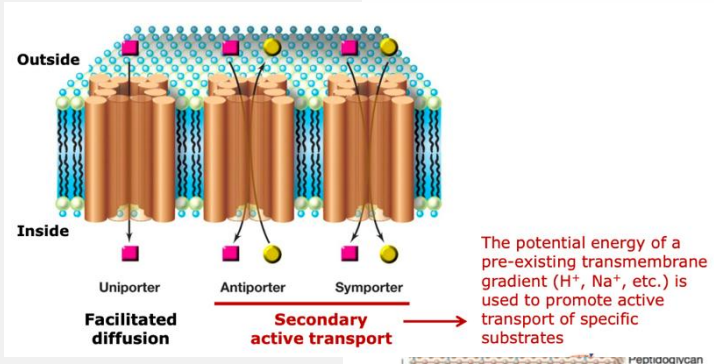
Compared to eukaryotes, bacteria generally have a higher concentration of membrane proteins, which makes since because it carries out all the membrane functions of the cell, which in eukaryotes is spread out between the membrane bound organelles. Membrane proteins are either *peripheral* (20-30%), or *integral* (70-80%). Peripheral proteins associate with the membrane indirectly through interactions with integral proteins, or directly through *lipid linkages* to the polar head groups. Integral proteins are imbedded in the lipid membrane, or cross it completely, and form *channels, transporters, sensors* and *structural components of bacterial appendages*. Integral can be single, or *multi-pass proteins*, and generally cross using a *a-helix*, but b-barrels can form too. Integral proteins can diffuse laterally but cannot invert their configuration.

The cytoplasmic membrane in bacteria plays 3 key roles. Acting as a *selectively permeable* barrier, energy production and storage, and an anchor site for proteins involved in transport, *signal transduction*, *detoxification* and *motility*.

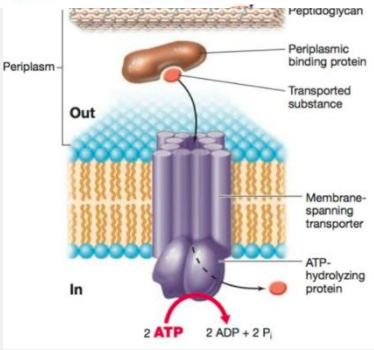
Proton Motive Force = ΔpH + ΔΨ

Due to its selective permeability, especially the impermeability to *charged ions* allows the cell the create an *ion concentration gradient*, important in pumps and energy production. The selective permeability also allows for the increase in nutrients inside the cell, which requires active transport. The *protein motive force (PMF*) is the sum of the difference in pH across the membrane, and the difference in electrical potential, and together determines the electrochemical gradient of the cell. This *electrochemical gradient* allows the cell the pair the selective import of protons with the mechanical synthesis of ATP through ATP-ase. The gradient force is created through the electron transport chain, which used the energy derived from glucose metabolism, to high energy electron carries (NADPH, NADH) allow the ETC to pump protons out of the cell.

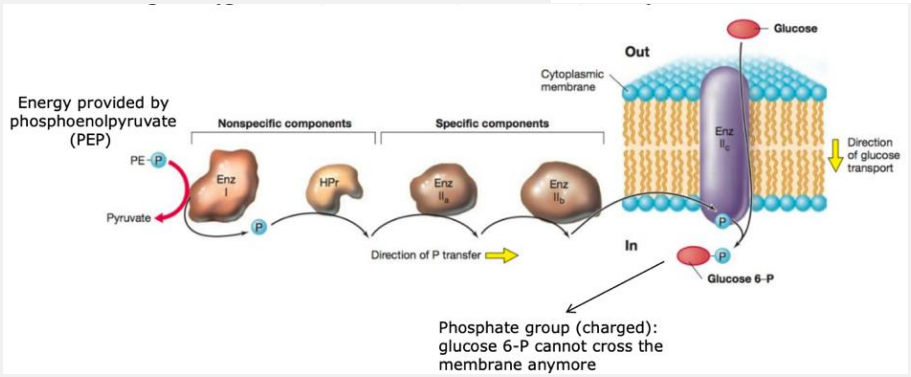




Transporter proteins mediate either passive (*facilitated diffusion*) or *active transport* across the membrane. Transporters are *selective* to a specific molecule, or a family or chemically related molecules. *Uniports, Antiport*, and *Symports* contain 12 transmembrane a-helices which form the pore. *Secondary active transporters* exploit the potential energy of the electrochemical gradient, while *primary active transporters*, and *PEP group translocators* use ATP or PEP respectively.



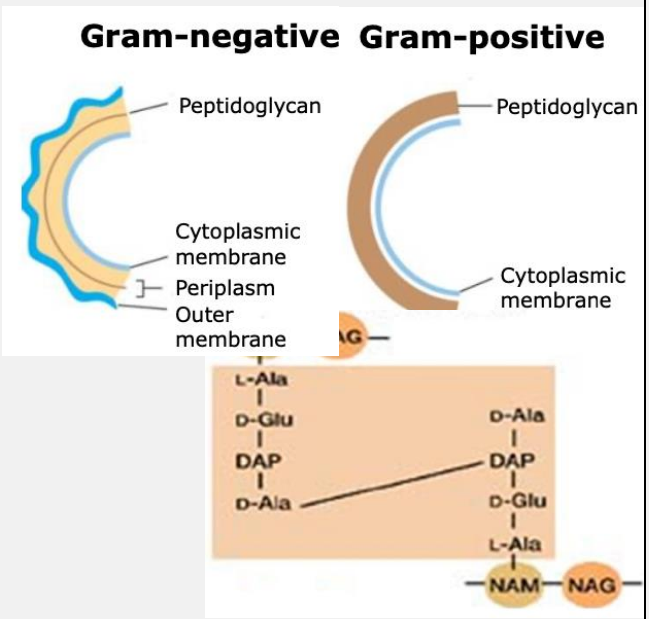
ABC transporters (ATP-binding cassette) pair the hydrolysis of ATP with the import of a substance through the periplasmic binding protein. *PEP group translocators* (phosphotransferase system) chemically modify the substance during import, usually phosphorylation once inside. PEP (*phosphoenolpyruvate*) is hydrolyzed and the phosphate is relayed through the chain. *PEP translocators* are involved in the transport of sugars such as glucose, mannose, fructose etc.. PEP translocators work on a *phosphorylation cascade*, beginning with PEP, and ending with *Enz-IIc*, the transmembrane protein that actually transports glucose, and immediately phosphorylating it (*Glucose-6-phosphate),* blocking it from leaving the cell, and locking it inside.



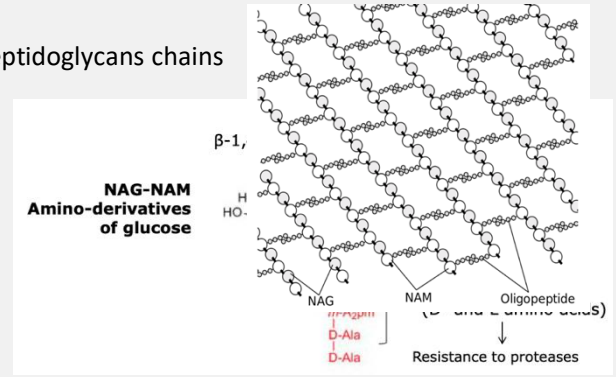
Enz I, and Hpr are nonspecific components in the phosphotransferase system, while Enz-II(a, b and c) are specific to each substrate. There are more than 15 different Enz-II-a,b,c complexes in E. coli

Cell Wall

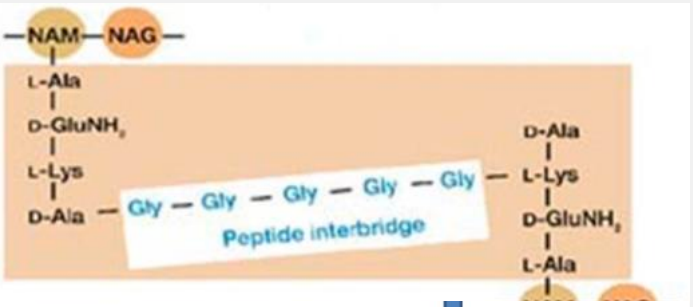
Cell walls contribute to the shape of the cell and provide support. It also resists damage due to osmotic pressure, and some degree of resistance to diffusion of molecules. It is a single, bag-like molecule made of polysaccharide chains made of glycans, and cross linked with peptide components, short amino acid chains. Cell calls can be grouped into gram-negative, or gram-positive. The difference is mainly in the thickness of the Pepto glycan layer, as well as the presence of outer membrane in gram-negative. The outer membrane provides a higher resistance to diffusion of molecules, such can convey a resistance to antibiotics.



Gram-positive have many more layers of peptidoglycans, but these peptidoglycans chains are chemically identical. The gram staining test can be used to see the difference between gram positive and negative, in which in gram-negative, the dye is easily washed out. It is important to remember the gram-positive and gram-negative cells react differently to the staining due only to physical differences rather than chemical differences in the wall.

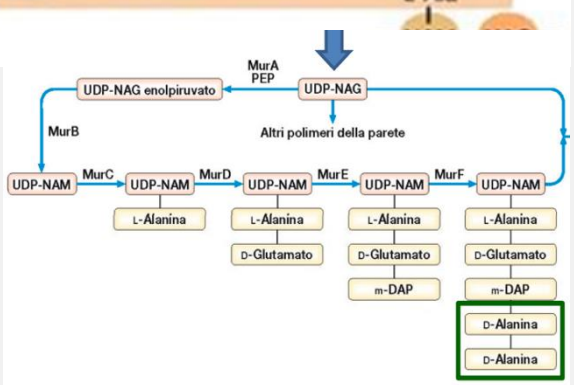


Gram+ and gram- have a similar peptidoglycan. The peptidoglycan in gram- is formed into a complex heteropolymer, made of glycan tetrapeptide monomers. This monomer is made of NAG (N-acetal-glucosamine) and NAM (N-Acetyl-momonic acid), linked to a tetrapeptide, which gives a resistance to proteases. A chain of this glycan tetrapeptide is formed through B-1,4 glycosidic linkages containing dozens to hundreds of monomers. The D-Ala can form a cross linkage to a neighboring DAP, and in this case the second D-Ala is removed to form the cross link, by two tetrapeptides.



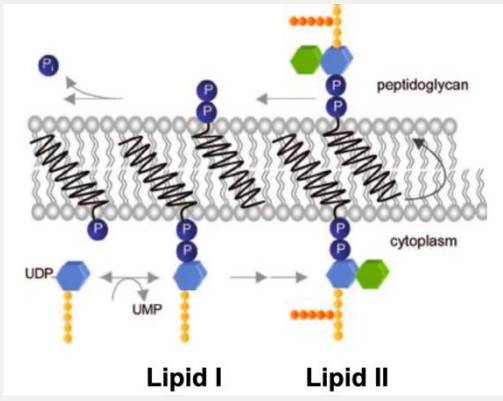
In gram positive instead, we have a slightly different amino acid chain, containing L-Lys instead of m-A2pm, which changes the way the chains are crosslinked. In gram+ we have a peptide bridge made of 5-Gly instead of the interridge from Ala to DAP.

This is the standard gram+ and gram- bacteria, but many exceptions exist, especially differences in the 3rd amino acid. On the same idea, the pentaglycine bridge can be substituted by other pentapeptides, or even single amino acids. The bridge is usually a 4,3 bridge but can also be a 3,3 bridge when the cell is stressed.



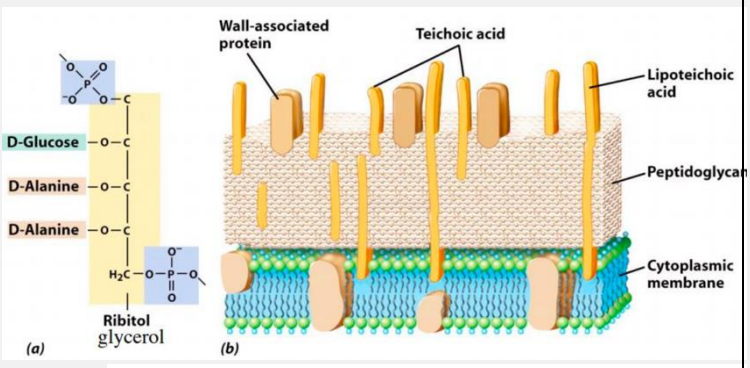
Peptidoglycan biosynthesis:

The formation begins with fructose-6-phosphate, imported by the PEP translocator, an amine is added, a phosphate is moved, and the N-acetylglycasamine-1-P is formed. The NAG is then attached to a UDP. This is NAG-UDP is modified by Murine enzymes (MurA, MurB…MurF) to form UDP-NAM. Therefor we can see everything in the peptidoglycan monomer is synthesized by the fluctose-6-phospate. The UDP-NAM and the NAG-UDP are connected by a bactoprenol, a highly hydrophobic membrane bound protein, NAM-NAG is formed, through the removal of UDP .
The bactoprenol is responsible for the flip-flop motion of the peptidoglycan to the outside of the cytoplasm. In the last step, the monomers are polymerized by trans-glycosylases enzymes, who form the B-1,4 glycosidic bond, followed by the transpeptidation, the formation of a new bridge between the two peptides. The energy for these reactions is taken from the breakage of the last covalent linkage between the last two D-Ala.

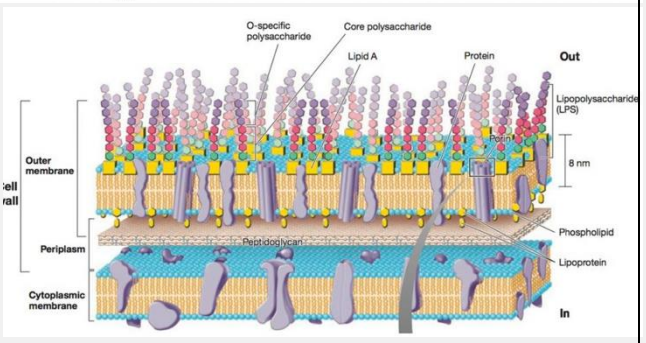


Penicillin Binding Proteins (PBB): enzymes the catalyze the trans-peptide and trans-glycosylation. Many bacteria, especially pathogens have 4-8 different PBP's. Penicillin therefor blocks the transpeptidase bond formation. PBP can be high or low molecular weight, where HMWPBP has trans-peptidation and trans-glycosylation activity, while LMWPBP only blocks trans-peptidation.

Autolysins instead are the enzymes able to cleave the B-1,4 cross linkage, which extends the peptidoglycan chain. The peptidoglycan assembly process is dependent on these autolysins and must maintain the seamless structure of the cell wall by breaking one peptidoglycan and simultaneously forming three new chains. The inability to maintain a seamless structure results in the lysis of the cell due to the osmotic pressure increase. Many enzymes exist for the formation and remodeling of the peptidoglycans, many involving the B-1,4 bond breakage. Amidases work specially on the peptidoglycan amino chains. Peptidoglycans outside the cytoplasm can be recycled, or even used as signal molecules.

