Anatomy of a Cell :

A Very Brief Overview

Since this entire course is devoted to understanding the workings of the cell, it is almost superfluous to dedicate a chapter to identifying the parts of the cell and their functions. However, because it is easy to get lost in the intricacies of the molecules and chemical reactions within the cell, consider this chapter more of a framework or map for the course, giving context to the minutiae.

The cell is the smallest unit of life, so all cells, whether they are unicellular organisms or just a tiny part of a multicellular organism, have certain characteristics in common: they must contain genetic information and the mechanisms to regulate and use that information to produce its own parts and to reproduce new cells, they must be able to use energy in chemical reactions and physical actions, they must be able to regulate those activities, and they must respond to stimuli.

Cells use DNA (deoxyribonucleic acid) for their genetic material, and all cells contain the transcriptional and translational enzymes to read it and use the information to construct more cell components. However, simply having genetic material does not define life: viruses have genetic material containing all the information necessary to make a complete virus, but it does not contain the enzymes necessary to do so, nor the ability to obtain the raw molecular material needed to do so. It is absolutely dependent on the machinery inside whatever cell it infects. Therefore, a virus is not a living organism.

The genome is not only an instruction set for making a cell (or an organism, for that matter), it is also replicable itself. Roughly speaking, during part of its life cycle, the cell makes an extra copy of its genome and increases the numbers of all the other "stuff" (proteins, fats, etc.) of which it is made, and then it reproduces by division: the mother cell splits into two daughter cells, each with the same complement of genetic information, and with approximately the same cellular components. Thus we see that while the genome is often considered the blueprint for a cell/organism, in fact cells are not built up from scratch directly from DNA. Every cell comes from another cell. The

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This chapter, as a very simple introductory chapter, has no advanced material, and anything inthe right column should be considered basic information.

DNA can then be used to customize that cell for specific purposes as determined by its environment. When a particular component of the cell is needed, the information for making that component is read from the DNA and copied into RNA which is used as a program from which ribosomes can manufacture the proteins needed. A living cell needs all these things: the genetic information, the mechanisms and machinery to use the information to build cell parts, and the ability to harness energy to do so.

As we will see in chapter 3, the physical laws of nature require that everything tends towards its simplest, least organized, state unless there is an input of energy to work against that tendency. Since cells are a highly ordered collection of very complex molecules, they must therefore require energy to remain as cells. Thus, life requires the ability to obtain energy, either from sunlight or food, and the ability to convert that energy into forms that can be readily used by the cell to maintain itself by building or rearranging necessary molecules and macromolecular structures.

How do cells know when to carry out these activities? This leads us to the next characteristic of living cells, the ability to respond to stimuli. In other words, they are self-regulating. If glucose levels run low and the cell needs energy, glucose transport proteins are made, or if the cell needs to move to an area of higher food concentration, the cell cytoskeleton rearranges to move the cell. The cell has the ability to initiate repair processes if it detects lesions in its genome, it can pause the cell cycle to allow such repair processes time, and it can even initiate its own death if repairs are repeatedly unsuccessful.

In addition to responding to internal signals, living cells are also able to respond to external stimuli. Whether it is contact with a neighboring cell, binding a hormone released from a cell far away, or simply interacting with non-cellular environmental objects, a cell is able to respond to such stimuli. Responses may include making new proteins, destruction of existing proteins, moving away from the stimulus, moving towards the stimulus, initiation of reproduction, and many other possibilities.

There are two basic types of cells: prokaryotes and eukaryotes. The difference is simple and readily recognizable under light microscopy. Eukaryotic cells contain intracellular membrane-bound compartments (called organelles). Prokaryotic cells do not contain any such compartments (fig. 1). There is only one membrane in prokaryotes, the cell membrane, and only one compartment in prokaryotic cells, the cytoplasm. That does not preclude a certain level of organization in prokaryotes, but it is not as complex as eukaryotes. The genomic DNA is usually organized in a central nucleoid. There are not intracellular membranous organelles, but the cell is defined by a cell membrane. Outside of the cell membrane, prokaryotes have a cell wall. This wall is relatively rigid

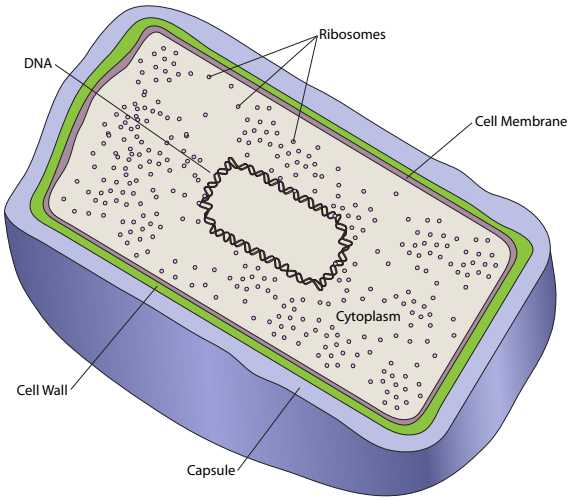

Figure 1. A Prokaryotic Cell.

and confers shape to the cell. Depending on the type of bacteria, the thickness of the wall varies (thick = "gram positive", thin = "gram negative"). Some, but not all bacteria also secrete another layer outside of the cell wall. This is a relatively tight matrix called a capsule that helps protect the cell from dessication in dry environments. A comparatively loose matrix of the same types of molecules may be secreted, and instead of the capsule, the result is called a slime layer. The slime layer is important in bacterial attachment and formation of biofilms (see chapter 13, Extracellular Matrix).

Eukaryotic cells are considerably more complex. Eukaryotic organisms are currently classified into four kingdoms: animal, plant, fungus, and protists. The animal cell in figure 2 depicts has many features in common with cells of the other three kingdoms.

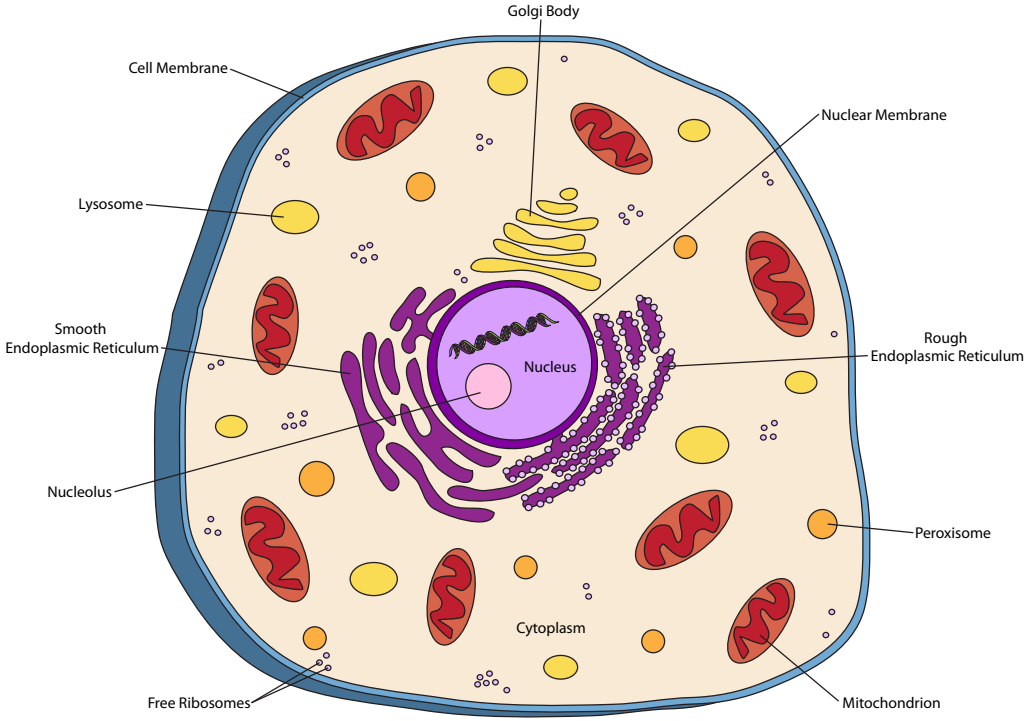

Figure 2. A prototypical animal cell.

Obviously, the biggest difference between the animal cell (or any eukaryotic cell) and prokaryotic cells is the presence of internal membrane-bound compartments, or organelles. The most prominent of these is the nucleus, which houses the DNA. Traditionally, it has been assumed that most eukaryotic genomes can range from 10 to 100

x 10^6 nucleotides (10-100 Mb) in total length, over two or more chromosomes (DNA molecules) of roughly similar size. In contrast, prokaryotic genomes have traditionally been viewed as a single circular chromosome, and mostly under a megabase (10^6 nucleotides) in length.

The nucleus is bounded by a double-layered membrane (most other organelles are bounded by a single membrane) that is continuous with the the endoplasmic reticulum (ER). The endoplasmic reticulum is subdivided into the rough ER (RER) and the smooth ER (SER) based on appearance in electron micrographs. The "studs" on the RER are ribosomes, which are the molecular machinery for making proteins in the cell. There are also free-floating ribosomes - the difference is that the free ribosomes make proteins that stay in the cytoplasm, while ribosomes attached to the RER are synthesizing proteins that are destined to insert into a membrane, localize inside an organelle, or be secreted out of the cell entirely. The RER makes modifications to the proteins as well as compartmentalizing them. The SER counts lipid synthesis (e.g. to make membranes) and detoxification reactions among its duties. It should be noted that ribosomes on the RER are not permanently attached, and after they have produced a protein, they dissociate from the RER and rejoin the general pool of free ribosomes in the cytoplasm.

The Golgi complex, or Golgi bodies, while physically independent, are a functional extension to the protein processing and sorting that occurs in the ER. Proteins leave the Golgi in vesicles bound for the cell membrane or other organelles. Vesicles, while membrane-bound, are not generally counted as organelles: they are simply small transport packages.

Mitochondria are complex organelles that are not only bounded by a membrane, but also contain a second membrane that is highly crenulated. Mitochondria make aerobic respiration possible, using oxygen as an oxidizer to produce chemical energy (i.e. ATP) far more efficiently than the anaerobic processes used by most prokaryotes. This ability to produce more energy from the same amount of food allows eukaryotic cells to grow larger than prokaryotes.

Lysosomes are acidic and contain digestive enzymes that break down large food molecules — particularly proteins and fats — to make them usable by the rest of the cell. These enzymes work optimally in acidic conditions, which acts as a sort of safety mechanism: if a lysosome breaks and releases its enzymes into the cytoplasm, they will not break down cellular components willy-nilly because the cytoplasmic pH is close to neutral and the enzymes do not work well. Once thought to be exclusive to animal cells, lysosomes have now been described in all cells from all eukaryotic kingdoms.

Recent and better methods for genome mapping and sequencing, and a broadening of the sample organisms has shown those numbers to be inaccurate. In fact, eukaryotic genomes range from ~3 Mb to over 4000 Mb. Prokaryotic genomes vary from 0.5 Mb to a little over 10 Mb (0.5 to 6 Mb for Archaea, 0.6 to 10 Mb for Bacteria) and may be spread over multiple DNA molecules that may be either linear or circular.

Peroxisomes also break down or convert molecules, but they generally act on smaller molecules by oxidation. For example, some peroxisomes in human liver cells are used to break down alcohol (ethanol). Processes like this often produce H_2O_2, hydrogen peroxide, as a byproduct. Since H_2O_2 in high concentrations is harmful, peroxisomes often contain an enzyme, catalase, that converts it into water and molecular oxygen.

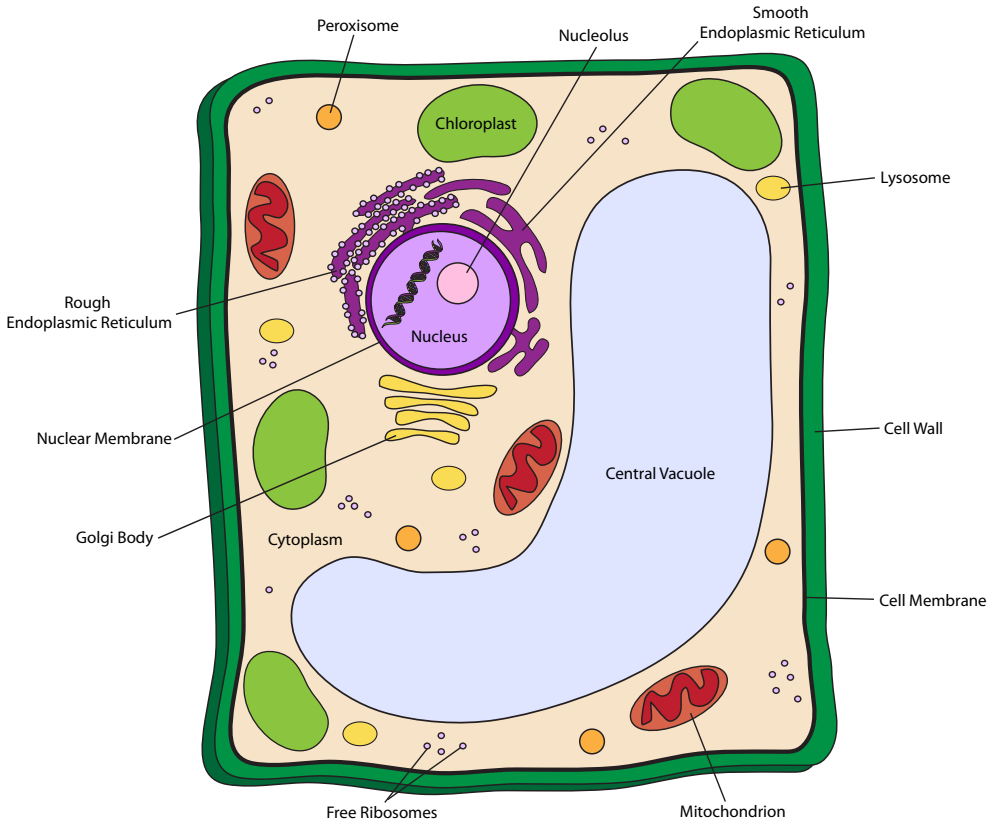

Figure 3. A typical plant cell.

Plant cells have all of the above named organelles, but additionally may also bear two other types of organelles: chloroplasts and vacuoles. In addition to this, plant cells also have a rigid cell wall external to the cell membrane. Chloroplasts are similar to mitochondria in shape and structure (membranes within the outer membrane). However, its function is very different: chloroplasts absorb light energy from the sun (or other light sources) and convert them into chemical energy in the form of simple sugars for the cell to store and use later, in essence turning the cell into a solar energy warehouse and distribution center. Since plants and some photosynthetic bacteria are the only

organisms capable of converting solar energy into a form useful to living cells, they are crucial to the survival of all other life.

Vacuoles are essentially storage units. They may store starches for use as energy sources when sunlight is unavailable or when immediate photosynthesis alone is not sufficient to provide for the energy needs of the cell. Other vacuoles, such as the one depicted above in fig. 3, store water, which helps the cell to maintain rigidity in combination with the cell wall.

Plant cell walls are composed of very different materials than the previously mentioned bacterial cell walls. Plant cell walls are primarily composed of the glucose polymer, cellulose, but contain other polysaccharides as well. Depending on the type of plant cell, there may be multiple layers of cellulose composing the cell wall. The wood and bark of trees, for example have both a primary (thin) cell wall and a secondary (thick) wall, while the leaves would have only a primary wall. Fungi also have cell walls, and they too are different from bacterial cell walls. True fungi have cell walls that are composed primarily of the polysaccharide chitin, and no cellulose.

Finally, consider the cytoplasm. Once considered merely the aqueous environment in which the "important" molecules or organelles floated, it is now better understood to be filled with important structural and transport elements (fig. 4). The cytoskeleton provides not only an internal physical structure but also a transport system to move molecules, vesicles, and even organelles to where they are needed.

All of the cell parts introduced in this chapter will be explained in much greater detail in subsequent chapters. More importantly, the intertwined relationships between many of the molecules and organelles will be discussed and elucidated. As you go through this course, you will notice that the same species come up over and over as examples. These are the model organisms upon which the great majority of molecular cell biology research is based. Most prokaryotic research has been based on *Escherichia coli* (*E. coli*), which is a Gram-negative rod-shaped bacterium commonly found in the gut of many higher animals. The Gram-negative soil bacteria, *Bacillus subtilis*, is a spore-forming organism that has also been used in research because, like *E. coli*, its genome is easily manipulated for experimentation, and is also relatively easy to grow in the lab. On the eukaryotic side, yeast (*Saccharomyces cerevisiae*, or *Schizosaccharomyces pombe*) are very commonly used for simpler intracellular processes due to simple genetics and very fast generation times. *Caenorhabditis elegans* (a nematode) and *Drosophila melanogaster* (fruit fly) are popular invertebrate model organisms, especially for developmental and genetic studies due to the small number of cells, mostly with traceable lineage, and fast generation time (for metazoans). Frogs, particularly the South African clawed

Interestingly, two groups once classified as fungi: oomycetes and dictyostelids, have cell walls composed of cellulose (and some have both). These organisms have been reclassified in Protista.

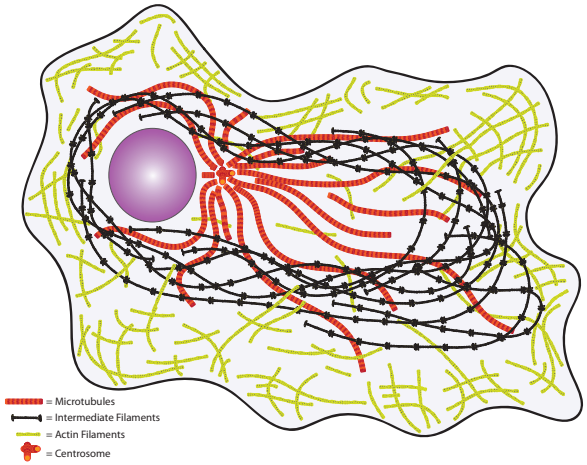

Figure 4. Animal cell cytoskeleton

frog, *Xenopus laevis*, and the Northern Leopard frog, *Rana pipiens*, are popular for certain types of developmental and cell cycle studies because they have huge oocytes that are amenable to many kinds of genetic and physiological manipulation not possible in other cells. *Arabidopsis thaliana* is the most commonly used model organism for the study of plant genetics. Finally, because they are mammals like us (humans), but breed quickly and can be genetically manipulated with relative ease, mice (*Mus musculus*) are very commonly used in the study of more complex intra- or inter-cellular mechanisms. More recently, the near complete sequencing of the genome and development of techniques to manipulate it, have made the rat (*Rattus norvegicus*) another viable research organism for the study of mammalian genes.

The commonalities that make all of these organisms excellent models for the study of the molecules of the cell and the interactions between them that constitute life, are a relatively short generation time, well-described (and in most cases fully sequenced) genome, and ease of experimental manipulation. Most of the molecules and mechanisms you will learn in the course were discovered in the simpler model mechanisms, and then found again, often with elaboration in the more complex ones.

Figure 5. Model Organisms. The Northern Leopard frog, *Rana pipiens* (left), the mustard-family plant, *Arabidopsis thaliana* (center), and the common rat, *Rattus norvegicus* (right). All images released to the public domain by the United States Goverment (USGS, NPS, NIH).

Further information on model organisms can be found at the United States' National Institutes of Health web site:

http://www.nih.gov/science/models/

Basic Cell Chemistry :

A Review of Chemical Compounds and their Interactions

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In this chapter, there are few "advanced" concepts, and the right side text should be considered clarification and review.

Water

There is no life without water. In this chapter, water will be used to review some very basic ideas in chemistry, particularly as applies to cell and molecular biology. What is water? H_2O. Two hydrogen atoms and one oxygen atom (Fig. 1). Together they form a molecule of water. They are defined as a molecule by the presence of strong chemical bonds connecting each atom. In this case, each atom is connected to another by a *covalent* bond. These are the strongest type of chemical bonds, and form when two atoms are *sharing electrons* in order to fill their outermost (valence) electron shell and increase stability. In the case shown here, hydrogen (H) has only one electron, and for maximal stability of that electron shell, it should have two. Oxygen, on the other hand, has six electrons in its outer shell, and a filled shell would have eight. Thus, it would "like" to pull in two more electrons for maximal stability. As shown in Fig. 1B,

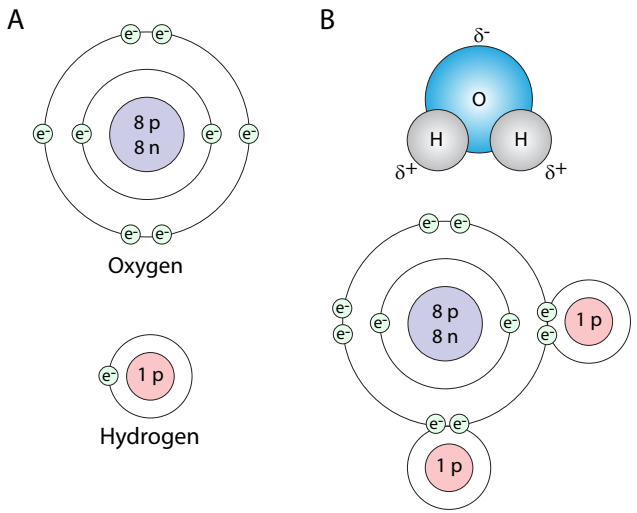
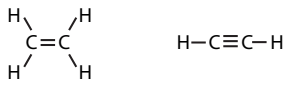


Figure 1. (A) Oxygen and hydrogen (B) Water.

If any of the first five pages is not a review, then your high school chemistry class failed to prepare you properly for college. Go take an intro chemistry class before proceeding with this course. Then go complain to your high school administrators and tell them to stop dumbing down courses and teaching to the lowest common denominator.

The volume of an atom is defined by electrons in a very fast and energetic obit around a nucleus. The electrons are very small negatively charged particles, and the nucleus is composed of neutrons (electrically neutral) and protons (positively charged), both relatively massive in comparison to electrons. The electrons' orbits around the nucleus can be approximated by "shells" or levels. These shells characteristically have limitations on the number of electrons that can fit within them: the first shell (closest to nucleus) holds only 2 electrons, while the second shell holds 8, and the third shell holds 18. The atom is most stable when its outer shell (and by extension, all inner ones also) is filled. The energy of the electrons also varies by level - innermost electrons have the least energy while the outermost electrons have the most.

both of those requirements are fulfilled when each of the hydrogen atoms shares an electron with the oxygen, which also shares an electron each with the hydrogen. The water molecule can also be written as H—O—H, in which the single solid line indicates a *pair of shared electrons,* i.e. a single covalent bond. The energy of an average single covalent bond is about 80 kcal/mol. However, as shown at left, double and even triple covalent bonds are possible. The strength of those types of bonds is slightly less than double (~150 kcal/mol) or triple (~200 kcal/mol) the energy of the single bonds.



Sharing electrons is not the only way to create bonds between atoms. *Ionic bonds* are created when an atom donates or receives an electron, rather than sharing one. When an atom gives up an electron, the electrical balance between the numbers of positively charged protons in its nucleus and negatively charged electrons is upset, and the overall atom now has a positive electrical charge. Similarly when an atom receives an extra electron, the balance in a neutral atom is upset, and the atom becomes negatively charged. An ionic bond is formed when one atom donates an electron to an adjacent atom, creating an ionic pair, one positively and one negatively charged. The electrical attraction between the oppositely charged atoms holds them together. Ionic bonds are weaker than covalent bonds, with an average bond energy of ~5.5 kcal/mol. Both covalent and ionic bonds are thermodynamically stable in dry, room temperature conditions (25°C, 298 K, 77°F). The average energy imparted when molecules collide at this temperature is only ~0.6 kcal/mol, far less than the energy needed to break a covalent or ionic bond.

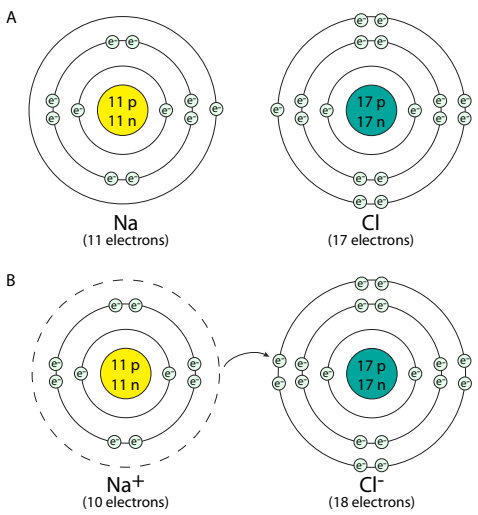


Figure 2. (A) Individually, the Na atom and the Cl atom are electrically neutral. However, they are both very reactive chemically because both need only get rid of (Na) or take in (Cl) one electron to have a full outer shell. (B) Because an electron is completely transferred, the Na becomes Na^+ and Cl becomes Cl^-, reflecting the new charge imbalance. Although electrically no longer neutral, the thermodynamic enhancement from filling the outer shells makes both of these ions very stable.

Bond energy is a measure of the strength of the bond between two covalently joined atoms, and is proportional to the bond distance, which is determined by the atomic radii. It is not the same thing as bond dissociation energy, which is the energy released in a homolytic reaction (bond is split with electrons equally distributed) taking place at absolute zero, but they are similar in being measures of bond strength.

Although salts (such as NaCl) are ionic compounds, not all ionic compounds are salts. The chemical definition of a salt requires that the compound be formed by the substitution of a hydrogen ion (H^+) in the original compound. This usually occurs in neutralization reactions, such as the neutralization of hydrochloric acid, HCl (or H^+Cl^-) with sodium hydroxide ($Na^+(OH)^-$), which yields the salt NaCl, and water (HOH = H_2O).

Covalent and ionic bonds between atoms are the only way to make molecules, which are stable collections of chemically bonded atoms. However, other attractive interactions between atoms and molecules exist, but they are significantly weaker, and can be disrupted with relatively small changes in temperature or environmental conditions. These are van der Waal's forces. They are very short-range interactions, requiring close apposition of the two atoms. As mentioned, an individual hydrogen bond (a specific type of van der Waal's force described below) or other van der Waal's interaction can be easily disrupted, but these types of interactions generally occur *en masse*. In a sense, they are like molecular Velcro® - each individual little plastic hook and individual loop of nylon could barely hold two hairs together, but a suit of velcro can hold a person on a vertical wall (a la Late Night with David Letterman, 1984).

In the case of hydrogen bonds, these occur when there is permanent asymmetric electron sharing within a covalently bonded molecule so that the shared electrons spend more time around one nucleus (thus imparting a negative character), than the other (which is therefore somewhat positive in character) to create a permanent electrical dipole. These dipole moments can interact with oppositely charged moments on other molecules or the same molecule. Van der Waals forces also include induced (non-permanent) dipole-dipole interactions in which a temporary shift in electron density as they orbit the nucleus forms a minute charge differential, that can induce an opposite and attractive charge differential in a very close neighboring atom. In fact, some texts define van der Waals forces exclusively as such, leaving hydrogen bonds as a separate category altogether. One of the arguments for that idea is that the bond length of the average H-bond is smaller than the sum of the van der Waal's radii of the two atoms.

As noted above, hydrogen bonds result from severely uneven sharing of electrons that generate permanent dipoles. In biological systems, this generally means that a hydrogen is covalently bound to either an oxygen or a nitrogen atom, which are both highly electronegative atoms, strongly attracting the shared electrons away from the hydrogen. Common hydrogen-bonding pairs are OH ···· :O, OH ···· :N, NH ···· :N, and NH ···· :O. Dotted lines are a common method for depicting hydrogen bonds in printed text and diagrams.

Water is a molecule that has a permanent dipole (i.e. it is a polar molecule), with the highly electronegative oxygen nucleus taking the lion's share of the shared electrons' time, leaving the hydrogen nuclei stripped bare down to their protons. The geometry of the water molecule (Fig. 1B) makes one side of the molecule somewhat negative with two pairs of free electrons, and the opposite side positive, because the shared electrons are only rarely near the hydrogen nuclei. This gives water the ability to hydrogen bond, and is the basis for several of water's most important qualities. The ability to form

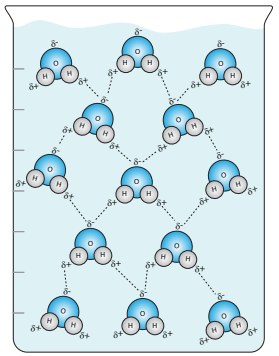


Figure 3. The hydrogen bonding of water molecules to one another is an important determinant of the physical properties of water.

many hydrogen bonds leads to a high specific heat of water, and enables it to act as a generous heat buffer. In order to get enough molecules of water moving faster and increase the temperature of the water, the energy put into the water must first be used to break apart the hydrogen bonds without generating heat. This is unlike most other liquids, which do not link internally with H-bonding. So the water is able to absorb more heat (energy) without a phase change than many other liquids.

Another important and unique characteristic of water is that the solid phase (ice) is less dense than the liquid phase. With most other liquids, as the temperature drops, the molecules have less energy, so they move less, and they stay closer together, increasing the density. Only part of that holds true with water. Again, the ability to form hydrogen bonds is directly related to this: as the temperature is lowered, the molecules move around less, affording them more opportunities to form hydrogen bonds. However, even though they are attractive, the H-bonds also act as spacers separating the water molecules more than if they were allowed to tumble about together in a liquid without forming H-bonds.

This aspect of water chemistry is actually more important to life in a geologic sense than at the cellular level. At the cellular level, the consequence is that freezing cells causes the water in them to expand and burst, killing them at low temperatures unless the cell has chemicals that act as antifreeze and lower the freezing temperature of the cytoplasm. On the other hand, at the geological level, when a pond or lake freezes in winter, the ice is less dense than water, thus staying on top of the pond, insulating deeper layers, and helping them stay liquid and able to support life (many organisms migrate deeper down in the winter). If water became more dense as it froze, as many other molecules, ice would sink, and eventually the entire pond would be completely solid, killing off most life in it once a year!

From a chemical standpoint, the polar nature of water makes it an excellent solvent for ionic and polar molecules. As you can see in the figure, the hydrogen side of water interacts with the negatively charged chloride ion, while the oxygen side of water interacts with the positively charged sodium ion, thus easily dissolving the salt. However, the polarity of water also makes it repel nonpolar molecules or by non-polar regions of molecules. This property, known as hydrophobicity, is crucial to life, since it is the basis for the formation of the biological membranes that define a cell. In general terms, the H-bonding between water molecules is very stable. Non-polar molecules cannot participate in H-bonding, and therefore create areas of instability wherever they are touching aqueous (water-based) solutions. The resolution to this problem is for hydrophobic molecules to aggregate, thus lowering the total surface area in contact with water. In living organisms, many protein and lipid molecules are amphipathic, with some portions hydrophobic, while other parts of the molecule are hydrophilic.

Acids and Bases

While it is easiest to think of water as H_2O, it is in fact in an equilibrium between the ionized molecules H^+ (which is simply a proton) and OH^- (the hydroxyl ion). The H^+ itself can be subsequently bound to a water molecule to form a hydronium ion, H_3O^+. The release of H^+ and OH^- are not limited to water molecules, and many compounds do so in aqueous solutions. These compounds can be classified as acids (raising the free H^+ concentration) or bases (increasing the free hydroxyl concentration]. The ex-

Water can dissociate from H_2O into the ions H^+ and OH^-, in which the departing hydrogen leaves its electron with the oxygen. However, H^+ is extremely reactive and almost immediately attaches to a nearby water molecule, forming the hydronium ion H_3O^+.

tent to which acids and bases donate or remove protons is measured on the pH scale, which is a logarithmic scale of relative H^+ concentration. Thus the Coca-Cola® that I am drinking, and which counts phosphoric, carbonic, and various other acids among its ingredients, has a pH around 3, which means that it liberates 10^4 times more H^+ than water, which has a pH of 7. Inside cells, the pH range is tightly restricted to slightly above neutral (neutral = pH 7), although in eukaryotes, various intracellular organelles (e.g. lysosomes) may have significantly different internal acidity/alkalinity. This is important biologically because changes in acidity or alkalinity can alter hydrogen and ionic bonds, thus potentially changing the shape and activity of enzymes and other biomolecules.

Sometimes, this can be used to an organism's advantage. For example, cells lining the stomach of an animal such as yourself secrete the enzyme pepsin into the stomach to help digest proteins. Pepsin has a pH optimum close to pH 2, which is great because stomach pH is also around 2. However, considering that cells themselves contain a lot of proteins, and we don't want pepsin-containing cells to digest themselves away, what is the solution? Because the pH inside the cell is close to 7.2, far above the pH optimum for pepsin, it is inactive inside the cell, and only works after it has been secreted into an acidic environment.

Carbon

The major constituent molecules in all living organisms are based on carbon. Carbon has versatility stemming from its four outer shell electrons allowing the possibility of four covalent bonds with a variety of partners, incuding very stable carbon-carbon covalent bonds. Because of this, long carbon chains can form the backbone of more complex molecules, and makes possible the great diversity of macromolecules found in the cell. The carbon chains themselves are not very reactive, but they often have reactive chemical groups attached to them.

Common groups are the hydroxyl (—OH), carbonyl (—CO), carboxyl (—COOH), and phosphate ($—PO_4$). Carbon chains may even have other carbon chains attached to them: the smaller ones behave and are named as groups also: methyl ($—CH_3$), ethyl, ($—C_2H_5$), propyl ($—C_3H_7$), and so forth. Figure 4B (right) depicts several functional groups that can be found in the simple molecule, acetic acid (very dilute acetic acid is the primary component of vinegar).

Carbon is also the basis for the four major classes of biological molecules: sugars, nucleotides, amino acids, and fatty acids. The first three are classes of molecules that can

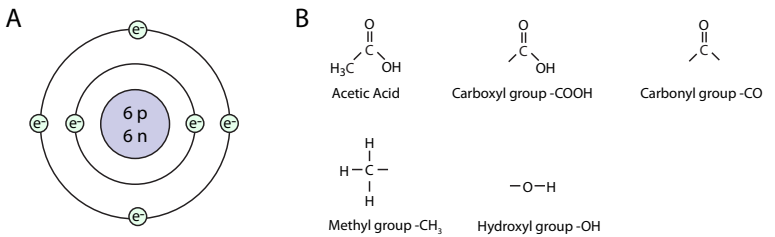


Figure 4. (A) The carbon atom has four electrons in its outer shell. (B) Functional groups that can be identified from a molecule of acetic acid.

be strung together by covalent bonds to make important large biomolecules: simple sugars can form large polysaccharides such as starch, cellulose, or glycogen, nucleotides can form RNA (ribonucleic acids) or DNA (deoxyribonucleic acids), and amino acids can form proteins. Fatty acids, on the other hand, are acid derivatives of long chains of carbons linked to one another, with hydrogens taking up most of the other bonding positions.

Sugars

Sugars, and glucose in particular, are important molecules for cells because they are the primary energy source. Sugars have the general chemical formula CH_2O, and can be joined together almost infinitely for storage. However, because they are hydrophilic, they allow water molecules to intercalate between them, and cannot pack as efficiently as fats, which are hydrophobic and thus exclude water. On the other hand, the sugars can be mobilized for use more quickly. Therefore, polysaccharides are usually short-term reservoirs of energy for an organism, while fats are used for longer-term storage.

The general chemical formula cannot fully define a particular sugar, because the same set of atoms, e.g. $C_6H_{12}O_6$ can refer to glucose, fructose, mannose, or galactose, and that doesn't even include the stereoisomers. Isomers are rearrangements of the same atoms, such as with glucose and fructose (fig. 5), while stereoisomers are much more similar: they are mirror-images of one another. Thus glucose can exist as L-glucose or D-glucose, depending on whether it is a "left-handed" or "right-handed isomer. This may seem like an esoteric distinction, but it becomes important in intermolecular interactions, because many are based on recognition of specific shapes, so an L-conformation molecule may not be recognized by an enzyme that recognizes its D- isomer.

Another important aspect of sugar chemistry is whether it is an aldose or a ketose, based on the type of carbonyl group it carries. This is easiest to understand looking at the position of the carbonyl group in the linear structure: put simply, an aldehyde is a terminal carbonyl group, while a ketone is an internal carbonyl group. Sugars in aqueous solution exist in an equilibrium between the linear form and the ring form, which is formed by intramolecular attack by a hydroxyl group on the carbonyl. Technically, the cyclic sugar is a pyranose (6-membered ring) or a furanose (5-membered ring), so that D-glucose cyclizes into D-glucopyranose. However, in most cell biology courses, the cyclic sugar will still be referred to as its non-cyclic alter ego. Note that due to the difference between the $C_6H_{12}O_6$ aldose glucose, and the $C_6H_{12}O_6$ ketose fructose, cyclization generates a pyranose in one case, and a furanose in the latter (fig. 5), although the

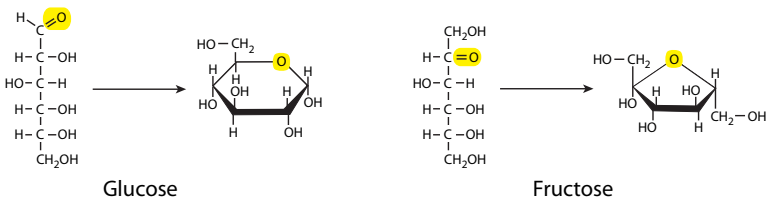


Figure 5. Glucose is an aldose (terminal carbonyl) that cyclizes into a pyranose, fructose is a ketose (internal carbonyl) that cyclizes into a furanose.

number of carbons (and other atoms) are the same. These two molecules are therefore recognized differently by the enzymes of the cell, leading to different metabolic pathways.

Simple sugars can be joined together by *condensation reactions* to form glycosidic bonds. These reactions are called condensation reactions because they form water as a by-product. The glycosidic bond is an –O– linkage between carbons of two sugars. The bond is usually named with the specific linkages: for example in cellulose, glucoses are linked by β(1,4) linkages, which means in a standard ring diagram, the upward-facing β-hydroxyl on the 1-carbon interacts with the –OH on the 4-carbon of a neighboring glucose (fig. 6B). [Technically, since only two glucoses are shown here, this is a molecule of cellobiose, not cellulose.] In contrast, the maltose shown in the same figure (fig. 6A), while also showing two glucoses linked together, is an α(1,4) linkage, with a downward-facing α-hydroxyl on the 1-carbon.

Large polysaccharides generally have one of two functions: as a very strong structural component of a cell, and as a storage molecule for readily accessible energy. The two major structural polysaccharides made by cells are *cellulose* and *chitin*. Cellulose is primarily synthesized by plants, while chitin is mostly synthesized by invertebrates (think crab shells), though it is also made by many fungi and algae. As we just saw, cellulose is an array of parallel lengths of glucose monomers joined together by β(1,4) glycosidic bonds (fig. 7). These long glucans are stacked closely on one another so that many H-bonds can form along their lengths, which are virtually limitless, determined by the needs of the organism. Interestingly, chitin is also a homopolymer linked by β(1,4) glycosidic bonds, but instead of glucose, the monosaccharide used is N-acetylglucosamine (often abbreviated GlcNAc, see chapter 11). However, the macromolecular structure is very similar to cellulose, and like cellulose, it is very strong.

