The natural universe

Part II: The present – Biochemistry, cells and energy

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7. What biochemistry and cellular biology tell us

We have seen how the universe grew from a tiny point to become the enormous – probably infinite – place which surrounds us. Focusing on only a small part of this huge entity, we saw how our solar system formed and then our planet; how the Earth evolved to reach its current, temporary state of support of life; when life was born and how it evolved from bacteria to plants and marine creatures, then land creatures like dinosaurs, then mammals and primates and – currently – us.

So now what? A complete study of the subject of us is well beyond the domain of this document, so let's concentrate on a limited subset of it. As a former physicist and *informaticien*, and so naturally interested in energy and communications, my goal (hope?) is to emphasize those two threads in studying the human body. This route should lead to the ultimate and most subjective-seeming domain, cognitive science – the study of the brain.

We must start small, though, with cells, as all else living follows from them. In order to understand them, we need to know some chemistry.

7.1. Some basic biochemistry

Understanding physiology and neuroscience requires knowing a certain amount of biochemistry. Most of the building blocks of our bodies are ***macromolecules***, composed of proteins (long chains of amino acids), polysaccharides (carbohydrates), lipids (fats) and nucleic acids (which make up DNA and RNA).

7.1.1. Amino acids and proteins

Amino acids are the basic building blocks for proteins. In a way, they are quite simple, all being variations on the same basic formula.

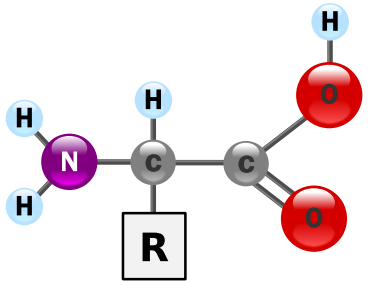


Figure 7.1: Common formula for amino acids, by GyassineMrabetTalk via Wikimedia Commons[1]

Each amino acid consists of a central carbon atom, an amino group (NH_3^+), a carboxyl group (COO^-) and a variable group, designated by the letter “R”, for residue. In the figure, the third H in NH_3 has been transferred to one O on the COO^- to make COOH and balance out the total charge.

Although there are over 500 amino acids, only 20 are manufactured from the genetic code. Figure 7.2 shows 21, but this includes selenocystein, which is incorporated into proteins by another mechanism and so is usually omitted from the list.

1 https://commons.wikimedia.org/wiki/File:AminoAcidball.svg

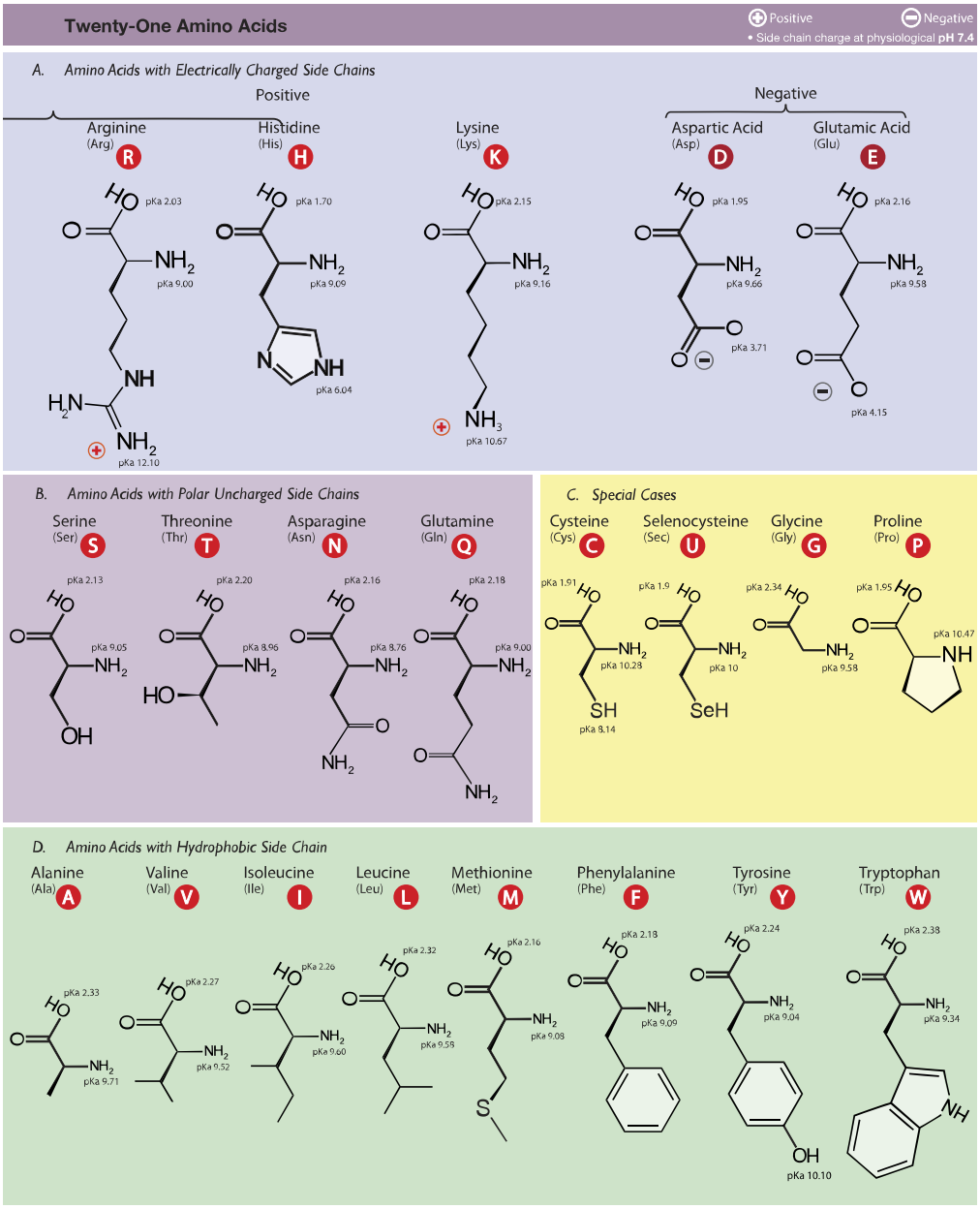


Figure 7.2: The amino acids, by Dan Cojocari via Wikimedia Commons[2]

Amino acids are the basic building blocks of proteins. A ***protein*** is a ***polypeptide***, that is a ***polymer*** (a chain of linked subunits) formed by ***condensation*** (ejection of water molecules) so as to link the amino acids by ***peptide bonds***. Schematically, it looks like the example in Figure 7.3, which shows the OH^- on the left combining with an H^+ on the right to make a water molecule and leave the two amino acids connected by a peptide bond. Actually, the process is not that direct, but goes through several enzyme-assisted steps in order to achieve the peptide bond. (We'll get to enzymes in a page or two.)

2 https://commons.wikimedia.org/wiki/File:Molecular_structures_of_the_21_proteinogenic_amino_acids.svg

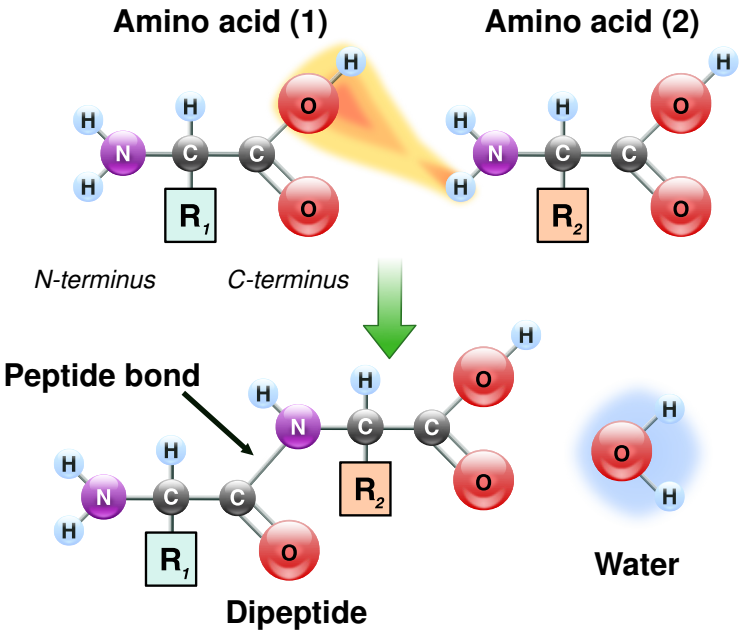


Figure 7.3: Peptide formation by condensation of two amino acids, by GyassineMrabet via Wikimedia Commons[3]

The bonding properties of proteins depend largely on their shape. The shape of the protein depends first on the sequence of amino acids, which constitutes the protein's ***primary structure***, which is therefore linear (one-dimensional). But proteins then curl up into ***secondary structures*** and again into tertiary structures. Imagine a protein backbone on which every N-H group hydrogen bonds[4] to the C=O group three or four residues earlier – 1 to 4, 2 to 5 and so on. The result is a helix, the ***alpha helix***, or ***α-helix***. Alpha helices and ***beta sheets*** are the most frequent secondary protein structures, although there are others. The R groups also may interact among themselves, bringing about a change in the 3-dimensional shape, or conformation, the ***tertiary structure***. Different polypeptide chains then may bind to form a ***quaternary structure***. In this way, proteins can take on very intricate shapes.

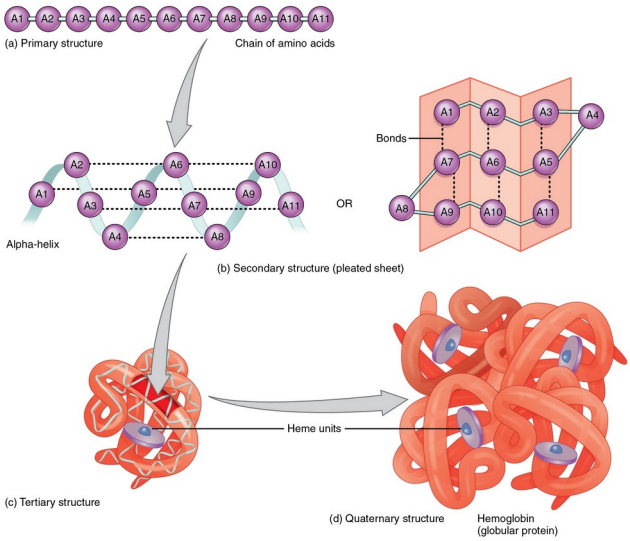
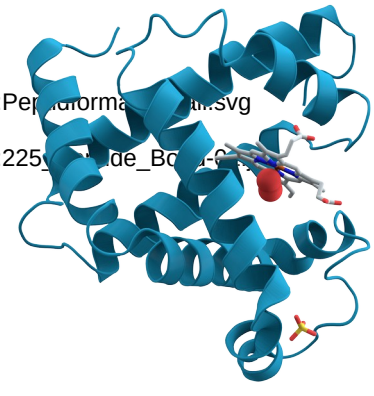


Figure 7.4: Four hierarchical structures of hemoglobin, by OpenStax College via Wikimedia Commons[5]

Symmetry does not seem to be well respected in biology. A helical protein with a right-handed twist can not



3 https://commons.wikimedia.org/wiki/File:Pep[illegible].svg
4 See water section in chapter 3.
5 https://commons.wikimedia.org/wiki/File:225[illegible]

generally be substituted for one with a left-handed twist: It just will not work the same way. This differing of the two versions is called ***chirality***.

Figure 7.5: 3D structure of myoglobin protein. Alpha helices are turquoise. By AzaToth via Wikimedia Commons[6]

Proteins may be enormously long polypeptide chains.

7.1.2. Enzymes

Enzymes, which usually are proteins[7], serve as organic catalysts, meaning that they help to bring about reactions that otherwise would not happen or would happen far too slowly. They only bring about reactions which are energetically possible but which nevertheless need a “push” to get started. Enzymes provide the push by lowering the ***activation energy*** of the reaction. Complete equations for different reactions would include the enzymes on both sides, but they usually are omitted. Every physiological process in the body depends on enzymes. Enzymes themselves only work under rather strict conditions of temperature and acidity. If the pH or temperature is not just right, the enzymes will not work, the reactions will not take place and the organism will suffer. We saw in chapter 3 how the conjugate pair of carbonic acid and bicarbonate constitute a buffer to maintain blood pH within the required limits.

The names of enzymes generally end in *-ase*, for example, lactase.

An enzyme can do its work because of its shape. Its folding forms a pocket called the ***active site***. A molecule which fits into the active site is called a ***substrate***. The enzyme can then usher the substrate through the reaction. This “***lock and key***” model of enzyme-substrate interaction is refined further in the ***induced-fit model***, wherein dynamic modifications in the enzyme's structure enable it to exactly fit the substrate, like a glove stretching to fit a hand..

The body can regulate the rate of such reactions by regulating the efficiency of the enzymes which catalyze it. One way to do this is to have a molecule similar in shape to the substrate and use it to block the active site. Or a molecule can bind to what is called an ***allosteric site*** on the enzyme, meaning a site which is not the active site. Binding to such a site changes the shape of the molecule and thereby renders it ineffective for binding with its usual substrate. It’s like a switch. If the enzyme catalyzes a reaction too much, so that there is an excedent of end products, the end products themselves may attach to an allosteric site and block further reactions, resulting in a feedback mechanism which reduces the rate of the reaction.

Reactions catalyzed by enzymes generally take place in a number of small steps rather than all at once. This has a double advantage:

- At each step, the enzyme can bring the reactants together, reducing the activation energy, the amount of energy needed for the reaction to begin.
- The energy output from each small step will not be so much as to harm the cell.

The sum of all the small steps is referred to as a ***metabolic pathway***.

7.1.3. Carbohydrates

Carbohydrates are molecules composed of carbon, hydrogen and oxygen, usually with the latter two elements in the same relative amounts as in water. So a generic “carb” could be represented by the formula

$$C_n(H_2O)_n$$

Carbohydrates are ***saccharides***, or sugars, and referred to as monosaccharides or polysaccharides, depending on the length of the molecule.

The most important ***monosaccharides*** in the body are: two, ribose and deoxyribose, based on rings of five carbon atoms (pentoses) and three, glucose, fructose and galactose, based on rings of six carbon atoms (hexoses).[8]

6 https://commons.wikimedia.org/wiki/File:Myoglobin.png
7 RNA can also function as an enzyme and it is not a protein.
8 In fact, glucose, galactose and fructose all have the same formula, $C_6H_{12}O_6$, but differ in their conformations. Similarly, ribose and deoxyribose share the same formula, $C_5H_{10}O_5$, but different conformations.

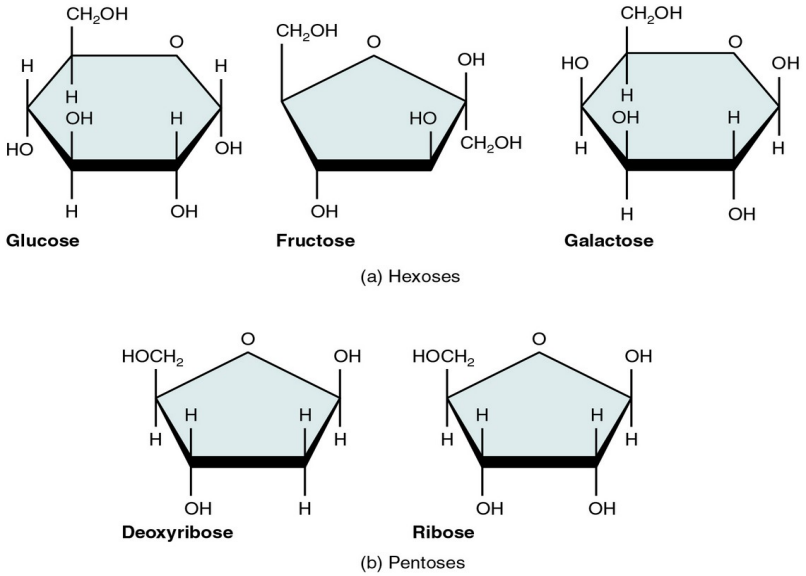


Figure 7.6: The five common monosaccharides, from Openstax College[9]

In order to interpret these diagrams. i.e., to count the carbon atoms, one needs to know that when an atom is designated without being connected by a line, as in the O at the top of fructose, it means the O is what is at that vertex, instead of C in this case. When the element or radical is joined by a line, it means the element or radical is joined – added to -- to a C at that vertex. Understanding this, it is clear that glucose, fructose and galactose all have the same formula, $C_6H_{12}O_6$, but differ in their conformations. Similarly, ribose and deoxyribose share the same formula, $C_5H_{10}O_5$, but different conformations.

Saccharides formed from two monosaccharides are called ***disaccharides***. Important ones for the human body are sucrose (table sugar), lactose (milk sugar) and maltose (malt sugar).

Polysaccharides may contain thousands of monosaccharides. Common ones are starches (polymers of glucose found in plant foods), glycogen (a polymer of glucose used for storage in the body) and cellulose ("fiber", found in the cell walls of plants).

We will be considering the importance of carbohydrates in the body's production of energy from food.

7.1.4. Lipids

Lipids are mostly hydrocarbons with very little oxygen and so forming only non-polar C-C or C-H bonds, making them hydrophobic. They consist of triglycerides, phospholipids, cholesterol and small quantities of other substances. Lipids are necessary for the formation of cell membranes and for other functions within cells.

Triglycerides

The commonest form of lipid ("fat") in the body is ***triglyceride***, consisting of a glycerol nucleus covalently bonded to the ends of three fatty-acid chains, long hydrocarbon chains terminated at one end by a carboxyl group (COO^-) and at the other by a methyl group (CH_3). The C=O link to the glycerol is an ester linkage.

9 http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@7.28:15/Organic-Compounds-Essential-to

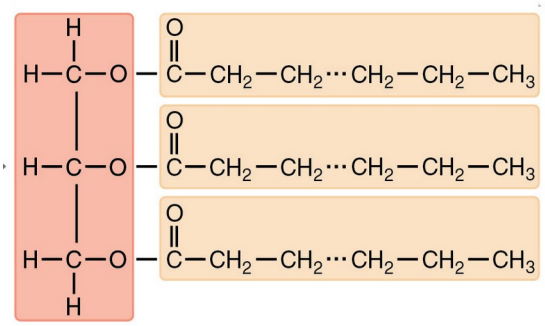


Figure 7.7: Triglyceride structure, with three fatty acids (orange background) attached to glycerol (pink), adapted from Openstax College.[10]

Fatty acids may be ***saturated*** or ***unsaturated***, meaning saturated in bonds with hydrogen. A saturated fatty acid has only single bonds between carbon molecules, leaving two bonds free to connect with hydrogen. An unsaturated acid may have a double bond between carbons, meaning each one can only bond with one hydrogen. The double bonds between carbons may change the shape of the fatty acid.

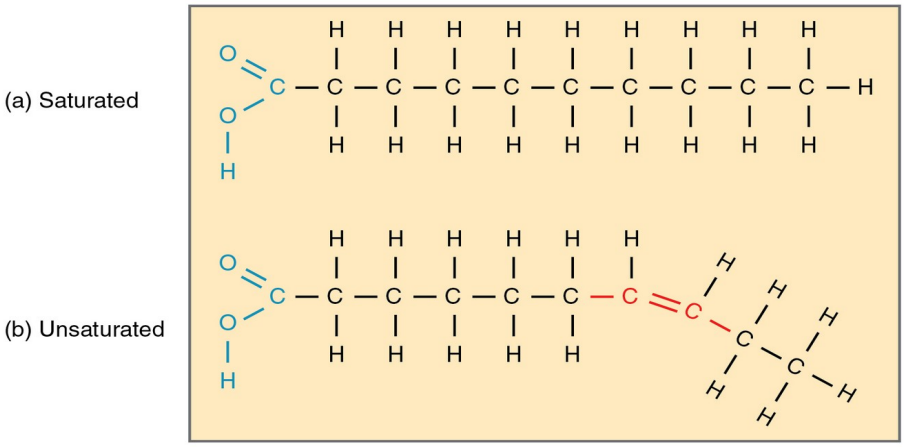


Figure 7.8: Saturated and unsaturated fatty acids, from Openstax college[11]

Saturated fatty acids pack tighter and so exist generally as semi-solid substances called ***fats***. Unsaturated fatty acids pack more loosely (because of the kinks) and are the constituents of more liquid ***oils***.

It is currently thought[12] that saturated fats lead to increased risk of heart disease, relative to unsaturated fats. The worst, though, is thought to be so-called ***trans fats***.[13] In order to ensure a longer shelf life, food producers sometimes convert unsaturated fats into saturated ones by hydrogenation, the addition of hydrogen atoms.[14] Trans fats are those which have only been partially hydrogenated.[15] On the other hand, there is some evidence that ***omega-3*** unsaturated fats are effective at reducing the risk of heart disease and perhaps beneficial in other ways. They are called omega-3 because the word “omega” is used in biochemistry to refer to the methyl end of the fatty acid chain and the double carbon bond is the third from that end.

Phospholipids

Phospholipids are similar to triglycerides, but the glycerol is attached to only two fatty acids, the third being replaced by a “head group” containing phosphate.

10 http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@7.28:15/Organic-Compounds-Essential-to
11 http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@7.28:15/Organic-Compounds-Essential-to
12 Or, at least, recently. It's hard to keep up with what nutritionists tell us.
13 The word trans comes from biochemistry and indicates functional groups on opposite sides of the carbon chain.
14 The first such hydrogenated shortening was marketed under the brand name Crisco. It was partially hydrogenated cottonseed oil.
15 In more detail, a cis double bond is converted to a trans double bond, hence the trans.

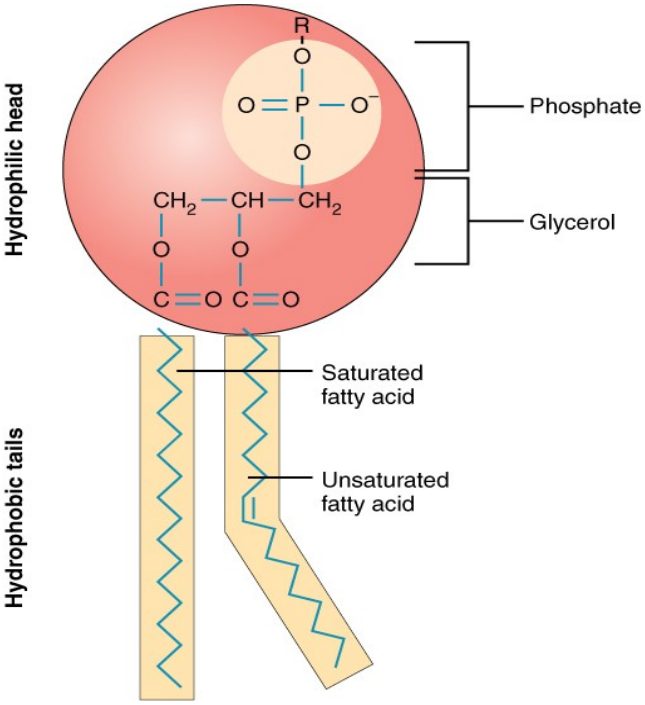


Figure 7.9: Phospholipid structure[16]

The phosphate “head” is negatively charged and therefore hydrophilic but the fatty acid tails are hydrophobic, so the molecule is ***ampiphatic*** (as discussed in the chemistry chapter) and forms micelles or membranes in an aqueous environment. Of major importance for life, phospholipids are the principal component of cell membranes.

7.1.5. Nucleotides

Just as proteins are polymers formed from chains of amino acids, ***nucleic acids*** – DNA and RNA – are polymers made up of chains of linked ***nucleotides***. A nucleotide is composed of a pentose (five-carbon) sugar molecule like deoxyribose (which gives the “D” in DNA) or ribose (in RNA), a nitrogenous base (or ***nucleobase***) and one phosphate group.[17] Different nucleotides contain different bases.

There are five possible nucleobases in two groups:

- ***pyrimidines*** – cytosine, thymine and uracil, with a single-ring structure; and
- ***purines*** – adenine and guanine, with two rings and therefore two nitrogen atoms.

16 Openstax *Anatomy & Physiology*, via Wikimedia Commons https://commons.wikimedia.org/wiki/File:0301_Phospholipid_Structure.jpg

17 Common usage employs the term nucleotide for those with more than one phosphate group.

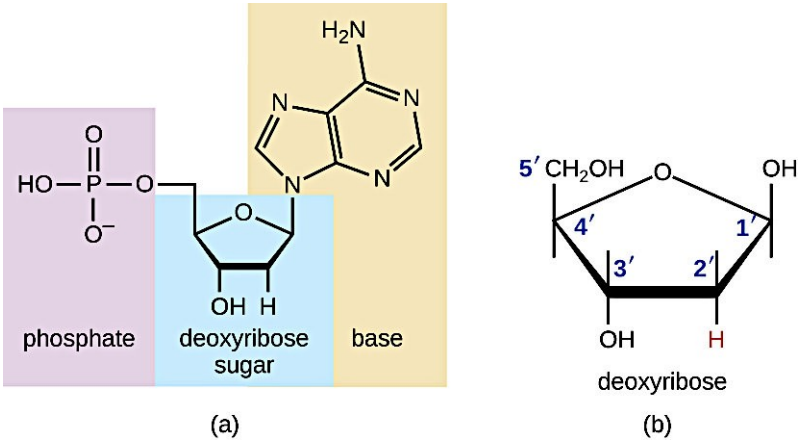


Figure 7.10: Deoxyribonucleotide, the backbone of DNA[18]

Another, very special nucleotide is ***adenosine monophosphate***, or ***AMP.*** When a second phosphate group is added to AMP, it makes ***ADP*** (adenosine diphosphate); addition of a third phosphate group makes ***adenosine triphosphate***, or ***ATP***, the "energy currency" or energy carrier in cells of all living organisms. (Much more, later.)

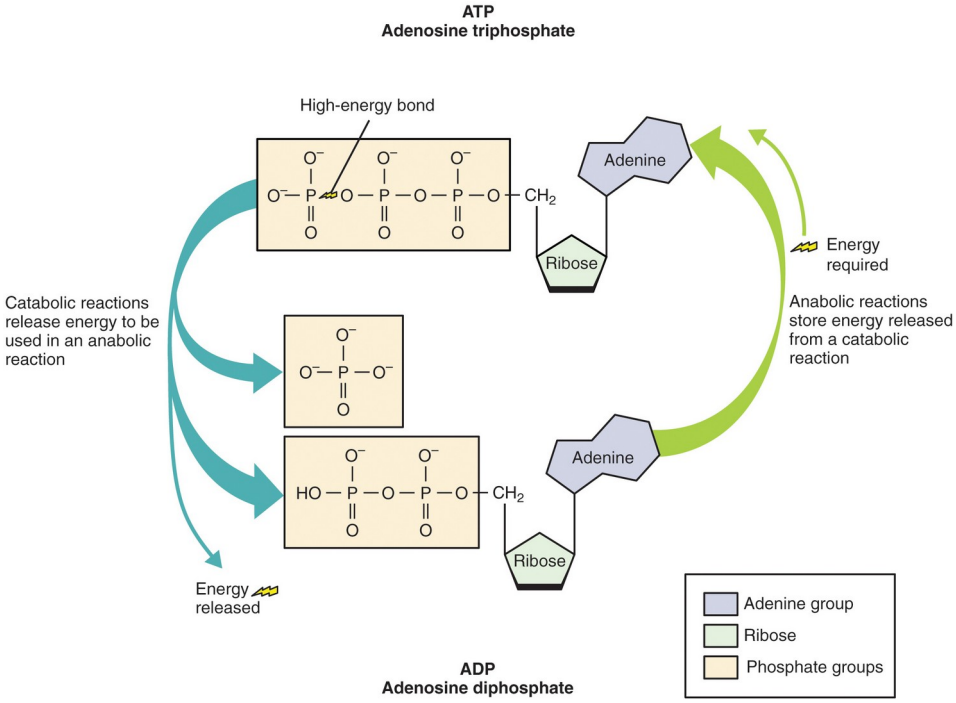


Figure 7.11: ATP and ADP[19]

Like all nucleotides, AMP consists of a nitrogenous base, adenine, attached to a pentose sugar, ribose. It takes energy to add a Pi (phosphate) to make ADP or a second Pi to make ATP. This energy is stored in the ATP molecule as chemical potential energy and can be recovered later to do useful biological work, such as flexing muscles or powering action potentials.

A nucleotide without the phosphate group is called a ***nucleoside***, so ATP may also be referred to as a nucleoside triphosphate. Nucleoside triphosphates are the raw materials for building RNA molecules.

18 From LibreTexts Biology, https://bio.libretexts.org/Courses/City_College_of_San_Francisco/Introduction_to_Microbiology_OER_-_Ying_Liu/11%3A_DNA_and_RNA/11.03%3A_Structure_and_Function_of_DNA.

19 From Openstax Anatomy and Physiology, http://cnx.org/contents/FPtK1zmh@7.30:wJt-Gj_K@4/Overview-of-Metabolic-Reaction.

7.1.6. Nucleic acids – DNA and RNA

The nucleic acids, DNA and RNA, are assembled from nucleotides. They differ in three ways:

- ***DNA***, ***deoxyribonucleic acid***, contains deoxyribose as its sugar; ***RNA***, ***ribonucleic acid***, contains ribose.
- The “allowed” nucleobases for DNA are ***cytosine*** (referred to in this context as ***C)***, ***guanine*** (***G***), ***adenine*** (***A***), and ***thymine*** (***T***); in RNA, T is replaced by ***uracil*** (***U***).
- DNA molecules form a double strand; RNA molecules, a single one.

The IUPAC (International Union of Pure and Applied Chemistry) has a rather hairy set of rules for numbering carbon atoms in organic compounds. In the case of the sugar in a nucleotide, the 1' carbon (one-prime, prime to denote sugar) is the one attached to the nitrogenous base. The count moves around the ring away from the oxygen apex.

Nucleic acids are formed by dehydration (or condensation, removal of a water molecule) between a pentose sugar of one molecule (the 3' carbon) with the phosphate (on the 5' carbon of the pentose) of another. The result is called a ***phosphodiester bond***. The chain is thus held together by a sugar-phosphate backbone, independently of attached nucleobases, which protrude out from the chain.

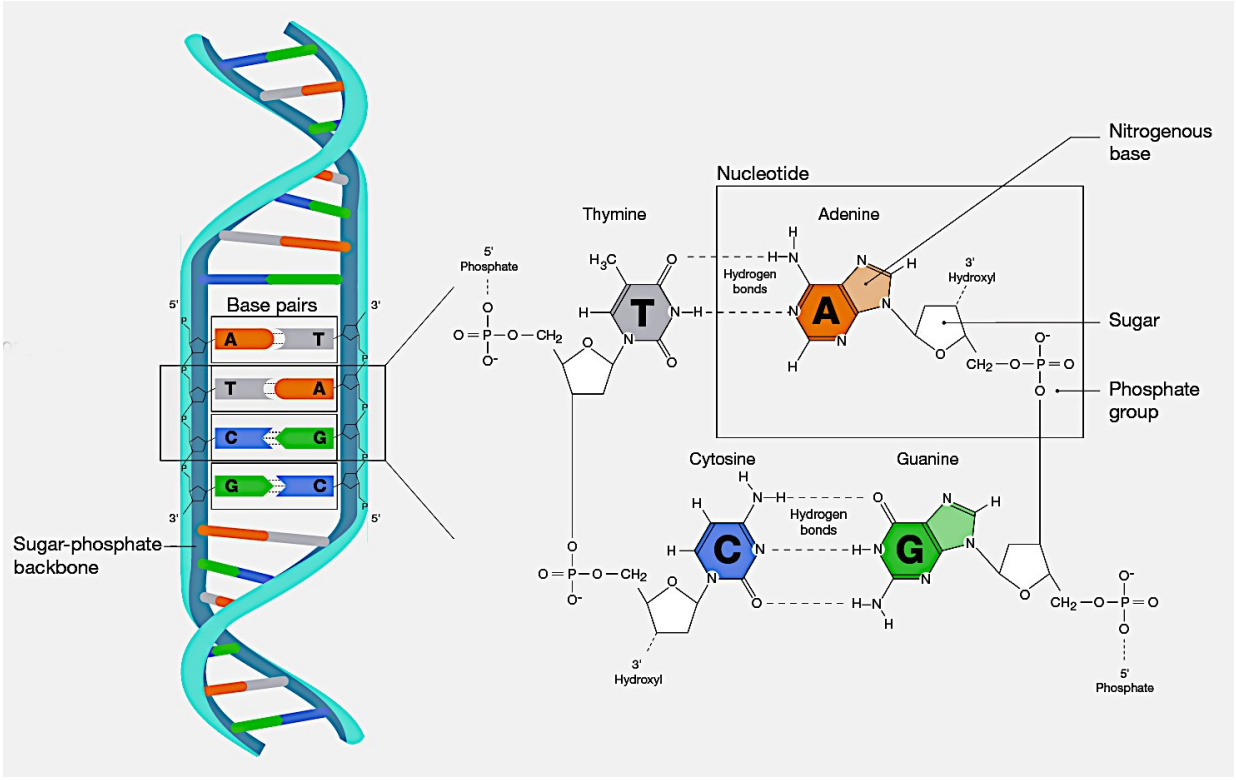


Figure 7.12: DNA chemical structure, from National Human Genome Research Institute[20]

DNA chains form double strands due to hydrogen bonds between nucleobases on each chain, with C bonding only to G and A only to T. So a purine (A or G) is always bonded to a pyrimidine (C, T or U). The result forms a double helix, like a twisted ladder. Note from figure 7.12 that there are three hydrogen bonds between guanine and cytosine, but only two between adenine and thymine.

The combination of two DNA strands into a double helix offers the advantage that the nucleobases are not sticking out into the cytoplasm where they may be more easily mutated. Rather, the bases of the two strands are “holding hands” (through hydrogen bonds) to protect each other from mutation. This increased security may explain why genetic information is stored in DNA, a double helix, rather than in RNA.