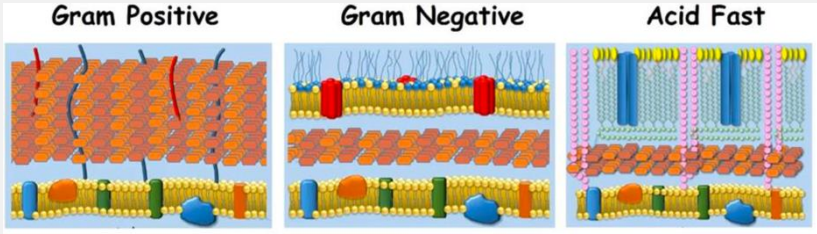


Teichoic acids are found only in gram+ bacteria and is a peptidoglycan imbedded element. Teichoic acids influence porosity, elasticity, strength as well as interaction with cations. IT also works as a phosphate storage mechanism, and the lipoteichoic acid connects the peptidoglycans to the cytoplasmic membrane.



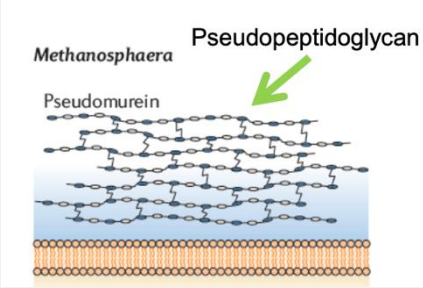
Gram- also contain an outer membrane containing lipopolysaccharides, (LPS), which is an asymmetrical with extracellular leaflets and phospholipids in the periplasmic leaflets. The LPS is formed of three layers 1) Lipid A: is a dimer of NAG with two phosphate groups, as well as 4-7 fatty acid tails 2) Core polysaccharide: composed of the inner with (1-3 molecules of Keto-deoxyoctulosonate) and outer core (Different in species, but usually hexose and heptose) 3) O-specific polysaccharide (or o-antigen) is 4-5 sugars, and highly immunogenic. LPS contributes to structure, protects the membrane, increases negative charge of the cell membrane, and induces strong immune responses in animals.

The periplasmic space is found between the inner and outer membrane and is a gel like matric containing peptidoglycans and different proteins. The periplasmic space is important for conducting reactions that cannot occur inside the cell, such as proteasomes and is continuous with the extracellular space. This connection is made by PORIN proteins, which perforate the outer membrane. PORINS form a basket and allow the passage of small molecules. Other outer membrane proteins are used for active transportation, and lipoproteins which connect the outer membrane to the periplasmic space, and serve structural, transport, and signal transduction.



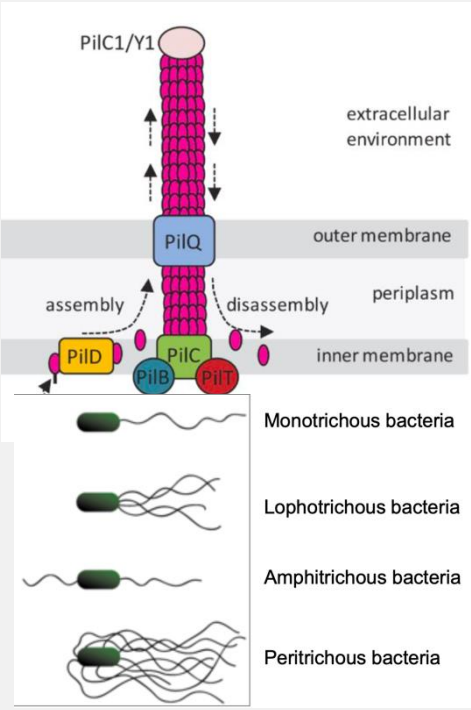
Some bacteria lack the cell wall completely, and usually work in an osmotically controlled environment. They instead use sterols to stabilize the plasma membrane. Other bacteria lack the peptidoglycan but still have an exoskeleton like structure to resist the osmotic pressure. Another cell wall exception is in mycobacterium, in which we have a cytoplasmic membrane, but instead a cell wall consisting of a basal layer, and an outer layer. The inner later is composed of peptidoglycan and arabinogalactan (branches polysaccharide) and the outer later is made of mycolic acid and other lipids. These mycobacteria can be considered a third type of cell wall other than Gram+ and Gram-. Mycobacteria are only studied so well due to their harsh effects on humans.

There are additional coats outside the cell wall, mainly glycocalyx and s-layer. Glycocalyx is present in some bacteria help in protection, adhesion, and resistance to dryness. Glycocalyx can be a well-organized capsule or a slime. S-Layer instead form a protein layer outside the cell wall.



Cell wall in Archaea is much more variable, most commonly and simply the Methanosphaera formed of a pseudopeptidoglycan similar to NAM, but instead NAT, which links to different peptides. In this form, we lack D-amino acids, only have L.

Appendages and Motility:



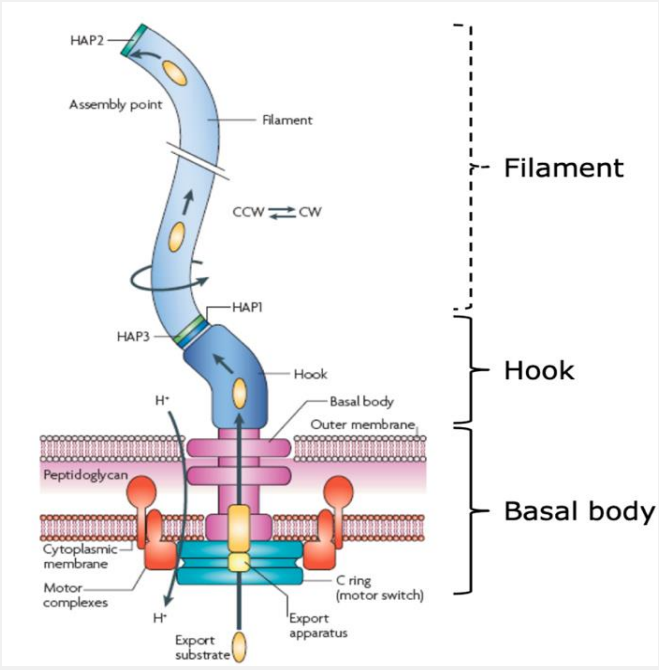
The main appendages in bacteria are pili, fimbriae and flagellum. Pili and fimbriae are very similar, differing mostly in size. Both are thin, protein tubes originating from the inner membrane, common in gram-, sometimes in gram+. Pili are typical longer, and fewer in number, while fimbria are short and numerous. Both serve to stick bacteria to a surface, allowing them to resist flushing and colonize environmental surfaces. Fimbriae and pili have shaft proteins called Pilin, and at the top have a variety of proteins that interact with the surface glycoproteins or glycolipids, can serve as adhesive to other surfaces, in pathogens the target cells.

Function of Pili: 1) Genetic exchange: the sex pilus is involved in the genetic exchange between cells in the process of conjugation. 2) Pathogenicity: Pili interact with surface proteins on the host cell, allowing the pathogens to attach to the target cell. 3) Movement: some pili assist in movement, specifically twitching. Twitching is created by the type 4 pilus(right) .

The pilus increases in length due to the polymerization of pilin (pink), a process performed by two ATPases, PilQ and PilC in pilus 4. The twitching movement, produced by pilin 4 is a steplike movement from attachment, retraction, unattachment, extension.

Another movement, called *gliding*, we include all bacterial movement not involving flagella or pili that require energy. The gliding class is large varied, and mostly found in flavobacterium species. Another example is seen in the Myxoccoccus Xanthus, considered the fastest bacterium due to its diversity of techniques used to move. One movement called AGL-GLT, evolves focal adhesion complexes attached to the cytoskeletal filaments(MreB).

The most common and known movement technique is from the flagellum. Flagella are long and thin hollow filaments composed by proteins, and bacterial cells can have one or more flagellum, usually indicative of the species but can also change due to environmental factors.



Structure of eubacterial flagellum:

The external part is formed by flagella, attached to the hook. The filament is composed of a cap protein(green) and the curved filament made of over 20000 single protein subunits, called flagellin. The flagellin is added to the flagellum filament at the cap end, because the flagellum is hollow, and is therefore transported up, and is assisted in placement by the cap protein. The hook is a flexible structure made of about 120 subunits of a single protein and is allowed to polymerize due to the hook proteins similar to the cap proteins. The basal body is composed of four protein rings L-pipololysacharide, P-perapexic space, MS-Membrane spanning and C- cytoplasmic ring anchor the basal body to the cell membrane. The IM rings (MS and C) represent rotor while MotA and MotB represent stator. Fili proteins (c ring) are involved in the regulation of the direction of the flagella rotation. The force is created by the proton motor force, the selective entry of H+ (Na+ in some marine bacteria) rotates the flagella. In Gram+, we see the absence of L and P rings due to the absence of the outer membrane.

Flagellum biogenesis: grows from the inner part to the outer part. The basal body is assembled first, and the basal body allows the formation of the hook, and in turn the filament. Each of these steps contains checkpoints, in which the cell can stop the production of components.

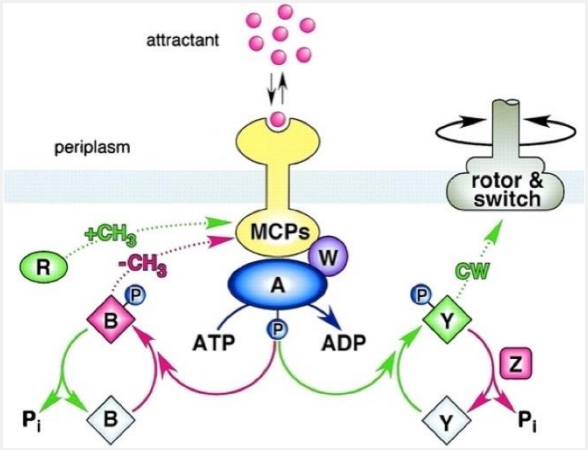
Flagella dependent movement is divided into swimming, and swarming. The Counterclockwise movement is associated to the forward movement called the run, in which the flagella line up and focus on one direction. On the other hand, a clockwise movement called the tumble movement, involves the disruption of the coordination of flagella, and slows movement. In single monoicous flagella organism, the movement can be forwards and backward in this way. Swimming speeds are extremely fast, per body size faster than the cheetah.

An interesting case of flagella is seen in endoflagella in spirochetes, the corkscrew shaped bacterial cells, which contain a corkscrew shaped flagella contained inside its periplasmic space.

The second flagella dependent movement is seen on the surface of water. This is considered faster than swimming, and are peritrichous (many flagella), and is a social movement, many bacteria cells do it together.

Flagellum of bacteria is functionally similar to eubacterial flagella. The difference is that the filament is not hollow, and therefor grows from the base instead of the top. We therefor lack a secretory pathway to the top of the flagellum. Instead of the proton motor force, they use ATP.

Quorum Sensing



Bacterial movement is based on Taxis, the movement in response to a specific chemical or physical stimulus, and can be attractive, or repellent:

– Chemotaxis: response to chemical compounds
– Phototaxis: response to light
– Aerotaxis: response to oxygen
– Hydrotaxis: response to water
– Osmotaxis: response to ionic strength

The response to stimulus, and the shifting between run and tumble, seems to be random in direction, but overtime the directional movement is obvious. The rate of tumble and run is controlled by the chemotaxis machinery. The first step in the pathway is signal recognition, in the MCP receptor protein, which are membrane localized. The MCP proteins can bind either attractant, repellents or both. The MCP induces the autophosphorylation of ChA and ChW. The ChW activates the regulator molecule ChY which increases clockwise motion. ChR and ChB are activated by a repellent signal, which act as movement suppressors and block the phosphorylation of CheY.

Cell Metabolism:

Catabolism yields energy through the digestion of complex molecules into smaller subunits, while anabolism costs energy and builds complex molecules from smaller parts. Bacteria must uptake nutrients from their environment for catabolism, these nutrients are macro or micro. Macronutrients are needed in large quantities, mainly formed of C,H,O,N,P,S, as well as Ca, Fe, Mg, K in smaller amounts. Bacteria also require micronutrients in trace amounts, including Zn, Mo, and Mn.
Certain active transporters selectively uptake these nutrients, and can be broken into:

1. Simple transporters - driven by proton motive force (antiport)
2. Group translocators -chemical modification of import molecule, driven by phosphoenolpyruvate (Glucose import, PEP transporters)
3. ABC transporters - uses periplasmic binding proteins, fueled by ATP hydrolysis (most transport proteins)

Different bacteria take energy from different sources. The main division is between the source of their carbon, heterotrophs consumer carbon already fixed by autotrophs in the form of sugars, while autotrophs fix their own carbon through CO_2. Therefore, heterotrophs derive energy from the oxidation of sugars synthesized by autotrophs, and in return recycle materials autotrophs depend on, and degrade harmful byproducts.

Cells 'produce' high energy battery molecules by coupling the oxidation of exergonic reactions to the formation high energy phosphate bonds(ATP, GTP, PEP) , or indirectly using electron carriers (NAD+/NADHNADP+/NADPH, FAD/FADH2). This is achieved through the stepwise reduction of the high energy electron through various electron acceptors and donors, passing

the electron from one carrier to the next, decreasing energy with each pass. The electron transport chain ends with the best electron acceptor, O2 where we form H2O.

Energy carriers, such as Pyruvate, ATP, Glucose-6-Phosphare, Acetyl-CoA are all classified by the free energy produced by the hydrolysis of their high energy group. Electron carries (NAD+) called coenzymes are free to diffuse, and accept an electron from the electron donor substrate, which will accept the electron by binding to the active site of a specific enzyme. After acquiring an electron, the charged NADH can then associate with a second enzyme, who will reduce the NADH to NAD+, using this free energy to transfer an electron to the product. In this way, electron carriers allow the redox reaction to occur without the direct contact of the primary electron donor, and the final electron acceptor.

Compound	$G^{0\prime}$ kJ/mol
$\Delta G^{0\prime} > 30$kJ	
Phosphoenolpyruvate	−51.6
1,3-Bisphosphoglycerate	−52.0
Acetyl phosphate	−44.8
ATP	−31.8
ADP	−31.8
Acetyl CoA	−31
$\Delta G^{0\prime} < 30$kJ	
AMP	−14.2
Glucose 6-phosphate	−13.8

Energy Synthesis:

Glycolysis happens in all conditions, aerobic and anaerobic. It consists of two phases, the investment, and the payoff phases. In the investment, two ATP is hydrolyzed, while in the payoff phase, 4 ATP is created, as well as 2 NADH, and 2 pyruvate. ATP is produced in step 7 and step 10, and since there are two molecules of glycealdegyde-3-phosphate, we gain 4 ATP. Not important to remember enzymes and reactions for his oral exam.