As with structural polysaccharides, there are also two primary energy-storage polysaccharides: *starch*, which is synthesized by plants, and *glycogen*, which is synthesized by animals. Starch is actually a mixture of two slightly different polysaccharides. One is α-amylose, which is a glucose homopolymer like cellulose, but connected by α(1,4) glycosidic linkages, which makes it completely different structurally. Unlike the linear and highly stackable cellulose polysaccharides, α-amylose takes on a twisting α-helical shape. The other starch polysaccharide is amylopectin, which is like α-amylose with the addition of branches formed from α(1,6) glycosidic bonds every 24-30 residues (fig. 6C). The storage polysaccharide for animals, glycogen, is essentially amylopectin with a higher frequency of branching, approximately every 8-14 residues. Whereas the tight packing of the structural polysaccharides renders them waterproof, this is certainly not the case for starch or glycogen, both of which can interact with many water molecules

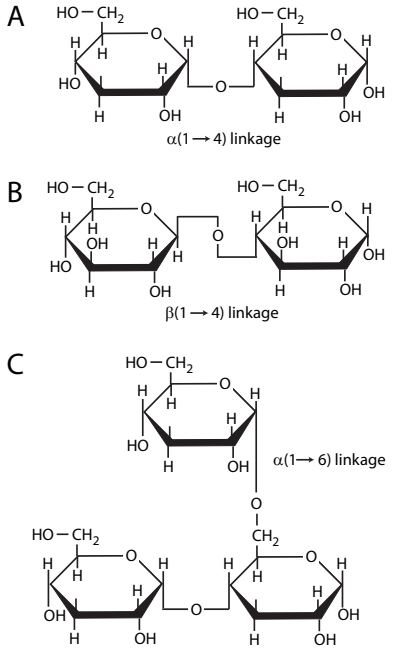


Figure 6. (A) the α(1,4) glycosidic bond of maltose, (B) the β(1,4) bond of cellobiose, and (C) the α(1,6) bond in branching glycogen.

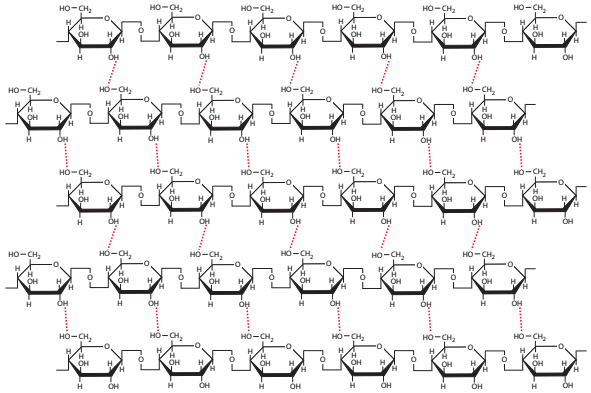


Figure 7. Cellulose is a very strong material due to the many hydrogen bonds (in red) possible when strands of β(1,4)-linked glucoses are aligned.

simultaneously, and swell up with the hydration, as any cook who has ever made a pudding (the thickening ingredient is starch from corn) can attest.

Nucleotides

Nucleotides, the building blocks of RNA and DNA, are themselves composed of a pentose sugar attached to a nitrogenous base on one side and a phosphate group on another. The sugar is either the 5-carbon sugar ribose or its close cousin, deoxyribose (the "deoxy" refers to a "missing" hydroxyl group on the 2-carbon, which has an H instead). The attached nitrogenous base can be a *purine*, which is a 6-member ring fused to a 5-member ring, or a *pyrimidine*, which is a single 6-membered ring. These bases are usually adenine (purine), guanine (purine), thymine (pyrimidine), and cytosine (pyrimidine) for DNA, with a substitution of uracil for thymine in RNA bases. However, there are also some unconventional and modified bases that show up in special situations, such as in tRNAs. In addition to being the monomer components of DNA and RNA, nucleotides have other important functions as well. The best known, adenosine triphosphate, or ATP, is the primary "instant" energy source for the cell by the energy released through hydrolysis of its terminal phosphate group.

DNA or RNA are built from nucleotides through linkages of the sugars, and the polymerization occurs by condensation reactions, but these bonds are not glycosidic bonds like with polysaccharides. Instead, bonds form between the 5' phosphate group of one nucleotide and the 3' hydroxyl group of another. These are phosphodiester bonds, and a quick glance at the structure (fig. 8) explains the naming: an ester bond is a carbon-oxygen linkage, and the phosphodiester bond is a C-O-P-O-C, so there are two esters with a phosphorus linking them. With the purine or pyrimidine base on the 1-carbon, this arrangement places the bases on the opposite side of the sugar from the polymerizing phosphodiester bonds. This forms a sugar-phosphate backbone to the DNA/RNA, which then has the bases projecting out from it.

The bases will then likely interact with the bases of other nucleotides, whether part of another nucleic acid strand or free-floating. Not only do they interact, but they interact with great specificity and consistency: adenines base-pair with thymines (or uracils) through two hydrogen bonds, while guanines interact with cytosine through three H-bonds. Note that while one extra hydrogen bond does not appear to be particularly significant, the attraction between G-C is 50% stronger than between A-T, and over long stretches of DNA, areas high in G-C content are significantly more difficult to unzip (separate strands) than areas high in A-T pairs. This specific base-pairing, known as *Chargaff's rules*, is the basis for life: base-pairing is needed to make DNA double-

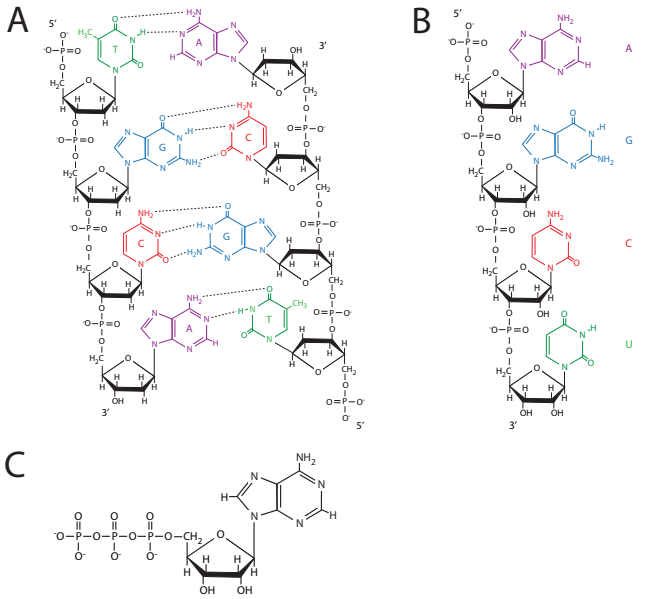


Figure 8. (A) DNA and (B) RNA differ by the presence of –OH on the 2-carbon of ribose but not deoxyribose and the use of uracil in RNA instead of thymine. Both are constructed from nucleotides like adenosine triphosphate (C).

stranded which gives an organism a built-in backup of genetic information and it is also the basis for transforming that information into proteins that form the bulk of a cell.

Nucleic acids, the long polymers of nucleotides, exist in either single or double stranded forms in vitro. However, in the cell, most RNA is single-stranded, and most DNA is double-stranded. This difference is important to their function: RNA is a temporary information transfer molecule for a particular gene, DNA is the permanent repository of all genetic information needed to make an organism. Therefore, RNA needs to be easily read, meaning that the bases need to be accessible, and not locked to a complementary strand. Its long-term stability is not particularly important because when it is made, usually many copies are made at the time, and it is only needed while the cell needs to make the protein it encodes. Conversely, the same strand of DNA is read over and over to make the RNA, and since there are only two copies of each chromosome (a chromosome is a single double-stranded DNA molecule) in a cell, the ability to maintain the integrity of the DNA is crucial. Because of base pairing, each strand of DNA contains all the information necessary to make a complete exact copy of its complementary strand.

Of course, the point of the genetic information in DNA is to encode the production of proteins that can then carry out the functions that define cellular life. Some of those functions, such as DNA replication, gene regulation, transcription, and translation, require the proteins to interact with a nucleic acid. Usually, part of the recognition process involves apposition of a positively charged region of the protein to the DNA (or RNA), which is a very negatively charged molecule, as expected from all the phosphates in the sugar-phosphate backbone. RNA, but not DNA (with some exceptions), can also interact with itself by complementary base-pairing. If a stretch of RNA sequence comes into contact with a stretch of RNA with a complementary sequence on the same molecule, then base-pairing can occur. Depending on the number of nucleotides between the complementary areas, secondary structures such as stem-and-loops and hairpins can form.

Amino Acids

Most of the major molecules of the cell - whether structural, like cellular equivalents of a building's girders and beams, or mechanical, like enzymes that take apart or put together other molecules, are proteins. Proteins interact with a wide variety of other molecules, though any given interaction is usually quite specific. The specificity is determined in part by electrical attraction between the molecules. So, what determines the charge of different regions of a protein?

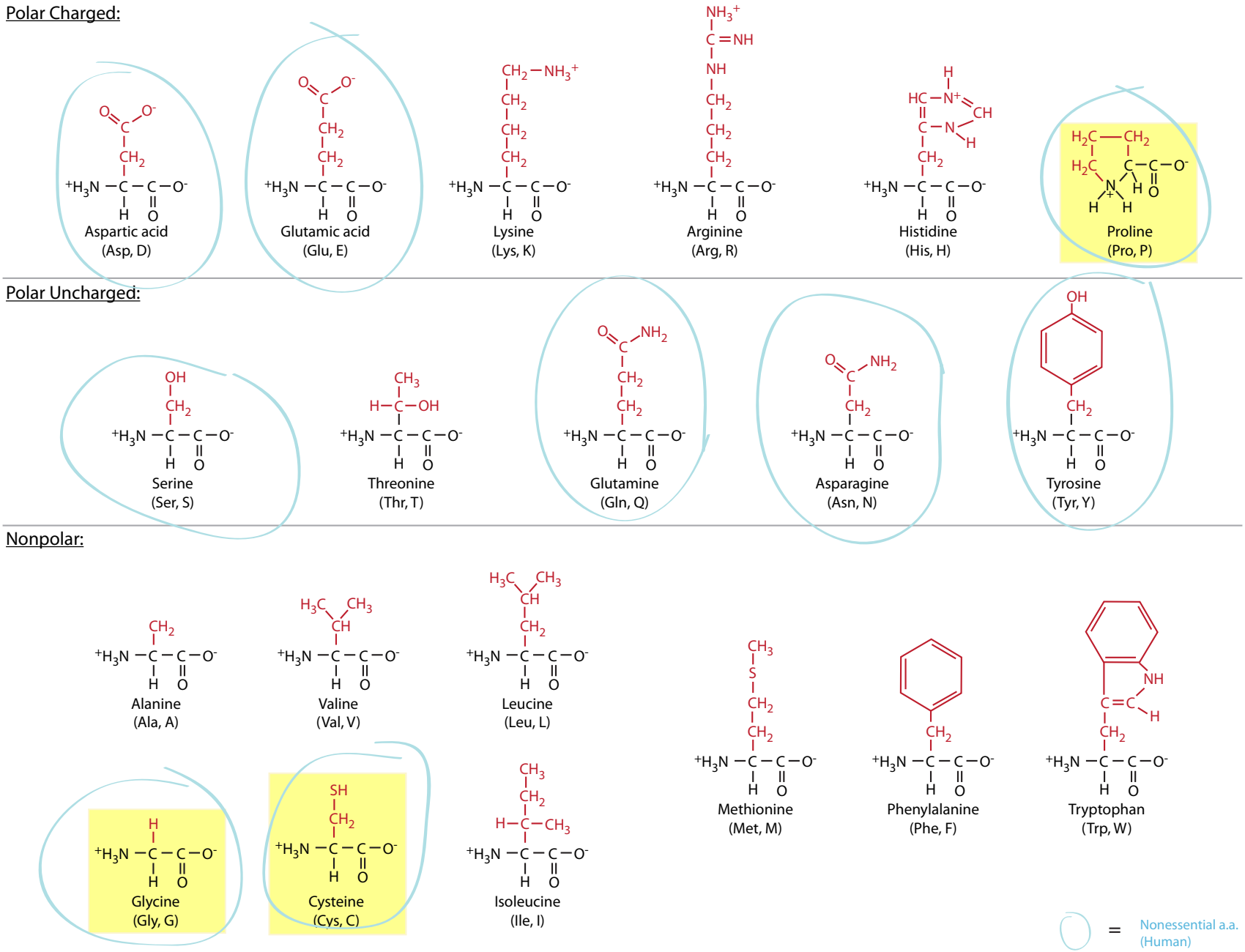
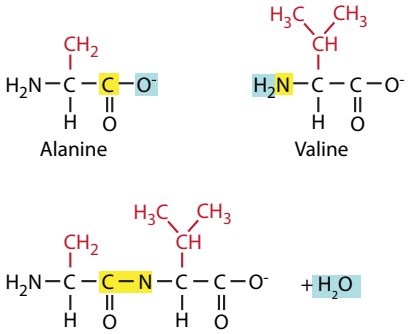


Figure 9. The Amino Acids. The backbone is shown in black, while the side chains are colored red. Amino acids circled in blue can be synthesized by humans, while the uncircled amino acids must be ingested. Amino acids with a yellow background have unique structural considerations: the extremely small side chain of glycine allows it to fit into tight spaces, the sulfhydryl group of cystein allows the formation of disulfide bonds, and the cyclic structure of proline introduces a forced bend in the polypeptide chain.

Amino acids (figure 9), which are joined together to make proteins, may be positively charged (basic), negatively charged (acidic), polar, or nonpolar, based on the characteristics of their side chains. The charge on the amino or carboxyl end of each amino acid does not play a role in the overall character of any particular region of the protein, because they are effectively neutral, having been linked, the amino group of one amino acid to the carboxyl group of another, by a peptide bond. Note the figure of the amino acid: it is one carbon, called the α carbon, linked to amino and carboxyl groups on opposite sides, and to hydrogen, and a side chain, denoted by R. These side chains, of which there are twenty common ones, can be as simple as a hydrogen atom (glycine), or could be quite complex, involving extended ring structures (histidine, phenylalanine). The variety in their size, shape, and charge all add up to an extremely versatile set of building blocks for some of the most important working molecules of the cell.
In the cell, a peptide bond is formed between two amino acids with enzymatic help from the ribosome. Like the previous two polymerizing reactions, formation of peptide bonds is a condensation reaction in which the carbon of the carboxyl group and the nitrogen from the amino group of their respective amino acids are bonded together (fig. 10). This is a very stable bond due to resonance of the amide group. In the cell, peptide bonds are mostly nonreactive, except when attacked by proteolytic enzymes.

Figure 10. Peptide Bond Formation. A condensation reaction between the carboxyl group of alanine and and the amino group of valine generates a peptide bond linking the two amino acids, with a molecule of water as a byproduct.



A peptide is an inexact term used for relatively few (usually <30) amino acids joined together. Each amino acid in a polypeptide or protein may also be referred to as a "residue" which can sometimes be confusing because the same term is also applied to monomers of nucleic acids and of polysaccharides. Larger polymers are known as polypeptides or as proteins, although polypeptide has more of a structural connotation and may be used to indicate an unfinished or not-yet-functional state, whereas protein generally implies some physiological function. On of the key characteristics of proteins is the ability to form secondary, tertiary, and for proteins, quaternary structure by means of specific folding patterns. If you think of a long piece of thread, yarn, or rope, you

Chirality

Almost all amino acids (glycine is the exception) are optically active, which means that they are asymmetric in such a way that it is impossible to superimpose the original molecule upon its mirror image. There is a "handed-ness" about them, much as your right hand cannot be superimposed on your left hand if both palms must face the same direction. In fact, in the figure here, you can also understand why glycine is an exception, since its R-group is a simple hydrogen atom.

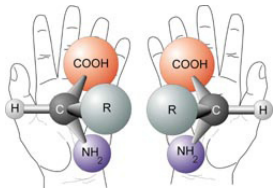


figure released to public domain by NASA

Chiral pairs, or enantiomers, not only have the same atomic components like all isomers, they also have the same bonds and bond order. The term "optically active" comes from the discovery that polarize light is rotated in different directions by enantiomers. Amino acids are often labeled as either D- (dextrorotatory) or L- (levorotatory) depending on their atomic configuration in relation to the enantiomers of glyceraldehyde. This is a common naming system, but not always logical, in that almost half of the L-amino acids are in fact dextrorotatory (clockwise rotation of light), but their molecular configurations resemble the levorotatory isomer of glyceraldehyde.

Ribosome-created proteins and peptides are all constructed with L-amino acids. However, D-amino acids do exist in nature, and can be incorporated into peptides through non-ribosomal means. An excellent example is found in the cell walls of some bacteria. Because most proteolytic enzymes only act on proteins with L-amino acids, the incorporation of D-amino acids into the cell wall can protect the bacteria from harm. These D-amino acids are incorporated by transpeptidase. Transpeptidase is also the target of the antibiotic, penicillin, which is an irreversible inhibitor of that enzyme.

can probably imagine an infinite number of different ways to arrange it, from spirals to loops to random tangles. This is essentially what can happen with a protein with the constraints put upon it by the size and charge of the amino acids that compose it.

The *primary structure* of a protein is simply the sequence of amino acids that compose the protein. These amino acids are joined by peptide bonds from the carboxyl terminal of one amino acid to the amino terminal of the next. Secondary structure refers to the localized, simple, shapes that can be formed, such as alpha-helices, or beta-sheets. These come about primarily through hydrogen bonding to nearby (relative to the primary structure) residues.

Tertiary structure is 3-dimensional structure that is built upon arrangements of second-

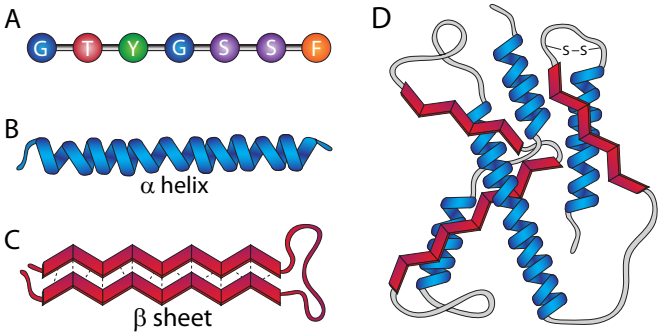


Figure 11. (A) Primary structure, (B) Secondary structure: an α-helical region, (C) Secondary structure: a β-pleated sheet region, (D) Tertiary structure.

ary structures, often through disulfide bonds and hydrophobic interactions in addition to hydrogen bonding. In the context of structural stability, cysteine plays a special role. Beyond the primary structure, most protein folding is held in place by hydrogen bonds. Although strong enough in most situations, they can be disrupted without extraordinary energy. Disulfide bonds (–S–S–) are covalent bonds that form between the sulfhydryl groups of two cysteines that effectively locks the local protein structure in place, making the protein extremely stable.

Finally, quaternary structure is the arrangement of different individual polypeptides (subunits) into a functional protein. Obviously, only multi-subunit proteins have a quaternary structure.

Not surprisingly, the proteins of thermophilic archaebacteria such as Thermophilus aquaticus (Taq) or Pyrococcus furiosus (Pfu) have a high proportion of cysteines and disulfide bonds, since they live in deep sea volcanic ocean vents under high pressure and temperatures.

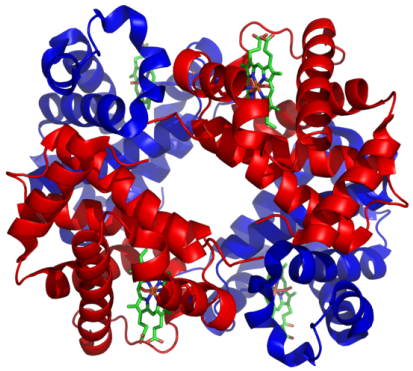


Figure 12. Quaternary structure is illustrated here by hemoglobin, which is composed of four independent polypeptide subunits that come together in a specific conformation to make a functional hemoglobin protein.

Fatty Acids

Unlike monosaccharides, nucleotides, and amino acids, fatty acids are not monomers that are linked together to form much larger molecules. Although fatty acids can be linked together, for example, into triacylglycerols or phospholipids, they are not linked directly to one another, and generally no more than three in a given molecule. The fatty acids themselves are long chains of carbon atoms topped off with a carboxyl group. The length of the chain can vary, although most are between 14 and 20 carbons, and in higher order plants and animals, fatty acids with 16 and 18 carbons are the major species. Due to the mechanism of synthesis, most fatty acids have an even number of carbons, although odd-numbered carbon chains can also be generated. More variety can be generated by double-bonds between the carbons. Fatty acid chains *with no double bonds are saturated*, because each carbon is saturated with as many bonded hydrogen atoms as possible. Fatty acid chains with double bonds are unsaturated (fig. 13). Those with more than one double bond are called polyunsaturated. The fatty acids in eukaryotic cells are nearly evenly divided between saturated and unsaturated types, and many of the latter may be polyunsaturated. In prokaryotes, polyunsaturation is rare, but other modifications such as branching and cyclization are more common than in eukaryotes. A table of common fatty acids is shown below.

Myristic Acid	14:0 (14 carbons, no double bonds)
Palmitic Acid	16:0
Stearic Acid	18:0
Arachidic Acid	20:0
Palmitoleic Acid	16:1
Oleic Acid	18:1
Linoleic Acid	18:2
Arachidonic Acid	2:4

There are significant physical differences between the saturated and unsaturated fatty acids due simply to the geometry of the double-bonded carbons. A saturated fatty acid is very flexible with free rotation around all of its C-C bonds. The usual linear diagrams and formulas depicting saturated fatty acids also serve to explain the ability of saturated fatty acids to pack tightly together, with very little intervening space. Unsaturated fatty acids, on the other hand are unable to pack as tightly because of the rotational constraint impoarted by the double bond. The carbons cannot rotate around the double bond, so there is now a "kink" in the chain. Generally, double-bonded carbons in fatty acids are in the cis- configuration, introducing a 30-degree bend in the structure.

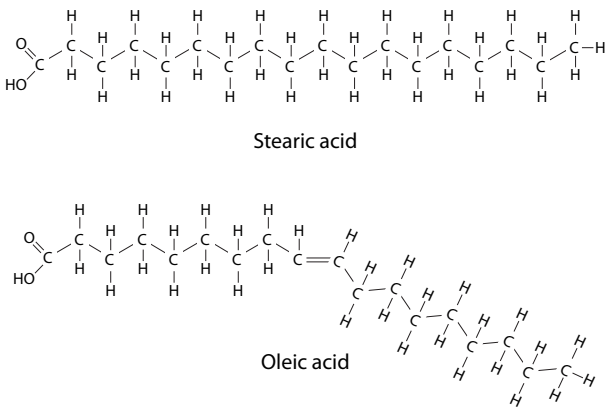


Figure 13. Fatty acids. (Top) Stearic acid is a fully saturated fatty acid with no carbon-carbon double bonds. (Bottom) Oleic acid is an unsaturated fatty acid.

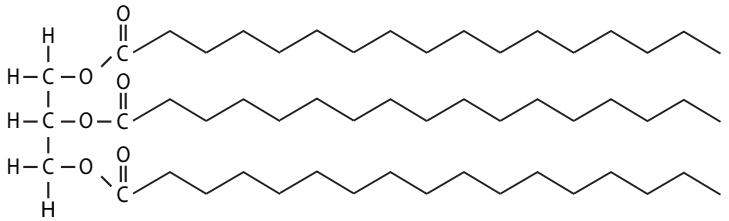


Figure 14. Triglycerides. These lipids are formed by conjugation of a glycerol to three fatty acyl chains through ester bonds from each glycerol oxygen.

Fatty acids inside cells are usually parts of larger molecules, rather than free acids. Some of the most common lipids derived from fatty acids are triacylglycerols, phosphoglycerides, and sphingolipids. Triacylglycerols, as the name implies, is three fatty acid (acyl) chains connected to a glycerol molecule by ester bonds (fig. 14). Triacylglycerols, also known as triglycerides, may have fatty acids of the same (simple triacylglycerols) or varying types (mixed triacylglycerols). Mixtures of these are the primary long-term energy storage molecules for most organisms. Although they may be referred to colloquially as fats or oils, the only real difference is the degree of saturation of their constituent fatty acids. Mixtures with higher percentages of saturated fatty acids have a higher melting point and if they are solid at room temperature, they are referred to as fats. Triacylglycerol mixtures remaining liquid at room temperature are oils.

In human medicine, a common test for heart disease risk factors is measurement of triglyceride levels in the blood. Although various cell types can make and use triglycerides, most of the triglycerides in people are concentrated in the adipose tissue, which is made up of adipocytes, or fat cells, though liver is also a significant fat store. These cells have specialized to carry fat globules that take up most of the volume of the cell. When triglyceride levels in the blood are high, it means that fat is being produced or ingested faster than it can be taken up by the adipocytes.

Phospholipids (also called phosphoglycerides or glycerophospholipids), are also based on attachment of fatty acids to glycerol. However, instead of three fatty acyl tails, there are only two, and in the third position is a phosphate group (fig. 15). The phosphate group also attaches to a "head group" . The identity of the head group names the molecule, along with the fatty acyl tails. In the example figure, 1-stearoyl refers to the stearic acid on the 1-carbon of the glycerol backbone; 2-palmitoyl refers to the palmitic acid on the 2-carbon of the glycerol, and phosphatidylethanolamine refers to the phosphate group and its attached ethanolamine, that are linked to the glycerol 3-carbon. Because of the negatively-charge phosphate group, and a head group that is often polar or charged, phospholipids are amphipathic - carrying a strong hydrophobic character in the two fatty acyl tails, and a strong hydrophilic character in the head group. This amphipathicity is crucial in the role of phospholipids as the primary component of cellular membranes.

Sphingolipids (fig. 16) are also important constituents of membranes, and are based not upon a glycerol backbone, but on the amino alcohol, sphingosine (or dihydrosphingosine). There are four major types of sphingolipids: ceramides, sphingomyelins, cerebrosides, and gangliosides. Ceramides are sphingosine molecules with a fatty acid tail attached to the amino group. Sphingomyelins are ceramides in which a phosphocholine or phosphoethanolamine are attached to the 1-carbon. Cerebrosides and ganglio-

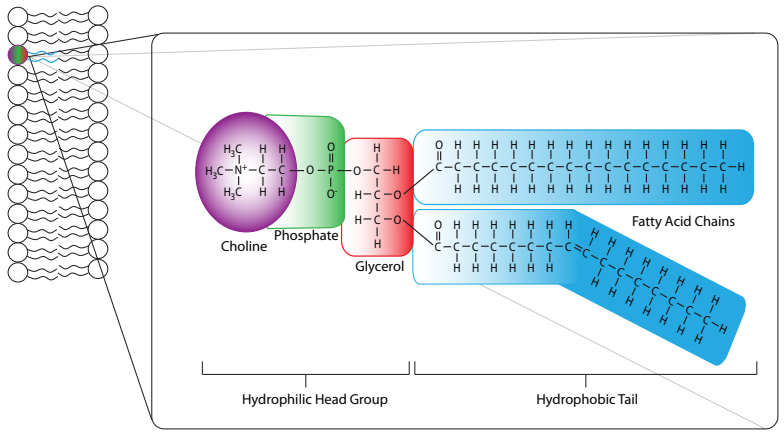


Figure 15. A phospholipid: the glycerol backbone (red) connects to two fatty acids and to a phosphate and polar head group.

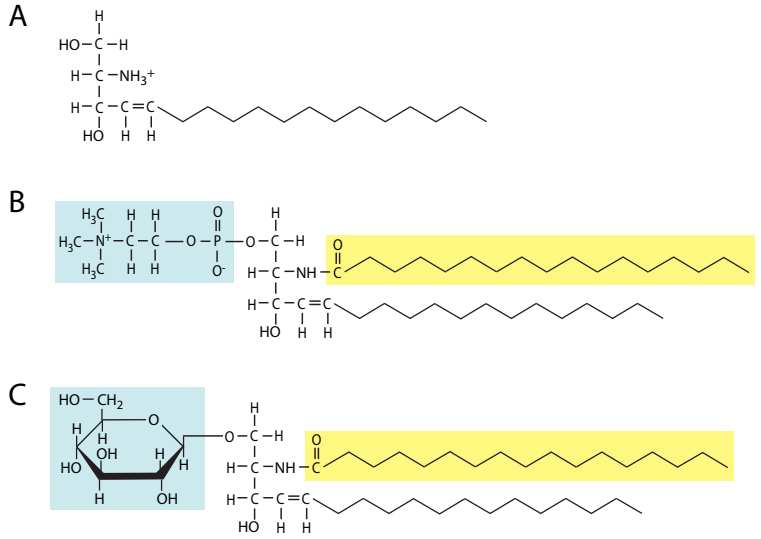


Figure 16. Sphingolipids are based on the amino alcohol, sphingosine (A). Ceramides have a fatty acid tail attached, and a ceramide with a phosphocholine head group is a sphingomyelin (B). If the head group is a sugar, then the molecule is a cerebroside. (C).

sides are glycolipids - they have a sugar or sugars, respectively, attached to the 1-carbon of a ceramide. The oligosaccharides attached to gangliosides all contain at least one sialic acid residue. In additional to being a structural component of the cell membrane, gangliosides are particular important in cell to cell recognition.

Lipids are vaguely defined as biological compounds that are insoluble in water but are soluble in organic solvents such as methanol or chloroform. This includes the fatty acid derivatives listed above, and it includes the final topic for this chapter, cholesterol. Cholesterol (fig. 17) is the major biological derivative of cyclopentanoperhydrophenanthrene, a saturated hydrocarbon consisting of four fused ring formations. It is an important component of plasma membranes in animal cells, and is also the metabolic precursor to steroid hormones, such as cortisol or β-estradiol. Plant cells have little if any cholesterol, but other sterols like stigmasterol are present. Similarly, fungi have their particular sterols. However, prokaryotes do not, for the most part, contain any sterol molecules.

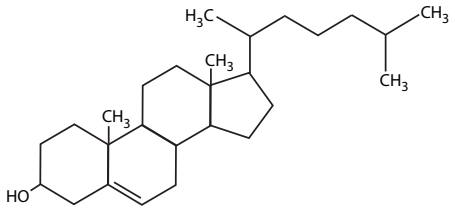


Figure 17. Cholesterol is an important lipid both as a membrane component and as a steroid precursor.

Bioenergetics:

Energy, Thermodynamics, and Enzymes

Using this book: This book is designed to be used in both introductory and advanced cell biology courses. The primary text is generally on the left side of the vertical divider, and printed in black. Details that are usually left to an advanced course are printed in blue and found on the right side of the divider. Finally, additional biomedically relevant information can be found in red print on either side of the divider.

Two fundamental concepts govern energy as it relates to living organisms: the First Law of Thermodynamics states that total energy in a closed system is neither lost nor gained – it is only transformed. The Second Law of Thermodynamics states that entropy constantly increases in a closed system.

More specifically, the First Law states that energy can neither be created nor destroyed: it can only change form. Therefore, through any and all processes, the total energy of the universe or any other closed system is constant. In a simple thermodynamic system, this means that the energy is transformed either by the transfer of heat energy (i.e. heating and cooling of a substance) or by the production of mechanical work (i.e. movement). In biological and chemical terms, this idea can be extended to other forms of energy such as the chemical energy stored in the bonds between atoms of a molecule, or the light energy that can be absorbed by plant leaves.

Work, in this case, need not imply a complicated mechanism. In fact, there is work accomplished by each molecule in the simple expansion of a heated mass of gaseous molecules (as visualized by expansion of a heated balloon, for example). This is expressed mathematically as the Fundamental Thermodynamic Relation:

$$dE = T\,dS - p\,dV$$

in which E is internal energy of the system, T is temperature, S is entropy, p is pressure, and V is volume.

The Second Law dictates that entropy always seeks to increase over time. Entropy is simply a fancy word for chaos or disorder. The theoretical final or equilibrium state is one in which entropy is maximized, and there is no order to anything in the universe or closed system. Spontaneous processes, those that occur without external influence, are always processes that convert order to disorder. However, this does not preclude the imposition of order upon a system. Examining the standard mathematical form of the Second Law:

$$\Delta S_{system} + \Delta S_{surroundings} = \Delta S_{universe}, \text{ where } \Delta S_{universe} > 0$$

shows that entropy can decrease within a system as long as there is an increase of equal or greater magnitude in the entropy of the surroundings of the system.

Unlike the First Law which applies even to particles within a system, the Second Law is a statistical law – it applies generally to macroscopic systems. However, it does not preclude small-scale variations in the direction of entropy over time. In fact, the Fluctuation theorem (proposed in 1993 by Evans et al, and demonstrated by Wang et al in 2002) states that as the length of time or the system size increases, the probability of a negative change in entropy (i.e. going against the Second Law) decreases exponentially. So on very small time scales, there is a real probability that fluctuations of entropy against the Second Law can exist.

The phrase "in a closed system" is a key component of these laws, and it is with the idea encapsulated in that phrase that life can be possible. Let's think about a typical cell: in its lifetime, it builds countless complex molecules - huge proteins and nucleic acids formed from a mixture of small amino acids or nucleotides, respectively. On its surface,

The "universe" is a closed system by definition because there is nothing outside of it.

this example might seem to be a counterexample to the second law - clearly going from a mixture of various small molecules to a larger molecule with bonded and ordered components would seem to be a decrease in entropy (or an increase in order). How is this possible with respect to the second law? It is, because the second law applies only to closed systems. That is, a system that neither gains nor loses matter or energy.

A living cell is not a closed system: it has inputs and outputs. However, the second law is still useful if we recognize that the only way that it can be bypassed is through the input of energy. If a cell cannot take in food (input of matter and energy into the system) it dies, because the second law requires that everything eventually breaks down into more random/chaotic collections of smaller components. The order required to sustain life (think about all the different complex molecules that were mentioned in the previous chapter) is phenomenal. The same thing applies on the organismal level (fig. 1) - without an input of energy (in the form of food molecules for animals or in the form of light for plants), the organism will die and subsequently decompose.

Creating molecules from atoms costs energy because it takes a disordered collection of atoms and forces them, through chemical bonds, into ordered, non-random positions. There is likewise an energy cost to formation of macromolecules from smaller molecules. By imposing order in the system, there must be an associated input of energy. This happens at every level of the system: atoms to molecules, small molecules to macromolecules, groups of molecules to organelles, etc.

Where does that energy go? It ends up in the bonds that are holding the molecules or macromolecules in their ordered state. When such a bond is broken, and a molecule is turned back into a collection of atoms, energy is released. The energy in a chemical bond is thus *potential* energy - it is stored energy that, when released, has the ability to do work. This term, if you recall your high school physics, is usually learned along with *kinetic* energy, which is energy that is being used in the process of actually doing work (i.e. moving an object from one place to another). The classic example is the rock on the top of a hill: it has potential energy because it is elevated and could potentially come down. As it tumbles down, it has kinetic energy as it moves. Similarly in a cell, the potential energy in a chemical bond can be released and then used for processes such as putting smaller molecules together into larger molecules, or causing a molecular motor to spin or bend - actions that could lead to pumping of protons or the contraction of muscle cells, respectively.

Coming back to the second law, it essentially mandates that breaking down molecules releases energy and that making new molecules (going against the natural tendency towards disorder) requires energy. Every molecule has an intrinsic energy, and therefore

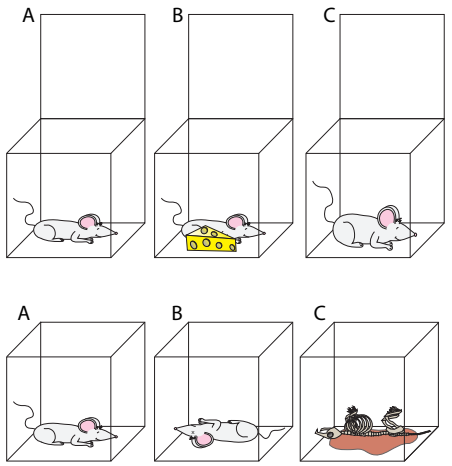


Figure 1. In the top panel, depicting an open system in which there are inputs for matter and energy (in the form of food), the box is open and air can be exchanged, food dropped in, etc, thus allowing the mouse to grow. However, in the bottom panel, depicting a closed system, the mouse does not have ready access any more oxygen than is in the box, nor does it have access to food. Without these inputs, the second law takes effect, and the mouse dies and decomposes into many smaller molecules.

As the polymerizing reaction reduces entropy, it requires energy generated (usually) by the breakdown of ATP into AMP and PPi, which is a reaction that increases entropy.

whenever a molecule is involved in a chemical reaction, there will be a change in the energy of the resulting molecule(s). Some of this change in the energy of the system will be usable to do work, and that energy is referred to as the free energy of the reaction. The remainder is given off as heat.

The Gibbs equation describes this relationship as $\Delta G=\Delta H-T\Delta S$. ΔG is the change in free energy, ΔH is the change in enthalpy (roughly equivalent to heat), T is the temperature at which the reaction takes place, and ΔS is the change in entropy. As a matter of convention, release of free energy is a negative number, while a requirement for input of energy is denoted with a positive number. Generally, a chemical reaction in which $\Delta G < 0$ is a spontaneous reaction (also called an *exergonic* reaction), while a chemical reaction in which $\Delta G > 0$ is not spontaneous (or *endergonic*). When $\Delta G = 0$, the system is in equilibrium. ΔG can also be expressed with respect to the concentration of products and reactants:

$$\Delta G = \Delta G^\circ + RT \ln ([P1] [P2] [P3] \ldots / [R1] [R2] [R3] \ldots)$$

Terms in square brackets denote concentrations, ΔG° is the standard free energy for the reaction (as carried out with 1M concentration of each reactant, at 298K and at 1 atm pressure), R is the gas constant (1.985 cal $K^{-1}mol^{-1}$), and T is the temperature in Kelvin. In a simpler system in which there are just two reactants and two products:

$$aA + bB \rightleftharpoons cC + dD$$

the equation for free energy change becomes

$$\Delta G = \Delta G^\circ + RT \ln \left(\frac{[C]^c [D]^d}{[A]^a [B]^b} \right)$$

This is important to us as cell biologists because although cells are not very well suited to regulating chemical reactions by varying the temperature or the pressure of the reaction conditions, they can relatively easily alter the concentrations of substrates and products. In fact, by doing so, it is even possible to drive a non-spontaneous reaction ($\Delta G > 0$) forward spontaneously ($\Delta G < 0$) either by increasing substrate concentration (possibly by transporting them into the cell) or by decreasing product concentration (either secreting them from the cell or by using them up as substrates for a different chemical reaction).

Changes in substrate or product concentration to drive a non-spontaneous reaction are an example of the more general idea of *coupling reactions* to drive an energetically unfavorable reactions forward. Endergonic reactions can be coupled to exergonic reactions as a series of reactions that ultimately is able to proceed forward. The only requirement is that the overall free energy change must be negative ($\Delta G < 0$). So, assuming standard conditions ($\Delta G = \Delta G^{\circ\prime}$), if we have a reaction with a free energy change of +5

kcal/mol, it is non-spontaneous. However, if we couple this reaction, to ATP hydrolysis for example, then both reactions will proceed because the standard free energy change of ATP hydrolysis to ADP and phosphate is an exergonic -7.3 kcal/mol. The sum of the two ΔG values is -2.3 kcal/mol, which means the coupled series of reactions is spontaneous.

In fact, ATP is the most common energy "currency" in cells precisely because the -7.3 kcal/mol free energy change from its hydrolysis is enough to be useful to drive many otherwise endergonic reactions by coupling, but it is less costly (energetically) to make than other compounds that could potentially release even more energy (e.g. phosphoenolpyruvate, PEP). Also, much of the -14.8 kcal/mol (ΔG°') from PEP hydrolysis would be wasted because relatively few endergonic reactions are so unfavorable as to need that much free energy.

