Basic Biochemistry

University notes for chemical engineers

András Szarka
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To my nephew, Peter
Preface

This booklet, as the title tells us, does not aim at giving comprehensive biochemistry knowledge. Instead it would like to give a short overview of the biochemical pathways and their connections. Hence the reader can read this booklet as an essential or an extraction of classical detailed handbooks. More details can be found in the handbooks in the list of reference.

András Szarka

Budapest, 13th of December 2012.
Chapter 1. Basic chemical and biological principles

The unity and diversity of organisms become apparent even at the cellular level. The smallest organisms consist of single cells and are microscopic. Larger, multicellular organisms contain many different types of cells, which vary in size, shape, and specialized function. Despite these obvious differences, all cells of the simplest and most complex organisms share certain fundamental properties, which can be seen at the biochemical level.

1. 1.1. Cells are the structural and functional units of all living organisms

Cells of all kinds share certain structural features (Fig. 1.1 and 1.2). The plasma membrane defines the periphery of the cell, separating its contents from the surroundings.

It is composed of lipid and protein molecules that form a thin, tough, flexible, hydrophobic barrier around the cell. The membrane is a barrier to the free passage of inorganic ions and most other charged or polar compounds.

Transport proteins in the plasma membrane allow the passage of certain ions and molecules. Receptor proteins transmit signals into the cell, and membrane enzymes participate in some reaction pathways. Because the individual lipids and proteins of the plasma membrane are not covalently linked, the entire structure is remarkably flexible, allowing changes in the shape and size of the cell. As a cell grows, newly made lipid and protein molecules are inserted into its plasma membrane. Cell division produces two cells, each with its own membrane. This growth and cell division (fission) occurs without loss of membrane integrity.

Since living organisms are so complex large communities of biologists have become dedicated to studying different aspects of the same model organism.

1.1. 1.1.1. Prokaryotes

Prokaryotes (cells without a distinct nucleus) are biochemically the most diverse organisms and include species that can obtain all their energy and nutrients from inorganic chemical sources. Most bacteria and archaea are small unicellular organisms with compact genomes comprising 1000-4000 genes.

In the enormously varied world of bacteria, the spotlight of molecular biology has for a long time focused intensely on just one species: Escherichia coli, or E. coli (Fig. 1.1.).

Figure 1.1. E. coli and its genome - http://www.ncbi.nlm.nih.gov/books/NBK26866/figure/A66/?report=objectonly
This small, rod-shaped eubacterial cell normally lives in the gut of humans and other vertebrates, but it can be grown easily in a simple nutrient broth in a culture bottle. Evolution has optimized it to cope with variable chemical conditions and to reproduce rapidly. Its genetic instructions are contained in a single, circular molecule of DNA that is 4,639,221 nucleotide-pairs long, and it makes approximately 4300 different kinds of proteins (Fig.1.1). Most of our understanding of the fundamental mechanisms of life for example, how cells replicate their DNA to pass on the genetic instructions to their progeny, or how they decode the instructions represented in the DNA to direct the synthesis of specific proteins has come from studies of E. coli. The basic genetic mechanisms have turned out to be highly conserved throughout evolution: these mechanisms are therefore essentially the same in our own cells as in E. coli.

1.2. 1.1.2. Eukaryotes

Eukaryotic cells, in general, are bigger and more elaborate than prokaryotic cells, and their genomes are bigger and more elaborate, too. The greater size is accompanied by radical differences in cell structure and function (Fig. 1.2).

Figure 1.2. The major features of eukaryotic cells - http://www.ncbi.nlm.nih.gov/books/NBK26909/figure/A70/?report=objectonly
By definition, eukaryotic cells keep their DNA in a separate internal compartment, the *nucleus*. The DNA is separated from the cytoplasm by the nuclear envelope, which consists of a double layer of membrane. Their cells are, typically, 10 times bigger in linear dimension, and 1000 times larger in volume. They have a *cytoskeleton* a system of protein filaments crisscrossing the cytoplasm and forming, together with the many proteins that attach to them, a system of girders, ropes, and motors that gives the cell mechanical strength, controls its shape, and drives and guides its movements (Fig. 1.2).