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$$\text{Glucose} \longrightarrow 2\ \text{Pyruvate} + 2H_2O$$
$$2\ \text{ADP} + 2\text{Ⓟ}_i \longrightarrow 2\ \text{ATP}$$
$$2\ \text{NAD}^+ \longrightarrow 2\ \text{NADH} + 2\text{H}^+$$

Type	Reaction	Organisms
Alcoholic	Hexose → 2 Ethanol + 2 CO_2	Yeast, *Zymomonas*
Homolactic	Hexose → 2 Lactate$^-$ + 2 H^+	*Streptococcus*, some *Lactobacillus*
Heterolactic	Hexose → Lactate$^-$ + Ethanol + CO_2 + H^+	*Leuconostoc*, some *Lactobacillus*

After glycolysis, the bacterial cell can either enter the citric acid cycle (aerobic) or enter fermentation reactions (anaerobic) . In fermentation, the goal is only to regenerate NAD+ from NADH by the reduction of pyruvate, in order to run the glycolysis reactions again.

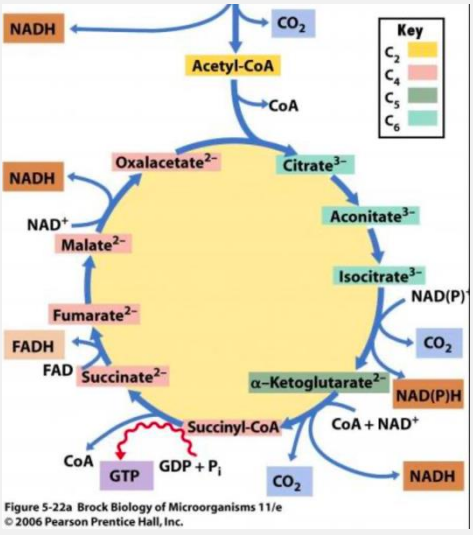

Figure 5-22a Brock Biology of Microorganisms 11/e
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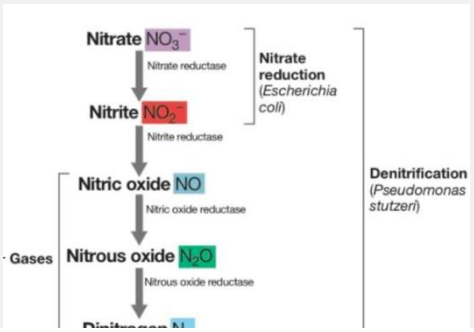
Fermentation varies in different organisms, the most important are listed above. In aspergillus bacteria as well as lactobacillus form lactic acid and give is soy sauce and cheese.

Respiration instead occurs in the presence of the final electron acceptor, oxygen in aerobic respiration, otherwise anerobic respiration. The final energetic product is ATP, NADH, NADPH and FADH2 in plants. The Krebs cycle begins with the conversion of pyruvate into acetyl-CoA, when then undergoes various reactions. The important thing to know is the number of carbons during the cycle, and the products. In the end, the complete oxidation of the substrate (glucose) to CO_2 directly produces 1 GTP (=1 ATP) for each pyruvate 2 ATP for each glucose. Also produced, is 4 NADH + 1 FADH for each pyruvate 8 NADH + 2 FADH for each glucose.

The electron carriers must then enter the electron transport chain, where their stepwise oxidation will be used to pump out protons from the inner membrane, thus increasing the membrane potential, which is later used to mechanically synthesis ATP through the ATP-synthase complex. The electron carries move through 4 complexes. Anaerobic respiration differs from aerobic in that the last electron acceptor is not oxygen. It is common in prokaryotes, rare in eukaryotes.

In summary, glycolysis produces 2 ATP per glucose molecule, 2 ATP from Krebs cycle, and 32 ATP from electron transport chain. Therefor in aerobic conditions we yield 36 ATP per glucose, while in anaerobic fermentation we only get 2 ATP.

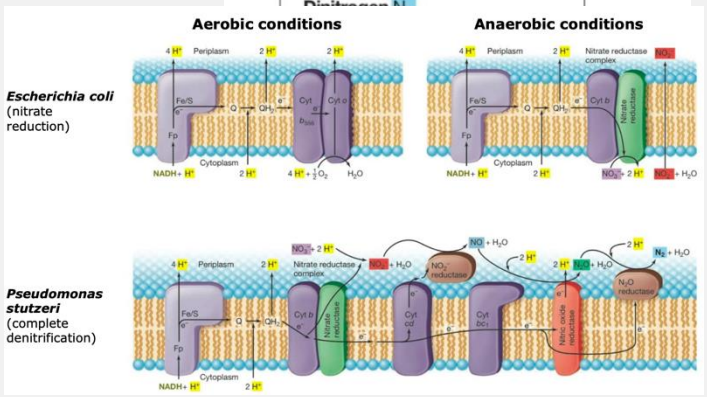
Anaerobic Respiration



One of these anaerobic respiration pathways in prokaryotes is *denitrification*, where nitrate is redacted to nitrate, nitric oxide, and dinitrogen (complete denitration) depending on the oxidative strength or the organism. These bacteria are known as *nitrifying bacteria*.

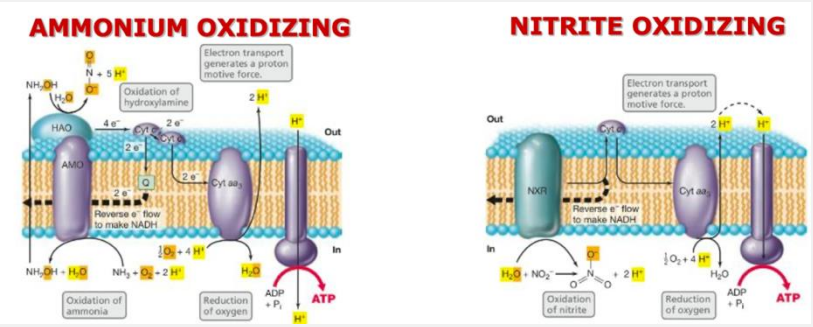
Another example is the *Sulfate-reducing* bacteria, mostly found in obligate anaerobic bacteria meaning oxygen is toxic for them.

Lastly, *methanogenesis* uses CH4, and is found strictly in anaerobic archaea.



Chemolithotrophs are widespread in nature and take energy from oxidation of inorganic compounds. They are mostly autotrophs, fixing CO_2 through Calvin cycle, are able to grow in the dark, and have a generally low metabolic rate. Most use O2 as their final electron accepter, but some use sulfur, or sulfate.

Nitrifying bacteria are common in water and soil, are important for the nitrogen cycle. Their electron acceptor is always O2. Their reducing power comes from the reverse electron transport chain. Chemolithotroph is broken into ammonium oxidizing and nitrite oxidizing, which coverts ammonia to nitrate. Both evolve specific electron transport chains in the cytoplasm. These processes are both very low yield compared to aerobic respiration.

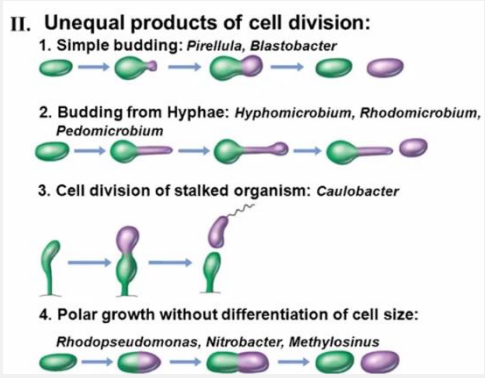


Anabolism

Glycogenesis is the reverse of glycolysis, which can be used to build the sugar bases of nucleotides in the pentose phosphate pathway. Amino acids are synthesizing through the citric acid cycle, mostly from Krebs cycle intermediates breaking off from the cycle. Glycolysis can produce other amino acids in the same manner. Fatty acid biosynthesis starts with the condensation of the Acetyl-ACP, the fatty acid is then extended though the addition of the subunits. He went through all this super-fast I don't think he cares about it very much.

Bacterial Growth:

Bacteria growth is a result of cell division, and there for growth means an increase in number of cells, population. Most bacterium divides through binary fission and is therefore called equal cell division. The first step is growth, and elongation, followed by the formation of a septum, and finally the completion of the septum and the formation of new cell walls. Each Binary fission event doubles the population and intercellular components. Therefor each generation has to double the contents of its cell, the proteins, amino acids, nucleotides and ribosomes. The time it takes to realize a new generation is called the generation time. Generation time is dependent on nutritional conditions, genetic factors, and temperature. In E. coli, the generation time is 20 minutes.



Unconventional divisions produce unequal cell division products. In equal products, we don't consider the concept of aging, while in unequal, we can because the green cell (mother) keeps all the old ribosomes, nucleic acids etc. While the purple

(daughter) receives the newly synthesized components. The daughter cell is produced in stalked organisms to explore new environments.

Types of Growth

Organisms can be grown in labs into *colonies*, a visible growth of billions of cells all starting from a single cell. We can also observe *turbid suspensions* in the lab and in nature, in which a clear, nutrient rich broth is transformed into a turbid suspension of millions to billions of cells per ml. Lastly, we can see the formation of a *biofilm*, in labs and nature, which is a thin layer on an inert surface, obtaining nutrients from the surrounding fluids.

We can therefor distinguish different colony morphologies, which can be used to describe the type of bacteria, metabolic rate. Form, elevation, margin as well as color all contribute to the colony's morphology.

Organized *colonies* and *biofilms* are called sessile growth, while turbid suspensions are called planktonic growth. Planktonic growth can tell us what type of bacteria we have based on the area where the bacteria grow, for example when bacteria grows only on the surface, we have obligate aerobes, where if they are absent at the surface they are obligate anaerobes. Sessile growths grow into communities, while turbid growths are distributed in a medium. Biofilms are a sessile growth beginning with the attachment of a bacteria to a surface, and the subsequent addition of more cells. Biofilms can only be removed through mechanical means, such as the removal of dental plaque. Biofilms prevent harmful chemicals from penetrating, dehydration and washing away of cells. *Marine biofilms* can form multilayer sheets with different organism present in each layer, called microbial mats.

Culture media contain nutrition factors such as water, macronutrients, micronutrients, and growth factors. Culture media are broken into *complex media*, in which many nutrients are available and it therefor allows the growth of various types of bacteria, whereas *chemically defined media* are formed with a precise chemical composition for the promotion of a specific kind of growth, usually for *fastidious bacterium*, bacteria that are hard to grow (called *enriched growth media* for fastidious bacteria) . We can also use *selective growth media*, which contains components that inhibit the growth of some bacteria, while *differential growth media* highlights the specific chemical reactions of interest with different colored dyes, such as use of lactose or not.

Parameters of bacterial growth

Growth is the increase in number of cells, growth rate is the change in number of cells per unit of time, while generation time is the time required for one cell to become two. This leads to an exponential increase in population growth rate. Generation time is highly variable, ranging from 10 minutes to days, and depends on environmental conditions as well as innate genetic conditions. Bacterial growth can be measured by change in number of bacteria, as well as change in specific cell components, as well as dry weight.

We can measure number of bacteria (direct count), number of colonies (viable count), or turbidity of bacteria (indirect total count). For direct count, we use a counting chamber in which the cells are laid on a grid, and tiles in the grid are marked either yes containing a cell or no meaning no cell. The problem with direct count is we can't distinguish between living and dead bacteria in the direct count. Viable count instead counts the colonies formed from single bacteria cells after a set time, and we can therefor count the number of cells present before the colonies were produced. For viable count we have to dilute the starting solution in order to distinguish separate colonies, and the CFU (colonies forming units) are counted. Viable counts from environmental samples must be mixed into many different media, and culture conditions in order to retain the highest number of different species as possible. This minimizes the risk that fast growing bacteria will out compete slow growing bacteria.

Measuring the cultural turbidity in the indirect count method is achieved through the light penetration rate, in terms of optical density values. This Is extremely fast and does not damage the sample. The drawbacks are that we need to already know the standard curve to calculate turbidity values, as well as the need for dense bacterium populations.

Batch cultures grow in a fixed shape, exponential increase, plateau called the stationary, and then slow decline. Batch cultures are created by a fixed about of nutrients. Diauxic growth involves the growth after the stationary phase, when the bacteria can switch from one carbon source to another such as glucose, then lactose. This can be measured by the appearance of B-galactosidase when the glucose supply is finished.

Continues cultures can be cultivated through a chemostat, which instead is an open system, and continuously adds fresh medium, as well as continuously releasing cells + medium. In this way, the bacterial cells are frozen in a perpetual exponential growth state. The dilution rate (concentration of limiting nutrient) modulates growth rate, and cell density.

FACTORS INFLUENCING MICROBIAL GROWTH:

Temperature: We can consider three important temperatures for each microorganism, the minimum, maximum, and optimum temperature. The minimum is the lowest temperature the bacteria can replicate at. Usually minimum is the threshold in which the membrane turns to gel, and membrane transport cannot occur. The growth rate increases slowly to the optimal temperature, which is the highest enzymatic rate, and highest growth rate. If we continue to increase after optimum, we pass the max, where protein denaturation, cytoplasmic membrane thermal lysis occurs. The maximum threshold lies soon after the optimum, and sharply drops off after the optimum.

Bacteria can be classified based on these three temperatures. Psychrophile(4 degrees C), Mesophiles (39), thermophiles(60), hyperthermophiles (88), and hyperthermophiles (106) have different optimal temperatures. Eukaryotic microorganisms, Animals and plants have a much smaller region of habitable temperatures. Sulfur dependent, and methane dependent archaea are the extreme examples of heat, while Himalayan midge, a larvae of an insect, can be observed living far below freezing, an exception to the rule.

Psychrophiles are environmental microorganisms which presents problems especially in their ability to contaminate foods which we keep in low temperatures. These psychrophilic bacteria are obligate, or facultative (or psychrotolerant). Obligate survive between 15-0 degrees C, but up to -12C with an optimum temperature around 10. Facultative can grow at 0C, but their optimum is 20-30C, and therefor facultative are much more dangerous as a pathogen to humans.

These microorganisms use enzymes that function optimally in the cold, a property given by many a-helixes, and few b-sheets, as well as the content of many polar amino acids, and less hydrophobic amino acids, which increase flexibility and catalytic activity at low temps. Secondly, their cytoplasmic membrane has a high concentration of unsaturated fatty acid. Lastly, they protrude special antifreeze proteins called cryoprotectants, which prevent the formation of ice crystals in the cell.