20 National Human Genome Research Institute, Nucleic acids. https://www.genome.gov/genetics-glossary/Nucleic-Acids

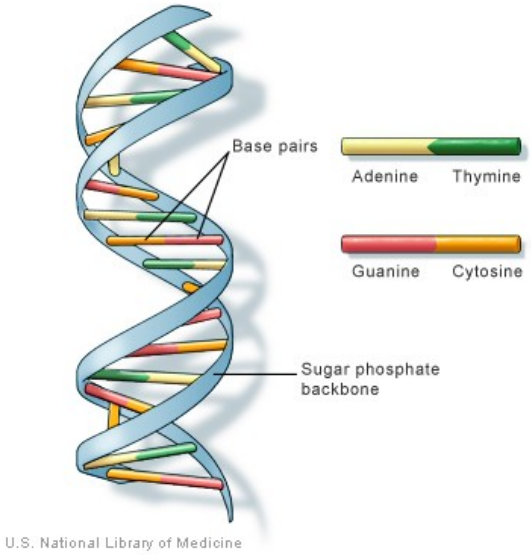


Figure 7.13: DNA structure[21]

Some detail: The nucleic acid strand is polar, i.e., the ends are not the same. One end has a phosphate group attached to the 5' carbon of the sugar; this is called the 5' end. The other end has a hydroxyl group (OH^-) attached to the 3' carbon of the sugar, so this is called the 3' end. When combining into a double helix, the ends are reversed, i.e., the 3' end of one is opposite the 5' end of the other.

Since the total length of all the DNA strands in a human nucleus would equal 2-3 m, it must be compacted in order to fit into the nucleus. The helical strand is wrapped around ***histone*** proteins to form ***nucleosomes***. The string of nucleosomes is then twisted and re-twisted, like a piece of cord, until it forms a compact string called ***chromatin***. The chromatin will be used to form chromosomes (only) when needed for reproduction.

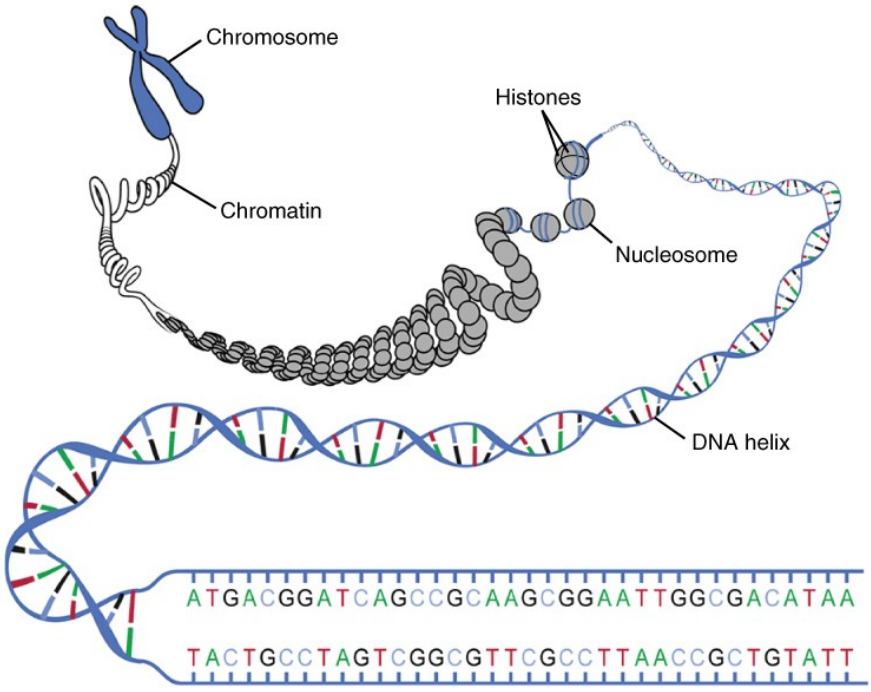


Figure 7.14: DNA compaction, from Openstax College[22]

7.1.7. Oxidation-reduction and electron carriers

The concept of oxidation and reduction is essential to biochemistry, so let's beat on it a while. Actually, it

21 From U.S. National Library of Medicine, http://ghr.nlm.nih.gov/handbook/basics/dn.
22 http://cnx.org/contents/FPtK1zmh@7.30:9TxHOD3O@4/The-Nucleus-and-DNA-Replication.

also is important to other domains of chemistry. Oxidation and reduction occur together in ***oxidation-reduction***, or ***redox***, ***reactions***.

An entity which loses electrons is said to be ***oxidized***; if it gains electrons, it is ***reduced***. Think of its charge, which is reduced (becomes more negative) as it gains an electron. Oxygen likes to gain electrons, it is said to be ***electronegative***, so when it pinches one from another substance, that substance is oxidized. A simple example is Na and Cl interacting in water:

$$Na + Cl \rightarrow Na^+ + Cl^-$$

The Na loses an electron and becomes positive; it is an electron donor and is oxidized, even without oxygen in the reaction. The Cl gains an electron, becoming negative, so is reduced. We can say symbolically,

$$Xe^- + Y \rightarrow X + Ye^-.$$

When an electron transfers from a less to a more electronegative atom, it loses potential energy, just like a falling massive object in a gravitational field. This potential energy is released and can be used for work.

A substance which is oxidized, i.e., gives up electrons, is an electron donor or ***reducing agent*** or ***reductant***. One which is reduced, i.e., gains electrons, is an electron receptor or **oxidizing agent** or ***oxidant***. When a donor (reductant) releases an electron which is then taken up by a receptor (oxidant), the reductant and oxidant together are said to constitute a ***conjugate redox pair***.

The term oxidation is understood perhaps most clearly in a reaction like the rusting (oxidation) of copper:

$$2\ Cu + O_2 \rightarrow 2\ CuO$$

One can see that:

- At the same time as oxygen receives electrons from Cu and so is ***reduced***,
- copper, in releasing electrons to oxygen, is ***oxidized*** to become copper oxide (rust).

So we can see that

- adding *oxygen* is also oxidation and releasing it is reduction.

Again,

$$H_2 + F_2 \rightarrow 2\ HF$$

which is perhaps not easy to recognize as an oxidation of hydrogen. But consider the two half-reactions, the obvious oxidation part

$$H_2 \rightarrow 2\ H^+ + 2\ e^-$$

and the reduction part

$$F_2 + 2\ e^- \rightarrow 2\ F^-$$

Put them together to get

$$H_2 + F_2 \rightarrow 2\ H^+ + 2\ F^- \rightarrow 2\ HF.$$

But now, look at combustion (oxidation) of propane. It can be complete combustion

$$C_3H_8 + 5\ O_2 \rightarrow \ \ 3\ CO_2 + 4\ H_2O$$

or partial combustion with some carbon left over

$$C_3H_8 + 2\ O_2 \rightarrow \ \ 3\ C + 4\ H_2O.$$

Carbon is obviously oxidized in complete combustion, since it takes on oxygen. Partial combustion, even though it does not add oxygen, is still considered oxidation. Electrons in covalent bonds of propane are about equally shared because carbon and hydrogen are close in their degrees of electronegativity. The same is true of the electrons in the oxygen molecule. But oxygen is one of the most electronegative elements, so in CO_2 and H_2O, the electrons are much closer to the oxygen atoms than to the C or H atoms, meaning that partial oxidation has taken place.

An even less obvious example is oxidation of copper oxide:

$$2\ Cu_2O + O_2 \rightarrow 4CuO.$$

The thing to notice here is that the first oxide of copper is ***cuprous*** oxide, where copper has a valency state

of +1. On the right, though, it has valency state of +2 and so this is ***cupric*** oxide. Copper has changed valency states and in so doing has given up an electron, which indicates its oxidation.

Chemists also talk about oxidation-reduction in term of ***oxidation states*** which describe the degree of oxidation of an atom in a chemical compound, but those are quite complicated, so we will ignore them.

Getting back to biochemistry, the basic energy-providing reaction is the oxidation of glucose:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O.$$

As in the case of propane, the electrons pulled out of glucose by the extremely electronegative oxygen are drawn into lower energy states and the energy released can be harvested to power metabolism. However, if the reaction is allowed to take place all at once, it releases a useless and dangerous amount of energy. To get around this problem, the reaction is broken up into “smaller” intermediate steps catalyzed by coenzymes acting as ***cofactors,*** intermediate oxidizing and reducing substances.

The ***coenzymes***[23] involved are nicotinamide adenine dinucleotide and flavin adenine dinucleotide, better and more simply known as ***NAD*** and ***FAD***. These two molecules are ***electron carriers*** and it is through redox reactions that they pick up and leave off their electrons.

The oxidized forms of NAD and FAD are NAD^+ and FAD. By gaining electrons, they are reduced to NADH and $FADH_2$.

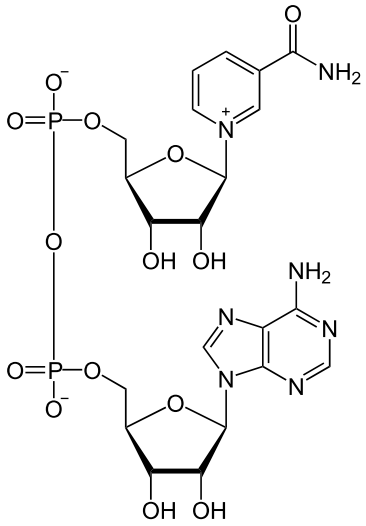


Figure 7.15: NAD molecule by NEUROtiker via Wikimedia Commons[24]

NAD is a dinucleotide, being composed of two nucleotides, which are joined by a phosphate group. One nucleotide has an adenine base (the lower, in the figure), the other, nicotinamide.

During cellular respiration (explained later), a molecule referred to as the substrate gives up two H atoms, bringing about the ***reduction*** of NAD^+ in the following way, where R means “residue” and indicates a part of the substrate:

$$RH_2 + NAD^+ \rightarrow NADH + H^+ + R$$

What is happening here is that a dehydrogenase, an enzyme, removes two hydrogen atoms, complete with their two electrons, from the residue and delivers them to NAD^+. The removal of two electrons tells us that the substrate is oxidized. Ignoring R on both sides

$$NAD^+ + 2H \rightarrow NAD^+ + 2\,e^- + 2\,H^+ \rightarrow NADH + H^+, \qquad (7.1)$$

where it is clear that NAD^+ has been reduced. In different detail,

$$NAD^+ + 2H \rightarrow NAD^+ + H^- + H^+ \rightarrow NADH + H^+$$

which shows that one of the H atoms is in the form of ***hydride***, H^-, with two electrons. The NAD^+ absorbs

23 A coenzyme is a non-protein compound that is necessary for the functioning of an enzyme. Enzymes are macromolecular catalysts, most of which are proteins.

24 NEUROtiker at https://commons.wikimedia.org/wiki/User:NEUROtiker, file from Wikimedia Commons at https://commons.wikimedia.org/wiki/File:NAD%2B_phys.svg.

the hydride, equivalent to two electrons and one proton, thereby gaining electrons and so being reduced to NADH.[25]

$$NAD^+ + H^- \rightarrow NAD^+ + H^+ + 2\ e^- \rightarrow NADH.$$

In a later step, the H atoms will be used for energy transfer and the NADH will give up its electrons and so be re-oxidized to NAD^+. In this way, NAD transports electrons from one reaction to another.

When an electron is transferred from glucose to NAD^+, it loses very little potential energy, so most of this energy remains available for later use in respiration.

Note that the + sign on NAD^+ is not an indication of its charge, which is in fact negative.[26] The H in NADH indicates refers to the added hydride ion. NAD^+ indicates the oxidized form of the nicotinamide ring with a positive charge on the N atom, shown on the left side of Figure 7.16.

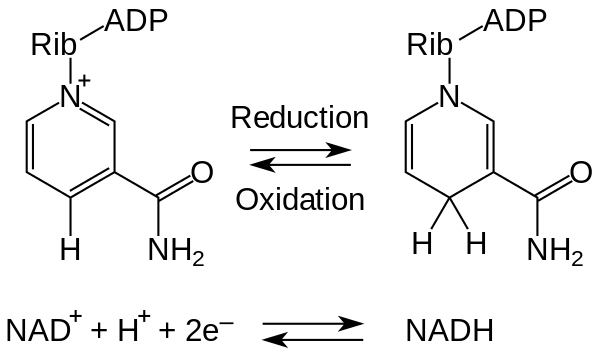


Figure 7.16: NAD oxidation-reduction from Wikimedia Commons[27]

We will later meet NADP, which is a close analog of NAD. In fact, you can substitute NADP for NAD in equation (7.1):

$$NADP^+ + 2H \rightarrow NADP^+ + 2\ e^- + 2\ H^+ \rightarrow NADPH + H^{+\cdot} \qquad (7.2)$$

FAD is a more complicated molecule. The equivalent formula for the reduction of FAD goes in two steps:

$$FAD + e^- + H^+ \rightarrow FADH$$

$$FADH + e^- + H^+ \rightarrow FADH_2$$

to make

$$FAD + 2H \rightarrow FAD + H^- + H^+ \rightarrow FADH_2$$

which shows that FAD is reduced to $FADH_2$, since it gains electrons.

7.2. Cell structure

Dogs and cats, butterflies and crayfish, strawberry plants and poison ivy are all organisms. ***Organisms*** are composed of ***organ systems*** (digestive, circulatory or immune systems, to mention three examples). These systems are in turn composed of ***organs***, which are composed of ***tissues*** which are composed of ***cells***. The cell is where the action starts!

The definition of life usually includes the requirement that whatever lives is capable of viable reproduction, which makes it subject to natural selection. All life is composed of cells and reproduction takes place both at the levels of the organisms and of the cells.

There are two kinds of cells, which have already been mentioned in the geology (!) chapter:

- ***Eukaryotic*** cells contain structures called organelles, which are not found in prokaryotic cells. This makes them bigger than prokaryotic cells. One such organelle is the nucleus, which houses the DNA.

25 Remember, NAD^+ and NADH are abbreviations, not chemical formulas.
26 Nelson and Cox, 517.
27 Reduction and oxidation of the coenzyme NAD, by Fvasconcelllos, https://commons.wikimedia.org/wiki/File:NAD_oxidation_reduction.svg.

- ***Prokaryotic*** cells are usually smaller. They do not contain organelles, so their DNA is floating around in the cytoplasm. They contain ribosomes to carry out protein synthesis, but their composition is different in eukaryotic and prokaryotic cells.

Cells come in a huge variety of shapes and functions. They are the basis of all living beings. Whether they are nerve cells, muscle cells, skin cells, bone cells or any other kind of cells; whether they come from humans or cats or grasshoppers or oak trees or bacteria, they all are based on common features like a membrane filled with cytoplasm, DNA or RNA along with ribosomes for reproduction, and the fabrication by cellular respiration of ATP for energy. This fact alone strongly suggests a common origin of all life.

7.2.1. Plasma membrane

As we saw in the discussion of water in chapter 3, lipid bilayers, in this case, ***phospholipid bilayers***, can form membranes. All cells are surrounded and protected by such membranes, also called ***plasma membranes***. The membrane contains various proteins which serve to pass substances across the membrane, to identify it or to fulfill other functions. (More on that shortly.) This view of the cell membrane, as a mosaic of elements, is called the ***fluid mosaic mode***l.

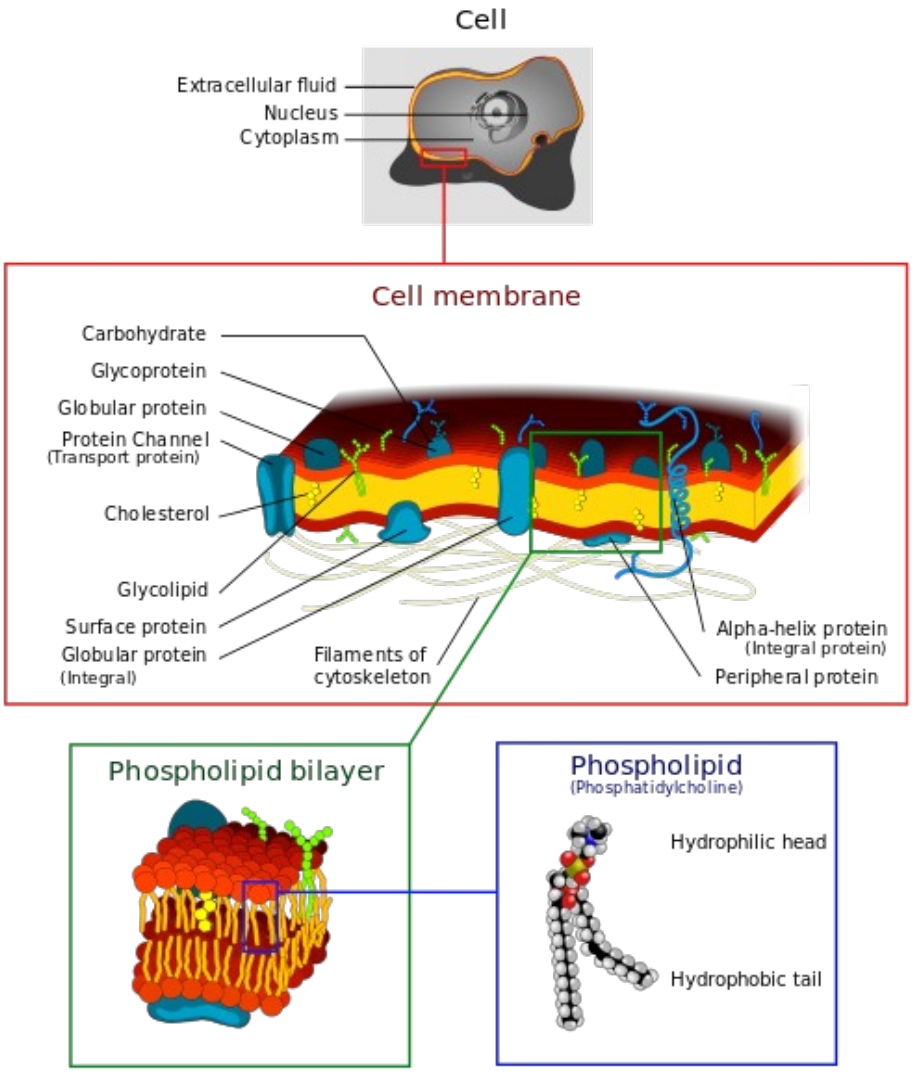


Figure 7.17: Structure of cell membrane, by "LadyofHats" via Wikimedia Commons.[28]

Everything inside the cell except the nucleus is the ***cytoplasm.*** The ***cytosol*** is the jelly-like fluid in which other structures float, the ***organelles***, including the the nucleus and mitochondria.

The membrane is capable of forming pockets called ***vesicles***. They can form on the inside of the cell membrane and surround material, then close and reopen on the outside in order to eject the material stored

28 https://commons.wikimedia.org/wiki/File:Cell_membrane_detailed_diagram_4.svg

in them. In the other direction, they may form around external material and carry it into the cell.

7.2.2. The cell nucleus

The nucleus holds the DNA, normally wrapped with other proteins into strings of ***chromatin***. Chromosomes are only constituted when needed to facilitate reproduction. The nucleus is surrounded by its own *double* membrane. Between the two membrane bilayers is the nuclear ***lumen***, which is continuous with that of the endoplasmic reticulum. The ***nucleolus*** within the nucleus serves to synthesize ribosomes, which in turn are sites for synthesis of proteins. The greater the number of proteins produced by a cell, the larger its nucleolus.

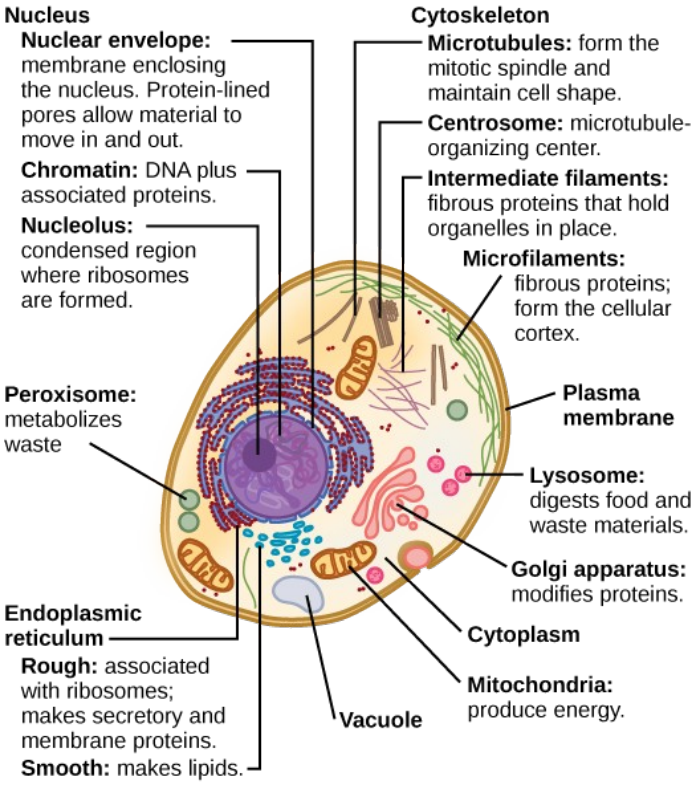


Figure 7.18: Typical animal cell, from Openstax College[29]

7.2.3. Mitochondria

Mitochondria are where the final products of digestion, mainly glucose, are converted into ATP, the body's “energy currency”, which transports energy to whatever metabolic process may need it. A ***mitochondrion*** has both an external and an internal membrane. ATP is produced by processes taking place along the inner membrane. The interior of the mitochondrion is called the ***matrix***. Cellular respiration (energy production) will be discussed in detail in chapter 9 on anatomy and physiology.

According to ***symbiogenesis***, or ***endosymbiotic theory***, mitochondria originally were prokaryotic bacteria which moved into other cells and formed a symbiotic relation with them. Similarly, choroplasts would have been prokaryotic bacteria which have moved into plant cells. Mitochondria still have their own DNA, independent of that in the cell nucleus, although they have abandoned or given up much of their original DNA.

The current model of eukaryotic evolution starts with an anaerobic eukaryote which absorbed an aerobic bacterium which later became a mitochondrion. Subsequently, some of these now aerobic eukaryotes absorbed photosynthetic bacteria, probably cyanobacteria, which became chloroplasts. The latter eukaryotes joined to form plants, whereas those which did not absorb the photosynthetic bacterium formed animals.

29 For this and next figure, http://cnx.org/contents/s8Hh0oOc@8.57:EygPBNl9@9/Eukaryotic-Cells.

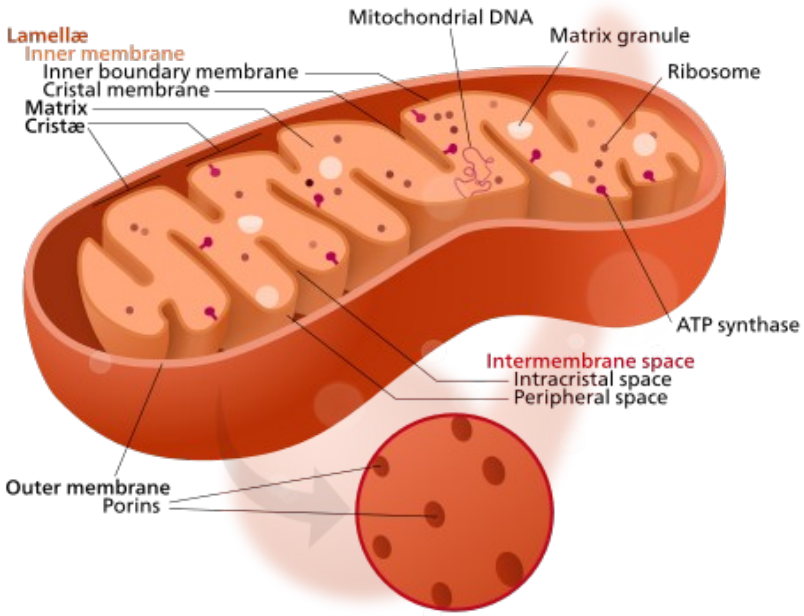


Figure 7.19: Mitonchondrion structure[30]

7.2.4. Ribosomes

Ribosomes are where proteins are built following instructions contained in DNA; the instructions are transmitted to the ribosome in the form of messenger RNA, mRNA, about which more later. Ribosomes consist of two subunits, large and small, both of which are constructed in the nucleolus from ribosomal RNA (~75%) and proteins.

7.2.5. Endomembrane system

The endomembrane system has been referred to as the "post office" of the cell, but it's also like a factory, as its components produce, package and export certain cell products, such as proteins or lipids. It has several components.

- ***Endoplasmic reticulum (ER)*** – This is actually an extension of the outer membrane of the nucleus outwards to form folds, so the space between the two membranes of the nuclear wall is connected to the ***lumen*** (cavity) of the ER. Functions of the ***smooth ER*** include synthesis of lipids, such as the phospholipids which make up cell membranes, metabolism of carbohydrates, detoxification of drugs and poisons, and calcium storage. The ***rough ER*** (***RER***) is so called because it is studded with ribosomes, to make proteins such as insulin which will be secreted by the cell. As such polypeptides are produced by the ribosomes of the rough ER, they enter the ER lumen where they fold into their functional shape. The ER lumen keeps them separate from the cell's cytosol and ejects them in transport vesicles formed from the membrane of a specialized region of the ER. Many are so transported to the Golgi apparatus. Portions of that are then sent elsewhere in transport vesicles.
- ***Golgi apparatus*** – The Golgi serves to dispatch the various products to their final destinations. It is composed of a series of flat sacs which have been compared to pita bread.[31] The are directional in the sense that transport vesicles from the ER deliver their contents to the *cis* face of the Golgi, closer to the ER, and the output leaves from the *trans* face. Most products passing through the Golgi are modified in steps across different *cisternae* (interior cavities) of the Golgi sacs. Transport vesicles exiting from the *trans* face may have added external molecules to make their shape correspond to "docking sites" on their destination.[32]

30 By Kelvinsong via Wikimedia Commons, https://commons.wikimedia.org/wiki/File:Mitochondrion_structure.svg.
31 Campbell et al., 176.
32 Campbell et al., 176.

- ***Lysosomes*** – These “garbage-collection” modules contain enzymes which can break down and digest molecules and even cells. In the process of ***phagocytosis***, for example, ***macrophages***, a type of white blood cell and part of the immune system, gobble up pathogens and then deliver them to their own lysosomes for destruction. Lysosomes do not exist in plant cells.
- ***Peroxisomes*** – These also contain enzymes for decomposing various molecules by transferring hydrogen to oxygen to produce hydrogen peroxide, H_2O_2. In the liver, they break down ethanol (alcohol from alcoholic beverages).

An example of how these components of the endomembrane system work together is the sequence of events during production and exportation of a protein:

- A ribosome attaches itself to the rough ER and begins to make a protein. (Details in section 7.3.2.)
- As the protein is made, it is pushed into the lumen of the RER, where carbohydrate markers are attached to it.
- The RER pinches around the finished protein and forms a sealed vesicle which is transported from the RER to the *cis* side of the Golgi.
- The vesicle seals to the *cis* face of the Golgi and delivers the protein, which may be modified there.
- Another vesicle transports the protein from the *trans* face of the Golgi, where it fuses with the cell membrane so that the protein can be released outside the cell.

A reminder: Proteins are special, as it is they which make up most of the enzymes necessary for metabolism, the set of chemical reactions which keep us alive. (The exception is RNA, which is a nucleotide.) Recipes for making them, as well as transcription factors modifying the conditions for their fabrication, are stored in the nuclear DNA. The RER and its attached ribosomes are where the RNA derived from the DNA is used to actually fabricate the proteins, which are then delivered via the RER and the Golgi from the cell.

“*Form follows function*” in biology. Cells which produce large amounts of proteins have voluminous endoplasmic reticulums and those which secrete much have large amounts of Golgi bodies. Those which, like muscles, need great amounts of energy contain many mitochondria.

7.2.6. Cytoskeleton

Proteins of the cytoskeleton support the cell and give it shape, and provide “tracks” for transport of proteins. They are of three varieties.

Microtubules are made of the protein ***tubulum*** and provide structure, like scaffolding, which allows the cell to resist compression. They also form something like a railroad track for RNA to flow along during protein synthesis, rather than letting it float loose in the cytoplasm.

Microtubules make up two important structures:

- ***flagella***, the tail-iike appendages existing in humans only on sperm, and which allow them to move about;
- ***cilia***, fine, hair-like structures which wave continuously and move such things as waste in the respiratory system or egg cells in fallopian tubes. They also detect sound waves in the ear.

Microtubules form the ***centrioles***, the principal part of ***centrosomes***, which play an essential role in chromosome splitting during reproduction (explained later in this chapter).

Microfilaments are made of ***actin.*** They are thinner than microtubules and form chains responsible for muscle contraction with the cooperation of ***myosins***. (See section 9.7.1.)

Microtubules and microfilaments are like cables and work with ***motor proteins*** which pull themselves along the cable. A family of motor proteins, ***kinesins***, attach to vesicles carrying, for instance, RNA and crawl along the microtubules similarly to the way myosin crawls along actin in muscle cells.

Since motor proteins move along the support structure of the cell, they are probably evolved forms of the cytoskeleton of bacteria.[33] Bacteria can be motile in their way too, since they change by adding on elements at one end and leaving them off at the other, effectively moving. The evolution of motility was an essential step in the spread of plants and – especially – animals some 250-or-more million years ago.

33 This was only observed in the mid-1990s. Lane (2009), 167.

Intermediate filaments are made of ***keratin*** and serve, like microtubules, for maintaining cell shape, but, contrary to microtubules, they resist tension which tries to pull apart the cells.

In addition to the support they receive from the cytoskeleton, many animal cells have an extracellular matrix of ***connective tissue*** composed of long proteins like ***collagen*** which help support the cell and bind cells together. Plants have cell walls composed of a reinforcing layer of cellulose.

7.2.7. Plant cells

Plant cells differ from animal cells in that they have cell walls outside the cell membrane, and contain a central vacuole and chloroplasts (Figure 7.20).

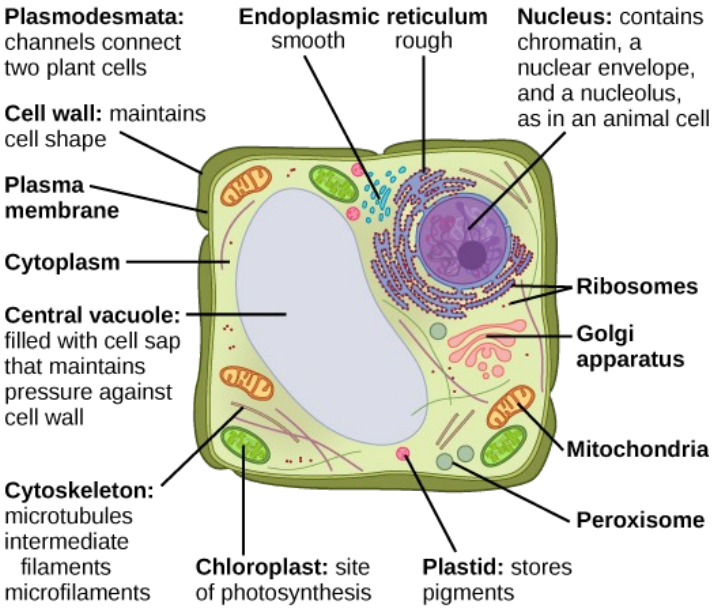


Figure 7.20: Typical plant cell, from Openstax College

The ***cell wall*** is a stiff outer layer which supports and protects the plant. The large ***central vacuole*** contains liquid which exerts pressure to maintain the plant's standing position, just like air in a balloon makes the balloon stiff. The liquid also stores proteins.

The ***chloroplasts*** are where photosynthesis takes place. Like mitochondria, they contain their own simple form of DNA as well as ribosomes, because, like mitochondria, they originated as bacteria which moved into another cell, felt at home and stayed.

The structure of chloroplasts and their use of energy from sunlight to convert CO_2 and H_2O into sugar will be presented in the next chapter.

7.2.8. Viruses

Viruses are not cells because they do not contain all the good things described above. A virus consists of a protective protein coat called a ***capsid***, which contains the viral DNA (or RNA). It may also be surrounded by an envelope which closely resembles a cell membrane, because the virus has stolen it from a cell and adapted it to its own nefarious ends – a virus in cell's clothing. Viruses have no ribosomes or other organelles to synthesize proteins, so they are dependent for reproduction on normal cells which they invade, substituting their own genetic material for that of the cell and then letting the cell do the job of protein synthesis – but using the virus's recipe. Real Trojan horses! After the proteins are synthesized, they reassemble into viruses and escape by making the cell explode. Since they cannot reproduce independently, they are usually not considered to be alive.

7.3. DNA expression – protein synthesis

DNA, RNA and ribosomes, in that order, are essential components in the synthesis of proteins. DNA

contains the *information* necessary not only for reproduction, but also for daily cell growth and maintenance. Messenger RNA carries the information to the ribosomes. With the help of yet another kind of RNA, the ribosomes assemble the proteins. All this depends on gene regulation.

The use of DNA to initiate protein synthesis is called **DNA expression**.

This sequence of events is summed up in the so-called ***central dogma of molecular biology*** of Francis Crick, often paraphrased as "DNA makes RNA and RNA makes protein." Crick's idea was more precise than this, as shown in the following diagram.[34]

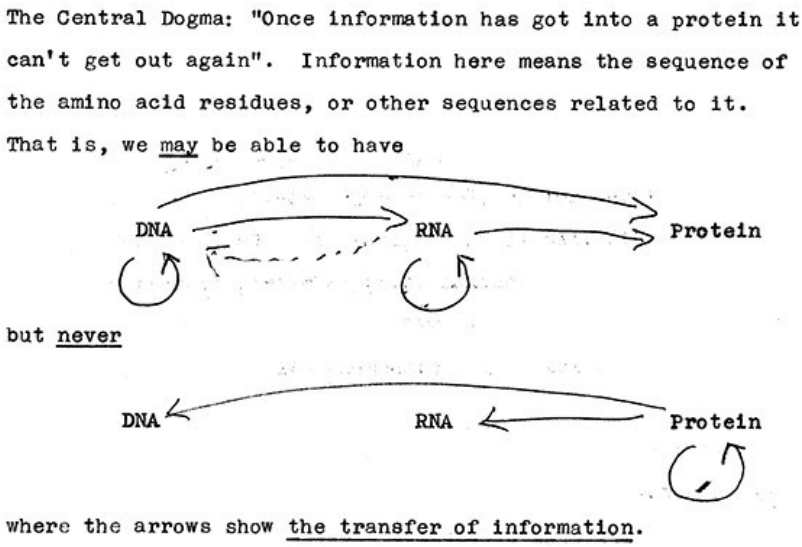


Figure 7.21. Crick's central dogma.

