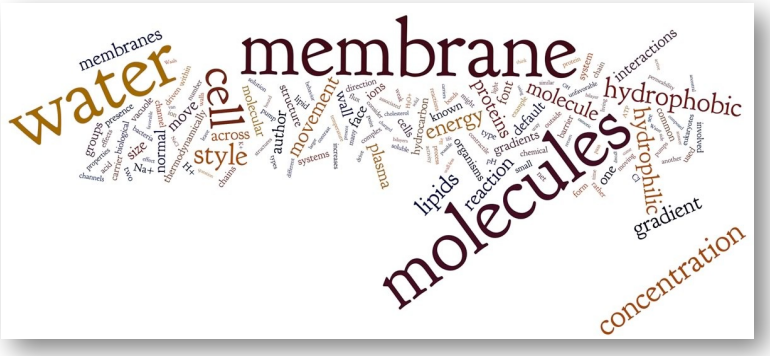
6. Membrane boundaries and capturing energy

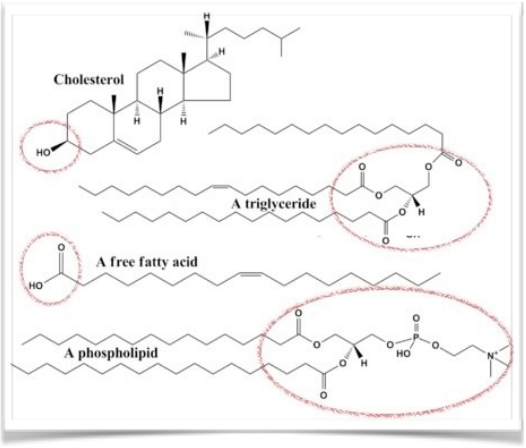
In which we consider how the aqueous nature of biological systems drives the formation of lipid-based barrier membranes and how such membranes are used to capture and store energy from the environment and chemical reactions. We consider how coupled reactions are used to drive macromolecular synthesis and growth.



Defining the cell's boundary

A necessary step in the origin of life was the generation of a discrete barrier, a boundary layer that serves to separate the living non-equilibrium reaction system from the rest of the universe. This boundary layer, the structural ancestor of the plasma membrane of modern cells, serves to maintain the integrity of the living system and mediates the movement of materials and energy into and out of the cell. Based on our current observations, the plasma membrane of all modern cells appears to be a homologous structure derived from a precursor present in the last common ancestor of life. So what is the structure of this barrier (plasma) membrane? How is it built and how does it work?

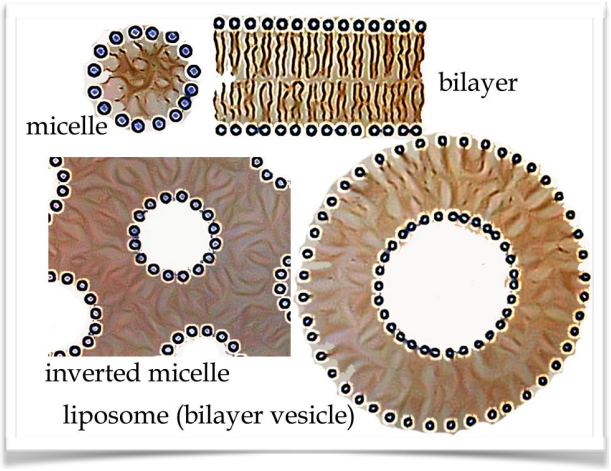
As we have already seen, when a new cell is formed, its plasma membrane is derived from the plasma membrane of the parental cell. As the cell grows, new molecules must be added into the membrane to enable it to increase its surface area. Biological membranes are composed of two general classes of molecules, proteins (which we will discuss in much greater detail in the next section of the course) and lipids. It is worth noting explicitly here that, unlike a number of other types of molecules we will be considering, such as proteins, nucleic acids, and carbohydrates, lipids are not a structurally coherent group, that is they do not have one particular basic structure. Such apparently diverse molecules as cholesterol and phospholipids, are both considered lipids, and while there is a relatively small set of common lipid types, there are many different lipids found in biological systems and the characterization of their structure and function(s) has led to a new area of specialization known as lipidomics.[140]



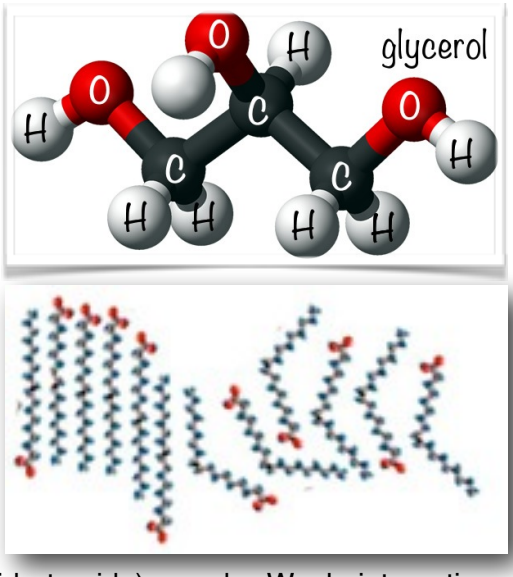
All lipids have two distinct domains: a hydrophilic (circled in red in this figure →) domain characterized by polar regions and hydrophobic/hydroapathetic domains that are usually just made up of C and H and are non-polar. Lipids are **amphipathic**. In aqueous solution, entropic effects will drive the hydrophobic/hydroapathetic parts of the lipid out of solution. But in contrast to totally non-polar molecules, like oils, the hydrophobic/hydroapathetic part of the lipid is connected to a hydrophilic domain that is soluble in water. Lipid molecules deal with this dichotomy by associating with

[140] On the future of "omics": lipidomics: http://www.ncbi.nlm.nih.gov/pubmed/21318352 and Lipidomics: new tools and applications http://www.ncbi.nlm.nih.gov/pubmed/21145456

other lipid molecules in multimolecular structures in which the interactions between the hydrophilic parts of the lipid molecule and water molecules are maximized and the interactions between the lipid's hydrophobic/hydroapathetic parts and water are minimized. Many different multi-molecular structures can be generated that fulfill these constraints. The structures that form depend upon the details of the system, including the shapes of the lipid molecules and the relative amounts of water and lipid present, but the reason these structures self-assemble is because their formation leads to an increase in the overall entropy of the system, a somewhat counterintuitive idea. For example, in a micelle the hydrophilic region is in contact with the water, while the hydrophobic regions are inside, away from direct contact with water. This leads to a more complete removal of the hydrophobic domain of the lipid from contact with water than can be arrived at by a purely hydrophobic oil molecule, so unlike oil, lipids can form stable structures in solution. The diameter and shape of the micelle is determined by the size of its hydrophobic domain. As this domain gets longer, the center of the micelle becomes more crowded. Another type of organization that avoids "lipid tail crowding" is known as a bilayer vesicle. Here there are two layers of lipid molecules, pointing in opposite directions. The inner layer surrounds a water filled region (the lumen of the vesicle), while the outer layer interacts with the external environment. In contrast to the situation within a micelle, the geometry of a vesicle means that there is significantly less crowding as a function of lipid tail length. Crowding is further reduced as a vesicle increases in size to become a cellular membrane. Micelles and vesicles can form a colloid-like system with water, that is they exist as distinct structures that can remain suspended in a stable state. We can think of the third type of structure, the planar membrane, as simply an expansion of the vesicle to a larger and more irregular size. Now the inner layer faces the inner region of the cell (which is mostly water) and the opposite region faces the outside world. For the cell to grow, new lipids have to be inserted into both inner and outer layers of the membrane; how exactly this occurs typically involves interactions with proteins. For example, there are proteins that can move a lipid from the inner to the outer domain of a membrane (they flip the lipid between layers, and are known as flipases), but the molecular details are beyond our scope here. While there are a number of distinct mechanisms that are used to insert molecules into membranes they always involve a pre-existing membrane – this is another aspect of the continuity of life. Totally new cellular membranes do not form, membranes are built on pre-existing membranes. For example, a vesicle (that is a spherical lipid bilayer) could fuse into or emerge from a planar membrane. These processes are typically driven by thermodynamically favorable reactions involving protein-based molecular machines. When the membrane involved is the plasma (boundary) membrane, these processes are known as exocytosis and endocytosis, respectively. These terms refer explicitly to the fate of the material within the vesicle. Exocytosis releases that material from the vesicle interior into the outside world, whereas endocytosis captures material from outside of the cell and brings it into the cell. Within a cell, vesicles can fuse and emerge from one another.

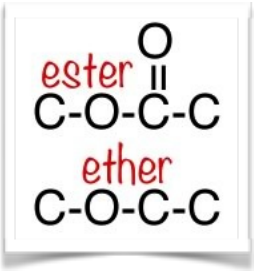


As noted above, there are hundreds of different types of lipids, generated by a variety of biosynthetic pathways catalyzed by proteins encoded in the genetic material. We will not worry too much about all of these different types of lipids, but we will consider two, the glycerol-based lipids and cholesterol, because considerations of their structures illustrates general ideas related to membrane behavior. In bacteria and eukaryotes, glycerol-based lipids are typically formed from the highly hydrophilic molecule glycerol combined with two or three fatty acid molecules. Fatty acids contain a long chain hydrocarbon with a polar (carboxylic acid) head group. The nature of these fatty acids influences the behavior of the membrane formed. Often these fatty acids have what are known as saturated hydrocarbon tails. A saturated hydrocarbon contains only single bonds between the carbon atoms of the tail domain. While these chains can bend and flex, they tend to adopt a more or less straight configuration. In this straight configuration, they pack closely, which maximizes the lateral (side to side) van der Waals interactions between them. Because of the extended surface contact between the chains, lipids with saturated hydrocarbon chains are typically solid around room temperature. On the other hand, there are cases where the hydrocarbon tails are "unsaturated", that is they contain double bonds (–C=C–) in them. These are typically more fluid and flexible. This is because unsaturated hydrocarbon chains have permanent kinks in them (because of the rigid nature and geometry of the C=C bonds), so they cannot pack as regularly as saturated hydrocarbon chains. The less regular packing means that there is less interaction area between the molecules, which lowers the strength of the van der Waals interactions between them. This in turn, lowers the temperature at which they change from a solid (no movement of the lipids relative to each other within the plane of the membrane) to a liquid (much freer movements). Recall that the strength of interactions between molecules determines how much energy is needed to overcome a particular type of interaction. Because these van der Waals intermolecular interactions are relatively weak, changes in environmental temperature influence the physical state of the membrane. The liquid like state is often referred to as the fluid state. The importance of membrane state is that it can influence the behavior and activity of membrane proteins. If the membrane is in a solid state, such proteins will be immobile, while in the liquid state they move by diffusion, that is, by thermally driven movement. Alternatively, since lipids are closely associated with proteins in the membrane, the physical state of the membrane can influence the activity of a protein embedded within it (a topic to which we will return).