Thermophiles instead gain their properties through enzymes that work optimally at high temps, which are similar in amino acids sequence. Secondly, the cells produce a lots of solutes, such as diglycerol phosphate, which could be involved in protein stabilization ay high temps. Lastly, the cytoplasmic membrane has a high concentration of saturated fatty acids.

Osmolarity: Activity of water (aw) Relationship between the quantity of water, that is not involved in an interaction with other solutes, that is the water that cannot evaporate. Cells have a higher concentration of solutes, and therefore have a positive water balance. When the cell is in an environment in which Aw is very low, water flows out if the cytoplasm, dehydrating the cell. This concept is also called osmoregulation, hypertonic solutions, cause cell dehydration, isotonic solutions maintain the turgor pressure of the cell, while hypertonic solutions cause the lysin of the cell from too much water entering.

Bacteria can be classified by their osmolarity, called their salt tolerance. Nonhalophile (%NaCl = 0), Halotolerant (0-5) , Halophile (6-7), and Extreme halophiles (18+) have different ranges in which their optimal activity is achieved. Extreme Halophiles interestingly increase intercellular osmolarity through compatible solute accumulation, which imports the solutes that do not affect their cellular functions. The compatible solutes can be Sucrose, glycerol, and other organic compounds such as amino acid derivatives, sugar, and even ion in some Archaea and Bacteria.

Oxygen: Bacteria can be Aerobes (obligate, faculties, microaerophilic) or Anaerobes (Aerotolerant, obligate). Obligate need oxygen to survive, while facilitative can use oxygen, or not. Microaerophilic bacteria require only low levels of oxygen, while aerotolerant do not require oxygen, but frow better with it, while obligate anaerobes are negatively affected by oxygen. As we mentioned before, the type of bacteria can be identified in the lab based on the environment they grow in inside the lab.

Oxygen is reduced to water through aerobic respiration, through the addition of four electrons. All of the intermediated are highly reactive and toxic, because they are strong oxidizers. For this reason, reactive oxygen species(ROS; Hydrogen peroxide, hydroxyl radical) can be accidentally generated by other metabolic reactions. ROS molecules create oxidative stress, and therefor in anaerobic organisms, we have a family of ROS-detoxifying enzymes, such as the catalase, which

reduces hydrogen peroxide, the superoxide reductase which reduces the O2 to Hydrogen peroxide. These enzymes are present in the majority of aerobic bacteria(obligate and facultative) and these enzymes are absent in the obligate Anaerobes, who are quickly harmed by these radicals. Anaerobic growths can be cultured by removing oxygen from a gel.

pH: pH provides a value describing the concentration of protons [H+] and conjugatively the concentration of OH-. Neutrophiles prefer a neutral pH, while acidophiles prefer strong acid solution and low pH, and Alkaliphiles prefer basic solution with a high pH. No matter what the pH of the external pH is, bacterial cells are able to maintain a neutral pH close to 7, regardless of if they are alkaliphiles or acidophiles.

Controlling bacterial growth:

Decontamination is the complete cleaning of an object or surface in order to make it safe to handle. Disinfection instead refers specifically to only the removal of pathogens, while sterilization is the killing and removal of all living microorganism and viruses. Heat sterilization is the increase of heat in order to kill all microorganisms, and the thermal death time of an individual is given by the Decimal reduction time (D) which is the time we need to reduce the original population to $1/10^{th}$ at a given temp. D values vary greatly between mesophiles and thermophiles.

Heat sterilization is influence by the type of heat, where wet heat is more effective than dry heat. Also, we must consider if the cell is a mesophile or thermophile, as well as the type of cell (endospore) can decrease effectiveness, as well as the pH of the medium, specifically acidity increases cell death. The medium concentration of sugars and proteins increases the resistance of microorganisms to heat, as well as the salt concentration, which can either increase or decrease resistance depending on the organism. The most common method of heat sterilization is through a device called an autoclave, which uses steam (wet heat) to kill all microorganisms including spores. Sterilization time depends on the organism's present, but must be at least 10-12 minutes, and is standard at 121C. Pasteurization is the heat sterilization for edible liquids, usually in short, flash-parturitions, in Ultra High Temperature Treatment (UHT) is extremely high heat for only 1-2 seconds.

Radiation can also be used to sterilize, specifically the treatment with UV light (220-300nm) which penetrates DNA inducing mutations and death of the exposed organisms. Due to the low penetration power of UV, it can only kill organisms on the surfaces and air, but enough time of exposure increases efficiency. Another form of radiation sterilization is ionization radiation, x-ray and gamma rays. The electromagnetic radiation produces ions and reactive molecules that harm the cell. Units of radiation are roentgens, where the standard of sterilization is the absorbed radiation dosage in rads, that reduces the initial population to $1/10^{th}$(D). Radiation is good because it can easily reach all parts of the object, and permits sterilization of heat sensitive material, as well as the sterilization is instantaneous. The cons, are that its dangerous, requires well trained staff, and special equipment to remain safe.

Sterilization by filtration instead is based on the sieve effect (selective permeability) , and is the most common in labs, where different filters are used to sift particles by size of liquid samples. Different filters can be used as different cut offs for the filtration, examples include membrane filter (complex mesh) and nucleopore filter (small holes all the same size). Filtration is effective, safe, and fast when they filtered compounds can be differentiated by size.

We can also use *chemical antimicrobial agents*, which can be broken into: Sterilant: Destroys all forms of microbial life, Disinfectants: Can only be used on nonliving objects (high concentration alcohols), Sanitizer: Reduces microbes to a safe level, Antiseptics: Chemical agent that kills or inhibits, and can be safely used on living tissues. Antimicrobial agents can be natural or synthetics, and comprise disinfectants, antimicrobial drugs, and antibiotics. When it inhibits growth, it ends in -static (bacteriostatic). When is kill, -cidal (fungicidal) when it kills by lysing, -lysitc (bacteriolytic)?

Antibiotics:

Treating infectious diseases

Antimicrobials drugs can be broken into antimicrobial agents, which inhibits or kills microorganism, while antibiotics are produced by a microorganism which kills or inhabits growth of microorganism. Many antibiotics are synthesis chemically simply because it is cheaper than the naturally occurring alternative. These antibiotics can also be improved or modified chemically to form semi-synthetic antibiotics. Both of these drugs principle on selective toxicity, hitting the pathogens while leaving the healthy microorganism.

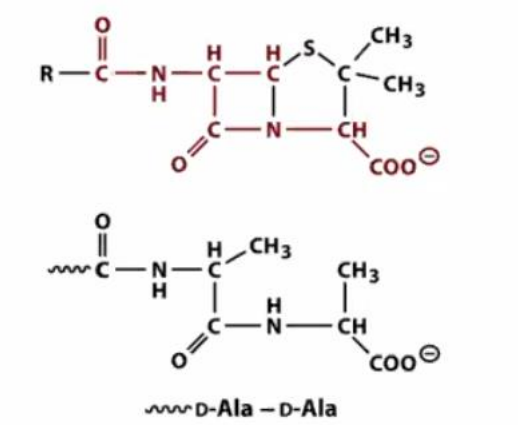
Paul Ehrlich was interested in the idea of biological magic bullets, that is designer molecules able to target and disable a specific disease-causing agent. He created Salvarsan, an arsenobenzol which is highly toxic. Fleming instead, discovered a fungi that could inhibit the growth of strains of bacteria, called later penicillin, which can be purified and used as a treatment therapy. Other antibiotics were discovered in a similar fashion, through the studying of molds.

Antibiotics can be broken down by their source, Gram positive rods, Actinomycetes, and fungi. Thousands of antibiotics have been discovered in organic compounds, only a small percentage can be used due to the selective toxicity principle, most are simply too toxic for humans. The problem shows that discovering new molecules with novel antibiotics mechanisms, is sadly more expensive than modifying existing antibiotics, which inevitably leads to faster resistance to antibiotics.

We also need to consider the therapeutic index, that is the blood concentration when the drug becomes toxic, and when it is effective, if this window is small, its hard and risky to correctly dose. As a general rule, antibiotics are less toxic when the specific target is absent or structurally different than the host, that is, very effective antibiotics target cellular components that the pathogen has and the host cells do not. We also need to consider the range and type of activity of the antibiotic.

The range of activity is the range of bacteria species that the microbial agent is used to kill. A large range of activity means a wide range variety of agents it can kill, such as tetracycline. No agents can stop all pathogens, and the wider the range of the agent, the lower dose must be used as to not kill all the useful bacteria.

Antibiotics can be broken into bacteriostatic, bactericidal, and bacteriolytic. Bacteriostatic stop growth, and keep the number of living bacteria constant, bactericidal kill the bacteria, but the total number of bacteria remains constant, and the viable count drops. Finally, the bacteriolytic kills the cells through lysing them, so the bacterial count, and the viable count drop. A test to assess the antimicrobial activity is measured by the minimal inhibitory concentration (MIC) which is the smallest concentration that inhibits the growth of a microorganism. MIC varies based on both the antibiotic, and the target organism. This can be studied the test-tube concentration test, or the Kirby-Dauer disc diffusion method. These methods both have advantages and drawbacks, chiefly the test-tube concentration test is easiest to standardize, while the disc method lets us study many different antibiotic compared to each other. A hybrid of this is the E-Test diffusion method, where a strip containing a gradient of one antibiotic is laid in a medium, and therefor we can compare many different antibiotics, as well as needed concentration.

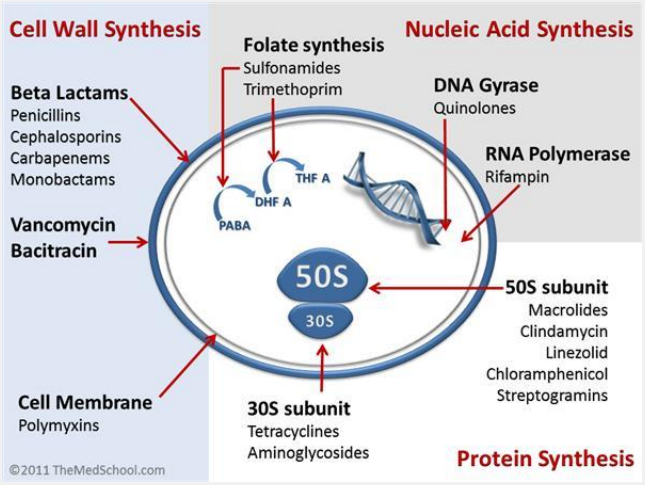


Antibiotic targets

Cell wall: Antibiotics that target the cell wall biogenesis are called b-lactams. B-lactams interfere with the cell wall, specifically the inhibition of the transpeptidation of the peptidoglycans. Penicillin targets the transpeptidase enzyme (PBPs) , by imitating the D-Ala D-Ala as a B-lactams structure is very similar. This leads to the acylation of the serine residues in the PBPs site, irreversibly inactivating the enzyme. The R group of the B-lactams(top) have been industrially modified in order to create penicillin derivatives such as Methicillin, oxacillin, ampicillin which each have slight advantages, such as oxacillin's resistance to acidity, increasing bioavailability due to decreased breakdown in the stomach acids.

Another antibiotic targeting the cell wall is vancomycin, a glycoprotein antibiotics. While b-lactams inactivate the PBPs, vancomycin's attach to the D-Ala D-ala residues, therefor blocking the assembly of the peptidoglycan by hiding the residues, the same result of B-Lactams, but different methods of action.

Translation and Transcription: Macrolides bind to 50S(large) subunit and prevent continuation of protein synthesis, by blocking the active site, while chloramphenicol also works on the large subunit, but by blocking the formation of the glycosylic bond. Tetracyclines interfere with the attachment of tRNA to mRNA-ribosomes complex. Aminoglysides change the shape of the 30s(small) subunit of the ribosome causing the code on the mRNA to be misread.



Other antibiotics such as Rifamycin's interact with the b-subunit of RNA polymerase, inhibiting RNA synthesis and blocking transcription. Quinolones instead inhibit bacteria DNA gyrase, the enzyme responsible for supercoiling.

Metabolism: Sulfonamides are not antibiotics because they do not derive from a natural source, therefor technically they should be called anti-microbial agents. Sulfanilamide imitates the structure of p-aminobenzoic acid, an important metabolic element involved in the synthesis of folic acid, which is important for the biosynthesis of purines and pyrimidines. Therefor when the sulfanilamide is introduced, inactive folic acid will be unable to be synthesized efficiently. Humans do not synthesis folic acid, we consume it in the diet, and therefore sulfanilamide is not toxic at all to humans

Antivirals and Antimycotics

Viruses are harder to target with toxic compounds because viruses often use the hosts cells components to multiply, so targeting the versus is targeting the hosts own cells in some way, therefor its much harder to find antiviral and antimycotic drugs. In fungi and protozoa, the problem instead is that they are eukaryotes and therefor share many more metabolic and enzymatic similarities with humans.

Some antiviral drugs block the attachment and entry process into the cell, such as hydroxychloroquine blocking the formation of clathrin buds. Antiviral can also target some unique components of the virus; in retro viruses we can block reverse transcriptase. Antimycotic drugs target cell wall biosynthesis, sterol synthesis (ergosterols instead of cholesterol), and nucleic acid synthesis, again exploiting the differences between host and parasite. Polyenes for example interact with the ergosterols in the membrane, which is not present in mammals, therefore disrupting the membrane. Azoles instead target the synthesis of this molecule (ergosterols), while Griseofulvin disrupts the microtubules during mitosis, but is also inherently toxic to mammals because we use the same microtubules. Polyoxins can target chitin synthesis, and 5-Florocytosine instead targets nucleic acids, and is very toxic to mammals.

The search for antifungal activity is again chiefly limited by toxicity, but sometimes it can be seen as a opportunity, especially in the case of Vincristine, Taxol which disrupts the polymerization of microtubules. This is innately toxic, but is much more toxic for rapidly dividing cells, allowing them to kill cancerous cells faster than they kill healthy cells.

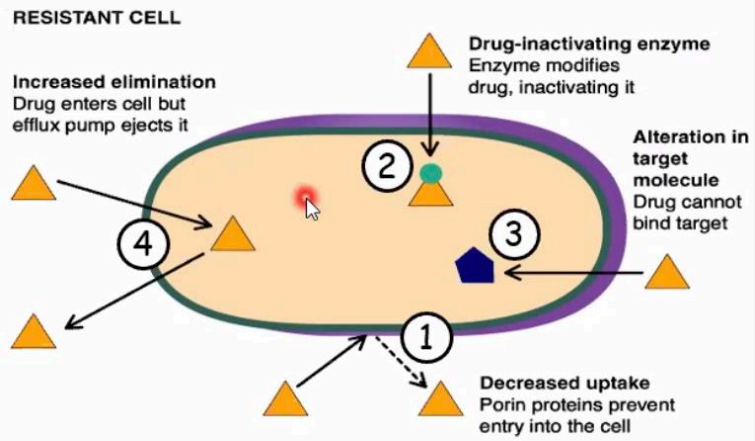
Antibiotic Resistance

Antibiotics radically changed the reason people died, the top three causes of death in 1900 were all infectious diseases, while today only two of the top ten are infectious diseases. This revolution has radically improved life quality, but also created the problem of antibiotic resistance and superbugs. There are two types of resistance, intrinsic resistance occurs is all strains and species and is caused naturally by absence of a target, low affinity target, or low permeability. Acquired resistance appears as a mutation or the accusation of a gene (horizontal transfer), causing the target to be modified and the inactivation of the drug, or the efflux mechanism (pumps) is changed. Acquired resistance is accelerated by human antibiotic activity, and importantly differs between strains vs intrinsic resistance which occurs in all strains in a species.