As Crick's diagram shows, it's all about information flow, which according to him (and, seemingly, to the data) flows from DNA/RNA to proteins, but not back in the opposite directions.

More precisely, DNA is transcribed inside the nucleus to make mRNA, which is expelled from the nucleus to the cytoplasm, where it is translated to protein by ribosomes.

DNA –> ***transcription*** (nucleus) → mRNA → ***translation*** (ribosome) → protein.

The recipe in DNA is expressed in "bytes" of three nucleobases; one three-base byte is referred to as a ***code-word***. When transcribed to its complementary form in mRNA, it is called a ***codon***. Since each base can have one of four values (C, G, A or T, in DNA; C, G, A or U in RNA), the codon can take on 64 values. Their correspondance with amino acids or start/stop codons is given in Figure 7.22.

34 Cobb on Crick: The "central dogma". Why evolution is true. https://whyevolutionistrue.com/2024/12/02/cobb-on-crick-the-central-dogma/. Original at https://wellcomecollection.org/works/xmscu3g4/items. CC-BY license.

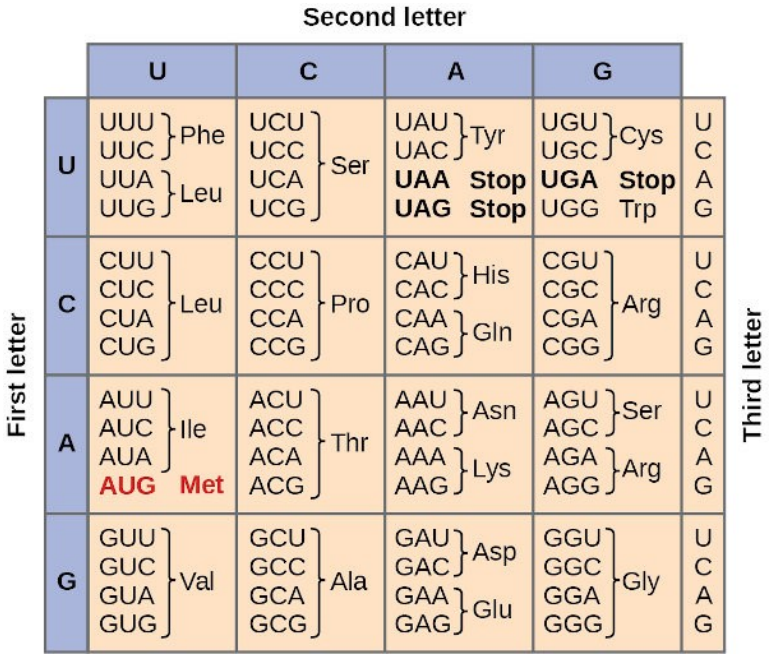


Figure 7.22. Table of RNA codons, from Openstax College[35].

7.3.1. RNA transcription

The enzyme which does the work of "reading" a gene on the DNA and building a corresponding gene of RNA is called ***RNA polymerase***[36]. There are at least four types of RNA and transcription makes them all. For protein synthesis, the RNA constructed is called ***mRNA***, or ***messenger RNA***. The DNA recipe begins with a sequence called the ***promoter***. RNA polymerase contains a complementary sequence which binds to the promoter and launches transcription. As will soon be seen, transcription is started only if it is allowed by gene regulation. RNA polymerase unwinds a part of the DNA chain and reads code-words, starting with the promoter. As it reads the DNA, it constructs a complementary chain, called ***pre-mRNA***, from nucleotides. It is complementary in the sense that if the DNA contains a C (or A or G or T) then the pre-mRNA contains a G (or U or C or A – remembering that RNA replaces T by U).

The raw materials RNA polymerase uses to construct RNA are ***nucleoside triphosphates*** (***NTPs***). An NTP molecule has two phosphate groups which contain a significant amount of energy from ATP. This energy is used to bond the nucleotides together to form RNA.

The RNA polymerase moves along the DNA, unwinding sections as it goes, reads the code words and assembles the appropriate pre-mRNA codons from NTPs. The separated DNA strands recombine in its wake. Eventually, it reaches a ***transcription-terminator*** sequence in the DNA and ends transcription. It now has gone through three steps, known as initiation, elongation (of the produced pre-mRNA) and termination. The pre-mRNA then is released into the nucleoplasm.

35 Openstax Biology. openstax.org/books/biology-2e/pages/15-1-the-genetic-code
36 In fact, there are several forms of RNA polymerase, but that complexity is well beyond the scope of this document.

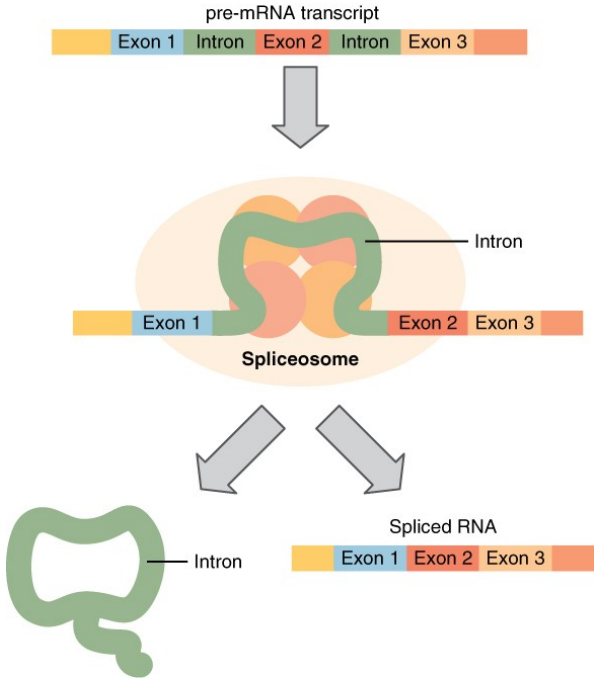


Figure 7.23: Splicing mRNA, from Openstax College[37]

Before leaving the nucleus, the pre-mRNA must be cleaned up. This is needed because DNA contains non-coding, or junk, sequences. The codons which should be kept are called ***exons*** (like "expressed") and those which should be deleted are called ***introns*** (like "interrupted").[38] Small particles called "snurps" (for snRPS, or small nuclear ribonucleoproteins), made up of RNA and proteins, bind together to form ***spliceosomes***, which remove introns and splice the exons back together again, resulting in a cleaned-up form of mRNA.[39]

The mRNA is then shipped out of the nucleus for the next step.

7.3.2. Protein synthesis – translation

After the mRNA leaves the nucleus, it is used to provide the input data for the synthesis of proteins. This takes place on ribosomes.

There are two sorts of ribosomes in eukaryotic cells, depending on their location.

- Free ribosomes float in the cytoplasm and make proteins which will function there.
- Membrane-bound ribosomes are attached to the rough endoplasmic reticulum; they are what makes it look "rough". Proteins produced there will either form parts of membranes or be released from the cell.

In most cells, most proteins are released into the cytoplasm.

Ribosomes are made of ***ribosomal RNA***, or ***rRNA*** (one more kind of RNA), and proteins. They are constructed within the nucleolus as two subunits, which are released through the nuclear pores into the cytoplasm.

In addition to the mRNA and the ribosome subunits, a method Is needed for supplying the appropriate amino acids to be linked by peptide bonds to make up the protein or enzyme being

37 http://cnx.org/contents/FPtK1zmh@7.30:lyDdZqp4@6/Protein-Synthesis.

38 I would have preferred for exon to mean "exclude" and intron to mean "include", but some contrary biologist decided otherwise. He could at least have taken a vote!

39 Are you wondering how the snurps can recognize the introns an exons? So am I. All I can say is that it is quite complicated and has something to do with methylation of the DNA strands. It is currently not completely understood why there are introns at all, but there are indications that they may be of importance.

constructed. Enter still one more kind of RNA, ***transfer RNA***, or ***tRNA***.

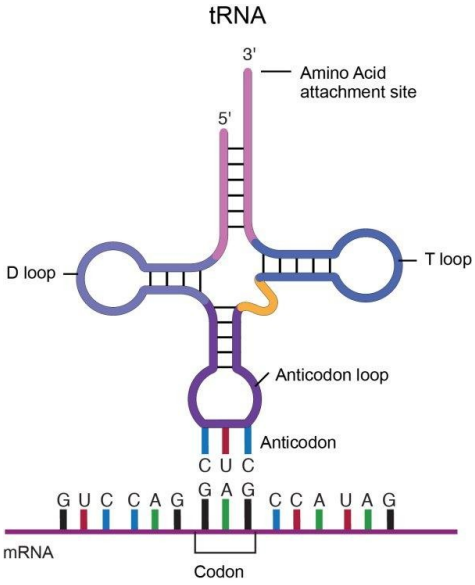


Figure 7.24: Transfer RNA, tRNA, from National Human Genome Research Institute[40]

A molecule of tRNA is a molecule of RNA folded into a double strand with loops which give it a precise 3-dimensional shape. The loop on one end has an ***anticodon***, the function of which is to match its complement codon on mRNA. The other end has a binding site (adenylic acid) for a specific amino acid. So the tRNA is the "dictionary" which converts codons into amino acids.[41] A tRNA molecule is "charged" with an amino acid molecule by a ***tRNA-activating enzyme*** which uses energy from ATP to covalently bond the appropriate amino acid from molecules in the cytoplasm. Such tRNA molecules, carrying an amino acid, are called ***aminoacyl tRNA***.

The ribosome itself contains three assembly areas or spaces called, in order of occupation, the A-site, the P-site and the E-site. Initially, the ribosome subunits are floating independently in the cytoplasm or attached to the RER. The ***initiation*** of translation begins when the small subunit binds at its P-site to the START codon of the mRNA strand. Then the corresponding tRNA (methionine) binds to the START codon and the large ribosome subunit is attached, completing assembly of the ribosome. Now the first tRNA is in the P-site and next mRNA codon in the A-site. The methinone constitutes the beginning of the peptide chain which will become the protein. Then a cycle takes place in which the ribosome reads in the mRNA strand, like computers of my youth read in paper tape, each new codon arriving in A.

40 https://www.genome.gov/dmd/img.cfm?node=Photos/Graphics&id=85250.
41 This of course poses the question, where do the tRNA molecules come from? Good question.

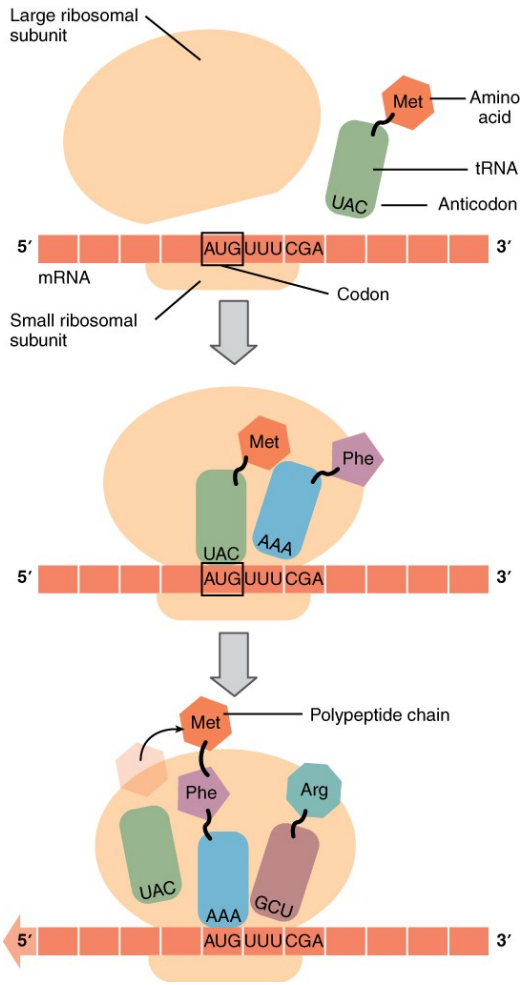


Figure 7.25: Gene translation in the ribosome, from Openstax College[42]

The process then pursues the ***elongation*** stage of translation. The aminoacyl tRNA for the codon in the A-site is carried in, so the first two amino acids are now in the P and A sites. The ribosome then catalyzes the formation of a peptide bond between these two amino acids. The ribosome then moves the mRNA so the P-site amino acid enters the E-site, the A-site one enters the P-site, and a new one enters the A-site. It continues like that until a STOP codon enters the A-site and brings about ***termination*** of translation and release of the completed peptide chain.

All these steps of transcription and translation require energy, so protein synthesis is one of the most energetically costly of cell processes. Much of this energy is used to make enzymes essential to the functioning of the cell. Most enzymes are proteins.

Once part of a strand of mRNA has left one ribosome, it can enter another. One strand may actually be in 3 to 10 ribosomes at once, in a different step of translation in each one. Such clusters of ribosomes translating the same mRNA strand are called ***polyribosomes***.

7.3.3. Regulation of gene expression

Every cell in an organism has the same complete genome in its nucleus and so has access to all the same protein “recipes”. But, for example, heart cells should not produce proteins used only by the liver and no cell should produce proteins in quantities beyond what it can use. Cells change over time too: Think of the adaptation to pregnancy or disease.

42 http://cnx.org/contents/FPtK1zmh@7.30:lyDdZqp4@6/Protein-Synthesis

Cells are specialized and so express different genes: Differential gene expression leads to cell differentiation. Controlling which proteins to express and when is called ***regulation***. Note that this is one more instance of communication in the body, telling genetic machinery what to express when.

Regulation of prokaryotic cells

Regulation in prokaryotic cells is relatively simple, as there is no nucleus. so transcription and translation take place almost in the same place and at the same time. Regulation in prokaryotic cells, though, almost always concerns transcription.

An example from a prokaryotic cell will show how this works – and introduce some new terminology.

The bacterium *E. Coli* normally uses glucose for energy. But if glucose is absent and lactose is present, it can use the lactose. Bacteria arrange groups of genes to be controlled together into a structure called an ***operon***. The set of proteins necessary for the use of lactose are part of the ***lac operon***. The operon begins with a ***promoter***, which indicates the beginning of the operon and is the site where RNA polymerase binds to begin the transcription. In between the promoter and the set of genes, of which there may be any number, is a sequence called the ***operator***, which is where DNA-binding genes bind to regulate transcription.[43]

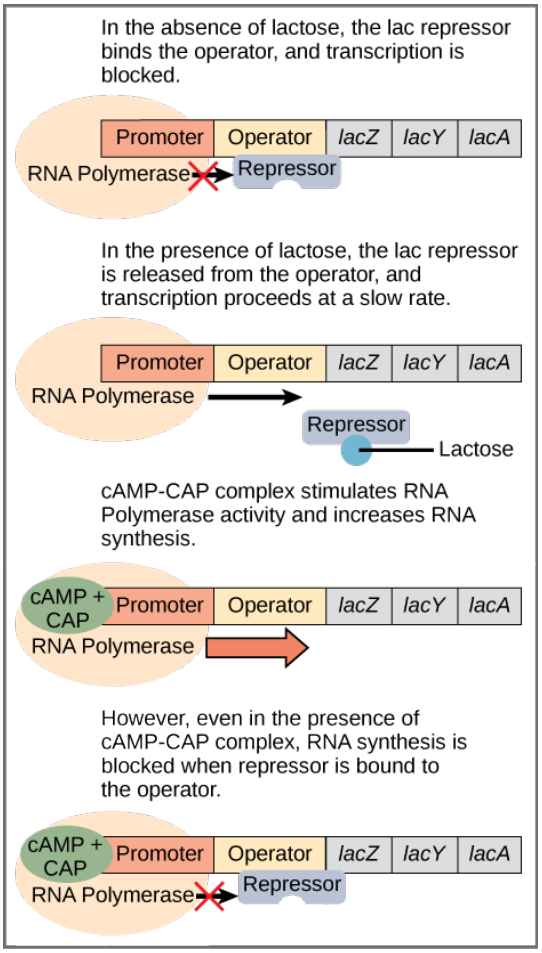


Figure 7.26: Regulation of the lac operon, from Openstax College

When no lactose is present, a protein called the ***lac repressor*** is bound to the operator and the state of the lac operon is "off". (Figure 7.26) This is because the repressor blocks access to the rest of the operon.

43 Look out for the terminology: Sean B. Carroll refers to the operator as a genetic switch, a term we will meet with in the disussion of regulation in eukaryotes.

The gene for the lac repressor is a ***constitutive gene***: It is always expressed because it is the recipe for an essential protein. On the other hand, a ***regulated gene***, is expressed selectively.

In addition to the binding site for the operator, the lac repressor has a second, ***allosteric***, binding site. When lactose is present, an isomer of lactose binds to the allosteric site of the repressor, which causes it to change its form and unbind from the operator. The lac operon is now in the "on" state. This form of regulation is called ***induction***: Lactose is said to be the ***inducer*** of the lac operon and acts through the allosteric site of the lac repressor. Transcription now occurs, but slowly. Because some glucose still may be present, it is not certain that the proteins mapped by the lactose-digesting genes are needed. This depends on how much glucose is lacking.

The second part of this process depends on the presence of glucose and is regulated by a second DNA-binding protein, ***CAP*** (catabolite activator protein). CAP is also an allosteric protein with one DNA-binding site and one allosteric site which binds to ***cyclic AMP*** (***cAMP***). CAP is only active when it is bound to cAMP. You guessed it, cAMP levels are high when glucose levels are low.[44] In that case, cAMP-CAP binds to the promoter and enhances transcription of the genes. So lactose can be considered the "on-off" switch for transcription of genes for lactose-digestion and cAMP-CAP, the "volume control".

We have already pointed out that AMP is "related to" ADP and ATP, the three differing in the number of phosphate groups – one, two or three. Cyclic AMP is AMP with the phosphate attached to the 3' and 5' carbons. Take ATP, remove two phosphates and link the 3rd to 3' and 5' and you have cAMP.

Regulation of eukaryotic cells

In eukaryotic cells, transcription occurs inside the nucleus and translation outside, so mRNA is shuttled across the nuclear membrane in between the two processes. Regulation in eukaryotic cells therefore may take place inside or outside the nucleus or at any step in the expression pathway, including control of access to the gene in the DNA, control of transcription, pre-mRNA processing, mRNA lifetime and translation, and modification of the final proteins. Even the activity levels of enzymes which facilitate expression can be controlled.

Pre-transcription regulation

Inside the nucleus, histones, around which chromatin is wound to make nucleosomes, can wind or unwind to change spacing of the nucleosomes and thereby allow or deny access to genes. This process is a form of ***epigenetic regulation***.[45] Since histones are positively charged and DNA, negatively, modifying the charge by adding chemical "tags" to either modifies the configuration of the DNA.

Transcription regulation

The most prevalent regulation of expression in eukaryotes is during transcription. Control of transcription by the prokaryotic lac operon is a relatively simple process: In order to begin transcription of a gene, RNA polymerase must bind with the gene's promoter but cannot do so if a lac repressor is bound to the operator region which follows the promoter on the gene.

Eukaryotic gene transcription is regulated similarly, but with more of everything. Instead of a repressor which binds to an operon, eukaryotes have a slew of ***transcription factors*** which bind to multiple ***regulatory sequences***. Regulatory sequences, sometimes referred to as ***switches***, may be

44 If we go one step back, we see that glucose binds to an allosteric site on the enzyme adenylate cyclase, which makes cAMP from ATP, and disables it. So lack of glucose stimulates production of cAMP, which binds to CAP, which binds to the promoter to enhance synthesis.

45 Look out, epigenetic regulation is used for somewhat different notions, too, and they are not all necessarily so.

almost anywhere on the DNA strand, even far from the gene. The existence of multiple switches for each gene allows the gene to be present In different types of cells, but activated selectively in each by different switches.

There are two types of *transcription factors*.

- ***general transcription factors*** affect any gene in all cells and are part of the transcription-initiation complex;
- ***regulatory transcription factors*** affect genes specific to the type of cell.

The two types of transcription factors work together with three types of *regulatory sequences* (transcription-factor binding sites).

- ***promoter proximal elements*** are, of course, near the promoter and turn transcription on;
- ***enhancers*** are far from the regulated genes or in more than one place and also turn the transcription on;
- ***silencers*** are also far away from the regulated genes but turn transcription off.

Activator transcription factors are those which bind to enhancers to promote expression, ***repressor*** transcription factors, to silencers to decrease expression.

The promoter in eukaryotic cells is more complex too, The basal promoter begins with the ***TATA box***, recognized by its beginning which contains the seven-nucleotide sequence TATAAAA, followed by a set of transcription-factor binding sites.

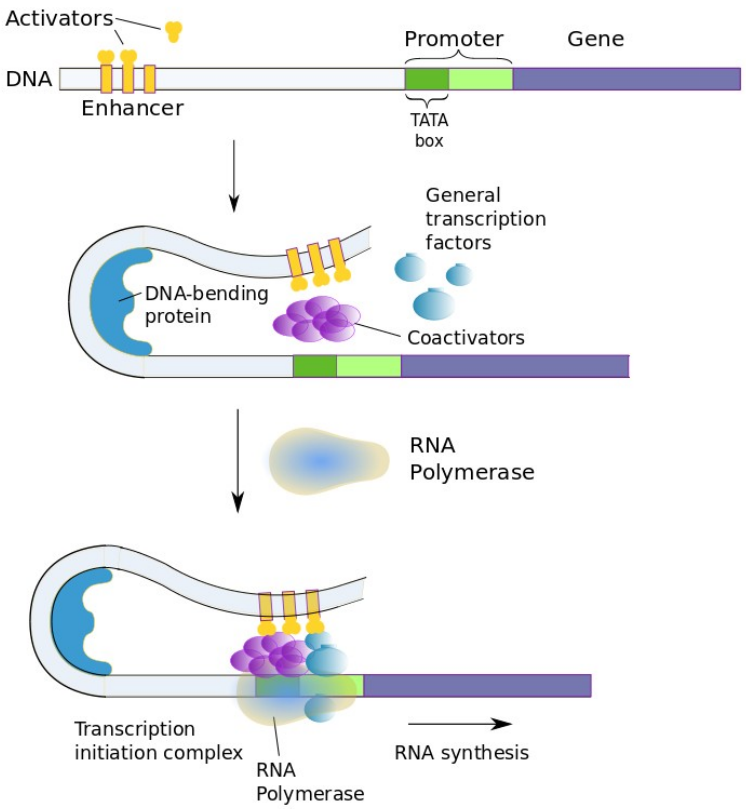


Figure 7.27: Transcription factors in eukaryotic cells

The whole set of transcription factors is summed ***combinatorially*** to determine whether or how much the gene will be expressed. Selective promotion or inhibition at combinations of these sites can therefore bring about tissue-specific gene expression. Each tissue type may have its own specific

enhancer or silencer sequence for the same gene. For instance, the ***neuron-restrictive silencing element*** (***NRSE***) is a repressor which prevents genes from being expressed in any cells which are not neurons. In addition, environmental changes may bring about different gene expression according to current, perhaps temporary needs.

Coactivator proteins bind with general and regulatory transcription factors to form the ***transcription-initiation complex***. RNA polymerase only binds to the transcription-factor complex.

Figure 7.27 shows the case of an enhancer bound by activator transcription factors.[46] The enhancer, on the left, originally is quite far from the promoter, until DNA bending causes it to change its shape, allowing the enhancer to come in contact with the promoter and the rest of the transcription-initiation complex.

Since transcription factors are proteins, they too are coded by genes and these genes are regulated in turn by other transcription factors. Eventually, it is the original cell (such as a fertilized egg) plus the environment which start the chain going. Of course, only genes which are present can be influenced; it's nature *and* nurture.

Transcription factors and signaling elements coded by some of these genes make up the genetic toolkit, as we will see in a moment.

Splicing regulation

In between transcription and translation, proteins may interfere with spliceosomes to modify splicing of pre-mRNA. Different intron selections can allow different mRNAs to be produced from the same pre-mRNA, a phenomenon known as ***alternative splicing***.

Pre-translation and translation regulation

RNA does not hang around forever, nor should it. Eventually, it is degraded and is no longer functional. So controlling its lifetime is another way of regulating its activity.

Yet another type of RNA, very short-stranded ***microRNA*** or ***miRNA***, can bind with complementary mRNA before it is translated and signal that it should be destroyed by the cell. For this purpose, miRNA also associates with ***RISC*** (***RNA-induced silencing complex***).

Other proteins, ***RNA-binding proteins*** (***RBP***s), can bind with the 5' cap or the 3' tail of the mRNA and either increase or decrease its stability.

Phosphorylation or attachment of other chemicals to the mRNA protein initiator complex also inhibit translation.

Similar bindings may take place on the protein after translation and modify its stability, lifetime or function.

7.3.4. The development genetic toolkit – what evo-devo tells us

Development, meaning embyronic development, is the process in which a genotype, a set of genes, becomes a phenotype, a particular living organism. Mutation works on genes, but natural selection works on phenotypes, the results of development. So evolution and development work hand in gene, so to speak, and the branch of biology which studies them together is called "***evo-devo***".

The ***homeobox*** is a genetic sequence of DNA about 180 bases long. It is a sequence of DNA, of genetic material. It codes for about 60 amino-acid residues and these proteins are the ***homeobox domain***, or the ***homeodomain***. The reason the homeobox is really special is that it is "conserved" across most

46 The bobby-pin curl is an idealization; DNA shapes are far more complex than that.

species of eukaryotes, meaning they all have similar homeobox sequences. Some such sequences are the same in frogs and mice by up to 59 of 60 base pairs. It is thought that there are about two dozen types of homeodomains, therefore of homeoboxes. The ubiquity of the sequence is fairly astounding in itself. It means that the sequence could not have evolved independently all those many times – think of the number of species concerned. So the homeobox must be on the order of 500 millions years old.

But there's more. The homeobox is not a gene by itself, but exists within many different, much larger genes – indeed, hundreds of times larger. Since they all contain the homeobox, they are called ***homeobox genes***.

Many animals have a disposition of body parts along an axis, such as the antennae, wings and legs along the body axis of a fruit fly, or the existence or not of ribs along the vertebrae of a vertebrate animal. It turns out that the choice of body part at each segment along the axis is regulated by a transcription factor coded by a single gene – the ***Hox gene***. Hox genes are an example of homeobox genes; they contain the homeobox sequence. These Hox "master" genes control the developmental differentiation of, for instance, a fruit fly's serially homologous body parts[47]; in simpler terms, its body pattern. They are "master" genes because they determine whether a given part will form or not, leaving the details to genes farther down the chain. But such "detail" genes will not function at all without the "master" gene, which therefore regulates quite a large number of genes. Hox genes are sufficiently similar that introduction of mouse Hox genes into a fly can cause the growth of the indicated organ -- in fly format. They also control the very different serial structure of snakes.

Homeotic genes occur in clusters. One more amazing fact is that the genes of a cluster are in the same order as that of the body segments they control. It is sufficient to replace the gene in a given cluster, say at the antenna position on a fruit fly, with another, say a leg gene, and a leg develops at the antenna position on the fly. Since the transcription factors coded by such genes can change the cells they regulate into something else, they are called ***homeotic***[48] transcription factors and their genes are ***homeotic genes***. The protein domain they express is therefore a ***homeotic domain***, ***Hox***, for short.

Other homeobox families also exist, as we will see a few in a moment. Hox genes are just one family of them.

It is remarkable that quite similar homeodomains have been found in almost all animals. Such conservation of homeobox genes across species shows that embryonic development of most animals, fungi and plants is controlled at some level by approximately the same genes. They must have been around since animals diverged from each other over 500 Mya. The original Hox gene was duplicated and then each copy took on slightly different functions. Subsequent duplications and modifications have led to the diversity of animals today. Comparison of the genes can contribute to building at least a partial tree of life.

Because the homeobox genes code for transcription factors, each of which is used in so many organisms in similar ways, the proteins coded by homeobox genes are constituents of what some biologists call the ***genetic toolkit.*** It's like using a common screwdriver to drive screws in different contexts. Just as one screwdriver serves in many contexts, so does each type of homeobox gene. A homeobox gene is therefore in some way a "master" gene. The toolkit is common to almost all animals, with only little variation from one to another. It contains genes not only for transcription factors, but also for various molecules which are signaling elements. They play important roles in embryonic development, or ***embryogenesis***.

In other animals also, the genes exist in clusters, with the gene order in the cluster corresponding to

47 The front legs of a cat and our arms are considered ***homologous*** body parts. Structures along a body axis, similar but different, are called ***serially homologous*** with respect to each other.

48 Homeosis is the transformation of one organ into another.

that of the organism's parts. Different Hox genes, being similar but slightly different, bind to different regulatory sequences on DNA and therefore regulate different genes. One homeobox protein may regulate many genes and a number of homeobox proteins may work together to refine selection. Because of this possibility of multiple binding, a small change in activation of toolkit genes can bring about a large change in the phenotype. So the genetic toolkit may explain development more simply than if all genes had to be specific to each different part, location and development time of an organism.

Toolkit genes themselves have multiple switches. Switches are the means by which a relatively limited set of toolkit genes may be used differently in different regions, or even different animals, or at different times in embryonic development – which furnishes material for evolution.

The existence of different layers of transcription factors also explains how a small genetic change (in a transcription factor) can bring about a relatively important change in the phenotype of the organism.

A specific bodily environment (liver, heart, blood, ...) contains some set of organic molecules specific to that environment. These molecules or a sub-set thereof will serve as transcription factors to activate a particular sub-set of the toolkit. In other words, the environment chooses which tools to use.[49] The proteins expressed by toolkit genes will activate or suppress expression of body-part proteins at that place and time.

Environmental molecules ==> toolkit proteins ==> body parts

Each arrow indicates that the object to the left switches on expression of the object to the right.

Some terminology helps to understand the evo-devo literature.

- Transcription factors are proteins and so are not on the DNA string, therefore not on the same molecule as the DNA which is regulated. They therefore are called ***trans-acting regulatory elements*** (***TRE***).[50]
- Switches are on the same string of DNA as the regulated gene and are called ***cis-acting regulatory elements*** (***CRE***).

So one can say that TREs bind to CREs to regulate gene expression. Got that?

Let's resume ... again:

- A ***homeobox*** is s sequence of genes about 180 base-pairs long, corresponding to about 60 amino acids, which encode a protein domain which consists of transcription factors for genes. Homeoboxes are found, for instance, in bacteria, fruit flies, mice, frogs, cows and humans. There are different kinds, or families, of homeoboxes.
- A ***homeodomain*** is a protein domain corresponding to a family of homeobox genes.[51] Homeodomains can be seen as the building blocks of development and evolution.
- The set of homeoboxes comprises the ***genetic toolkit***.
- ***Hox genes*** are a subgroup of homeobox genes. They occur in similar forms within genes for morphogenesis in animals, fungi and plants. The objects transcribed are regulated by a common homeobox, but correspond in their nature to the specific species.[52]

The following table lists just a few of the homeobox gene families and the organism components they

49 I see this nicely in an analogy with a computing program. A program to construct an organism will contain a higher-level library of homeobox routines for making, say, eyes or legs. Another library will contain the specific routines for that organism. A mouse organism will pass control at a specific place to a Pax-6 gene which will pass control to the appropriate eye routine.

50 In Latin, "cis" means "this side of" and "trans" means "the other side of". Think of cis-Alpine (this side of the Alps) and trans-Alpine.

51 A domain is a conserved part of a protein sequence which can exist independently of the rest of the protein chain.

52 Some authors use the term homeobox only for Hox genes.

regulate. As is clear, they regulate quite different structures.

Protein name	Phenotype or organ regulated
Hox	body regions (e.g., head, thorax or abdomen)
Pax-6	eyes
Distal-less (Dll)	limbs
Sonic	organogenesis (tissue patterns)
Ulrabithorax (Ubx)	represses insect wing formation
Tinman	heart

In all animals, there exist similar gene sequences corresponding to protein domains which are transcription factors for that animal's version of some phenotype. A Pax-6 gene from a mouse makes an eye form in a fruit fly – a fruit-fly eye, not a mouse eye.

7.3.5. Stem cells and cell differentiation

Stem cells are those which may divide and form other kinds of cells, including those of all three tissue types – ectoderm, endoderm and mesoderm. They usually divide to form another, identical stem cell and another cell, which may or may not be a stem cell. In the early embryo, the first cells produced by the egg are ***totipotent***, capable of producing cells both within and without the body, such as placental cells. These quickly disappear after producing ***pluripotent*** stem cells, capable of producing any kind of cell in the body. Most of their daughter stem cells are ***multipotent***, capable of producing many, but not all, cell types. Once a specific type of cell is made, it can only do certain things. This is because it no longer has access to the entire recipe book (genome), but only those recipes which it needs. The cell is then said to be ***differentiated*** and the process for making it is ***differential gene expression***. Such gene regulation or differentiation depends on the cell's environment. We have seen an example where the presence of lactose induces the expression of the lac operon.

Gene regulation is a vast subject which is still under intense study by scientists.

7.4. Cell division – the cell cycle

In order for cells to increase in number, either for growth or replacement of old cells, they go through the process of cell division – the cell cycle. Although the cycle is generally considered to consist of three major phases, there is a fourth phase to cell life. Cell cycles in various parts of the human body may last anywhere from 10 hours (bone marrow cells) to a lifetime (neurons).

G_0 is the “fourth” state, where the cell is not waiting to divide but is just doing its cellular thing. It may stay in this state indefinitely or eventually enter into the first phase of the cell cycle.