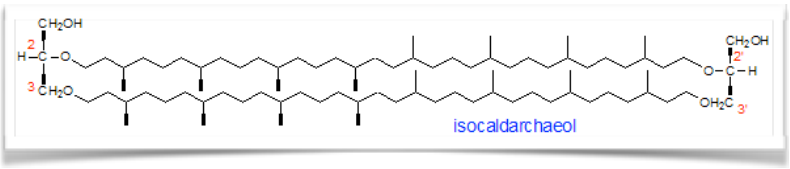


Cells can manipulate the solid-to-liquid transition temperature of their membrane by altering the membrane's lipid composition. For example, by altering the ratio of saturated to unsaturated chains present. This level of control involves altering the activities of the enzymes involved in saturation/desaturation reactions. That these enzymes can be regulated implies a feedback mechanism, by which either temperature or membrane fluidity acts to regulate metabolic processes. This type of feed back mechanism is part of what is known as the homeostatic and adaptive system of the cell (and the organism) and is another topic we will return to toward the end of the course.

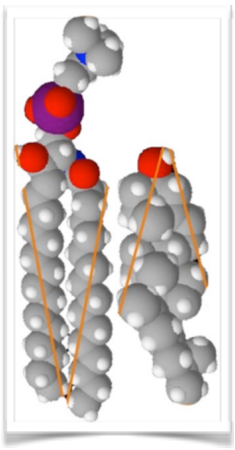
There are a number of differences between the lipids used in bacterial and eukaryotic organisms and archaea.[141] For example, instead of hydrocarbon chains, archaeal lipids are constructed of isoprene (CH_2=C(CH_3)CH=CH_2) polymers linked to the glycerol group through an ether (rather than an ester) linkage. The bumpy and irregular shape of the isoprene groups (compared to the relatively smooth saturated hydrocarbon chains) means that archaeal membranes will tend to melt (go from solid to liquid) at lower temperatures.[142] At the same time the ether linkage is more stable (requires more energy to break) than the ester linkage. It remains unclear why it is that while all organisms use glycerol-based lipids, the bacteria and the eukaryotes use hydrocarbon chain lipids, while the archaea use isoprene-based lipids. One speculation is that archaeal were originally adapted to live at higher temperatures, where the greater stability of the ether linkage would provide a critical advantage.



At the highest temperatures, thermal motion might be expected to disrupt the integrity of the membrane, allowing small charged molecules (ions) through.[143] Given the importance of membrane integrity, we will (perhaps) not be surprised to find "double-headed" lipids in organisms that live at high temperatures (thermophiles and hyperthermophiles).These lipid molecules have a two distinct hydrophilic glycerol moieties, one located at each end of the molecule; this enables them to span the membrane. The presumption is that such lipids act to stabilize the membrane against the disruptive effects of high temperatures - important since some archaea live (happily, apparently) at temperatures up to 110 ºC[144]. Similar double-headed lipids are also found in bacteria that live in high temperature environments.



That said, the solid-fluid nature of biological membranes, as a function of temperature, is complicated by the presence of cholesterol and structurally similar lipids. For example, in eukaryotes the plasma membrane can contain as much as 50% (by number of lipid molecules present) cholesterol. Cholesterol has a short bulky hydrophobic domain that does not pack well with other lipids (**FIG**: a hydrocarbon chain lipid (left) and cholesterol (right)). When present, it dramatically influences the solid-liquid behavior of the membrane. The diverse roles of lipids is a complex subject that goes beyond our scope here.[145]



[141]A re-evaluation of the archaeal membrane lipid biosynthetic pathway: http://www.nature.com/nrmicro/journal/v12/n6/full/nrmicro3260.html

[142]The origin and evolution of Archaea: a state of the art: http://rstb.royalsocietypublishing.org/content/361/1470/1007.full

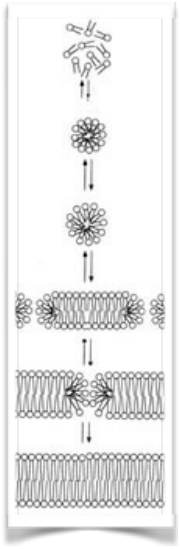
[143] Ion permeability of the cytoplasmic membrane limits the maximum growth temperature of bacteria and archaea. : http://www.ncbi.nlm.nih.gov/pubmed/8825096

[144] You might want to consider how this is possible and under want physical conditions you might find these "thermophilic" archaea.

[145] At this point, such a search recovers 636 papers (and there are many more than concern lipid function but do not contain lipidomics in the title.

The origin of biological membranes

The modern cell membrane is composed of a number of different types of lipids. Those lipids with one or more hydrophobic “tails” have tails that typically range from 16 to 20 carbons in length. The earliest membranes, however, were likely to have been composed of similar, but simpler molecules with shorter hydrophobic chains. Based on the properties of lipids, we can map out a plausible sequence for the appearance of membranes. Lipids with very short hydrophobic chains, 2 to 4 carbons in length, can dissolve in water (can you explain why?) As the lengths of the hydrophobic chains increases, the molecules begin to self-assemble into micelles. By the time the hydrophobic chains reach ~10 carbons in length, it becomes increasingly more difficult to fit the hydrocarbon chains into the interior of the micelle without making larger and larger spaces between the hydrophilic heads. Water molecules can begin to move through these spaces and interact with the hydrocarbon tails. At this point, the hydrocarbon-chain lipid molecules begin to associate into semi-stable bilayers. One interesting feature of these bilayers is that the length of the hydrocarbon chain is no longer limiting in the same way that it was limiting in a micelle. One problem, though, are the edges of the bilayer, where the hydrocarbon region of the lipid would come in contact with water, a thermodynamically unfavorable situation. This problem is avoided by linking edges of the bilayer to one another, forming a balloon-like structure. Such bilayers can capture regions of solvent, that is water and any solutes dissolved within it.



Bilayer stability increases further as hydrophobic chain length increases. At the same time, membrane permeability decreases. It is a reasonable assumption that the earliest biological systems used shorter chain lipids to build their "proto-membranes" and that these membranes were relatively leaky.[146] The appearance of more complex lipids, capable of forming more impermeable membranes must therefore have depended upon the appearance of mechanisms that enabled hydrophilic molecules to pass through membranes. The process of interdependence of change is known as co-evolution. Co-evolutionary processes were apparently common enough to make the establishment of living systems possible. We will consider the ways through a membrane in detail below.

Questions to answer & to ponder:

- Draw diagrams to show how increasing the length of a lipid's hydrocarbon chains affects the structures that it can form.
- How are the effects at the hydrophobic edges of a lipid bilayer minimized?
- What types of molecules might be able to go through the plasma membrane on their own?
- In the light of the cell theory, what can we say about the history of cytoplasm and the plasma membrane?
- Why do fatty acid and isoprene lipids form similar bilayer structures?
- Speculate on why it is common to see phosphate and other highly hydrophilic groups attached to the glycerol groups of lipids?
- Are the membranes of bacteria and archaea homologous or analogous? What type of data would help you decide?
- Why is the movement of materials through the membrane essential for life?
- Why do membrane lipids solidify at low temperature? How are van der Waals interactions involved? Are H-bond type electrostatic interactions involved?

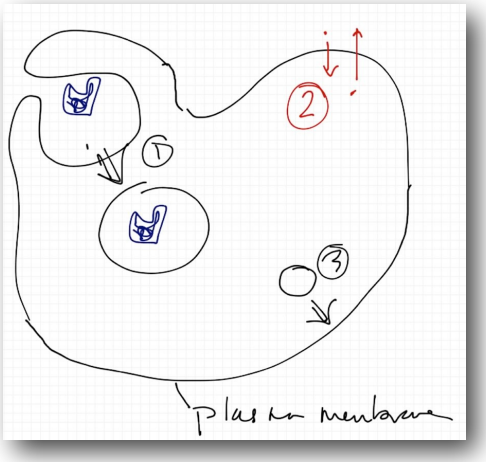
[146] http://astrobiology.arc.nasa.gov/workshops/1996/astrobiology/speakers/deamer/deamer_abstract.html

- Predict (and justify) the effect of changing the position of a double bond in a hydrocarbon chain on the temperature of membrane solidification.
- Would a membrane be more permeable to small molecules at high or low temperatures and why?

Transport across membranes

As we have said before (and will say again), the living cell is a continuous non-equilibrium system. To maintain its living state both energy and matter have to move into and out of the cell, which leads us to consider both the intracellular and extracellular environments and the membrane that separates them. The differences between the inside and the outside of the plasma membrane are profound. Outside, even for cells within a multicellular organism, the environment is generally mostly water, with relatively few complex molecules. Inside, the membrane-defined space, is a highly concentrated (> 60 mg/ml) solution of proteins, nucleic acids, smaller molecules, and thousands of interconnected chemical reactions, known collectively as cytoplasm. Cytoplasm (and the membrane around it) is inherited by the cell when it was formed, and represents an uninterrupted continuous system that first arose billions of years ago.