Antibiotic resistance appears shortly after antibiotics enter the clinical practice. Many strains which are resistant to all available antibiotics (pan-resistant strains) have been isolated, meaning we can find these strains, and call them superbugs, very rare, but very dangerous. Plasmids can be horizontally transfer, and some of these plasmids confer antibiotic resistance.

The R-Plasmid was a plasmid containing resistance to six different antibiotics, and if spread these multiresistant plasmids are able to confer antibiotic resistance rapidly and irreversibly.

Antibiotic resistance in induced by selective pressures that by nature only let antibiotic resistant mutants to survive. These mechanisms generally work by:



1) Reducing permeability: Reduced permeability can cause the reduced expression or loss of function of specific transport proteins(porins) or modification of cell wall component (LPS) as well as mutation of the binding site of the antibiotic.
2) Antibiotic inactivating mechanism(b-lactamases break the penicillin ring, forming penicilloic acid) Clavulanic acid is able to inhibit b-lactamase enzymes, destroying resistance to penicillin
3) Modification of molecular target
4) Antibiotic efflux (pumping out) can be accusation or overexpression of efflux pumps which are either specific or wide range
5) Development of an alternative metabolic pathway, such as in the case of resistance to Vancomycin, which took a long time because it involved mutations in more than one metabolic events causing D-Ala D-Ala to be changed to D-Ala D-Ser or D-Ala D-Lac in PEP protein cell wall synthesis

Drug resistance develops from spontaneous mutations, or horizontal gene transfers. This is accelerated by humans due to the over prescription, use in agriculture, and use of broad-spectrum antibiotics when knowledge is limited. Resistance increases morbidly and mortality rate, as well as increased cost of treatment. To fight these problems, we can use Antivirulence drugs, or phage therapy. Anti-virulence drugs are not toxic for 'good' bacteria, and do not affect the survival of pathogenic bacteria, instead inactivate their pathogenic parts. This means there is no selective pressure and therefore no resistance, and can work by inhibiting quorum sensing, toxin excretion, or secretion systems. Phage therapy began in 1921, and is still used today, although it was mostly abandoned after the discovery of antibiotics, and now is resurging due to antibiotic resistance. Bacteriophages are limited because they cannot be administered systemically, they are ineffective against intercellular pathogens, and treatment is limited by host range. We also encounter the same problem as antibiotics, as we select for bacteriophage insensitive mutants (BIM). Bacteriophages work by receptor recognition, therefor receptor modification, masking of receptor as well as EPS (exopolysaccharide) obstruction and EPS modification creates resistance to bacteriophages.

Biofilms are heavily resistant to antibiotics, because they can only penetrate the peripheral later. Longer time in saturation increases the penetration, but always contains a safe zone for the bacteria. In the horizon of the safe zone, the bacteria are slowly introduced to low concentrations of the antibiotic, creating the perfect resistance to the antibiotic. Therefor we have to define MBEC (minimum biofilm eradicating concentration) instead of MIC If the bacterium forms biofilms. The MBEC is always much higher than the MIC

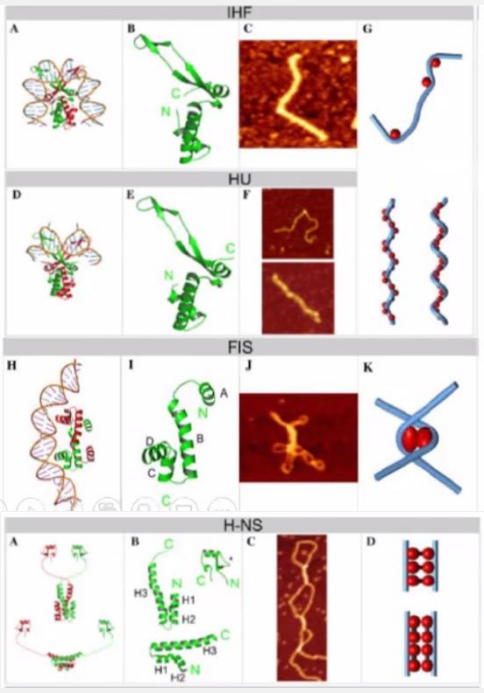
Bacterial Genetics

Central dogma of biology tells us DNA replicates to two new molecules before division, is transcribed into RNA by RNA Polymerase, and this RNA is translated into proteins by ribosomes. Retro viruses are an exception to the dogma, with the reverse transcriptase enzyme converting RNA to DNA.

Genome Structure

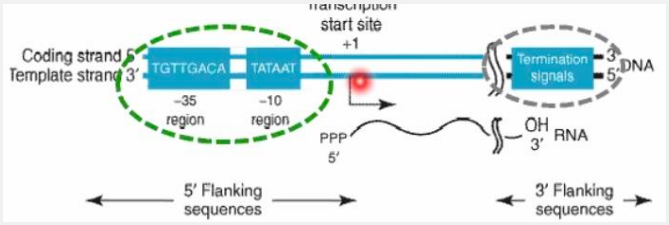
Prokaryotes contain two important sources of genetic information, chromosomes and plasmids. Chromosomes contain essential genes as well as non essential, while plasmids carry non-essential genes, or genes only essential in certain environments. Both of these contain transposable elements, chromosomal islands, and integrated phages. In most prokaryotes the genome consists of a single circular chromosome, with many notable exceptions such as Vibrio Cholerae which has two circular chromones, one containing all the essential genes, while chromosome two contains genes giving

resistance to antibiotics. Some other rare cases such as Streptomyces have a single linear nucleoid, presenting problems in replication.



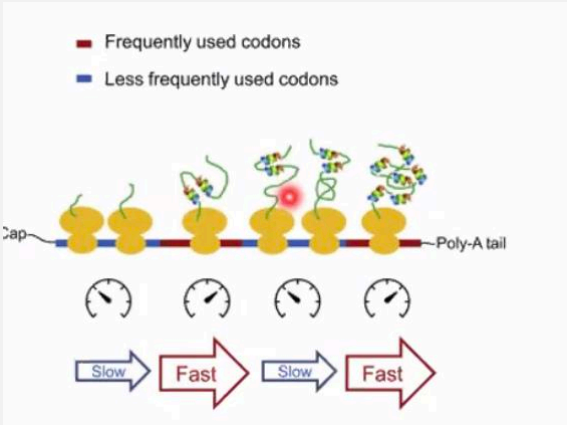
The E. coli chromosome is many times larger than the cells body, therefore we know they must have a packaging mechanism to compact the DNA at least 1000 fold. Two factors contribute to the compaction of nucleoids; the supercoiling of DNA and NAPS (nucleoid associated protein, previously called histone-like-proteins). Supercoiling allows the bacterial chromosome to loop around itself, reducing the space that it occupies. This supercoiling follows the topo isomeric principles we learned in molecular biology, the system must be opened, modified, then closed. Two classes of enzymes exist, DNA gyrase, which induces negative supercoiling, and topo-I, which relaxes supercoiling, negative in bacteria. NAP proteins instead contribute to the organization of the nucleoid and the control of gene expression similarly to histone proteins in eukaryotes, different proteins important in different activities. NAP proteins also play a structural role in the bacterial genome. Examples include H-NS, IHF, HU, FIS, all contributing to the packing shapes of the chromosome.

Bacterial genomes have smaller genomes in general, but the number of ORF's does not vary as greatly compared to eukaryotes. This can be explained by the so-called genome efficiency of prokaryotes, while they have the same number of genes as eukaryotes, the amount of non-coding DNA is vastly higher in eukaryotes. Coding and non-coding sequences in bacterial genomes differ from those of eukaryotes because eukaryotes contain introns important for alternative splicing, while bacterial genes generally do not contain introns, with few exceptions.



Coding sequences can be identified by looping for exceptionally long regions in which, in random DNA, we would expect to fine a stop codon. If these stop codons appear significantly less than 3/64 codons, its likely we have found an open reading frame, a long sequence with no stop codons which likely represents a coding sequence. Another important denature which helps us identify a potential coding sequence is the Shine-Dalgarno sequence or Ribosome Binding Site (RBS), an element on the mRNA telling the ribosome to start. We can also look for known promoter sequences (-35, -10), as well as transcriptional terminator. The best promoter sequences would contain -35 region, and -10Tatabox in a perfect theoretical consensus sequence, but is impossible to find in because the expression for ANY gene would be too high, it would necessarily be toxic. Terminators can be RHO dependent, or RHO independent, the former depends on the attachment of a Rho protein, which is what induces termination. In Rho independent, a steam loop structure drives the termination. Software's can be used to find ORFs based on these techniques.

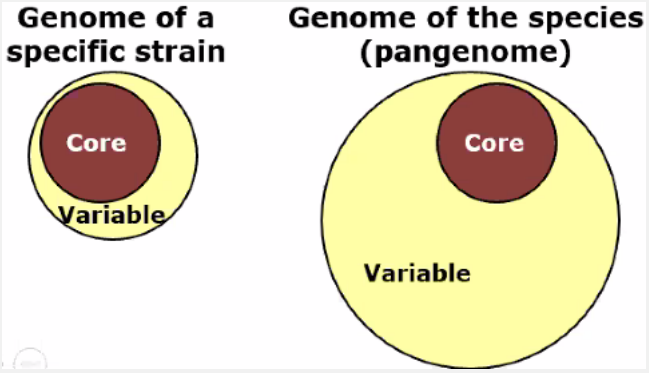
Bacterial genomes have a unique element not contained in eukaryotes, called an operon. Operon are groups of adjacent genes which are transcribed by a single RNA, and are regulated by a single regulatory region (promoter). The promoter and terminator are only present once each per operon, but the number of genes can be 2-15, all which work in a pathway and need to be transcribed together. Some amino acids can be coded by different codons, because there are 22 amino acids in the code and 64 combinations. Therefore, some codons are used more frequently and some and used less frequency, where frequently used codons are able to be translated much faster due to aminocyta-tRNA concentration. In this way, the bacterial cell can regulate the expression of DNA based on the time it takes to translate specific codons corresponding to the same amino acid, as in this time different folding patters can occur.



Once we encounter an ORF, we need to define its product. In order to do this, we compare the amino acid sequence to a database of proteins, allowing us to predict a function in cases of high fidelity. This analysis must be considered on a domain basis, each functional part should be compared separately, using basic local alignment tools (BLASTS). Even in the best studied bacteria E. coli, we do not know the function of 38% if the genes in its genome.

Complexity of Genome

In Bacteria and viruses, the genome size is proportional to the number of genes, because of their high genome efficiency. Large genomes are found is mainly high metabolic and adaptively versatile bacteria. Small genomes are represent in bacteria with well defines ecological niches, such as intercellular pathogens who don't need to synthesize their own machinery. Some genes, called essential genes, remain constant through bacteria. These genes controlling replication, translation, transcription and other necessary function remain constant in number, so their relative percentage decreases as genome size increases, while other genes involved in metabolism or gene regulation increase in proportion as genome size increase. These essential genes cannot be deleted or modified, or the cell will die or not reproduce. Essential genes can form almost 65% in small genomes such as M. genitalium, while in E. Coli it can be less than 10%, and together can be called the *essential genome* of the microorganism.

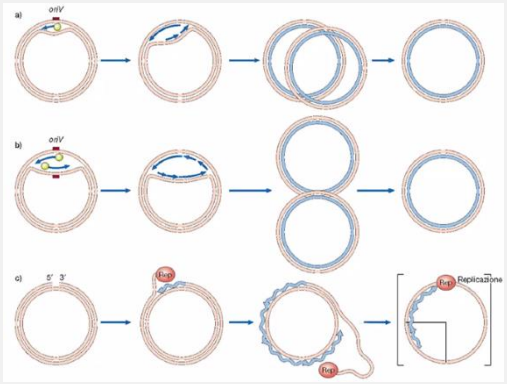


From this, we can see that within a species share around 80% of the same common genome, with another 20% strain specific sequences relevant for the survival of that strain vs another. This brings us the idea of genome vs pangenome, which is the full set of genes that can be present in a species. Core genomes are present in ALL strains of the given species, while accessory/variable genome is the genes present in one or more but not all strains of a species. Accessory genes are acquired through gene transfer. Therefore, we can say as pangenome increases, the proportion of the core genome decreases.

Open pangenomes are predicted to increase in size as novel genomes are sequenced, because bacterial species who share genetic elements are increasingly adaptable, while the closed pangenome will remain constant as we sequence more genes, because they will likely operate in a well-defined environments.

Accessory genetic elements

Plasmids are extracellular mobile genetic elements which can replicate autonomously in the cell. Transposable elements can change their location in the replicon in which they are inserted (chromosome or plasmid) and just from one region to another.



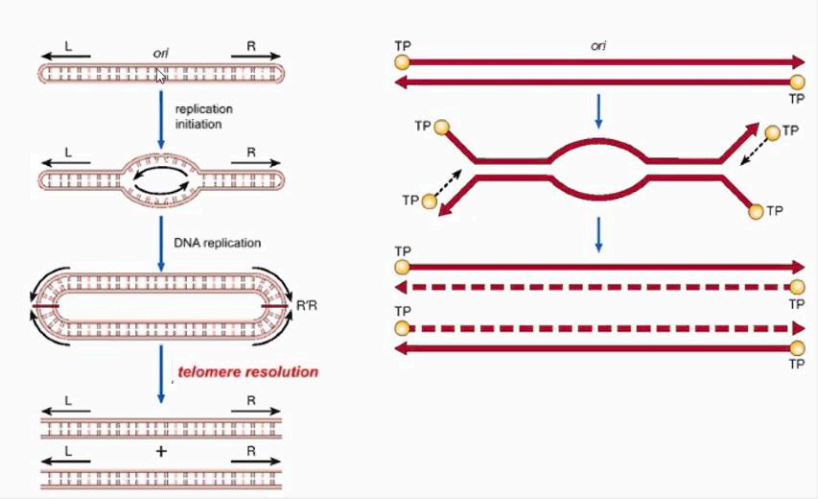
Plasmids are small extracellular elements, some can be 25-200 kilobases, some are linear, but most are linear, and some mega plasmids exist that are larger than the genome. Because of this, the structural definition of differentiating (based on size) between plasmids and chromosomes no longer applies. Instead, we use a functional differentiation, that is plasmids encode for accessory function, generally not essential for the livability (exceptions include antibiotic resistance, virulence etc.) The term chromide can be used in the case where a mega plasmid has at least one essential gene.