The nuclear envelope is only one part of an elaborate set of *internal membranes*, each structurally similar to the plasma membrane and enclosing different types of spaces inside the cell, many of them involved in processes related to digestion and secretion. Lacking the tough cell wall of most bacteria, animal cells and the free-living eukaryotic cells called protozoa can change their shape rapidly and engulf other cells and small objects by phagocytosis (Fig. 1.3).

**Figure 1.3. Phagocytosis**

http://www.ncbi.nlm.nih.gov/books/NBK26909/figure/A71/?report=objectonly

Almost all eukaryotic cells also contain *mitochondria* (Fig. 1.2 and 1.4). These are small bodies in the cytoplasm, enclosed by a double layer of membrane, that take up oxygen and harness energy from the *oxidation of food molecules* such as sugars to produce most of the ATP that powers the cell's activities.
Mitochondria originated from free-living oxygen-metabolizing (aerobic) eubacteria that were engulfed by an ancestral eukaryotic cell that could otherwise make no such use of oxygen (that is, was anaerobic) (Fig. 1.5). This partnership between a primitive anaerobic eukaryotic predator cell and an aerobic bacterial cell is thought to have been established about 1.5 billion years ago, when the Earth's atmosphere first became rich in oxygen.
Many eukaryotic cells specifically, those of plants and algae also contain another class of small membrane-bounded organelles somewhat similar to mitochondria: the chloroplasts (Fig. 1.6). Chloroplasts perform photosynthesis, using the energy of sunlight to synthesize carbohydrates from atmospheric carbon dioxide and water, and deliver the products to the host cell as food. Like mitochondria, chloroplasts have their own genome and almost certainly originated as symbiotic photosynthetic bacteria, acquired by cells that already possessed mitochondria (Fig. 1.7).

Figure 1.6. The chloroplasts

![Chloroplast diagram](http://www.ncbi.nlm.nih.gov/books/NBK26909/figure/A77/?report=objectonly)

Figure 1.7. The origin of chloroplasts - http://www.ncbi.nlm.nih.gov/books/NBK26909/figure/A77/?report=objectonly
Fungi represent yet another eukaryotic way of life. Fungal cells, like animal cells, possess mitochondria but not chloroplasts. In contrast with animal cells and protozoa, they have a tough outer wall that limits their ability to move rapidly or to swallow up other cells. Fungi, it seems, have turned from hunters into scavengers: other cells secrete nutrient molecules or release them upon death, and fungi feed on these leavings performing whatever digestion is necessary extracellularly, by secreting digestive enzymes to the exterior.

The molecular and genetic complexity of eukaryotes is daunting. Even more than for prokaryotes, biologists need to concentrate their limited resources on a few selected model organisms to fathom this complexity.

To analyze the internal workings of the eukaryotic cell, without being distracted by the additional problems of multicellular development, it makes sense to use a species that is unicellular and as simple as possible. The popular choice for this role of minimal model eukaryote has been the yeast *Saccharomyces cerevisiae* (Fig. 1.8) the same species that is used by brewers of beer and bakers of bread.

Figure 1.8. *Saccharomyces cerevisiae* or baker’s yeast - http://www.ncbi.nlm.nih.gov/books/NBK26909/figure/A89/?report=objectonly
2. 1.2. Basic cell chemistry

The incredible diversity of living forms, their seemingly purposeful behaviour, and their ability to grow and reproduce all seem to set them apart from the world of solids, liquids, and gases that chemistry normally describes. Until the nineteenth century it was widely accepted that animals contained a Vital Force that was uniquely responsible for their distinctive properties.

We now know there is nothing in living organisms that disobeys chemical and physical laws. However, the chemistry of life is indeed of a special kind.

First, it is based overwhelmingly on carbon compounds, whose study is therefore known as organic chemistry.

Second, cells are 70 percent water, and life depends almost exclusively on chemical reactions that take place in aqueous solution.