A lipid bilayer membrane poses an interesting barrier to the movement of molecules. First for larger molecules, particles or other organisms, it acts as a physical barrier. Typically when larger molecules, particles (viruses), and other organisms enter a cell, they are actually engulfed by the membrane, in a range of processes from pinocytosis (cell drinking) to endocytosis (cell entry) and phagocytosis (cell eating)(process 1). A superficially similar process, running in "reverse", known as endocytosis (process 3), is involved in moving molecules to the cell surface and releasing them into the extracellular space. Both endocytosis and exocytosis involve membrane vesicles emerging from or fusing into the plasma membrane. These processes leave the topology of the cell unaltered, in the sense that a molecule within a vesicle is still "outside" of the cell, or at least outside of the cytoplasm. These movements are driven by various molecular machines that we will consider only briefly; they are typically considered in greater detail in courses on cell biology. We are left with the question of how molecules can enter or leave the cytoplasm, this involves passing directly through a membrane (process 2).



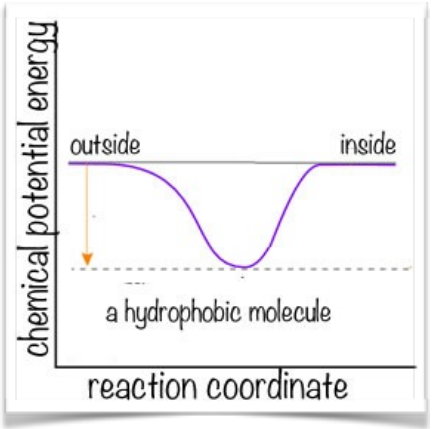
Transport to and across the membrane

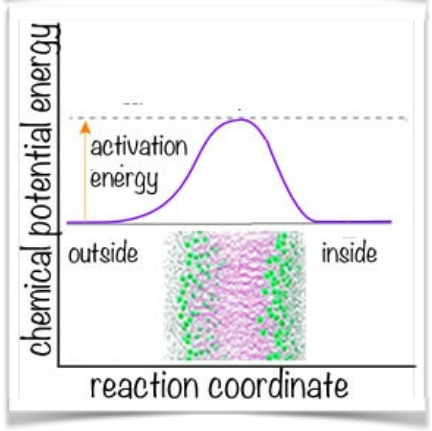
So the question is, how does the membrane "decide" which molecules to allow into and out of the cell. If we think about it, there are three possible general mechanisms (let us know if you can think of more). Molecules can move on their own through the membrane, they can move passively across the membrane using some type of specific "carrier" or "channel", or they could be moved actively using some kind of "pump". In particular, which types of carriers, channels, and pumps are present will determine what types of molecules move through the membrane. As you might deduce pumps require a source of energy to drive them. As we will see, in the vast majority of cases, these carriers, channels,

and pumps are protein-based molecular machines, the structure of which we will consider in detail later on. We can think of this molecular movement reaction generically as:

$$Molecule_{outside} \rightleftharpoons Molecule_{inside\ membrane} \rightleftharpoons Molecule_{inside}.$$

As with standard chemical reactions, movement through a membrane involves an activation energy, which amounts to the energy needed to pass through the membrane. So, you might well ask, why does the membrane, particularly the hydrophobic center of the membrane, pose a barrier to the movement of hydrophilic molecules. Here the answer involves the difference in the free energy of the moving molecule within an aqueous solution, including the hydrophilic surface region of the membrane, where H-bond type electrostatic interactions are common between molecules, and the hydrophobic region of the membrane, where only van der Waals interactions are present. The situation is exacerbated for charged molecules, since water molecules are typically organized in a dynamic shell around an ion. Instead of reactants and products we can plot the position of the molecule relative to the membrane. We are considering molecules of one particular substance moving through the membrane and so the identity of the molecule does not change. If the concentrations of the molecules are the same on both sides of the membrane, then their Gibbs free energies are also equal, the system will be in equilibrium with respect to this reaction. In this case, as in the case of chemical reactions, there will be no net flux of the molecule across the membrane, but molecules will be moving back and forth at an equal rate. The rate at which they move back and forth will depend on the size of the activation energy associated with moving across the membrane.



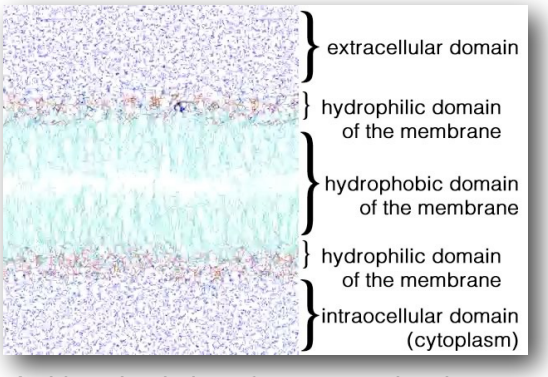


If a molecule is hydrophobic (non-polar) it will be more soluble in a hydrophobic environment in the center of the membrane than it is in an aqueous environment. In contrast the situation will be distinctly different for hydrophilic molecules. By this point, we hope you will recognize that in a simple lipid-only membrane (a biologically unrealistic case), the shape of this graph, and specifically the height of the activation energy peak will vary depending upon the characteristics of the molecule we are considering moving as well as the membrane itself. If the molecule is large and highly hydrophilic, for example, if it is charged, the activation energy associated with crossing the membrane will be higher than if the molecule is small and uncharged. Just for fun, you might consider what the reaction diagram for a single lipid molecule might look like; where might it be located, and what energy barriers are associated with its movement (flipping) across a membrane.

http://youtu.be/JxtnneWWzHo

Let us begin with water itself, which is small and uncharged. When a water molecule begins to leave water and enter the hydrophobic (central) region of the membrane, there are no H-bonds to take the place of those that are lost, no strong handshakes, and often the molecule is “pulled back” into the water. Nevertheless, there are so many molecules of water outside (and inside) the cell, and water molecules are so small, that once they enter the membrane, they can pass through it. The activation

energy for the $Water_{outside} \rightleftharpoons Water_{inside}$ reaction is low enough that water can pass through a membrane (in both directions) at a reasonable rate.

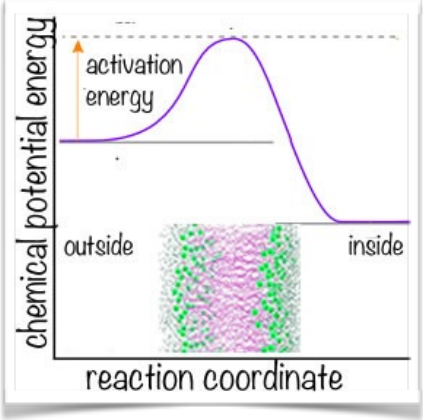


A video simulation of a water molecule moving through a membrane: http://youtu.be/ePGqRaQiBfc

Small non-polar molecules, like O_2 and CO_2, can (very much like water) pass through a biological membrane relatively easily. There is more than enough energy available through collisions with other molecules (thermal motion) to provide them with the energy needed to overcome the activation energy and pass through the membrane. However now we begin to see changes in free energies of the molecules on the inside and outside of the cell. For example, in organisms that depend upon O_2 (obligate aerobes), the O_2 outside of the cell comes from the air (it is generated by plants that release O_2 as a waste product.) Once O_2 enters the cell, it takes part in the reactions of respiration (we will get back to both processes further on.) The result is that the concentration of O_2 outside the cell will be greater than the concentration of O_2 inside the cell. That means that the free energy of O_2 outside will be greater than the free energy of O_2 inside. The reaction $O_{2\ outside} \rightleftharpoons O_{2\ inside}$ is now thermodynamically favorable and there will be a net flux of O_2 into the cell. We can consider how a similar situation applies to water. The intracellular domain of a cell is a concentrated solution of proteins and other molecules. Typically, the concentration of water outside of the cell is greater than the concentration of water inside the cell. Our first order presumption is that the reaction:

$$H_2O_{\ outside} \rightleftharpoons H_2O_{\ inside}$$

is favorable, so water will flow into a cell. So the obvious question is, what happens over time? We will return to how cell's (and organisms) resolve this important problem shortly.



Channels and carriers

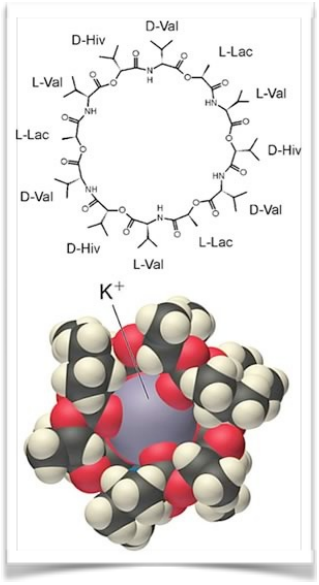
Beginning around the turn of the last century, a number of scientists began working to define the nature of cell's boundary layer. In the 1930's it was noted that small, water soluble molecules entered cells faster than predicted based on the assumption that the membrane acts like a simple hydrophobic barrier - an assumption known as Overton's Law. Collander et al., postulated that membranes were more than simple hydrophobic barriers, specifically that they contained features that enabled them to act as highly selective molecular sieves. Most of these are proteins (never fear, we are getting closer to a more thorough discussion of proteins) that can act as channels, carriers, and pores. If we think about crossing the membrane as a reaction, then the activation energy of this reaction for highly hydrophilic and larger molecules will be quite high, we will need a catalyst to reduce it. There are two generic types of membrane permeability catalysts available: carriers and channels.