Even when a reaction is energetically favorable (ΔG < 0), it may not occur without a little "push", chemically speaking. The "push" is something called *activation energy*, and it overcomes thermodynamic stability. Consider glucose, for instance. This simple sugar is the primary source of energy for all cells and the energy inherent within its bonds is released as it breaks down into carbon dioxide and water. Since this is large molecule being broken down into smaller ones, entropy is increased, thus energy is released from reaction, and it is technically a spontaneous reaction. However, if we consider a some glucose in a dish on the lab bench, it clearly is not going to spontaneously break down unless we add heat. Once we add sufficient heat energy, we can remove the energy source, but the sugar will continue to break down by oxidation (burn) to CO_2 and H_2O.

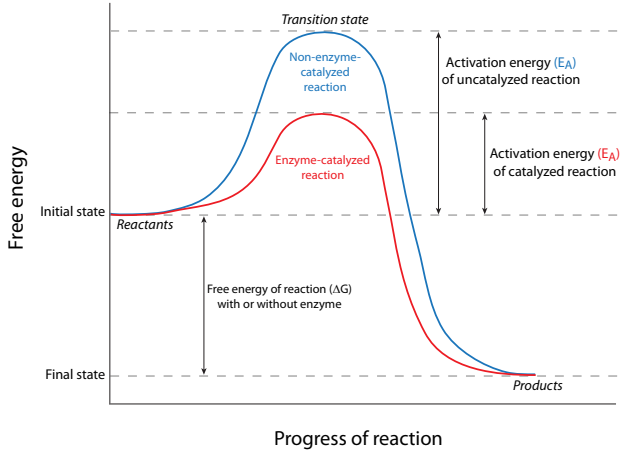


Figure 2. Catalysts lower the activation energy barrier to chemical reactions without altering the free energy change for that reaction.

Why is ATP different from other small phosphorylated compounds? How is it that the γ-phosphoanhydride bond (the most distal) of ATP can yield so much energy when hydrolysis of glycerol-3-phosphate produces under a third of the free energy? The most obvious is electrostatic repulsion. Though they are held together by the covalent bonds, there are many negative charges in a small space (each phosphate carries approximately 4 negative charges). Removing one of the phosphates significantly reduces the electrostatic repulsion. Keeping in mind that ΔG is calculated from the equilibrium of both reactants and products, we also see that the products of ATP hydrolysis, ATP and phosphate, are very stable due to resonance (both ADP and P_i have greater resonance stabilization) and stabilization by hydration. The greater stability of the products means a greater free energy change.

Put another way, the reactant(s) must be brought to an unstable energy state, known as the transtion state (as shown at the peak of the graphs in fig. 2). This energy requirement barrier to the occurrence of a spontaneous thermodynamically favored reaction is called the activation energy. In cells, the activation energy requirement means that most chemical reactions would occur too slowly/infrequently to allow for all the processes that keep cells alive because the required energy would probably come from the chance that two reactants slam into one another with sufficient energy, usually meaning they must be heated up. Again, cells are not generally able to turn on some microscopic bunsen burner to generate the activation energy needed, there must be another way. In fact, cells overcome the activation energy problem by using catalysts for their chemical reactions. Broadly defined, a *catalyst* is a chemical substance that increases the rate of a reaction, may transiently interact with the reactants, but is not permanently altered by them. The catalyst can be re-used because it is the same before the reaction starts, and after the reaction completes. From a thermodynamic standpoint, it lowers the activation energy of the reaction, but it does not change the ΔG. Thus it cannot make a non-spontaneous reaction proceed; it can only make an already spontaneous reaction occur more quickly or more often.

Enzymes

Biological catalysts are called enzymes, and the overwhelming majority of enzymes are proteins. The exceptions are a class of RNA molecules known as ribozymes, of which most act upon themselves (i.e. part of the RNA strand is a substrate for the ribozyme part of the strand). In this book (and most textbooks in this field), unless otherwise specified, the term enzyme refers to one made of protein. Enzymes confer extraordinary specificity to a chemical reaction: a reaction that might occur between a variety of potential substrates in an uncatalyzed situation may only be allowed between two specific substrates when catalyzed by an enzyme. Enzymes allow cells to run chemical reactions at rates from a million to even a trillion times faster than the same reactions would run under similar conditions without enzymes. In some cases, the enzymes allow reactions to proceed that would normally (i.e. sans enzyme) require more extreme temperature, pressure, or acidity/alkalinity. Finally, and perhaps most importantly for life, enzymes can be regulated. This is crucial for the cell, since it must be able to react to different situations, such as availability of energy, accumulation of toxic byproducts, the need to reproduce, etc. Not only can enzymes be modified either covalently or non-covalently to increase or decrease their activity, the cell can also regulate production of the enzymes, providing another level of control over particular cellular biochemical reactions.

Enzymes are the most diverse type of protein in a cell. They vary not only in size, but also in the number of independently manufactured subunits that must come together to form an active enzyme, or *holoenzyme*. Part of the reason for requiring so many different enzymes is that they are usually very specific for their substrate molecules, and that specificity is based upon a combination of shape and charge. The interactions between substrate and enzyme are often likened to a lock and key or pieces of a jigsaw puzzle. If the substrate fits the shape of the enzyme's active site (the part of the enzyme that carries out the actual catalytic reaction), and the charges interact (e.g. positively charged amino acids on the enzyme lining up with negative charges on the substrate), then there may be further stabilization of the interaction by Van der Waals and hydrogen bond interactions. In fact, formation of a stable Enzyme-Substrate (ES) intermediate is energetically analogous to the transition state (fig. 2) of reactions.

The specificity of enzymes is such that stereoisomers may not be recognized by some enzymes: for example, a protease (enzymes that chop up proteins into smaller pieces by hydrolyzing the peptide bonds between specific amino acids) such as trypsin can be stymied by the presence of a D-amino acid in place of the usual L-amino acid in a protein, even though it is a mirror image of the very same amino acid. This specificity means that enzymes are highly selective with respect to the reactions they catalyze, which means that specific reactions can be greatly enhanced without causing a general increase in many related chemical reactions. Another implication of the high specificity is that enzymes can (and often do) have high affinity for their substrates without the problem of binding non-substrate molecules (other than specific inhibitors - see below).

If most biochemical reactions would proceed extremely slowly, if at all, without catalysis, enzymes are needed to lower the activation energy needed for chemical reactions to support life. Exactly how does an enzyme lower the activation energy of a reaction? What exactly does "activation energy" mean in the context of a cell? To understand this, there are two principles to keep in mind: first, when we talk about chemical reactions, generally, we are concerned with populations of substrate, product, and enzyme molecules, not individuals; and second, the reactions are generally taking place between molecules dissolved in the aqueous cytoplasm of the cell.

Consider a reaction in which substrates A and B interact to form product C (fig. 3). If this reaction is not catalyzed, it depends on the happenstance that a molecule of A runs into a molecule of B in just the right orientation, and with the right amount of energy, to react and form the new molecule. We can conceptualize "activation energy" as the difficulty in getting A and B together perfectly so the reaction can proceed. How might an enzyme lower this activation energy? By making it easier for A and B to find each

Enzyme Classification

Enzymes have been catalogued and classified since the 1950's, during which time there was an explosion of enzyme discoveries and a need for a unified nomenclature and catalog. An International Commission on Enzymes was established (yes, of course I'm serious, why do you ask?) and thus started the Enzyme List. This list is now kept up-to-date online at http://www.chem.qmul.ac.uk/iubmb/enzyme.

All enzymes now have both recommended names for common usage, often reflecting historical naming, and a systematic name, which is highly specific. They also have a classification number based on their activity. The major classes of enzymes are (1) Oxidoreductases, which carry out oxidation-reduction reactions, (2) Transferases, which transfer functional groups, (3) Hydrolases, which carry out hydrolysis reactions, (4) Lyases, which eliminate groups to form double bonds, (5) Isomerases, which rearrange the bonds in a molecule but do not add or remove atoms, and (6) Ligases, which form bonds in reactions coupled to ATP hydrolysis.

As an example, DNA ligase (recommended name) catalyzes the formation of a phosphodiester bond between the 3' end of one DNA fragment and the 5' end of another. Its rather long and tedious systematic name is "poly(deoxyribonucleotide):poly(deoxyribonucleotide) ligase (AMP-forming)" and its classification number is 6.5.1.1. As a ligase, it is class 6; because it forms phosphoric ester bonds, it is subclass 5; the sub-subclass of 1 in this case is meaningless because it is the only sub-subclass of phosphoric-ester bond-forming ligases, but the final number designates the DNA ligase separately from other 6.5.1 enzymes such as RNA ligase, which is 6.5.1.3.

other with the right orientation and energy. So it could have binding sites for molecule A and molecule B, and once it has bound these two molecules, it changes its conformation, bringing A and B together under exactly the right conditions to react and form C. Once the reaction is complete, the product floats off because the enzyme has no affinity for it, and the enzyme returns to its initial shape, ready to bind more substrates.

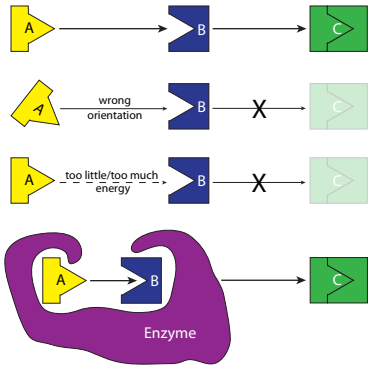


Figure 3. Enzymes can lower activation energy by binding substrates individually and bringing them together under optimal conditions to react.

Another example may be found with enzymes that break apart a molecule (figure 4). In order for a molecule to break apart, it may need to collide with another molecule with sufficient energy to break one or more of its covalent bonds. An enzyme that catalyzes

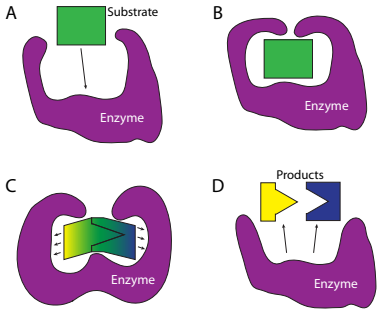


Figure 4. The enzyme (A) binds a specific substrate, leading to a conformational change (B) that stresses the molecule (C) until it breaks down into two smaller component molecules (D), which are released.

the breakdown reaction might bind to the molecule, and in binding it, undergoes a conformational shift that bends or twists the molecule in such a way that the bonds in the substrate molecule are weakened or broken. These two examples oversimplify the chemistry of enzyme activity into a mechanical idea, but the general relationship in how an enzyme lowers activation energy for a reaction is accurate.

Enzymes may also facilitate a chemical reaction by acting as a temporary holding site for an active group being transferred from one substrate to another. Alternatively, temporary formation of hydrogen bonds or even covalent bonds between the enzyme and substrate can alter chemical characteristics of the substrate to make it react more easily. An example of enzyme mechanisms on the molecular level is shown in Chapter 5: Figure 1.

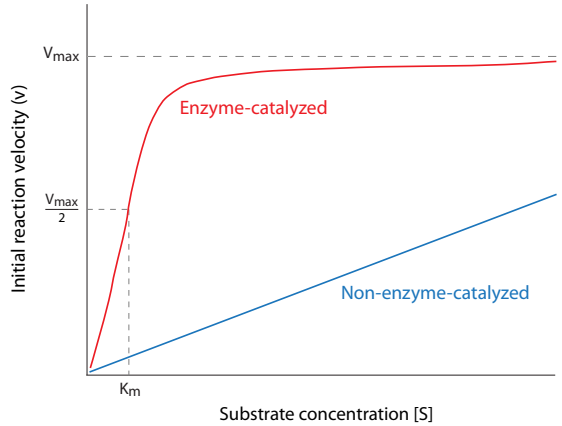


Figure 5. Saturation curve for a single-substrate enzyme-catalyzed reaction.

Enzyme Kinetics

Unlike uncatalyzed (but readily occurring) reactions, in which the rate of the reaction is dependent only on the concentration of the reactants, the rate of enzyme-catalyzed reactions is limited by the number of enzyme molecules available. This maximal rate of turnover from substrate to product is a function of the speed of the enzyme and the number of enzyme molecules. V_{max}, this theoretical maximal rate or reaction, is approached when there is such a high concentration of substrate molecules that not only is every available enzyme at a given time occupied, but as soon as an enzyme finishes converting substrate to product, it immediately binds a new substrate. Another term, K_m, is related to V_{max} in that K_m (the Michaelis constant) is the concentration of substrate at which half-maximal reaction rate ($V_{max}/2$)occurs. These two terms are related in the Michaelis-Menten equation, which describes the reaction rate *v* with respect to the substrate concentration [S].

$$v = \frac{V_{max}[S]}{K_M + [S]}$$

Figure 6. Michaelis-Menten Equation

The Michaelis-Menten equation assumes a simple reaction of the form:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

where E is an enzyme, S is the substrate, and P is the product. Note the formation of the intermediate enzyme-substrate complex, ES, which is a transition state (recall fig. 2) in which the substrate is unstable and associated with the enzyme. In fact, ES could as

The Michaelis-Menten equation was derived by Leonor Michaelis and his graduate student Maud Menten in 1913, based on work by Victor Henri, and is applicable only to simple enzyme kinetics in which there is only one substrate that is changed immediately to a product during the reaction without forming any intermediate compound, the enzyme in question shows no allostericity, and the reaction is unidirectional.

It should be noted that the reaction rate *v* is actually the initial reaction rate at a particular substrate concentration, and is sometimes denoted v_o. Naturally, as the reaction continues, the substrate concentration decreases, along with the reaction rate.

easily be considered EP, since this state is essentially the tipping point between the conversion from substrate to product. In this construction, the Michaelis constant, K_M, of an enzyme-catalyzed reaction is $(k_2 + k_3) / k_1$. That is the rate of ES dissociation over the rate of ES association. K_M, of course, varies not only depending on the enzyme, but also with respect to the identity of the substrate. Some enzymes can work with multiple substrates, and the K_M of that enzyme for the different substrates is usually different. Because the saturation curve in Fig. 5 can be difficult to work with, linearizations of the Michaelis-Menten equation were developed. The most common is the double reciprocal plot, better known as the Lineweaver-Burk plot. On this type of graphical representation of enzyme kinetics, the reciprocal of the substrate concentration is plotted against the reciprocal of the reaction velocity. This generates a line in which the x-intercept is then $-1/K_m$, the y-intercept is $1/V_{max}$, and the slope of the line is K_m/V_{max}.

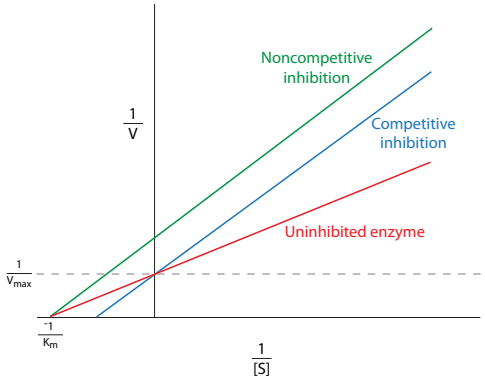


Figure 7. Lineweaver-Burk plot of enzyme kinetics and the effect of competitive and non-competitive inhibitors at constant concentrations.

Regulation of Enzyme Activity

Figure 7 (and 9) also illustrates the effects of two different types of inhibition on the different components of enzyme kinetics. Enzymes can be slowed down or even prevented from catalyzing reactions in many ways including preventing the substrate from entering the active site or preventing the enzyme from altering conformation to catalyze the reaction. The inhibitors that do this can do so either reversibly or irreversibly. The irreversible inhibitors are also called inactivators, and either bind to the enzyme with such high affinity as to be virtually irreversible, or they actually form covalent bonds with the enzyme. Reversible inhibitors are generally grouped into two basic types: competitive and non-competitive.

Obtaining V_{max} and K_m from a direct plot of v against [S] can be difficult because even at very high substrate concentrations, experimental data may still be significantly under the V_{max}. This leads to underestimation of the V_{max}.

The Lineweaver-Burk plot addresses this concern, but has some shortcomings of its own. Because it is easier to obtain data at high concentrations, most of the data points are near 0, and fewer data points are available further out (to the right of the graph). Because these are reciprocals, under these low [S] conditions, small errors in measured values of v turn into large errors in $1/v$, and therefore large errors in K_M and V_{max}. This is evident on examination of the Lineweaver-Burk equation:

$$\frac{1}{v} = \left(\frac{K_M}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

Figure 8. Lineweaver-Burk Equation

Finasteride (trade names include Propecia and Proscar) is an irreversible inhibitor that binds very tightly to the enzyme 5-α-reductase, used in converting testosterone to dihydrotestosterone. It is used in the treatment of male pattern baldness, benign prostatic hyperplasia, and prostate cancer.

Aspirin is an example of an irreversible inhibitor that actually forms a covalent bond with the enzyme. The aspirin (acetylsalicylic acid) transfers its acetyl group onto a serine residue on cyclooxygenase-2 (COX-2). This stops the production of inflammation-producing prostaglandins and thromboxanes by COX-2.

Competitive inhibition is perhaps the simplest to understand. The inhibitor molecule competes directly with the substrate for the active site of an unbound enzyme. If an inhibitor binds to the active site, the substrate is unable to do so until the inhibitor has vacated the site. Thus, one could potentially overwhelm competitive inhibition with sufficiently larger concentrations of substrate so that the probablility that the enzyme bumps into a substrate to bind becomes exceeding large compared to the probablility of bumping into an inhibitor. Normal, uninhibited V_{max} is then achieved despite the presence of the competitive inhibitor, which has only affected the K_m, that is, the concentration of substrate needed to reach $V_{max}/2$. This is the kinetic signature of competitive inhibitors: with increasing inhibitor concentrations, K_M is increased but V_{max} is unaffected.

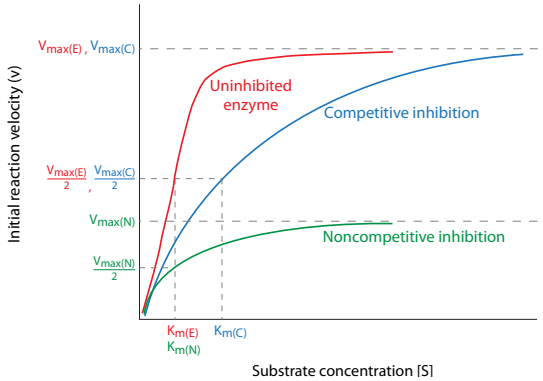


Figure 9. Saturation curves for enzyme-catalyzed reactions without inhibitor (red) with competitive inhibitor (blue) at a constant concentration, and with noncompetitive inhibitor (green) at a constant concentration.

Non-competitive inhibition involves inhibiting the enzyme by altering its ability to complete the catalyzed reaction through binding of the enzyme at a position that is not the active site. When the inhibitor binds to the enzyme, it causes a change, usually conformational, that may either prevent the enzyme from binding the substrate, or prevent the enzyme from acting upon a bound substrate. In either case, increasing the availability of substrate will not ultimately overcome the effect of the inhibitor. Thus, V_{max} is reduced because some proportion of the enzymes are no longer usable, but because the enzymes that are available have the same access to substrate as it would without inhibitor (that is, it is not in competition with an inhibitor), the K_m is not affected.

Non-competitive regulation is one example of *allosteric regulation* of enzymes. Allosteric interactions occur when the binding of a ligand (not necessarily a substrate) to a protein influences the binding of another ligand to the protein at a separate binding

Methotrexate is a competitive inhibitor of dihydrofolate reductase (DHFR), an enzyme that synthesizes tetrahydrofolate, which is a precursor for purine synthesis, and therefore for DNA and RNA. It has a very similar molecular structure for folic acid, the natural substrate of DHFR. Methotrexate is used as an anti-cancer drug because it affects rapidly reproducing cells (which need to make DNA sooner than other cells) more than non-cancerous cells.

Many of the enzymes in metabolic pathways (chapters 5 and 6) are regulated by a naturally occurring non-competitive inhibitor. One example is phosphofructokinase (PFK), which is involved in glycolysis, which produces ATP for the cell. However, if there are high enough levels of ATP in the cell that other cellular processes aren't using, the it can bind to phosphofructokinase outside of its active site (it binds fructose-6-phosphate), and turn it off. This blocks glycolysis and production of excess ATP when the cell does not need it. As ATP is used up, there is less available to inhibit PFK, and glycolysis starts back up.

There are two models for allosteric interactions. The symmetry model, also known as the concerted model, or MWC model (Monod, Wyman, and Changeux, 1965), proposes that the allosteric enzyme is an oligomer of several subunits, each of which are symmetrically related, and can be in either a "tensed" or "relaxed" state, but all of the subunits are in the same state and at equilibrium. When a ligand binds, it changes the state of the subunit(s) to which it binds, and to maintain equilibrium, that in turn causes the state of the other subunits to match, thus altering binding properties for a subsequent ligand. The sequential model, or the KNF model (Koshland, Nemethy, and Filmer, 1966), proposes

site. These kinds of interactions can be either positive (activating) or negative (inhibitory), and either homotropic (both ligands are identical) or heterotropic (ligands are different). Interestingly, sometimes the regulator ligand may actually be a product of the catalyzed reaction. In this kind of feedback mechanism, the progress of a reaction is self-regulating.

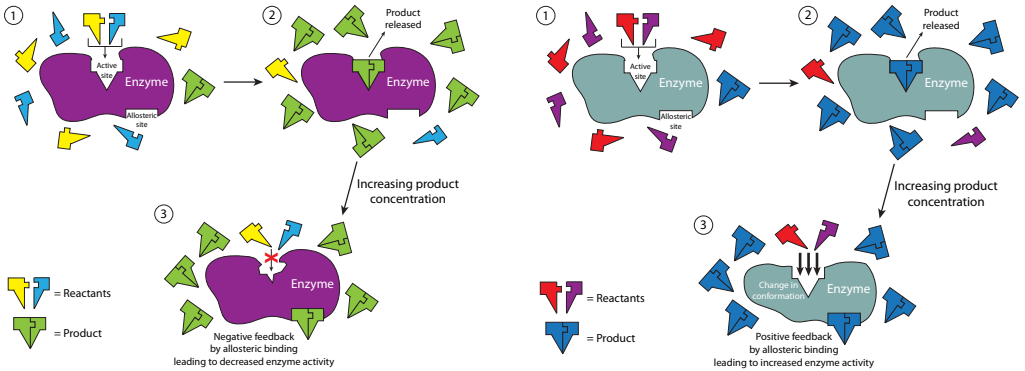


Figure 10. Feedback control loops. (Left) Negative feedback is a common biological mechanism for self-regulating processes. The product of the reaction, either by itself or inconjunction with other molecules, acts as an allosteric inhibitor of the enzyme. (Right) Positive feedback is less common because it can lead to rapid expansion of the scope of a reaction, and requires an external (relative to the enzymatic reaction) mechanism for slowing or stopping the reaction.

While important, especially pharmaceutically, the use of enzyme inhibitors is not the only way to regulate enzymes. There are numerous examples of one type of enzyme activating or inhibiting another. The most common general example are the protein kinases. These enzymes phosphorylate (transfer phosphate group to) other enzymes and thereby activate them. Kinases are generally fast and very specific, and this is an efficient method for activating large numbers of particular enzymes quickly. Conversely, protein phosphatases are enzymes (also quite fast, but much less specific than kinases) that remove the phosphate groups from phosphorylated proteins, thereby turning off those enzymes. Keep in mind that this is a generalization, and that not all phosphorylations are activating. In addition to enzymatic inhibition of enzymes, there is also inhibition by binding and sequestration of the substrates. In fact, the antibiotic vancomycin works just this way, binding to the substrate peptide for transpeptidase and preventing the enzyme from recognizing it. Transpeptidase normally helps stabilize the cell wall of certain bacteria by altering some of the proteins, and without its activity, the protection of the cell wall is compromised and the bacteria may be more easily killed.

something quite different: although it also supposes subunits in either tensed or relaxed states, it does not require all subunits to be connected in such a way as to mandate that all subunits be in the same state, and therefore conformational changes in one subunit need not be propagated to all. Instead, using an induced-fit model of ligand binding rather than the more rigid basic lock and key mechanism, it suggests that when a ligand binds to the enzyme, it induces a slight conformational change in the active site that increases its affinity for the ligand. The conformational change may slightly alter the conformation of other subunits of the enzyme, but may not constitute a state change between relaxed and tensed depending on how tightly the subunits are interacting. However, the change is enough to increase substrate affinity in adjacent subunits.

Most cell/molec courses stop the discussion of enzyme inhibitors at competitive vs non-competitive based on their kinetic profiles. However, it should be noted that if you take a biochemistry course, you may encounter the terms *uncompetitive* inhibitor and *mixed* inhibitor. These terms are defined not just by the enzyme kinetics, but the mechanism of interaction: *Uncompetitive inhibitors* bind only to the enzyme-substrate (ES) complex, and not to the enzyme before it has encountered substrate. This leads to decreased V_{max} and decreased K_m. *Mixed inhibition* means that the inhibitor can bind to either enzyme alone or the enzyme-substrate complex. Because the affinities of the inhibitor for the two forms of the enzyme are different, and because part of it depends on substrate concentration while the other kind of binding does not, generally, V_{max} decreases and K_m increases. Non-competitive inhibition is a special case of mixed inhibition in which the catalytic activity of the enzyme is diminished or abolished, but the ability to bind substrate is unaltered.

The activity of enzymes is greatly influenced by both pH and temperature, as expected from the discussion of protein structure in the previous chapter. Activity profiles of most enzymes shows a peak of activity that tails off on either side, whether it is pH or temperature. This is an innate characteristic of the enzyme. For example, pepsin, a digestive enzyme secreted into the stomach (pH 2) does not function when the pH > 5. On the other hand, another digestive enzyme, trypsin, which is secreted into the duodenum (proximal small intestine) where the pH is ~8, does not work in acidic environments. Changes in pH can change ionization of amino acid side chains that can thereby alter interaction with the substrate, or lead to changes in tertiary structure.

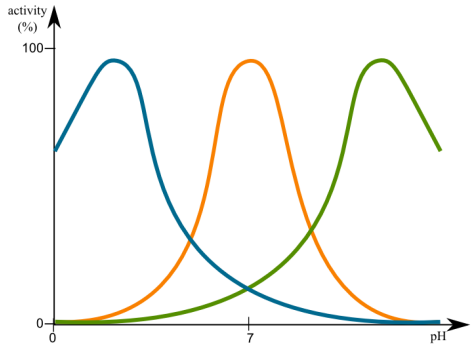


Figure 11. pH dependence of enzymatic activity. This graph depicts three hypothetical enzymes with acidic, neutral, and basic pH optima.

Similarly, at suboptimal temperatures, the likelihood of protein-substrate interaction is low but above the optimal temperature, the increased energy can lead to breaking of hydrogen bonds within the structure of the enzyme, resulting in changes that inactivate the catalytic ability of enzyme or prevent it from binding substrate with sufficient affinity. The temperature optimum of most enzymes is very close to its typical environment. Thus, a human enzyme would operate optimally around 37°C, while an enzyme from bacteria that live in deep-sea volcanic vents (e.g. Thermophilus aquaticus) might have temperature optima over 90°C. This is one of the reasons that refrigeration can slow down growth of microorganisms (which obviously have no ability to regulate their temperature), and why most microorganisms are killed (enzymes permanently denatured) when put into sustained high temperature environments. Interestingly, the DNA polymerase from the T. aquaticus bacteria, also commonly called Taq polymerase, is used in a rapid DNA-amplifying lab technique known as PCR (polymerase chain reaction, see Methods chapter) in which samples are repeatedly heated to high temperatures to separate DNA strands in preparation for making copies of them. DNA polymerases from most prokaryotic or eukaryotic species would be denatured and inactivated by the high heat, but Taq has evolved (with respect to its tertiary structure) for extraordinary structural stability even in heat extremes.

Well over half of the enzymes discovered so far do not act in the simplistic Michaelis-Menten one-substrate-one-product mechanism, but rather operate with two substrates and two products, usually with the transfer of an active group. These types of reactions are sometimes known as Bi Bi reactions. There are two major classes of these reactions: the *sequential reactions*, in which all substrates bind with the enzyme before the reaction proceeds, and the *ping pong reactions*, in which one or more products are created and released before all of the substrates have been bound. In fact, unlike sequential reactions, the two substrates do not interact with one another while bound to the enzyme.

Finally, many enzymes require a molecular partner that has no catalytic activity of its own, but like a catalyst, is not permanently altered by the chemical reaction. These molecules are *cofactors*. Some are simple: elemental, in fact, including metal ions such as Zn^{++} or Ca^{++}. Others are slightly more complex: small organic cofactors are called *coenzymes*, and accomplish the same thing, acting as a required partner to the enzyme in catalyzing a reaction. The interaction with the enzyme itself varies and may be only transient, as in NAD^{+}/NADH which are coenzymes used in redox reactions, or permanently bound to the enzyme by covalent bond like the heme group of hemoglobin. Often the function of the coenzyme is to provide an active group to facilitate the catalyzed reaction. Coenzyme A, in various metabolic pathways such as glycolysis or the tricarboxylic acid cycle, can be bound to a substrate to form a stable product that then acts as an intermediate. The Co-A is released from the molecule as it undergoes the next step in a series of reactions in the metabolic pathway (see Chapter 5).

From a human health standpoint, it is interesting to note that many coenzymes are vitamins, or derived from vitamins. These are the B vitamins biotin (B_7), cobalamin (B_{12}), folic acid (B_3), niacin/nicotinamide (B_9), pantothenic acid (B_5), pyridoxine (B_6), riboflavin (B_2), and thiamine (B_1). Vitamins are small organic compounds that are not synthesized by an organism and must therefore be ingested. They are generally needed only in small quanitities, but necessary nonetheless. Naturally, the vitamins we are familiar with are those required by humans. The specific roles of these vitamins and the consequences of not having enough of them are discussed later in this textbook, as the enzymes that they work with are introduced in detail.

Membranes:

Structure, Physical and Chemical Properties, and Function

Using this book: This book is designed to be used in both introductory and advanced cell biology courses. The primary text is generally on the left side of the vertical divider, and printed in black. Details that are usually left to an advanced course are printed in blue and found on the right side of the divider. Finally, additional biomedically relevant information can be found in red print on either side of the divider.

Biological membranes are the basis for many important properties of the cell, not the least of which is to physically define the cell boundary, and in eukaryotes, the boundaries of each intracellular organelle. However, they are not completely impermeable boundaries, and through embedded proteins, the membrane serves as the gatekeeper for the passage of specific molecules into (e.g. nutrients) and out of (e.g. waste) the cell. Other embedded proteins can identify the cell to other cells, and participate in numerous interactions with the environment or other cells. Finally, the membrane, or more precisely, the chemical gradients across the membrane, is an important energy source for the cell.

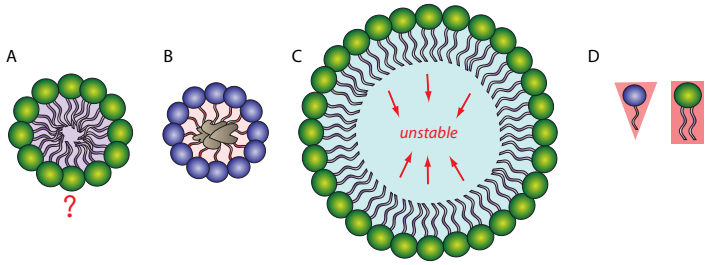
Membrane Structure and Composition

Since most cells live in an aqueous environment and the contents of the cell are also mostly aqueous, it stands to reason that a membrane that separates one side from the other must be *hydrophobic* to form an effective barrier against accidental leakage of materials or water. In the earlier chapter on the basic biomolecules, cellular membranes were partially defined as being composed primarily of phospholipids: molecules consisting of a phosphorylated polar head group attached to a glycerol backbone that has two long hydrocarbon tails. The composition of the hydrocarbons can vary in length and degree of saturation, and there is also variation in the head groups. It is also important to remember that although we concentrate on the phospholipids as the primary components of the membrane, there are other significant parts: other lipids, including cholesterol, integral and peripheral membrane proteins, and glycosylated lipids and proteins.

Because the phospholipids are amphipathic, meaning they have a hydrophilic head and hydrophobic tail, the simplest conformation for a small group of phospholipids in aqueous solution might be expected to be a micelle (fig. 1A), but is this actually the case? Mixtures of hydrophobic molecules and water are thermodynamically unstable, so this structure would protect the hydrophobic fatty acyl tails from the aqueous environment with which the head groups interact. Micelles can form with other amphipathic lipids, the most recognizable being detergents such as SDS (sodium dodecyl sulfate, also called

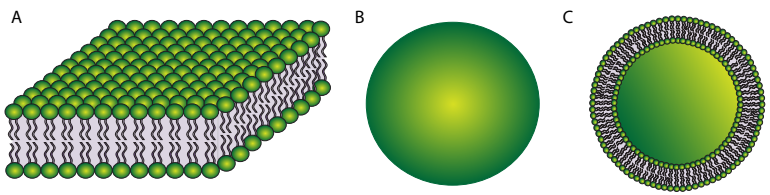
sodium lauryl sulfate), used in common household products such as shampoos. The detergents act by surrounding hydrophobic dirt (1B) and holding it in solution within the micelle to be rinsed away with the water. At smaller sizes, the micelle is fairly stable; however, when there are a large number of phospholipids, the space inside the micelle becomes larger and can trap water in direct contact with the hydrophobic tails (1C). This renders the micelle unstable, so a large single layer of phospholipid is unlikely to serve stably as a biological membrane. Micelles form easily with SDS and other single-tailed lipids because their overall shape (van der Waals envelope) is conical (1D), which lends itself to fitting tight curvatures. However, phospholipids are more cylindrical, and it is harder to fit them into a tight spherical micelle. If they do form micelles, they tend to be larger, and likely to collapse.

Figure 1.



On the other hand, a phospholipid bilayer (figure 2A) could form a fatty acyl sandwich in which the polar head groups face outward to interact with an aqueous environment, and the fatty acids are sequestered in between. However, this does not resolve the problem on the edges of the sandwich. Sometimes, a collapsed micelle can form a closed bilayer in which the edges appear to be sealed, but due to the shape of the phospholipids, there is poor contact between acyl chains. Such a tight bend is unstable and the edge phospholipids are likely to break apart from one another. So, the solution to the ideal phospholipid structure in an aqueous environment is a spherical phospholipid bilayer (2B and cutaway in 2C) : no edges mean no exposed hydrophobicity.

Figure 2.

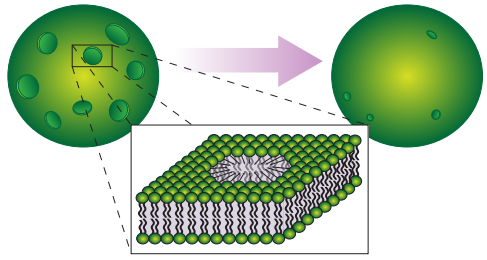


Just as overly large micelles will be unstable, there is also a minimal concentration of lipid needed for a micelle to form. This is known as the critical micelle concentration (cmc) and is a property of each particular lipid. Below the cmc, there are not enough amphipathic lipids to mutually shield their hydrophobic tails from the water, and the more likely position of the lipids is on the surface of the aqueous solution, hydrophilic heads in contact, with the hydrophobic tails in the air.

Liposomes are artificial spherical bilayers (Fig.2) that are used both in research for study of both membrane lipids and integral membrane proteins. They are also used to deliver drugs and other macromolecules into cells for either research of therapeutic purposes. A common method for getting DNA, a large molecule that is not normally transported into cells, into a cell is called lipofection. This technique involves creating liposomes within a solution containing the DNA of interest. This traps the DNA into the liposome, which can then be applied to cells. The liposomes then fuse with the plasma membrane and deliver the DNA into the cell.

Another technique for introducing foreign DNA into a cell is electroporation, which illustrates the self-sealing property of the phospholipid bilayer (figure 3). The cell (usually thousands of cells, actually) is placed in a DNA solution and subjected to an electric current. This transiently pulls on the cell membrane in all directions, causing many small holes to open up. DNA then moves into the cell, and the holes spontaneously heal. Of course, the process is not perfect, and due to variations in the cells and in the electric field, some cells will be unable to reseal the holes, or for other reasons may not survive the process.

Figure 3.



The stability of the spherical phospholipid bilayer does not imply that it is static in its physical properties. In most physiologically relevant conditions, the membrane is cohesive, but fluid. It can mold its surface to the contours of whatever it is resting on, and the same thermodynamic and hydrophobic properties that make it so stable also allow it to seal up minor tears spontaneously. Maintaining a working range of fluidity is important to the cell: if the membrane is too rigid, then it may be unable to move or undergo necessary processes such as endocytosis, in which a cell takes up large extracellular molecules by enveloping them with the cell membrane and pinching it off in a vesicle; while if it becomes too fluid, it may lose integrity and fall apart.

There are three major factors that govern the fluidity of the membrane: (1) degree of saturation of the fatty acyl chains, (2) the temperature, and (3) the concentration of cholesterol. Fully saturated fatty acyl chains can rotate freely around any bond and therefore can pack together very tightly, thus decreasing membrane fluidity. As more unsaturated fatty acyl chains are introduced into the membrane, the more space there is between some of the fatty acyl tails, and there is an increase in fluidity. Similarly at higher temperatures, even saturated fatty acyl chains, with their increased energy, move more and create more space between the chains, also increasing fluidity. Finally, cholesterol, as a small planar lipid molecule, can intercalate between the fatty acyl tails. Interestingly, in normal physiological temperatures, the effect of cholesterol is dependent on its concentration. At normal concentrations, the cholesterol restricts acyl tail movement and decreases fluidity. However at very low concentrations, cholesterol has the opposite effect, separating the hydrophobic tails and slightly increasing fluidity, especially in the innermost regions of the membrane. [In case you were wondering, human medical problems with cholesterol are unrelated to the cholesterol in the cell membranes, and refers to cholesterol (bound to lipoprotein carriers) in the bloodstream. That cholesterol can build up in the blood vessels, decreasing the internal diameter and along with it, blood flow. When this happens to the heart, which uses a lot of oxygen, the oxygen deprivation can cause necrosis and a heart attack.]

In addition to the three factors noted above, phospholipid composition can also alter membrane fluidity: shorter acyl chains lead to greater fluidity, while longer chains, with more surface area for interaction, generate membranes with higher viscosity. The phospholipid composition of biological membranes is dynamic and can vary widely. The table below shows the differences in the ratios of major phospholipid species phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and sphingomyelin (SM) in plasma membranes from two different cell types. As you might expect based on their differing functions, the ratios of plasma membrane lipids of a myelinating Schwann cell are very different from the lipids in the plasma membrane of a red blood cell.

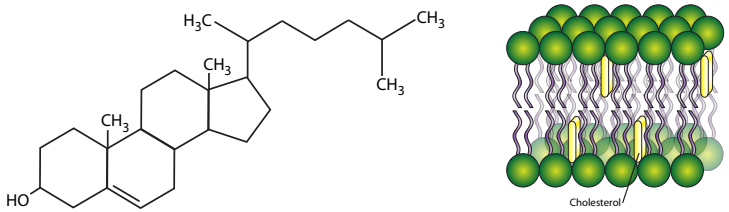


Figure 4. Cholesterol is a small planar molecule that can fit in between the fatty acyl tails of phospholipids in the membrane.