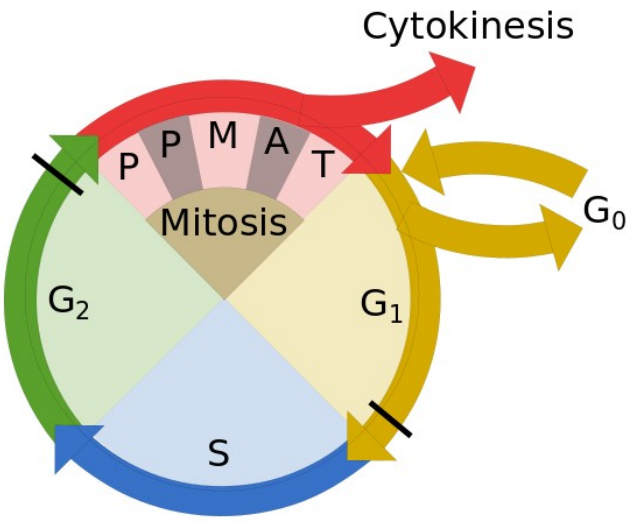


Figure 7.28: The cell cycle. PPMAT = Prophase, Prometaphase, Metaphase, Anaphase and Telophase. Thick black lines are checkpoints.[53]

7.4.1. The interphase

The first major phase of the cell cycle is the so-called ***interphase***, in which the cell fulfills its normal activities while preparing for cell division. The interphase is itself divided into three steps.

1. During ***G_1***, or the first gap, the cell procures and stores proteins and energy for the task ahead. Near the end of G_1, a checkpoint controls the cell's readiness for the next phase.
2. During the ***S*** (synthesis) phase, DNA replication takes place. Chromatin is condensed and each chromosome is duplicated into two copies, ***sister chromatids***, which are attached at a point called the ***centromere***. DNA replication takes place in much the same way as RNA transcription except that both strands are duplicated, entirely. The cell's centrosome, the organizing center for microtubules, is also duplicated.
3. The ***G_2*** (gap 2) phase is another moment for storing energy and making proteins. Also, the cytoskeleton is dismantled and some organelles are replicated. Near the end of the phase, another checkpoint is performed.

7.4.2. Mitosis

The mitosis phase does all the rest of the replication and localization of organelles, leaving only the actual division of the cell in two. It too is understood to take place in a number of steps.[54]

1. During the first phase, ***prophase***, the two centrosomes move towards opposite sides of the cell and microtubules form between them an array of links called the ***mitotic spindle***. The chromosomes condense more and the nuclear envelope and the nucleoli break down.
2. The main event of the ***prometaphase*** is the connection of the sister chromatids to the microtubules of the spindle by protein complexes called ***kinetochores*** .
3. During the ***metaphase***, the still condensing chromosomes are lined up in the equatorial plane of the cell, the ***metaphase plate***.

53 Author's own work.
54 Some sources omit the prometaphase. There is also disagreement as to what goes into each step. For instance, Katz and Openstax say the nuclear envelope breaks down in the prophase; Bear et al., in the prometaphase.

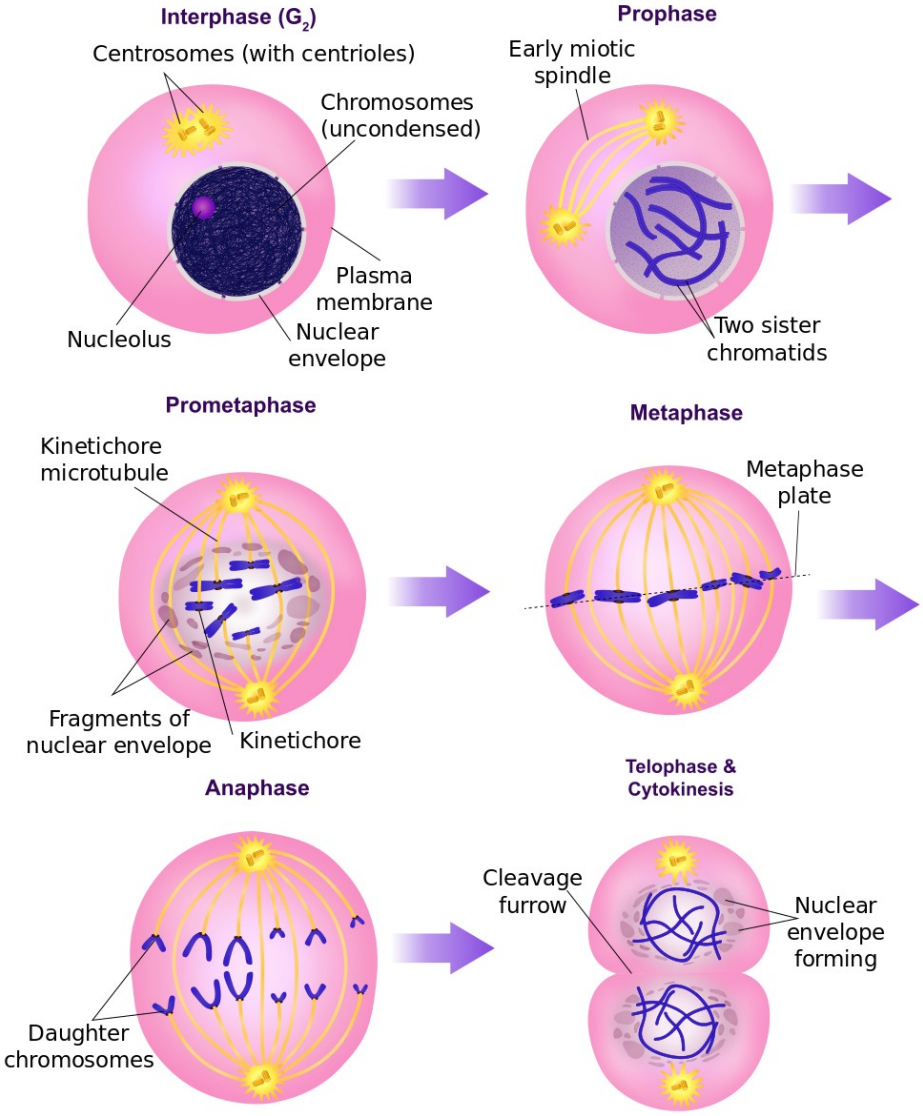


Figure 7.29: Stages of mitosis, after Ali Zifan via Wikimedia Commons[55]

4. The actual split of chromosomes takes place in the ***anaphase***[56], with sister chromatids pulled towards opposite poles of the cell by the kinetochores, which crawl along the spindle tubules much as myosin crawls along actin during muscle contraction.[57]
5. During the ***telophase***, chromosomes decondense and the mitotic spindle breaks down in preparation for making new cytoskeletons. Portions of the endoplasmic reticulum are used to form new nuclear envelopes around the two separate chromosomes.

7.4.3. Cytokinesis

The actual division of the cell into two takes place differently in plant and animal cells, since plant cells are surrounded by a stiff outer cell wall.

In animal cells, a ring of actin filaments contracts around the center of the cell and pinches it in two. The contraction of the ring is probably due to actin and myosin acting as in muscles.

55 File Mitosis_stages.svg, https://commons.wikimedia.org/wiki/File:Mitosis_Stages.svg
56 Why is it not called the cataphase?
57 See the section on muscles later in this document.

In plant cells, the Golgi apparatus has stored up enzymes, proteins and glucose molecules during the interphase. During the telophase, these components move along microtubules to the metaphase plate. There, they grow and fuse and eventually form a new cell wall.

7.4.4. Checkpoints

At several points in the cell cycle, there are checkpoints at which the readiness of the cell to enter the next part of the cycle is controlled. The fresh copies are compared to the original strands and any divergent segments replaced. If the DNA is not intact, the whole process may be restarted or canceled altogether. The cell may actually be instructed to commit ***apoptosis***, cell suicide.

If ever, due to a mutation or bad gene copy, for instance, a checkpoint does not function properly, defective DNA could be passed on to daughter cells. This may lead to the unchecked replication of such cells and genes – ***cancer***.

7.5. Reproduction – meiosis

Most animals and plants are ***diploid***, meaning they have two sets pf chromosomes, one copy from each parent. A cell containing only one copy of its chromosomes is called ***haploid***. Cell growth and doubling take place through the cell cycle and mitosis, but reproduction of the organism depends on another cycle, ***meiosis***.

Eukaryotes participate in both mitosis and meiosis:

- Mitosis grows new cells identical to the original. A diploid (or 2n) cell duplicates itself into two diploid (2n) cells.

  diploid cell → (mitosis) → two diploid cells

- Meiosis, for reproduction, creates two haploid cells from one diploid cell.

  diploid cell → (meiosis) → two haploid cells

  Then two haploid cells, one from each parent, join to make a child diploid cell.

Meiosis is preceded by an ***interphase***, composed of G1, S and G2 phases, which travels essentially the same path as in mitosis. Eukaryotic meiosis takes place in two cyclical parts**:**

- ***Meiosis I*** splits the cell's DNA into two diploid pairs.
- ***Meiosis II*** splits the pairs into haploid sister chromatids.

The splitting is illustrated in Figure 7.31. How it all works on the chromosomal level is sketched in Figure 7.30,

At the top of the figure (a) are shown two ***homologous chromosome***s, meaning that they have the same chromosome number (between 1 and 23, in humans) and so correspond to the same genes. For clarity, they are labeled 1 and 2. Suppose that one (colored blue) comes from the father and the other (pink), from the mother.

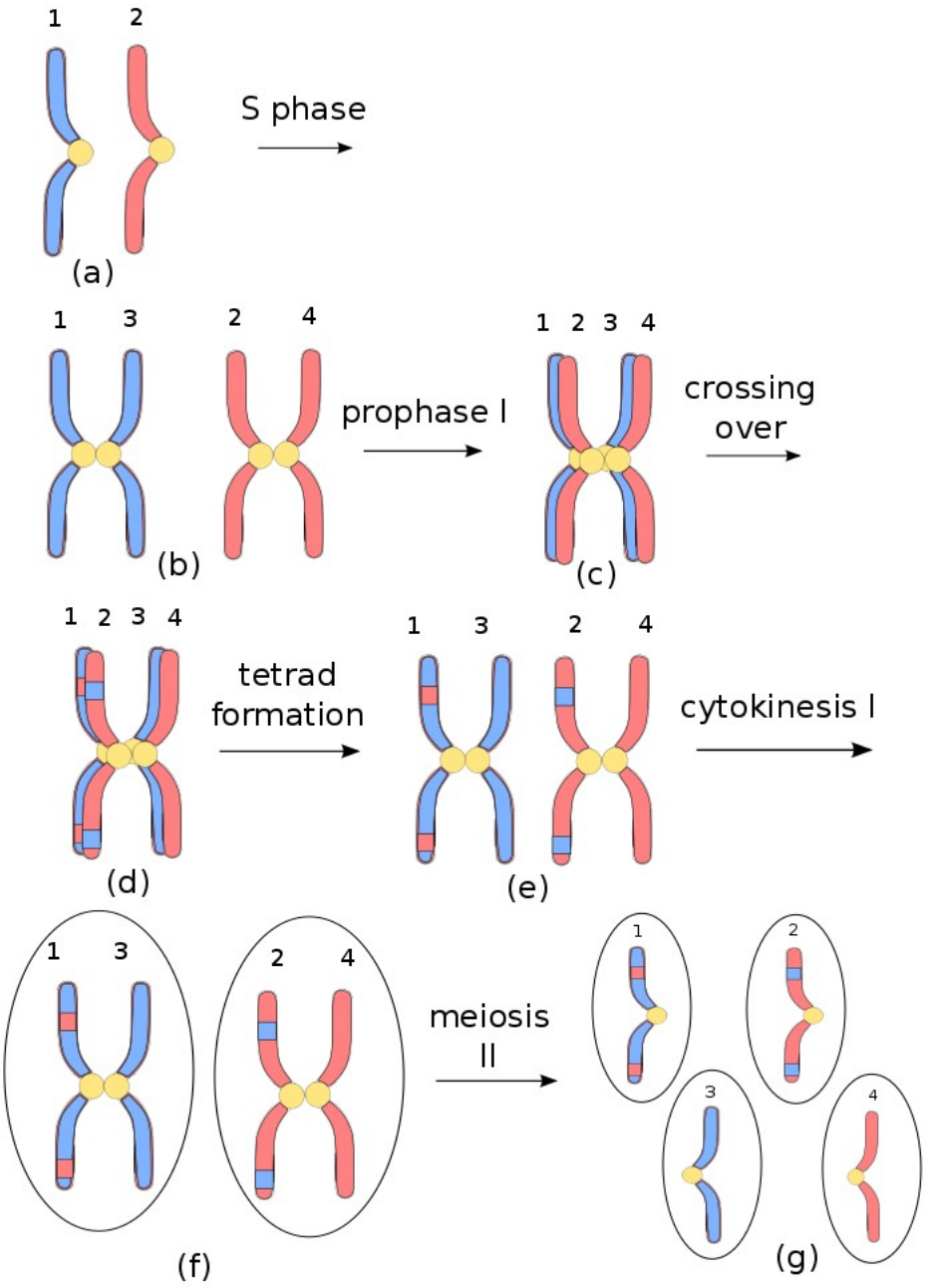


Figure 7.30: Chromosome mixing in meiosis in animals[58]

During the S part of interphase, duplicates of all chromosomes are made, called, as in mitosis. ***sister chromatids***, as in (b). The two blue chromosomes (1 and 3) are sisters and so are the two pink ones (2 and 4). All four are homologous. Sister chromatids are bound together at the ***centromere*** and will remain so bound until meiosis II.

During ***prophase I*** (prophase of meiosis I), the two pairs, which are now visible under a microscope, are closely and precisely aligned, a pairing called ***synapsis*** (c). Being homologous, a gene on a pink chromosome is aligned with the corresponding gene on a blue one. The proximity of the two allows ***crossing over***, or ***recombination***, to take place (d), the reciprocal exchange of homologous sequences of DNA between two non-sister chromosomes, in this case numbers 1 and 2. Genes are exchanged between the chromosomes of the father and those of the mother. Since this is random, it is the first source of randomness in the resulting chromosome. The synapsis then loosens (e), but the pairs

58 Author's own work.

remain linked at the ***chiasmata*** (singular = ***chiasma***), the loci where crossing over has taken place (not shown). The four chromosomes are said to constitute a ***tetrad***. In general, a sister chromatid is no longer composed of genes from only one parent, but a mix of maternal and paternal genes.

It can happen that a piece of one chromosome may break loose and join on to another. Such ***translocations*** have been observed in persons suffering from a specific kind of leukemia.

At the end of prophase I, homologous, non-sister chromatids are held together at chiasmata; sister chromatids, at centromeres.

During ***prometaphase I*** and ***metaphase I,*** microtubules of the spindle from the centrosomes, which are now on opposite side of the cell, attach via kinetochores to the centromeres of the chromosomes, i.e., to sister pairs. These connections are also random. Any pair of the 23 chromosomes may be pulled to one side and the other pair to the other side. This introduces a second source of randomness in the final product. In the case of human beings with 23 chromosomes, there are 2^{23} – over eight million – possibilities.

Next, during ***anaphase I***, the spindle fibers pull the chromosomes to either side, with sisters breaking apart at the chiasmata, but remaining together, since the kinetochores are attached to the centromeres.

In the ***cytokinesis*** phase, the two cells separate. In our case, chromosomes numbers 1 and 3 remain together, as well as 2 and 4. Each cell contains two chromosomes, but they are sisters, so the cell is considered to be haploid.

In summary, at the end of meiosis I, parental genes have been exchanged on chromosomes by crossing over, and the distribution of parental gens is mixed by their random distribution to the two resultant cells, rather like cards which are shuffled and then dealt into two decks.

During ***meiosis II***, a kinetochore is formed on each sister chromatid. Phases similar to those of meiosis I take place until, in ***anaphase II***, the chromatid pairs of each cell are pulled apart and nuclear envelopes form around each. ***Cytokinesis*** finishes the separation, leaving only one chromosome in each cell, which is therefore haploid. Each one contains a random mix of maternal and paternal genes and forms a unique ***gamete*** (egg or sperm cell) of the species.

One more time: Mitosis assures growth and cell replacement and serves reproduction in some simple organisms. Sexual reproduction, as in animals, requires meiosis. The process is shown in the following figure.

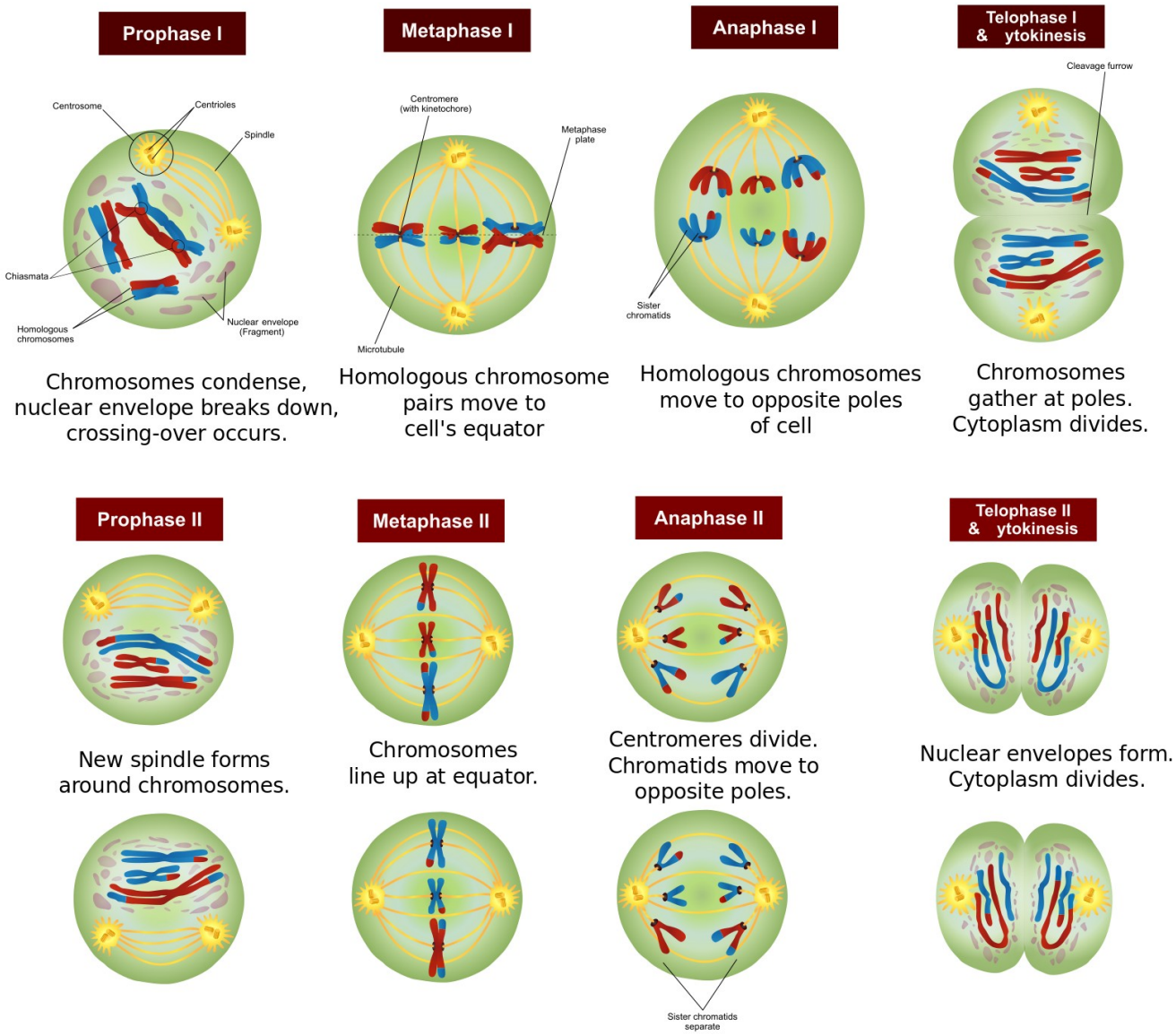


Figure 7.31: Stages of meiosis, after Ali Zifan via Wikimedia Commons[59]

7.6. Plant life cycles – alternation of generations

Cell growth and reproduction employ three processes:

- mitosis: diploid cell → two diploid cells, or haploid cell → two haploid cells
- meiosis: diploid cell → two haploid cells (gametes or spores)
- fertilization: two haploid cells (gametes) → one diploid cell (zygote)

Mitosis can duplicate cells for growth or for reproduction, depending on the organism; meiosis only serves reproduction, and fertilization is its inverse.

Cells of different organisms may spend different relative amounts of time in haploid and diploid states. Humans cells spend most of their time in the diploid state, going haploid only for the short period required for sexual reproduction. This is not true for plants. Biologists use the following terms to classify organisms by the relative duration spent in stages of their life cycle.

59 File Meiosis_stages.svg, https://commons.wikimedia.org/wiki/File:Meiosis_Stages.svg.

- ***haplontic*** – The organism's life cycle is comprised of a dominant haploid stage.
- ***diplontic*** – The diploid stage is dominant (in humans, for example).
- ***haplodiplontic*** – The two stages alternate more or less equally (the case for most plants).

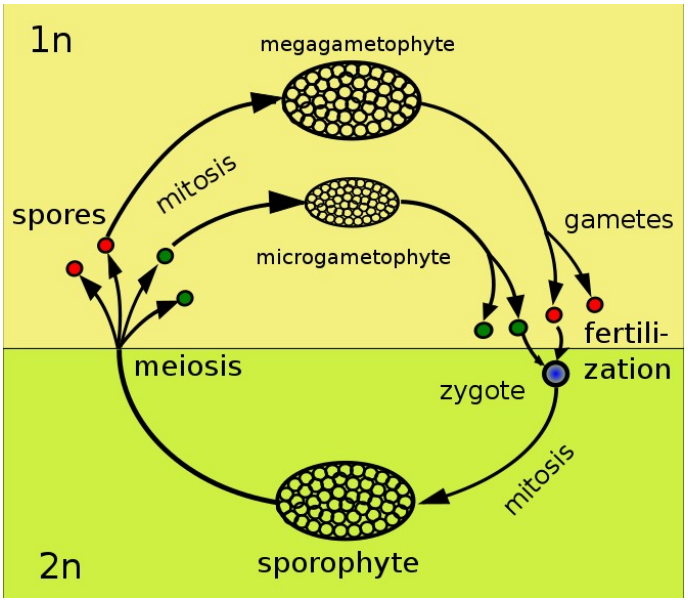


Figure 7.32: Alternation of generations (heterosporous)[60]

The alternating stages of haplodiplontic organisms is called the ***alternation of generations***. Figure 7.32 illustrates the heterosporous case. We use the notation ***1n*** for haploid and ***2n*** for diploid, meaning one or two times the number, n, of chromosomes in the organism.[61] Note that:

- ***spores*** and ***gametes*** are 1n;
- ***zygotes*** are 2n;
- ***sporophytes*** and ***gametophytes*** are, respectively, the 2n and 1n multicellular forms of the organism and produce spores and gametes, respectively.

The steps in alternation of generations are the following.[62]

- Fertilization of two haploid gametes produces a diploid ***zygote***, which duplicates and grows by mitosis to form the ***sporophyte***, the diploid multicellular form of the organism.
- The sporophyte carries ***sporangia*** (spore-containing sacs), in which diploid ***sporocytes*** produce haploid ***spores*** by meiosis. The spores may be ***homosporous*** (containing male and female parts) or ***heterosporous*** (different spores for male and female).
- The spores multiply by mitosis to form the haploid multicellular form of the organism, the ***gametophyte***. In the case of heterosporous organisms, separate male and female gametophytes are formed.
- Inside the appropriate gametophyte, ***antheridia*** produce sperm and ***archegonia*** produce eggs, haploid ***gametes*** which join through fertilization to form the diploid zygote, bringing us[63] back to the beginning of the cycle. Generically, antheridia and archegonia are called ***gametangia***.

60 Author's diagram, based on Openstax Biology, itself a modification of work by Peter Coxhead.
61 For humans, n = 23, of course.
62 Note that this discussion concerns land plants only.
63 "...by a commodius vicus of recirculation..."

In plants, multicellular individuals exist in both the diploid and haploid states, as in Figure 7.32. In the life cycle of a diplontic individual such as a human, there are neither a gametophyte stage nor spores. An individual produces gametes by meiosis, which are quickly fertilized (or lost) to become a diploid zygote from which a new individual will grow.

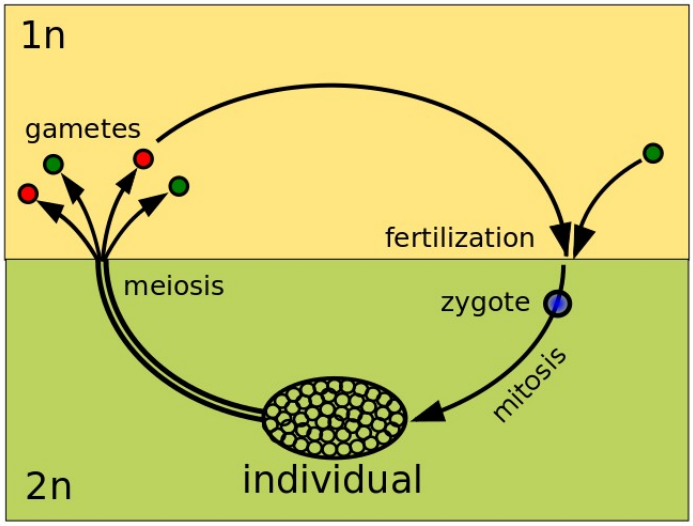


Figure 7.33: Life cycle of diplontic individual

Plants vary in several significant ways.

- Different plants have different dominant states: A moss's dominant stage is the gametophyte; a fern's, the sporophyte. All seed plants (***angiosperma***) are sporophyte-dominant.
- Plants may be homosporous or heterosporous. Homosporous plants produce both male and female gametangia from the same gametophyte. Heterosporous plants may have the, male and female spores occurring on the same flower or plant (monoecious), on different flowers or cones (pine) or even on different plants (dioecious, like gingkos … or most animals). Seed plants are heterosporous and the seed grows from the female spore; the pollen, from the male.
- Plants may be ***vascular***, containing cells which conduct water and solutes throughout the plant, or non-vascular.
- Plants may or may not flower.

In haplodiplontic plants, alternation of generations refers to the reproductive cycle. The plant does not exist at one time as a sporocyte and then disappear to reappear as a gametophyte, or vice versa. The less dominant phase exists on the dominant stage. A tree appears as a tree, which is its sporophyte stage. The gametophyte stage takes place on the various parts of the flowers or in the cones.

Trees and bushes are vascular plants. Their dominant phase is the sporophyte. Heterosporous plants produce male ***microspores***, so called because of their small size, which then form pollen grains. The much larger female ***megaspores*** form ovules. After fertilization by pollen, ovules become seeds, but this takes time, maybe two years.[64]

The differences between spores and seeds (both haploid) are summarized in the following table.

64 Openstax, “Concepts of biology”, 346.

	spore	seed
size	microscopic	large
cell	unicellular	multicellular
type plant	non-flowering	flowering
dissemination	gravity, wind	animals

Land plants are commonly divided into four major divisions, as shown in the following figure.

Embryophytes: The Land Plants						
Nonvascular Plants "Bryophytes"			Vascular Plants			
			Seedless Plants		Seed Plants	
			Lycophytes	Pterophytes	Gymno-sperms	Angio-sperms
Liverworts	Hornworts	Mosses	Club Mosses	Whisk Ferns		
			Quillworts	Horsetails		
			Spike Mosses	Ferns		

Figure 7.34: Major divisions of plants, from Openstax College[65]

The group of non-vascular plants, commonly called ***bryophtyes***, includes moss, whose dominant stage is the haploid gametophyte. The sporophyte stage grows on the gametophyte plant. Moss is heterosporous and so produces male and female spores.

Figure 7.35: Moss with sporophytes, by James919 via Wikipedia[66]

A fern is a seedless vascular plant and its dominant state is the diploid sporophyte. The tiny dark-colored objects under the fronds are spore-containing vessels called ***sori***.[67] Ferns are homosporous and their gametophytes are independent of the sporophytes.

65 http://cnx.org/contents/s8Hh0oOc@9.4:afcPXo2z@4/The-Plant-Kingdom
66 Moss with sporophytes on brick, slightly cropped from original. https://en.wikipedia.org/wiki/Moss#/media/File:Winter_moss.jpg.
67 Singular, sorus.

Gymnosperms are non-flowering seed plants, like conifers or the gingko.[68] The tree is the diploid sporophyte phase. Conifers are heterosporous: The male gametophytes lives in the smaller, pollen cones lower on the tree; the female gametophyte is in the form of the harder, upper ovulate cones. Wind carries the pollen from the male cones to the female ones.

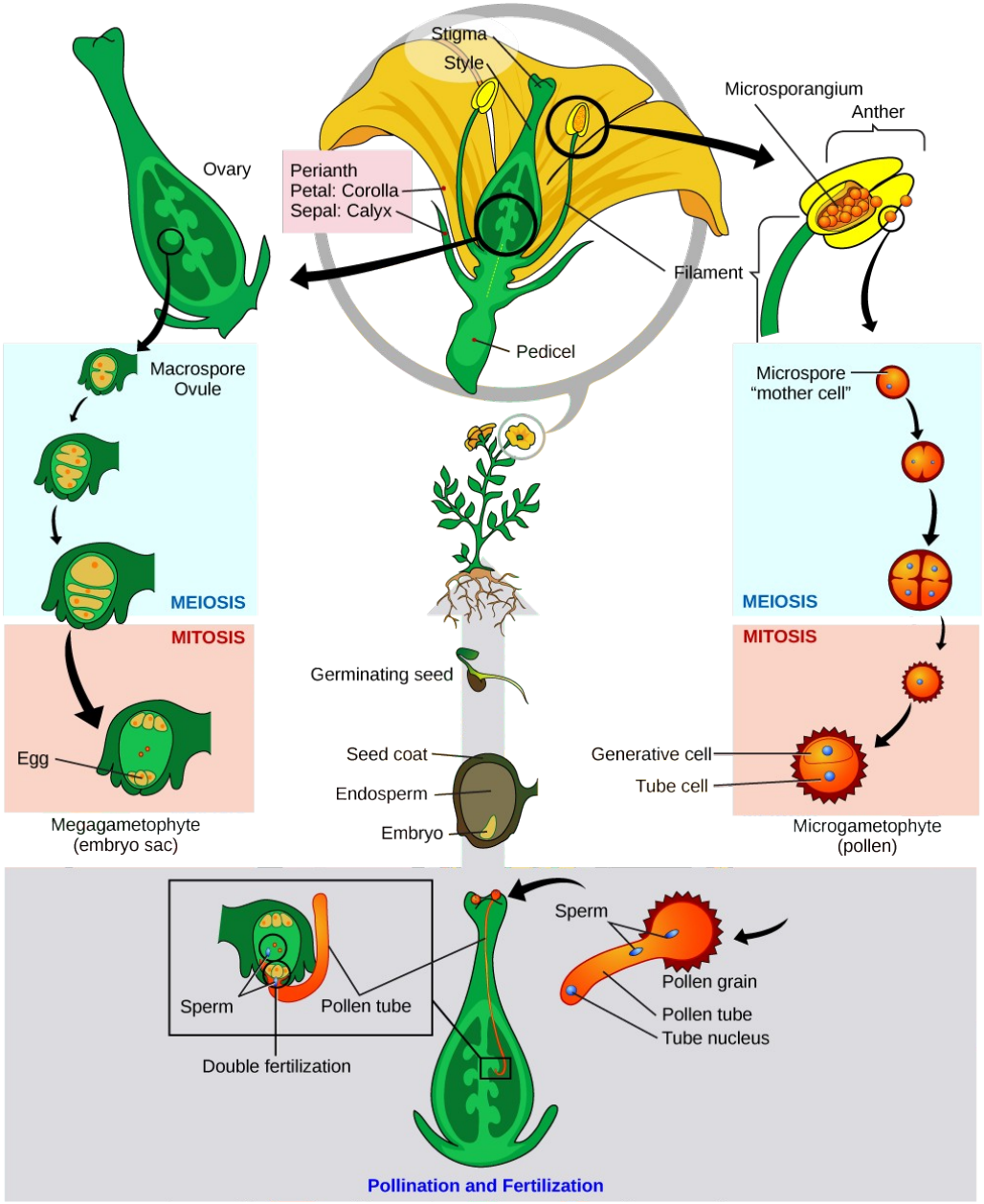


Figure 7.36: Angiosperm life cycle, by Mariana Ruiz Villareal, via Wikimedia Commons[69]

Angiosperms, flower-bearing vascular plants, are also heterosporous and their dominant phase is also the diploid sporophyte. The male and female gametophytes are found in the flowers, in the anther and the ovary, as in the following figure.

Angiosperms are fertilized through the rather complex process of ***double fertilization***.

In the anther, on the stamen, male microsporocytes divide by meiosis to produce haploid

68 Also called at least in French, l'arbre des quarante écus.
69 https://commons.wikimedia.org/wiki/File:Angiosperm_life_cycle_diagram-en.svg.

microspores, which grow by mitosis to produce pollen grains, each of which contains two cells: one with two sperm and one which will grow a pollen tube.

In the ovule, female megasporocytes develop similarly through division by meiosis and growth by mitosis to form the female gametophyte, the ***megagametophyte***, or ***embryo sac***. Typically, the embryo sac contains seven cells, one of which contains two nuclei and so is diploid. Of the other six haploid cells, one is the egg.

When the pollen grain enters in contact with the embryo sac, it germinates, sending forth a pollen tube which extends into the embryo sac and allows passage of the two sperm cells. One fertilizes the egg. The other fertilizes the double-nucleus cell, forming a ***triploid*** nucleus, which later develops into food for the zygote and Is called the ***endosperm***. On the wheat plant, the endosperm is the part we grind to make bread.

Such categories of plants have of course evolved over time. The group of non-vascular plants, or ***bryophytes***, is the oldest. Spores attributed to bryophytes date from the Ordovician, c. 490 Mya.[70] Vascular plants appeared later, in the Silurian, c. 440 Mya. By the Late Devonian period (385 Mya) vascular plants had developed leaves and root systems. During the Carboniferous period (359-299 Mya), they grew to immense heights, and when they died, they were the basis for the fossil fuels we are burning today. Ferns became enormous during the Carboniferous. Gymnosperms probably appeared during the Carboniferous and were dominant in the Mesozoic Era (251-65.5 Mya). Angiosperms had become dominant by the middle of the Cretaceous period (145.5-65.5 Mya) and are still the most abundant of the plant groups.