Third, and most importantly, cell chemistry is enormously complex:

Even the simplest cell is vastly more complicated in its chemistry than any other chemical system known. Although cells contain a variety of small carbon-containing molecules, most of the carbon atoms in cells are incorporated into enormous polymeric molecules chains of chemical subunits linked end-to-end. It is the unique properties of these macromolecules that enable cells and organisms to grow and reproduce as well as to do all the other things that are characteristic of life.

2.1. 1.2.1. Cells Are Made From a Few Types of Atoms

Atoms are so small that it is hard to imagine their size. An individual carbon atom is roughly 0.2 nm in diameter, so that it would take about 5 million of them, laid out in a straight line, to span a millimeter. One proton or neutron weighs approximately $1/(6 \times 10^{23})$ gram, so one gram of hydrogen contains $6 \times 10^{23}$ atoms. This huge number ($6 \times 10^{23}$, called Avogadro's number) is the key scale factor describing the relationship between everyday quantities and quantities measured in terms of individual atoms or molecules. If a substance has a molecular weight of X, $6 \times 10^{23}$ molecules of it will have a mass of X grams. This quantity is called one mole of the substance.

There are 92 naturally occurring elements, each differing from the others in the number of protons and electrons in its atoms. Living organisms, however, are made of only a small selection of these elements, four of which carbon (C), hydrogen (H), nitrogen (N), and oxygen (O) make up 96.5% of an organism's weight. This composition differs markedly from that of the non-living inorganic environment and is evidence of a distinctive type of chemistry.

2.2. 1.2.2. Chemical bonds

Protons and neutrons are welded tightly to one another in the nucleus and change partners only under extreme conditions during radioactive decay, for example, or in the interior of the sun or of a nuclear reactor. In living tissues, it is only the electrons of an atom that undergo rearrangements. They form the exterior of an atom and specify the rules of chemistry by which atoms combine to form molecules.

The electrons closest on average to the positive nucleus are attracted most strongly to it and occupy the innermost, most tightly bound shell. This shell can hold a maximum of two electrons. The second shell is farther away from the nucleus, and its electrons are less tightly bound. This second shell can hold up to eight electrons. The third shell contains electrons that are even less tightly bound; it can also hold up to eight electrons. The fourth and fifth shells can hold 18 electrons each. Atoms with more than four shells are very rare in biological molecules.

The electron arrangement of an atom is most stable when all the electrons are in the most tightly bound states that are possible for them that is, when they occupy the innermost shells. Therefore, with certain exceptions in the larger atoms, the electrons of an atom fill the orbitals in order the first shell before the second, the second before the third, and so on. An atom whose outermost shell is entirely filled with electrons is especially stable and therefore chemically unreactive. Atoms found in living tissues all have incomplete outer electron shells and are therefore able to donate, accept, or share electrons with each other to form both molecules and ions (Fig. 1.9).
Figure 1.9. Filled and unfilled electron shells in some common elements - http://www.ncbi.nlm.nih.gov/books/NBK26883/figure/A172/?report=objectonly

<table>
<thead>
<tr>
<th>Element</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
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<tr>
<td>Hydrogen</td>
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<td><img src="image2" alt="Unfilled shell" /></td>
<td><img src="image3" alt="Filled shell" /></td>
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<tr>
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<td>Nitrogen</td>
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<tr>
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<tr>
<td>Potassium</td>
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<tr>
<td>Calcium</td>
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</table>

Because an unfilled electron shell is less stable than a filled one, atoms with incomplete outer shells have a strong tendency to interact with other atoms in a way that causes them to either gain or lose enough electrons to achieve a completed outermost shell. This electron exchange can be achieved either by transferring electrons from one atom to another or by sharing electrons between two atoms. These two strategies generate two types of chemical bonds between atoms: an ionic bond is formed when electrons are donated by one atom to another, whereas a covalent bond is formed when two atoms share a pair of electrons (Fig. 1.10).