The effect of cholesterol is actually a little more complicated than explained to the left. Cholesterol is not only planar but rigid, and it intercalates close between acyl tails near the head region. Because cholesterol is somewhat shorter than many acyl tails, while the parts of the hydrocarbon chains nearer the head groups are stabilized and restricted in movement, the cholesterol actually acts as a spacer for the other (methyl) end of each chain, so in the center domain of the hydrophobic core of the bilayer, there is actually increased fluidity around cholesterols. Cholesterol concentration varies greatly among organelles of the same cell, and can even be dynamically regulated in response to temperature changes. Membrane samples taken from fish living in warmer temperatures contain more cholesterol than samples from the same species acclimatized to a lower temperature.

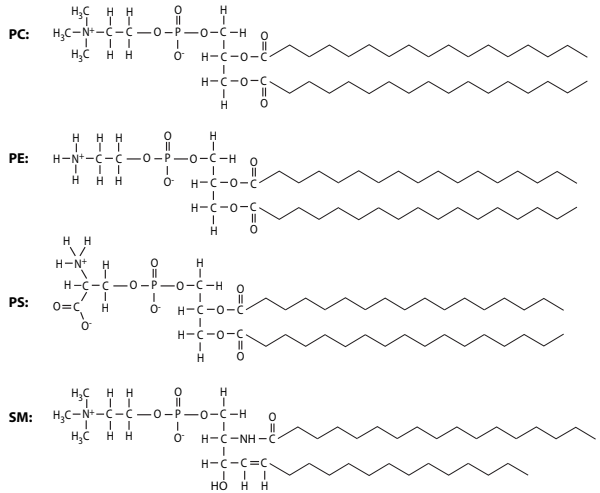


Figure 5. The molecular structures of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and sphingomyelin (SM). The acyl chains of all four molecules are of variable length except the 13-C chain of SM.

Lipid	*Schwann Cell PM*	*Erythrocyte PM*
Phosphatidylcholine	44%	19%
Phosphatidylethanolamine	14%	18%
Phosphatidylserine	3%	8%
Sphingomyelin	29%	17%

Even within a single cell, the composition of the plasma membrane differs from that of intracellular organelles. There is even heterogeneity within a membrane itself - the lipids are not simply distributed randomly in the membrane. Research over the last two decades have identified lipid "rafts" that appear to be specific for embedding particular proteins. Since they are unanchored lipids, the rafts can move laterally within the membrane just like most individual lipid molecules. Finally, there are different ratios of the lipids between the two layers of the bilayer. The cytoplasmic face of every membrane will have different associations and functions than the non-cytoplasmic face, so why should we expect the lipid composition to be the same?

Though lipid rafts were considered a possibility in the fluid-mosaic membrane model proposed by Singer and Nicholson in 1972, only in the last two decades has the idea been researched seriously, but there have been technical difficulties with visualizing a small domain of lipids within a virtual ocean of lipids. In broad terms, the rafts are considered small areas of ordered lipids within a larger undirected membrane. Lipid rafts most often form in association with specific membrane proteins while excluding others. Some of the proteins are peripheral membrane proteins such as src or the GPI-linked adhesion molecule Thy-1, while others are transmembrane proteins such as the T-cell receptor. Usually the included proteins have signaling-related functions, and one model proposes that these proteins may direct the organization of selected lipids around them, rather than the other way around.

Although some phospholipids are directly linked to proteins and the cytoskeleton, most are not, and are therefore free to move within the plane of its layer of the bilayer. In the experiment shown in figure 6 below, the cell surface has been labeled with a covalently bound dye that fluoresces red when excited by green light. When the dye molecules are exposed to high intensity light for an extended period, they no longer fluoresce, a phenomenon known as photobleaching. Photobleaching is a permanent effect, and therefore it can be inferred that if a photobleached spot fluoresces again, then other, non-bleached, phospholipids must have moved into the spot. This experiment clearly proves the lateral mobility of phospholipids in a membrane.

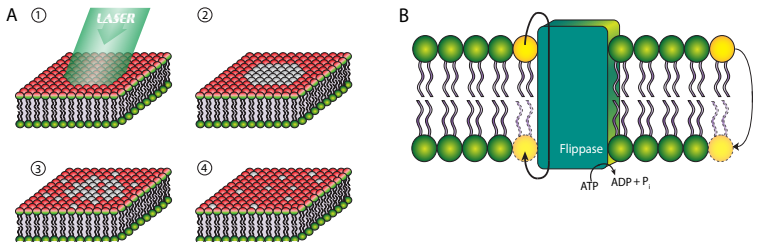


Figure 6. (A) photobleaching experiments can demonstrate lateral mobility of lipids in a bilayer. But, transverse mobility is severely limited and is usually facilitated by a flippase enzyme (B).

However, this is not the case with transverse mobility from one face of the membrane to the other. Due to the highly unfavorable energetics of pushing a polar head group through the lipid tail layers, phospholipids very rarely move from one layer to the other within a membrane (fig. 6B, far right yellow phospholipid). Such movement is greatly facilitated by a flippase enzyme, which hydrolyzes a molecule of ATP for the energy to push a phospholipid from one face of the bilayer to the other.

For over a century since Meyer (1899) and Overton (1901) first suggested it, the prevailing theory for the mechanism of gaseous general anesthesia (e.g. ethyl ether, halothane, nitrous oxide, cyclopropane) has been that they partition into and interact with the lipids of the plasma membranes of neurons and by altering the physical membrane properties accomplish the anesthesia. However, in 1985, Franks and Lieb published a report in Nature that, for the first time, showed that an enzyme could be directly affected by a gaseous anesthetic. Since that report, the evidence has been building against the old model of altering membrane lipid properties, and now the current model of direct gas-protein interaction is assumed.

In a pure phospholipid bilayer, membrane proteins as well as lipids have lateral mobility, but in living cells, the proteins are generally constrained either by preferential association with certain kinds of lipids, by direct attachment to cytoskeletal elements, by indirect attachment to the cytoskeleton, or by being "fenced in" by cytoskeletal gridwork directly underlying the cell membrane. Since 1972, the Singer-Nicholson "fluid mosaic" model of membrane structure has been accepted as a general model for biological membranes. It proposes that integral membrane proteins as well as membrane lipids have lateral freedom of movement. This has since been refined with the recognition of lipid rafts and clustered membrane protein patches, but is still viable as a basic model.

Membrane Permeability

A pure phospholipid bilayer, whatever the lipid composition, is a semi-permeable membrane that is generally repellent to large molecules and to ions. Small polar molecules can sometimes pass easily (e.g. ethanol), but more often pass at low rates if at all (e.g. water). However, small nonpolar molecules are able to pass through the membrane with relative ease. The reasons should be self-evident: larger molecules simply cannot fit between the lipid molecules to make their way through. Small molecules

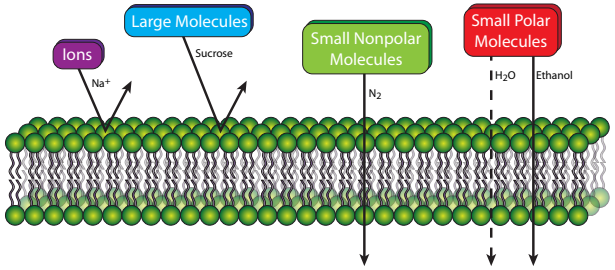


Figure 7. A pure phospholipid bilayer is inherently semi-permeable.

that can fit must be hydrophobic, otherwise the fatty acyl core of the membrane will repel them and block them from proceeding. Higher concentrations of cholesterol, by filling in gaps between phospholipid tails, decreases permeability even for small molecules that can normally pass through the membrane easily. Cells need far more than small nonpolar molecules for their material and energy requirements. Fortunately for life on earth, the membranes of living cells are not purely phospholipids, and as we will see, proteins embedded in the phospholipid bilayer can form conveyances for the transport of many different molecules in and out of the membrane.

In fact, the observation of saturation kinetics in glucose transport in erythrocyte membranes was the first indication of protein-mediated transport (the GLUT1 glucose transporter). Another telling observation was the finding that glucose permeability through erythrocyte membranes is a million times greater than that through an artificial lipid bilayer. The concentration of glucose in the blood is relatively high compared to that inside of most cells, so this is mediated transport, but passive transport since it is going down the concentration gradient. To facilitate the process by preventing a buildup of glucose concentration in the cell, the first step of glucose metabolism is phosphorylation to convert it into a different molecule, glucose-6-phosphate. Thus the concentration of glucose stays very low, and it flows readily from the bloodstream into the cell.

There are some obvious differences between transport of molecules directly through the lipid bilayer (nonmediated transport) and transport using a protein facilitator embedded in the membrane (mediated transport). *Nonmediated transport* is governed by diffusion: the solute moves from areas of high concentration to areas of low concentration, thereby eliminating the gradient. As long as a solute (A) can get through the membrane, its flux (J) is determined solely by concentration difference and the permeability (P) of the membrane: $J_A = P_A ([A]_{out} - [A]_{in})$ and the relationship between the flux across the membrane and the concentration differential is linear.

This is not the case in *mediated transport*. As the name implies, a protein intermediary is required, and alarm bells should be going off in your head saying, "there's a limit," to the number of available transport proteins at any given time. Therefore, just as we saw with enzyme kinetics in chapter 3, the flux of solutes going through a transporter is not linearly related to the concentration differential across the membrane, though there is still a concentration effect. Instead, the relationship is logarithmic, reaching a saturation plateau once all the available transport proteins are in use. At that point, increasing the concentration of the solute will not increase its flux across the membrane. Thus for simple unidirectional mediated transport of a solute (B), the flux (J) can be expressed as a value of the affinity of the transporter for the solute (K_M) and the concentration of the solute:

$$J_B = (J_{max}[B])/(K_M + [B])$$

Membrane permeability allows for the possibility of concentration gradients across membranes, which in turn have potential energy associated with the concentration differential across the membrane. This turns out to be a phenomenally important source of cellular energy, and is the basis for aerobic synthesis of ATP by oxidative phosphorylation (chapter 5). However, to have a meaningful discussion of how concentration differences across semipermeable membranes store energy, we should review some basic concepts first.

If a point source (e.g. a "glob") of a solute (e.g. honey) is placed into a solvent (e.g. tea), it starts to dissolve, and as it does so, the concentration of solute near the point source will start out much higher than the concentration towards the periphery of the container (e.g. teacup). Over time, the solute then diffuses from the point source outward in all available directions, and eventually the concentration of solute is equal at any point in teacup-space. This behavior is governed by the Second Law of Thermodynamics. The solute is initially concentrated, which means that its constituent molecules are relatively organized. By the second law, these molecules will tend toward chaos, moving away from the constraints of the initial point toward an area with lower concentrations of the solute.

In case you have already forgotten and are too lazy to flip back to chapter 3 to check, this is the law that states that the entropy of the universe (or any closed system) tends toward maximum.

Now, imagine a temporary wall around the point source. The natural tendency is for the solutes to spread out, so by preventing that movement, you have bottled up some potential energy. Of course, this is only potential energy if there is some chance that the solutes can eventually go through the barrier (e.g. the wall has windows that can be opened). If the solutes have absolutely zero chance of passing through, then there is no potential energy because there is no potential to get out and about. Recalling the Energy chapter in which the second law was introduced, the chemical potential energy of a solute is $G=RT\ \ln[A]+G'$, so the *chemical potential* difference across a membrane is then $\Delta G= RT\ \ln([A_i]/[A_o])$.

Now imagine this as something like a hydroelectric dam, where there is a great deal of pressure building up behind the dam, which can be utilized when some of the water is allowed through, powering turbines that generate electricity. In the biological case, there is concentration pressure building up both inside and outside the cell because the natural thermodynamic tendency is to bring the inside and outside concentrations of each solute to equilibrium. When this pressure is released by allowing the ions or other molecules to flow across the membrane, energy is released, and may be captured and used. The most direct example of this the proton-gradient-driven ATP synthase in the inner mitochondrial membrane (see Chapter 5, Metabolism), which contains a direct molecular equivalent to the turning of a water wheel with the flow of water. For another example, if we look at $[Na^+]$ in an animal cell, the extracellular concentration is much higher than that intracellularly. When a Na^+ channel is opened, the Na^+ ions rush inward to try to equalize the concentration of Na^+ inside and outside of the cell. Equilibrium is not actually reached in a living cell because Na^+ channels are tightly regulated and only open for short periods of time.

In cells, concentration gradients of ions are great energy sources because the lipid part of the membrane is strongly repellent to ions, preventing them from passing through, but the membrane is embedded with channels and transporters that can allow the ions through if and when they are open. Because ions have both concentration differentials and charge differentials across the membrane, the *electrochemical potential* difference across the membrane is represented by a modification of the chemical potential difference equation with a term that takes that electrical charge into account:

$$\Delta G=RT\ \ln([A_i]/[A_o]) + ZF\Delta\Psi$$

Z is the charge of the ion (e.g. +1 for Na^+, -1 for Cl^-, +2 for Ca^{++}), F is the Faraday constant (9.6485×10^5 C/mol), and $\Delta\Psi$ is the membrane potential. In an average animal cell, the membrane potential is approximately -70mV. The number is negative to show that the inside of the cell is negative with respect to the outside. Thus, again considering Na^+, not only is there a chemical gradient of more Na^+ ions outside the cell than inside, there

Potassium leak channels are structurally as well as functionally different from other potassium channels. Where most K^+ channels have one pore domain, the leak channels have two. While leak channels, by definition are not voltage gated, nor appreciably activated or inactivated, this is not true of all members of the tandem pore domain family of potassium channels. Interestingly, some (e.g. TASK-1) are mechanoreceptors, opening in response to membrane stretch, and others act as thermoreceoptors, with heat-sensitive activation (e.g. TREK-1).

is also a charge gradient of more positive charges outside the cell to inside, so both forces contribute to the energy of Na^+ flow into the cell. The equilibrium potential of one ion (e.g. Na^+) across a membrane is determined by the Nernst equation:

$$E_m = (RT/zF) \ln ([Na^+]_{out}/[Na^+]_{in})$$

which is extended in the Goldman equation (also Goldman-Hodgkin-Katz equation) that calculates membrane potential based on multiple ion gradients. For most animal cells, a good approximation of the overall membrane potential can be calculated using the three major gradients: Na^+, K^+, and Cl^-. There are, of course, other ion gradients, but their contributions are normally much smaller than these three.

$$V_m = (RT/F) \ln \frac{P_{Na+}[Na^+]_{out} + P_{K+}[K^+]_{out} + P_{Cl-}[Cl^-]_{out}}{P_{Na+}[Na^+]_{in} + P_{K+}[K^+]_{in} + P_{Cl-}[Cl^-]_{in}}$$

The membrane potential is relatively stable in non-excitable cells, but in neurons and muscle cells, the membrane potential is quite dynamic, so the membrane potential in a non-excited state is referred to in these cells as the resting potential. The membrane (resting) potential in most animal cells is around -70mV. This is due in large part to the presence of K^+ leak channels. These channels leak K^+ from the cell down the concentration gradient until the chemical potential difference of K^+ is at equilibrium with the membrane potential. In other words, the gradient pushing K^+ out will eventually be stopped by an equal force from the gradient pushing positive ions (including K^+) back in. There are also Na^+ and Cl^- leak channels, but there are far fewer and they contribute far less to the resting potential than K^+.

Although we most commonly think of water as the solvent in which "interesting" molecules (e.g. ions) diffuse, its concentration and movement across membranes has important biological consequences. *Osmosis* is a term that specifically refers to the diffusion of water across a membrane. In this case, water is considered a solute rather than a solvent, so that if a water-permeant liposome, embedded with aquaporin channels to allow the passage of water, is placed in a very salty saline solution, the cell will shrink because there is a lower ratio of water to dissolved salts outside of the cell than inside. This is a *hypertonic* solution relative to the cell. Therefore the water flows from the cell (higher water concentration) out into the saline (with lower water concentration). Conversely, a cell placed in distilled and deionized water will swell and potentially burst because the water rushes from the highest possible concentration (pure water) to a cytoplasm with lower water concentration (because dissolved in it are various ions and other molecules). This is an example of a *hypotonic* solution. An *isotonic* solution will have the same concentration of water inside and outside of the cell.

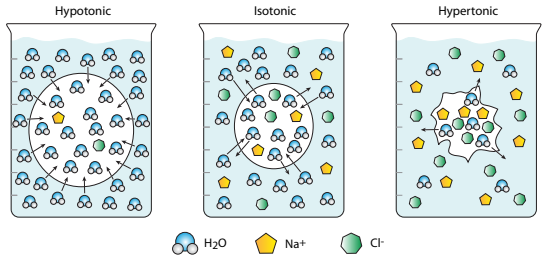


Figure 8. Osmosis. An artificial "cell" that is permeable to water but not to Na^+, K^+, or Cl^- is placed in a aqueous solutions of varying salinity.

Membrane Transport Proteins

Membrane proteins come in two basic types: integral membrane proteins (sometimes called intrinsic), which are directly inserted within the phospholipid bilayer, and peripheral membrane proteins (sometimes called extrinsic), which are located very close or even in contact with one face of the membrane, but do not extend into the hydrophobic core of the bilayer. Integral membrane proteins may extend completely through the membrane contacting both the extracellular environment and the cytoplasm, or they may only insert partially into the membrane (on either side) and contact only the cytoplasm or extracellular environment. There are no known proteins that are completely buried within the membrane core.

Integral membrane proteins (fig. 9) are held tightly in place by hydrophobic forces, and purification of them from the lipids requires membrane-disrupting agents such as organic solvents (e.g. methanol) or detergents (e.g. SDS, Triton X-100). Due to the nature of the bilayer, the portion of integral membrane proteins that lie within the hydrophobic core of the membrane are usually very hydrophobic in character, or have outward-facing hydrophobic residues to interact with the membrane core. These transmembrane domains usually take one of the two forms depicted in figures 8 and 14: alpha helices - either individually or in a set with other alpha helices, or barrel-shaped insertions in which the barrel walls are constructed of beta-pleated sheets. The hydrophobic insertions are bounded by a short series of polar or charged residues that interact with the aqueous environment and polar head groups to prevent the hydrophobic portion of the protein from sliding out of place. Furthermore, proteins can have multiple membrane-spanning domains.

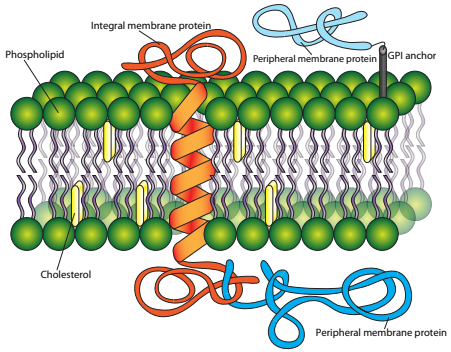


Figure 9. Integral (orange) and peripheral (blue) membrane proteins embedded in a phospholipid bilayer.

Peripheral membrane proteins (also shown in figure 9) are less predictable in their structure, but may be attached to the membrane either by interaction with integral

membrane proteins or by covalently attached lipids. The most common such modifications to peripheral membrane proteins are fatty acylation, prenylation, and linkage to glycosylphosphatidylinositol (GPI) anchors. Fatty acylation is most often a myristoylation (a 14:0 acyl chain) and palmitoylation (a 16:0 chain) of the protein. A protein may be acylated with more than one chain, although one or two acyl groups is most common. These fatty acyl chains stably insert into the core of the phospholipid bilayer. While myristoylated proteins are found in a variety of compartments, almost all palmitoylated proteins are located on the cytoplasmic face of the plasma membrane. Prenylated proteins, on the other hand, are primarily found attached to intracellular membranes. Prenylation is the covalent attachment of isoprenoids to the protein - most commonly isoprene (a C5 hydrocarbon), farnesyl (C15), or geranylgeranyl (C20) groups (fig. 10). GPI anchors (fig. 11) are found exclusively on proteins on the outer surface of the cell, but there does not appear to be any other commonality in their structures or functions.

Of course, not all membrane proteins, or even all transmembrane proteins, are transporters, and the many other functions of membrane proteins - as receptors, adhesion molecules, signaling molecules, and structural molecules - will be discussed in subsequent chapers. The focus here is on the role of membrane proteins in facilitating transport of molecules across the cell membrane.

Transport across the membrane may be either passive, requiring no external source of energy as solute travels from high to low concentration, or active, requiring energy expenditure as solute travels from low to high concentration (fig. 12). Passive transport can also

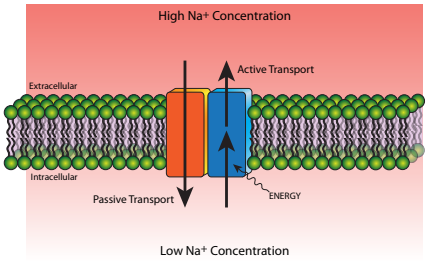


Figure 12. For Na^+ ions and animal cells, passive transport is inward, sending Na^+ from the high concentration outside the cell to the low concentration inside. Active transport requires energy such as ATP hydrolysis to push a Na^+ ion from the low concentration inside the cell to the higher concentration outside.

be divided into nonmediated transport, in which the movement of solutes is determined solely by diffusion, and the solute does not require a transport protein, and mediated passive transport (aka facilitated diffusion) in which a transport protein is required to help a solute go from high to low concentration. Even though this may sometimes involve a change in conformation, no external energy is required for this process. Nonmediated passive transport applies only to membrane-soluble small nonpolar molecules, and the kinetics of the movement is ruled by diffusion, thickness of the membrane, and the electrochemical membrane potential. Active transport is always a mediated transport process.

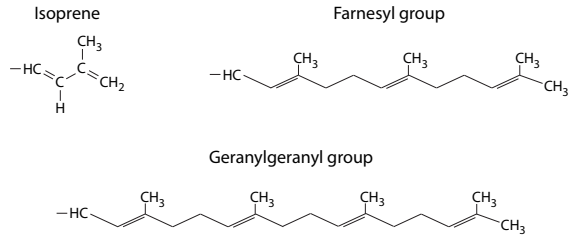


Figure 10. Prenylation

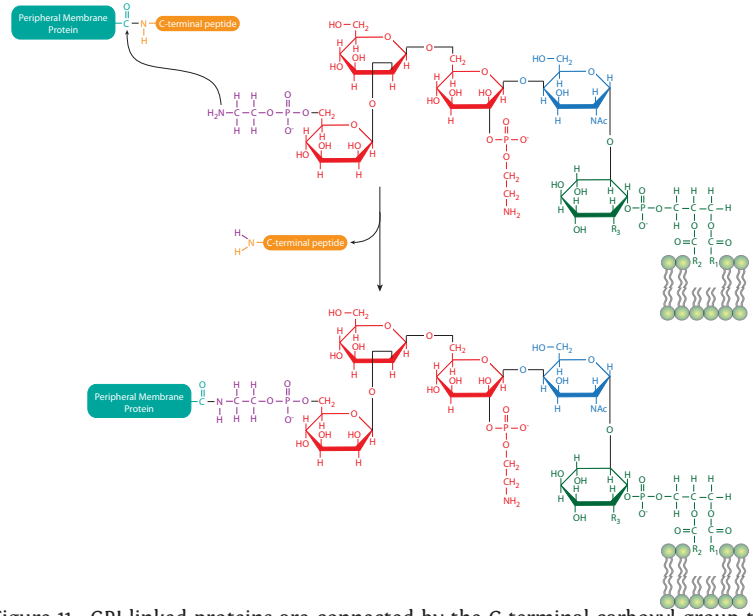


Figure 11. GPI-linked proteins are connected by the C-terminal carboxyl group to phosphoethanolamine, which is linked to a core tetrasaccharide of three mannose residues and one N-acetylglucoasmine, the latter of which is bound by glycosidic linkage to a phosphatidylinositol.

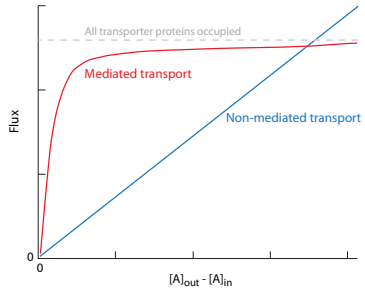


Figure 13. Non-mediated and Mediated transport: flux vs concentration.

Comparing the solute flux vs initial concentration in figure 13, we see that there is a linear relationship for nonmediated transport, while mediated passive transport (and for that matter, active transport) shows a saturation effect due to the limiting factor of the number of available proteins to allow the solute through. Once there is enough solute to constantly occupy all transporters or channels, maximal flux will be reached, and increases in concentration cannot overcome this limit. This holds true regardless of the type of transporter protein involved, even though some are more intimately involved in the transport than others.

Channels are essentially hands-off transport systems that, as the name implies, provides a passage from one side of the cell to another. Though channels may be gated - able to open and close in response to changes in membrane potential or ligand binding, for example - they allow solutes through at a high rate without tightly binding them and without changes in conformation. The solute can only move through channels from high to low concentration. The potassium channel depicted below (fig. 14A) is an example: there is a selectivity filter (14B) of aligned carbonyl oxygens that transiently

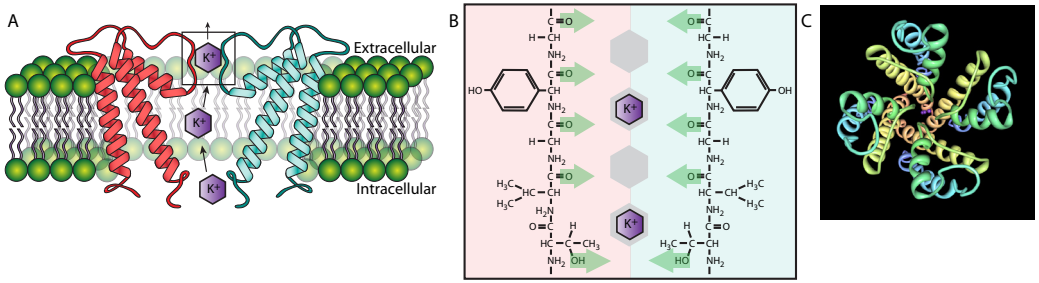


Figure 14. (A) Half of the tetrameric K+ channel showing two subunits. (B) Detail of the selectivity filter boxed in A. (C) Top-down image generated from data from the RCSB Protein Data Bank.

positions the K^+ ions for rapid passage through the channel, but it does not bind the K^+ for any significant period, nor does the channel undergo any conformational changes as a result of the interaction. Smaller Na^+ ions could (and on rare occasion do) make it

In addition to protein transporters, there are other ways to facilitate the movement of ions through membranes. Ionophores are small organic molecules, often (but not exclusively) made by bacteria, that help ions move through membranes. Many ionophores are antibiotics that act by causing the membranes to become leaky to particular ions, altering the electrochemical potential of the membrane and the chemical composition inside the cell. Ionophores are exclusively passive-transport mechanism, and fall into two types.

The first type of ionophore is a small mostly-hydrophobic carrier almost completely embedded in the membrane, that binds to and envelopes a specific ion, shielding it from the lipid, and then moves it through the cell membrane. The most studied carrier-type ionophore is valinomycin, which binds to K^+. Valinomycin is a 12-residue cyclic depsipeptide (contains amide and ester bonds) with alternating D- and L- amino acids. The carbonyl groups all face inward to interact with the ion, while the hydrophobic side chains face outward to the lipid of the membrane. Carrier ionophores are not necessarily peptides: the industrial chemical 2,4-dinitrophenol is an H^+ carrier and important environmental waste concern, and nystatin, an antifungal used to treat *Candida albicans* infections in humans, is a K^+ carrier.

The second type of carrier forms channels in the target membrane, but again, is not a protein. Gramicidin is a prototypical example, an anti-gram-positive antibacterial (except for the source of gramicidins, the gram-positive *Bacillus brevis*) and ionophore channel for monovalent cations such as Na^+, K^+, and H^+. It is impermeable to anions, and can be blocked by the divalent cation Ca^{++}. Like valinomycin, gramicidin A is also a made of alternating D- and L- amino acids, all of which are hydrophobic (L-Val/Ile-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp). Gramicidin A dimerizes in the membrane to form a compressed β-sheet structure known as a β-helix. The dimerization forms N-terminal to N-terminal, placing the Trp residues towards the outer edges of the membrane, with the polar NH groups towards the extracellular and cytoplasmic surfaces, anchoring the pore in place.

through the K^+ channel, but because they are too small to be properly positioned by the K^+ filter, they usually pop back out. It should be noted that this channel is a tetramer (14C) and the cutaway diagram in (14A) only shows half of the channel for clarity.

While most proteins called "channels" are formed by multiple alpha-helices, the porins are formed by a cylindrical beta sheet. In both cases, solutes can only move down the concentration gradient from high to low, and in both cases, the solutes do not make significant contact with the pore or channel. The interior of the pore is usually hydrophilic due to alternating hydrophilic/hydrophobic residues along the beta ribbon, which places the hydrophobic side chains on the outside, interacting with the membrane core.

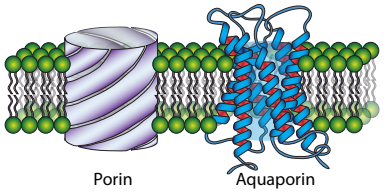


Figure 15.

Porins are primarily found in gram-negative bacteria, some gram-positive bacteria, and in the mitochondria and chloroplasts of eukaryotes. They are not generally found in the plasma membrane of eukaryotes. Also, despite the similarity in name, they are structurally unrelated to aquaporins, which are channels that facilitate the diffusion of water in and out of cells.

Transport proteins work very differently from channels or pores. Instead of allowing a relatively fast flow of solutes through the membrane, transport proteins move solutes across the membrane in discrete quanta by binding to the solute on one side of the membrane, changing conformation so as to bring the solute to the other side of the membrane, and then releasing the solute. These transport proteins may work with individual solute molecules like the glucose transporters, or they may move multiple solutes. The glucose transporters are *passive transport* proteins, so they only move glucose from higher to lower concentrations, and do not require an external energy source. The four isoforms are very similar structurally but differ in their tissue distribution within the animal: for example, GLUT2 is found primarily in pancreatic β cells, while GLUT4 is found mostly in muscle and fat cells.

On the other hand, the classic example of an *active transport* protein, the Na^+/K^+ ATPase, also known as the Na^+/K^+ antiport, utilizes the energy from ATP hydrolysis to power the conformational changes needed to move both Na^+ and K^+ ions against the gradient. Referring to the figure 16, in its resting state, the Na^+/K^+ ATPase is open to the cytoplasm and can bind three Na^+ ions (1). Once the three Na^+ have bound, the

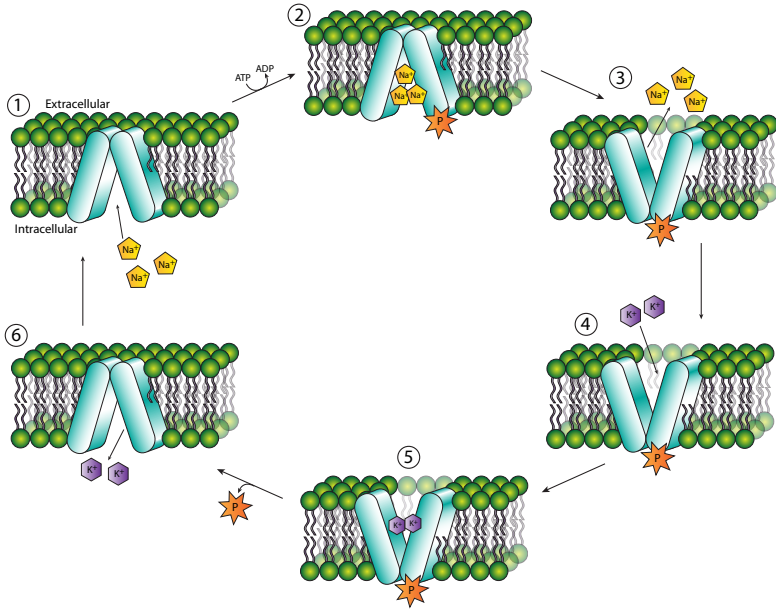


Figure 16. Active Transport by Na^+/K^+ ATPase. This enzyme pushes three Na^+ ions out of the cell and two K^+ ions into the cell, going against the gradient in both directions and using energy from ATP hydrolysis. [Note: some texts diagram this enzyme activity with separate binding sites for Na^+ and K^+, but recent crystallographic evidence shows that there is only one ion binding site that changes conformation and specificity.]

transporter can catalyze the hydrolysis of an ATP molecule, removing a phosphate group and transferring it onto the ATPase itself (2). This triggers a conformational change that opens the protein to the extracellular space and also changes the ion binding site so that Na^+ no longer binds with high affinity and drops off (3). However, the ion binding site specificity is also altered in this conformational change, and these new sites have a high affinity for K^+ ions (4). Once the two K^+ bind, the attached phosphate group is released (5) and another conformational shift puts the transporter protein back into its orginial conformation, altering the K^+ binding sites to allow release of the K^+ into the cytoplasm (6), and revealing Na^+ affinity once again.

The Na^+/K^+ ATPase is a member of the P-type family of ATPases. They are named because of the autophosphorylation that occurs when ATP is hydrolyzed to drive the transport. Other prominent members of this family of ATPases are the Ca^{++} -ATPase that pumps Ca^{++} out of the cytoplasm into organelles or out of the cell, and the H^+/K^+ ATPase, though there are also P-type H^+ pumps in fungal and plant plasma membranes, and in bacteria.

Unlike Na^+ or K^+, the Ca^{++} gradient is not very important with respect to the electrochemical membrane potential or the use of its energy. However, tight regulation of Ca^{++} is important in a different way: it is used as an intracellular signal. To optimize the effectiveness of Ca^{++} as a signal, its cytoplasmic levels are kept extremely low, with Ca^{++} pumps pushing the ion into the ER (SR in muscles), Golgi, and out of the cell. These pumps are themselves regulated by Ca^{++} levels through the protein calmodulin. At low Ca^{++} levels, the pump is inactive, and an inhibitory domain of the pump itself prevents its activity. However, as Ca^{++} levels rise, the ions bind to calmodulin, and the Ca^{++}-calmodulin complex can bind to the inhibitory region of the Ca^{++} pump, relieving the inhibition and allowing the excess Ca^{++} to be pumped out of the cytoplasm.

There are three other families of ATPases: the F-type ATPases are proton pumps in bacteria and mitochondria and chloroplasts that can also function to form ATP by running "backwards" with protons flowing through them down the concentration gradient. They will be discussed in the next chapter (Metabolism). Also, there are V-type ATPases that regulate pH in acidic vesicles and plant vacuoles, and finally, there are anion-transporting ATPases.

Hydrolysis of ATP, while a common source of energy for many biological processes, is not the only source of energy for transport. The active transport of one solute against its gradient can be coupled with the energy from passive transport of another solute down its gradient. Two examples are shown in figure 17: even though one is a *symport* (both solutes crossing the membrane in the same physical direction) and one is an

Cardiac glycosides (also cardiac steroids) inhibit the Na^+/K^+ ATPase by binding to the extracellular side of the enzyme. These drugs, including digitalis (extracted from the purple foxglove plant) and ouabain (extracted from ouabio tree) are commonly prescribed cardiac medications that increase the intensity of heart contractions. The inhibition of Na^+/K^+ ATPase causes a rise in $[Na^+]_{in}$ which then activates cardiac Na+/Ca++ antiports, pumping excess sodium out and Ca^{++} in. The increased $[Ca^{++}]_{cytoplasm}$ is taken up by the sarcoplasmic reticulum (see chap. XX), leading to extra Ca^{++} when it is released to trigger muscle contraction, causing stronger contractions.

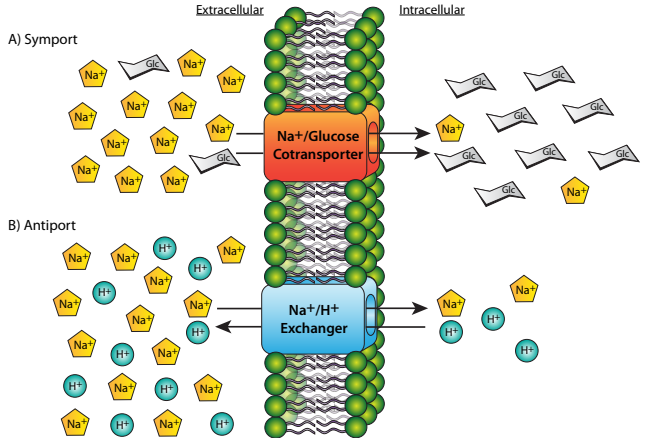


Figure 17. Symport and Antiport. The terms refer only to direction of solutes in or out of cell, not to energetics. In this symport, the energy release from passive transport of Na^+ into the cell is used to actively transport glucose in also. In the antiport example, Na^+ transport is again used, this time to provide energy for active transport of H^+ out of the cell.

Acetylcholine receptors (AchR), which are found in some neurons and on the muscle cells at neuromuscular junctions, are ligand-gated ion channels. When the neurotransmitter (acetylcholine) or an agonist such as nicotine (for nicotinic type receptors) or muscarine (for muscarinic type receptors) binds to the receptor, it opens a channel that allows the flow of small cations, primarily Na^+ and K^+, in opposite directions, of course. The Na^+ rush is much stronger and leads to the initial depolarization of the membrane that either initiates an action potential in a neuron, or in muscle, initiates contraction.

antiport (the two solutes cross the membrane in opposite physical directions), they both have one solute traveling down its gradient, and one solute traveling up against its concentration gradient. As it happens, we have used Na^+ movement as the driving force behind both of these examples. In fact, the Na^+ gradient across the membrane is an extremely important source of energy for most animal cells. However this is not universal for all cells, or even all eukaryotic cells. In most plant cells and unicellular organisms, the H^+ (proton) gradient plays the role that Na^+ does in animals.

The Action Potential in Neurons

The transport of solutes in and out of cells is critical to life. However, in neurons, the movement of ions has another crucial function in metazoan animals: production of action potentials used for neurotransmission. This specialization allows for extremely rapid transmission of information across long distances. An example my mentor would use when teaching basic neuroscience to schoolchildren was a bipolar neuron that extends from the toe to the brain. For information from the toe to be useful, it must reach the brain for processing very quickly. This signal may have to travel several meters in a giraffe, far more in whales. No chemical signal can move that quickly, and despite the popular simplification and dramatic depictions on television, electricity does not flow through neurons as though they were copper wires.

The reality does involve rapid changes to membrane potential. Since that is measured in volts and is a difference in electrical charge across the membrane, it is sometimes thought of colloquially as electrical transmission, but the mechanism is completely different from electrons flowing through a wire. Instead, there is a moving change in membrane potential due to sequential opening of ion channels along the length of the axon (the long thin part of the nerve cell). Generally, the signal starts with an

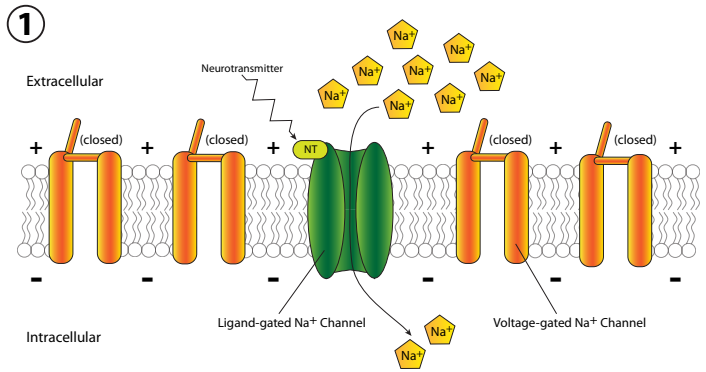


Figure 18.