Carrier proteins are membrane proteins that can shuttle back and forth across the membrane. They can bind to specific hydrophilic molecules when they are located in the hydrophilic region of the membrane, hold on to the bound molecule as they traverse the hydrophobic region of the membrane, and then release their "cargo" when they again reach the hydrophilic region of the membrane. These movements of carrier and cargo across the membrane are driven by thermal motions, so no other energy source is necessary. We can write this class of reactions as:

$$Molecule_{outside} + carrier_{empty} \rightleftharpoons carrier\text{–}\ Molecule_{outside} \rightleftharpoons carrier\text{–}\ Molecule_{inside} \rightleftharpoons Molecule_{inside} + carrier_{empty}.$$

There are many different types of carrier molecules and each type of carrier has a preferred cargo molecule. Related molecules may be bound and transported, but with much less specificity (and so at a much lower rate). So exactly which molecules a particular cell will allow to enter will be determined in part by which carrier protein genes it expresses. Mutations in a gene encoding a carrier can change (or abolish) the range of molecules that that carrier can transport across a membrane.

Non-protein carriers: An example of a carrier is a class of antibiotics that carry ions across membranes. These molecules are known generically as ionophores. They kill cells by disrupting the normal ion balance across the membrane and within the cytoplasm, which in turn is thought to disrupt normal metabolic activity.[147] One of these is valinomycin (→), a molecule made by *Streptomyces* type bacteria. The valinomycin molecule has a hydrophobic periphery and a hydrophilic core. It binds K^+ ions approximately 10^5 times more effectively than it binds Na^+. It shuttles (with the bound ion) back and forth across the membrane. In the presence of a K^+ gradient, that is a higher concentration of K^+ on one side of the membrane compared to the other, the presence of valinomycin will produce a net flux of K^+ across the membrane. Again, to be clear, in the absence of a gradient, K^+ ions will still move across the membrane (in the presence of the carrier), but there will be no net change in the concentration of K^+ ion inside the cell. For the experimentally inclined, you might consider how you could prove that movements are occurring even in the absence of a gradient. In a similar manner, there are analogous carrier systems that move hydrophobic molecules through water.



Channel molecules sit within a membrane. They contain a channel that spans the membrane's hydrophobic region. Hydrophilic molecules of particular sizes and shapes can pass through this "aqueous" channel and their movement involves a much lower activation energy than would be associated with moving through the lipid part of the membrane. Channels are generally very selective in terms of which particles pass through them. For example, there are channels in which 10,000 potassium ions will pass through for every one sodium ion.

The channels in these proteins can be regulated; they can exist in two or more distinct structural states. For example, in one state the channel can be open and allow particles to pass through or it can be closed, that is the channel can be turned on and off. The transition between open and closed states

[147] That said, there is little data in the literature on exactly which cellular processes are disrupted by which ionophore; in mammalian cells (as we will see) these molecules are by disrupting ion gradients in mitochondria and chloroplasts, apparently.

can be regulated through a number of processes, including the reversible binding of small molecules and various other molecular changes (which we will consider when we talk about proteins) or changes in electrochemical gradients across the membrane.

Another method of channel control depends on the fact that channel proteins are embedded within a membrane and are contain of charged groups. As we will see, cells can (and generally do) generate ion gradients, that is a separation of charged species, across their membranes. For example if the concentration of K^+ ions is higher on one side of the membrane, there will be an ion gradient where the natural tendency is for the ions to move to the region of lower K^+ concentration[148]. The ion gradient in turn can produce electrical fields across the plasma membrane. As these fields change, they can produce (induce) changes in channel structure, which can switch the channel from open to closed and vice versa. Organisms typically have many genes that encode specific channel proteins which are involved in a range of processes from muscle contraction to thinking. As in the case of carriers, channels do not determine the direction of molecular motion. The net flux of molecular movement is determined by the gradients of molecules across the membrane, with the thermodynamic driver being entropic factors. That said, the actual movement of the molecules through the channel is driven by thermal motion.

Questions to answer & to ponder:

- What does it mean to move up a concentration gradient?
- Are there molecules that can move up their concentration gradients spontaneously?
- Where does the energy involved in moving molecules come from? Is there a "force" driving the movement of molecules "down" their concentration gradient?
- If there is no net flux of A, even if there is a concentration gradient between two points, what can we conclude?
- What happens to the movement of molecules through channels and transporters if we reverse the concentration gradients across the membrane?
- Is energy needed to maintain gradients across a membrane (what is your thermodynamic logic)?
- Why do we need to add energy to maintain gradients?
- Which (and why) would you think would transport molecules across a membrane faster, a carrier, a channel, or a pump?

Generating gradients: using coupled reactions and pumps

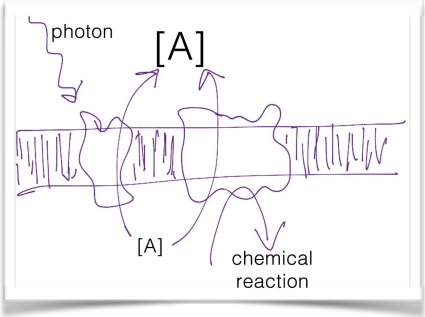
Both carriers and channels can allow the directional movement (net flux) of molecules across a membrane, but only when a concentration gradient is present. If a membrane contains active channels and carriers (as all membranes do), without the input of energy eventually concentration gradients across the membrane will disappear (disperse). The $[molecule]_{outside}$ will become equal to $[molecule]_{inside}$. Yet, when we look at cells we find lots of concentration gradients, which raises the question, what produces and then maintains these gradients.

The common sense answer is that there must be molecules (proteins) that can move specific molecules through a membrane against their concentration gradient. We will call this type of molecule a pump and write the reaction it is involved in as:

$$[Molecule]_{low\ concentration} + pump \longleftrightarrow [Molecule]_{high\ concentration} + pump$$

[148] In fact this tendency for species to move from high to low concentration until the two concentrations are equal can be explained by the Second Law of Thermodynamics. Check with your chemistry instructor for more details

As you might already suspect this is a thermodynamically unfavorable reaction. Like a familiar macroscopic pump, it will require the input of energy. We will have to "plug in" our molecular pump into a source of energy. What energy sources are available to biological systems? Basically we have two choices: the system can use electromagnetic energy, that is light, or it can use chemical energy. In a light-driven pump, there is a system that captures (absorbs) light which is then coupled to the pumping system. Where the pump is driven by a chemical reaction, the thermodynamically favorable reaction is often catalyzed by the pump itself and coupled to the movement of a molecule against its concentration gradient. An interesting topological point is that for a light or chemical reaction driven pump to work to generate a concentration gradient, all of the pump molecules within a membrane must be oriented in the same direction. If the pumps were oriented randomly there probably would be no overall flux (the molecules would move in both directions) and no gradient would develop.



Chemical-reaction driven pumps are also oriented within membranes in the same direction. A number of chemical reactions can be used to drive such pumps and these pumps can drive various reactions (remember reactions can move in both directions). The most common ones are the movement of energetic electrons through a membrane-bound, protein-based "electron transport" system, leading to the creation of an H^+ electrochemical gradient. The movement of H^+ down its concentration gradient through the pump then drives the synthesis of ATP:

$$H^+ \text{ from } [H^+]_{high} \rightleftharpoons H^+ \text{ to } [H^+]_{low}$$

which is coupled to

$$\text{adenosine diphosphate (ADP) + phosphate} \rightleftharpoons \text{adenosine triphosphate (ATP) + } H_20$$

or through the hydrolysis of adenosine triphosphate, a highly thermodynamically favorable reaction:

$$H^+ \text{ from } [H^+]_{low} \rightleftharpoons H^+ \text{ to} [H^+]_{high}$$

is coupled to

$$\text{adenosine triphosphate (ATP) + } H_20 \rightleftharpoons \text{adenosine diphosphate (ADP) + phosphate.}$$

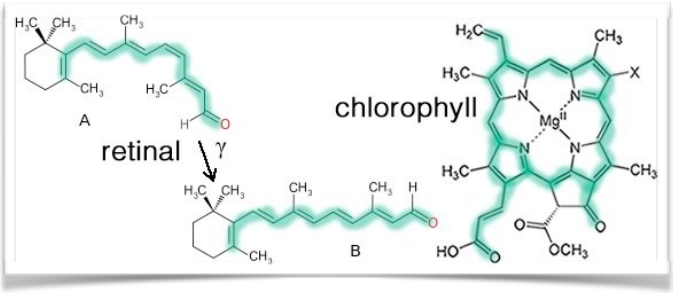
By coupling a ATP hydrolysis reaction to the pump, the pump can move molecules from a region of low concentration to one of high concentration, a thermodynamically unfavorable reaction.

Simple Phototrophs

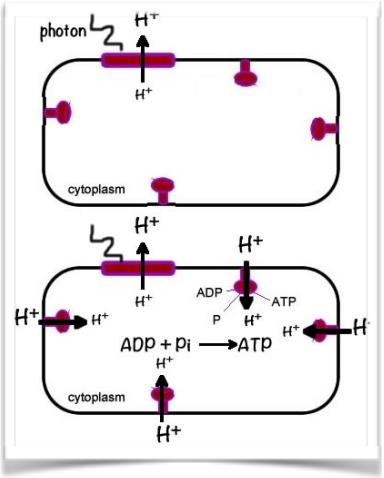
Phototrophs are organisms that capture light particles (photons) and transform their electromagnetic energy into energy stored in unstable molecules, such as ATP and carbohydrates. Light can be considered as both a wave and a particle (that is quantum physics for you) and the wavelength of a photon determines its color and the amount of energy it contains. Again, because of quantum mechanical factors, a particular molecule can only absorb photons of specific wavelengths - in fact, we can identify molecules based on the photons they absorb (this is the basis of spectroscopy). Our atmosphere allows mainly visible light from the sun to reach the earth's surface, but most biological molecules do not absorb visible light very effectively (or at all). To capture this energy, organisms have evolved the ability to synthesize special molecules, known as pigments, to capture and therefore allow

organisms to use visible light. The color we see for a typical pigment is the color of the light that is not absorbed, but rather is reflected. For example chlorophyl appears green because light in the red and blue regions of the spectrum is absorbed and green light is reflected. The question we need to answer is how does the organism use the electromagnetic energy that is absorbed?