Figure 1.10. Comparison of covalent and ionic bonds - http://www.ncbi.nlm.nih.gov/books/NBK26883/figure/A173/?report=objectonly
Often, the pair of electrons is shared unequally, with a partial transfer between the atoms. This intermediate strategy results in a polar covalent bond.

Most covalent bonds involve the sharing of two electrons, one donated by each participating atom; these are called single bonds. Some covalent bonds, however, involve the sharing of more than one pair of electrons. Four electrons can be shared, for example, two coming from each participating atom; such a bond is called a double bond.

2.3. 1.2.3. Water, the most abundant part of cells

As it was mentioned earlier, water accounts for about 70% of a cell's weight, and most intracellular reactions occur in an aqueous environment. In each water molecule (H₂O), the two H atoms are linked to the O atom by covalent bonds (Fig. 1.11). The two bonds are highly polar because the O is strongly attractive for electrons, whereas the H is only weakly attractive.

Consequently, there is unequal distribution of electrons in a water molecule, with a preponderance of positive charge on the two H atoms and of negative charge on the O. When a positively charged region of one water molecule (that is, one of its H atoms) comes close to a negatively charged region (that is, the O) of a second water molecule, the electrical attraction between them can result in a weak bond called a hydrogen bond.

Figure 1.11. Three representations of a water molecule - http://www.ncbi.nlm.nih.gov/books/NBK26883/figure/A185/?report=objectonly
When the polar molecule becomes surrounded by water molecules, the proton is attracted to the partial negative charge on the O atom of an adjacent water molecule and can dissociate from its original partner to associate instead with the oxygen atoms of the water molecule to generate a hydronium ion (H$_3$O$^+$) (Fig. 1.12, panel A). The reverse reaction also takes place very readily, so one has to imagine an equilibrium state in which billions of protons are constantly flitting to and from one molecule in the solution to another.

Substances that release protons to form H$_3$O$^+$ when they dissolve in water are termed acids. The higher the concentration of H$_3$O$^+$, the more acidic the solution is. H$_3$O$^+$ is present even in pure water, at a concentration of 10$^{-7}$ M, as a result of the movement of protons from one water molecule to another (Fig. 1.12, panel B).

By tradition, the H$_3$O$^+$ concentration is usually referred to as the H$^+$ concentration, even though most H$^+$ in an aqueous solution is present as H$_3$O$^+$. To avoid the use of unwieldy numbers, the concentration of H$^+$ is expressed using a logarithmic scale called the pH scale (Fig. 1.13). Pure water has a pH of 7.0.
Because the proton of a hydronium ion can be passed readily to many types of molecules in cells, altering their character, the concentration of H$_3$O$^+$ inside a cell (the acidity) must be closely regulated. Molecules that can give up protons will do so more readily if the concentration of H$_3$O$^+$ in solution is low and will tend to receive them back if the concentration in solution is high.

The opposite of an acid is a base. Just as the defining property of an acid is that it donates protons to a water molecule so as to raise the concentration of H$_3$O$^+$ ions, the defining property of a base is that it raises the concentration of hydroxyl (OH$^-$) ions which are formed by removal of a proton from a water molecule.

**2.4. 1.2.4. Four types of non-covalent interactions**

In aqueous solutions, covalent bonds are 10 to 100 times stronger than the other attractive forces between atoms, allowing their connections to define the boundaries of one molecule from another. But much of biology depends on the specific binding of different molecules to each other. This binding is mediated by a group of non-covalent attractions that are individually quite weak, but whose bond energies can sum to create an effective force between two separate molecules.

Because of their fundamental importance in all biological systems, we shall summarize their properties:

1. **Ionic bonds.** These are purely electrostatic attractions between oppositely charged atoms. These forces are quite strong in the absence of water. However, the polar water molecules cluster around both fully
charged ions and polar molecules that contain permanent dipoles (Fig. 1.14). This greatly reduces the potential attractiveness of these charged species for each other.