As we said, plasmids are generally circular, self-replicating elements, which vary in copy number with low of (1-5) and high of (10-100) . They can also be conjugative or non-conjugative, having a wide or narrow range or hosts, based on where who they can be transferred and integrated to. Therefore, plasmids contain two kinds of genetic information; genes responsible for plasmid replication, maintenance, and partition, as well as genes encoding for additional functions which vary heavily among plasmids.

Circular plasmid replication happens in three ways:

1. Unidirectional replication: Has a single replication fork, common
2. Bidirectional replication: Has two replication forks, common
3. Rolling circle replication: Rep protein nicks DNA, producing 3' end which is polymerized by Pol, spinning the original and producing two separated plasmids, ligase seals. Rare, but interesting.

Linear plasmids can replicate though covalent attachment of linear ends (requires protelomerase) or covalent binding to a terminal protein (TP).

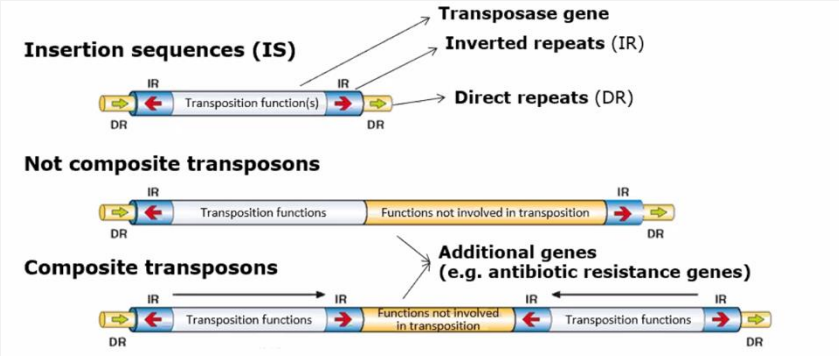


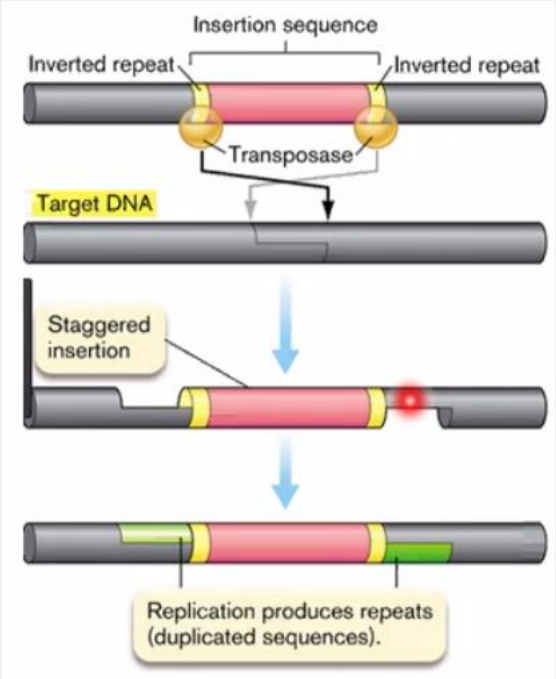
Plasmid incompatibility refers to the inability of a plasmid sharing the same replication system to coexist in the same cell. Different plasmids can be differentiated to incompatibility groups, two plasmids are incompatible if they share the same system of replication control. Plasmids with different control systems and replicate independently are both maintained in the daughter cell, while plasmids with the same systems influence their replication/partition, and results in the loss of one or two plasmids from daughter cells. Coexistence of two plasmids belonging to the same incompatibility group can be forced in the lab environment by treating them in conditions which need both plasmids to survive

Transposable elements

Transposable elements are genetic elements able to change localization in the replicon in which they are (chromosome or plasmid) or to jump from one replicon to another. Transposable elements have a few common features:

- Contain a gene encoding for transposase, the protein that allows the element to move around
- Contain a terminal inverted repeat sequence, which serve as targets for the transposes, by cutting before the IR on the left and after the IR on the right
- Generate direct repeats (duplications) at the insertion site

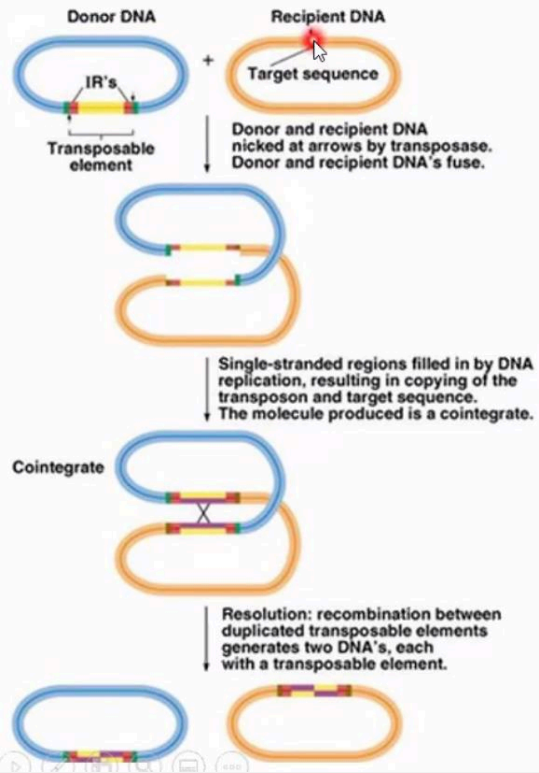




Transpositions are broken into three classes, insertion is the simplest, only containing the DR, IR and transposon function. Composite transposons has two IS's, with a fragment of DNA in between that codes for other genes such as antibiotic. In a composite, only one of the IS is functional. Non-composite has additional genes, and only one IS. Retro transposons are another class that uses an RNA intermediate, we won't discuss it in this summary. Target sequences are a random sequence of 4-8 bases that is targeted by the transposable element, each target sequence is recognized by a different transposable element.

There are two mechanisms of transposition, *conservative* in which the copy number of the transposons remains constant, or *replicative*, where the copy number of transposons increases every jump. In conservative the element is cut and pasted, in replicative, the element is copied and pasted. Conservative transposition begins with the recognition of the inverted repeat sequence by transposases, who bluntly cut the insertion sequence. The insertion sequence is then moved from its original site, where the transposases cut the target DNA in a staggered fashion, inserting the insertion sequence, sealing the single strands with repetitive single stranded DNA, from DNA Pol-I. The staggered insertions both have 5' protrusions, since the first cut was blunt (not staggered) the replication produces duplication sequences to fill the gaps, called direct repeats.

In the replicative model, we instead have a Donor DNA and a recipient DNA, and the transposase generates staggered ends both in the transposon and the target site, differing from conservative. The protruding strands of the transposon and the target site are then joined, and the single stranded regions are filled by DNA Pol-I, leading to duplication of the transposon, and generating the cointegrate intermediate. The cointegrate intermediate is resolved by recombination, mediated by an enzyme called resolvase. The resolution ends with the copy and pasting of the transposable element.



The transposase is transcribed and translated by the cells own machinery, and orchestrates the cutting, looping, insertion in both mechanisms. The major difference in the models is the number of cuts the transposase makes. Cut-paste cuts both strands, copy-paste cuts only one. In both models the cut 5' end of the target DNA is joined to the 3' end of the transposon, as well as the 3' end of the target are used as primers for replication that proceeds until a free 5' end is reached.

Transposons are responsible in an important number of mutations that occur when they jump into either a regulatory sequence, or an actual gene. This evolutionary significance is largely negative in the individual, leading to a hyperactive or non-functional gene, but in rare cases the effects will help the organism. The acquisition of a novel transposons can also lead to the insertion of a new gene, especially in bacteria antibiotic resistant genes. Transposons have also played a crucial role in the evolution of the prokaryotic genome, especially in the lab where they are used as mutagens.

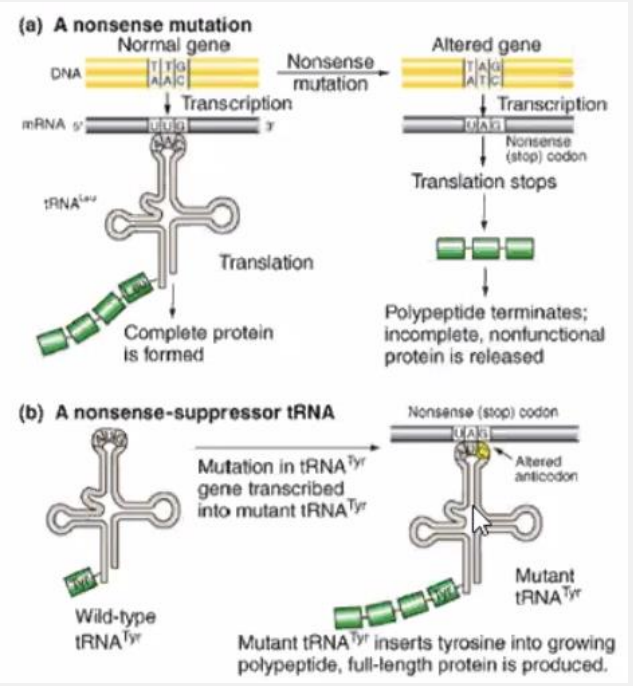
Genome Evolution

Mutations and Damage

Since there is no sexual reproduction, genetic variation in prokaryotes comes from mutations, and HGT(horizontal gene transfer). These are the sources of new variants, different members of a species who are genetically and phenotypically different, who are selected by selective pressures. Mutations can be spontaneous in DNA replication and homologous recombination, or can be induced by chemical mutagens, as well as radiation.

Mutations can be broken into two types:

- Micro-lesion: including point mutations, frameshifts
- Macro-lesions: includes the deletion, inserts, and duplicates of a sequence, or inversions and translocations



Mutations can be either advantageous, disadvantages, or neutral. Advantageous mutations will be more likely to spread in the population, disadvantageous will likely be deleted. Point mutations can be either transitions or transversions, the former is a purine-purine or pyrimidine-pyrimidine, while a later is purine-pyrimidine or vise versa. Point mutations can be silent, nonsense (loss of function) or missense (no effect or loss or gain of function). Mutations often have no effect on genotype, but some can change the phenotype in ways such as temperature sensitivity, drug resistance, virus resistance, loss of cellular components such as flagella, which can radically change the metabolism and survivability of the cell.

Non selectable phenotypes can be selected for by using specific treatments in the median, while nonselective phenotypes such

as auxotroph are harder to select for due to the complex interactions involved in the selective mutation's role in the cell. Back mutations can also occur, in which a mutation is reversed directly in the same site (reversion) or a mutation in another site that restores the wild type phenotype (suppressor mutations). Suppressor mutations are therefor called intergenic compensation mutations, as they can restore the original function of the gene that was mutated. One example of a suppressor is the 'amber mutation', a mutation in the tRNA gene that enables the ribosome to put the amino acid at the UAG codon, in the presence of a mutated stop codon. The amber suppressor is allele specific but gene nonspecific.

Mutagens are chemicals that interact with DNA or related proteins in a way that increases the rate of mutations in its presence. The level of mutation from a substance can be measured by the Ames test, which is based on the frequency of back mutations in auxotrophic strains for specific nutrients. Hypermutable strains occur from mutations in critical cell cycle control processes, or DNA repair such that the cell line accumulates more mutations than usual.

Darwin vs Lamar : The Lauia-Dalbruck experiment (or Fluctuation Test) validates the spontaneous mutation hypothesis, which tells us that mutants always exist in a population, they are just selected under specific conditions. By splitting an original strain inoculated flask between dozens of tubes, and then dozens of trays -which select for the same mutation- the number of surviving colonies were very different, indicating that the mutated cells existed before splitting of the median. The fluctuation test experiment

Repair systems

Direct repair: by photolyase, a class of flavoproteins uses blue light to repair two type of UV-induced DNA damage. Use blue light as the energy source for the repair, reversing the point mutation and correcting the mis paired element.

NER: Nucleotide excision repair first involves a UvrAB protein complex sliding along the DNA, looking for thymine dimers. Once detected, the complex is replaced by UvrC, an enzyme able to create a single stranded cut upstream and downstream of the dimer. A thirst enzyme, UvrD helicase with help from DNA pol-I and ligase replace the thymine dimer containing region with correct base pairs.

BER: Base excision repair occurs when only one base needs to be replaced, such as a uracil being detected in DNA. In this case, a glycosylase enzyme recognizes the incorrect nitrogenous base, cutting the bind that links the nitrogenous base to the pentose sugar, not effecting the backbone of the DNA. After this elimination, an apurinic site is formed, recognized by an AP endonuclease which cuts the phosphate scaffold at the site of the 5' AP site. In the end DNA pol-I and ligase polymerize and close the excision.

MMR: Mismatch repair corrects mismatches nucleotides that are non-canonical complements arising from DNA replication. In this process, MutS recognizes the mismatched base pair, then recruiting MutL(uses ATP) and MutH. The three enzymes then move alone the DNA, looking for the mismatch on the unmethylated (newly synthesized) strand and assumes that this strand should be correct. The unmethylated strand is then nicked upstream of the methylation element and downstream of the incorrectly paired base. Lastly, UvrD helicase and DNA Pol-III fill in the single stranded gap, fixing the mutation. The important part is to know how MMR knows which strand is wrong.

Recombination repair: In case the repair systems cannot act before a replicative fork reaches a mutation site. In this case, the DNA Pol-III must 'jump' this region -skipping about 1000 bps- and starts synthesis again after. This process produces single stranded DNA with 5' and 3' free ends, activating the RecA protein and SOS repair systems. This is an emergency mechanism implemented in order to not stop the synthesis of DNA, at the cost of losing genetic information. The information can however be recovered retrospectively by the formation of a holiday junction, similar to a crossing over even, in which a crossing over event from an unmutated strand replaces the single strand gap. In this way, we have at least one healthy strand on each chromosome, and DNA Pol-III and other repair systems can fix the damage with no information lost.