7.7. Taxonomy – classification of species

Biologists classify all life in a hierarchical system of classifications, or ***taxa***. These classification schema have varied over time. Current stages of the classification for animals are shown in Figure 7.37.

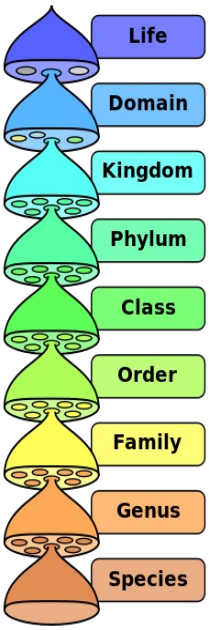


Figure 7.37: Biological taxonomic classification system, by Pengo (Public domain), via Wikimedia Commons[71]

From the original two suggested kingdoms of 1735, we are now up to six in the latest suggested

70 There is a diagram of geological periods in the geology chapter.
71 https://commons.wikimedia.org/wiki/File:Biological_classification_L_Pengo_vflip.svg

version.[72] This document will stick with the three – Bacteria, Archaea and Eucarya – we have been using so far. The system is most simply explained by some examples. Plant taxonomy is very different, using different terms for Domain, Phylum and Class, so we will skip them.

Domain	Animalia				
Kingdom	Eukarya				
Phylum	Chordata				
Class	Mammalia				Actinopterygii
Order	Primates	Carnivora			Salmoniformes
Family	Hominidae	Felidae		Canidae	Salmonidae
Genus	Homo	Felis	Felinae	Canis	Salmo
Species	H. sapiens	F. catus (domestic cat)	A. jubatus (cheetah)	C. lupus (common dog)	S. salar (Atlantic salmon)
Sub-species	H. sapiens sapiens (you know who)				

Table 7.1Example of taxonomic classification.

8. What genetic anthropology tells us

We now consider what may be called ***genetic anthropology***. At its core is genetics, in particular, ***population genetics***, which deals with how genetic variation is distributed in populations and how it changes over time. It is an extraordinarily useful tool for studying not just evolution, but also the history and movement of peoples (which links it to historical linguistics) and popular family genealogy. It's what, in addition to archaeology, allowed us to talk about the movement of peoples like the Yamnaya back in chapter 6. This shows it to be a kind of applied genetics. It's is a big topic, far more than we can consider here.

Our genome has historical significance, for we can consider it not just as the repository for recipes to make proteins for use in the present, but also as containing the story of our inheritance. It's like a long ancient manuscript, copied down through thousands of generations. As for any document that is copied and recopied, errors slip in, in the form of mutations. They may occur anywhere on a DNA strand, not necessarily in a part which contains something "useful" like a recipe for a protein. Although some of the mutations may have good or bad evolutionary effects, most of them have no such effects and are harmless. Nevertheless, since some of them become long-lasting, study of their geographical and chronological presence allows us to deduce information about the history of the genome.

We have already talked about clades. A ***clade*** is simply a group of organisms that descend from a common ancestor and includes all his descendants. It is defined by shared derived characteristics which are not necessarily genetic. As such, one clade may be contained, or nested, within another. Within a phylogeny, or family tree, each node defines a descendant tree and that sub-tree may be considered a clade. If all the descendants of my 5th great grandfather define something analogous to a clade on an ancestor tree, then all the descendants of my grandfather define another, smaller one, nested within. It's all about shared ancestry of organisms and so a clade can be enormous or minuscule. Clades allow us both to classify species (taxonomy) and to discern the sequence of events generated by evolution through natural selection

72 There are various mnemonics for remembering the order. My favorite is "King Phillip came over for good spaghetti". Other versions vary in the word "spaghetti"...

based on genetic mutations.

Clades are trees of organisms, but it is also fruitful to look at trees of mutations of DNA, regardless of any eventual evolutionary influence. In order to do this, we need some new vocabulary. There are somewhat differing ways of defining some of these terms, depending on where we start. For clarity and cohesion, I will base at least most of mine on those of the ISOGG.[73]

Geneticists study genetic ***markers***, short sequences of DNA scattered across regions of known addresses on a chromosome. Different versions of the markers are called ***alleles*** and may vary in several ways. Their position on the genome is indicated using the standard reference.

The standard reference is a product of the Human Genome Project (1990-2023). It was derived from the genomes of a small group of anonymous volunteers but since has been augmented and refined. It is a composite and does not represent one person's genome. It serves essentially as a coordinate system or blueprint which allows numeric indication of placement of nucleotides on the genome, like geographic coordinates on a map.

A ***SNP*** (***single nucleotide polymorphism***, pronounced "snip") is "… a DNA sequence variation occurring when a single nucleotide adenine (A), thymine (T), cytosine (C), or guanine (G) in the genome (or other shared sequence) differs between members of a species or paired chromosomes in an individual." It is a substitution of one nucleotide for another (A → T, for instance). Deletions or insertions of nucleotides are called ***indels***.

A SNP must satisfy certain criteria: It must be inherited, must be found in a significant portion of population members, and must remain stable over generations so as to mark a distinct and traceable lineage. Some but not all definitions require that a SNP be present in a certain fraction of the genome, typically 1%. A SNP is referenced in terms of the changed nucleotide (A, C, G or T) and its position on the genome relative to the sequence of the genome as defined by the standard reference. A mutation X at position 1751 of the full mitochondrial genome would be referred to as 1751X.

An ***STR*** (***short tandem repeat***) "...in DNA occurs when a pattern of two or more nucleotides are repeated and the repeated sequences are directly adjacent to each other." It is generally a short sequence of 2 to 12 base pairs, often in non-coding genes, which is repeated a number of times, such as in "CATACATACATA".

STRs mutate faster than SNPs and therefore are useful for genealogical studies of recent ancestry, but not for deep history. Autosomal STRs are the chief variants used in forensic cases.

During recombination (crossing over) in meiosis, genes close to each other on a chromosome are not as likely to be separated as those farther apart, so they tend to be inherited together. This phenomenon is called genetic ***linkage***. Linkage is responsible for the existence of haplotypes.

A ***haplotype*** (haploid genotype) is a set of specific genetic variants—typically SNPs or short tandem repeats (STRs)—that are inherited (linked) together from a single parent on the same chromosome segment.

They are long enough to be statistically significant, but close enough that their linkage is not easily broken up. More simply, a "... haplotype is a set of specific DNA sequences that are inherited as a unit."[74] Haplotypes can occur anywhere in the genome and are especially useful for tracking recent shared ancestry, identifying population structure, or studying inheritance patterns.

Over time, recombination breaks up the chains of linked sequences, so any remaining long and common haplotypes are interesting as perhaps being of evolutionary significance. Such is the case of the haplotype conferring lactose tolerance in Europeans and south Asians. It is so favored by natural selection that it's frequency has increased faster than its rate of annihilation by recombination.

Since presence of a haplotype in different organisms means they are likely descended from a common ancestor, the concept is useful in genealogy. The more similar the haplotypes of individuals, the more closely related they are and the closer in time their most recent common ancestor (MRCA). This similarity can be used to deduce the number of generations and so furnish a rough estimate of the duration of time back to that ancestor.

Mitochondrial DNA (mtDNA), transmitted only by mothers to both daughters and sons, and (most) Y-chromosome DNA, transmitted from fathers to sons but not to daughters, are not subject to recombination.

73 international Society of Genetic Genealogy. Licensed via Deed-Attribution-NonCommercial-ShareAlike 4.0 International, https://creativecommons.org/licenses/by-nc-sa/4.0/legalcode.en.

74 Archibald, John. Genomics: A very short introduction. 53.

In such uniparental DNA, a haplotype may be shared by others with close genetic ties.

A ***haplogroup*** is a genetic lineage defined by a specific set of (SNPs) that originated in a common ancestor. All individuals belonging to the haplogroup carry this defining set of SNPs, passed down unchanged (except for further mutations) through either the maternal or paternal line. Haplogroups are identified only in mitochondrial DNA (mtDNA) or the non-recombining portion of the Y chromosome (Y-DNA), where inheritance is uniparental and recombination during meiosis is absent or negligible. These lineages form distinct branches of a phylogenetic tree and are used to trace human ancestry and migration over thousands of years.

Within a haplogroup, subsequent generations may be accompanied by another mutation, anywhere in the genome, thus forming a new haplogroup within the old one. Across time, there can be many nested sub-haplogroups within the original or each other.

Each new haplogroup is defined by a small number of additional mutations which mark its branch point on an ancestral tree. Top-level haplogroups – A for Y chromosomes and L for mtDNA -- are defined by the absence of those mutations which define the next level. The next, upper-level haplogroups – L0-L6 and eventually M and N (those who left Africa), A1-A3, B, C-T, etc. --- typically are distinguished by an additional 1-5 mutations.

A haplogroup can define and even be a clade, specifically a unilineal genetic clade. Since only a small subset of the genome defines the haplogroup and the other genes may vary significantly, there can be tremendous variation among members of a haplogroup and even more among members of a clade. It is rather like defining a group of people by hair color while ignoring other, more detailed characteristics. A haplogroup therefore usually represents a more limited group of people than a clade. The inverse is not necessarily true, since a clade may include many different haplogroups. For instance, each male human is automatically a member of two haplogroups, that of his mtDNA and that of his Y chromosome. So as a means of distinguishing descendant trees, haplogroups are far more precise than clades. They are a kind of “fine tuning”.

If the boundary between a haplotype and a haplogroup seems a bit fuzzy, it’s because it is. This can be seen by considering the history and use of the two concepts. Historically, haplotypes came first, originating in population genetics of the 1970s and 1980s and used to track variation in small sets of markers, often with specific uses in mind – like medicine or forensics. Haplotypes were originally sets of alleles, short but large enough to be statistically significant, inherited together because of low recombination.

Haplogroups came later, in the 1990s, due greatly to studies of mtDNA and Y-chromosomes. They were devised to organize haplotype data into phylogenetic trees. So one can see haplotypes as raw materials and haplogroups as the branches made from them. One thing came after another.

In order to do this, geneticists compare DNA (especially from mitochondria or Y chromosomes) from thousands of people and group them on the basis of sets of SNPs commonly inherited together within individuals. They may call some of these haplotypes. It would be logical for them to then move “up” a notch and look for common sets of mutations among the haplotypes to find a broader set in which the haplotypes are nested -- a haplogroup. They then repeat that among the set of haplogroups to find higher-level haplogroups. And so on.

But … it isn’t always that easy. Geneticists often face non-unique sets of haplogroups and so are required to employ mathematical algorithms and statistical models in order to construct a model tree. Due to multiple sources of ambiguity (coincidental independent mutations, gaps in the data, reversion and variations in mutation rates), the result is often a best-fit model, where several good solutions are about equally valid. Then criteria like parsimony (aka Occam’s razor) are used to choose a final model – when this is possible. The results evolve as new data and new analysis methods become available.

A haplotype is a candidate for defining a haplogroup if it becomes widespread across populations, lasts long enough and defines a new branch on the phylogenetic tree, in which case it is promoted to a full haplogroup. So a haplogroup starts with the renaming of a haplotype: They are the same thing at different moments in their/its lifetime. In different studies, the same short string of SNPs may be referred to as a haplotype in one and a haplogroup in the other.

In fact, this “tuning” has three levels:

- Clades. based on shared ancestry, define major branches of evolutionary descent over millions of years.
- Haplogroups, based on shared SNPs in non-recombining regions, trace uniparental branches on time scales from tens to hundreds of thousands of years.

- Haplotypes, based on specific combinations of alleles or SNPs, identify recent ancestry and sub-structure within a haplogroup over hundreds to thousands of years.

If we view haplogroups as the major branches of a hypothetical tree, haplotypes are the twigs and leaves on that branch.

So, a quick review summary, from ISOGG[75]:

- ***Haplotype***: A set of markers (polymorphisms) on a single chromosome that tend to be inherited together. A haplotype can refer to a combination of alleles, to a set of short tandem repeats (STRs), or to a set of single nucleotide polymorphisms (SNPs). Haplotype is a contraction of the term haploid genotype, and is also known as a DNA signature or a genetic signature.
- ***Haplogroup***: A genetic population group of people who share a common ancestor on the patriline or the matriline. Top-level haplogroups are assigned letters of the alphabet, and deeper refinements consist of additional number and letter combinations.

It is generally true that we can achieve greater understanding of the results of scientific studies if we know how they were discovered in the first place. In order to reconstruct genetic lineages, we are interested in the top-down view from ancient ancestor to more recent species, but geneticists must start at the “bottom” (current, more varied) and work their way “upwards” to older, less numerous groups.

This was how mtDNA and Y-DNA were analyzed to find convergence of haplogroups of each onto a single ancestral lineage representing the most recent common ancestors of that lineage

- mtDNA haplogroup L of mitochondrial Eve, or the “lucky lady”, around 155 Kya, our matrilineal most recent common ancestor, from whom we are all descended.
- Y-DNA haplogroup A, “Adam“,living longer ago, around 200-300 Kya.

Of course, Eve was not the only woman around at the time, but the descendants of all the others have died out by now. Thus, haplogroups provide a stable framework for mapping deep ancestry and human migration

75 Genetics glossary. https://isogg.org/wiki/Genetics_Glossary

9. What anatomy and physiology tell us – energy

9.1. Thermodynamics and metabolism – an overview of bioenergetics

The human body (like other living organisms) is a complex, multi-level symbiosis of parts. The most basic constituent of any organism is the atom, because ***atoms*** make up chemical ***molecules***. In biology, molecules can be of an astonishing and enormous complexity. Molecules join to make ***cells***, which in turn make up ***tissue***, such as skin, bone or muscle tissue. Tissues comprise ***organs***, like the heart or liver, which are grouped into ***systems***, such as the nervous or digestive systems. The systems in turn make up the ***organism***. All those different levels of organization add up to a very complicated yet highly organized system.

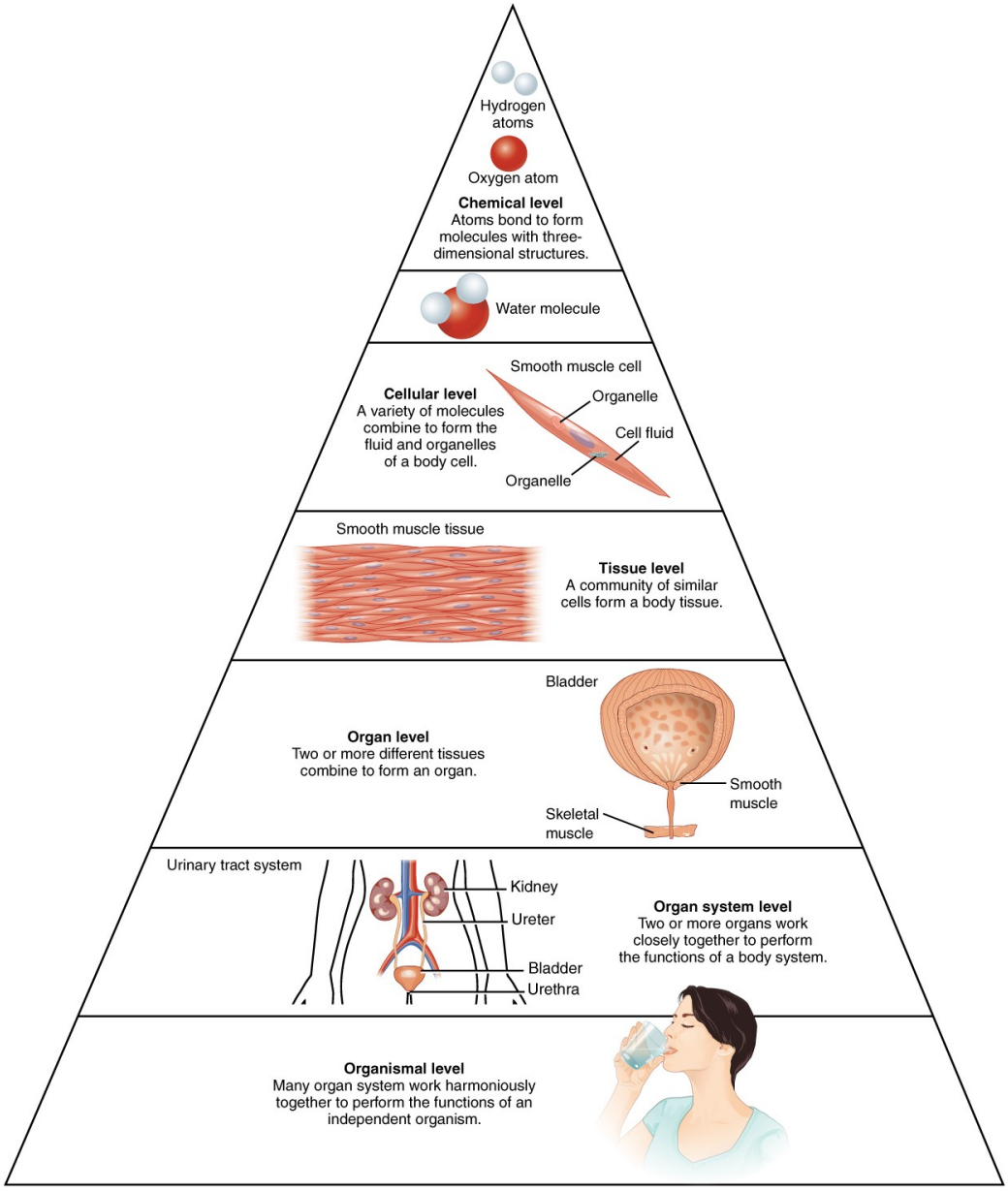


Figure 9.1: Levels of organization of the body, from Openstax College[76]

76 http://cnx.org/contents/FPtK1zmh@8.24:Xh_25wmA@7/Structural-Organization-of-the

Organisms are maintained in working order by a group of processes called ***metabolism***, the set of all the chemical reactions which take place in the body in order to ensure life, including the maintenance of homeostasis. ***Homeostasis*** is the environment necessary to ensure proper functioning. It includes temperature, blood pressure and glucose level. At the same time it maintains the ordered state of the body, this energy is fighting against entropy increase in the body. In doing so, it is increasing the entropy of the universe. Food entering the body is highly organized, so in a state of low entropy, whereas waste products are in a state of much higher entropy. Energy is also dissipated as heat, as in any machine, and this is equivalent to an increase of universal entropy.

Metabolism is a two-faced affair, composed of two groups of processes:

1. ***Catabolism*** is the breaking down of large molecules into smaller ones, a good example being the breaking down of food molecules to obtain energy. In general, a larger number of molecules tends to be more disordered and so catabolic reactions tend to increase entropy.
2. ***Anabolism*** is the opposite of catabolism, so it is the combining of smaller molecules to produce larger ones, such as the formation of proteins from amino acids. Anabolic reactions tend to decrease entropy locally.Reactions inside the body may occur spontaneously or not. Biochemists use the concept of ***free energy***, or ***Gibbs free energ***y, the change of which is defined by

   $\Delta G = \text{energy}_{prod} - \text{energy}_{reac}$,

i.e., the energy of the products minus the energy of the reactants. But input (reactant) and output (product) energies are related by

$$\text{energy}_{reac} \rightarrow \text{energy}_{prod} + \Delta E,$$

where ΔE is the energy released. So

$$\Delta E = \text{energy}_{reac} - \text{energy}_{prod} = -\Delta G$$

Since nature (physics) likes systems to go towards states of lower energy, the reaction will take place spontaneously if the emitted energy ΔE is positive and therefore ΔG is negative.[77] Such reactions are said to be exergonic. So we have two possible cases:

- The energy of the products is less than the energy of the reactants ($\Delta G < 0$), so the reaction is ***exergonic*** and takes place spontaneously, or
- the energy of the reactants is greater than the energy of the reactants ($\Delta G > 0$), so the reaction is ***endergonic*** and does not occur spontaneously.

9.2. Overview of some interesting (astounding) facts about life

Life processes require an input of energy in order to sustain the organization which is essential – or identical? – to it. To drive the analogy into the ground, the entropy tax must be paid constantly until the failing organism defaults and entropy forecloses – and the organism loses.

Here are two extraordinary facts[78]:

- All life depends on ***oxidation-reduction*** (***redox***) reactions, in which electrons are pulled away from one atom or molecule, which is said to be ***oxidized***, to another, which is said to be ***reduced***, since its net charge is reduced by the negative charge of the electron. The receptor can pull the electron away from the donor because it exerts a stronger attractive force on the electron. The degree of this attraction is its ***electronegativity***.
- All life – well almost, excepting several rare bugs which use fermentation instead – depends on ***proton pumps***. This method of energy extraction and storage is common to bacteria, archaea and

77 It seems to me the sign is backward.

78 This section is based mainly on the wonderful presentation of Nick Lane, *The vital question*, Chapter 2.

eukaryotes, the three basic forms of living things.

The proton pump, or ***electron transport chain***, which will be discussed in section 9.6.3, depends on

- an electron donor which is oxidized to furnish an electron;
- a series of structures (three or four, depending on how you count) each of which is partially reduced, using some of the electron's energy to pump protons across a membrane;
- an electron receptor, which is the final resting point for the electron and is therefore reduced; and
- an incredible molecular machine which the electrical gradient of the isolated protons powers so as to store energy in a molecule called ATP (adenosine triphosphate).

It is the energy of the ATP molecule which is then used to combat the ravages of entropy. This schema inspired one biologist to say that life is nothing but an electron looking for a place to rest.[79]

The source and sink of the electrons may vary. For instance, three common sources are water, hydrogen sulfide (H_2S) or ferrous iron (Fe^{2+}). And the final oxygen may instead be nitrate or nitrite, sulfate or sulfite.

Since archaea, bacteria and eukarya use the same process for energy, it must be very old on an evolutionary scale.

9.3. Global bioenergetics

Movement is a form of energy (kinetic) and everything which moves (steam engines or animal muscles) requires input of the energy it expends or loses as heat. Animals get their energy from the nutrients they ingest – food. Plants are ***autotrophs***, making their own nourishment, converting energy from the sun into mostly carbohydrates, which we can then eat. Humans are ***heterotrophs***, "hetero" meaning "other". We must get our nourishment from others, meaning plants and other animals.

On a global scale, energy transformations are cyclic. Sunlight strikes plants and, in doing so, provides energy to power photosynthesis which produces carbohydrates using CO_2 from the atmosphere and water from the ground, ejecting O_2 back into the atmosphere. Some plants are eaten directly by us and some indirectly, as we eat the animals which have consumed the plants. Our digestive system and cells convert what we eat mostly into sugar and cellular respiration combines this with oxygen we breathe to produce usable energy (the chemists' free energy). Our waste products include CO_2 and water, which are then reused by plants. The details go way beyond this overly simple illustration, but the idea is the same – a cycle of substances from the atmosphere through plants, then animals, then back to the atmosphere. At each step of the cycle, some of the initial solar energy is dissipated into space as heat and is lost. New solar energy must continually be pumped in to keep the cycle running, in accordance with the Second Law of thermodynamics.

79 Albert Szent-Györgyi, cited by Nick Lane, *The vital question*, 28.

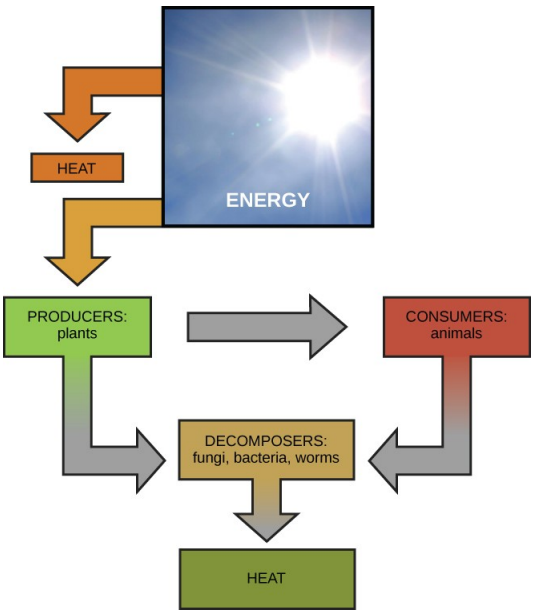


Figure 9.2: Global bioenergetic cycle, from Openstax College[80]

Calculation shows that the Earth obeys the Second Law of Thermodynamics.[81] Energy from the Sun is at high temperature and therefore has relatively low entropy, whereas the energy radiated back into space from the Earth is at a lower-temperature and so has higher entropy, which guarantees that the entropy of the whole system increases.[82]

9.4. Energy flow in the human body

Two main energy input paths exist in the human body, respiration and digestion – air and food.

Oxygen is distributed by the ***circulatory system***. When we breathe, air enters through the nose, then passes through the pharynx, the larynx, the trachea and the bronchi and into the lungs. There, it passes into the alveoli where oxygen is transferred to the blood which has been pumped into the lungs from the right ventricle of the heart through the pulmonary artery. After oxygenation in the alveoli, the blood is pumped through the pulmonary vein[83] into the left atrium of the heart, then out from the left ventricle through the rest of the body's arterial system finally passing from tiny capillaries into the cells. De-oxygenated blood is pumped back through the veins into the right atrium of the heart and then the process starts over.

80 Openstax College, “Concepts of Biology”,http://cnx.org/contents/s8Hh0oOc@8.57:TlvTuW6C@6/Energy-and-Metabolism
81 Carroll (2010), 192-3.
82 Mathematically, entropy is heat emitted divided by the temperature.
83 The pulmonary veins are the only veins to convey oxygenated blood, so it is convenient to think of veins as conveying blood toward

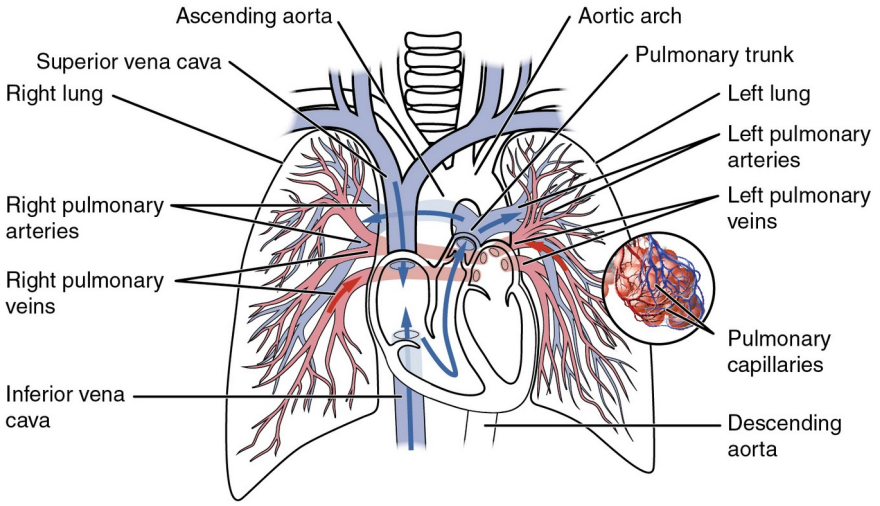


Figure 9.3: Pulmonary circuit, by Openstax College via Wikimedia Commons[84]

The blood circulates all kinds of things, not just oxygen. For instance, it carries products of digestion such as glucose to the cells and removes waste products.

The second energy-input pathway, ***digestion***, takes place as food enters the body through the mouth.

Food is first ground by the teeth, which facilitates subsequent digestion. ***Ptyalin***, a digestive enzyme found in saliva, begins hydrolysis. Only about 5% of hydrolysis takes place in the mouth before the food is moved by the tongue into the pharynx (throat). The epiglottis ensures that it moves past the tracheal opening and into the esophagus (a process which sometimes functions less well in older people), through which it descends into the stomach. Hydrolysis continues there until the food becomes mixed with enough gastric secretions that the mounting acidity renders the enzyme inactive. After being kneaded in acidic gastric juice, the resulting pasty ***chyme*** passes into the small intestine, which secretes mucus, hormones and digestive enzymes. There, it is digested by various processes using different enzymes depending on composition (carbohydrates, lipids or proteins). The result is principally glucose, which enters the blood from which it may be used immediately or stored for later use.

When the cell needs energy, a series of processes called ***cellular respiration*** takes place, mostly in the mitochondria. Glucose from digestion is combined with oxygen from the lungs to liberate energy. This is the fun part! What happens is this.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2 \ + \text{energy}$$

which in plain English reads

$$\text{glucose} + \text{oxygen} \rightarrow \ \text{water} + \text{carbon dioxide} \ + \text{energy}$$

This is where the two input paths come together. Glucose from digestion is combined with oxygen from the lungs to make water, carbon dioxide and much energy. It is ***glucose catabolism***, or breakdown. The liberated energy supports metabolism.

84 https://commons.wikimedia.org/wiki/File:2119_Pulmonary_Circuit.jpg

9.5. Digestion – energy and metabolic pathways

Our nutriments are composed almost entirely of three types of substances – carbohydrates, proteins and lipids (fats); the rest consists of small amounts of minerals and vitamins (organic nutrients required in small amounts, but which the organism cannot synthesize). The sequences of biochemical processes which constitute use of these three types of molecules are called ***metabolic pathways***.

The very notion of metabolic pathways is somewhat of an idealization, since science is not that simple when we look closely. In fact, as the image shows, the pathways are connected at multiple points. In addition, there are inputs of enzymes and hormones from other organs.

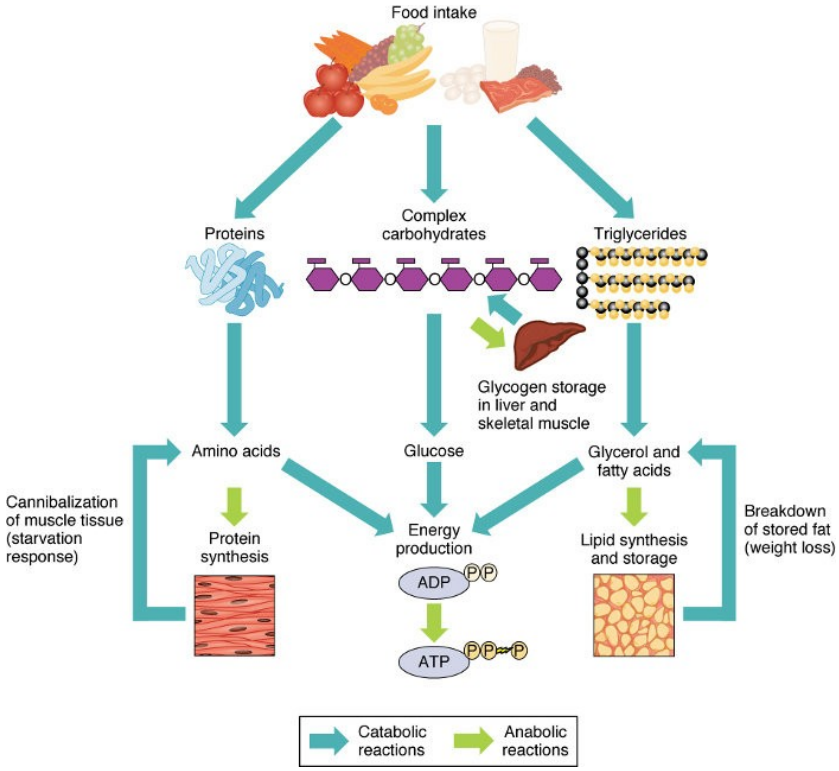


Figure 9.4: Sources of ATP, from Openstax College[85]

In addition, there are inputs of enzymes and hormones from other organs. The ***liver*** is essential in energy regulation and storage and produces ***bile***, which is needed for digestion of fats and lipids. Bile can be stored in the ***gallbladder*** to be delivered to the ***duodenum*** (the first part of the small intestine) when needed. The ***pancreas*** secretes enzymes essential for the digestion of all three food types.

Digestion in the small intestine is similar for all three principal food types. Carbohydrates, fats and proteins are all broken down by ***hydrolysis***, adding a water molecule to separate the long molecules into shorter ones. For a simple disaccharide represented by R″-R′, this can be represented chemically by:

$$R''\text{-}R' + H_2O \rightarrow R''OH + R'H,$$

where the hydrolysis process indicated by the arrow requires the use of an enzyme.

Basic digestion of the three food types goes like this:

- Carbohydrates are composed of poly- and monosaccharides. These are reduced by digestion

85 From Openstax *Anatomy and Physiology*, http://cnx.org/contents/FPtK1zmh@7.30:wJt-Gj_K@4/Overview-of-Metabolic-Reaction.

to monosaccharides, which are used for energy or stored in cells, especially liver and muscle cells.

- Proteins are polypeptide chains of amino acids, which are broken down into the separate amino acids which will be used to make more proteins during gene expression.
- Fats are composed mainly of triglycerides, which are separated into fatty acids.

Different enzymes are used in the three cases.

The following sections discuss digestion of each type in more detail.[86]

9.5.1. Digestion of carbohydrates

Carbohydrates are composed mainly of sucrose, lactose, long-chain starches and cellulose. Cellulose is not digestible and just passes through the body in the form of fibers of which nutritionists are so fond.

By the time food enters the small intestine, 40-50% of carbohydrates have been hydrolyzed, principally to maltose. Further digestion of carbohydrates takes place in the small intestine as ***pancreatic amylase***, an enzyme secreted by the pancreas, breaks them down into maltose or small glucose polymers, large molecules composed of repeated subunits. Different enzymes then split these into their constituent ***monosaccharides***, the simplest forms of carbohydrates, which are soluble and so can cross the intestinal wall to enter the blood stream and be carried to the liver.

- Maltose from starches is broken down by ***maltase*** and a-dextrinase to glucose.
- Lactose from milk is converted by ***lactase*** into glucose and ***galactose***.
- Sucrose (table sugar) is converted by ***sucrase*** into ***fructose***.

The products are therefore glucose (about 80%), fructose and galactose. The last two substances are converted by the liver into glucose, which is therefore the common final form of carbohydrates arriving in cells. It is either used right away for energy, being sent to the glycolytic pathway and thence to the Krebs cycle, or converted to glycogen (by the process of ***glycogenesis***) and stored.