Figure 1.14. How the dipoles on water molecules orient to reduce the affinity of oppositely charged ions or polar groups for each other - http://www.ncbi.nlm.nih.gov/books/NBK26883/figure/A193/?report=objectonly

2. **Hydrogen bonds.** The structure of a typical hydrogen bond is illustrated in Fig.1.15. This bond represents a special form of polar interaction in which an electropositive hydrogen atom is partially shared by two electronegative atoms. Its hydrogen can be viewed as a proton that has partially dissociated from a donor atom, allowing it to be shared by a second acceptor atom. Unlike a typical electrostatic interaction, this bond is highly directional being strongest when a straight line can be drawn between all three of the involved atoms. As already discussed, water weakens these bonds by forming competing hydrogen-bond interactions with the involved molecules.

Figure 1.15. Hydrogen bonds - http://www.ncbi.nlm.nih.gov/books/NBK26883/figure/A195/?report=objectonly
3. van der Waals attractions. The electron cloud around any nonpolar atom will fluctuate, producing a flickering dipole. Such dipoles will transiently induce an oppositely polarized flickering dipole in a nearby atom. This interaction generates an attraction between atoms that is very weak. But since many atoms can be simultaneously in contact when two surfaces fit closely, the net result is often significant. These so-called van der Waals attractions are not weakened by water.

4. The fourth effect that can play an important part in bringing molecules together in water is a hydrophobic force. This force is caused by a pushing of nonpolar surfaces out of the hydrogen-bonded water network, where they would physically interfere with the highly favourable interactions between water molecules. Because bringing two nonpolar surfaces together reduces their contact with water, the force is a rather nonspecific one.

### 2.5. 1.2.5. A cell is formed from carbon compounds

If we disregard water, nearly all the molecules in a cell are based on carbon.

Carbon is outstanding among all the elements in its ability to form large molecules. Because it is small and has four electrons and four vacancies in its outermost shell, a carbon atom can form four covalent bonds with other atoms. Most important, one carbon atom can join to other carbon atoms through highly stable covalent C-C bonds to form chains and rings and hence generate large and complex molecules with no obvious upper limit to their size. The small and large carbon compounds made by cells are called organic molecules.

Certain combinations of atoms, such as the methyl (-CH3), hydroxyl (-OH), carboxyl (-COOH), carbonyl (-C=O), phosphate (-PO₃⁻), and amino (-NH₂) groups, occur repeatedly in organic molecules. Each such chemical group has distinct chemical and physical properties that influence the behaviour of the molecule in which the group occurs.

The small organic molecules of the cell are carbon-based compounds that have molecular weights in the range 100 to 1000 and contain up to 30 or so carbon atoms. They are usually found free in solution and have many different fates.

Some are used as monomer subunits to construct the giant polymeric macromolecules the proteins, nucleic acids, and large polysaccharides of the cell. Others act as energy sources and are broken down and transformed into other small molecules in a maze of intracellular metabolic pathways.

Many small molecules have more than one role in the cell for example, acting both as a potential subunit for a macromolecule and as an energy source.

All organic molecules are synthesized from and are broken down into the same set of simple compounds. Cells contain four major families of small organic molecules: the sugars, the fatty acids, the amino acids, and the nucleotides(Fig. 1.16). Although many compounds present in cells do not fit into these categories, these four families of small organic molecules, together with the macromolecules made by linking them into long chains, account for a large fraction of cell mass.

**Figure 1.16. The four main families of small organic molecules in cells** - [http://www.ncbi.nlm.nih.gov/books/NBK26883/figure/A202/?report=objectonly](http://www.ncbi.nlm.nih.gov/books/NBK26883/figure/A202/?report=objectonly)
Basic chemical and biological principles

**building blocks of the cell**
- SUGARS
- FATTY ACIDS
- AMINO ACIDS
- NUCLEOTIDES

**larger units of the cell**
- POLYSACCHARIDES
- FATS, LIPIDS, MEMBRANES
- PROTEINS
- NUCLEIC ACIDS
Chapter 2. Enzymes

1. 2.1. The catalysed reactions

The chemical reactions that a cell carries out would normally occur only at temperatures that are much higher than those existing inside cells. For this reason, each reaction requires a specific boost in chemical reactivity. This requirement is crucial, because it allows each reaction to be controlled by the cell. The control is exerted through the specialized proteins called enzymes, each of which accelerates, or catalyses, just one of the many possible kinds of reactions that a particular molecule might undergo. Enzyme-catalysed reactions are usually connected in series, so that the product of one reaction becomes the starting material, or substrate, for the next (Fig. 2.1).