A number of potent toxins have been used in the study of the AchR, including histrionicatoxin, an arrow poison extracted from a South American tree frog, *Dendrobates histrionicus*, curare (d-tubocurarine) isolated from certain plants, and α-bungarotoxin, which is isolated from the Taiwanese snake, the banded krait. All three act by competitive inhibition, blocking the opening of the AchR channel.

Acetylcholine spontaneously dissociates from AchR in about a millisecond, closing the channel. It could then be reactivated by the acetylcholine, but this is tightly regulated by the enzyme acetylcholinesterase (AchE), which is a GPI-anchored protein on the membrane of the target cell, and prevents overstimulation by rapid degradation of the acetylcholine. The military nerve gas sarin, which gained notoriety in a 1995 attack on the Tokyo subway system, works by inactivating AchE, thus causing overstimulation of AchR.

excitatory chemical signal, or neurotransmitter (fig. 18), that binds to a receptor on the neuron. This receptor may be linked to an ion channel or it may itself be a ligand-gated ion channel. In either case, the channel opens and Na^+ comes into the cell.

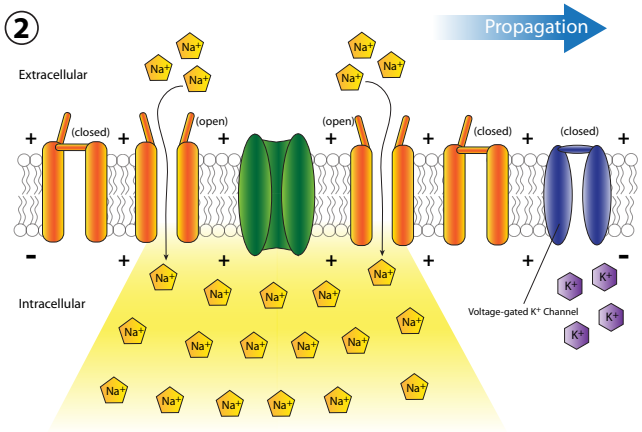


Figure 19.

The sudden influx of positive charges transiently and locally depolarizes the membrane (more + charges along the inside of the membrane than outside). The depolarization of the membrane near the channel causes all nearby voltage-gated Na^+ channels to transiently open (fig. 19), thus allowing a new rush of Na^+ ions into the cell that can then depolarize another small section of membrane. Although technically, channels open up on either side of the receptor, normally, the receptor is located on the main cell body, or soma, while most of the ion channels are lined up along the axon. Therefore, there is a de facto directionality to the propagation of the depolarization.

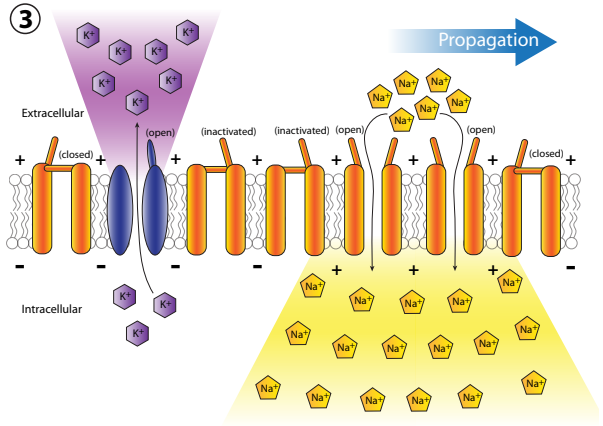


Figure 20.

Since neuronal transmission of information is critical to many systems, it is not surprising that a variety of organisms have evolved toxins that can paralyze or kill an attacker or prey. Interestingly, the primary target of the toxins is the voltage-gated Na+ channel, and not the K+ channel.

The toxin most widely used in research, is tetrodotoxin (TTX), which is derived from the skin and certain organs of the puffer fish (*Fugu rubripes*). This fish is considered a delicacy (primarily in Japan), and even when prepared by expert and specially-trained chefs, the small amount of residual TTX in the flesh causes a tingling numbing of the tongue as neural excitation is blocked. Interestingly (but unrelated to ion channels) fugu has a highly compressed genome - where most other eukaryotes have large swaths of non-coding regions, fugu has little, and its genes are very close together. In fact, it has almost as many predicted genes as the human genome in only 1/8th the DNA!

Saxitoxin (STX) is another poison that acts on the voltage-gated Na^+ channel. It is produced by the plankton that cause "red tide" (dinoflagellates of the genus *Alexandrium*, *Gymnodium*, and *Pyrodinium*) and is concentrated by filter-feeders such as mussels, oysters, and other shellfish. Interestingly, STX was once investigated by the US military as a nerve agent for chemical warfare, under the designation TZ. Both STX and TTX bind to the external surface of the closed voltage-gated Na^+ channel near the mouth of the pore, preventing opening, and thereby blocking Na^+ flow.

Batrachotoxin, on the other hand, binds to voltage-gated Na^+ channels when they are open rather than closed, so there is a massive continuous influx of Na^+. A product of another South American tree frog, *Phyllobates aurotaenia*, this toxin is used as a hunting poison by aboriginal tribes in the area, and is the most potent venom currently known. With a lethal dose of less than 2 µg/kg, batrachotoxin is roughly ten times more deadly than TTX. However, to put this in perspective, botulinum toxin, used pharmacologically as Botox© and the cause of botulism, is well over a hundred times more potent (though acting through a completely different mechanism - prevention of neurotransmitter release).

More voltage gated Na^+ channels are opened up quickly after their neighboring channels have opened and depolarized their section of the membrane, leading to a long chain reaction of voltage-gated depolarizations all the way down the axon (fig. 20). Once the signal is propagating down the axon, there are two questions that should come into your head. First, neurons are not disposable cells, so how are all these opened channels reset and the membrane re-polarized for the next action potential? And second, why wouldn't the depolarization wave become bidirectional since each little depolarization area spreads out in both directions from the opened Na^+ gate?

In fact, the answers to both questions are closely related, and has to do with the gating mechanism of voltage-gated ion channels (fig. 21). Voltage-gated ion channels have two gates: one that opens in response to an increase in membrane potential, and one that closes the channel after a short period of time. This means that there are three potential states for most voltage-gated ion channels: closed, open, and inactivated.

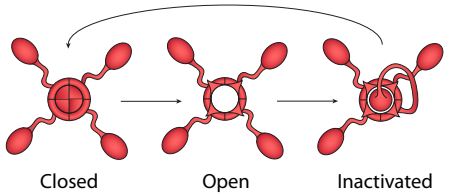


Figure 21. Three states of a voltage-gated K^+ channel.

The inactivated state occurs when the second gate (more like a plug, really) closes, because this is voltage-insensitive. The plug eventually comes out of the pore and the voltage-sensitive gate is set back in place to the closed state. Voltage-sensitive K^+ channels are tetramers, as shown here, and each subunit carries a potential inactivating domain. The choice of which domain appears to be random. Voltage-sensitive Na^+ channels, on the other hand, also have four transmembrane regions, but they are all part of a single protein, and the channel only has one inactivation gate/domain. However, the three-state gating mechanism is still the same.

Back to the action potential: each new depolarization opens the next adjacent set of voltage gated Na+ channels, and so on. In a neuronal axon, which is where action potentials occur, the movement of the depolarizations happens very quickly and unidirectionally. It happens quickly because the axon is a very long thin projection of the cell, so the volume is small and therefore the influx of Na+ can quickly depolarize a long section of membrane. It happens unidirectionally because the previously opened voltage-sensitive Na+ channels go into a refractory inactivated state that cannot be reopened immediately.

Thus when the adjacent wave of depolarization hits, the previously opened channels do not open again, and the opening of more voltage-gated channels continues unidirectionally away from the start.

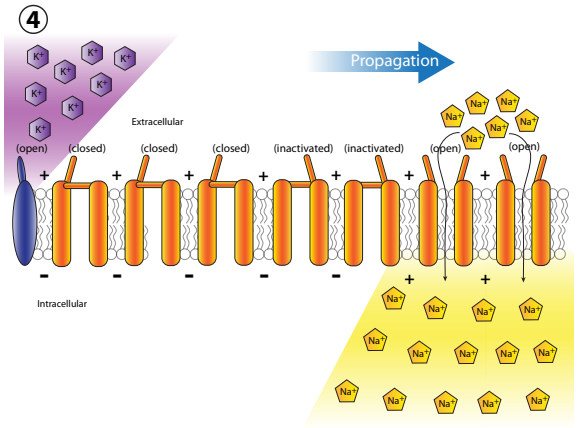


Figure 22.

But wait, there's more! Just because the Na^+ channels are locked closed doesn't mean that the membrane goes back to normal. What repolarizes the membrane behind the action potential? The voltage-gated potassium channels, which are interspersed among the Na^+ channels (note purple ions and channels in figs. 20, 22). They open more slowly than the sodium channels (fig. 23) and thus reach peak flux shortly after the peak flux of Na^+. Thus, the recovery phase, or repolarization back towards the resting potential, is helped by both cessation of the inward Na+ movement and continued outward K+ movement.

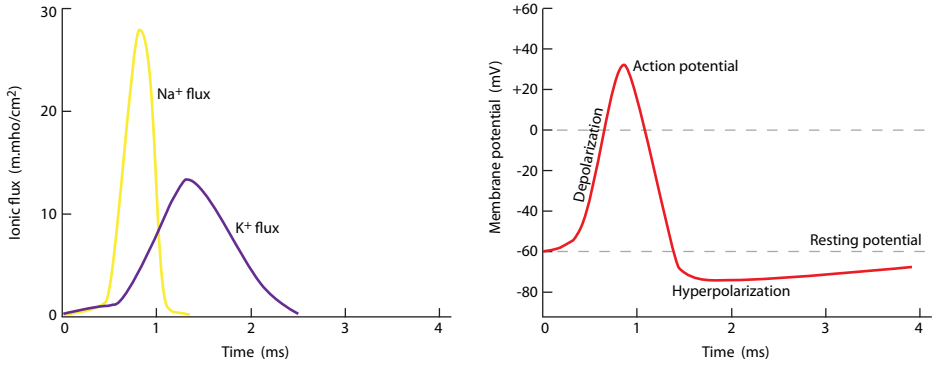


Figure 23. The contributions of Na^+ flux (depolarizing) and K^+ flux (repolarizing) to the neuronal action potential. Remember that the Na^+ movement is into the cell, while K^+ is moving out.

Since all action potentials behave the same way from the standpoint of changes in membrane potential, the difference between stronger and weaker nerve signals is in the frequency/rate of action potential firing, not in magnitude of the ion flux.

Metabolism 1 :

Catabolic Reactions of the Cell

Using this book: This book is designed to be used in both introductory and advanced cell biology courses. The primary text is generally on the left side of the vertical divider, and printed in black. Details that are usually left to an advanced course are printed in blue and found on the right side of the divider. Finally, additional biomedically relevant information can be found in red print on either side of the divider.

Life requires energy. As our discussion of biomolecules pointed out, the major functional components of the cell are mostly polymers - long chains of smaller individual molecular units. Each addition of a small link to the chain costs energy. Chemical reactions that build up complex molecules from simple ones are known as *anabolic* reactions. Conversely, heterotrophic organisms such as animals ingest food made up of these large polymers, which, when broken down in the digestive process, release energy for use in maintaining and building that organism. Such chemical reactions, in which complex molecules are broken down to simpler components, are classified as *catabolic* reactions. Taken as a group of reactions within a cell or even an organism, they can be referred to as the cell's or organism's anabolism or catabolism. The sum total of both types of reactions is the metabolism. Nearly all metabolic reactions are catalyzed by enzymes in order to keep up with the energy and material demands of the cell. In fact, the discussion of some of the metabolic processes in this chapter will almost seem to be laundry lists of enzymes. We will begin with one such list in describing the catabolism of the simple sugar, glucose, through the process of glycolysis.

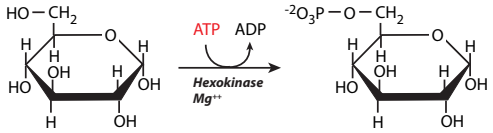
Glycolysis

Whether the cell is prokaryotic or eukaryotic, one of its basic methods for generating usable energy is glycolysis. This process uses glucose, which is the most common energy source for most cells. However, glucose cannot be directly broken down to provide energy for the cell, and glycolysis is a process that breaks it down in a series of reactions to create adenosine triphosphate (ATP), which is the most common energy "currency" of the cell. That is, ATP *can* release usable energy in a single reaction.

Glucose, being a 6-carbon sugar, has a large amount of potential energy stored in its bonds. However, since it is thermodynamically stable, it would take the investment of a lot of external energy to release the energy of glucose in one step (i.e. lighting it on fire to break it down into CO_2 and H_2O), and not only is it impossible for cells to

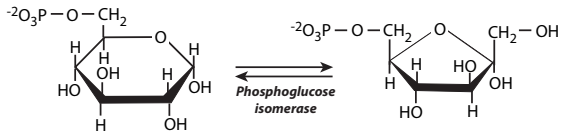
generate that kind of energy at once, the cell has no mechanism to use all the energy released at one instant in time. Most of it would be wasted as excess heat. Instead, the cell uses enzymes to destabilize and break down the sugar through a series of conversions into intermediate compounds. The basic process and enzymes involved are as follows.

1. Glucose is phosphorylated by hexokinase to make Glucose-6-Phosphate. The enzyme is so named because it is a kinase (puts a phosphate group on) that acts on a hexose (six-carbon sugar). In this case, it places the phosphate on the 6-carbon of glucose. However, it can also phosphorylate other hexoses such as fructose and mannose (all in the D- conformation). There are two major reasons this is good for the cell. Since glucose concentration is higher inside the cell than outside, there is pressure for it to move back out of the cell. By converting it to G6P, it is no longer part of the glucose concentration gradient, and it has a charged phosphate group making it nearly impossible to leak out of the membrane. The addition of the phosphate also increases the energy in the molecule, making it less thermodynamically stable, so that it can be broken down. This reaction requires the use of ATP as a phosphate donor and the energy needed to attach it. That is, energy is used in this step, not producing it. Consider it more of an investment of energy though, since by the end of glycolysis, more ATP is produced than used.



Glucose Glucose-6-phosphate

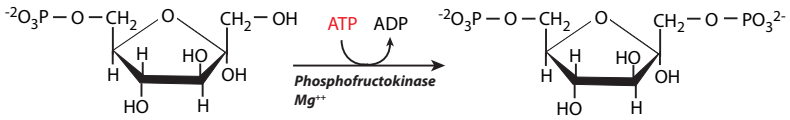
2. Glucose-6-Phosphate is converted to Fructose-6-Phosphate by phosphoglucose isomerase. As the name implies, the isomerase simply rearranges the existing atoms within the G6P to make the F6P without removal or addition of any atoms.



Glucose-6-phosphate Fructose-6-Phosphate

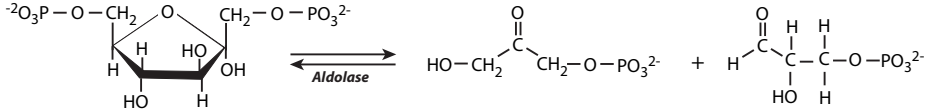
Hexokinase requires ATP in the form of a complex (to the 2nd and 3rd phosphate groups) with a divalent cation, typically Mg^{++} in vivo. ATP alone is actually a competitive inhibitor of hexokinase. The product, G6P, also functions as an inhibitor, thus providing some measure of feedback regulation. In fact, muscle cells using glycogen stores convert the glycogen directly to G6P, so hexokinase activity is very low in those cells.

3. Fructose-6-Phosphate is phosphorylated by phosphofructokinase (PFK) to Fructose-1,6-bisphosphate. There is again an investment of an ATP to provide the phosphate group and the energy to attach it.



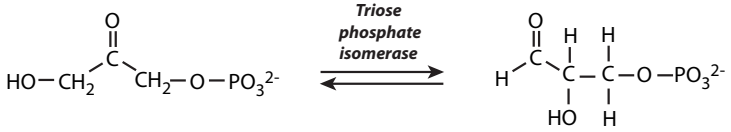
Fructose-6-Phosphate Fructose-1,6-bisphosphate

4. The Fructose-1,6-bisphosphate is cut in half by aldolase, yielding a molecule of dihydroxyacetone phosphate and a molecule of glyceraldehyde-3-phosphate.



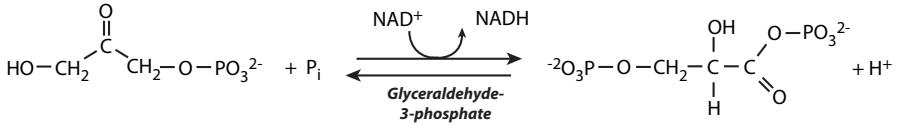
Fructose-1,6-bisphosphate Glyceraldehyde-3-phosphate Dihydroxyacetone phosphate

5. The G3P can participate in the next reaction, but the dihydroxyacetone phosphate, despite its similarity, cannot. So, it needs to be rearranged by triose phosphate isomerase, which converts it to another molecule of glyceraldehyde-3-phosphate.



Glyceraldehyde-3-phosphate Dihydroxyacetone phosphate

6. Each of the two molecules of G3P generated from the glucose molecule now undergo oxidation catalyzed by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the presence of NAD+ and inorganic phosphate (P_i). Each of these reactions produces 1,3-bisphosphoglycerate, which has a high-energy phosphate group, and NADH. NADH is a high energy electron carrier (electron comes from G3P). In eukaryotes with an aerobic environment, this NADH will likely be used to help generate ATP through the tricarboxylic acid cycle (aka Krebs cycle or citric acid cycle). In anaerobic situations, the NADH will participate in fermentation for reasons discussed in the next section.

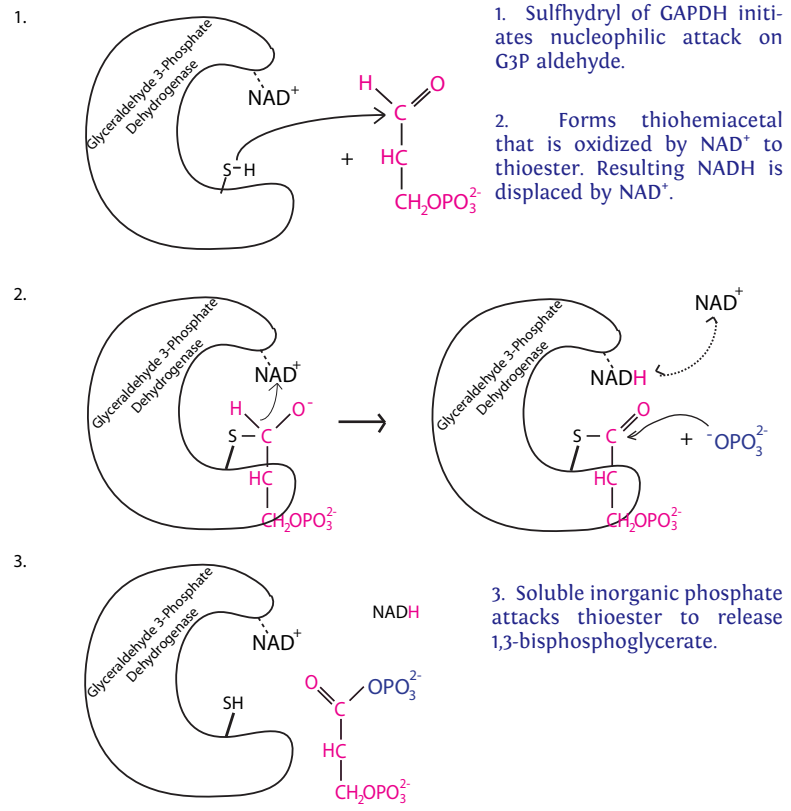


Glyceraldehyde-3-phosphate 1,2-Bisphosphoglycerate

3. PFK is an important regulator of glycolysis. It is a tetrameric protein, and each subunit has two binding sites for ATP: one is the normal substrate site, the other is an inhibitory site such that binding of ATP lowers the enzyme's affinity for F6P. ATP is not the only regulator of PFK activity: AMP is also a positive regulator of PFK, and can increase it up to 5-fold.

4. There are two classes of aldolases: class I are found in animals and plants, while class II are found in fungi and bacteria. Class I require no cofactors, but class II require a divalent cation (physiologically usually Fe^{++} or Zn^{++}).

5. Triose phosphate isomerase is a "perfect enzyme" that catalyzes the formation of product as fast as the enzyme and substrate can make contact in solution (i.e. rate is purely diffusion-limited).



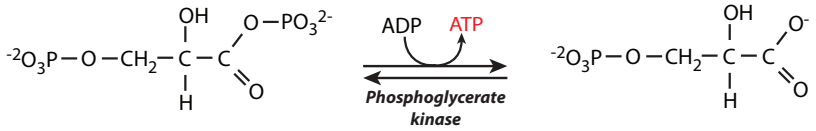
1. Sulfhydryl of GAPDH initiates nucleophilic attack on G3P aldehyde.

2. Forms thiohemiacetal that is oxidized by NAD^+ to thioester. Resulting NADH is displaced by NAD^+.

3. Soluble inorganic phosphate attacks thioester to release 1,3-bisphosphoglycerate.

Figure 1. NAD^+ is reduced by glyceraldehyde-3-phosphate dehtdrogenase to form NADH. The NADH is released by substitution with another NAD^+.

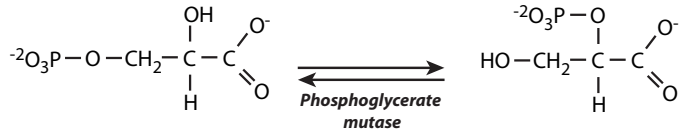
7. The phosphate group on the 1-carbon of 1,3-bisphosphoglycerate is transferred to ADP by phosphoglycerate kinase to make 3-phosphoglycerate and ATP (finally!). From the two molecules of G3P entering step 6, we get two molecules of ATP to provide energy for the cell in this step. Recalling the earlier investment of ATP (in steps 1 and 3), the reaction has only "broken even" at this point. 2 in, 2 out.



1,2-Bisphosphoglycerate 3-Phosphoglycerate

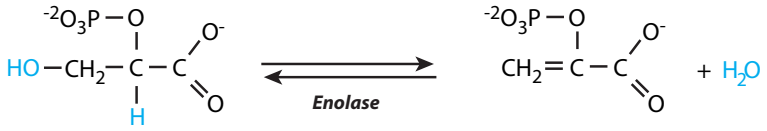
The name of the enzyme suggests that a phosphate is added to phosphoglycerate. This is not a mistake: remember that enzymes can catalyze reactions in either direction, depending on reaction conditions. Under conditions of high phosphoglycerate and ATP, phosphorylation of phosphoglycerate would occur. However, the physiological conditions are a relatively high concentration of the 1,3-bisphosphoglycerate in comparison to relatively low levels of phosphoglycerate thus driving the reaction "backwards" with respect to the naming of the enzyme.

8. The 3-phosphoglycerate is then rearranged by phosphoglycerate mutase to make 2-phosphoglycerate. This molecule has a higher free energy of hydrolysis than when the phosphate group is on the 3-carbon.



3-Phosphoglycerate 2-Phosphoglycerate

9. That energy is used to create ATP, as the 2-phosphoglycerate undergoes dehydration by enolase to make phosphoenolpyruvate (PEP).



2-Phosphoglycerate Phosphoenolpyruvate

The action of phosphoglycerate mutase is not just the intramolecular phosphate group transfer that it seems to be at first glance. The enzyme must first be activated by phosphorylation, and it is the enzyme's phosphate that is added to the 2-carbon of 3PG. The doubly-phosphorylated intermediate then transfers its 3-phosphate to the enzyme, and 2PG is released.

PEP is made because hydrolysis of the phosphate from 2PG does not release enough energy to drive phosphorylation of ADP to ATP. PEP hydrolysis, on the other hand, releases significantly more than needed.

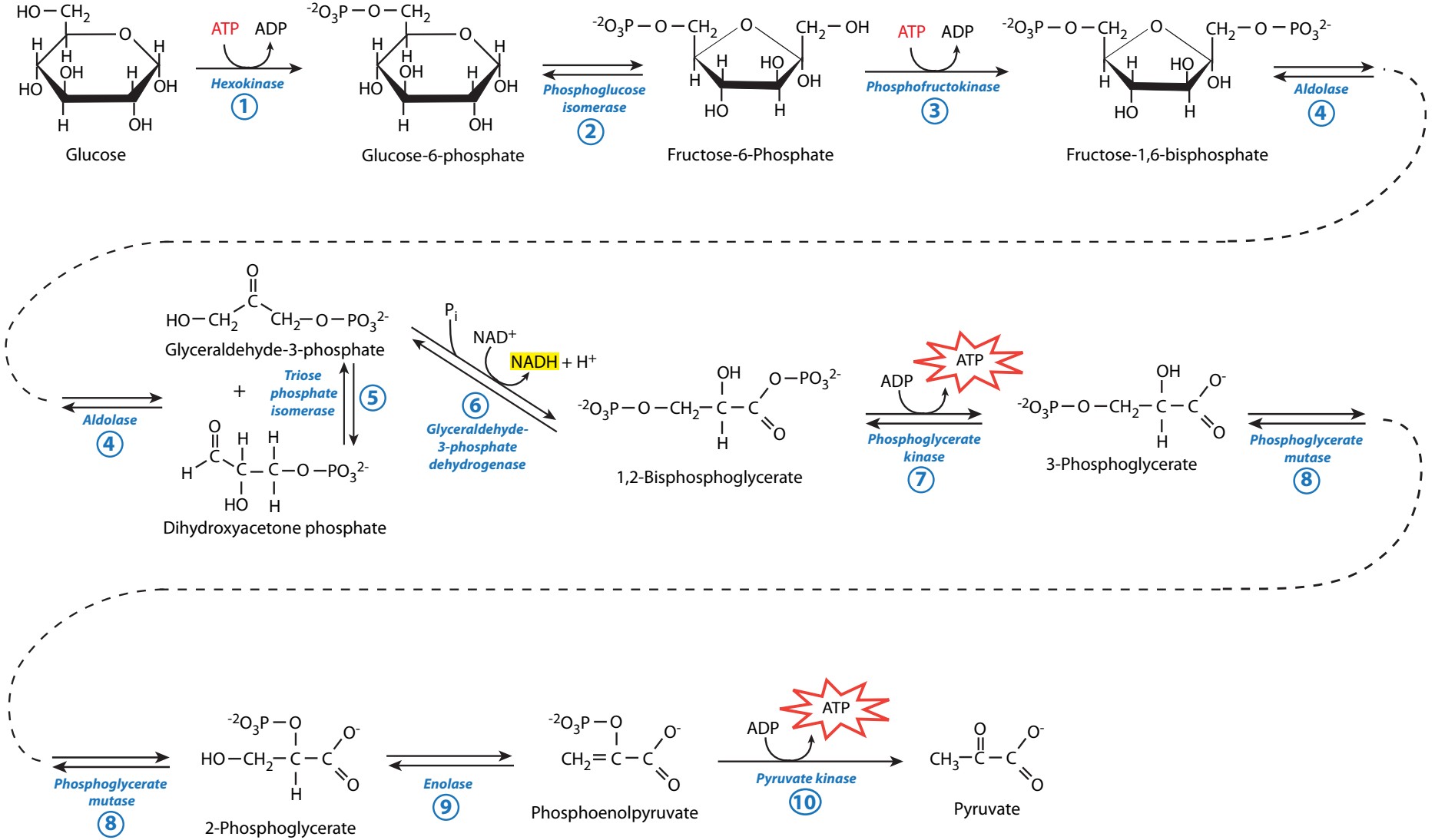
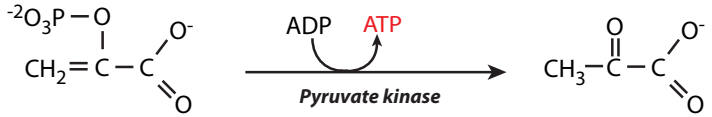


Figure 2. Overview of Glycolysis.

Bidirectional arrows indicate enzymes used for both glycolysis and gluconeogenesis. Unidirectional arrows indicate enzymes that only function in glycolysis. Note that reactions 6-10 are occurring in duplicate (two G3P from one glucose).

10. Pyruvate kinase then transfers a high energy phosphate group from PEP to ADP, producing an ATP for use by the cell, and pyruvate.



Phosphoenolpyruvate Pyruvate

Keeping in mind the doubling of reactions from steps 6-10 (splitting of fructose-1,6-bisphosphate generates two G3P), the total usable energy production from glycolysis of a single molecule of glucose is 4 ATP and 2 NADH. However, the net ATP production is only 2 ATP if we remember the initial investment of two ATP in the early steps. *Not really anything to write home about*. Furthermore, although the NADH and pyruvate can participate in the tricarboxylic acid cycle in aerobic eukaryotic situations to generate a significant amount of ATP, in anaerobic situations, they do not produce usable energy.

Thus anaerobic ATP production, i.e. glycolysis, is far less efficient at extracting energy from a glucose molecule than aerobic ATP production, which can generate approximately 38 ATP per glucose. On the other hand, when a lot of ATP must be generated quickly, glycolysis is the mechanism of choice, in cells such as the fast-twitch fibers of skeletal muscle. These cells actually have very few mitochondria because glycolysis can produce ATP at a much higher (up to 100 times) rate than oxidative phosphorylation.
What happens to the pyruvate and NADH? In aerobically metabolizing cells, they go to the mitochondria for the TCA cycle and oxidative phosphorylation. In anaerobes, they undergo fermentation.

Fermentation

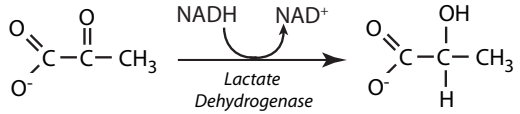
When the average person hears the word "fermentation" he probably thinks about alcohol. As you no doubt recall, glycolysis gave us some usable energy in the form of ATP, and then there are the other products, NADH and pyruvate. As we shall see in the next section, if the cell is eukaryotic and oxygen is available, then those molecules can help make more ATP. If no oxygen is available or the cell is just a lowly prokaryote, it undergoes fermentation to produce either lactate or ethyl alcohol. Why does the cell need lactate or ethanol? It doesn't, although the lactate can contribute to overall metabolism. What the cells do need is NAD^+, so that glycolysis can continue beyond step 6. Without fermentation, continued glycolysis would convert all of the NAD^+ to NADH, and then be stuck, unable to continue. So the primary reason for fermentation, whichever path it takes, is to regenerate NAD^+ from the NADH.

Pyruvate kinase requires not only divalent Mg^{++} as with most other kinases, but also K^+. The enzyme works in two steps: the ADP attacks the PEP phosphorus to make ATP and enolpyruvate. Enolpyruvate is then converted to its keto- tautomer.

Note that the NADH produced by glycolysis in the cytoplasm does not *directly* participate in oxidative phosphorylation in the mitochondria since the inner mitochondrial membrane is impermeable to it, but it sends a "virtual equivalent" into the mitochondria via one of two pathways: the aspartate-malate shuttle combines malate-α-ketoglutarate antiports, aspartate-glutamate antiports, and metabolite interconversion by transaminase with malate dehydrogenase to oxidize NADH cytoplasmically and use the energy generated to reduce NAD+ in the mitochondrial matrix; the other pathway is a DHAP shuttle system, in which NADH is used to reduce dihydroxyacetone phosphate to glycerol-3-P using a cytoplasmic glycerol-3-phosphate dehydrogenase, and the cycling the DHAP to glycerol-3-P via a flavoprotein dehydrogenase embedded in the inner mitochondrial membrane. This flavoprotein dehydrogenase takes the electrons from glycerol-3-P to make $FADH_2$, which can participate in the electron transport chain.

The DHAP or glycerophosphate shuttle is less efficient than the malate-aspartate shuttle, generating approximately 2 ATP vs 2.7 ATP per NADH. However, it can operate even when the concentration of cytoplasmic NADH is low, as happens in tissues/cells with a very high metabolic rate (including skeletal muscle and brain), while the malate-aspartate shuttle (prevalent in liver and heart) is sensitive to the relative concentration of NADH and NAD^+.

In lactate fermentation, the pyruvate is converted to lactate by lactate dehydrogenase. This reaction requires the oxidation of NADH, which thus provides NAD^+ to the cell for continued glycolysis.

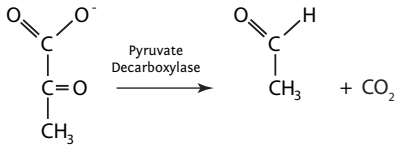


Pyruvate Lactate

For many cells, the lactate is a waste product and excreted. In fact, this is the case with most muscles: the lactate is carried by the blood from the muscle cells to the liver, where it can be converted to glucose. Thus, although lactate is formed at high rates when muscles are overworked and become fatigued, it is not directly the cause of muscle fatigue. As oxygen availability cannot keep up with aerobic ATP production, and larger and larger proportions of the ATP generated come from glycolysis with fermentation. The current model of muscle fatigue posits that it is due to acidification of the muscle cell as it undergoes rapid glycolysis.

However, in some tissues and cell types, particularly in the heart and brain of higher animals, cell membranes are highly permeable to lactate, the cells can readily convert the lactate to pyruvate, and since these are highly oxygenated tissues, the pyruvate is then used for the TCA cycle and oxidative phosphorylation to generate ATP. In fact, some non-neuronal support cells in the brain (astrocytes) generate and excrete copious lactate which is taken up by the neighboring neurons to fuel ATP production.

In alcohol fermentation, pyruvate is first acted upon by pyruvate decarboxylase, which liberates a CO_2 molecule and produces acetaldehyde. Acetaldehyde is then acted upon by alcohol dehydrogenase, using NADH, generating NAD^+ and ethanol. Here, like with lactate fermentation, the desired product is the regenerated NAD+. Ethanol is excreted, and in most animals, is converted to acetaldehyde and then acetic acid, before finally ending up as acetyl-CoA.



As with glycolysis, fermentation can and does take place in cells that are able to make ATP by oxidative phosphorylation. The relative contribution of glycolysis and oxidative phosphorylation to the cellular ATP pool is determined dynamically by physiological conditions.

The TCA cycle

So you're a hot young eukaryote sporting all kinds of fancy internal membranous organelles, with a need to prove yourself better than the old guard prokaryotes – what do you do? Well, make scads of ATP, of course, and seemingly effortlessly at that, using only the dregs left over after glycolysis has taken its pass at a glucose molecule: NADH and pyruvate. Glycolysis in eukaryotes, as befits its prokaryotic origins, happens in the cytoplasm. The TCA cycle happens inside the matrix of the *mitochondria*, a double-membraned organelle.

The pyruvate needs to make its way from the cytoplasm, through both outer and inner mitochondrial membranes, and into the mitochondrial matrix. How does this work? The outer membrane is porous, being riddled with large relatively nonspecific anion channels known as voltage-dependent anion channels (VDACs), and will readily admit pyruvate. In contrast, the inner mitochondrial membrane is highly impermeable, and entry of pyruvate is specifically regulated by a pyruvate transporter protein.

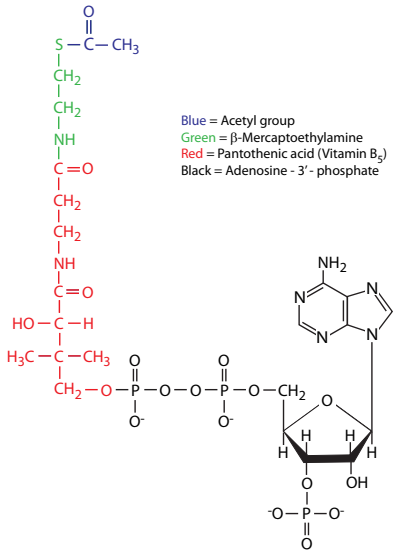
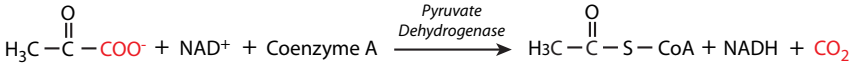


Figure 3. Acetyl-Coenzyme A is composed of four distinct molecular parts.

1. Once the pyruvate enters the mitochondrial matrix, the pyruvate dehydrogenase complex (consisting of three enzyme subunits E1, E2, and E3) converts it to acetyl-CoA (fig. 3) for entry into the tricarboxylic acid cycle (TCA). This reaction generates NADH and liberates CO_2.



Now consider the breakdown of glucose. Recall that the complete breakdown of that six-carbon sugar should yield six single-carbon molecules of carbon dioxide. In glycolysis, the glucose is broken down into two molecules of three-carbon pyruvate. As the pyruvate is converted to acetyl-CoA, one CO_2 is generated per molecule of pyruvate. That leaves just four carbons (in two 2-carbon molecules of acetyl-CoA) out of the original glucose 6. The TCA cycle will liberate each of those carbons as CO_2 as well. Knowing the reactions in which the remaining carbons are released is a good way to study the first half of the TCA cycle.

As an integral part of coenzyme A, vitamin B_5, or pantothenic acid, is needed for the TCA cycle, and therefore, for normal efficient generation of ATP. However, unlike some other vitamins, B_5 deficiency is rare, and usually associated with deficiency in other vitamins or general malnourishment. On the other hand, deficiency in another B vitamin involved in pyruvate dehydrogenase activity (fig. 4), thiamine (B_1), can lead to disease symptoms known as beriberi.

Arsenic, or more specifically arsenic-containing compounds such as arsenite and arsenate, are poisonous to cells by interfering with this reaction. The arsenic compound can interact with dihydrolipoamide, resulting in cyclization by bonding of both sulfhydryl sulfurs to the arsenic atom. This prevents E2 from working, and acetyl-CoA cannot be generated for ATP production via TCA cycle and oxidative phosphorylation. It should be noted that these arsenic compounds also affect other sulfhydryl-containing compounds, and within the context of the TCA cycle, it can also inactivate α-ketoglutarate dehydrogenase, which is similar to the pyruvate dehydrogenase.

Beriberi (also beri-beri) symptoms are classified in two groups: wet beriberi affects the cardiovascular system with symptoms such as enlarged heart, lung congestion, shortness of breath, swelling of lower legs, congestive heart failure; dry beriberi (also known as Wernicke-Korsakoff syndrome)affects the nervous system: symptoms include polyneuritis in both central and peripheral nervous system, leading to pain, tingling, loss of sensation in extremities, loss of muscle function or paralysis of the lower legs, vomiting, nystagmus, and eventually mental confusion, speech difficulties, coma and death.

1. Pyruvate dehydrogenase complex (fig. 4) is actually an amalgamation of three enzymes. That is, there are three subunits to the complex: pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3). These three subunits are associated by noncovalent bonds. The pyruvate dehydrogenase subunit E1 acts first, using the cofactor thiamine pyrophosphate (TPP) to help remove a CO_2 from the pyruvate to generate hydroxyethyl-TPP.

This is immediately used as a substrate by E2, resulting in regeneration of TPP and reactivation of pyruvate dehydrogenase, and also making the intermediate acetyl-dihydrolipoamide. Coenzyme A, which is also a substrate for E2, has a sulfhydryl group that attacks the acetyl group of acetyl-dihydrolipoamide. The acetyl group is immediately transferred to Coenzyme A to form the Acetyl-CoA that enters the TCA cycle.