Glycogen is a large polymer of glucose molecules and is an efficient form for storage. Storing glycogen instead of the individual glucose molecules allows more storage without substantial modification of the cell's osmotic pressure.[87] All the body's cells can store small amounts of glycogen, but the champions are the liver (5-8% of its weight) and the muscles (1-3%).

When the body's glycogen storage capacity is filled, excess glucose is converted to acetyl-CoA and thence into triglycerides (lipids), which are stored in adipose tissues. Yes, too much sugar turns to fat. Fat contains ~2.5 times the energy of an equal weight of glycogen. So since much more fat than glycogen can be stored in the body, an average person stores around 150 times as much energy in fat as in carbohydrates.[88]

9.5.2. Digestion of proteins

Proteins are partially broken down in the stomach by the enzyme ***pepsin***, important because it is capable of breaking down the protein collagen in the connective tissue of meat. This enzyme prefers a pH of 2.0-3.0, which is maintained by hydrochloric acid secreted at pH 0.8 by gastric glands in the stomach. Although it is actually the enzyme pepsin which is primarily concerned with breakdown of proteins, it can only do so once inactive pepsinogen is converted by HCl into active pepsin. HCl also

86 Principal source of information, Guyton and Hall (2011), chaps. 67-69.
87 Since osmotic pressure depends on the number of molecules in a solvent. See the discussion of osmosis and related possible danger for cells in the section on water.
88 Guyton and Hall, 824.

plays other roles, such as unfolding protein structures. When the resulting chyme passes into the small intestine, the pancreas excretes sodium bicarbonate to lower its acidity. As in the case of carbohydrates, the pancreas contributes enzymes, ***pancreatic proteolytic enzymes*** or ***proteases***, to the small intestine to continue the breakdown of polypeptides into amino acids, although most remain as relatively small di- or tripeptides. These are taken up by ***enterocytes*** on the intestinal wall, columnar epithelial cells whose large surface area facilitates transfer of molecules from the intestine. There, various ***peptidases*** break down what is left into single amino acids which then pass into the blood. These are carried to the cells to be used as raw materials for the construction of new proteins in ribosomes. Any remaining peptides or whole proteins may cause serious health problems.

In order to prevent proteolytic enzymes from destroying needed bodily proteins, they exist in unactivated precursor forms called ***zymogens*** and are only turned on when needed.

9.5.3. Digestion of lipids

Lipids (fats) consist of triglycerides, phospholipids, cholesterol and small quantities of other substances. Lipids are *necessary* for the formation of cell membranes and for many functions within cells. Like carbohydrates, they can provide energy. Most ingested fats are neutral fats called triglycerides, consisting of a glycerol nucleus attached to the ends of three fatty-acid side chains (Figure 7.7). Phospholipids are composed of only two fatty acids, attached to a hydrophilic phosphate "head group".[89] Cholesterol is not a fat but has chemical properties similar to those of fatty acids and is digested similarly.

Fat digestion occurs mainly in the small intestine.[90] The problem is that fats are not soluble in water. The hydrophobic fat globules are relatively large and must be broken up by emulsification before they can be effectively acted upon by enzymes. First, fat is mixed with bile (bile salts and, especially, lecithin) with polar and non-polar parts. The non-polar parts attach to the fats, leaving their hydrophilic polar parts pointing outwards. When they are agitated, they break up to form an ***emulsion***, a mixture of one normally non-miscible liquid in another (like well-stirred *vinaigrette* salad dressing, or detergent in dish water). The smaller units of the fat present a much greater surface area than before and so can be attacked by water-soluble ***pancreatic lipase*** and split into ***free fatty acids*** (***FFAs***) and monoglycerides (glycerol linked to a fatty acid by an ester bond).

Again, the products are surrounded by bile salts with their non-polar ends towards the fats and their polar ends pointing outwards into the water. They then form small spheres called ***micelles***, globs of which are water-soluble. The micelles then ferry the FFAs and monoglycerides through the aqueous solution to the epithelial cells. Since these are also fatty, the lipids can diffuse into them, leaving the bile behind behind to form more micelles.

The same processes of hydrolyzation and ferrying by micelles takes place for phospholipids and cholesterol using different pancreatic lipases.

Inside the intestinal membrane, the FFAs are re-synthesized into triglycerides. The triglycerides cholesterol, phospholipids and some apoprotein B (about 9% phospholipids, 3% cholesterol and 1% apoprotein B) are packaged into tiny (0.08-0.6 microns) phospholipid vesicles called ***chylomicrons*** which leave the intestinal cells. They are too big for capillaries but can flow via lacteals into the lymphatic system.[91] The chylomicrons transport the fats and cholesterol through the aqueous lymph and then into the venous blood via the thoracic duct.[92] From the blood, they are stored either in the liver or in adipose tissue.

89 This subject was discussed in some detail in section 7.1.4.
90 This topic based mainly on Guyton and Hall, 792.
91 Guyton and Hall, 819.
92 Explained in the chapter on the lymphatic system.

Triglycerides are converted into FFAs and back because triglycerides cannot cross the intestinal cell membranes, but FFAs can. They then enter the lymphatic system. Only small amounts of water-soluble, short-chain fatty acids, like butterfat, can pass directly from the small intestine into the blood without making a detour through the lymphatic system.

In short, fats are emulsified by bile salts so that they can be broken up by pancreatic lipase into FFAs. FFAs can cross the intestinal membrane and are reconverted to triglycerides. In order for them to be transported in the aqueous lymphatic system, FFAs and cholesterol are enclosed in phospholipid vesicles called chylomicrons.

In this manner, almost all ingested fats (except short-chain fatty acids) are absorbed from the intestine into the intestinal lymphatic system in chylomicrons. These then are passed through the thoracic duct into venous blood. Several types of tissues, especially skeletal muscles, adipose tissues and heart tissues, synthesize ***lipoprotein lipase*** which hydrolyzes the triglycerides from the chylomicrons. The resulting fatty acids are either used for energy or stored in the cells until needed.

After breakup of the chylomicrons and their removal by the liver, most of the lipids left in blood plasma (>95%) are in the form of tiny particles called ***lipoproteins***, partly lipid and partly protein, which are like miniature chylomicrons. The lipoproteins are processed by the liver from lipids and may contain triglycerides, phospholipids, cholesterol or proteins. Like chylomicrons, lipoproteins serve to transport lipids in the blood. Originally, use of a centrifuge to separate their components led to the definition of four classes of lipoproteins based on their densities.

In the body, though, no centrifuge exists to distinguish among the different densities of lipoproteins. Lipoproteins sent out by the liver carry triglycerides and are referred to as VLDLs (very-low density lipoproteins), since triglycerides are lighter than water. As tissues absorb the triglycerides, the lipoproteins become denser, effectively becoming IDLs (intermediate-density) and then LDLs (low-density). LDLs serve essentially to deliver cholesterol to tissues. But HDLs (high-density) are made up of different proteins. Their function is to carry cholesterol away from tissues and back to the liver.[93] Hence, the slightly incorrect practice of calling LDLs “bad cholesterol” and HDSs “good cholesterol”. They are not cholesterol, they transport it.

The fat intake of the world's peoples is quite variable, being 10-15% in parts of Asia and 35-50% in western countries. Part of this is converted to triglycerides and stored.

9.5.4. Accessory organs of digestion

We have seen that the liver, gall bladder and pancreas are essential for digestion. They are not part of the digestive system, since no breaking down of nutrients takes place in them, so they are referred to as accessory organs of digestion.

The liver

The liver is one of the most important organs in our bodies and we have seen many examples of its actions. Let's try to collect them together to get a coherent look at liver functioning.

The liver is essential to digestion of fats. It produces ***bile***, an extremely alkaline substance, which is either sent through bile ducts to the duodenum or stored in the gall bladder until it is needed. In the duodenum, it serves to emulsify fats, breaking them up so that they may either be transported or subjected to chemical reactions. It also processes lipids into lipoproteins.

All blood from the digestive processes passes through the liver.

There are two input paths to and two output from the liver.

- The hepatic artery brings oxygenated blood from the heart (the aorta).

93 Engle, *Pain-free biochemistry,* 124.

- The hepatic portal vein transports partially deoxygenated blood (more than the hepatic artery) from the small intestine, containing nutrients as well as drugs and toxins.
- Processed and filtered blood is released through the hepatic vein.
- Bile is released through the hepatic duct, which merges with that exiting the gall bladder.

The liver also carries out many other functions, including the following.

- It processes and filters toxins, such as alcohol, and metabolic waste, including dying blood cells.
- It converts fructose and galactose into glucose or triglycerides (fats).
- It converts much glucose to glycogen (glycogenesis) and stores that. It can also convert stored glycogen back into glucose (glycogenolysis) or synthesize glucose (gluconeogenesis) if needed.
- If glucose and fats are lacking for energy production, acetyl-CoA accumulated in the liver is combined in pairs to form acetoacetic acid, a ketone body. The acetoacetic acid can be carried by the blood to other cells where it can be reconverted to acetyl-CoA and enter the Krebs cycle

And much more.

The gall bladder

The gall bladder stores bile from the liver and releases it when it is needed for the digestion of fats.

The pancreas

The pancreas has both digestive and endocrine functions. We have seen its two main functions. It produces and secretes enzymes essential to digestion. And it produces insulin and glucagon, essential to blood sugar regulation.

- Insulin tells the liver and kidneys to store more glucose as glycogen, thus reducing its concentration in the blood.
- Glucagon does the opposite, stimulating the release of glucose from glycogen.

9.6. Making energy available – cellular respiration

Although products of the digestion of all three food types – carbohydrates, proteins and lipids – all follow some common pathways for energy conversion, one usually attacks this subject by considering carbohydrate digestion, as this allows a linear presentation of the entire sequence – glycolysis, the Krebs cycle and chemiosmotic phosphorylation (the electron transport chain). Afterwards, we will consider how the other two types enter into the same processes.

Glucose is the body's principal fuel supply and is carried by the blood to all cells. In each cell's mitochondria, glucose is broken down by cellular respiration, a series of reactions which use various enzymes to convert the glucose into water and CO_2 and release the energy needed by metabolism. The overall reaction forming the basis of cellular respiration is

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2 + \text{energy}$$

i.e.,

$$\text{glucose} + \text{oxygen} \rightarrow \text{water} + \text{carbon dioxide} + \text{energy}$$

where the energy is principally stored in molecules of ATP, the body's “energy currency”. ATP serves as a transport agent, carrying the energy from mitochondria to elsewhere in the cells, which need it in order to function.

There are several sets of reactions capable of breaking down glucose to store energy in ATP molecules. The three main ones are

- glycolysis,
- the Krebs cycle (or citric-acid cycle, or TCA cycle) and
- oxidative phosphorylation (the electron transport chain).

Each set is called a ***pathway***. Taken in sequence, they together are referred to as ***cellular respiration***. This step-by-step process has two functions:

1. Ensure that energy is released in small, controlled quantities; and
2. make the activation energy necessary for each step small enough to be adequately lowered by an appropriate enzyme.

9.6.1. Glycolysis

The first pathway, glycolysis, is a series of reactions which take place in the cytoplasm of the cell and convert one six-carbon (C6) glucose molecule into two three-carbon (C3) pyruvate molecules. The overall equation is:[94]

$$\text{glucose} + 2\ \text{ATP} + 2\ \text{NAD}^+ + 4\ \text{ADP} + 2\ \text{P}_i \rightarrow 2\ \text{pyruvate} + 4\ \text{ATP} + 2\ \text{NADH} + 2\ \text{H}^+$$

We see that glycolysis has the effect of reducing NAD^+ to NADH. The latter will be furnished to the electron transport change where the electrons relinquished by its oxidation will provide the energy needed for ATP synthesis. Each step of this pathway (and all the others) is brought about by an enzyme. The following table indicates the steps of glycolysis in some detail.

94 Coenzyme electron carriers NAD and FAD were discussed in the last chapter.

Step	Input	Output	Enzyme
1	glucose + ATP	glucose-6-phosphate (G6P)	hexokinase (or glucokinase, if in the liver)
2	glucose-6-phosphate	fructose-6-phosphate (F6P)	glucose-6-phosphate polymerase
3	fructose-6-phosphate + ATP	fructose-1,6-biphosphate	phosphofructokinase
4	fructose-1,6-biphosphate (split)	glyceraldehyde-3-phosphate + dihydroxyacetone phosphate	aldelase
5	dihydroxyacetone phosphate	glyceraldehyde-3-phosphate	triosephosphate isomerase
End energy-consuming, start of energy-yielding phase: Glucose now converted into two glyceraldehyde-3-phosphate			
6	2 glyceraldehyde-3-phosphate + 2 P_i + 2 NAD^+	2 1,3-biphosphoglycerate + 2 NADH	glyceraldehyde-3-phosphate dehydrogenase
7	2 1,3-biphosphoglycerate + 2 ADP (dephosphorylation)	2 3-phosphoglycerate + 2 ATP	phosphoglycerate kinase
8	2 3-phosphoglycerate	2 2- phosphoglycerate	phosphoglycerate mutase
9	2 2- phosphoglycerate	2 phosphoenolpyruvate (PEP)	enolase
10	2 phosphoenolpyruvate + 2 ADP + 2 H^+ (dephosphorylation)	2 pyruvate + 2 ATP	pyruvate kinase

Step (6) is important for two reasons. First, it is the one where the P_i necessary to convert ADP into ATP comes into the action. After step (6), the P_i is no longer free and the conversion is now energetically favorable and takes place in the step (7). A similar step produces another pair of ATP molecules in step (10). Step (6) also includes the reduction of NAD^+, whereby it collects electrons which it will carry to oxidative phosphorylation..

The glycolysis pathway is used by almost every organism on Earth, including prokaryotes, so it must have evolved very early in the history of life.

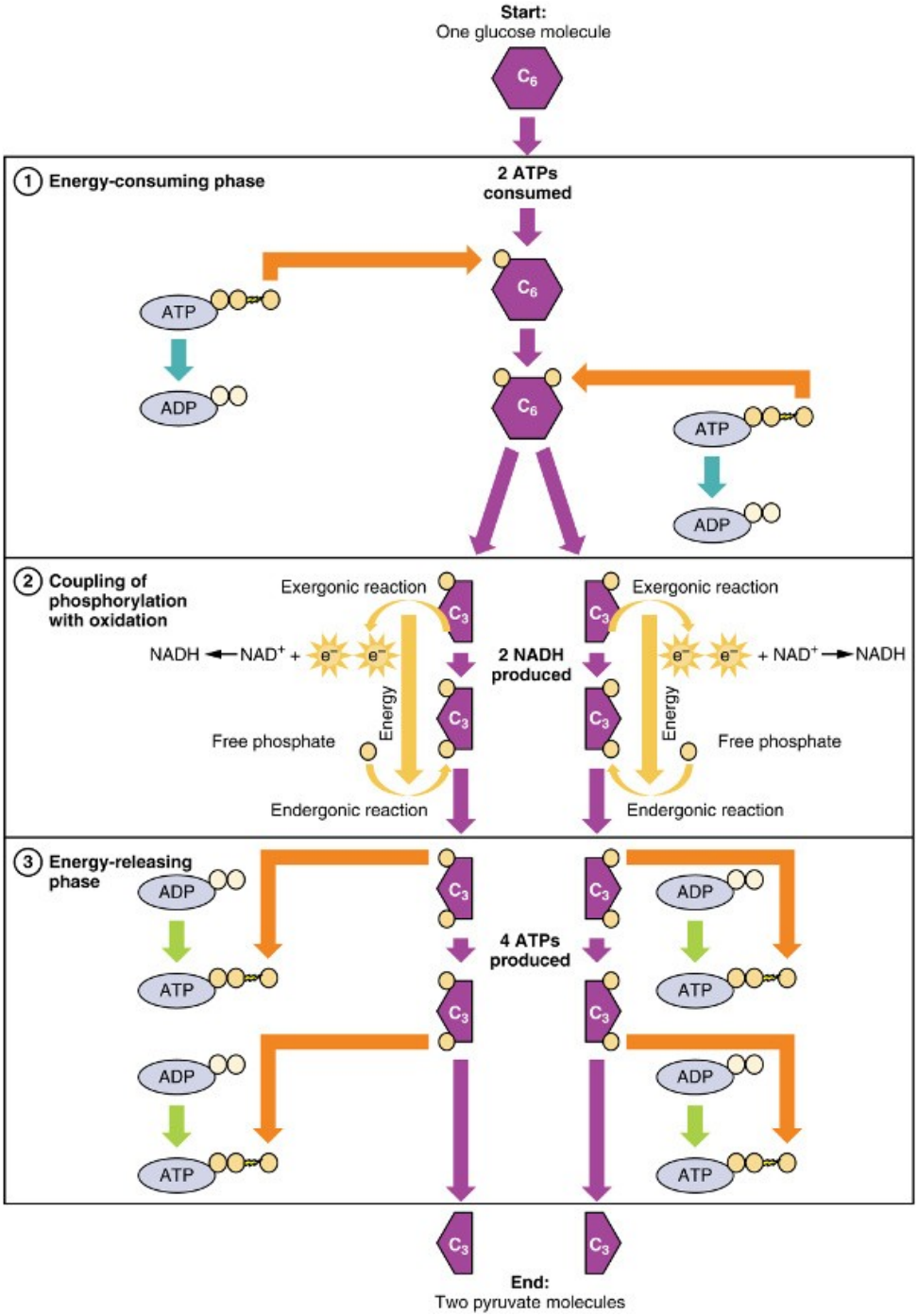


Figure 9.5: Glycolysis overview[95]

Notice that glycolysis does not require oxygen in order to proceed; it is an example of ***anaerobic respiration***.

Ignoring those complex chemical names, the table shows that each step is chaperoned, so to speak, by a different enzyme. It also shows that although the overall process is to convert glucose to pyruvate, it requires two ATP to get started (input column) but produces four (output column), for a net gain of two ATP.

95 From Openstax *Anatomy and Physiology*, http://cnx.org/contents/FPtK1zmh@7.30:nWir-Uwu@4/Carbohydrate-Metabolism.

9.6.2. The Krebs cycle

Recall the overall view of cellular respiration:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2 + \text{energy}$$

i.e.,

glucose + oxygen → water + carbon dioxide + energy

The ***Krebs cycle*** (or ***citric acid cycle***, or ***TCA, tricarboxylic acid cycle***) takes place within the matrix of a mitochondrion. The pyruvate molecules formed by glycolysis in the cytoplasm of the cell pass into the mitochondrial matrix, but pyruvate can not enter directly into the Krebs cycle. It is first converted by the enzyme pyruvate dehydrogenase (PDH) as follows:

- one C and two O atoms are removed as CO_2 (decarboxylation);
- the pyruvate is oxidized and its electrons serve to reduce NAD^+ to NADH + H^+;
- finally, coenzyme-A is added to produce ***acetyl-CoA***, which enters the Krebs cycle.

This is shown at the top of Figure 9.6. This step has been referred to variously as the linking step, the grooming step or the bridging step, but I prefer the ***preparation step*** – or prep step.

The steps of the Krebs cycle are the following:

1. The citrate synthase enzyme (always enzymes) joins the 2-carbon acetyl-CoA with a 4-carbon molecule of oxaloacetate to form a 6-carbon molecule, citrate (or citric acid, hence one of the names of the cycle). To balance things out, the reaction absorbs one molecule of H_2O and ejects one of CoA-HS. As it traverses the cycle, this C6 molecule will lose carbon atoms to become once again a C4 oxaloacetate molecule, which can start the cycle over again with the addition of some acetyl-CoA. But along the way, Good Things will be produced.
2. Another enzyme, aconitase, converts citrate into isocitrate by a “simple” rearrangement of its bonds.
3. A further enzyme, isocitrate dehydrogenase, oxidizes C6 isocitrate into C5 α-ketoglutarate.[96] Carbon is released as CO_2 and electrons serve to reduce NAD^+ to NADH + H^+, with a gain of one NADH + H^+.
4. Yet another enzyme α-ketoglutarate dehydrogenase converts α-ketoglutarate into C4 succynal CoA. Once again, CO_2 is released and oxidized electrons reduce NAD^+ to NADH + H^+, for a gain one more NADH + H^+.
5. The enzyme succynal CoA dehydrogenase converts succynal CoA into succinate. This reaction is exergonic and the released energy serves to form GTP, guanosine triphosphate, similar to ATP, which in turn furnishes energy to convert ADP into ATP. A different enzyme would produce ATP directly. That makes a gain of one ATP.
6. Succinate dehydrogenase converts succinate into fumarate. The oxidized electrons are passed to the electron carrier FAD which is reduced to $FADH_2$, so there is a gain of one $FADH_2$.
7. Fumarase catalyzes the addition of a water molecule to fumarate to form malate.
8. Malate dehydrogenase oxidizes malate back to oxaloacetate (back to step 1). The electrons reduce NAD^+ to $NADH^+$ + H^+., so one more NADH + H^+ is gained.

Note that one glucose molecule makes two pyruvates and so brings about two “turns” of the Krebs cycle. The oxidation steps in one cycle result in a gain of three NADH + H^+, one $FADH_2$ and one ATP.

96 Gulp!

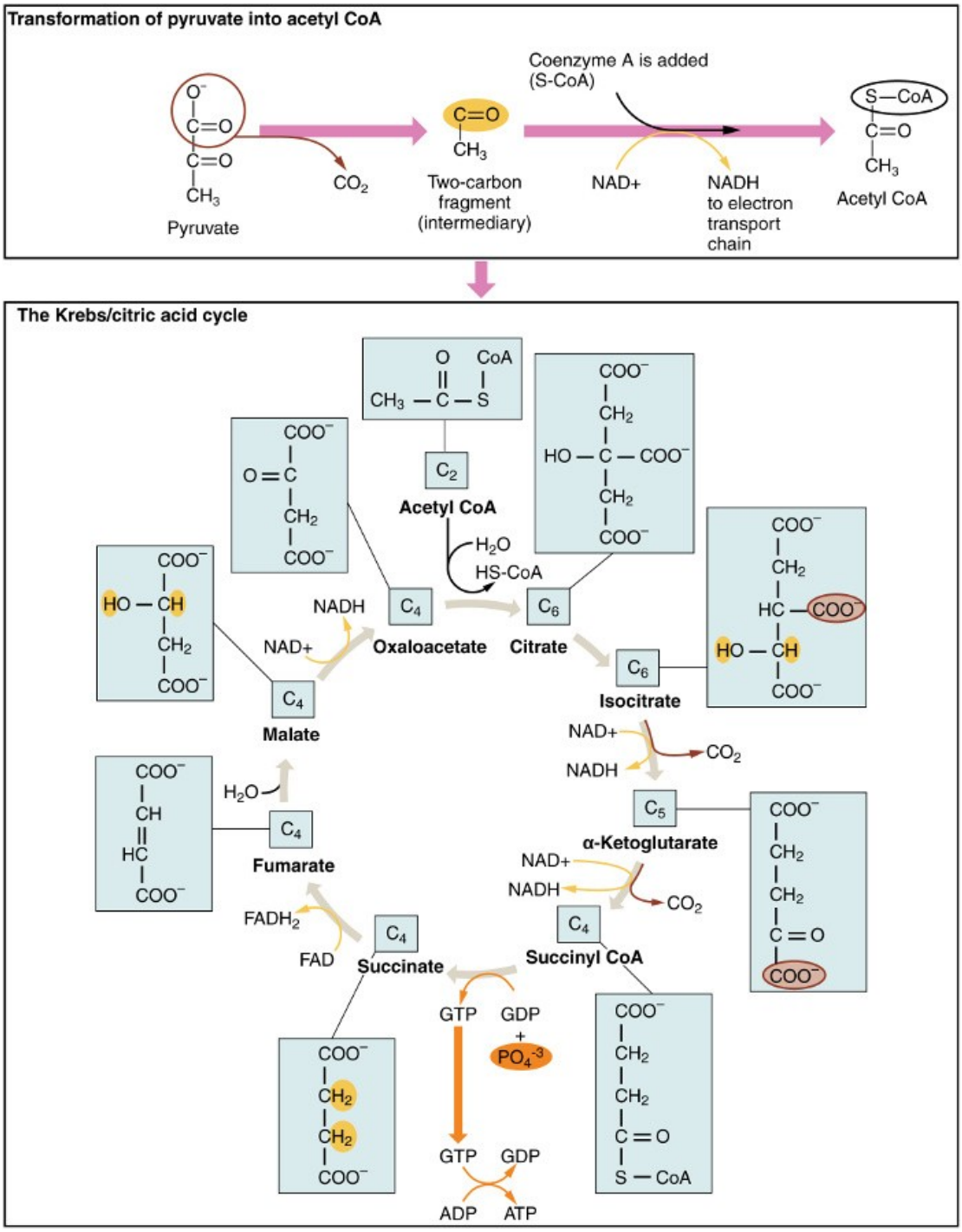


Figure 9.6: The Krebs cycle[97]

In words now. The Krebs cycle is a temporal cycle, with one reaction taking place after another in time. There is no point in looking inside a mitochondrion with a super microscope, you won't see a whole bunch of rings like in the usual diagrams. Since each element of a ring is produced from the preceding one, only one element exists at a given moment, which is why you won’t see a ring. What you may see is oxaloacetate waiting for acetyl CoA. That does not take long to appear, as glucose molecules are constantly broken down into two pyruvate molecules each, and these are moved into the mitochondrion where they are transformed into acetyl CoA. (The mitochondrion is like another

97 From Openstax *Anatomy and Physiology*, http://cnx.org/contents/FPtK1zmh@7.30:nWir-Uwu@4/Carbohydrate-Metabolism.

country to the rest of the cell. It requires a passport to enter and some kinds of immigrants are not allowed in.) In the mitochondrion, the reaction between the acetyl CoA and oxaloacetate takes place, aided and abetted by the appropriate enzyme. A (very brief) moment later, the whole sequence of reactions has taken place and the acetyl CoA is used up, having absorbed some water and some NAD^+ and FAD from the mitochondrial matrix. The NAD^+ and FAD are reduced to NADH, $FADH_2$ and some ATP is produced, as well as … a new molecule of oxaloacetate, fresh and waiting eagerly for another molecule of acetyl CoA to go 'round again. Aside from those NAD^+, $FADH_2$ and H_2O, nothing has changed, so the whole cycle is acting as a catalyst to convert acetyl CoA, NAD^+ and FAD into NADH, $FADH_2$ and ATP. The equation looks like this:

$$\text{Acetyl-CoA} + 3\ NAD^+ + FAD + GDP + P_i + 2\ H_2O \rightarrow$$
$$\text{CoA-SH} + 3\ NADH + FADH_2 + 3\ H^+ + GTP + 2\ CO_2$$

The Krebs cycle has been likened to a traffic roundabout, where molecules are able to enter or exit at any point. In this way, the rate can be heightened by addition of Krebs-cycle molecules or slowed by their removal, for instance, so they can be exported from the mitochondrion and used in other metabolic processes. The cycle has one overall effect of breaking down molecules from food into energy, so it is a set of catabolic reactions. *Even more importantly, it reduces NAD^+ and FAD to NADH and $FADH_2$ which will carry electrons into the mitochondria. There, their oxidation in the electron transport chain will use the electrons' energy to produce much ATP.*

After many years of debate, it seems biologists are finally (mostly) agreed that there exists a version of the Krebs cycle which is "reversed". This means that instead of oxidizing carbohydrates to produce energy in the form of ATP, the reverse cycle uses energy, CO_2 and water to produce organic compounds. Figure 9.7 shows how it is done and how it is, in fact, not the exact reversal of the Krebs cycle. In the figure, Fd denotes ***ferrodoxin***, a protein bound to one or two iron-sulfur clusters, and which is a powerful at transferring electrons from one molecule to another. It is needed in light reactions, as we will see in Figure 9.19, and essential in enabling the C2-to-C3 and C4-to-C5 steps of the reverse Krebs cycle.

Note that every tour of the reverse Krebs cycle also produces an additional molecule of oxaloacetate at the C6-to-C2 step. This molecule is also an intermediate in the cycle and so can generate any of the other intermediates, effectively creating another, new cycle. In other words, the cycle is autocatalytic.[98]

In the figure, the notation +CO2 stands for the addition of one molecule of that substance, which changes the C count of the preceding element. Similarly, +2H stands for the reduction of NAD^+ and its 2 protons to NADH, thereby adding two protons to the preceding element.

The biosynthesis process usually mentioned in the context of plants is the Calvin-Benson cycle (the reductive pentose phosphate, or simply the ***pentose cycle***), which we will discuss in section 9.8.3. The reverse Krebs cycle, the reductive carboxylic acid or ***carboxylic cycle*** is also one of six pathways for fixing carbon. The Calvin-Benson cycle and the acetyl CoA cycle are two others. The reverse cycle is used by some bacteria. Since it may pre-date the Calvin cycle, it is of interest to research into the origin of life.

98 Lane (2022), 100-101.

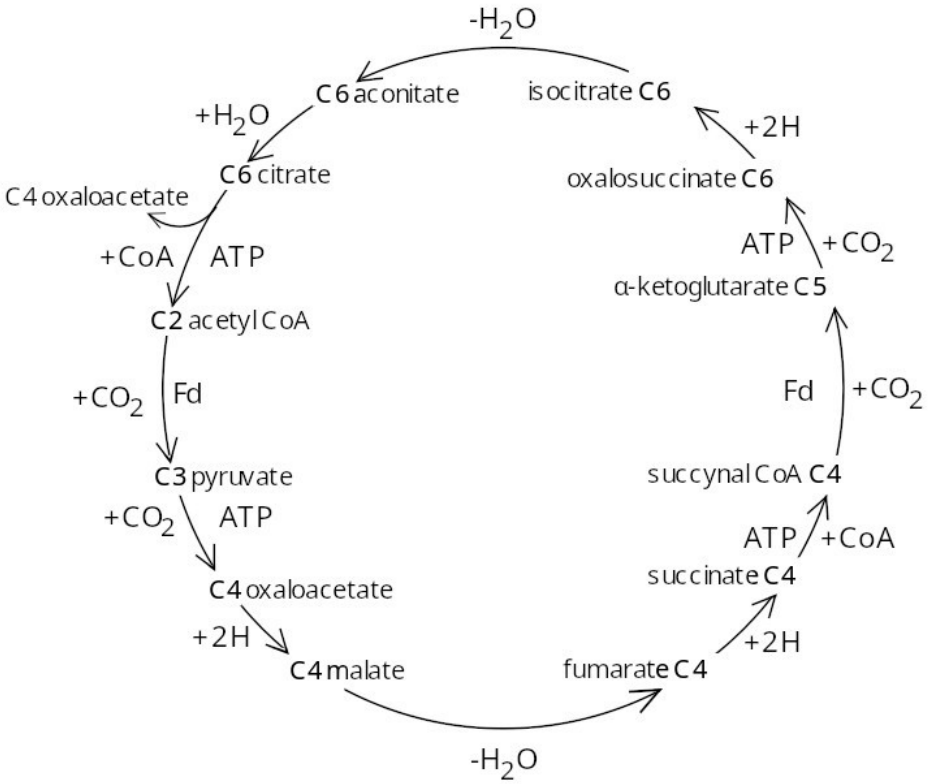


Figure 9.7. The “reverse” (reductive carboxylic acid) Krebs cycle, after Lane[99]

The Krebs cycle is also of importance in the study of cancer. For instance, with age, complex I of the ETC functions less efficiently, leading to an excess of NADH. This then may push a part of the Krebs cycle into reverse, accumulating succinate and thereby signaling a non-existing oxygen deficit. This in turn causes the cell to privilege ***aerobic glycolysis***, fermentation in the presence of oxygen, and this is the preferred environment of cancer cells (the Warburg effect), the goal of which is not to produce more ATP but to grow.[100]

Lane’s recommendations for the ageing: “… the best we can do is keep our mitochondria active … oxidize NADH in your mitochondria as far as possible. Nothing is failsafe, but there’s no doubt that regular aerobic exercise and a healthy diet will help protect you against cancer. .. [So] nurture your mitochondria. Don’t let cell respiration run down, which is the underlying cause of cancer as we age.”[101] I can imagine a T-shirt with “Keep your mitochondria active”.

9.6.3. Chemiosmotic theory of oxidative phosphorylation – electron transport chain

The Krebs cycle may be a great advantage over glycolysis in terms of efficiency of ATP production, but the ***electron transport chain*** (***ETC***), where oxidative phosphorylation takes place, has them both beat hollow.[102] Oxidative phosphorylation takes the electrons carried by NADH and $FADH_2$ produced in the Krebs cycle and uses them to produce more ATP – much more.

99 Lane (2022), 128.
100 Lane (2022), chapter 5, appropriately entitled “To the dark side”, gives an illuminating, although difficult, explanation of this.
101 Lane (2022), 232.
102 When I first read about it, I was giggling with joy.

Recall that NAD and FAD are electron carriers, picking up electrons as they are reduced to NADH and $FADH_2$ as follows:

$$NAD^+ + 2e^- + 2H^+ \rightarrow NADH + H^+$$

and

$$FAD + 2e^- + 2H^+ \rightarrow FADH_2$$

and leaving them off as they are oxidized to NAD^+ and FAD in the opposite reactions (just invert the arrows).

In the electron transport chain, the NADH and $FADH_2$ produced in the Krebs cycle, with the help of two coenzymes, transport electrons to the first of a series of four enzyme complexes in the inner mitochondrion membrane. Each complex is reduced as it receives an electron and oxidized as it passes it on to the next complex, somewhat like a bucket brigade (or a relay race). The electron is passed along because each successive complex is more electronegative (electron loving) than the preceding one. (One also says it goes from a complex of higher reducing potential to a lower one.) The energy produced by the redox reactions at each complex serves to pump protons across the inner mitochondrial membrane into the inter-membrane matrix.

At the end, the electrons are passed to oxygen which uses them to combine with the residual hydrogen to produce water. The left-over re-oxidized NAD^+ and FAD can return to glycolysis and the Krebs cycle to pick up more electrons.

Here is the amazing part! The gradually built-up proton gradient becomes sufficiently strong to power an incredible-seeming object in the inner mitochondrion membrane, an enzyme called the ***ATP synthase***. The protons passing through it actually cause it to turn and to power the combination of ADP with phosphate radicals to produce ATP in large quantities. This synthesis of ATP by adding P_i to ADP with the energy from a proton gradient is called ***chemiosmotic phosphorylation***.[103]

In summary, the overall function of the ETC Is to use the energy of electrons from reduced NADH and $FADH_2$ to pump H^+ across the inner mitochondrial membrane into the inter-membrane matrix so that the built-up electrical potential can power the synthesis of ATP by ATP synthase.