Figure 2.1. A set of enzyme-catalysed reactions generates a metabolic pathway - http://www.ncbi.nlm.nih.gov/books/NBK26838/figure/A232/?report=objectonly

These long linear reaction pathways are in turn linked to one another, forming a maze of interconnected reactions that enable the cell to survive, grow, and reproduce (Fig. 2.2).

Figure 2.2. Some of the metabolic pathways and their interconnections in a typical cell - http://www.ncbi.nlm.nih.gov/books/NBK26838/figure/A233/?report=objectonly
Two opposing streams of chemical reactions occur in cells: (1) the catabolic pathways break down foodstuffs into smaller molecules, thereby generating both a useful form of energy for the cell and some of the small molecules that the cell needs as building blocks, and (2) the anabolic, or biosynthetic, pathways use the energy harnessed by catabolism to drive the synthesis of the many other molecules that form the cell. Together these two sets of reactions constitute the metabolism of the cell (Fig.2.3).
All biological macromolecules are much less thermodynamically stable than their monomeric subunits, yet they are kinetically stable: their uncatalysed breakdown occurs so slowly (over years rather than seconds) that, on a time scale that matters for the organism, these molecules are stable. Virtually every chemical reaction in a cell occurs at a significant rate only because of the presence of enzymes, biocatalysts that, like all other catalysts, greatly enhance the rate of specific chemical reactions without being consumed in the process.

The path from reactant(s) to product(s) almost invariably involves an energy barrier, called the activation barrier (Fig. 2.4), that must be surmounted for any reaction to proceed.
The breaking of existing bonds and formation of new ones generally requires, first, the distortion of the existing bonds, creating a transition state of higher free energy than either reactant or product. The highest point in the reaction coordinate diagram represents the transition state, and the difference in energy between the reactant in its ground state and its transition state is the activation energy, $\Delta G$.

2. 2.2. Most enzymes are proteins

With the exception of a small group of catalytic RNA molecules, all enzymes are proteins. Their catalytic activity depends on the integrity of their native protein conformation. If an enzyme is denatured or dissociated into its subunits, catalytic activity is usually lost. If an enzyme is broken down into its component amino acids, its catalytic activity is always destroyed. Thus the primary, secondary, tertiary, and quaternary structures of protein enzymes are essential to their catalytic activity.

Enzymes, like other proteins, have molecular weights ranging from about 12,000 to more than 1 million. Some enzymes require no chemical groups for activity other than their amino acid residues. Others require an additional chemical component called a cofactor, either one or more inorganic ions, such as Fe$^{2+}$, Mg$^{2+}$, Mn$^{2+}$, or Zn$^{2+}$, or a complex organic or metalloorganic molecule called a coenzyme. Coenzymes act as transient carriers of specific functional groups. Most are derived from vitamins, organic nutrients required in small amounts in the diet. Some enzymes require both a coenzyme and one or more metal ions for activity. A coenzyme or metal ion
that is very tightly or even covalently **bound to the enzyme protein** is called a **prosthetic group**. A complete, catalytically active enzyme together with its bound coenzyme and/or metal ions is called a **holoenzyme**.

The **protein part** of such an enzyme is called the **apoenzyme** or apoprotein. Finally, some enzyme proteins are modified covalently by phosphorylation, glycosylation, and other processes. Many of these alterations are involved in the **regulation of enzyme activity**.

### 3. 2.3. Enzymes are classified by the reactions they catalyse

Many enzymes have been named by adding the suffix “-ase” to the name of their substrate or to a word or phrase describing their activity. Thus urease catalyses hydrolysis of urea, and DNA polymerase catalyses the polymerization of nucleotides to form DNA. Other enzymes were named by their discoverers for a broad function, before the specific reaction catalysed was known.