SOS repair: Is an important repair system found in bacterial cells and is the last chance for the bacterial cell to salvage the damage concentrated on the same locus in a way that there is no longer any readable template. In this case, a special polymerase is transcribed and activated. This polymerase randomly inserts the missing nucleotides causing 3 over 4 times mutations. UmuD and UmuC gene encodes for the UmuDC protein, a complex recognized as DNA Pol-V in E. Coli. Pol-V, unlike pol-I and III does not need a template strand, only a primer and randomly inserts nucleotides, a risky but preferable solution to losing the whole chromosome/plasmid. Pol-V activity must be tightly controlled by RecA proteins. RecA interestingly recognizes the single stranded DNA damage, and also regulates the LexA regulatory sequence -by binding and

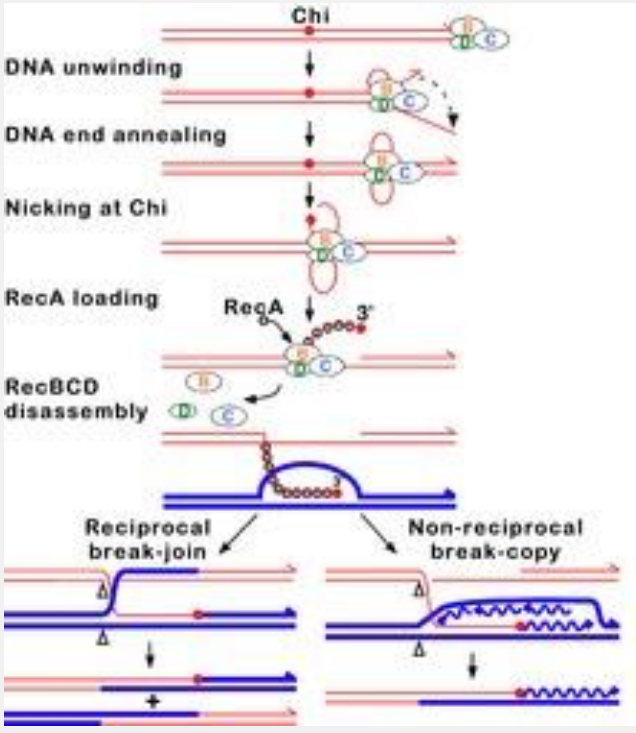
inducing a autocatalytic removal of the repressor LexA- which controls the activity of transcription for pro-UmuD, pro-UmuC and RecA. Pro-UmuD and C are also proteolytically activated by RecA. In summary, the RecA regulates the activity of the repressor LexA, repressing transcription of pro-UmuD and Pro-UmuC, while also activating these pro forms of the proteins when RecA stranded single stranded DNA damage.

DNA micro-lesions are heavily involved in genome evolution, deletion, insertion, duplication, insertion and translocation all increase the potential of genetic reshuffling, especially the duplication and divergence theory. Homologous recombination represents the exchange of DNA sequences between DNA molecules which contain identical or similar sequences. These homologous regions are made of >500 bp's, the longer the region the higher the probability of recombination's. RecA is the most important recombination protein in prokaryotes.

Homologous recombination

Homologous recombination represents the exchange of DNA sequences between DNA molecules which contain identical or very similar sequences. The identical DNA sequences represent 'homologous regions' and can be strand exchange, or cross over (depending how the holiday junction is resolved). The prerequisites to HR are the presence of the homologous region, the presence of the recombination proteins in the cell (RecA, RecBCD, RecQJ, RuvABC complexes) and the presence of a truncated single strand DNA.

Homologous recombination is a major DNA repair process in bacteria. It is also important for producing genetic diversity in bacterial populations. Homologous recombination has been most studied and is best understood for *Escherichia coli*. Double-strand DNA breaks in bacteria are repaired by the RecBCD pathway of homologous recombination. Breaks that occur on one of the two DNA strands, known as single-strand gaps, are thought to be repaired by the RecF pathway. Both the RecBCD and RecF pathways include a series of reactions known as branch migration, in which single DNA strands are exchanged between two intercrossed molecules of duplex DNA, and resolution, in which those two intercrossed molecules of DNA are cut apart and restored to their normal double-stranded state.



RecA is the most important recombination protein, it works as a polymer which assembles into a helical around a single stranded DNA region, protecting it. RecA's association with DNA major is based on its central role in homologous recombination. The RecA protein binds strongly and in long clusters to ssDNA to form a nucleoprotein filament. The protein has more than one DNA binding site, and thus can hold a single strand and double strand together. This feature makes it possible to catalyze a DNA synapsis reaction between a DNA double helix and a complementary region of single-stranded DNA. The RecA-ssDNA filament searches for sequence similarity along the dsDNA. A disordered DNA loop in RecA, Loop 2, contains the residues responsible for DNA homologous recombination. In some bacteria, RecA posttranslational modification via phosphorylation of a serine residue on Loop 2 can interfere with homologous recombination.

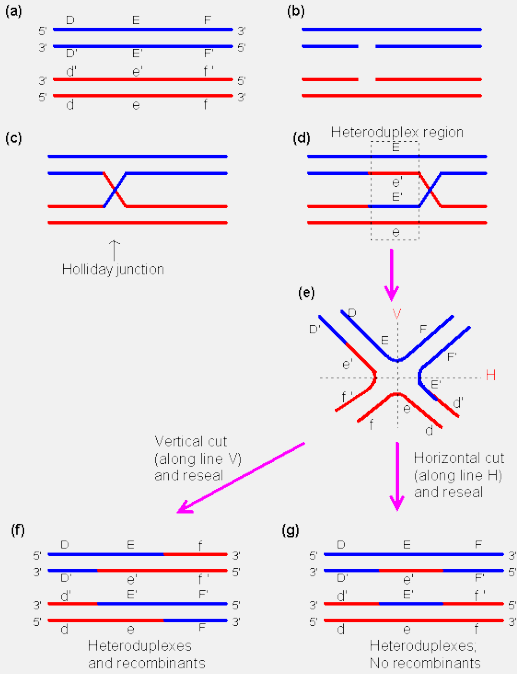
In this pathway, a three-subunit enzyme complex called RecBCD initiates recombination by binding to a blunt or nearly blunt end of a break in double-strand DNA. After RecBCD binds the DNA end, the RecB and RecD subunits begin unzipping the DNA duplex through helicase activity. The RecB subunit also has a nuclease domain, which cuts the single strand of DNA that emerges from the unzipping process. This unzipping continues until RecBCD encounters a specific nucleotide sequence (5'-GCTGGTGG-3') known as a Chi site.

Upon encountering a Chi site, the activity of the RecBCD enzyme changes drastically. DNA unwinding pauses for a few seconds and then resumes at roughly half the initial speed. This is likely because the slower RecB helicase unwinds the DNA after Chi, rather than the faster RecD helicase, which unwinds the DNA before Chi. Recognition of the Chi site also changes the RecBCD enzyme so that it cuts the DNA strand with Chi and begins loading multiple RecA proteins onto the single-stranded DNA with the newly generated 3' end. The resulting RecA-coated nucleoprotein filament then searches out similar sequences of DNA on a homologous chromosome. The search process induces stretching of the DNA duplex, which enhances homology recognition.

Upon finding such a sequence, the single-stranded nucleoprotein filament moves into the homologous recipient DNA duplex in a process called *strand invasion*. The invading 3' overhang causes one of the strands of the recipient DNA duplex to be displaced, to form a D-loop. If the D-loop is cut, another swapping of strands forms a cross-shaped structure called a Holliday junction.

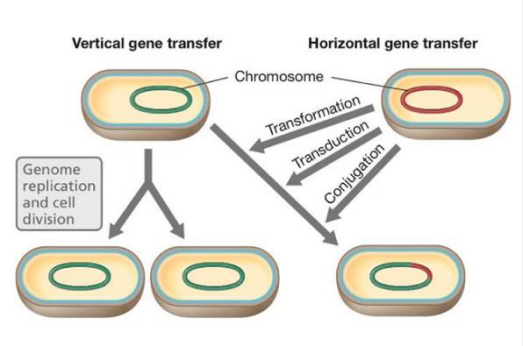
The holiday structure is a dynamic structure than can migrate up and down the DNA molecules, this process is called strand/branch migration. This process is mediated by RuvAB complex by improving flow rate of DNA strands while RuvC is the endonuclease responsible for cutting and resolving the junction.

Resolution of the Holliday junction by some combination of RuvABC or RecG can produce two recombinant DNA molecules with reciprocal genetic types, if the two interacting DNA molecules differ genetically. Alternatively, the invading 3’ end near Chi can prime DNA synthesis and form a replication fork. The resolution can be through a vertical cut, or a horizontal cut, the former producing heteroduplexes with recombination, and the later heteroduplexes without recombination, that is recombination/crossing over(f) and strand exchange/non-crossover recombination (g).



When crossing over occurs in plasmids, the crossing over patterns can result in a recombinant. Recombinants result in two plasmids with exchanges single stranded regions. Additionally, recombination’s can deleterious when multiple similar sequences exist, the recombination of two homologous sequences can even result in the excision of a portion of DNA, that may leave and itself become a plasmid. The same process of homologous recombination of two different sites can also add a sequence or gene, either as end-in recombination or end-out recombination. End-out recombination disrupts the target sequence on the chromosome, while end-in recombination generates the duplication of the target sequence.

Horizontal gene transfer



In labs, the MLST (Multi locus sequence typing) is used as a nucleotide-based approach for the characterization of microorganisms. MLST categorizes the sequences of variations in the housekeeping genes and evaluates the relationship to the genes in our sample vs references in a database, based on similarity of sequences. Comparing these housekeeping proteins, we are able to locate speciation points in the shared ancestor, however due to horizontal gene transfer, we can observe the reconnecting of two lost ancestors. Because of this we don’t consider a tree of life, but a web of life and even a circle of life.

Horizontal gene transfer can be transformation, transduction and conjugation. Transformation and transduction both involve the release of DNA into a median, and later the uptake by the receiving cell, only differing as transduction uses a bacteriophage as a carrier. Conjugation instead involves the contact of two cells. Cells with the ability to uptake DNA from the environment are called competent.

Transformations

Bacterial transformation may be referred to as a stable genetic change, brought about by the uptake of naked DNA (DNA without associated cells or proteins). Competence refers to the state of being able to take up exogenous DNA from the environment. There are two forms of competence: natural and artificial.

For transformation to happen, bacteria must be in a state of competence, which might occur as a time-limited response to environmental conditions such as starvation and cell density. About 1% of bacterial species are capable of naturally taking up DNA under laboratory conditions; more may be able to take it up in their natural environments. DNA material can be transferred between different strains of bacteria in a process that is called horizontal gene transfer.

Some species, upon cell death, release their DNA to be taken up by other cells; however, transformation works best with DNA from closely-related species. These naturally-competent bacteria carry sets of genes that provide the protein machinery to bring DNA across the cell membrane(s). The transport of the exogeneous DNA into the cells may require proteins that are involved in the assembly of type IV pili and type II secretion system, as well as DNA translocase complex at the cytoplasmic membrane.

Natural transformations differ in mechanism between gram+ and gram-, but the important components are conserved. Due to the differences in structure of the cell envelope between Gram-positive and Gram-negative bacteria, there are some differences in the mechanisms of DNA uptake in these cells. However, most of them share common features that involve related proteins. The DNA first binds to the surface of the competent cells on a DNA receptor and passes through the cytoplasmic membrane via DNA translocase. Only single-stranded DNA may pass through, one strand is therefore degraded by nucleases in the process, and the translocated single-stranded DNA may then be integrated into the bacterial chromosomes by a RecA-dependent process. In Gram-negative cells, due to the presence of an extra membrane, the DNA requires the presence of a channel formed by secretin's on the outer membrane. Pilin may be required for competence; however, its role is uncertain. The uptake of DNA is generally non-sequence specific, although in some species the presence of specific DNA uptake sequences may facilitate efficient DNA uptake.

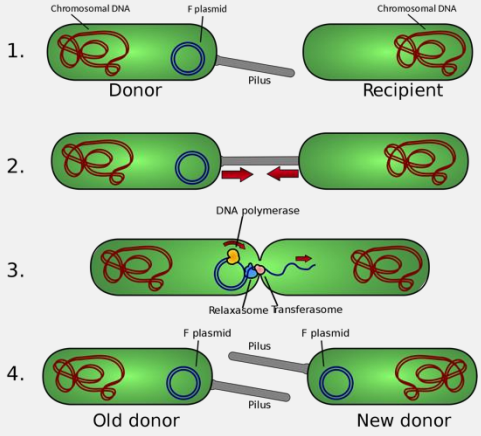
Artificial competence can be induced in laboratory procedures that involve making the cell passively permeable to DNA, by exposing it to conditions that do not normally occur in nature. Typically, the cells are incubated in a solution containing divalent cations; most commonly, calcium chloride solution under cold condition, which is then exposed to a pulse of heat shock. However, the mechanism of the uptake of DNA via chemically-induced competence in this calcium chloride transformation method is unclear.

The surface of bacteria such as E. coli is negatively-charged due to phospholipids and lipopolysaccharides on its cell surface, and the DNA is also negatively-charged. One function of the divalent cation, therefore, would be to shield the charges by coordinating the phosphate groups and other negative charges, thereby allowing a DNA molecule to adhere to the cell surface. It is suggested that exposing the cells to divalent cations in cold condition may also change or weaken the cell surface structure of the cells making it more permeable to DNA. The heat-pulse is thought to create a thermal imbalance on either side of the cell membrane, which forces the DNA to enter the cells through either cell pores or the damaged cell wall. Electroporation is another method of promoting competence. Using this method, the cells are briefly shocked with an electric field of 10-20 kV/cm which is thought to create holes in the cell membrane through which the plasmid DNA may enter. After the electric shock, the holes are rapidly closed by the cell's membrane-repair mechanisms.

The uptake of a genetic element can be either positive, neutral or negatively selective. Positively selecting elements will continue to be uptake by other cells, and its expression In a population will increase, if neutral it wont spread faster, and negatively selecting elements will decrease in expression in the population.

Conjugations

Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells. This takes place through a pilus/sex pilus. It is a parasexual mode of reproduction in bacteria. The Lederberg-Tatum experiment proved that genetic information could be transferred between bacteria by using a selective median, and two types of bacteria that each could survive in one median. The results were that the mixed colonies of A and B survived, and the individual colonies of A or B all died. The sex pilus structure promotes the contact between the donor and recipient cell, but is important to remember DNA doesn't not pass through the pilus, it is only required for establishing close cell-cell contact.