103 Gulp!

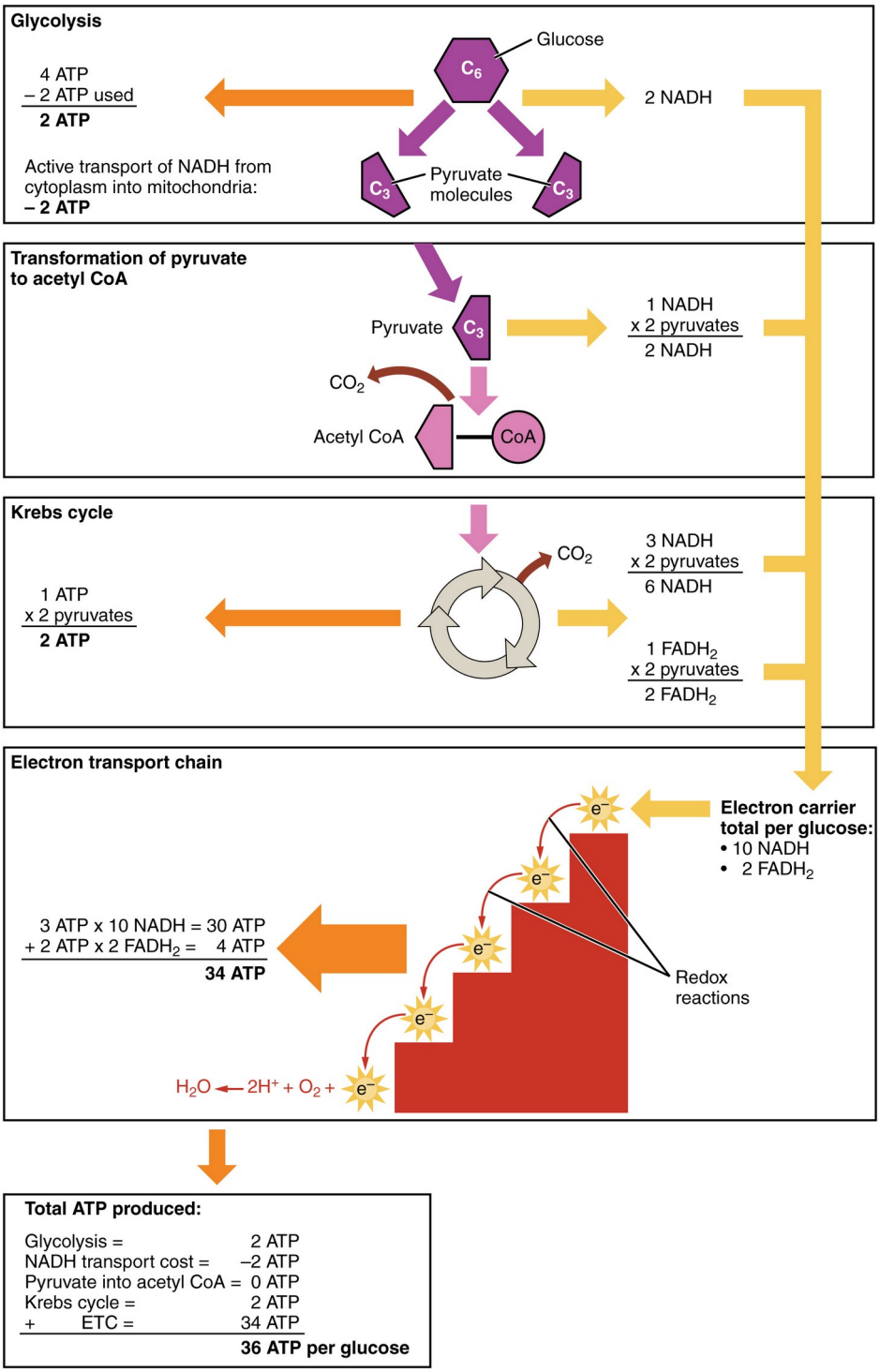


Figure 9.8: ATP production in cellular respiration[104]

Look at Figure 9.8 with the accounting data. The accounting is clear. Oxidative phosphorylation can produce up to 34 ATP molecules per glucose molecule entering the glycolysis pathway, for a total of 36 ATP in cellular respiration. This is where we get most of our energy.

The production of 36 ATP per glucose is theoretical. The ATP synthase is not perfectly efficient and some protons leak across the mitochondrial membrane, so observed yields of ATP by oxidative phosphorylation are ~2.5 ATP per NADH and ~1.5 ATP per $FADH_2$, leading to a total net production of

104 From Openstax *Anatomy and Physiology*, http://cnx.org/contents/FPtK1zmh@7.30:nWir-Uwu@4/Carbohydrate-Metabolism.

around 30 ATP per glucose.[105]

The system exists in one form or another in all eukaryotes and serves also in photosynthesis in plants to make the energy necessary for producing glucose.

The process can be broken down into the electron transport chain (ETC), the four complexes on the left in Figure 9.9, and ATP Synthase, the red complex on the right. We will ignore the details of the many redox reactions taking place in each complex.

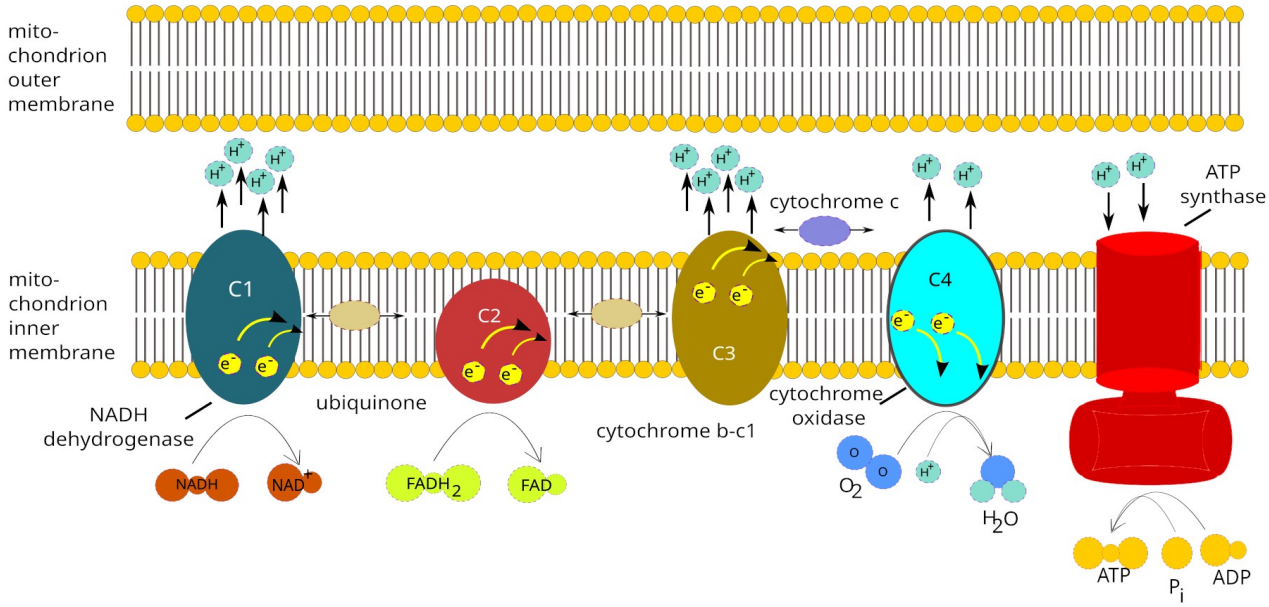


Figure 9.9: Oxidative phosphorylation (electron transport chain)

Consider the electrons transported to the ETC by one NADH. Note that only complexes 1, 3 and 4 pump protons across the membrane. Complex 1 accepts electrons from NADH, complex 2, from $FADH_2$.

1. One NADH is oxidized by complex 1 (C1), NADH dehydrogenase, to give up two e^- and one H^+. For each electron, two H^+ are pumped from the matrix across the inner membrane, so four per glucose molecule. Various reactions take place within the complex, which we will ignore.
2. Both e^- are transferred to ubiquinone (or coenzyme Q), a mobile transfer molecule. CoQ is hydrophobic and so can move about within the membrane, by which means it delivers the e^- to complex C3, cytochrome b-c1, bypassing complex 2. The e^- are passed one at a time to cytochrome c (another mobile transfer molecule). For each e^- accepted by cytochrome c, two more H^+ are pumped across the inner membrane, four per glucose molecule. Once again, more is taking place than meets the eye.
3. Cytochrome c moves back and forth along the inner side of the inner membrane and so delivers the e^- one at a time to complex C4, cytochrome c oxidase. Here, for each pair of electrons, only two H+ are pumped from the matrix across the membrane. After two NADH, C4 has received four e^-, which it uses with the four accumulated H+ to reduce an oxygen molecule, which is therefore the last electron acceptor in the chain. For one glucose molecule, four e^-, four H^+ and one O_2 form two water molecules, four H^+ having been pumped across the membrane into the inter-membrane space..
4. As shown in the figure, $FADH_2$ delivers its e^- directly to complex C_2, which does not pump H^+. The e^- are passed to CoQ and then follow the same path as the other e^-.

105 Wikipedia, Citric acid cycle. https://www.wikipedia.com/en/Citric_acid_cycle.

5. The electric potential brought about by the H^+ inside the cell membrane drives them across the membrane through the ATP Synthase, as the membrane itself is impervious to H^+. Three H^+ are necessary to make one molecule of ATP.

Many such chains exist in every mitochondrion's inner membrane, so multiple H^+ pumps are running all the time, converting glucose and oxygen into energy stored in ATP.

9.6.4. Other energy sources – energy production from metabolic pathways

We have considered glycolysis, the Krebs cycle and the ETC as components of the carbohydrate metabolic pathway. But we can also get energy from lipids and proteins using parts of the same pathway.

Lipids

In a recent episode, we left lipids inside chylomicrons or other lipoproteins in the blood, or in the liver or muscles. In order to use lipids for energy, triglycerides in the liver or muscle are converted by ***lipolysis*** to glycerol and fatty acyl CoA. Acyl CoA is then carried by ***carnitine*** into the mitochondria of the cells, where, it is converted back to fatty acids, which are degraded by the cyclic process of ***β-oxidation***. Each loop of this process converts a two-carbon segment into acetyl-CoA, shortening the chain by two carbon atoms at each cycle. The acetyl-CoA enters directly into the Krebs cycle of cellular respiration, bypassing the glycolysis stage necessary for glucose. Since the products of the Krebs cycle also feed the electron transport chain, converted lipids can furnish a lot of energy.

Compared to carbohydrates and proteins, triglycerides can furnish more than twice the energy per unit mass. So when glucose levels become inadequate, triglycerides stored in the liver or muscle are hydrolyzed to fatty acids which, again, must be transported through the blood, this time by ***albumins***, globular proteins in the plasma. The rate of conversion and transport of fatty acids is great enough that they can be oxidized to fulfill almost all the body's energy needs without the need of energy from carbohydrates. Most cells except brain tissue and red blood cells can get their energy from fatty acids – as long as they last.

But some cells need glucose for energy production. Red blood cells have no mitochondira for β-oxidation and fats cannot cross the blood-brain barrier to enter the brain. The brain only represents about 2% of body weight, but it consumes on the order of 20% of metabolic energy, the greater part of which is to maintain electric potentials. So the brain needs energy continuously and quickly, which explains why we tend to black out after only a few seconds lack of oxygen in the blood. If the body's supply of carbohydrates becomes too low, the liver goes to work, converting stored glycogen back into glucose (***glycogenolysis***) or synthesizing glucose from lactate, pyruvate, glycerol or amino acids alanine or glutamine (***gluconeogenesis***). This may happen when eating mostly fats or when fasting,

The liver stores a large quantity of fatty acids, but uses only a small part of these for its own energy requirements. So when carbohydrate stores (including glycogen) are low, great use is made of fats for energy. But, due to lack of intermediary substances, the Krebs cycle slows down. Excess acetyl-CoA accumulates in the liver, where it is combined in pairs to form ***acetoacetic acid*** which is then carried by the blood to other cells. Some of it is converted to β-hydroxybutyrate and acetone, large quantities of which can be transported rapidly to cells.[106] These two substances plus acetoacetic acid are collectively referred to as ***ketone bodies*** and their synthesis from fats is called ***ketogenesis***. On arriving in cells, all three are converted back into acetyl-CoA, which enters the Krebs cycle.

This is interesting because, in such circumstances, the brain may lack energy because fats can not cross the blood-brain barrier to supply it. But ketone bodies can. So the brain goes from glucose input

106 Differences between Guyton and Hall and Openstax about the order of these two processes. Which came first, acetoacetic acid or β-hydroxybutyrate and acetone? The end result is the same.

to ketone bodies without ever using fats. In fact, ketone bodies are produced all the time, even in a healthy body. They are not bodies, but water-soluble liquids which contain ketone groups. The brain normally uses glucose for ATP generation, but whenever that is scarce, it uses ketone bodies. In fact, This seems to depend mainly on their concentration in the blood. In neurodegenerative diseases, which entail a deterioration of the brains' glucose metabolism, any means of increasing ketone concentration may result in enhancement of the brain's energy metabolism.[107]

In brief, fats stored as triglycerides in the liver may be modified to ketone bodies and enter cells where they are transformed into acetyl-CoA and enter the Krebs cycle, including in the brain.[108]

Proteins

Proteins can also contribute to energy production. While some amino acids are used to re-form proteins (biosynthesis via gene expression) which are then used or stored, some remain. Most of these are converted by ***deamination***, removal of amino groups (-NH_2) from the acids, a process occuring almost entirely in the liver. Most of the time, the amino group is transferred to α-ketoglutanate, which thereby becomes an amino acid, glutamate. A >C=O group from the α-ketoglutanate replaces the original amino group, converting the amino acid into a keto acid. So the transfer is really ***transamination***. Here is the interesting part. The amino acid alanine is transaminated to pyruvate; aspartate, to oxaloacetate; and almost all the others, to molecules (pyruvate, acetoacetyl CoA, fumarate, succinyl CoA, oxaloacetate and α-ketoglutamate) which figure somewhere in the Krebs cycle. So all our major nutrients wind up sooner or later participating in the Krebs cycle to make ATP.[109] The number of ATP molecules obtained from a gram of oxidized protein is slightly less than that from a gram of oxidized glucose.

In order to avoid its poisoning us, ammonia left over from deamination must be removed and this is carried out by the Urea cycle.

Some of the keto acids can be modified to enter the Krebs cycle, like glucose or glucose products, and so are called ***glucogenic***; others form ketone bodies (***ketogenesis***) and are called ***ketogenic***. So ketone bodies may be the product of lipids or proteins.

Excess protein can turn to fat, too. If more protein than can be used for amino acids is consumed, much of the excess is converted and stored as fat or glycogen (gluconeogenesis).[110] From here on, it follows the course of any other fat or glycogen.

Order of use

The preferred order of nutrients for energy is simple: Carbohydrates come first. But even the glycogen stored mainly in the liver can only furnish the body in fuel for maybe a half day. If carbohydrates run out, fats and proteins start to be used, principally fats. Use of fats continues approximately linearly until they run out. At the same time, stored amino acids are converted by gluconeogenesis to glucose. When the easily-accessed amino-acid store is reduced, fat usage increases and some of this is converted to ketone bodies, which can enter the brain and supply energy to it. However, changing from a carbohydrate-rich to a fat diet may require several weeks for brain cells to switch to obtaining up to 75% of their energy from fats (via ketone bodies).[111]

We have seen that excess carbohydrates can be transformed into fat. Although such fats from carbohydrates also can be converted to acetyl-CoA to make energy, they can never be converted back into carbohydrates.

107 Effects of ketone bodies on brain metabolism and function in neurodegenerative diseases. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7699472/.
108 Engel, 133; Guyten and Hall, 823.
109 Engel, 143-4; Guyton and Hall, 834-5.
110 Guyton and Hall, 834.
111 Guyton and Hall, 2011, 824, 852.

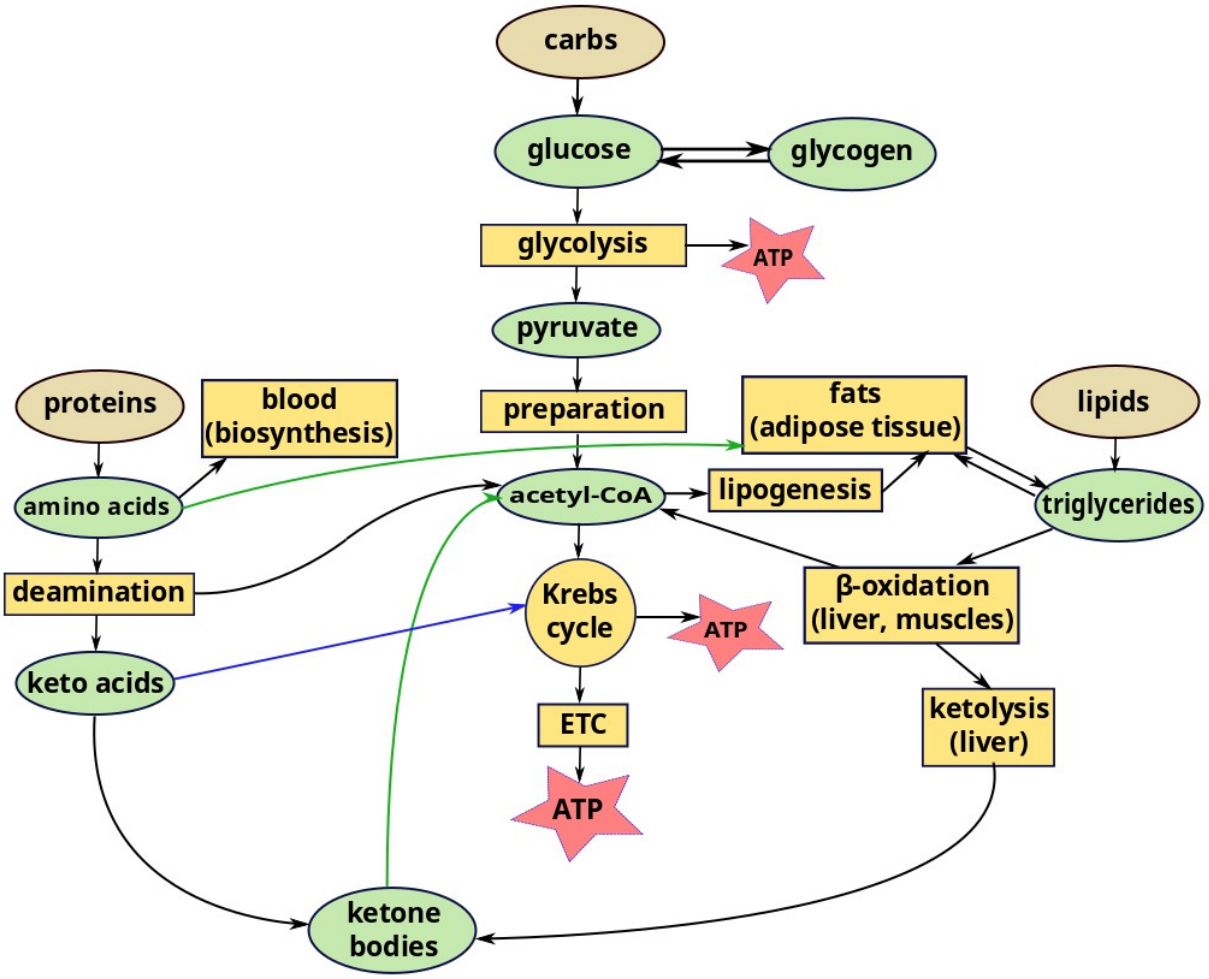


Figure 9.10: Metabolic pathways for ATP synthesis from foods

9.6.5. Anaerobic respiration

As its name implies, oxidative phosphorylation is an aerobic pathway, requiring oxygen. The electron transport chain requires oxygen for the penultimate step of adding H^+ ions to O_2 to make water. In the absence of oxygen, this cannot take place. If oxygen is lacking, energy must be obtained differently.

Glycolysis requires no oxygen other than what is present in the initial reactants and therefore is a form of anaerobic respiration. In case of rapid, intense energy consumption by muscles, the need may be fulfilled by glycolysis, which is much faster than the Krebs cycle, but can furnish energy for only about 15 seconds. Then another energy source must be found.

There is another, much less efficient means of creating energy from pyruvate by ***anaerobic respiration*** – ***fermentation***. One sort of fermentation exists within the body; the other, in yeasts (in bread or beer, for instance).

When oxygen is limited, lactic acid fermentation can take place. Enzymes use electrons from NADH to reduce the pyruvate to lactic acid plus a small amount of energy. In particular, muscle cells can can do this in order to get fast (but relatively little) energy, releasing the lactic acid into the muscles. The oxidized NADH (now NAD^+) can now be recycled to glycolysis.

In bread or beer, ethanol fermentation takes place. Enzymes bring about the decarboxylation of pyruvate to acetaldehyde, releasing CO_2 which makes the bubbles in beer or bread. Then enzymes use electrons from

NADH to reduce acetaldehyde to ethanol. The ethanol evaporates when cooking bread, but is carefully conserved in the making of beer or wine.

9.6.6. Regulation of glucose levels

The liver converts glucose into glycogen and stores that; it can be reconverted whenever the level of blood sugar becomes low. This function is mediated by the hormones ***insulin*** and ***glucagon***, both produced by the pancreas but having opposite effects.

Glucose concentration in the blood needs to be within a certain limited range in order to distribute enough energy to the body, but not so much as would harm organs and tissues, especially where blood vessels are tiny, as in the retina, the body's extremities and the kidneys. Most cells have receptors which bind ***insulin***, causing glucose transporters in the cell membranes to allow more glucose to enter the cell for storage, thereby reducing the glucose level of the blood. Abnormally high glucose levels in the blood are a sign of diabetes, which can be due to inadequate insulin production or to the cells' not reacting correctly to insulin presence.

If the blood glucose level becomes too low, the pancreas secretes ***glucagon*** which stimulates production of glucose from the breakdown of glycogen, from amino acids or from stored triglycerides (***lipolysis***).

9.7. Muscles – motility and energy

The most obvious distinction between plants and animals is animals' ***motility***, our ability to move around whenever we feel like it.

Scientists think that the basic mechanism of motility is similar in all complex cells. This mechanism and its evolutionary origins were referred to briefly in the section on the cytoskeleton in the biochemistry chapter, section 7.2.6.

Motility of large organisms requires muscles. There are three types of muscles:

- ***Skeletal muscles*** are attached to bones by tendons and contract to produce movement of the body. They are innervated voluntarily. (This is partly a question of definition. Even when they are innervated involuntarily, the innervation is said to be voluntary.)
- ***Cardiac muscles*** make the heart beat. In fact, the heart is pretty much a big muscle. The heart has its own clock, whose rate can be modified by the nervous system.
- ***Smooth muscles*** line the digestive and respiratory tracts, surround sphincters and control the opening of the iris of the eye, among other things. Their action is involuntary, triggered by hormones, the ANS and local conditions.

Let’s look at those in more detail.

9.7.1. Skeletal muscles

Skeletal muscles do not just make joints move. Muscles are rarely completely relaxed, but maintain ***muscle tone***, constantly contracting and relaxing by small amounts in order to stabilize joints and maintain balance. This is brought about by complex interactions with the nervous system.

During the development of skeletal muscles, many individual cells fuse together, usually retaining their nuclei and mitochondria. Multiple mitochondria are necessary in order to supply the great amounts of energy needed by muscles. Muscles which work more and contain more mitochondria are darker colored than those which contain fewer. Chicken legs serve to walk and are dark meat; chicken wings serve little purpose and are small and light-colored.

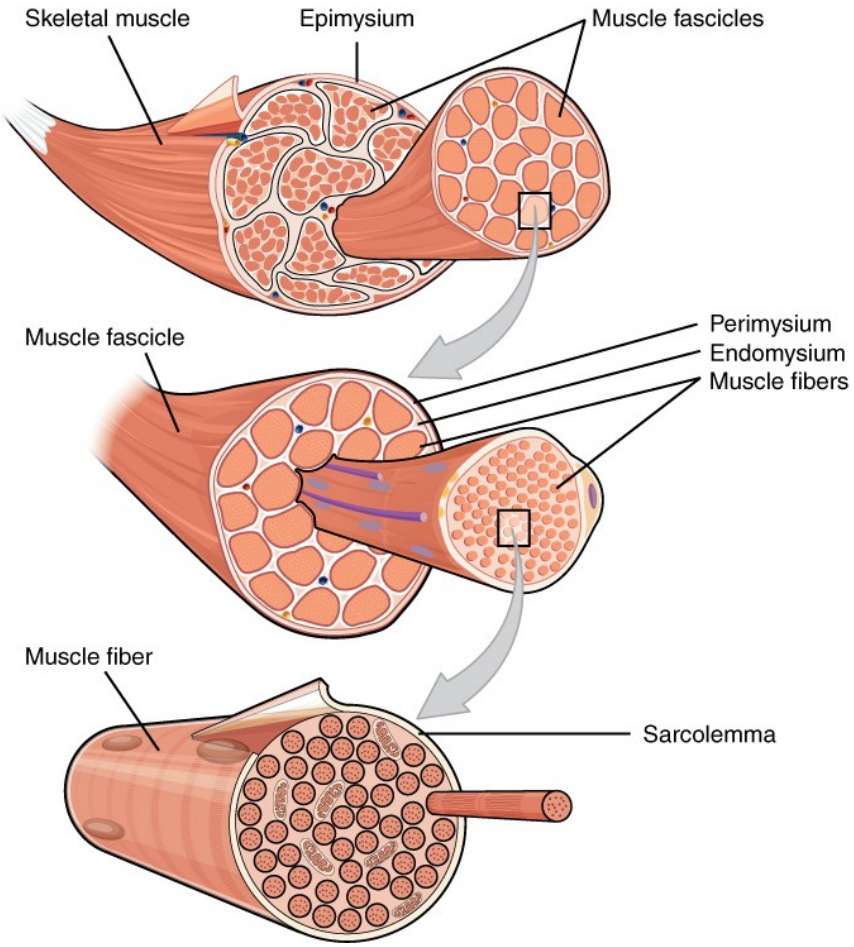


Figure 9.11: Three skeletal muscle structures, from Openstax.[112]

Looking from the outside in, muscle cells consist of numerous components, one nested within another, including three layers of connective tissue, or ***mysia*** (singular, ***mysium***).

- An outer layer, the ***epimysium***, holds the muscle together as it contracts and expands. It also connects to tendons.
- Inside the epimysium are bundles of cells called ***fascicles***, bounded by a second layer of connective tissue, the ***perimysium***.
- Inside each fascicle, the muscle cells themselves, called ***fibers***, are bundled in another layer of connective tissue, the ***endomysium***. The endomysium furnishes nutrition to the cells. Fibers can be quite long, up to 30 cm in the leg.
- The fibers in turn are bundles of elements, the ***myofibrils***, which run the length of the fiber.

In skeletal muscles, special groups of fibers called ***muscle spindles*** serve as stretch receptors which send information to the brain about changes in the length of the muscle. As such, they are part of ***proprioception***, the sense of the body's position and movement. They also contain motor neurons which can regulate muscular contraction. They can, for instance, unconsciously regulate the incremental muscular contraction necessary for holding a glass of water stationary as it is being filled.[113]

Within the fibers, skeletal muscle cell components have special names, beginning with "sarco" (Greek for "flesh"). The cell membrane is called the ***sarcolemma***; the cytoplasm, the ***sarcoplasm***; and the smooth endoplasmic reticulum, the ***sarcoplasmic reticulum*** (abbreviated ***SR***).

112 http://cnx.org/contents/FPtK1zmh@7.30:bfiqsxdB@3/Skeletal-Muscle.
113 O'Shea, 82.

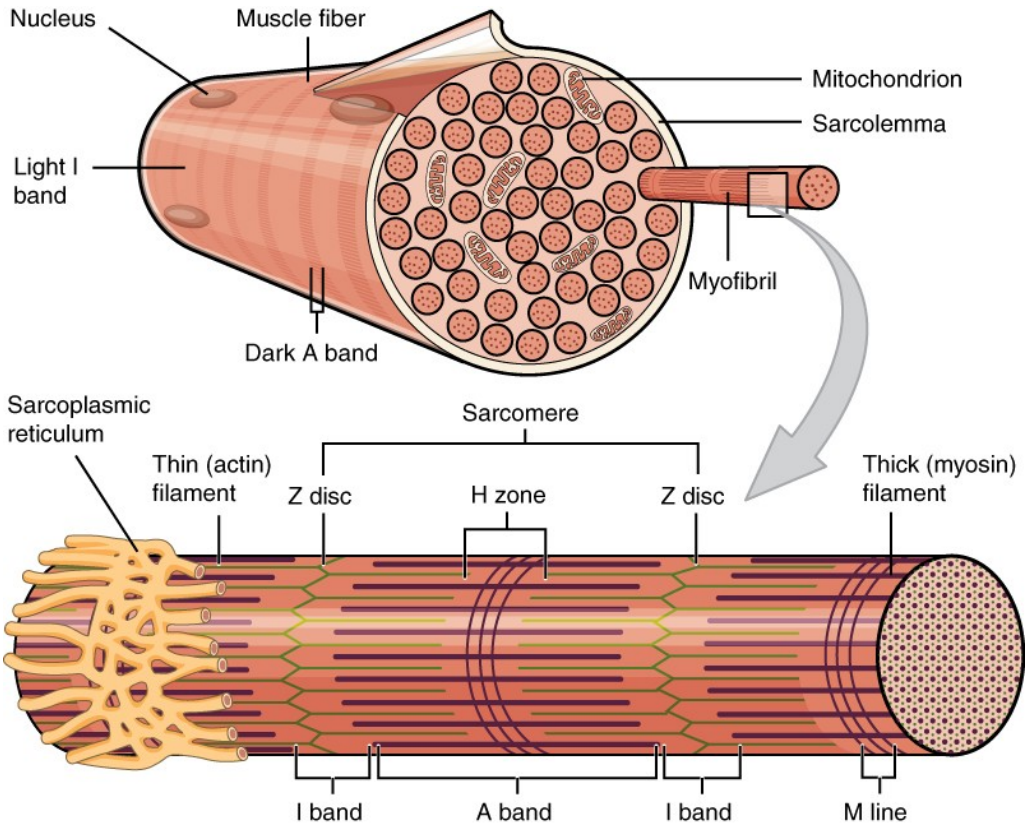


Figure 9.12: Muscle fibers, from Openstax.[114]

Skeletal muscles appear to be striated (or striped) because each myofibril is composed of a series of elements called ***sarcomeres***, which are where the action is. The sarcomeres are placed end to end along the myofibril and contain two types of filaments: thin filaments called ***actin*** and thicker ones called ***myosin***. The actin filaments are anchored to the ends of the sarcomeres (called ***Z disks*** or ***Z lines***) and do not meet in the middle. The myosin filaments are positioned at the mid-point of the sarcomere, called the ***M line***, and each end is bound to the nearest Z disc by elastic bands of the protein ***titin***, or ***connectin***, which act like springs, allowing the sarcomere to expand and contract while maintaining its form. The actin and myosin filaments overlap partially.

The myosin filaments are composed of multiple myosin molecules wound up together. Each molecule has a head on one end and this protrudes outward from the axis of the filament. Each head has a binding site for actin on the end and another one for ATP. In fact, the myosin head functions as an ATPase enzyme and can cleave ATP to recover the energy in it. The energy is then available for powering muscular contraction.

The actin filaments, consisting similarly of wound-up cables of actin molecules, contain binding sites for the myosin heads. When the muscle is relaxed, these sites are blocked by the protein ***tropomyosin***, which spirals around the actin and is in turn is bound with another protein, ***troponin***.

114 http://cnx.org/contents/FPtK1zmh@7.30:bfiqsxdB@3/Skeletal-Muscle

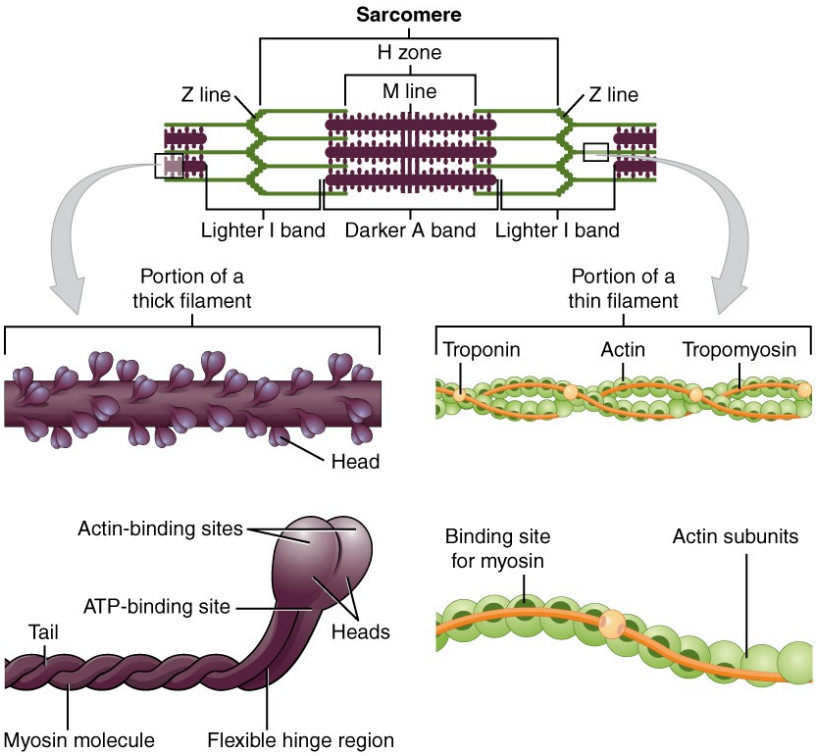


Figure 9.13: Thick and thin filaments, from Openstax College.[115]

The ***neuromuscular junction*** (***NMJ***) is the point at which the motor neuron from the peripheral nervous system innervates the fiber Each fiber is connected to one branch of a motor neuron axon[116]. If more than one fiber is connected to a neuron, the set of fibers for that neuron constitutes a ***motor unit***. This allows for precise innervation of muscle fibers: the smaller the motor unit, the more precise the control. In the thousands of muscle fibers which move our eyeballs, a motor unit is made up of only about six fibers, making for great precision of movement. In contrast, in our back or thigh muscles, a motor unit may include thousands of muscle fibers..