One of the simplest examples of a phototrophic system, that is, a system that directly captures the energy of light and transforms it into the energy stored in the chemical system, is provided by the archaea *Halobacterium halobium*.[149] *Halobacteria* are extreme halophiles or salt-loving organisms. They live in waters that contain up to 5M NaCl. *H. halobium* uses the membrane protein, bacteriorhodopsin to capture light. Bacteriorhodopsin consists of two components, a polypeptide, known generically as an opsin, and a non-polypeptide prosthetic group, the pigment retinal, a molecule derived from vitamin A.[150] Together the two, opsin + retinal form the functional bacteriorhodopsin protein.



Retinal absorbs visible light. This is because its electrons are located in extended molecular orbitals that have energy gaps between them that are of the same order as the energy of visible light. This extended molecular orbital (highlighted in the figure) is associated with a region of the molecule drawn as containing alternating single and double bonds (which we call a conjugated pi orbital system) between carbons. Similar conjugated pi systems are responsible for the absorption of light by other pigments, like chlorophyll and heme. When a photon of light is absorbed by the retinal group, it undergoes a reaction that leads to a change in the pigment molecule's shape and composition, which in turn leads to a change in the structure of the polypeptide to which the retinal group is attached. This is called a photoisomerization reaction.



The bacteriorhodopsin protein is embedded within the plasma membrane, where it associates with other bacteriorhodopsin proteins to form patches of proteins. These patches of membrane protein give the organisms their purple color and are known as purple membrane. When one of these proteins absorbs light, the change in the associated retinal group produces a light-induced change in protein structure that results in the movement of a H^+ ion from the inside of the cell to the outside of the cell, with the protein returning to its original low energy state, that is, its state before it absorbed the photon of light. Because all of the bacteriorhodopsin molecules are oriented in the same way in the membrane, as light is absorbed all of the H^+ ions move in the same direction, leading to the formation

[149] http://youtu.be/4OkN1QC4hyY

[150] As we will return to later, proteins are functional entities, composed of polypeptides and prosthetic group. The prosthetic group is essential for normal protein function. The protein without the prosthetic group is known as the apoprotein.

of a H^+ concentration gradient across the plasma membrane with $[H^+]_{outside} > [H^+]_{inside}$. This H^+ gradient is based on two sources. First there is the gradient of H^+ ions. As light is absorbed the concentration of H^+ outside the cell increases, and the concentration of H^+ inside the cell decreases. The question is - where is this H+ coming from? The answer lies in the energy absorbed by the pigment and its use to split a water molecule. As you (perhaps) learned in chemistry it takes energy to separate a water molecule into H^+ and ^-OH (the reaction is MUCH more favorable in the opposite direction:

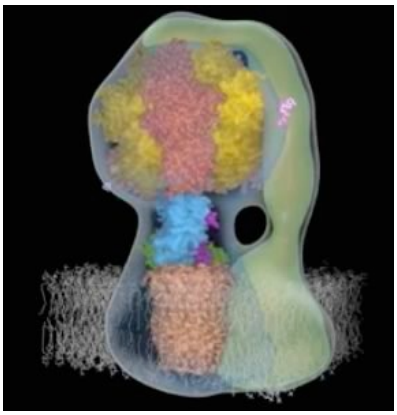
$$H_2O \rightleftharpoons H^+ + OH^-.$$

In addition to the chemical gradient in H^+ ions that forms as H^+ ions are pumped out of the cell by the bacteriorhodopsin + light + water reaction, an electrical field is also established. There are excess + charges outside of the cell (from H^+ being moved there) and excess – charges inside the cell (from –OH being left behind). As you know from your physics, positive and negative charges attract, but the membrane stops them from reuniting. The result is the accumulation of positive charges on the outer surface of the membrane and negative charges on the inner surface. This charge separation produces an electric field. Now, a H^+ outside of the cell will experience two forces. If there is a way across the membrane, the $[H^+]$ gradient will lead to its movement back into the cell. Similarly the electrical field will also drive the positively charged H^+ back into the cell. The formation of the $[H^+]$ gradient basically generates a battery, a source of energy, into which we can plug in our pump.

So how does the pump tap into this battery? The answer is a second membrane protein, an enzyme known as the H^+-driven ATP synthase. H^+ ions move through the ATP synthase molecule, which is a thermodynamically favorable reaction. The ATP synthase couples this favorable movement to an unfavorable chemical reaction, a condensation reaction:

$$H^+_{outside} + ADP + \text{inorganic phosphate} \xrightleftharpoons{\text{ATP synthase}} ATP + H_2O + H^+_{inside}$$

This reaction will continue as long as light is absorbed and bacteriorhodopsin acts to generate a H^+ gradient. It will also continue for a time after the light goes off (that is, night time) because it takes time for H^+ ions to move through the ATP synthase and for the H^+ gradient to dissipate, but after a short while (in the dark), net ATP synthesis will slow and stop. The point of this process is that, in the light, the cell generates (and stores for later use in various coupled reactions) ATP. ATP acts as a type of chemical battery, in contrast to the electrochemical battery of the H^+ gradient.



link: http://youtu.be/J8lhPt6V-yM

An interesting feature of the ATP synthase is that as H^+ ions move through it (driven by the electrochemical power of the H^+ gradient), it rotates. It is worth noting that there is no thermodynamic reason that the ATP synthase cannot run in the opposite direction. In fact, it can catalyze (and couple) the hydrolysis of ATP to the pumping of H^+ out of the cell:

$$ATP + H_2O + H^+_{inside} \xrightleftharpoons{\text{ATP synthase}} H^+_{outside} + ADP + \text{inorganic phosphate}$$

Because it catalyzes the hydrolysis of ATP, the enzyme can be called an ATP hydrolase. Again, when it catalyzes the hydrolysis of ATP, it rotates, although in the opposite direction compared to when it catalyzes the synthesis of ATP. Now its energy driven rotation (by either the electrochemical H^+ battery or ATP hydrolysis) raises in interesting possibility. This enzyme (or rather a variant) could be used to drive the swimming movement of cells (imagine connecting it to some kind of propeller.)

beSocratic exercise: Draw a membrane, place bacteriorhodopsin molecules in it, mark their orientation and the direction of the H^+ gradient that arises in the light. Draw the ATP synthase, indicate how movement of H^+ leads to ATP synthesis. Indicate how ATP hydrolysis or tapping into the H^+ gradient could lead to cell movement. Can you imagine and describe other mechanisms that could move cells?

Chemo-osmosis (an overview)

One of the most surprising discoveries in biology was the wide spread, almost universal use of H^+ gradients to generate ATP. It was originally known as the chemiosmotic hypothesis by the eccentric British scientist, Peter Mitchell (1920 – 1992).[151] Before the significance of H^+ membrane gradients was known, Mitchell proposed that energy captured through the absorption of light (by phototrophs) or the breakdown of molecules into more stable molecules (by various types of chemotrophs) relied on the same basic (homologous) mechanism, namely the generation of H^+ gradients across membranes (the plasma membrane in prokaryotes or the internal membranes of mitochondria or chloroplasts (intracellular organelles, derived from bacteria)(see below) in eukaryotes.

What makes us think that these processes have a similar evolutionary root, that they are homologous? It is that in both light and chemical based processes, captured energy is transferred through the movement of electrons through a membrane-embedded "electron transport chain." This chain involves a series of reactions, specifically reduction-oxidation or redox reactions (see below) during which electrons move from a high energy to a lower energy state. Some of this energy difference is used to move H^+ ions across the membrane and so generate a H^+ concentration gradient. The thermodynamically favorable movement of H^+ down this concentration gradient is then used to drive ATP synthesis (a thermodynamically unfavorable process.) ATP synthesis itself involves the rotating ATP synthase. The movement of H^+ ions down the H^+ gradient through the ATP synthase drives the reaction:

$$H^+_{outside} + ADP + Pi \rightleftharpoons ATP + H_2O + H^+_{inside},$$

where "inside" and "outside" refer to compartments defined by the membrane containing the electron transport chain and the ATP synthase. Again, this reaction can run backwards. When this occurs, the ATP synthase acts as an ATPase that can pump H^+ (or other molecules) against their concentration gradient. In fact the action of such pumping ATPases establishes many biologically important molecular gradients across membranes. In such a reaction:

$ATP + H_2O$ + molecule in low concentration region $\rightleftharpoons$ ADP + Pi + molecule in low concentration region.

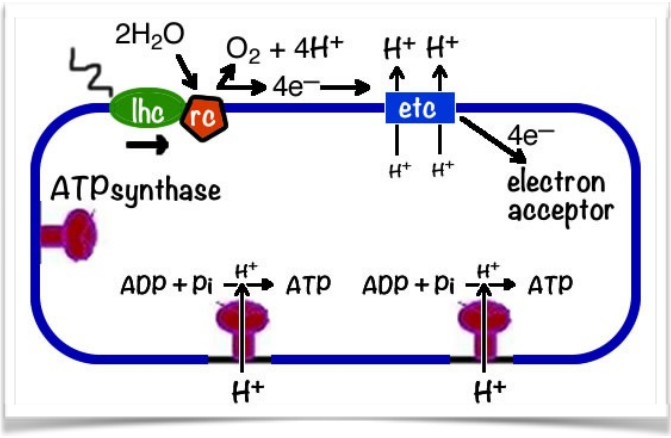
In an sense, the most important difference between phototrophs and chemotrophs is how high energy electrons enter the electron transport chain.