The final step is for the dihydrolipoamide to be oxidized back to lipoamide by E3. It is this oxidation step that generates the NADH from NAD+.

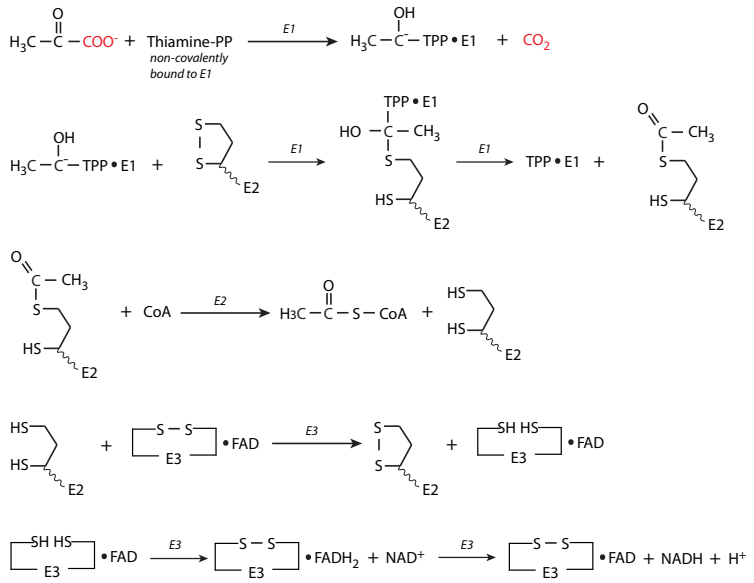
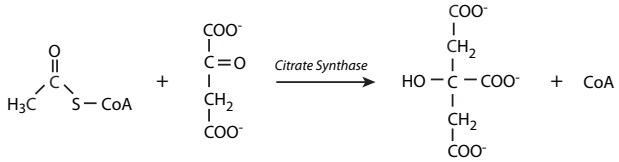


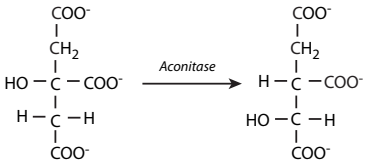
Figure 4. Pyruvate dehydrogenase is a complex of three enzymatic subunits, E1, E2, and E3, which work in sequence as depicted here.

Genetic deficiencies in the pyruvate dehydrogenase complex lead to similar, but more immediately severe problems. The most common mutation is an X-linked dominant mutation in the α subunit of E1. PDC loss-of-function mutations as well as mutations in pyruvate carboxylase and mutations in cytochrome oxidase, are considered causes of Leigh's disease, which is often neonatally fatal, though exceptions have survived a little over a decade. Severe lactic acidosis and the inability to generate sufficient energy, especially in neurons (which would normally be able to metabolize fat - see section of fatty acid catabolism - but cannot in these patients) and muscle cells, is the underlying cause of the symptoms.

2. Acetyl-CoA enters the tricarboxylic acid cycle as a substrate of citrate synthase, which adds it to oxaloacetate to make citrate. This is the reason that this cycle is also called the citric acid cycle. Citrate, having three carboxyl groups, is a tricarboxylic acid, leading to the name that this text will use. The other common name for this is the Krebs cycle, as it was first proposed by Hans Krebs in 1937.

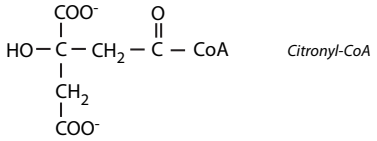


3. In the next step, aconitase rearranges citrate to make isocitrate.

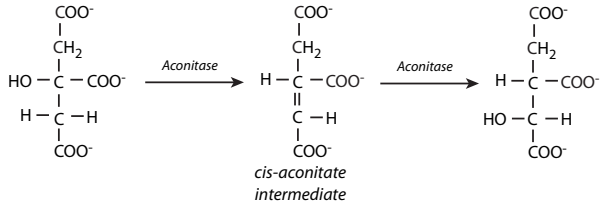


Sodium fluoroacetate, also known as compound 1080, is a common pesticide that is used primarily against rodent and other mammalian pests, and can act in humans if ingested. Once introduced to the organism, it can be converted to fluoroacetyl-CoA and then to fluorocitrate, which then acts as a competitive inhibitor of aconitase. As such, the poisoning most severely and quickly affects tissues with high energy needs. No effective antidotes are recognized for lethal doses of fluoroacetate poisoning.

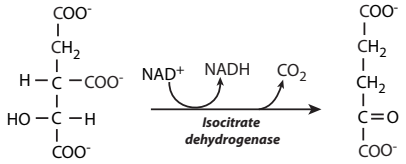
2. Citrate synthase is a dimeric enzyme that in its native form has a binding cleft for oxaloacetate. Binding of oxaloacetate causes a conformational shift closing the oxaloacetate binding site, locks it in and simultaneously reveals the acetyl-CoA binding site. The current model for this reaction involves three steps: Acetyl-CoA is converted to an enol intermediate, which attacks the oxaloacetate to form citronyl-CoA (S-citryl-CoA), which is then hydrolyzed to citrate and Coenzyme A.



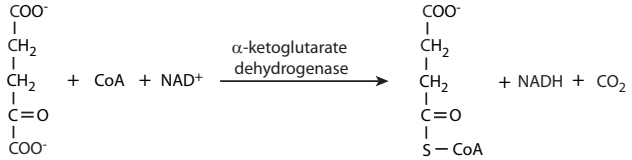
3. Aconitase pushes citrate into a cis-aconitate intermediate, which is then converted to isocitrate. Interestingly, while aconitase contains an Fe-S cluster, it does not appear to participate in redox reactions as is usually the case for such groups. Instead, its purpose is to hold the cis-aconitate in its place within the enzyme as it [the cis-aconitate] undergoes a bizarre molecular flip on its way to isocitrate.



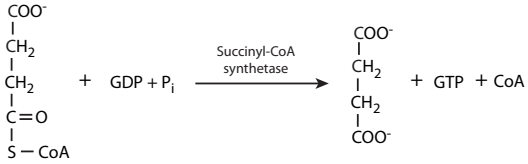
4. Isocitrate is a substrate for isocitrate dehydrogenase, which transfers a high energy electron from the isocitrate onto NAD+ to make NADH and α-ketoglutarate. This reaction also liberates one CO_2. For those keeping track at home, that leaves two more carbons from the six in glucose.



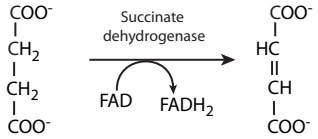
5. Alpha-ketoglutarate is also oxidized (by α-ketoglutarate dehydrogenase) generating NADH and succinyl-CoA. Like acetyl-CoA, this CoA-associated compound contains a high energy thioester bond. This reaction liberates the final CO_2 from the glucose.



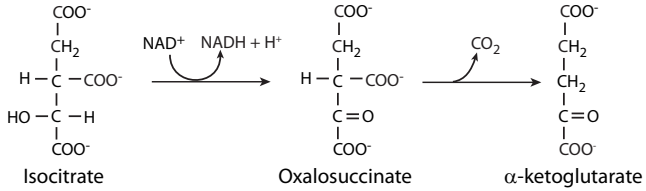
6. The CoA is regenerated by succinyl-CoA synthetase, which also forms succinate and GTP or ATP. This GTP is energetically an ATP equivalent, and made in animal cells. Bacterial and plant homologues of this enzyme use ADP and make ATP. Formation of this ATP/GTP is possible because the high-energy thioester bond of succinyl-CoA is broken.



7. Next, the succinate is oxidized. The enzyme that does this, succinate dehydrogenase, is a little different from the other dehydrogenases because this one happens to be embedded in the inner mitochondrial membrane, and instead of transferring the electron to NAD^+, the electron is transferred to FAD, making $FADH_2$, and fumarate. The energy in $FADH_2$ can also be used to power ATP production similar to the energy in NADH.



4. The NAD^+-dependent isocitrate dehydrogenase is actually found in two isoforms in mammals: an NAD+-utilizing isoform in the mitochondrial matrix, and an isoform that uses $NADP^+$ that is found in the cytosol as well as the mitochondria. The reaction starts with NADH-generating oxidation of isocitrate to oxoalosuccinate, which is then decarboxylated with the help of a Mn^{++} or Mg^{++} cofactor to release the carbon dioxide and form α-ketoglutarate.

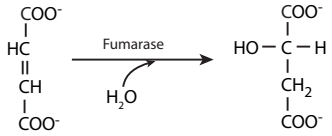


5. α-ketoglutarate dehydrogenase is very similar to pyruvate dehydrogenase complex structurally and mechanistically. There are three enzymes: the α-ketoglutarate dehydrogenase, a dihydrolipoyl transsuccinylase, and dihydrolipoyl dehydrogenase. Also similar to pyruvate dehydrogenase complex, the end product is a molecule containing a high energy thioester bond.

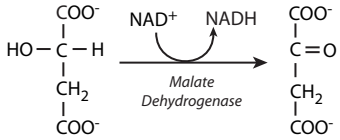
6. Succinyl-CoA synthetase first brings together the succinyl-CoA and inorganic phosphate (in solution within the mitochondrial matrix as well as the cytosol) to produce succinyl phosphate and liberate the CoA. Then the phosphate is transferred from the succinyl phosphate, to the enzyme itself temporarily, which then drops the succinate. And finally, the phosphate is transferred to GDP/ADP.

7. Even though the usual intro-class simplification is that $FADH_2$ is roughly an equivalent to NADH, the situation is actually more complicated. Unlike NAD^+, and for that matter, unlike most occurences of FAD, the FAD is covalently bound to the succinate dehydrogenase. Therefore, it is not a soluble metabolite, nor is it available to be reoxidized quite like NADH. It is, of course, reoxidized. But, this occurs within the context of the electron transport chain (where it is known as Complex II), with the help of Coenzyme Q.

8. Fumarase catalyzes the addition of water to the fumarate to generate malate.



9. Malate is oxidized by malate dehydrogenase in the final reaction of the TCA cycle to generate more NADH, and oxaloacetate, the latter of which can then be added to



acetyl-CoA to start the cycle all over again. Now that the complete cycle has been described, it should be noted that the regulation of this cycle is primarily through acetyl-CoA and oxaloacetate availability, as well as NADH concentration. As respiration rate increases, NADH levels drop as they are oxidized to make ATP (see next section). This drop in [NADH] then causes an increase in oxaloactetate, which is then used by citrate synthase. [Acetyl-CoA] is regulated by its synthesis by pyruvate dehydrogenase. On the reverse side of regulation, both NADH and succinyl-CoA are strong inhibitors of a-ketoglutarate dehydrogenase. Thus as NADH is used up, the enzyme is disinhibited and increases its production of more NADH.

Having taken the 2 pyruvates created during glycolysis through the TCA cycle to complete oxidation into CO_2, what is our intrepid eukaryotic hero left with? Two ATP-equivalents (GTPs) six NADH, and two $FADH_2$. This hardly seems to be a treasure trove of usable energy worth boasting about. Fortunately, the mitochondrion is not finished. Next, the high energy electrons from will take a ride on the electron transport chain, and via the magic of oxidative phosphorylation, produce ATP by the bucket.

8. The carbon double bond of fumarate is attacked by a hydroxyl (OH-) to form a carbanion transition, which then takes on a proton (H+) from the enzyme to form the malate. Fumarase is protonated as it also binds fumarase, and deprotonated at the end to form the malate.

9. Malate dehydrogenase is similar in structure to the lactate dehydrogenase and the alcohol dehydrogenase mentioned in the fermentation section. Energetically, the standard free energy change of this reaction is very highly positive (29.7 kJ/mol) but the oxaloacetate is quickly converted to citrate, so that more formation of oxaloactetate is favored over malate formation.

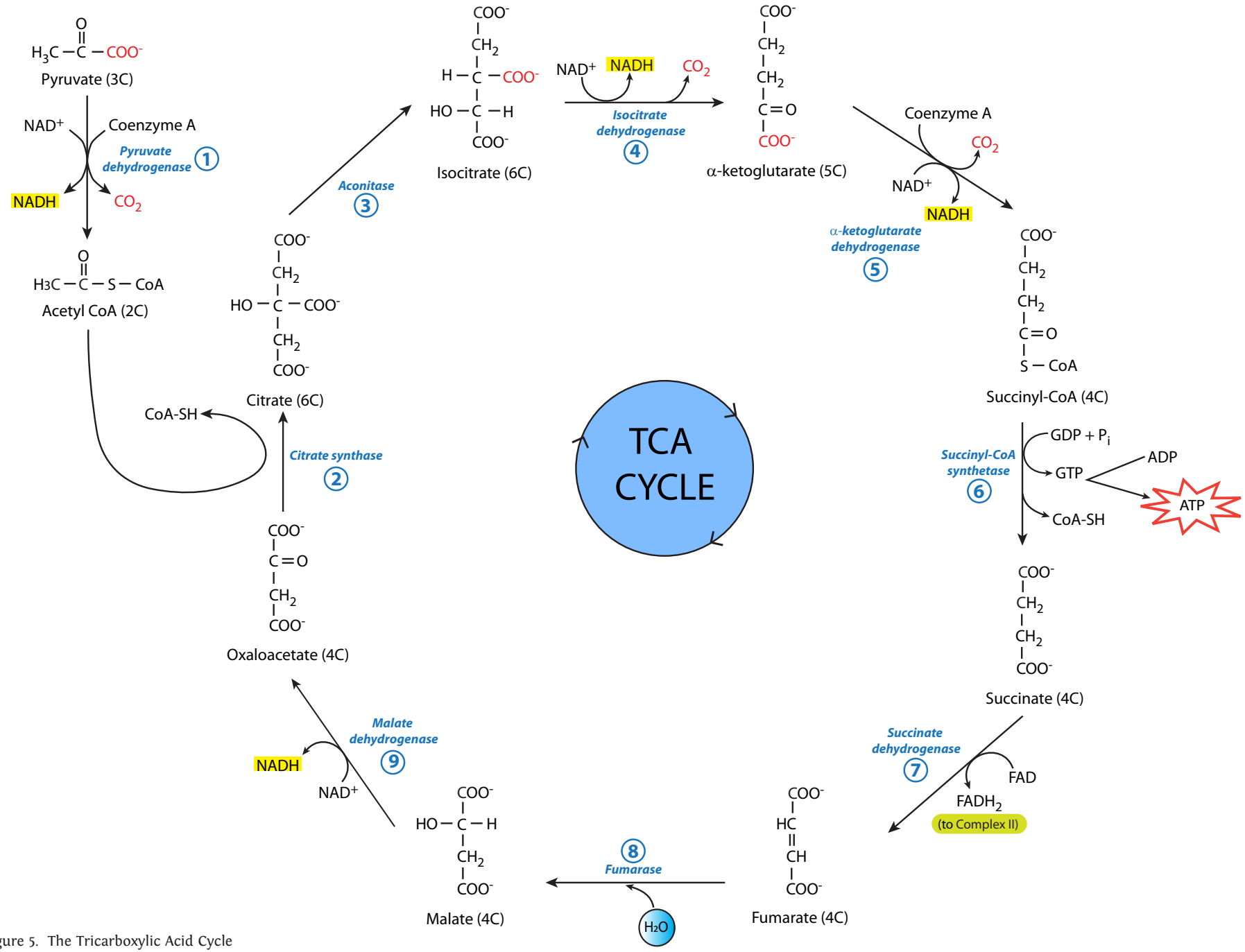


Figure 5. The Tricarboxylic Acid Cycle

Oxidative Phosphorylation

Oxidative phosphorylation denotes the phosphorylation of ADP into ATP, utilizing the energy from successive electron transports (hence the "oxidative"). The basic concept is that oxidation of NADH, being highly exergonic, can generate the energy needed to phosphorylate ADP. Since oxidation of NADH by oxygen can potentially release 52 kCal/mol (218 kJ/mol), and the energy needed to phosphorylate ATP is approximately 7.5 kCal/mol (30.5 kJ/mol), we should be able to expect the formation of several ATP per oxidized NADH. Indeed, this is what happens, although not directly. As noted with the breakdown of glucose, a one-step oxidation would generate too much energy for cellular processes to handle, and most would be wasted. So instead of oxidizing NADH directly with O_2, the electrons are transferred to a series of gradually lower-energy carriers until finally reaching oxygen. This sequence is the *electron transport chain*.

The electron transport chain is based on the activity of four major enzyme complexes (conveniently called complexes I-IV) embedded in the inner mitochondrial membrane, along with some small easily diffusible electron carriers to move the electrons from one complex to the next. These complexes are present in extremely high numbers as befits their necessity in generating energy, comprising nearly 75% of the inner membrane mass (in comparison, the plasma membrane of an average eukaryotic cell has a protein concentration closer to 50%). An overview of the process is shown in figure 6: as previously noted, electrons are stripped from NADH, and eventually end up on oxygen. As the electrons are moved to lower-energy carriers, energy is released and used to pump protons from the mitochondrial matrix into the intermembrane space.

Complex I is an NADH dehydrogenase. Shown in yellow in figure 6, its purpose is to remove a pair of electrons from NADH and transfer them onto ubiquinone (Coenzyme Q or CoQ), a small hydrophobic electron carrier that can then carry the electrons to complex III. This is a multistep process that involves first transferring the electrons onto an associated flavin mononucleotide (FMN) molecule, which then transfers the electrons to a set of iron-sulfur moieties connected to the enzyme complex itself (structure in fig. 7). Finally, the electrons are moved onto ubiquinone. As these transfers occur, the energy that is released during these transfers powers the pumping of 4 H^+ ions across the inner mitochondrial membrane. Complex I is inhibited by rotenone, a pesticide used primarily against insects and fishes.

We'll take a mental pass on complex II for now, and hit it at the end of this roll call. The reasons will be apparent then.

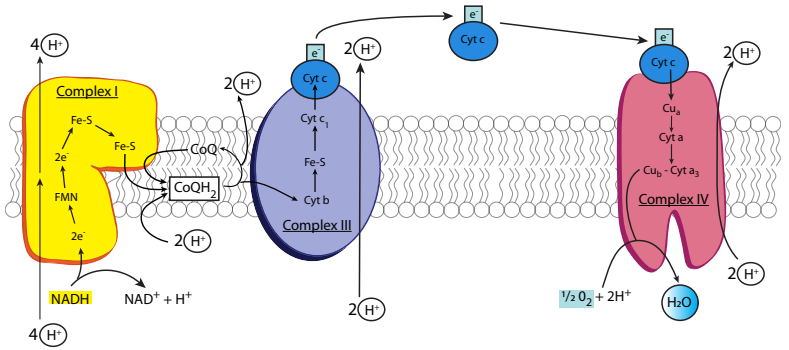


Figure 6. The primary electron transport pathway in mitochondria. Complexes I, III, and IV are shown. Complex II is pictured in fig. 10. The complexes are all buried in the inner mitochondrial membrane. Protons are being pumped from the matrix to the intermembrane space utilizing energy sapped from the high energy electrons as they move from a higher-energy carrier to a lower-energy carrier.

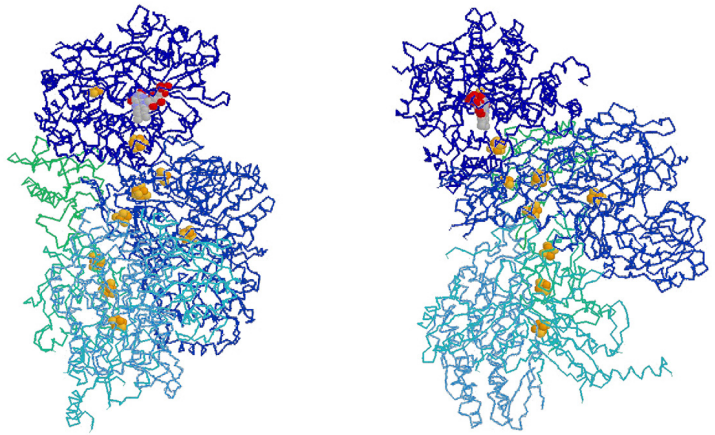


Figure 7. Although the size of complex I varies somewhat across species, the rough L-shaped three-dimensional conformation is constant. The FMN is located in the larger portion of the complex, while the ubiquinone docking site is located in the short branch. In the figure above, which depicts two aspects (rotated 90°) of NADH dehydrogenase complex, the FMN is shown in grey and red, while Fe-S centers are shown in orange and yellow. The figure was generated from data in the RCSB Protein Data Bank.

Complex III is also known as the cytochrome bc_1 complex (fig. 6, purple). The purpose of this complex is to pass the electrons from ubiquinone onto cytochrome c. The use of ubiquinone is important here, because it is stable with either two, or just one, extra electron. Cytochrome c, on the other hand, can only carry one electron. So, this complex docks ubiquinone, and holds it until it has passed its first electron onto cytochome c, which then moves onto complex IV, and then its second electron onto another cytochrome c. With each transfer, two protons are pumped across the membrane.

Finally, cytochrome c drops the electron off to complex IV, cytochrome c oxidase (fig. 6, red). Cytochrome c oxidase accomplishes the final step: transferring electrons onto oxygen atoms to make water. The really interesting thing about this process is that the enzyme must hold onto the electrons as they are transferred one at a time from cytochrome c, until it holds four electrons. Then, it can transfer one pair to each of the oxygen atoms in molecular oxygen (O_2). It is very important to do this because transferring any less than all four electrons would lead to the creation of reactive oxygen species (ROS) that could cause damage to the enzymes and membranes of the mitochondria.

In fact, some well known poisons act at exactly this point. Both cyanide and carbon monoxide can bind with higher affinity than oxygen at the heme in complex IV. Since neither can accept electrons, the effect is just as though no oxygen was available.

Although cytochrome c oxidase is sometimes abbreviated COX, it is *not* the target of the COX-2 inhibitors that are used pharmaceutically in pain management, e.g. Bextra, Celebrex, or Vioxx. That refers to a family of enzymes known as the cyclooxygenases.

Oxygen is absolutely required. If oxygen is not available, there is no place to transfer the electrons, and very quickly, the electron transport chain is halted and carriers such as cytochrome c and CoQ cannot release their electrons and eventually there are no more available carriers. Similarly, when that happens, NAD^+ is not regenerated, so the TCA cycle is also stuck. This leaves only the anaerobic non-oxygen-requiring glycolysis-fermentation cycle for generating ATP.

We now return to complex II (see fig. 10). We mentioned complex II as succinate dehydrogenase when discussing the TCA cycle. It also participates in the electron transport chain by passing electrons to ubiquinone. However, rather than transferring electrons that originated from NADH like the other three complexes of the electron transport chain, the electrons originate from the covalently bound electron carrier $FADH_2$ (flavin adenine dinucleotide), which received the electrons from succinate, as described in the TCA cycle section. Once the electrons have been passed to ubiquinone, it then moves on to complex III to drop off those electrons to cytochrome c, and the rest of the electron transport chain continues. FAD, the oxidized form of $FADH_2$, is then ready to participate in the next redox cycle.

The purpose of this electron transport chain, with respect to ATP generation, is the pumping of H^+ from the mitochondrial matrix into the intermembranous space. Since the concentration of protons is higher in the intermembrane space, it will take energy to move them against the concentration gradient, which is where our high-energy

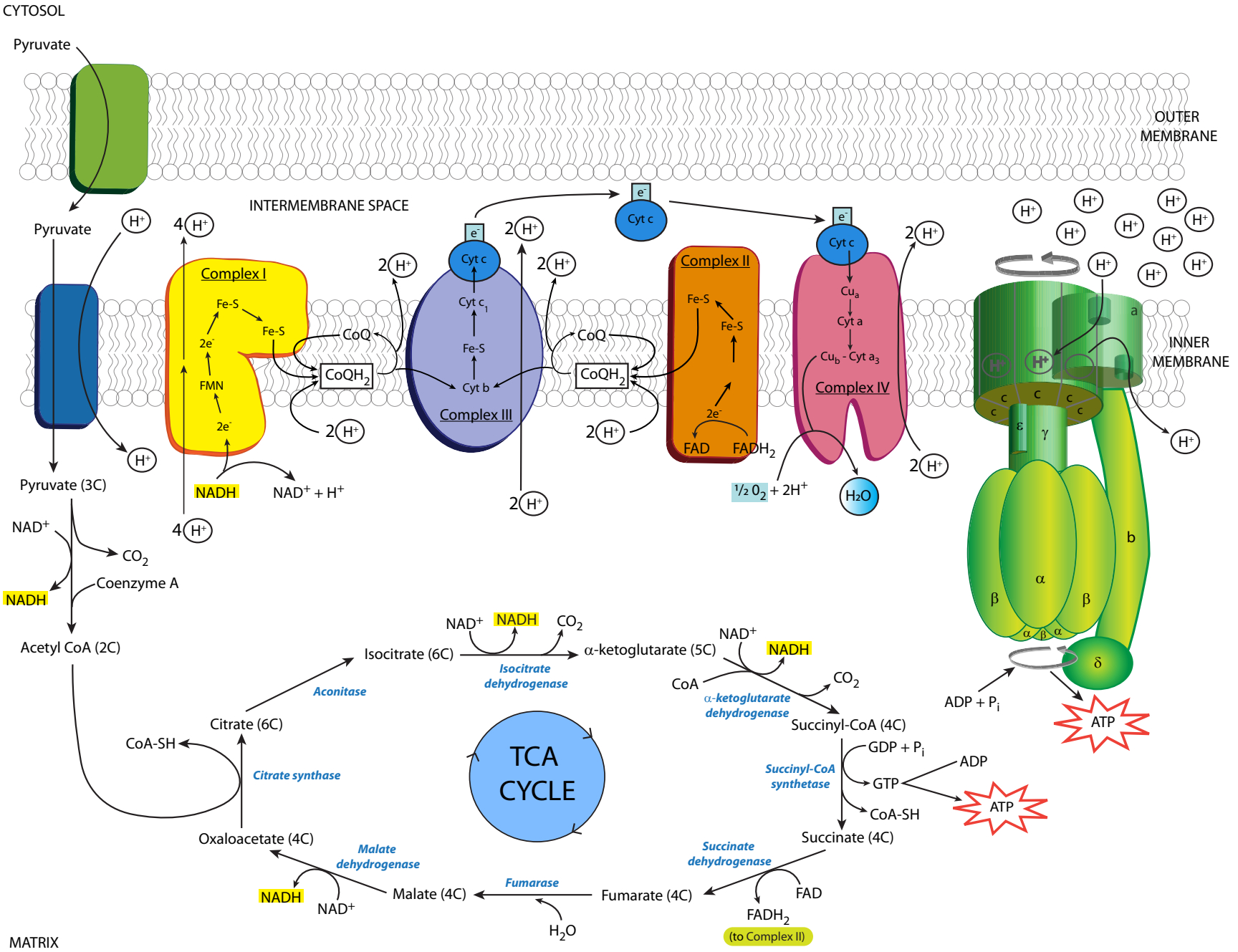


Figure 10. Catabolic reactions of the mitochondria.

electrons come into the picture. As they move from one carrier to the next, they are moving from a higher to a lower energy state. This implies that some energy is lost from the electron, and some of that energy is tapped by the enzymes of the electron transport chain to move protons from the matrix to the intermembrane space.

There are two methods by which the protons are moved: the redox loop, and the proton pump. The proton pump, which is the method by which complex IV moves protons, is the easier to understand: H^+ is bound on the matrix side of the enzyme in its reduced state (after it has received an electron), and a conformational shift occurs upon reoxidation to open the enzyme up to the intermembrane side, and the H^+ is released. The redox loop, which occurs in complex I, and in complex III in a variation called the Q cycle, essentially posits that an initial redox center requires the binding of both the high energy electron and a proton from the matrix side of the membrane. When the electron is transferred to the next redox center in the chain, a proton is released to the intermembrane space.

Whatever the mechanism, what is the point of all this proton pumping? As you might suspect, using up energy to pump an ion against its concentration gradient isn't done for the fun of it. Rather, this generates significant potential energy across the inner mitochondrial membrane. And, it so happens that there is an enzyme that can convert that energy into the physiologically useful chemical form of ATP. This enzyme is, not surprisingly, named ATP synthase (fig. 8). It is also referred to in some texts as the F_1F_0-ATPase, based on its reverse activity (at the expense of ATP, it can pump protons), and the fact that it can be broken down into two major functional units: F1 which can hydrolyze but not synthesize ATP and is a soluble protein, and F0 which is an insoluble transmembrane protein.

The ATP synthase is an extraordinary example of an enzyme that transforms the energy inherent in a concentration gradient across a membrane into mechanical energy, and finally into chemical bond energy. It is descriptively called a "rotary engine" because the very generalized sequence of events is as follows: protons flow down their gradient through a proton channel subunit of the ATP synthase, in flowing down the gradient, energy is released, this energy causes rotation of a multisubunit "wheel"-like subunit attached to a spindle/axle (γ subunit) which also spins. The spinning of this asymmetrically shaped spindle unit causes conformational changes in the catalytic subunit (made of the α and β subunits) it is attached to, changing an ADP+Pi binding site to a catalytic site that can "squeeze" the molecules together into an ATP, and then finally open up to release the ATP (fig. 9).

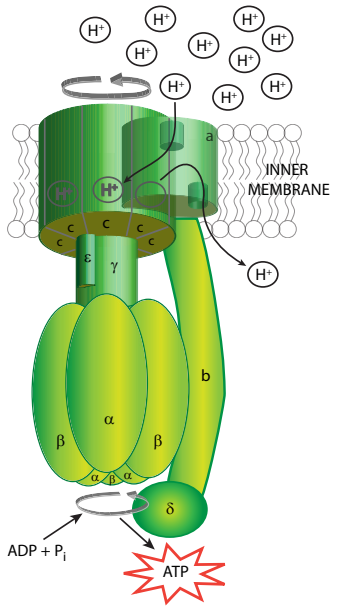


Figure 8. ATP synthase. As protons pass through the ATP synthase, they release energy by going from high concentration to low. This energy drives the rotational movement of the shaft and the generation of ATP.

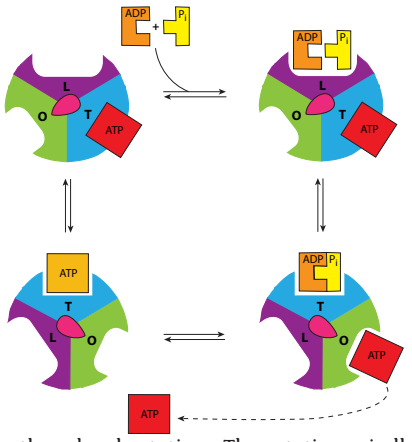


Figure 9. ATP synthase head rotation. The rotating spindle causes asymmetric changes to the shape of the three potential binding sites, cycling them through the loose (L) conformation that binds ADP and Pi, the tight (T) conformation that literally squeezes the two sustrates together into ATP, and the open (O) conformation that allows ATP.

Of course, it isn't quite that simple (fig. 8). Starting with the initial movement of protons, as they move from the intermembrane space into the ATP synthase, they enter a small hydrophilic channel (a) and then bind onto one of the c-subunits of the "water wheel" c-ring. Binding of the H^+ to the c-subunit causes it to lose affinity for the a-subunit, allowing it to spin, and simultaneously causes a conformational change that essentially pushes off against the a-subunit, initiating the movement. Once it has spun around almost a complete turn, the H^+ is positioned by another channel (b), which funnels it from the c-subunit into the matrix. The c-subunit structure is connected to an asymmetric spindle that is itself connected to the catalytic subunits.

Uncoupling Electron Transport from ATP Synthesis

So, that is oxidative phosphorylation. It productively utilizes the energy of the proton gradient across the inner mitochondrial membrane (created by oxidation-powered pumps) to drive ATP formation at an approximate rate of 3 protons to 1 ATP. The system is normally highly self-regulated due to impermeability of the inner mitochondrial membrane to H+. If the ATP is not used up quickly, then its concentration slows the action of ATP synthases, which slow the movement of protons out of the intermembrane space. This buildup of protons will eventually be enough that the free energy needed to transfer a proton into the intermembrane space (from the electron transport chain) will not be sufficient to overcome the concentration gradient. Electron transport is slowed, and working backwards, the chain reaction slows respiration rates in general. As the cell/organism requires more energy and uses up the ATP more quickly, protons flow more quickly and the electron transport chain is disinhibited. Thus there is a direct association between respiration rate and physiological energy need.

Interestingly, there is an exception to this tight coupling of the electron transport chain and formation of ATP. The purpose of brown fat (aka brown adipose tissue), which is most often found in newborn and hibernating mammals, is to generate non-shivering (non-movement-based) heat to keep the animal warm. This is accomplished by uncoupling the electron transport chain from the ATP synthesis. This uncoupling is a hormonally controlled process based on the presence of a mitochondrial proton channel called thermogenin. The hormone norepinephrine increases production of free fatty acids, which open the thermogenin channel. This allows protons to flow from the intermembrane space back into the matrix without having to go through ATP synthase. Because of this, the electron transport chain can keep chugging away, ATP levels do not build up, there is no reduction in respiration rate, and the excess energy not being used in ATP production is released as heat.

In fact, 2,4-dinitrophenol, which is used in a variety of research and industrial applications today, was at one time used as dieting drug (in the 1930's) because through a different mechanism, it too uncoupled electron transport from ATP synthesis. Its mechanism of action derived from its ability to carry and release protons as it freely diffused through the mitochondrial membrane (since it is a small hydrophobic molecule). As this continues, cells catabolize more and more stores of carbohydrates and fats, which is the reason for the interest by dieters. Unfortunately for some of those dieters, this pharmacological means of uncoupling the electron transport chain from the ATP synthesis had no regulation other than the amount of DNP taken. In cases of overdose, respiration rates could rise dramatically while producing little ATP and a great deal of heat. In fact, overdose illness and death are generally due to the spike in body temperature rather than lowered ATP availability. Strangely, there are still some dieters and bodybuilders who self-medicate with DNP despite the dangers.

Structure of Electron Carriers

Though they have been mentioned frequently in the earlier parts of this chapter, the structures of the electron transport chain participants, and particularly of the moieties that temporarily hold extra electrons, have not been addressed. So, now is the time to do so. The major players are the flavin mononucleotide (FMN) that plays a role in complex I, ubiquinone (Coenzyme Q), the lipid-soluble electron carrier, the heme groups of the cytochromes, and iron-sulfur clusters, found in complexes I, II, and III.

Flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD), are pictured in figure 11. Note the triple-ring structure and the three possible oxidation states. All three states are stable - the semiquinone state is not merely a transient form. This stability allows the conversion from carriers that can only handle one electron to carriers that can handle two electrons, and vice versa. The same holds true for ubiquinone - stable as ubiquinone (fully oxidized), semiubiquinone (radical state), and ubiquinol (fully reduced). Alternative nomenclature for these molecules is Coenzyme Q, $CoQH^+$, and $CoQH_2$. Note the aromaticity gained by ubiquinone when it is reduced. This enhances its stability and its suitability as a receiver of electrons from NADH.

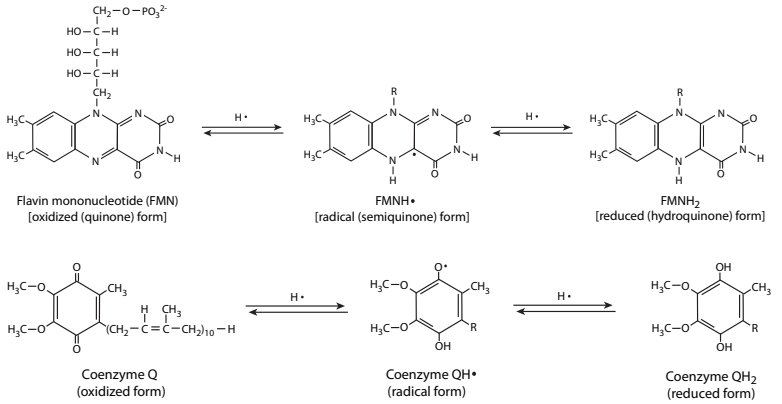


Figure 11. Flavin mononucleotide and Ubiquinone are electron carriers.

Heme groups (figure 12) are considerably larger, encompassing a porphyrin ring with an iron ion held in its center. This iron ion alternates between ferric (Fe^{3+}) and ferrous (Fe^{2+}) states as the heme group is oxidized and reduced, respectively. In the case of complex IV, the iron ion can form a complex with O_2, which can then receive the electrons being held by the ring structure. This large structure is particularly important because it needs to be able to transfer a total of 4 electrons to reduce O_2 to 2 H_2O.

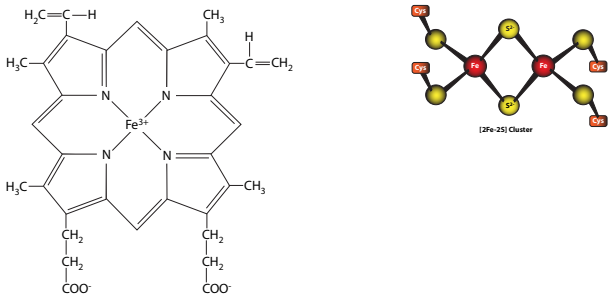


Figure 12. Left: The heme group shows one of the possible combinations of the groups attached to the outside of the porphyrin ring. Right: The Fe-S centers also have variations, such as 4 Fe and 4 S, or even just 1 Fe surrounded by the S atoms of the four cysteines.

Finally, Fe-S clusters (figure 12) can also act as electron carrying moieties. Like in the heme group, the iron atom can readily switch between the ferric and ferrous states.

Other Catabolic Reactions

Of course, for many organisms, the food used by cells is not in the form of simple glucose solutions, but made up of various polymeric biomolecules.

Starch and Glycogen Depolymerization

Glycogen and starch are long branched polymers of glucose that provide a rapidly available source of glucose molecules for glycolysis. In omnivores and herbivores, the primary source of carbohydrates (and thus glucose) is dietary starch. The catabolism of the amylose and amylopectin in humans begins in the mouth with salivary α-*amylase*. This enzyme breaks α(1-4) bonds of both starch molecules except at the ends and near branch points (in the case of amylopectin). Though the salivary enzyme is inactivated by the acidity of the stomach, a pancreatic α-amylase goes to work on starch that has reached the small intestine. The product of these digestions includes maltose, maltotriose, and dextrins. These are acted upon by other intestinal enzymes: α-*glucosidase* removes individual glucoses from oligosaccharides, and α-*dextrinase*, also known as debranching enzyme, can break α(1-6) bonds as well as the α(1-4) bonds.

Glycogen breakdown is different since most glycogen breakdown is occuring internal to the cells of an organism rather than in the digestive tract. The primary enzyme is *phosphorylase* (also known as glycogen phosphorylase), which breaks the bond of a terminal glucose to its neighbor by substituting a phosphate group. This generates glucose-1-phosphate, which can be converted to glucose-6-phosphate by *phosphoglucomutase*. The G6P, of course, can enter the glycolytic pathway. A *glycogen debranching enzyme* is also important, as the phosphorylase is unable to work closer than five glucose residues to a branch site.

The use of glycogen presents an interesting question: why use it as an energy storage molecule when fats are more abundant in most animals, and more efficient at packaging potential energy? As described in the next section, fatty acids can only be metabolized aerobically, so they cannot serve as a backup fuel source in anaerobic conditions. Furthermore, even in aerobic conditions, fatty acid catabolism cannot generate glucose, which is not only needed for cellular fuel, but in the bloodstream for feedback control mechanisms regulating organismic metabolism.