When the NMJ receives an action potential, it releases the neurotransmitter ***acetylcholine***, abbreviated ***Ach***. Reception of Ach on the fiber receptors brings about the propagation of an action potential along the sarcolemma and through so-called ***T tubules*** to the SR, which opens calcium channels and releases Ca++ into the cell.

In more detail, the Ach receptors contain Ach-gated cation channels which open when Ach binds to them. Since the cell is normally at a negative voltage relative to its surroundings, opening the channel lets positive ions flow through the membrane and into the cell, causing it to depolarize (become less negative). This causes the opening of voltage-gated sodium channels, so Na^{+} enters the cell and brings about an action potential. (For more information on action potentials, see the biochemistry chapter.) When the action potential crosses the T tubules and reaches the SR, the SR releases Ca^{++} ions into the cell. The Ca^{++} binds to the troponin, which in turn causes tropomyosin to re-configure in such a way that the myosin binding sites on the actin are unblocked.

115 http://cnx.org/contents/FPtK1zmh@7.30:bfiqsxdB@3/Skeletal-Muscle
116 An axon is the output arm of a neuron. This will be discussed in some detail in the neuroscience chapter.

What happens next is described by the ***sliding-filament model*** of muscular contraction. (Some details of this model are still hypothetical.)

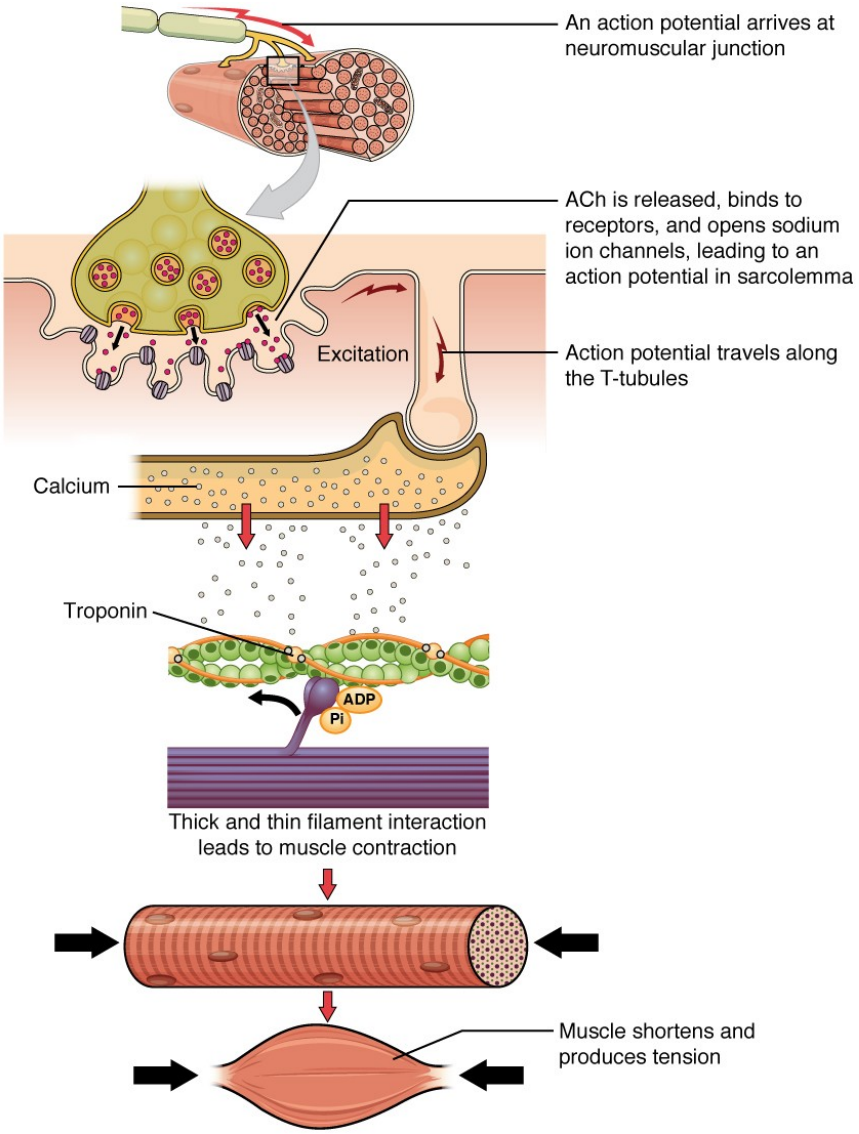


Figure 9.14: Muscle fiber contraction, from Openstax College[117]

1. The myosin heads already are bound to ADP and P_i. As the P_i is released, the myosin heads bind strongly to the actin, creating ***cross-bridges*** between the two filaments.
2. The myosin heads then execute the ***power stroke***: They bend toward the M-line (therefore in opposite directions on each side of the M-line), dragging the actin filaments with them (about 10 nm), increasing the overlap of the two filaments and compacting the sarcomere and, hence, the muscle.
3. The myosin head now can bend no more. ATP binds to it and causes it to detach from the actin. The head, using energy from conversion of the ATP to ADP and P_i, returns to its neutral position. It now is “cocked” and ready to launch another power stroke, As long as the binding sites on the actin are unblocked the cycle can repeat.

Eventually, the original Ca^{++} is stored back into the SR by a membrane pump. Then the cycle stops until more Ca^{++} unblocks the actin binding sites.

117 http://cnx.org/contents/FPtK1zmh@7.30:EtWWcJM-@3/Muscle-Fiber-Contraction-and-R

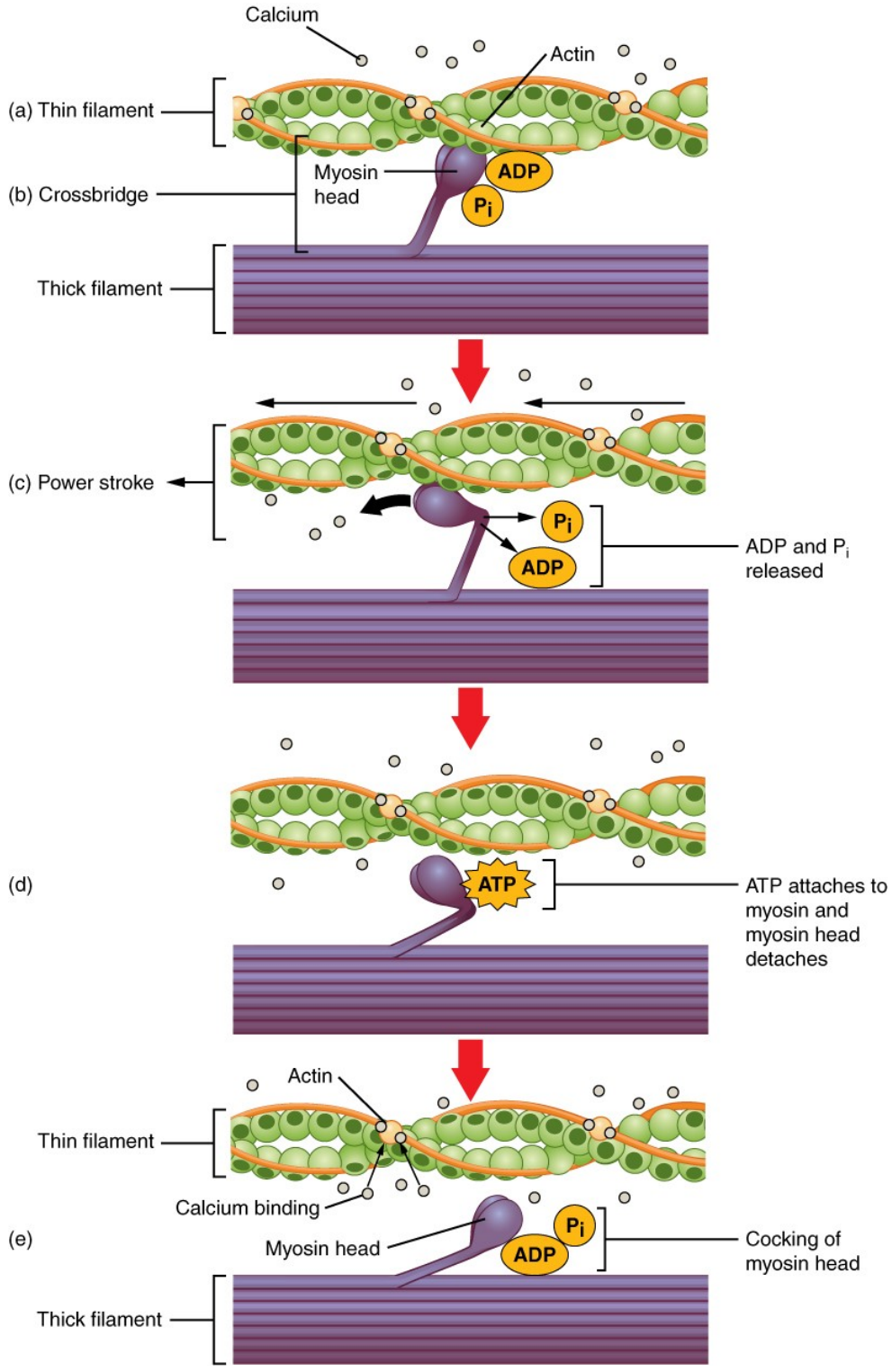


Figure 9.15: Sliding-filament steps, from Openstax College[118]

In the sarcomere, the myosin heads drag the actin filaments along, step by step, as they overlap and the sarcomere shortens.

118 http://cnx.org/contents/FPtK1zmh@7.30:EtWWcJM-@3/Muscle-Fiber-Contraction-and-R

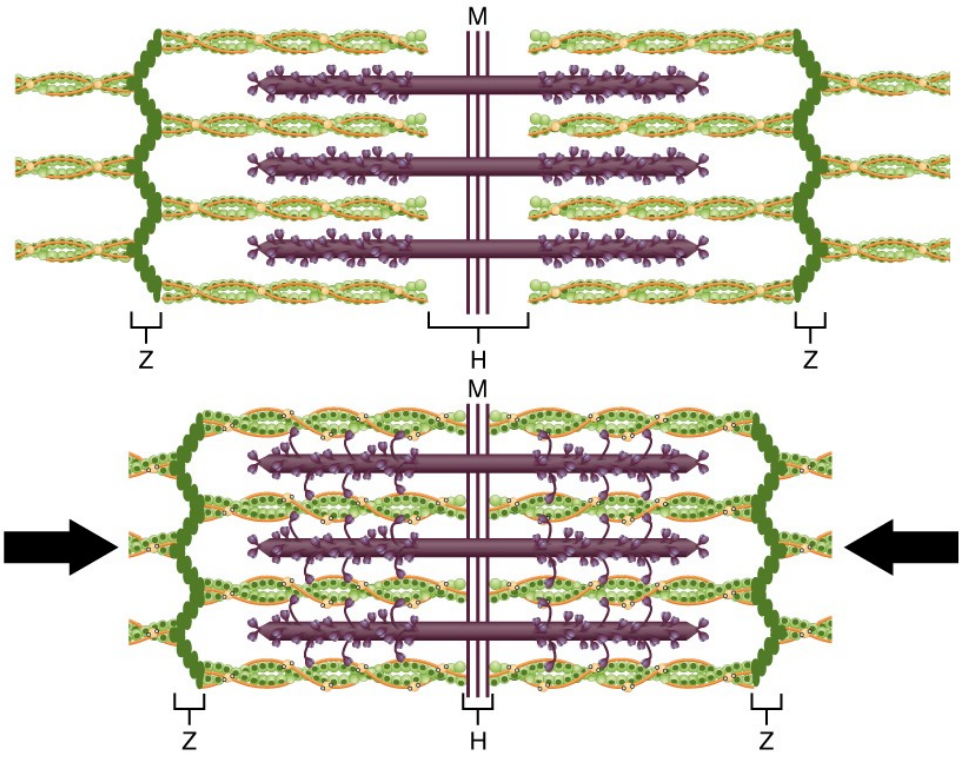


Figure 9.16: Sliding filaments, from Openstax College[119]

9.7.2. Energy for muscle action

Muscles need energy in order to function. This is why skeletal muscles have multiple mitochondria, energy factories. Energy can come from different sources and the muscles which use different sources differ in their type and function.

When a muscle is at rest, excess ATP produced may be transferred for storage to creatine phosphate (or ***phosphocreatine***) in the muscle. This molecule stores energy in its phosphate bonds and can give it back very quickly, but can not store more than about 15 seconds worth of energy. So it is a fast, but short-duration source of energy.

Energy can also be obtained without the need of oxygen by anaerobic respiration through the conversion of glucose into ATP by the process of glycolysis, which we have already considered in some detail. This procedure is slower and, as we have seen, relatively inefficient.

After this, if oxygen still is not available, fermentation, already discussed, can convert pyruvate into lactic acid with a small gain in energy.

If oxygen is present, then aerobic respiration (Krebs cycle + oxidative phosphorylation), which is very efficient, provides energy. But it does require a regular supply of oxygen and is slower than glycolysis. So intense energy needs by muscles will first be supplied by phosphocreatine and then by glycolysis.

When muscles need ATP from aerobic respiration but the oxygen supply is short, then there is what is called an ***oxygen debt***, which makes us breathe hard after an intense muscular effort.

9.7.3. Cardiac muscles

Heart muscles are striated like skeletal muscles, but are shorter and only have one nucleus per cell, although they still have many mitochondria. They are multi-branched and the branches are interconnected through intercalated discs. ***Gap junctions*** between fibers allow rapid transmission of action potentials for coordination of activity throughout the heart. The heart has its own clock and is not controlled by the nervous system.

Muscle contraction occurs through a sliding- filament scheme similar to the one in skeletal muscles. But cardiac muscles are autorhythmic: Their contractions are controlled by pacemaker cells within the heart

119 http://cnx.org/contents/FPtK1zmh@7.30:EtWWcJM-@3/Muscle-Fiber-Contraction-and-R

itself, although the ANS[120] may signal the heart to speed up or slow down. The pacemaker cells cause a group of cells, called a functional ***syncytium***, to auto-excite and create an action potential. This subject will be covered in the next chapter.

9.7.4. Smooth muscles

Smooth muscles are not striated and contain no sarcomeres. They occur in many organs and body structures, including the digestive, urinary, respiratory, circulatory and reproductive systems, so they are quite wide-spread. They are fusiform in shape – thick in the middle and tapered at the ends. Their action is involuntary, triggered by hormones, the ANS and local conditions.

They too contract through a system similar to the sliding-filament scheme of skeletal muscles, but with important differences. Smooth muscles possess no troponin-tropomyosin complex. In a process beginning with Ca^{++} ions binding to a protein called ***calmodulin***, the myosin heads are activated by being phosphorylated. The heads crawl along the actin thin filament, which is attached at its ends to dense bodies, the smooth-muscle equivalent of Z discs. But this pulls on a network of intermediate filaments, which in turn contracts the whole fiber along its length, causing it to get shorter as it swells up in the middle.

9.8. Photosynthesis – storage of solar energy by plants

We have seen how the body takes in energy and how it uses it. But before we can consume food to obtain the energy therein, that energy must have been stored there. This is the job of ***photosynthesis***. Photosynthesis employs energy from sunlight to produce organic molecules using water and carbon dioxide as raw materials – and with oxygen as a very important by-product. It is thus the opposite of respiration. As Nick Lane says[121]: “All our energy is a beam of sunlight set free from its captive state in food.”

Understanding photosynthesis requires that we consider another organelle, the ***chloroplast***.

9.8.1. Chloroplast structure

Chloroplasts are organelles, like the nucleus or mitochondria, which occur inside the cells of plants, algae and cyanobacteria. Like mitochondria, they contain their own simple form of DNA, because, like mitochondria, they probably originated as bacteria which moved into another cell, felt at home and stayed. Chloroplasts are surrounded by a double membrane enclosing a number of closed, disk-shaped membranes called ***thylakoids***, which are arranged in stacks, each of which is called a ***granum***. There is fluid inside all these spaces; that inside the membrane and surrounding the thylakoids is called the ***stroma***. Pigments called ***chlorophyll*** are embedded in the thylakoid membranes.

120 The autonomic nervous system.
121 Lane (2010), 63.

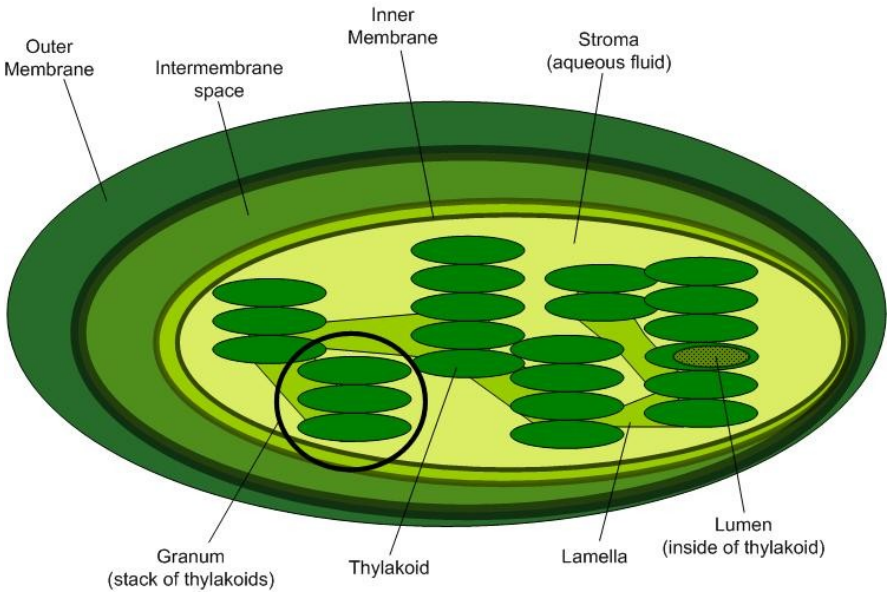


Figure 9.17: Chloroplast structure, from Wikimedia Commons

Photosynthesis takes place in two steps and two places: light reactions in the thylakoid membranes and the Calvin cycle in the stroma.

1. The ***light reactions*** (or light-dependent reactions) use energy from sunlight in two ways: to store energy as ATP; and to transfer electrons to form NADPH. Both are passed to the Calvin cycle.
2. The ***Calvin cycle*** uses the electrons and ATP plus CO_2 from the air to make glucose.

The light reactions thus furnish the energy and the fuel used by the Calvin cycle.

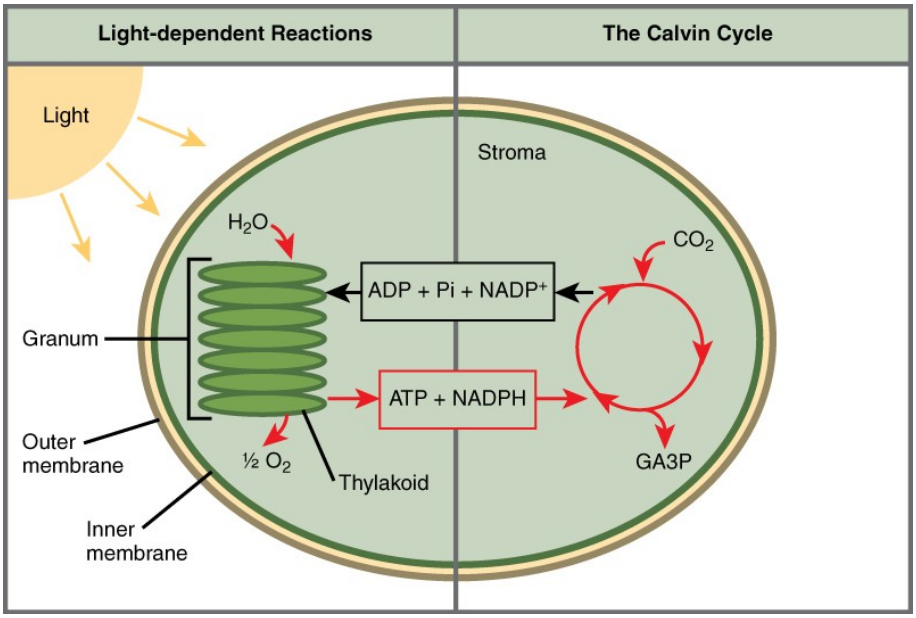


Figure 9.18: The two steps of photosynthesis, by Openstax College[122]

9.8.2. Light reactions

The light-reaction phase of photosynthesis is also called the ***Z-scheme***, but since the Z usually is shown lying on its side, it looks much more like an N-scheme. (Figure 9.19) Light reactions take place in three

122 http://cnx.org/resources/40d9ec867e76cf784a0c91b80652d4d16f5f2e02/Figure_08_01_06.jpg.

steps: (1) Photosystem II, (2) an electron transport chain and (3) Photosystem I.[123] Interestingly, the two photosystems seem to be more primitive schemes which existed separately.[124]

The photosystems (1) and (3) are all about the action of energy from sunlight on chlorophyll. In both photosystems, the energy from light “excites” an electron in chlorophyll by kicking it into a higher energy level. Although Photosystem II and Photosystem I are similar in operation, they differ in a number of ways. For one thing, their reaction centers contain different pigments: P680 in PII and P700 in PI. (The P numbers refer to the wavelength in nano-meters of maximum light sensitivity of each pigment.)

Since the most important form of chlorophyll, ***chlorophyll a***, absorbs red and blue light but reflects green, leaves are most often green. Other pigments may absorb light of other frequencies and so give different colors. Many such pigments, including chlorophyll a, chlorophyll b and carotenoids, are bound to proteins in the photosystem (collectively called the ***antenna complex***).[125] They absorb energy which pushes their outermost electron into an excited state called an ***exciton***, the term referring to the excited electron and the “hole” (ground-state energy shell) it normally occupies. One says the exciton is transferred when it is actually only energy which is transferred, and this is the essence of what is called an exciton. The energy is transferred from one antenna protein to the next as in a bucket brigade, until it reaches chlorophyll a in what is called the ***reaction center***. In this way, energy from light of different wavelengths can be harvested, extending the sensitivity range of the process. Only in the reaction center are excited electrons actually released so they can be passed to the next phase.

Many experiments and much discussion have taken place lately to explore how passage of exciton energy from one chromophore (light absorbing molecule) to another and on to the reaction center can take place with such efficiency and whether this capability requires or uses quantum effects (resembling Feynman’s phase integral technique) . It seems that the jury is still out.[126,127]

Let's consider the whole thing in sequence.

In PSII, (1) the excited electron is plucked off and sent "downhill" through the electron transport chain (2) to manufacture ATP. This leaves the chlorophyll from which the electron was ejected in a state of positive charge, so it wants another electron. The source of this electron is water, but the energy of one photon is inadequate to split a water molecule. So the chlorophyll picks up an electron from a sort of e^- buffer known as the ***oxygen-evolving complex*** (or ***water-splitting complex***). Simplifying some (a lot), one can consider that the e^- in the complex are associated with four manganese ions, one of which is requisitioned by chlorophyll each time it needs to replace the e^- it has lost to the ETC. For each electron removed, the Mn complex becomes more positive. When it reaches a charge of 4+, the Mn complex can seize upon 4 electrons from a water molecule:

$$[\text{Mn complex}]^{4+} + 2\ H_2O \rightarrow [\text{Mn complex}]^{0} + 4\ H^+ + O_2.$$

These are the protons pumped by the ETC to establish a gradient which will then be used to manufacture ATP from ADP + Pi. And this is the oxygen we breathe.

123 For historical reasons, photosynthesis II comes before photosynthesis I.
124 Hazen (2013), 162.
125 The light-dependent reactions. Khan Academy. https://www.khanacademy.org/science/ap-biology/cellular-energetics/photosynthesis/a/light-dependent-reactions.
126 Ball, Philipl “Is photosynthesis quantum-ish?”. Physics World, April 2018. https://physicsworld.com/a/is-photosynthesis-quantum-ish/.
127 Fassioli, F. et al. “Photosynthetic light harvesting: excitons, and coherence.” Journal of the Royal Society, March 2014. https://royalsocietypublishing.org/doi/10.1098/rsif.2013.0901

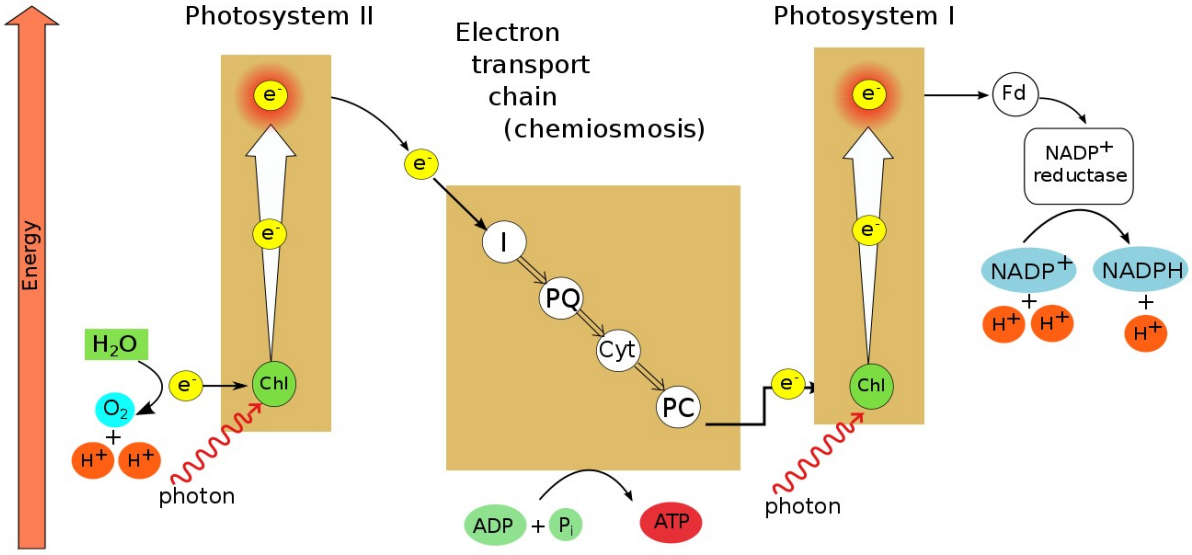


Figure 9.19: Photophosphorylation (Z-scheme), after Kratz[128]

All this takes place inside the thylakoid membrane.

Unfortunately, the oxygen-evolving process is not completely understood. If it were, it might enable us to extract hydrogen from water in an energy-efficient way, which could put an end to our energy problems.[129]

In step (2), the electron released by PII goes through ***photophosphorylation***, an electron transport chain similar to that in mitochondria, but now taking place in the thylakoid membrane of the chloroplast. At each step, some of the electron energy is used to pump protons across the thylakoid membrane. At the end of the chain, the electrochemical gradient of the protons across the membrane serves to turn ATP synthase which converts ADP into ATP by the process of ***chemiosmosis***. So at the end of this step, we have ATP and a free, but weak electron. The different processes of this chain are similar but not identical to those in mitochondria.

PI again uses solar energy to kick an electron up to higher energy where it is released, now replacing the lost electron by the one leaving the ETC. The electron released by PI has enough energy to go through a process which stores its energy on the electron carrier NADPH, a close relative of our old pal NADH. The solar energy is now stored in the NADPH and the ATP from the ETC and both move to the next step, the Calvin cycle.

In the light reactions, electrons and energy have different fates. Electrons from water wind up in NADPH; solar energy is transferred to ATP. So the overall effect of light reactions is to store solar energy in ATP for use by the plant or in the Calvin cycle, and to energize NADPH for the Calvin cycle. The complete chemical formula for the light reactions is the following.

$$2\ H_2O + 2\ NADP^+ + 3\ ADP + 3\ P_i + \text{light} \rightarrow 2\ NADPH + 2\ H^+ + 3\ ATP + O_2$$

9.8.3. The Calvin cycle

The second step of photosynthesis, the Calvin cycle, takes place in the stroma of the chloroplast. It takes in CO_2 and uses the chemical energy produced by the light reactions to make sugar molecules, usually glucose.

The Calvin cycles takes place in three stages, which are indicated in Figure 9.20.

128 Kratz (2009).
129 It could also completely shake up the world economic and political situation, but that is way beyond the scope of this document. But we can always hope.

In stage 1, ***carbon fixation***, the enzyme whose "much-needed nickname" is ***RuBisCO***[130], catalyzes the reaction of CO_2 and 5-carbon RuBP into a 6-carbon compound which immediately splits into two 3-carbon compounds called 3-PGA. Then, in the reduction step, ATP and NADPH from the light reaction photosystem I reduce 3-PGA to G3P. On each tour of the cycle, one G3P separates from the cycle and these molecules eventually (at the end of six tours of the cycle) form a carbohydrate molecule, usually glucose ($C_6H_{12}O_6$). The other G3P molecule and ATP regenerate RuBP, so the cycle can begin again. So it takes six tours of the Calvin cycle to convert CO_2 into glucose. The complete formula is therefore the following.

$$6\ CO_2 + 12\ (NADPH + H^+) \rightarrow C_6H_{12}O_6 + 12\ NADP^+ + 6\ H_2O$$

ignoring the energy from ATP going to ADP and P_i.

It is impossible to stress overly much the importance of these reactions. They are essential for life on Earth. Not only is our oxygen-rich atmosphere originally due to photosynthesis by cyanobacteria and stromatolites, the current maintenance of oxygen levels depends on it. And the very energy we live on, as we have seen, comes from the glucose made in the Calvin cycle.

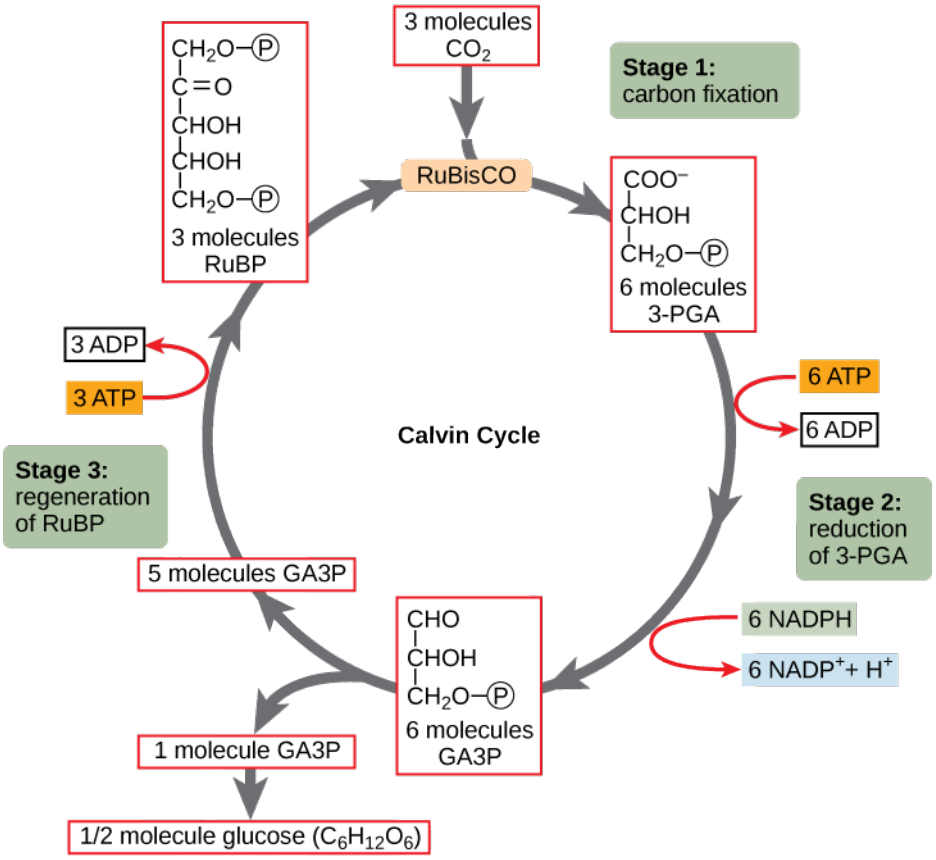


Figure 9.20: The Calvin cycle, from Openstax College[131]

This is worth repeating.

- Light reactions use the energy from sunlight to take in water and break it down into O_2, protons and electrons. The electrons are energized by light to go through chemiosmosis and form energy-rich products which are passed to the next step, the Calvin cycle.
- The Calvin cycles takes in CO_2 from the air and uses the energy-rich products of the light reactions to form glucose and prepare for the next tour of the cycle. This cycle depends on the enzyme RuBisCO, which therefore is essential to life on planet Earth.
- We and other animals eat the plants – and other animals which have eaten plants. After breakdown of food by digestion, the glucose originating in photosynthesis is used by cellular respiration to provide energy in the form of ATP which powers our muscles, our neurons and other metabolic functions. The waste from this conversion is CO_2, which goes back into the Calvin cycle

130 Kratz (2009), 197.
131 http://cnx.org/resources/820666f6f29a4fbee765e3dd678b10e87432b22e/Figure_08_03_02f.png.

Notice that CO_2 is produced as waste in cellular respiration, then taken in by the Calvin cycle to be reconverted into glucose and O_2. This process **must** remain in equilibrium.

Now for a word about evolution. Historically – and currently – other organisms used not water but ferrous iron or H_2S as electron sources. It is currently thought (without proof) that first there was only one photosystem, the one we call PI, and it was used by bacteria to derive electrons from H_2S.[132] Later, genetic duplication and mutation occurred and some PI evolved into PII, although the two were neither linked nor used at once. As for the oxygen-evolving complex, bacteria used Mn atoms to protect themselves against UV radiation. Somehow or other, Mn atoms formed non-organic crystals, perhaps in hydrothermal vents, which at some point were joined with PII. All that was left was for PII to be associated with PI and *voila*!

132 Lane (2010), 83-87.