[151] http://en.wikipedia.org/wiki/Peter_D._Mitchell

Oxygenic photosynthesis

Compared to the salt loving archaea *Halobium*, with its purple, bacteriorhodopin-rich membranes, photosynthetic cyanobacteria (which are bacteria), green algae, and higher plants (both eukaryotes) use more complex systems to capture and utilize light. In all of these organisms, their photosynthetic systems appear to be homologous, that is derived from a common ancestor, a topic we will return to later in this chapter. For simplicity's sake, we will describe the photosynthetic system of cyanobacterium; the system in eukaryotic algae and plants, while more complex, follows the same basic logic. At this point, we consider only one aspect of this photosynthetic system, known as the oxygenic or non-cyclic system (look to more advanced classes for more details.) The major pigment in this system, chlorophyll, is based on a complex molecule, a porphyrin (see above) and it is primarily these pigments that give plants their green color. As in the case of retinal, they absorb visible light due to the presence of a conjugated structure (drawn as a series of single and double) carbon-carbon bonds. Chlorophyll is synthesized by a conserved biosynthetic pathway that is also used to synthesize heme, which is found in the hemoglobin of animals and in the cytochromes within the electron transport chain present in both plants and animals (which we will come to shortly), vitamin B12, and other biologically important prosthetic (that is non-polypeptide) groups associated with proteins and required for their normal function.[152]

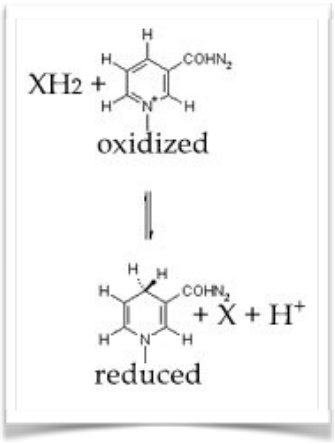
Chlorophyll molecules are organized into two distinct protein complexes that are embedded in membranes. These are known as the light harvesting and reaction center complexes. Light harvesting complexes (lhc) act as antennas to increase the amount of light the organism can capture. When a photon is absorbed, an electron is excited to a higher molecular orbital. An excited electron can be passed between components of the lhc and eventually to the reaction center ("rc") complex. Light harvesting complexes are important because photosynthetic organisms can compete with one another for light, so their presence can enable a photosynthetic organism to flourish at lower light levels.



In the oxygenic, that is molecular oxygen (O_2) generating (non-cyclic) photosynthesis reaction system, high energy (excited) electrons are passed from the reaction center to a complex of membrane proteins known as the electron transport chain ("etc"). As an excited electron moves through the etc its energy is used to move H^+s from inside to outside of the cell. This is the same geometry of H^+ movement that we saw previously in the case of the purple membrane system. The end result is the formation of a H^+ based electrochemical gradient. As with purple bacteria, the energy stored in this H^+ gradient is used to drive the synthesis of ATP within the cell's cytoplasm.

[152] Mosaic Origin of the Heme Biosynthesis Pathway in Photosynthetic Eukaryotes: http://mbe.oxfordjournals.org/content/22/12/2343.full.pdf

Now you might wonder, what happens to the originally excited electrons, and the energy that they carry. In what is known as the cyclic form of photosynthesis, low energy electrons from the electron transport chain are returned to the reaction center, where they return the pigments to their original (before absorbing light) state. In contrast, in the non-cyclic process that we have been considering, electrons from the electron transport chain are delivered to an electron acceptor. Generally this involves the absorption of a second photon, a mechanistic detail that need not trouble us here. This is a general type of chemical reaction known as an oxidation-reduction (redox) reaction. Where electrons are within a molecule's electron orbital system determines the amount of energy present in the molecule. In this light, it makes sense that adding an electron to a molecule will (generally) increase the molecule's overall energy, and make it less stable. When an electron is added to a molecule, that molecule is said to have been "reduced", and yes, it does seem weird that adding an electron "reduces" a molecule. If an electron is removed, the molecule's energy is changed and the molecule is said to have been "oxidized".[153] Since electrons, like energy, are neither created nor destroyed in biological systems (remember, no nuclear reactions), the reduction of one molecule is always coupled to the oxidation of another. For this reason, reactions of this type are referred to as "redox" reactions. During such a reaction, the electron acceptor is said to be "reduced". Reduced molecules are generally unstable, so the reverse, thermodynamically favorable reaction, in which electrons are removed (known as oxidation) can be used to drive various types of thermodynamically unfavorable metabolic reactions.



Given the conservation of matter in biological systems, if electrons are leaving the photosynthetic system (in the non-cyclic process) they must be replaced. So where do they come from? Here we see what appears to be a major evolutionary breakthrough. During the photosynthetic process, the reaction center couples light absorption with the oxidation (removal of electrons) from water molecules:

$$\text{light} + 2H_2O \rightleftharpoons 4H^+ + 4e^- + O_2.$$

The four electrons, derived from two molecules of water, pass to the reaction center, while the $4H^+$s contribute to the proton gradient across the membrane.[154] O_2 is a waste product of this reaction. Over millions of years, the photosynthetic release of O_2 changed the Earth's atmosphere from containing essentially 0% molecular oxygen to the current ~21% level. Because O_2 is highly reactive, this transformation is thought to have been a major driver of subsequent evolutionary change. However, there remain even today organisms that cannot use O_2 and cannot survive in its presence. They are known as obligate anaerobes (to distinguish them from organisms that normally grow in the absence of O_2 but which can survive in its presence, which are known as facultative anaerobes. In the past the level of atmospheric O_2 has changed dramatically based on how much O_2 was released into the atmosphere by oxygenic photosynthesis and how much was removed by various reactions, such as the decomposition of plant materials. When large amounts of plant materials are buried before they could decay, such as occurred with the formation of coal beds, during the Carboniferous period (from ~360 to

[153] you can review redox here: http://www.biologie.uni-hamburg.de/b-online/e18/18b.htm or in CLUE: http://besocratic.colorado.edu/CLUE-Chemistry/chapters/chapter7txt.html

[154] Photosystem II and photosynthetic oxidation of water: an overview: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1693055/

299 million years ago), the level of atmospheric O_2 increased dramatically, to an estimated level of ~35%. It is speculated that such high levels of molecular oxygen made it possible for organisms without lungs (like insects) to grow to gigantic sizes.[155]

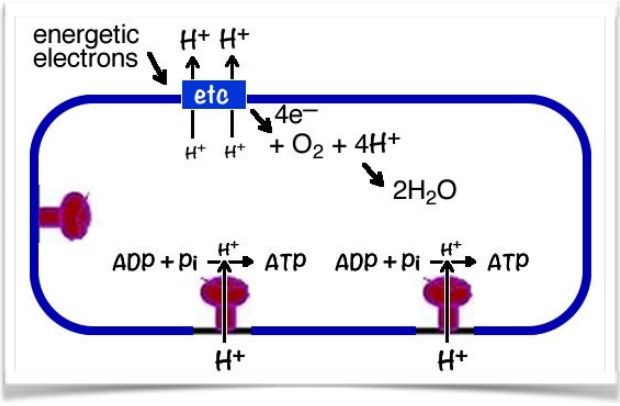
Chemotrophs

Those organisms that are not phototrophic capture energy from other sources, specifically by transforming thermodynamically unstable molecules into more stable species. These organisms are known generically as chemotrophs. They can be divided into various groups, depending upon the types of food molecules they use. They include organotrophs, which use carbon-containing molecules (you are an organotroph) and lithotrophs (or rock eaters), which use various inorganic molecules. In the case of organisms that can "eat" H_2, the electrons that result are delivered along with accompanying H^+ ions to CO_2, to form methane (CH_4) following the reaction:

$$CO_2 + 4H_2 \rightleftharpoons CH_4 + 2H_2O;$$

Because of this they are referred to as methanogens (methane-producers).[156] In the modern world methanogens (typically archaea) are found in environments with low O_2 such as your gut. In many cases, such reactions can occur only in the absence of O_2. In fact, O_2 is so reactive, that it can be thought of as a poison, particularly for organisms that cannot actively "detoxify" it. When we think about the origins and subsequent evolution of life, we have to consider how organisms that arose in the absence of molecular O_2 adapted to its introduction into their environment. It is commonly assumed that modern strict obligate anaerobes might still have features common to the earliest organisms.

The amount of energy that an organism can capture is determined by the energy of the electrons that the electron acceptor(s) they use can accept. If only high amounts of energy can be captured, then inevitably smaller amounts of energy have to be left behind. On the other hand, the lower the amount of energy that an electron acceptor can accept, the more energy can be captured from the original "food" molecules used and the less energy must be left behind. Molecular oxygen is unique in its ability to accept low energy electrons. For example, consider an organotroph that eats carbohydrates $[C_6H_{10}O_5]_n$, a class of molecules that includes various sugars, starches, and wood. In the absence of O_2, that is under anaerobic conditions, the end product of the breakdown of a carbohydrate leaves about 94% of the theoretical amount of energy present in the original carbohydrate molecule in molecules that cannot be broken down further by most organisms. However, when O_2 is present, the carbohydrate can be broken down completely into CO_2 and H_2O, a process known as glycolysis, from the Greek words meaning sweet (glyco) and splitting (lysis). In these organisms the energy stored in energetic electrons is used to generate a membrane-



[155] When Giants Had Wings and 6 Legs: http://www.nytimes.com/2004/02/03/science/when-giants-had-wings-and-6-legs.html

[156] http://en.wikipedia.org/wiki/Lithotroph

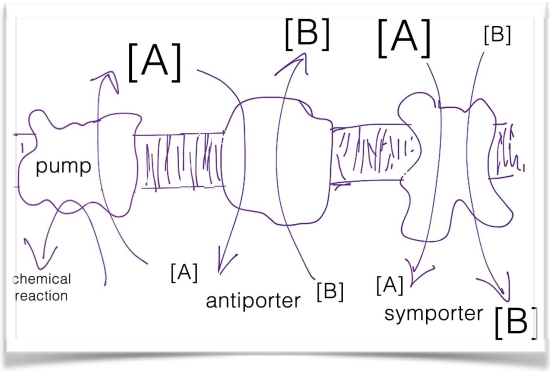
associated H+ based electrochemical gradient which in turn drives ATP synthesis, through the membrane-based ATP synthase. In an environment that contains molecular oxygen, organisms that use O_2 as an electron acceptor have a distinct advantage; instead of secreting energy rich molecules, like ethanol, they release the energy poor (stable) molecules CO_2 and H_2O.