Fatty Acid Breakdown

Hormone-sensitive lipase in adipose tissue hydrolyzes the stored fat in those cells into glycerol and fatty acids. Glycerol can enter the glycolytic cycle via conversion to dihydroxyacetone phosphate (a two-step conversion using glycerol kinase and glycerol-3-phosphate dehydrogenase). The fatty acids are secreted from the adipose cells into the bloodstream where they bind to a carrier protein, albumin. This complex can then be brought inside of other cells by endocytosis, where they can be broken down as an energy source.

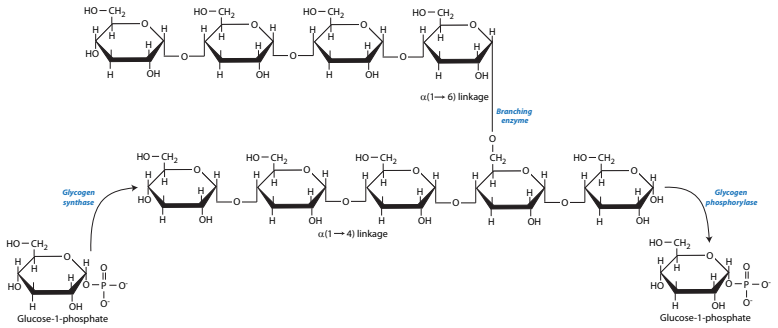


Figure 13. Glycogen is a storage form of glucose that is broken down by glycogen phosphorylase (linear α(1-4) bonds) and debranching enzyme (branchpoint α(1-6) bonds).

Phosphorylase is a homodimer that is allosterically controlled by glucose, G6P, and ATP negatively, and by AMP positively. In addition to allosteric binding sites for these molecules and a substrate binding site, phosphorylase also binds pyridoxal-5-phosphate as an essential cofactor. P5P is derived from pyridoxine, or vitamin B_6.

Much like the phosphoglycerate mutase in step 8 of glycolysis, phosphoglucomutase is a phosphorylated enzyme that temporarily transfers is phosphate group to the substrate to form a glucose-1,6-bisphosphate intermediate.

Debranching enzyme actually has two functions: it transfers a trisaccharide from a 4-sugar branch on the "1" side of an α(1-6) branching linkage to the end of a branch connected to the "6" side of the branchpoint. It then hydrolyzes the α(1-6) connecting the final glucose of the branch, leaving an unbranched chain of glucose for phosphorylase to attack.

The breakdown of fatty acids occurs by β oxidation inside the mitochondrial matrix (fig. 14). Since the inner mitochondrial membrane is impermeable to long-chain free fatty acids, they must first be activated to fatty acyl-CoA and linked to carnitine, an amino acid derivative synthesized from methionine and lysine (see Fig. 15). The first step is

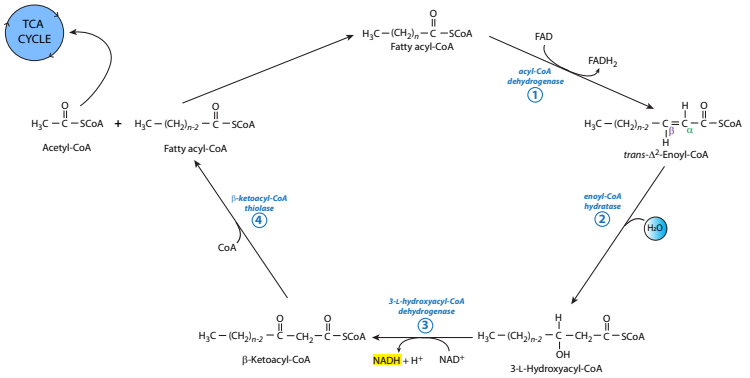
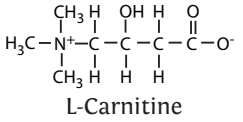


Figure 14. β oxidation of fatty acids happens in the mitochondrial matrix.

performed by one of a family of enzymes known as acyl-CoA synthetases or thiokinases, and requires Coenzyme A and ATP hydrolysis. These reactions occur either on the cytoplasmic surface of the mitochondrial outer membrane or the endoplasmic reticulum, where acyl-CoA synthetases are embedded. In the second reaction, carnitine palmitoyltransferase I on the outside of the inner mitochondrial membrane links the acyl chain to carnitine, releasing CoA. The acyl-carnitine is transported into the mitochondrial matrix where carnitine palmitoyltransferase II releases the fatty acyl chain from the carnitine and reattaches it to an molecule of CoA.

In the mitochondrial matrix, β oxidation occurs in four steps to yield an acyl-CoA chain that is shortened by two carbons, and an acetyl-CoA that can then enter the TCA. .
The β refers to the second closest carbon to the one attached to CoA. The bond that will be broken is the bond between the α and β carbons. All even-numbered, fully saturated, fatty acids can thus be completely oxidized. The presence of double bonds in unsaturated fatty acids introduces complications to this process that must be addressed using additional enzymes that either move the double bond or remove it.

Most animals and plants generate even-numbered fatty acids; however, some marine animals (e.g. smelt, mullet) and some plants and bacteria synthesize odd-chain fatty acids as well. The same enzymes responsible for β oxidation of even-numbered fatty acids can handle odd-numbered fatty acids as well, except that the final degradation yields propionyl-CoA instead of acetyl-CoA.


L-Carnitine

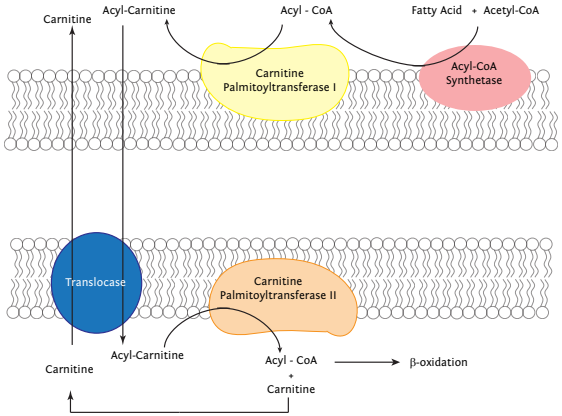
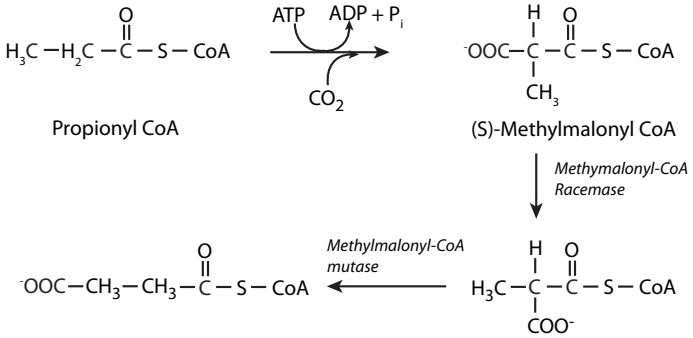


Figure 15. Fatty acid breakdown by Carnitine Palmitoyl Transferases.

Carnitine deficiency syndromes can occur when there is either a dysfunctional mutation of carnitine palmitoyltransferase or a severe deficiency of intracellular carnitine. Since most of the carnitine in the body is found in cardiac and voluntary muscle, the usual symptoms are muscle weakness and cardiac arrhythmias, as well as hypoketosis. In neonates, the arrythmias can lead to death. Carnitine supplementation is a successful treatment in systemic carnitine deficiency due to either low carnitine intake or defects in the carnitine transporter embedded in the cell membranes. However, if the defect is in the palmitoyltransferase, supplementation will be unsuccessful.

Carnitine is widely sold as a dietary supplement for increasing weight loss by enhancing fat catabolism. The basic idea is obvious: carnitine is needed for long-chain fatty acid breakdown, so more carnitine = more fat burned. However, that only holds true if carnitine levels are below saturation levels for the palmitoyltransferases. Because 75% of the carnitine in the body must be ingested (only 25% is synthesized), this is a mild possibility, depending on diet. Currently, the biomedical community has not reached a consensus on the efficacy of carnitine supplementation on fatty acid oxidation in carnitine-sufficient persons.



Propionyl-CoA is converted to succinyl-CoA through a series of three enzymes: propionyl-CoA carboxylase, methylmalonyl-CoA racemase, and methylmalonyl-CoA mutase. The succinyl-CoA could theoretically enter the TCA cycle, but recall that the succinyl-CoA is simply recycled and never actually consumed by the TCA cycle. Thus, in order for the succinyl-CoA to contribute to the energy needs of the cell, it must first be converted to malate (steps 6-8 of TCA cycle), which is then converted to pyruvate by malic enzyme, also known as decarboxylating malate dehydrogenase. Pyruvate can then enter and be consumed by the TCA cycle.

In addition to oxidation in the mitochondria, fatty acids also undergo β oxidation in peroxisomes. However, generally, the oxidation in peroxisomes is limited, and the purpose is to shorten long fatty acids in preparation for final degradation in the mitochondria.

In addition to the more common single-chain fatty acids, cells will also encounter branched fatty acids, which block β oxidation is alkyl group is on the β carbon. In these cases, phytanic acid for example, α oxidation is necessary to generate an intermediate with the alkyl group on the α carbon. This is then followed by β oxidation to completion.

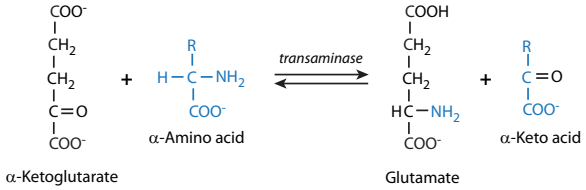
Finally (with respect to fatty acid catabolism), it must be noted that in liver especially, a large part of the acetyl-CoA generated by oxidation of fatty acids does not enter the TCA cycle. Instead, it is converted into acetoacetate or D-β-hydroxybutyrate, which along with acetone, are known, somewhat bizarrely, as ketone bodies. These molecules are water soluble, and transported through the bloodstream as energy sources for a variety of tissues, even including brain, which typically only uses glucose as fuel since fatty acids cannot pass through the blood-brain barrier. However, ketone bodies can penetrate and are used by brain cells under starvation conditions.

Vitamin B_{12}, or 5'-deoxyadenosylcobalamin, is a coenzyme component of methylmalonyl-CoA mutase, but it is not made by either plants or animals. It is only made by certain bacteria, some of which live in the intestinal tracts of herbivores. Herbivores thus absorb the B_{12} for their use, and carnivores obtain their B12 from eating herbivores. Defects in methylmalonyl-CoA mutase or severe deficiency in vitamin B_{12} (most often in vegetarians) can lead to methymalonyl aciduria/acidemia, that can be fatal in untreated infants due to acidosis. However, depending on the cause, it can be treated with high doses of B12 and/or by avoiding dietary odd-chain fats and proteins rich in isoleucine, leucine, or methionine, which also catabolize to propionyl-CoA. Pernicious anemia, in which usually elderly patients have very low levels of red blood cells and hemoglobin, as well as neurodegeneration, is also related to B_{12}. However, it is usually not due to a vitamin deficiency, but rather to the insufficient secretion of *intrinsic factor*, which binds B_{12} in the stomach and then is taken into intestinal cells by receptor-mediated endocytosis.

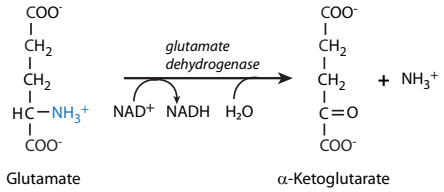
Ketoacidosis is a condition in which ketone bodies are being produced much faster than they are used. This leads to a buildup of the molecules in the bloodstream, which lowers the pH, since the molecules are acidic. An easy diagnostic of ketoacidosis is a sweet somewhat fruity smell (of acetone) on the breath. This condition can be an indication of diabetes, but may also occur when a person is consuming a high-fat/low-carb diet. When the body's metabolism is not using glucose/carbohydrates as the primary food source for either reason, fat is used instead, increasing production of ketone bodies. Left untreated, severe ketoacidosis can lead to cell damage as the blood acidifies, and compensation by increased exhalation of carbon dioxide and lead to respiratory failure in susceptible individuals.

Amino Acid Degradation

Proteins are broken down by a variety of proteases that hydrolyze the peptide bonds to generate smaller peptides and amino acids. Those amino acids that are not used for building new proteins may be broken down further to enter the metabolic processes discussed in this chapter. In their conversion to metabolic intermediates, the amino acids first undergo deamination. The primary goal of deamination is to excrete excess nitrogen (as urea) and then use or convert (to glucose) the remaining carbon skeleton. This deamination is a two-part process: the first step to deamination is usually a transamination catalyzed by an aminotransferase, in which the amino group of the amino acid is transferred to α-ketoglutarate which then yields a new α-keto acid of the amino acid and glutamate.



The amino group of glutamate could then transferred to oxaloacetate to form α-ketoglutarate and aspartate. That series of transaminations transforms the original amino acid, but does not get rid of the amino group nitrogen. The alternative pathway is deamination of the glutamate by glutamate dehydrogenase, which generates α-ketoglutarate and ammonia, using either NAD^+ or NADP as the oxidizing agent.



The amino acids break down into one of the following seven metabolic intermediates: pyruvate, acetyl-CoA, acetoacetate, α-ketoglutarate, succinyl-CoA, fumarate, and oxaloacetate as follows: 1) Ala, Cys, Gly, Ser, Thr, Trp break down to pyruvate; 2) Ile, Leu, Lys, Thr to acetyl-CoA; 3) Leu, Lys, Phe, Trp, Tyr to acetoacetate; 4) Arg, Glu, Gln, His, Pro to α-ketoglutarate; 5) Ile, Met, Val to succinyl-CoA; 6) Asp, Phe, Tyr to fumarate; 7) Asn, Asp to oxaloacetate.

There is a large variety of proteases, classified into one of six groups (as of 2008): serine proteases, metalloproteases, aspartic acid proteases, cysteine proteases, threonine proteases, and glutamic acid proteases. All of them work by forming a nucleophile at their active site to attack the peptide carbonyl group. They differ in the construction of their active sites, and the specificity of the target sequences to be cleaved. The MEROPS database (http://merops.sanger.ac.uk/) lists hundreds of enzymes and their specific recognition sites. As with other enzymes, recognition is based on formation of stabilizing hydrogen bonds between enzyme and target. In the case of proteases, many of the important stabilizing bonds must be formed right around the cleavage site, thus leading to specific recognition sequences.

Although cleavage is often thought of as a way of destroying the activity of a protein, in fact, specific cleavage of inhibitory parts of a protein can activate it. A prominent example of this (the caspase cascade) is discussed in the apoptosis section of the cell cycle chapter.

Some proteases are secreted and do their work extracellularly. These include digestive enzymes such as pepsin, trypsin, and chymotrypsin, as well as bloodstream proteases like thrombin and plasmin that help control clotting. The immune system also uses proteases to destroy invading cells and viruses.

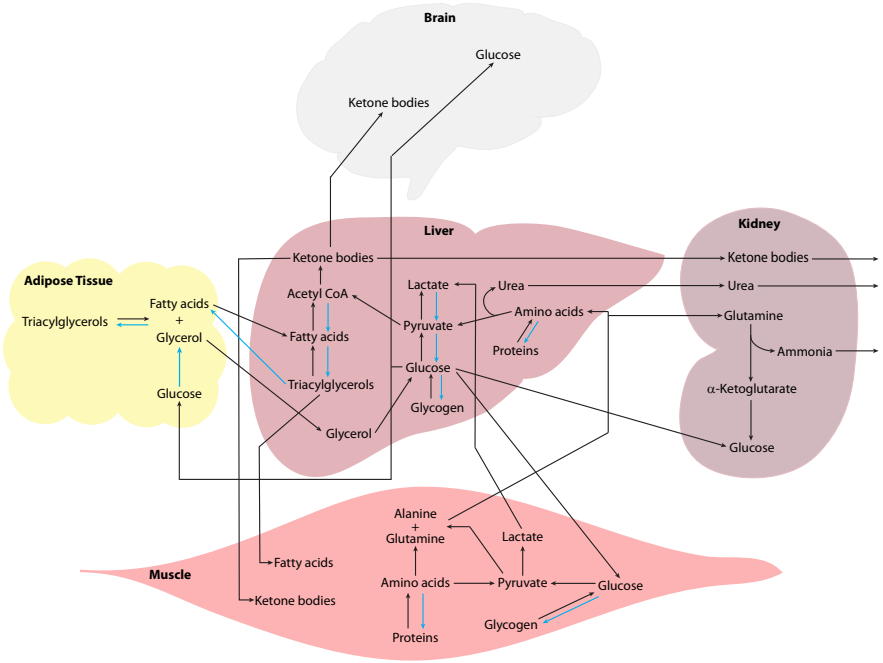


Figure 16. Overview of human major metabolites. Although most cells in the body carry out many of the metabolic activities described in this chapter and the next, the advantage of multicellular organisms is that certain cell types, tissues, or organs may become specialized to process particular metabolic reactions more efficiently than other cells, and thus take on a lot of that burden for the organism.

Metabolism 2 :

Anabolic Reactions of the Cell

Using this book: This book is designed to be used in both introductory and advanced cell biology courses. The primary text is generally on the left side of the vertical divider, and printed in black. Details that are usually left to an advanced course are printed in blue and found on the right side of the divider. Finally, additional biomedically relevant information can be found in red print on either side of the divider.

Anabolic Reactions

As pointed out at the beginning of this book, most of the energy for life on this planet originates from the sun. In the last chapter, the discussion was on the breakdown of complex molecules such as sugars and fats that hold great, but difficult to access, potential energy to produce molecules like ATP that can act as more readily accessible sources of cellular energy. This energy is then used to synthesize the more complex biomolecules necessary to build living cells. That synthesis, the formation of sugars, fatty acids, and amino acids, is the focus of this chapter. Although technically the polymerization of nucleic acids and proteins are anabolic processes, they are not included in this chapter and are examined in detail separately.

Photosynthesis

In one way or another, the energy of sugar and fat fuel molecules is derived from photosynthesis - the conversion of solar light energy into chemical bond energy, whether directly in photosynthetic plant cells and certain photosynthetic bacteria, or indirectly by the ingestion of those plants and bacteria. Photosynthesis is a simple idea: atmospheric carbon dioxide molecules are joined with water molecules to form sugars and oxygen:

$$CO_2 + H_2O \xrightarrow{light} CH_2O + O_2$$

The production of usable energy from sunlight and the fixation of atmospheric carbon dioxide are two separate sets of reactions. In plants, photosynthesis takes place only in cells containing chloroplasts. Chloroplasts are organelles with an evolutionary origin suspected to be similar to that of mitochondria, and like mitochondria, chloroplasts generate ATP and use a nicotinamide-based high-energy electron carrier. There are further similarities: they both have highly folded inner membranes, though in chloroplasts, there are three membranes in all, while mitochondria only have two. Finally,

an electron transport chain is embedded in the thylakoid membrane of chloroplasts, functioning very similarly to electron transport in the mitochondria. In addition to the electron transport components and ATP synthase (structurally and functionally almost identical to mitochondrial ATP synthase), the thylakoid membrane is also rich in a set of molecules that are not found in the inner mitochondrial membrane: light-absorbing pigment molecules.

In plants, these pigment molecules fall into two classes: the chlorophylls and the carotenoids (fig. 1) but only the chlorophylls can mediate photosynthesis. Photosynthetic bacteria do not contain chlorophyll, but do have carotenoid pigments that can carry out photosynthesis. Both are hydrophobic hydrocarbons that are held in place within the plane of the membrane by transmembrane proteins. Chlorophylls are easily recognizable by the very large Mg^{++}-containing porphyrin ring, while the carotenoids are long hydrocarbon chains that may or may not have small ring structures on the ends (e.g. β-carotene). While there is variation in the chlorophyll family, they all impart a green color to the leaf. Carotenoids, on the other hand have a much wider range of colors from yellows to reds. Both chlorophylls and carotenoids are able to absorb light energy of a particular wavelength/energy range and enter an unstable excited state. When the molecule returns to its ground state, the energy would be emitted as heat or light in an isolated situation. However within the context of the pigment arrays (antenna complex) in a living cell, most of the energy is shuttled to another pigment molecule of lower energy by resonance transfer. As described below, only one pair of chlorophyll molecules in an antenna complex will actually eject an electron as it drops from an excited state back to ground state. It is the transfer of that high-energy electron that powers photosynthesis.

Photosynthesis can be divided into two mechanisms: the *light reactions*, which use light energy to excite the electrons of certain chlorophylls, and participate in the electron transport chain to generate ATP and NADPH, and the *dark reactions*, which use that ATP and NADPH to fix carbon from CO_2 into organic molecules (carbohydrates). As the name implies, the light reactions require light energy to excite the chlorophyll and begin electron transport. Dark reactions, however, do not require darkness. They are technically light-independent, but in some plants, the dark reactions run better in the light for reasons to be discussed.

The light reactions are intimately tied to the anatomy of the thylakoid membrane; specifically, the arrangement of light-absorbing pigment molecules in antenna complexes, also called light-harvesting complexes (sometimes abbreviated LHC, not to be confused with the Large Hadron Collider). These pigments are held by proteins in ordered three-dimensional groups so that the pigments that absorb the highest energy

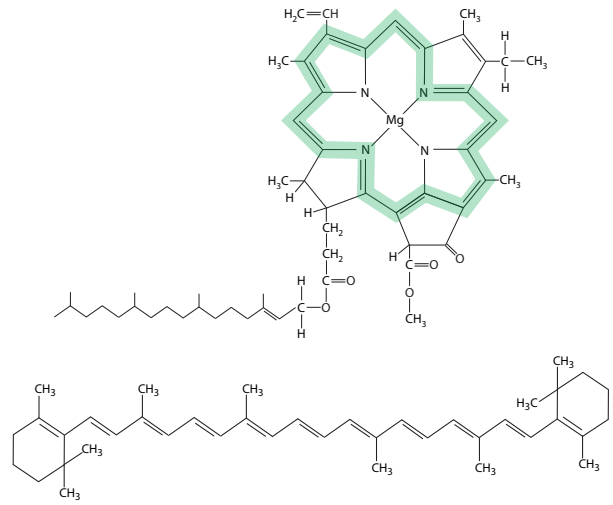


Figure 1. Chlorophyll (top) and β-carotene (bottom)

Chlorophyll molecules are made up of a phytol hydrocarbon tail that anchors the molecule within a membrane, and an electron-carrying porphyrin ring containing a magnesium cation. Note that the phytol tail is not drawn to scale with the porphyrin ring in figure 1. Among different types of chlorophyll, the chemical groups attached to the ring may vary, and this variation is responsible for differences in the absorption spectrum from one type of chlorophyll to the next. For example, chlorophyll a has absorption peaks at approximately 430 and 662 nm, whereas chlorophyll b has peaks at 453 and 642 nm. The difference between the two is small: at C7, there is a $-CH_3$ group on chlorophyll a, but a $-CHO$ group on chlorophyll b. Presently, there are five known chlorophylls: chlorophyll a is found in all photosynthetic organisms, chlorophyll b is only found in plants, chlorophylls c1 and c2 are found in photosynthetic algae, and chlorophyll d is found in cyanobacteria.

Carotenoids have two functions. As noted in the primary text at left, they can participate in energy transfer in toward the reaction center chlorophylls. They are also a protectant molecule, preventing reaction center auto-oxidation. Carotenoids can be highly efficient free radical scavengers due to the conjugation of alternating single-double carbon bond structures.

light are toward the periphery, and the lowest-energy-absorbing chlorophylls are in the center (fig. 2). Sunlight is composed of a broad range of wavelengths, some of which are transiently absorbed by the pigments. After a pigment molecule absorbs a photon, the energy is released and passed on to a pigment tuned to a slightly lower energy level (longer wavelength), and from there to an even lower-energy pigment, and so on until it reaches the reaction center chlorophylls. In this way, energy from a wide range of light wavelengths/energies can all contribute to the ATP and NADPH production by the light reactions. The antenna complex is crucial because it allows the use of a greater portion of the solar light spectrum. And, as a tightly-packed three-dimensional array, photons that pass by one pigment molecule may well hit another one on its way through the array. All of these characteristics combine to increase the efficiency of light use for photosynthesis. The reaction center chlorophylls (P680 for photosystem II, P700 for photosystem I) are the only chlorophylls that actually send excited electrons into the electron transport chain. The other chlorophylls and pigments only act to transfer the energy to the reaction center.

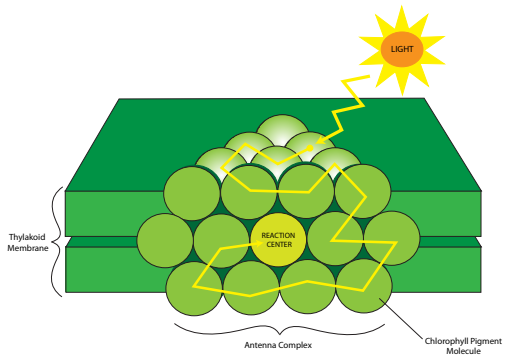


Figure 2. Pigment molecules are arranged in an antenna complex in the thylakoid membrane.

When excited, the reaction center chlorophyll of photosystem II (figure 3) begins the process of electron transport. This chlorophyll is part of a protein complex that also includes a Mn-based oxygen-evolving complex (OEC), pheophytin, and a docking site for plastoquinone. Although the chlorophyll electron is the one excited by the solar energy, the origin of the electrons to keep the chlorophyll replenished comes from the splitting (oxidation) of water to O_2 and 4 H^+.

The question of how a cell could generate the energy needed to split water was long a thorny issue because water is an exceptionally stable molecule. The current model suggests that the energy comes from an extremely strong oxidizer in the form of $P680^+$. After P680 is energized by light, an excited electron has enough energy to break away from the chlorophyll and jumps to pheophytin. Pheophytin becomes $Pheo^-$ temporari-

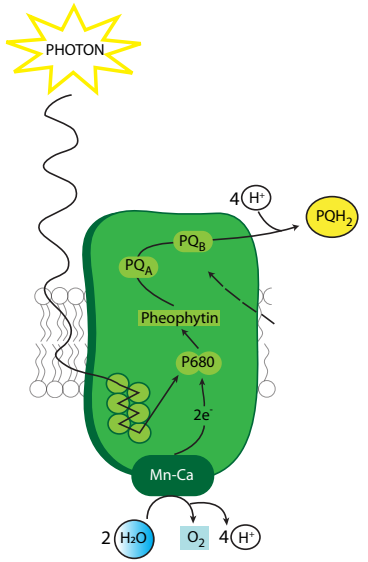


Figure 3. Photosystem II (which feeds electrons into photosystem I).

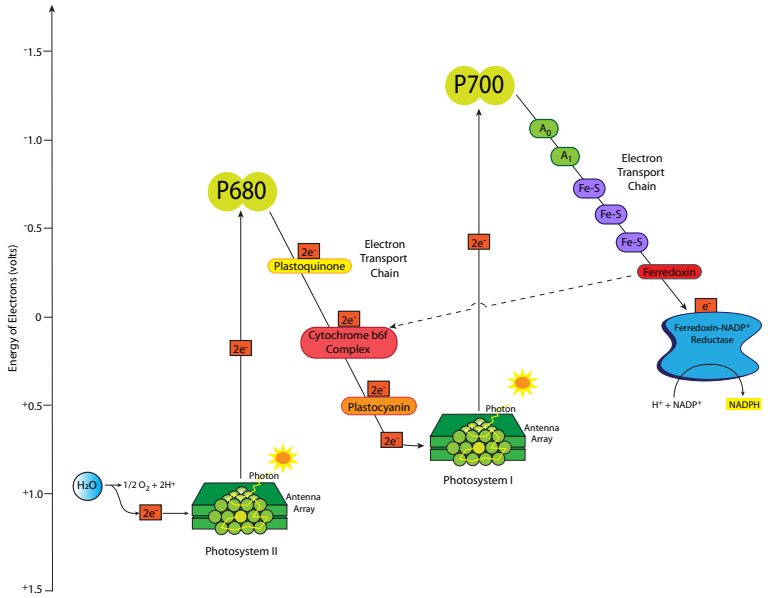


Figure 4. Change in electron energy moving through photosystems II and I. Light energy is needed in both photosystems to boost the electron energy high enough to move to the next electron carrier.

ly, and the charge separation in the complex between $P680^+$ and $Pheo^-$ helps to enhance the oxidative power of $P680^+$. That extraordinarily strong attraction for electrons is what allows the P680 chlorophyll to tear them away from H_2O and split the water. In fact, $P680^+$ is one of the strongest biological oxidizers known. Since four electrons must be taken to fully oxidize two water molecules and generate molecular oxygen, four photoexcitation events are needed. While the exact mechanism is still to be elucidated, it appears that the OEC helps to stabilize the water molecule during this process as well as holding onto each electron as it comes off.

The excited electrons, moving from the OEC to $P680^+$ to pheophytin, next move to the lipid-soluble carrier, plastoquinone. The similarity of the name with the mitochondrial carrier ubiquinone is not a coincidence. They function similarly, and as the plastoquinone takes on the electrons, it also takes on protons from the stromal side of the thylakoid membrane. The PQ moves within the membrane from pheophytin to cytochrome b_6f. As the electrons are transferred to cytochrome b_6f, the protons are then dropped off on the lumenal side of the membrane, increasing their concentration in the chloroplast lumen, and building a proton gradient to power ATP synthase. Cytochrome b_6f passes the electrons on to plastocyanin, an aqueous-phase carrier, which shuttles the electrons to the P700 reaction center chlorophyll of photosystem I. However, after all the transfers, the energy level of the electrons is now fairly low (fig. 4) and unable to power the upcoming reactions. Since it is now on a reaction center chlorophyll, the obvious answer is to re-energize it with a bit of sunlight. This raises the electron energy sufficiently to reduce ferredoxin. Now things get a little complicated.

This part of photosynthesis can take one of two directions, the linear pathway, which generates both NADPH and ATP, and the cyclic pathway which mostly generates ATP. Most of the time, the linear pathway is taken, with the electrons on ferredoxin transferred via ferredoxin-NADPH reductase (FNR) onto NADPH. However, sometimes the cell requires significantly more ATP than NADPH, in which case, the electrons from ferredoxin are transferred back to plastoquinone via ferredoxin-plastoquinone reductase. This acts just as described above, and pumps more protons across the membrane to power the ATP synthase. ATP synthesis goes up and NADPH synthesis goes down.

The ATP and NADPH generated by the chloroplast are almost exclusively used by the chloroplast itself (and not distributed to the rest of the cell) to power the dark reactions, which are energetically expensive. In fact, when the light reactions are not running due to darkness, some plant cells have mechanisms to prevent the dark reactions from using the limited resources of cellular, non-chloroplastic, respiration. The simplest method of such limitation is the pH sensitivity of *rubisco* (ribulose bis-phosphate carboxylase), at least in C3 plants (see below). Rubisco has a very sharp pH optimum

The OEC, or oxygen-evolving complex (also WOC, water oxidizing complex) is a metalloenzyme with a Mn_4O_XCa catalytic cluster, where X is the number of μ-oxo-bridges connecting the metal atoms, with surrounding amino acids, especially crucial tyrosines, also playing a role in the coordination sphere of the active site. The overall complex undergoes a series of 4 oxidation state changes as the P680 chlorophylls are excited by the light energy and transfer electrons, but at present it is not known what the exact oxidation state of any given Mn atom is through this series of state changes. The crucial reaction is the formation of the O-O bond to form O_2. There are two proposed models for this mechanism. One is that the O-O bond is formed when the OEC has reached its fully oxidized state, and an oxygen in a μ-oxo-bridge radical state interacts with a water molecule. The other proposed mechanism is that the O-O bond forms earlier as a complexed peroxide held by the OEC center.

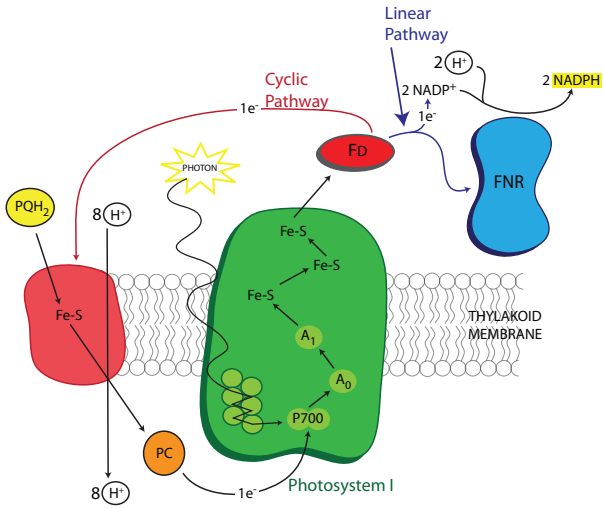


Figure 5. Photosystem I initiates electron movement through two pathways.

at about pH 8.0, so while the light reactions are running and the protons are being pumped, the pH rises to about 8 and rubisco works, but in the dark, the pH drops back to its basal level close to 7.0, inhibiting rubisco activity.

The Calvin Cycle

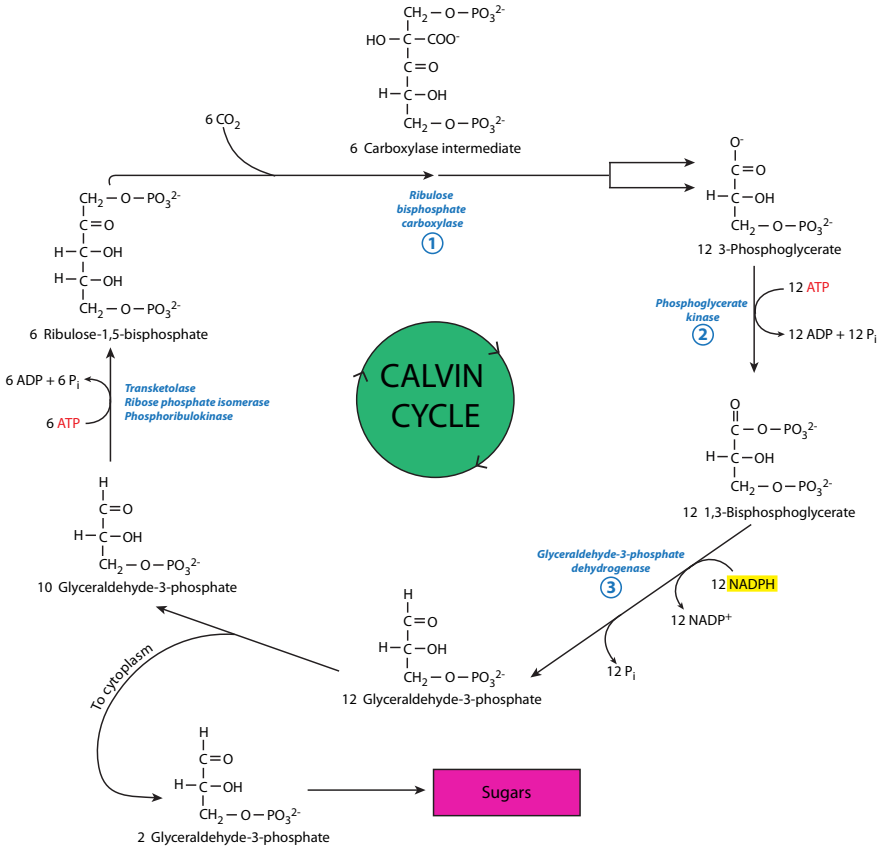


Figure 6. The Calvin cycle fixes atmospheric carbon to ribulose 1,5-bisphosphate to form the organic 3-carbon intermediate 3-phosphoglycerate for the formation of sugars.

The dark (carbon fixation) reactions vary depending on the type of plant. The most common set of carbon fixation reactions is found in C3-type plants, which are so named because the major stable intermediate is the 3-carbon molecule, glyceraldehyde-3-phosphate. These reactions, best known as the Calvin cycle (fig. 6), fix CO_2 onto the pentose, ribulose 1,5-bis-phosphate (RuBP). The production part of the cycle begins with formation of RuBP from glyceraldehyde-3-phosphate. Then, the rate-limiting step occurs:

Ribulose 1,5-bisphosphate and CO_2 are joined together by rubisco. Carboxylases are relatively slow enzymes as a family, and rubisco is one of the slowest. A 6-carbon intermediate is formed but it is unstable, and quickly breaks down to yield two molecules of 3-phosphoglycerate. Some familiar enzymes (from glycolysis, although this is happening in the stroma, not the cytoplasm) now come into play.

Phosphoglycerate kinase phosphorylates 3-PG to 1,3-bisphosphoglycerate. 1,3-BPG is then reduced by glyceraldehyde-3-phosphate dehydrogenase to form glyceraldehyde-3-P. This step requires the energy released from oxidation of NADPH. A small portion ($1/6^{th}$) of the GAP that is made is then exported from the chloroplast and will be used to form more complex carbohydrates. However, the majority is recycled through the recovery phase of the Calvin cycle to regenerate NADP.

As if having a central enzyme that moves at a snail's pace and needing to recycle the majority of its potential product was not bad enough, C3 plants also have to contend with the hijacking of rubisco for a competing, and energy-wasting, set of reactions known as *photorespiration*. Under conditions of low CO_2 and high O_2 in the local atmosphere, oxygen, instead of carbon dioxide, binds to rubisco and forms 3-PG and 2-phosphoglycolate from its reaction with RuBP. As detailed in figure 7, the 2-phosphoglycolate is dephosphorylated to glycolate and transported out of the chloroplast. From there, it undergoes a series of reactions in the peroxisomes and mitochondria to transform it to 3-PG, which can then go in the chloroplast and participate in the Calvin cycle. Unfortunately for the cell, in the course of these reactions, NADH and ATP are used, thus lowering the energy availability inside the cell.

This is a particular problem in hot climates, because the oxygenase activity of rubisco increases more than the carboxylase activity as the temperature increases. This leads to an interesting side effect: in C3 plants, as the temperature rises and CO_2 is outcompeted by O_2 for rubisco binding, the stomata of the leaves need to remain open for longer in order to allow for acquisition of enough CO_2 from the atmosphere. This in turn allows more water vapor from inside the cell to escape, leading to dehydration. C3 plants are thus at a competitive disadvantage in hot dry climates in comparison to plants that do not use rubisco for carbon fixation.