The basic mechanisms of conjugations are:

1. Donor cell produces pilus.

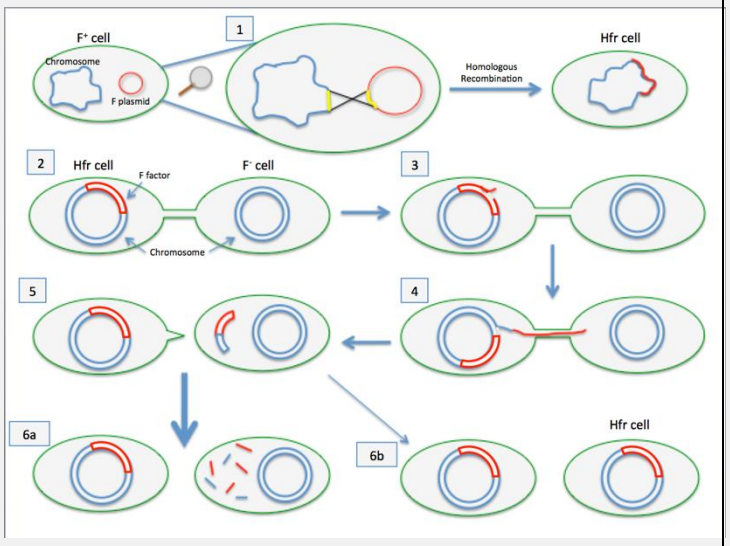
2. Pilus attaches to recipient cell and brings the two cells together.
3. The mobile plasmid is nicked and a single strand of DNA is then transferred to the recipient cell.
4. Both cells synthesize a complementary strand to produce a double stranded circular plasmid and also reproduce pili; both cells are now viable donor for the F-factor.

The F-plasmid is an episome (a plasmid that can integrate itself into the bacterial chromosome by homologous recombination) with a length of about 100 kb. It carries its own origin of replication, the *oriV*, and an origin of transfer, or *oriT*. There can only be one copy of the F-plasmid in a given bacterium, either free or integrated, and bacteria that possess a copy are called *F-positive* or *F-plus* (denoted F^+). Cells that lack F plasmids are called *F-negative* or *F-minus* (F^-) and as such can function as recipient cells.

Among other genetic information, the F-plasmid carries a *tra* and *trb* locus, which together are about 33 kb long and consist of about 40 genes. The *tra* locus includes the *pilin* gene and regulatory genes, which together form pili on the cell surface. Tra genes are generally related to the conjugation of the F-plasmid. The locus also includes the genes for the proteins that attach themselves to the surface of F^- bacteria and initiate conjugation. Though there is some debate on the exact mechanism of conjugation it seems that the pili are not the structures through which DNA exchange occurs. This has been shown in experiments where the pilus are allowed to make contact, but then are denatured with SDS and yet DNA transformation still proceeds. Several proteins coded for in the *tra* or *trb* locus seem to open a channel between the bacteria and it is thought that the traD enzyme, located at the base of the pilus, initiates membrane fusion.

When conjugation is initiated by a signal the relaxase enzyme creates a nick in one of the strands of the conjugative plasmid at the *oriT*. Relaxase may work alone or in a complex of over a dozen proteins known collectively as a relaxosome. In the F-plasmid system the relaxase enzyme is called TraI and the relaxosome consists of TraI, TraY, TraM and the integrated host factor IHF. The nicked strand, or *T-strand*, is then unwound from the unbroken strand and transferred to the recipient cell in a 5'-terminus to 3'-terminus direction. New DNA is synthesized in the donor cell through a replication called rolling circle replication. Conjugative replication may require a second nick before successful transfer can occur. Upon entering the recipient cell, a complementary RNA close to an okazaki fragment binds with the T-strand, stabilizing it. Once transfer is complete, the bridge degrades, and the cells separate.

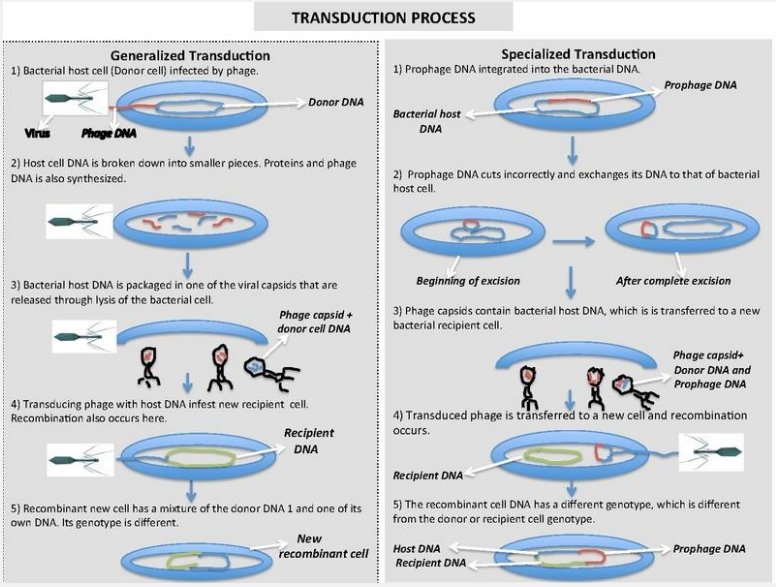
Integration of the F factor into the bacterial chromosome occurs at specific regions of DNA called insertion sequences (IS) The F factor carries several IS regions, such as, IS 2, IS 3. The E. coli chromosome carries 20 IS regions which are scattered throughout the chromosome with both, clock-wise and counter clock-wise orientations. The IS elements provide the loci for homologous pairing between the F factor and the bacterial chromosome and a single crossover event between them is sufficient for the integration of the F factor into the bacterial chromosome the chromosome carrying an F plasmid is called an Hfr chromosome. A high-frequency recombination cell (Hfr cell, also called an Hfr strain) is a bacterium with a conjugative plasmid (for example, the F-factor) integrated into its chromosomal DNA. The integration of the plasmid into the cell's chromosome is through homologous recombination. A conjugative plasmid capable of chromosome integration is also called an episome . When conjugation occurs, Hfr cells are very efficient in delivering chromosomal genes of the cell into recipient F^- cells, which lack the episome. Hfr cells can only conjugate with F^- cells.



Since Hfr strains are very efficient at integrating chromosomal genes, and the fact that transfer of chromosomal genes can stabilize the recipient cell by homologous recombination, we can observe the order of chromosomal genes such as Lac, Pro in Hfr cells are conserved. This linkage map allows the efficient study of the origins of genetic material based on the conserved order of the chromosomal genes. For this reason, genes on Hfr strains are referenced in location in minutes from another element. Because the F plasmid is transferred and integrated in order, the later elements are less likely to fit into the chromosome, regions like Tra which are near the end are almost never transferred a function Tra region. This means the recipient strain is not converted into a Hfr (F+) strain.

Phage Transduction

Phage transduction uses a bacteriophage as a carrier to transfer the genetic information, and can be generalized, or specialized transduction. In general transduction we see the transfer of all the genes of the donor strain to the recipient strain, regardless of location and function, while specialized transduction refers to the transfer of only genes located close to a single integration site, for example the Λ-Phage on E. coli only transfers genes called gal and bio genes.



Generalized transduction happens when a phage is in the lytic stage, at the moment that the viral DNA is packaged into phage heads. If the virus replicates using 'headful packaging', it attempts to fill the head with genetic material. If the viral genome results in spare capacity, viral packaging mechanisms may incorporate bacterial genetic material into the new virion. The new virus capsule that contains part bacterial DNA then infects another bacterial cell. When the bacterial DNA packaged into the virus is inserted into the recipient cell three things can happen to it:

1. The DNA is recycled for spare parts.
2. If the DNA was originally a plasmid, it will re-circularize inside the new cell and become a plasmid again.
3. If the new DNA matches with a homologous region of the recipient cell's chromosome, it will exchange DNA material similar to the actions in bacterial recombination.

Specialized Λ Transduction is a well-studied case of specialized transduction, in which the Λ phage is able to insert itself into the E. Coli DNA as a Λ prophage element. The phage can then catalyze its own release from the genome as a replicant. This cycle of excision, replication and lysine can be interrupted in bacteria that contain Λgal gene, which blocks the full excision of the λ-phage. The integration of phage λ takes place at a special attachment site in the bacterial and phage genomes, called att^{λ}. The sequence of the bacterial att site is called *attB*, between the *gal* and *bio* operons. The integration itself is a sequential exchange via a Holliday junction and requires both the phage protein Int and the bacterial protein IHF (*integration host factor*).

Gene Expression:

Some genes are needed at roughly the same level at all times in the cell, these genes are called constitutive and are transcribed under all conditions. Other genes however must be turned on and off during different conditions, these genes are called regulated genes. Regulation can happen at the level of transcription, post transcriptional, translation, or post translationally regulated. Transcriptional regulation is the least wasteful and is performed by DNA binding proteins called gene regulatory proteins which bind to gene regulatory sequences on DNA. Post transcriptional control relates to mRNA stability, and longevity. Translational control depends how many ribosomes transcribe the mRNA, and how fast the mRNA is degraded. Lastly, post transcriptional control deals with the activity of the proteins in the cell, turning on/off or permanently inactivation through proteases.

Gene regulation in bacteria aims to rapidly adapt the organism in the precedence of environmental changes. This is especially true for prokaryotes because their environment is free to change much more than cells within the stable multicellular tissues of eukaryotes. For this reason, bacteria use mostly transcriptional, and post-transcriptional regulation mechanisms over the others. Transcriptional control is seen from alternative sigma subunits, and regulatory proteins while post-transcriptional is modulated by srRNA, riboswitches and attenuation. Its important to consider that in prokaryotes the mRNA is being synthesized in the same compartment as the ribosomes are translating it, therefore the mRNA does not need to be packaged and exported from the nucleus.

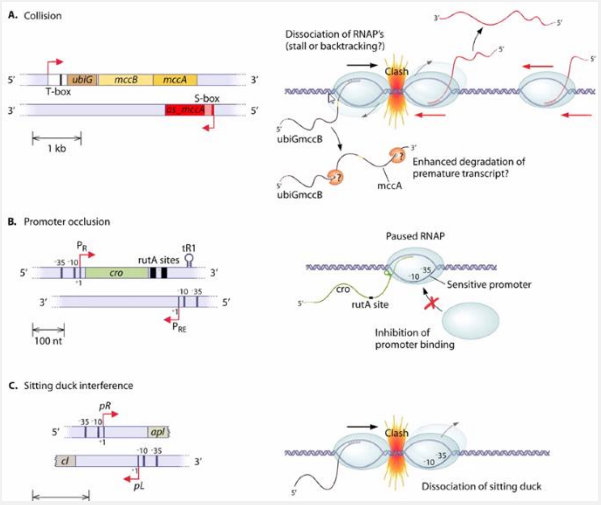
RNA-Pol in bacteria is present in two forms in bacterial cells, the RNAP core consists of two a subunits, and a B and B' subunits, and a RNAP holoenzyme made of RNAP core + sigma subunit. RNAP core has a polymerase activity, while the RNAP holoenzyme has the ability to recognize a specific promoter sequence and bind.

Transcription begins with the binding of the sigma factor to the promoter region. Terminators work in two different ways, intrinsic terminators ADD

Sigma 70 also known as vegetative sigma is responsible for the transcription of the majority of genes, however, many bacteria have different sigma factors, E Coli has seven. RpoD sigma factor is the most common, is used for growth related genes. Other sigma factors include N, S, H, F, E, FecI are activated during different environmental conditions, and are summarized to the right. When expressed, alternative sigma factors must compete with vegetative sigma factors which are consistently produced for the binding of the RNAP core. Two factors influence this competition; the number of subunits of each sigma/expression level, and secondly the activity of each sigma, that is the affinity for the RNAP core.

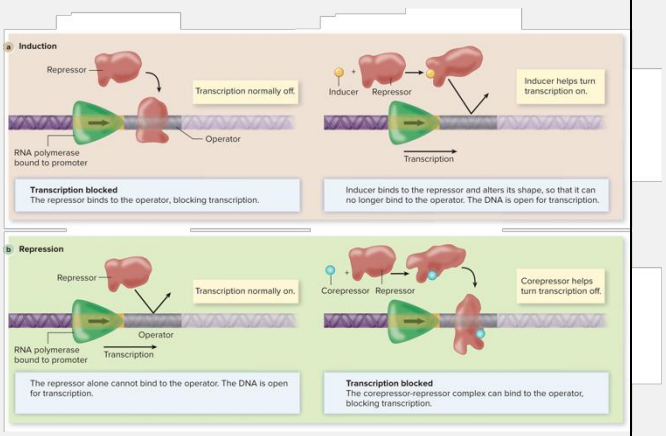
Sigma factor	Gene	Function
σ^{70}	*rpoD*	transcription of most genes during the exponential phase (*19*)
σ^{54}	*rpoN*	nitrogen-regulated gene transcription (*22*)
σ^{38}	*rpoS*	gene expression during the starvation and stationary phase (*23*)
σ^{32}	*rpoH*	heat-shock gene transcription (*24*)
σ^{28}	*rpoF*	expression of flagellar and chemotaxis genes (*25*)
σ^{24}	*rpoE*	response to the extracytoplasmic and extreme heat stress (*1*)
σ^{19}	*fecI*	regulation of the *fec* genes for iron dicitrate transport (*26*)

Promoters are selective for certain sigma factors, therefor when the RNAP core does not contain the proper sigma factor related to that promoter, it cannot efficiently bind. Each factor recognizes a specific consensus sequence, allowing us to easily see which sigma factor relates to a gene by studying the promoter. In this way, it is possible to regulate transcription by promoter competition through three means; collision when two promoters on opposite strands transcribe towards each other until they inevitably collide. Secondly, promoter occlusion involves the RNAP pausing after the transcription of a region in such a position that it blocks the binding of an additional RNAP on the complementary strand. Lastly, in the sitting duck model two promoters on complementary strands clash similarly to the collision model, but here the stronger polymerase will push the weaker polymerase off.



Transcriptional regulation can also be regulated by DNA binding proteins, generally binding in a dimer of equal proteins called a homodimer to a palindromic sequence of DNA. These proteins contain at least a domain for DNA binding, as well as a domain for protein-protein interactions. In prokaryotes the majority of transcriptional binding motifs are helix turn helix.

Transcriptional regulation can be negative, or positive. Negative control is mediated by transcriptional repressors either repression or induction, while positive control is always mediated by activators:



- Repression occurs on genes in the anabolic pathways, which are normally active, but can be inactivated by products of the same anabolic pathway which act as co-repressors. Corepressors bind to the repressor and induces a confirmational change, increasing its affinity for the target DNA.
- Induction instead generally affects catabolic processes which are normally inactive but can be activated by the presence of specific catabolites which act as inducers. The inducer binds to the repressor and induces a conformation chance, decreasing its affinity for the target DNA.
- Positive control is always mediated by an inducer and are mostly involved in catabolic genes pathways that are normally inactive, but can be activated by the presence of a specific catabolite called an inducer. The inducer binds to the transcriptional activator, inducing a conformational change increasing affinity of the target DNA. This binding site can be found far away from the promoter region, in which case the mediator complex allows association by folding the DNA regions close together.
- Some transcriptional regulators can serve as 'Dual Function' regulators, either increasing or decreasing transcription depending on which DNA sequence they bind to. AraC for example prevents transcription of arabinose catabolic enzymes when no arabinose is bound, while the same regulator bound to arabinose instead binds to different DNA regions that instead promote the transcription of said genes.