No matter how cells (and organisms) capture energy, to maintain themselves and to grow, they must make a wide array of various complex molecules. Understanding how these molecules are synthesized lies within the purview of biochemistry. That said, in each case, thermodynamically unstable molecules (like lipids, proteins, and nucleic acids) are built through series of coupled reactions that rely on energy capture from light or the break down of food molecules.

Questions to answer & to ponder

- In a phototroph, why does the H+ gradient across the membrane dissipate when the light goes off? What happens to the rate of ATP production?
- What would limit the "size" of the H+ gradient that bacteriorhodopsin could produce?
- What would happen if bacteriorhodopsin molecules were oriented randomly within the membrane?
- What is photoisomerization? Is this a reversible or an irreversible reaction?
- How (do you suppose) does an electron move through an electron transport chain? Make a graph that describes its energy as it moves through the chain.
- In non-cyclic photosynthesis, where do electrons end up?
- What would happen to a cell's ability to make ATP if it where exposed to an H+ carrier or channel?
- Why are oxidation and reduction always coupled?
- Why are carbohydrates good for storing energy?
- If "photosynthesis is glycolysis run backward", why does glycolysis not emit light?
- Which do you think would have an evolutionary advantage, an organism growing aerobically or anaerobically? How do environmental conditions influence your answer?

Using the energy stored in membrane gradients



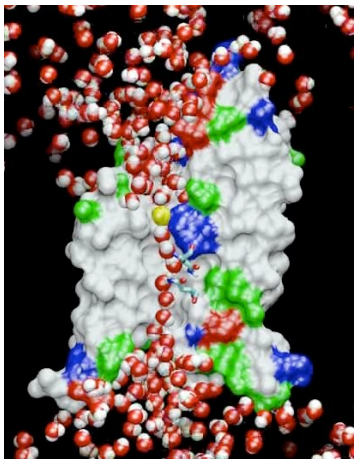
The energy captured by organisms (and their cells), is used to drive a number of processes in addition to synthesis reactions. For example, we have already seen that ATP synthases can act as pumps (ATP-driven transporters), coupling the favorable ATP hydrolysis reaction to the movement of molecules against their concentration gradients.The resulting gradient is a form of stored (potential) energy. This energy can be used to move other molecules, that is molecules that are not moved directly by a ATP-driven transporter. This involves what is known as coupled transport.[157] It uses membrane-bound proteins that allow a molecule to move down its concentration gradient. In contrast to simple carriers and channels, however, this thermodynamically favorable movement is physically coupled to the movement of a second molecule across the membrane and *against* its concentration gradient. When the two transported molecules move in the same direction, the transporter is known as a **symporter**,

[157] Structural features of the uniporter/symporter/antiporter superfamily: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2143070/

when they move in opposite directions, it is known as an **antiporter**. Which direction(s) the molecules move will be determined by the relative sizes of the concentration gradients of the two types of molecules moved. There is no inherent directionality associated with the transporter itself - the net movement of molecules reflects the relative concentration gradients of the molecules that the transporter can productively bind. What is important here is that energy stored in the concentration gradient of one molecule can be used to drive the movement of a second type of molecule against its concentration gradient. In mammalian systems, it is common to have Na^+, K^+, and Ca^{2+} gradients across the plasma membrane, and these are used to transport molecules into and out of cells. Of course, the presence of these gradients implies that there are ion-specific pumps that couple an energetically favorable reaction, typically ATP hydrolysis, to ion movement. Without these pumps (and the chemical reactions that drive them), the membrane battery would run down quite fast. Many of the immediate effects of death are due to the loss of membrane gradients and much of the energy needs of cells (and organisms) involves running such pumps.

Osmosis and living with and without a cell wall

Cells are packed full of molecules. These molecules take up space, space no longer occupied by water. The concentration of water outside of the cell $[H_2O]_{out}$ will necessarily be higher than the concentration of water inside the cell $[H_2O]_{in}$. This concentration gradient in solvent leads to the net movement of water into the cells[158]. Such a movement of solvent is known generically as osmosis. Much of this movement occurs through the membrane, which is somewhat permeable to water (see above). A surprising finding, which won Peter Agre a share of the 2003 Noble prize in chemistry. was that the membrane also contains water channels, known as aquaporins.[159] [This links to a molecular simulation of a water molecule (yellow) moving through an aquaporin →] It turns out that the rate of osmotic movement of water is dramatically reduced in the absence of aquaporins - they are important for cellular function. In addition to water, aquaporins can also facilitate the movement of other small uncharged molecules across a membrane.



http://www.ks.uiuc.edu/Research/aquaporins/waterpermeation.mpg

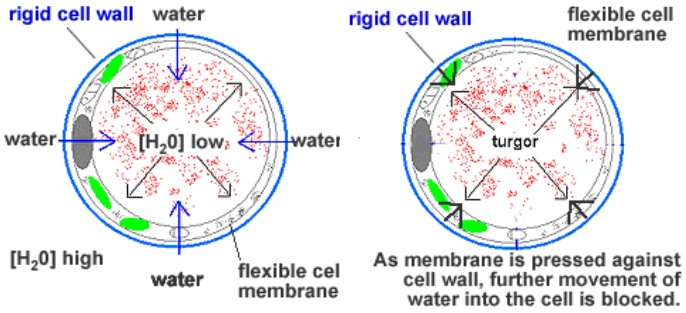
The difference or gradient in the concentrations of water (together with the presence of aquaporins) leads to a system that is capable of doing work, it can lift a fraction of the solution against the force of gravity. How is this possible? If we think of a particular molecule in solution, it will be moved around through collisions with its neighbors. These collisions drive the movement of particles randomly. But if there is a higher concentration of molecules on one side of a membrane compared to the other, then the random movement of molecules will lead to a net flux of molecules from the area of high concentration to that of low concentration, even though each molecule on its own moves randomly, that

[158] One important note here is that if you learn about osmosis in chemistry classes you will almost certainly be taught that water moves from a region of low SOLUTE concentration to a region of high SOLUTE concentration. These two definitions mean the same this but it is easy to get confused.

[159] Water Homeostasis: Evolutionary Medicine: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3540612/

is, without a preferred direction [this video [160] is good at illustrating this behavior]. At equilibrium, the force generated by the net flux of water moving down its concentration gradient is balanced by forces acting in the other direction.

The water concentration gradient across the plasma membrane of most organisms leads to an influx of water into the cell. As water enters, the plasma membrane expands (you might want to think about how that occurs, in terms of membrane structure). If the influx of water continued unopposed, the membrane would eventually burst like an over-inflated balloon, killing the cell. One strategy to avoid this lethal outcome, adopted by a range of organisms, is to build a semi-rigid "cell wall" exterior to the plasma membrane. The synthesis of this cell wall is based on the controlled assembly of macromolecules secreted by the cell through the process of exocytosis (see above). As water passes through the plasma membrane and into the cell (driven by osmosis), the plasma membrane is pressed up against the cell wall. The force exerted by the rigid cell wall on the membrane balances the force of water entering the cell. When the two forces are equal, the net influx of water into the cell stops. Conversely, if the $[H_2O]_{outside}$ decreases, this pressure is reduced, the membrane moves away from the cell wall and (because they are only semi-rigid) the walls flex. It is this behavior that causes plants to wilt when they do not get enough water. These are passive behaviors, based on the structure of the cell wall. They are essentially built into the wall as it is first assembled. Once the cell wall has been built, a cell with a cell wall does not need to expend energy to resist osmotic effects. Plants, fungi, bacteria and archaea all have cell walls. A number of antibiotics work by disrupting the assembly of bacterial cell walls. This leaves the bacteria osmotically sensitive, water enters these cells until they burst and die.



Questions to answer & to ponder:

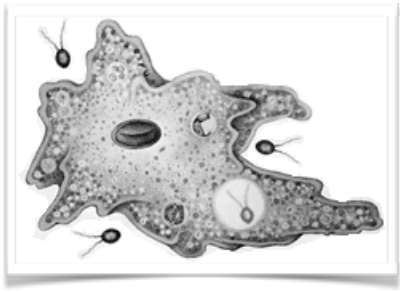
- Using the U-tube applet (in beSocratic), how would you get water to move from the right side to the left side of the membrane? How could such a system be used to purify water? (not currently active)
- Where does the energy involved in moving molecules come from?
- Plants and animals are both eukaryotes; how would you decide whether the common ancestor of the eukaryotes had a cell wall
- Why does an aquaporin channel not allow a Na+ ion to pass through it?
- If there is no net flux of A, even if there is a concentration gradient between two points, what can we conclude?

An evolutionary scenario for the origin of eukaryotic cells

When we think about how life arose, and what the first organisms looked like, we are moving into an area where data is fragmentary and speculation is often rampant. These are also, dare we remind you, events that took place billions of years ago. But these obstacles do not mean we cannot draw interesting conclusions – there is relevant data present in each organisms' genetic data (its

[160] http://youtu.be/ePGqRaQiBfc

genotype) and the structure of its cells and their ecological interactions that can be used as a basis for our speculations.



Animal cells do not have a rigid cell wall. This allows them to be active predators, moving rapidly and engulfing their prey whole or in macroscopic bits through phagocytosis (see above). They use complex "cytoskeletal" and "cytomuscular" systems to drive these thermodynamically unfavorable behaviors (again, largely beyond our scope here). Organisms with a rigid cell wall can't do that. Given that bacteria and archaea have cell walls, it is possible that cell walls were present in the common ancestral organism. But this leads us to think more analytically about the nature of the earliest organisms and the path to the common ancestor. A cell wall is a complex structure that would have had to be built through evolutionary processes before it would be useful. If we assume that the original organisms arose in an osmotically friendly (that is, non-challenging environment), then a cell wall could have been generated in steps, and once adequate it could enable the organisms that possessed it to invade new, more osmotically challenging (dilute) environments - like most environments today.