What about plants adapted to such climates? C4 plants, which include some grasses, corn, sugarcane, and weeds, utilize PEP carboxylase (which does not have the annoying photorespiratory capabilities of rubisco and a higher affinity for CO_2) to fix carbon dioxide to PEP, making oxaloacetate. In an interesting twist, the oxaloactetate, after conversion to malate, is decarboxylated to yield CO_2 again, which is fed to rubisco and the calvin cycle. The C4 mechanism, also called the Hatch-Slack pathway, utilizes two

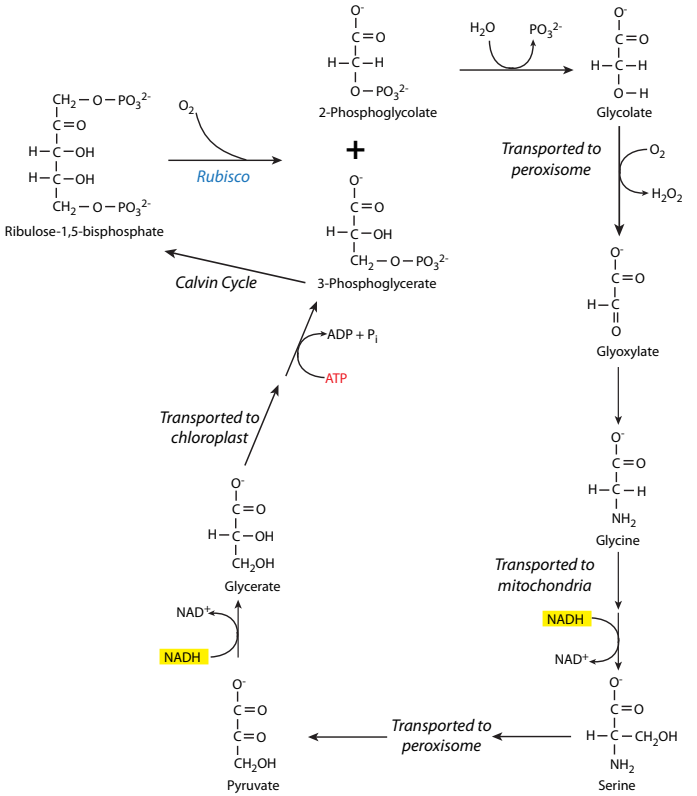


Figure 7. Photorespiration. Rubisco can catalyze the addition of O2 to RuBP, producing 3PG, which can be used by the Calvin cycle, and 2-phosphoglycolate, which is converted to glycolate, transported out of the chloroplast, converted to glycerate over several steps in the peroxisome and mitochondria, and shipped back to the chloroplast. This is an energetically expensive process (note NADH and ATP used).

PEP carboxylase actually fixes HCO_3^- to PEP rather than CO_2 directly. The atmospheric CO_2 is converted to the bicarbonate by carbonic anhydrase.

sets of cells, an outer layer (mesophyll) that takes in air and fixes the CO2 to PEP and produces malate, and an inner layer of cells (bundle sheath) that takes the malate, and decarboxylates it for its rubisco enzyme. The two cells are connected via plasmodesmata (see Cell-cell Interactions chapter). Although energetically more expensive than carbon fixation by C3 plants in cooler climates, the C4 pathway overtakes C3 in efficiency as temperatures rise and photorespiration increases.

Desert plants go one step further than C4 plants. Living in environments that are extremely hot and dry during the day, but relatively cool at night, many desert succulents (like cacti) are diurnal, and only open their stomata at night (when temperatures are significantly lower and water evaporates far more slowly) for CO_2 gathering, which is then fixed via the CAM pathway to malate. Then in the daylight hours, CO_2 is released from the malate and used in the Calvin cycle to generate carbohydrates.

Variations of this pathway have been found in which aspartate is transported to the bundle-sheath cells instead of malate.

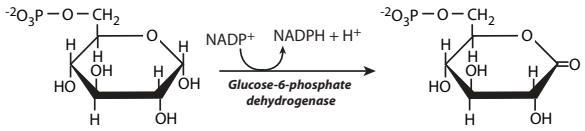
After decarboxylation of the malate by malic enzyme (NAD-dependent in some species, NADP-dependent in others) to release the CO_2 for rubisco, the resulting pyruvate is shuttled back to the mesophyll cell where it is phosphorylated by pyruvate-phosphate dikinase to generate PEP for re-entry into the C4 cycle.

The crassulacean acid metabolism (CAM) pathway is named for a carbon fixation pathway discovered in the Crassulaceae family of succulent plants including pineapples as well as various cactus species. It utilizes a similar biochemical mechanism as the C4 pathway, but occurs within a single photosynthetic cell. The major difference is that the CO_2 is only taken in at night, and it quickly turned into malate, which is stored in vacuoles until daytime. The malate is then released and decarboxylated to provide the RuBP carboxylase (rubisco) with a steady stream of CO_2 for fixation. Because there is such a rush of PEP carboxylase activity at night to fix the atmospheric CO_2 to PEP, there is a high rate of starch breakdown to provide the glucose for glycolytic generation of PEP. Interestingly, as the malate is decarboxylated in the day, its product, pyruvate, can then be used to re-synthesize glucose (see gluconeogenesis below) and then starch.

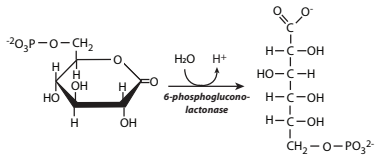
Pentose Phosphate Pathway

NADPH is found not only in plants, but in animal cells as well. Although our first discussion of NADPH was in the context of photosynthesis, it is also a general reducing agent in any cell. It is also crucial to note that though introductory texts often consider NAD^+/NADH and NADP/NADPH similarly as high energy electron carriers, and although they are structurally differentiated only by a phosphate group (on the 2'-OH of adenosine), they are *not* interchangeable in the metabolic pathways of a cell. NADP/NADPH is used in reductive metabolic pathways, whereas NAD+/NADH is used in oxidative pathways. With such an important role in biosynthesis, it is no surprise that its production is part of a major metabolic pathway, the pentose phosphate pathway (figure 7), also called the phosphogluconate pathway, and the hexose monophosphate shunt.

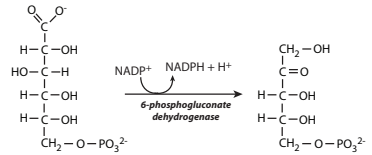
In step 1 of this pathway, glucose-6-phosphate and NADP+ are bound to glucose-6-phosphate dehydrogenase, which transfers a hydride ion from glucose-6-phosphate to NADP+ to form 6-phosphoglucono-δ-lactone and NADPH.



In step 2, the 6-phosphoglucono-δ-lactone is hydrolyzed to 6-phosphogluconate using 6-phosphogluconolactonase. This reaction actually proceeds fairly quickly even without the enzyme.



In step 3, the 6-phosphogluconate is decarboxylated by 6-phosphogluconate dehydrogenase, in the process producing more NADPH, as well as the five-carbon sugar, ribulose-5-phosphate. This metabolite is used by the cell as the basis for nucleotide synbthesis. This concludes the NADPH-producing portion of the pentose phosphate pathway.



However, it is useful, in the context of this chapter, to also consider the fate the Ru5P, which is converted to ribose-5-P by ribulose-5-P isomerase or it is converted to xylulose-5-phosphate using ribulose-5-P epimerase. The ribose-5-phosphate is used in nucleotide synthesis, so plays an important role in not only nucleic acid production, but general metabolism (e.g. for ATP).

Ribulose-5-phosphate and NADPH are the most significant products of this pathway. As mentioned earlier, NADPH is important as a general reducing agent. The mechanism for this involves glutathione and glutathione reductase. Glutathione is the primary scavenger of reactive oxygen species such as oxides and peroxides, and the key regulator of cellular oxidative stress. The reduced form of the glutathione tripeptide (Glu-Cys-Gly) dimerizes with another glutathione via disulfide bond as they donate electrons to oxidizers, and is regenerated by glutathione reductase. NADPH is a necessary cofactor for glutathione reductase activity, providing the electrons to reduce the G-S-S-G dimer.

A metabolic disorder known as G6PD deficiency manifests itself in erythrocytes, or red blood cells. NADPH in this case, is needed not for biosynthetic pathways, but to regenerate reduced glutathione (GSH). GSH is needed to detoxify peroxides through the action of glutathione peroxidase. Since the NADPH is in short supply (due to defect in G6PD), peroxides build up and cause damage to the membrane lipids. Extensive damage can lead to premature cell death by autolysis.

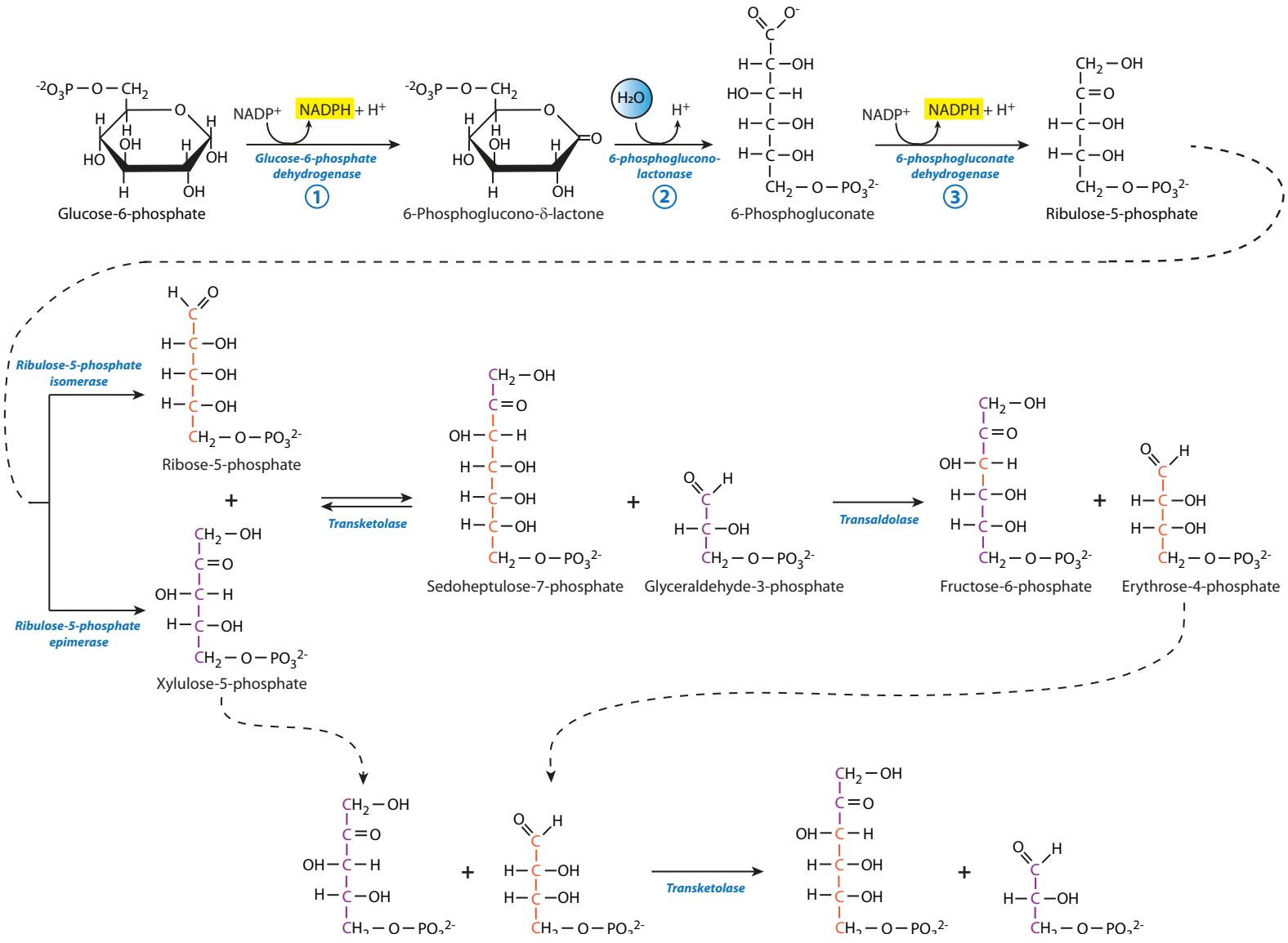


Figure 7. The pentose phosphate pathway. The first three reactions generate the energy carrier NADPH in the process of converting glucose-6-phosphate to ribulose-5-phosphate. The Ru5P is important as a precursor to nucleotide synthesis, as well as for production of other sugars and important metabolic intermediates, such as fructose-6-phosphate and glyceraldehyde-3-phosphate. Transketolase then transfers the terminal two carbons of ribulose-5-P to xylulose-5-P, making sedoheptulose-7-phosphate and G3P. Transaldolase comes up next. It transfers a 3-carbon unit from sedoheptulose-7-P to the G3P, forming erythrose-4-phosphate and fructose-6-phosphate.Transketolase is used again at this point, transferring a 2-carbon unit from xylulose-5-phosphate - to erythrose-4-phosphate and generating more G3P and fructose-6-P.

Gluconeogenesis

Having considered the initial anabolic reaction of life - carbon fixation by photosynthesis, we now turn our attention to utilizing the smaller metabolites to generate glucose and other sugars and carbohydrates. Glucose is the most important fuel for most organisms, and the only fuel for some cell types, such as brain neurons. Potential building blocks of glucose include many of the products and intermediates of glycolysis and the TCA cycle, as well as most amino acids. The key reaction is conversion of any of these compounds into oxaloactetate before using them to make glucose. In animals, the amino acids leucine and isoleucine, as well as any fatty acids, cannot be used to build glucose because they convert first to acetyl-CoA, and animals have no pathway for acetyl-CoA to oxaloacetate conversion. Plants, on the other hand, can push acetyl-CoA to oxaloacetate through the glyoxylate cycle, which will be discussed shortly.

The process of gluconeogenesis is in many ways the simple opposite of glycolysis, so it is not surprising that some of the enzymes used in glycolysis are the same as those used for gluconeogenesis. However, there are a few exceptions. These arose (and have probably evolved) for two major reasons - (1) the thermodynamics of the reaction are prohibitive, and (2) the need for independent control of the catabolic and anabolic processes. Since there is this parallel, we will explore gluconeogenesis first by starting with one of the major products of glycolysis, pyruvate. Pyruvate can be converted to oxaloacetate by pyruvate carboxylase, in a reaction requiring ATP hydrolysis. The oaxaloacetate is then converted to phosphoenolpyruvate (PEP) by PEP carboxykinase, which also uses nucleotide triphosphate hydrolysis for energy, though this time it is GTP.

As shown in the summary/comparison (figure 8), from the formation of PEP to the formation of fructose-1,6-bisphosphate the enzymes used in gluconeogenesis are exactly the same enzymes used in glycolysis. This works because the free energy change in those reactions is relatively small. However, in the dephosphorylation of fructose-1,6-bisphosphate to to fructose-6-phosphate, and subsequently in the dephosphorylation of glucose-6-phosphate to glucose, there is a large free energy change that works against the gluconeogenic reactions. Thus, the enzymes that drive these reactions are different from the enzymes that drive the reverse reactions in glycolysis (i.e. hexokinase, phosphofructokinase). These two hydrolytic reactions are catalyzed by *fructose bisphosphatase* and *glucose-6-phosphatase*, respectively. Full reversal of glycolysis in animals is limited, however, to liver and kidney, since they are the only tissues that express glucose-6-phosphatase. Other tissues use different mechanisms for generating glucose (e.g. glycogenolysis).

Interestingly, PEP carboxykinase (PEPCK) is unregulated at the protein level. There are no known activators or inhibitors of its activity. The only regulation of PEPCK appears to be at the level of transcription: glucagon can stimulate it (as can gluocorticoids and thyroid hormone), while insulin can inhibit it. The other gluconeogenic enzymes, though, do have direct activators and inhibitors. They are allosteric modulators, binding away from, but influencing the shape and efficacy of the substrate binding site. In examining the regulation of these enzymes, one important regulator stands out because it is not a metabolite of either glycolysis or gluconeogenesis. Fructose-2,6-bisphosphate (F2,6P) is an activator of phosphofructokinase, and an inhibitor of fructose bis-phosphatase. F2,6P levels are controlled by fructose-bisphosphatase-2 and phosphofructokinase-2, which are themselves controlled by levels of fructose-6-phosphate, as well as through a hormone-driven signaling cascade shown in the figure on the next page.

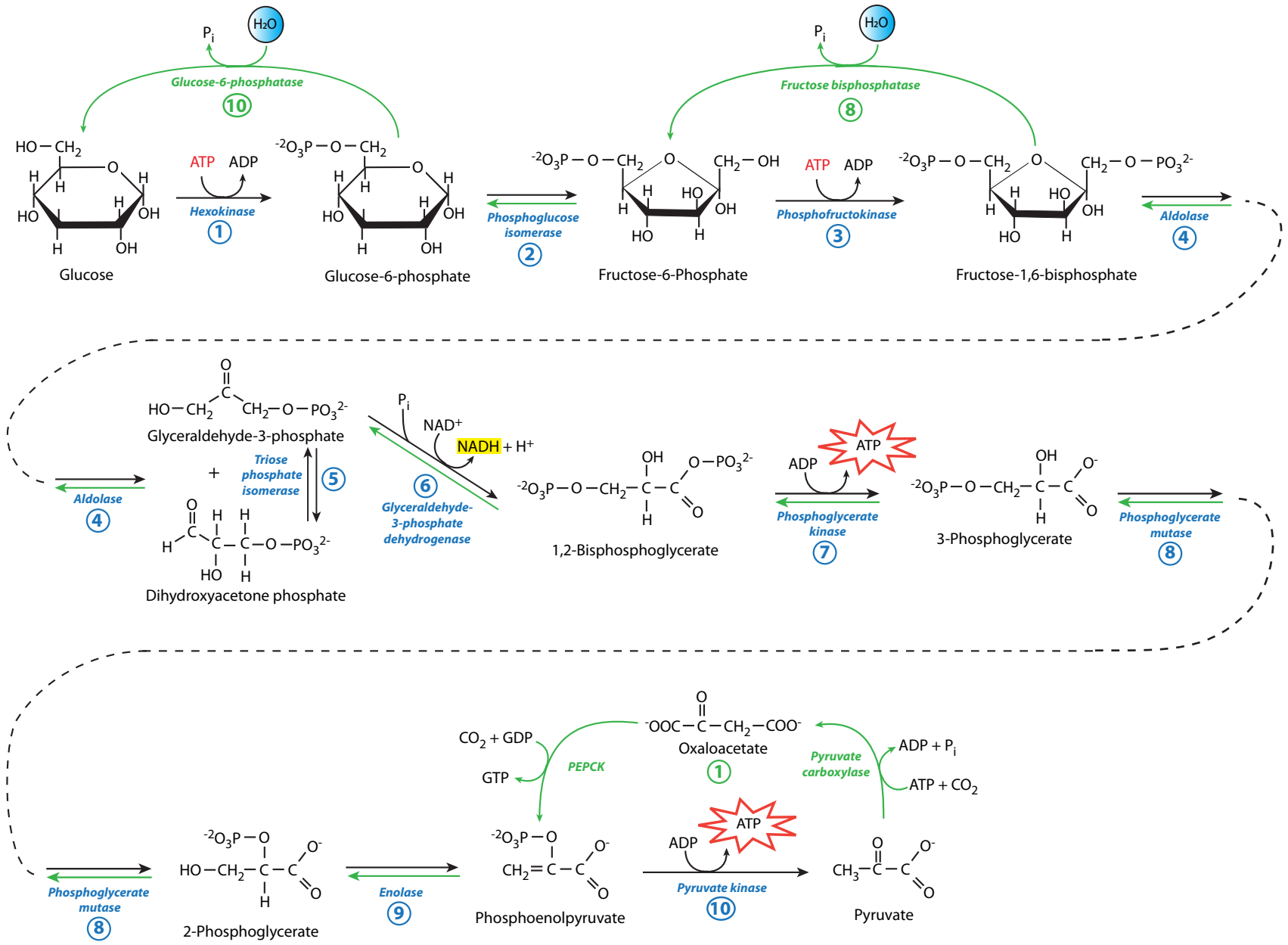


Figure 8. Gluconeogenesis (shown in green arrows) shares some, but not all enzymes with the reverse process, glycolysis (black arrows).

The glyoxylate cycle provides a mechanism for plants to convert acetyl-CoA into oxaloacetate, and therefore contribute to gluconeogenesis. This allows them to convert fatty acids and the hydrophobic amino acids leucine and isoleucine into glucose when necessary. The ability to do this comes from a plant-specific organelle called the glyoxysome, as well as some mitochondrial enzymes. The glyoxysomal part of the cycle consists of five steps, of which the first three contribute to the conversion, while the last two steps regenerate the glyoxysomal oxaloacetate (figure 9).

Once the macromolecules have been broken down to acetyl-CoA, they enter the glyoxysome and combine with oxaloacetate to make citrate. This is catalyzed by citrate synthase just as in the mitochondrial TCA cycle. The next reaction also uses a familiar enzyme: aconitase catalyzes the conversion of citrate to isocitrate. However, the aconitase is a cytosolic enzyme, so the citrate is transported out of the glyoxysome and then the isocitrate transported back in.

At this point, the glyoxysomal-specific enzyme, isocitrate lyase, hydrolyzes isocitrate to yield succinate and glyoxylate. The succinate is transported to the mitochondrion, where TCA cycle enzymes convert it to fumarate and then malate, which is transported out to the cytosol. In the cytosol, the malate is converted to oxaloactetate through malate dehydrogenase, and gluconeogenesis can proceed.

The glyoxylate is acted upon by another glyoxysomal enzyme, malate synthase, which adds it to acetyl-CoA to form malate.

The final step of the glyoxysomal portion of the glyoxylate cycle is oxidation of the malate to oxaloacetate by glyoxysomal malate dehydrogenase.

So, to summarize, the pool of oxaloacetate within the glyoxysome is used and regenerated within the glyoxysome. Acetyl-CoA is converted to succinate within the glyoxysome, but then goes to the mitochondrion for conversion to malate, and finally the cytosol for conversion to a separate pool of oxaloacetate that is then used in gluconeogenesis.

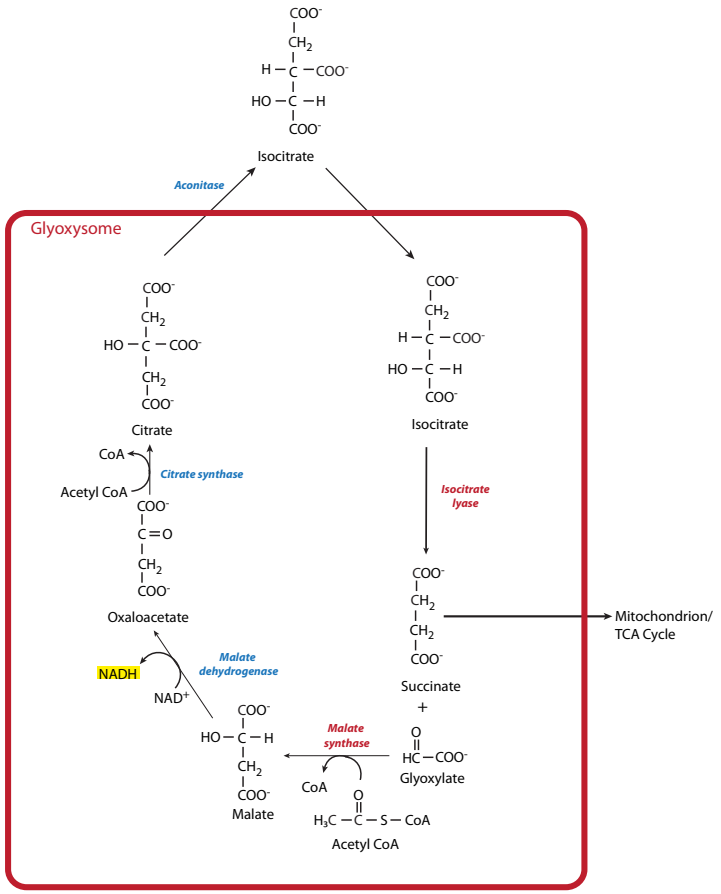


Figure 9. The glyoxylate cycle.

Glycogen synthesis

Although glucose is the primary fuel for cells, it is not an efficient molecule for long-term storage in complex (i.e. greater than single-celled) organisms. Therefore, in both plants and animals, the glucose molecules are linked together to form polysaccharides known as glucans. In animals, the glucan formed is glycogen, which consists of glucose molecules linked by α(1->4) glycosidic bonds, and branching α(1->6) bonds approximately between 8 to 14 residues apart. The average size of a glycogen unit is a cytoplasmic granule containing over 100000 glucose molecules. The addition of a glucose-1-phosphate to another (or to a glycogen chain) is energetically unfavorable, so it must be coupled with a sufficiently exergonic reaction to proceed.

Glycogen synthesis begins with UDP-glucose phosphorylase, which combines the nucleotide uridine triphosphate (UTP) with glucose-1-phosphate to release pyrophosphate (PPi) and form UDP-glucose.

The phosphoanhydride exchange reaction catalyzed by UDP-glucose phosphorylase is minimally exergonic. However, the pyrophosphate released is quickly hydrolyzed by inorganic pyrophosphatase, a ubiquitous cytosolic enzyme, in a highly exergonic reaction. This pyrophosphate hydrolysis is a mechanism utilized in many biosynthetic pathways to provide energy for otherwise endergonic reactions.

In the next step, glycogen synthase attaches the UDP-glucose to the pre-existing glycogen chain with an α(1->4) linkage. It cannot join two individual glucoses together, only add to a pre-existing chain. This means that there must be some workaround for the frst two glucoses: glycogenin is an enzyme that catalyzes the addition of UDP-glucose to itself, and can do so for up to seven UDP-glucose molecules, thus forming a short primer for glycogen synthase to work with. Furthermore, glycogen synthase can only add glucoses with an α(1->4) link. For branching to occur, a branching enzyme (specifically, amylo-(1,4->1,6)-transglycosylase is needed. This enzyme can transfer terminal chain segments to the 6-carbon hydroxyl of any glucose in a glycogen chain. However, the branches can only be added if there are at least 4 glucose residues between them, and if the originating chain was at least 11 residues in length.

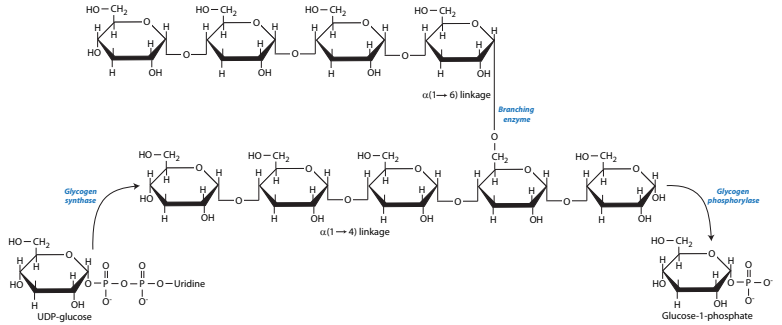


Figure 10. Glycogen synthesis

Oligosaccharide synthesis

Like glycogen synthesis, oligosaccharide synthesis also requires the initial step of coupling the sugar with a nucleotide. In mammals, a major disaccharide is lactose, which is the linkage of a galactose and a glucose, and the formation is catalyzed by lactose

synthase. However, before the lactose synthase is able to act, the galactose must first be in the form of a UDP-galactose. Similarly, in plants, the major disaccharide is sucrose, formed by the linkage of UDP-glucose and fructose-6-phosphate. This results in sucrose-6-phosphate, which is then readily dephosphorylated to sucrose. These kinds of mechanisms are also used in the glycosylation of proteins and lipids, which will be discussed primarily in the protein processing and trafficking chapter.

The major hexose species besides glucose are fructose, mannose, and galactose. Interconversion between these hexoses can occur via intermediates, as demonstrated in glycolysis (glucose-6-P to fructose-6-P). Mannose-6-P can be converted to fructose-6-P by phosphomannose isomerase. Galactose can be converted similarly, to galactose-1-P and then to glucose-1-P. The galactose to glucose conversion can also take place by epimerization of UDP-Glucose to UDP-galactose via intermediate redox using NAD^+/NADH.

Mutation of galactose-1-phosphate uridylyltransferase or mutations of other enzymes in this pathway (uridylyl transferase mutations are most common and usually most severe) can lead to galactosemia, a human genetic disease whose symptoms begin in infancy and may include mental retardation, liver damage, jaundice, vomiting, and lethargy. The cause of these symptoms is generally a buildup of galactose-1-phosphate, especially in the liver and nervous tissue. Fortunately, with early diagnosis, the symptoms can be prevented by avoiding milk products (lactose).

Fatty acid synthesis

This anabolic process is accomplished using a different set of enzymes than the catabolism of fatty acids discussed earlier. Fatty acid synthesis (fig. 11) starts with the formation of palmitic acid (C16) from acetyl-CoA and malonyl-CoA (which is itself a 3-carbon molecule formed from acetyl-CoA). Another difference between the catabolic and anabolic reactions for fatty acids is the location: whereas we saw that catabolism occurs largely in the mitochondria, fatty acid synthesis is run from a single large cytoplasmic enzyme complex. The fatty acid synthase system is comprised of seven enzymes linked together with an acyl carrier protein (ACP). As mentioned, this complex is found in the cytoplasm, so its substrates must be as well. The acetyl-CoA in the cytoplasm is primarily derived from the mitochondrial acetyl-CoA via a citrate-malate shuttle that couples deacetylation in the mitochondrion with acetylation in the cytosol.

The acetyl-CoA and malonyl-CoA are linked to the synthase and ACP, then there is a sequence of acetyl group transfers that runs a total of seven times to form palmitoyl-ACP, from which the palmitic acid is finally released. Palmitic acid is the precursor for variety of long-chain fatty acids such as stearic acid, palmitoleic acid, and oleic acid. Generally, there is either an elongation or sometimes a desaturation step. However, desaturation is a tricky process for vertebrates. The desaturation at C9 to form oleic acid from stearic acid can occur; however, other desaturations such as desaturation at C-12 to generate linoleic acid are not possible in vertebrates. Interestingly, they can be carried out in plant species. Furthermore, even though linoleic acid cannot be synthesized by vertebrates, it is nevertheless needed by vertebrates, which build arachidonic

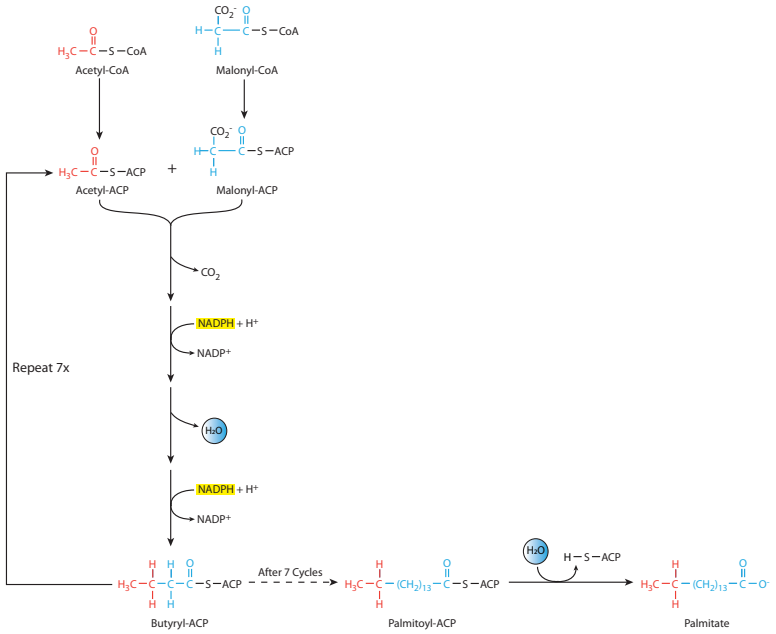


Figure 11. Fatty Acid Synthesis

acid, prostaglandins, and other molecules from it. Linoleic acid is therefore considered an *essential* fatty acid, since it must be ingested by the animal.

These fatty acids are then used to form the triacylglycerols that form the bulk of the energy storage molecules in most animals. Triacylglycerols are synthesized by the reaction of fatty acyl-CoA chains with glycerol-3-phosphate. Two rounds of this reaction yields diacylglygerol-3-phosphate (phosphatidic acid). After the action of phosphatidate phosphatase, the phosphatidic acid is converted to 1,2-diacylglycerol. This reacts with fatty acyl-CoA to form the final triacyglycerol.

Each of the fatty acyl chain additions generates an ester bond, which requires a significant energy input: that energy comes from a linked ATP hydrolysis reaction for each chain addition.

Amino acid synthesis

In humans, only half of the standard amino acids (Glu, Gln, Pro, Asp, Asn, Ala, Gly, Ser, Tyr, Cys) can be synthesized (fig. 12 and 13), and are thus classified the nonessential amino acids. Within this group, the first three, glutamate, glutamine, and proline, have a shared anabolic pathway. It begins with glutamate dehydrogenase, which adds ammonia to α-ketoglutarate in the presence of NADPH to form glutamate. This is a key reaction for all amino acid synthesis: glutamate is a nitrogen (amino group) donor for the production of all the other amino acids.

Glutamine synthetase catalyzes the formation of glutamine from glutamate and ammonia. This is an important biochemical reaction for a completely different reason: it is the primary route for ammonia detoxification.

Proline is synthesized from glutamate in a two-step process that begins with the reduction of glutamate to a semialdehyde form that spontaneously cyclizes to D-pyrroline-5-carboxylate. This is reduced by pyrroline carboxylate reductase to proline.

Alanine and Aspartate are the products of glutamate-based transamination of pyruvate and oxaloacetate, respectively.

Asparagine is synthesized through one of two known pathways. In bacteria, an asparagine synthetase combines aspartate and ammonia. However, in mammals, the aspartate gets its amino group from glutamine.

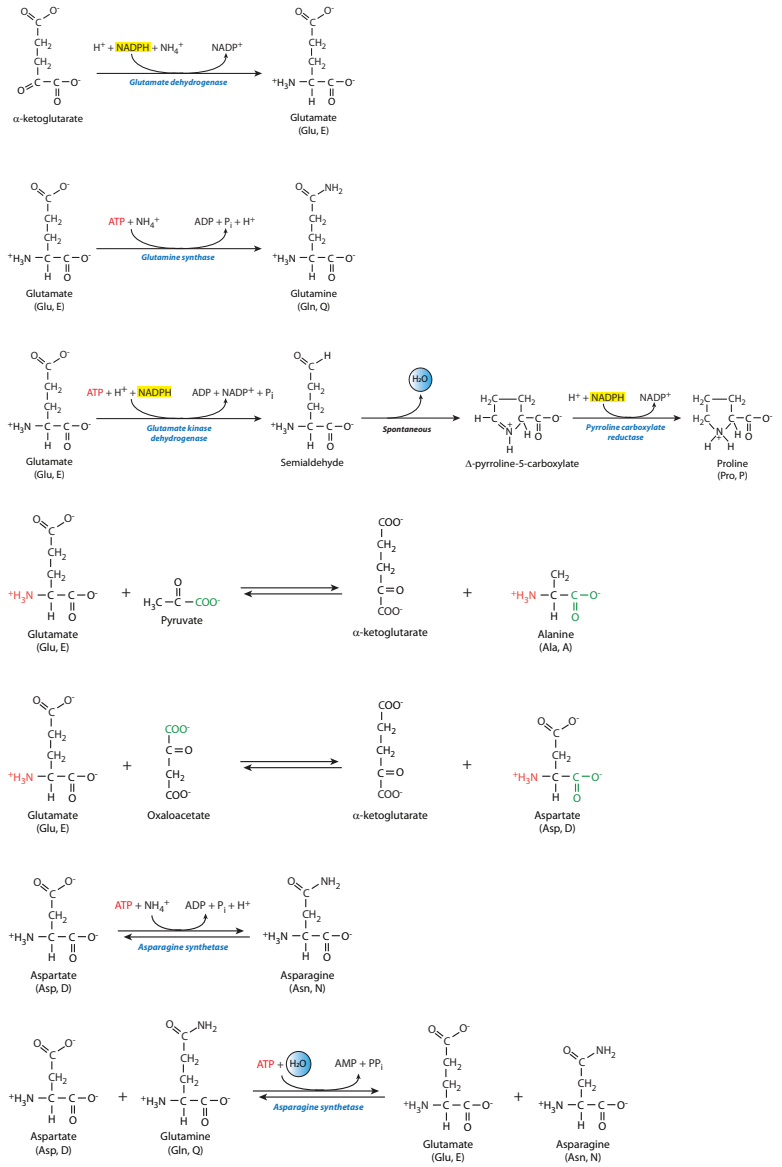


Figure 12. Synthetic reaction for amino acids: Glutamate, Glutamine, Proline, Alanine, Aspartate, Asparagine.

The synthesis of serine begins with the metabolic intermediate 3-phosphoglycerate (glycolysis). Phosphoglycerate dehydrogenase oxidizes it to 3-phosphohydroxypyruvate. An amino group is donated by glutamate in a reaction catalyzed by phosphoserine transaminase, forming 3-phosphoserine, and finally the phosphate is removed by phosphoserine phosphatase to produce serine.

Serine is the immediate precursor to glycine, which is formed by serine hydroxymethyltransferase. This enzyme requires the coenzyme tetrahydrofolate (THF), which is a derivative of vitamin B9 (folic acid).

Serine is also a precursor for cysteine, although the synthesis of cysteine actually begins with the essential amino acid methionine. Methionine is converted to S-adenosylmethionine by methionine adenosyltransferase. This is then converted to S-adenosylhomocysteine by a member of the SAM-dependent methylase family. The sugar is removed by adenosylhomocysteinase, and the resultant homocysteine is connected by cystathionine synthase to the serine molecule to form cystathionine. Finally, cystathionine-γ-lyase catalyzes the production of cysteine.

Tyrosine is another amino acid that depends on an essential amino acid as a precursor. In this case, phenylalanine oxygenase reduces phenylalanine to produce the tyrosine.

In general, the synthesis of essential amino acids, usually in microorganisms, is much more complex than for the nonessential amino acids and is best left to a full-fledged biochemistry course.

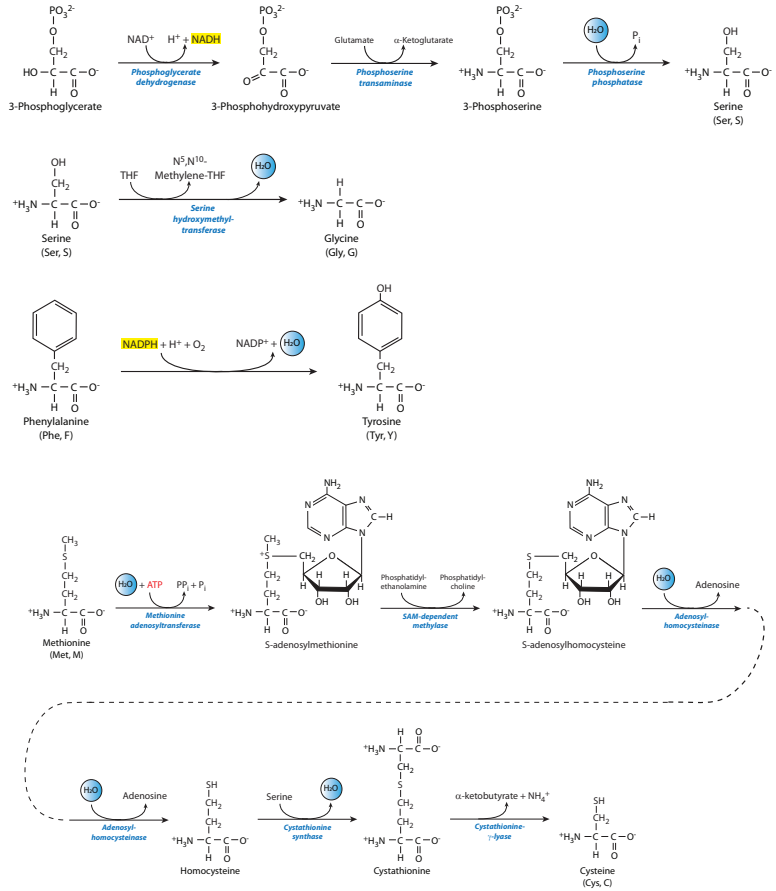


Figure 13. Synthetic reactions for amino acids: Serine, Glycine, Tyrosine, and Cysteine.