For example, one plausible scenario is that the ancestors of the bacteria and archaea developed cell walls originally as a form of protection against predation. So who were the predators. Where they the progenitors of the eukaryotes? If so, we would come to assume that the organisms in the eukaryotic lineage never had a cell wall, rather than that they once shared a cell wall with bacteria and archaea. In this scenario, the development of eukaryotic cell walls by fungi and plants represents an example of convergent evolution and these structures are analogous (rather than homologous) to the cell walls of prokaryotes.

But now a new complexity arises, there are plenty of eukaryotic organisms, including microbes like the amoeba, that live in osmotically challenging environments. How do they deal with the movement of water into their cells? They actively pump the water that flows in back out again using an organelle known as the contractile vacuole. Water accumulates within the contractile vacuole, a membrane-bounded structure within the cell, which inflates. To expel the water, the vacuole connects with the plasma membrane and is squeezed by cytomuscular systems within the cytoplasm. This squirts the water out of the cell. The process of vacuole contraction is an active one, it involves work and requires energy. One might speculate that this cytomuscular system was originally involved in predation, that is, enabling the cell to surround and engulf other organisms (phagocytosis). The resulting vacuole became specialized to aid in killing and digesting the engulfed prey. When digestion is complete, it can fuse with the plasma membrane to discharge the waste, using either a passive or an active "contractile system". It turns out that the molecular systems involved in driving active membrane movement are related to the systems involved in dividing the eukaryotic cell into two; distinctly different systems are used in the division of prokaryotes.[161] So a question is which came first, different cell division mechanisms, which led to differences in the membrane behavior of cells, one leading to a predatory active membrane and the other that led to a passive membrane, perhaps favoring the formation of a cell wall?

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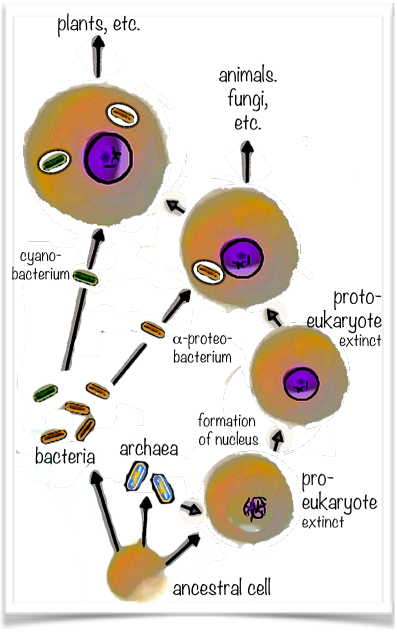
[161] The cell cycle of archael: http://www.ncbi.nlm.nih.gov/pubmed/23893102 and Bacterial cell division: http://www.ncbi.nlm.nih.gov/pubmed/17098054

Making a complete eukaryote

Up to this point we have only touched on a few of the ways that prokaryotes (bacteria and archaea) differ from eukaryote. The majors ones are the fact that eukaryotes have their genetic material isolated from the cytoplasm by a complex double-layered membrane/pore system known as the nuclear envelope (which we will discuss in some detail later on) and the location of chemo-osmotic and photosynthetic systems between the two types of organisms. In prokaryotes, these systems (light absorbing systems, electron transport chains and ATP synthases) are found either within the plasma membrane or within internal membranes clearly derived from the plasma membrane. In contrast, in eukaryotes (plants, animals, fungi, protozoa, and other forms) these structural components are not located on the plasma membrane, but rather within discrete intracellular structures. In the case of the system associated with aerobic respiration, these systems are located in the inner membranes of a double-membrane bound cytoplasmic organelles known as **mitochondria**. Photosynthetic eukaryotes (algae and plants) have a second type of cytoplasmic organelle (in addition to mitochondria), known as **chloroplasts**. Like mitochondria, chloroplasts are also characterized by the presence of a double membrane and an electron transport chain associated with the inner membrane and membranes apparently derived from it. These are just the type of structures one might expect to see if a bacterial cell were engulfed by the ancestral pro-eukaryotic cell (→), with the host cell's membrane surrounding the engulfed cells plasma membrane. A closer molecular analysis reveals that the mitochondrial and chloroplast electron transport systems as well as the ATP synthase proteins more closely resemble those found in one type of bacteria, rather than archaea. In fact, detailed analysis of the genes and proteins involved suggest that the electron transport/ATP synthesis systems of eukaryotic mitochondria are homologous to those of α-proteobacteria while the light harvesting/reaction center complexes, electron transport chains and ATP synthesis proteins of photosynthetic eukaryotes (algae and plants) appear to be homologous to those of a second type of bacteria, the photosynthetic cyanobacteria.[162] In contrast, many of the nuclear systems appear more similar to systems found in archaea. How do we make sense of these observations?



Clearly when a eukaryotic cell divides it must also have replicated its mitochondria and chloroplasts, otherwise they would eventually be lost. In 1883, Andreas Schimper (1856-1901) noticed that chloroplasts divided independently of their host cells. Building on Schimper's observation, Konstantin Merezhkovsky (1855-1921) proposed that chloroplasts were originally independent organisms and that plant cells were chimeras, really two independent organisms living together. In a similar vein, in 1925 Ivan Wallin (1883-1969) proposed that the mitochondria of eukaryotic cells were derived from bacteria. This "endosymbiotic hypothesis" for the origins of eukaryotic mitochondria and chloroplasts fell out of favor, in large part because the molecular methods needed to unambiguously

[162] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC138944/

resolve there implications were not available. A breakthrough came with the work of Lynn Margulis (1938-2011) and was further bolstered when it was found that both the mitochondrial and chloroplast protein synthesis machineries were sensitive to drugs that inhibited bacterial but not eukaryotic protein synthesis and by the discovery that mitochondria and chloroplasts contained DNA molecules that were organized like, and contained genes similar to genes found in bacteria (we will consider DNA and its organization soon).

All eukaryotes appear to have mitochondria. Suggestions that some eukaryotes, such as the human anaerobic parasites *Giardia intestinalis*, *Trichomonas vaginalis* and *Entamoeba histolytica* [163] do not failed to recognize cytoplasmic organelles known as mitosomes as degenerate mitochondria. Based on these and other data it is now likely that all eukaryotes are derived from an ancestor that engulfed an aerobic α-proteobacteria-like bacterium. Instead of being killed and digested, these (or even one) of these bacteria survived within the eukaryotic cell, replicated, and were distributed into the progeny cell when the parent cell divided. This process resulted in the engulfed bacterium becoming an endosymbiont, which over time became mitochondria. At the same time the engulfing cell became dependent upon the presence of the endosymbiont to initially detoxify molecular oxygen, and then to utilize molecular oxygen to break down molecules, and so maximize the energy that could be derived from their metabolism. All eukaryotes (including us) are descended from a mitochondria-containing eukaryote. This event is thought to have occurred around 2 billion years ago. The next step in eukaryotic evolution involved a second endosymbiotic event in which a cyanobacteria-like bacterium formed an endosymbiotic relationship with a mitochondria-containing eukaryote. This lineage gave rise to the glaucophytes, the red and the green algae. The green algae, in turn, gave rise to the plants.

As we look through modern organisms there are a number of examples of similar events, that is, one organism becoming inextricably linked to another through endosymbiotic processes. There are also examples of close couplings between organisms that are more akin to parasitism rather then mutually beneficial symbiosis.[164] For example, a number of insects have intracellular bacterial parasites, and some pathogens and parasites live inside human cells.[165] In some cases, even these parasites can have parasites. Consider the mealybug *Planococcus citri*; this organism contains cells known as bacteriocytes. Within these cells are *Tremblaya princeps* type β-proteobacteria. Surprisingly, within these bacterial cells, which lie within the eukaryotic mealybug cells, live *Moranella endobia*-type γ-proteobacteria.[166] In another example, after the initial endosymbiotic event that formed the proto-algal cell, the ancestor of red and green algae and the plants, there have been endocytic events in which a eukaryotic cell has engulfed and formed an endosymbiosis with a eukaryotic green algal cell, to form a "secondary" endosymbiont. Similarly, secondary endosymbionts have been engulfed by yet another

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[163] The mitosome, a novel organelle related to mitochondria in the amitochondrial parasite Entamoeba histolytica: http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2958.1999.01414.x/full

[164] Mechanisms of cellular invasion by intracellular parasites: http://www.ncbi.nlm.nih.gov/pubmed/24221133

[165] Intracellular protozoan parasites of humans: the role of molecular chaperones in development and pathogenesis. http://www.ncbi.nlm.nih.gov/pubmed/20955165

[166] Mealybugs nested endosymbiosis: going into the 'matryoshka' system in Planococcus citri in depth. http://www.ncbi.nlm.nih.gov/pubmed/23548081

eukaryote, to form a tertiary endosymbiont.[167] The conclusion is that there are combinations of cells that can survive better, in a particular ecological niche, than either could alone. In these phenomena we see the power of evolutionary processes to populate extremely obscure ecological niches in rather surprising ways.

Questions to answer & to ponder:

- Are the mitochondria of plants and animals homologous or analogous?
- Did the earliest eukaryote have a cell wall? why or why not? Where did this organism live?
- What advantage would the host cell get from the early proto-mitochondrial or proto-chloroplastic symbionts?
- Was there an advantage for the engulfed bacteria? If so, what could it be?
- Define the difference between a symbiotic and a parasitic relationship?
- Why does the number of membranes around an eukaryotic organelle matter? Where do these membranes come from?
- What evidence would lead you to suggest that there were multiple symbiotic events that gave rise to the mitochondria of different eukaryotes?
- Why might a plant cell not notice the loss of its mitochondria?

[167] Photosynthetic eukaryotes unite: endosymbiosis connects the dots: http://dblab.rutgers.edu/home/downloads/Files/Bhattacharya%20et%20al%20BioEssays%202004.pdf