Because of such ambiguities, and the ever-increasing number of newly discovered enzymes, biochemists, by international agreement, have adopted a system for naming and classifying enzymes.

This system divides enzymes into six classes, each with subclasses, based on the type of reaction catalysed (Table 2.1). Each enzyme is assigned a four-part classification number and a systematic name, which identifies the reaction it catalyses.

**Table 2.1. International classification of enzymes**

<table>
<thead>
<tr>
<th>Class no.</th>
<th>Class name</th>
<th>Type of catalysed reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oxidoreductases</td>
<td>Transfer of electrons (hydride ions or H atoms)</td>
</tr>
<tr>
<td>2</td>
<td>Transferases</td>
<td>Group transfer reactions</td>
</tr>
<tr>
<td>3</td>
<td>Hydrolases</td>
<td>Hydrolysis reactions (transfer of functional groups to water)</td>
</tr>
<tr>
<td>4</td>
<td>Lyases</td>
<td>Addition of groups to double bonds, or formation of double bonds by removal of groups</td>
</tr>
<tr>
<td>5</td>
<td>Isomerases</td>
<td>Transfer of groups within molecules to yield isomeric forms</td>
</tr>
<tr>
<td>6</td>
<td>Ligases</td>
<td>Formation of C-C, C-S, C-O, and C-N bonds by condensation reactions coupled to cleavage of ATP or similar cofactor</td>
</tr>
</tbody>
</table>

### 4. 2.4. How enzymes work

The enzymatic catalysis of reactions is essential to living systems. As it was stated earlier under biologically relevant conditions, uncatalysed reactions tend to be slow. Most biological molecules are quite stable in the neutral pH, mild temperature, and aqueous environment inside the cells. Furthermore, many common chemical processes are unfavourable or unlikely in the cellular environment, such as the transient formation of unstable charged intermediates or the collision of two or more molecules in the precise orientation required for reaction. Reactions required to digest food, send nerve signals, or contract a muscle simply do not occur at a useful rate without catalysis.

An enzyme circumvents these problems by providing a specific environment within which a given reaction can occur more rapidly. The distinguishing feature of an enzyme-catalysed reaction is that it takes place within the confines of a pocket on the enzyme called the **active site**. The molecule that is bound in the active site and acted upon by the enzyme is called the **substrate** (Fig. 2.5) encloses a substrate, sequestering it completely from solution. The enzyme-substrate complex is central to the action of enzymes. It is also the starting point for
mathematical treatments that define the kinetic behaviour of enzyme-catalysed reactions and for theoretical descriptions of enzyme mechanisms.

Figure 2.5. How enzymes work
http://www.ncbi.nlm.nih.gov/books/NBK26838/figure/A250/?report=objectonly

4.1. 2.4.1. Enzymes Affect Reaction Rates, Not Equilibria

A simple enzymatic reaction might be written:

\[ E + S \leftrightarrow ES \leftrightarrow EP \leftrightarrow E + P \]  

(2.1)

where E, S, and P represent the enzyme, substrate, and product; ES and EP are transient complexes of the enzyme with the substrate and with the product. To understand catalysis, we must first appreciate the important distinction between reaction equilibria and reaction rates. The function of a catalyst is to increase the rate of a reaction. Catalysts do not affect reaction equilibria. Any reaction, such as \( S \leftrightarrow P \), can be described by a reaction coordinate diagram (Fig. 2.4), a picture of the energy changes during the reaction. The equilibrium between S (X) and P (Y) reflects the difference in the free energies of their ground states. In the example shown in Fig. 2.4, the free energy of the ground state of P is lower than that of S, so \( \Delta G \) for the reaction is negative and the equilibrium favours P. The position and direction of equilibrium are not affected by any catalyst.

A favourable equilibrium does not mean that the \( S \rightarrow P \) conversion will occur at a detectable rate. The rate of a reaction is dependent on an entirely different parameter. There is an energy barrier between S and P: the energy required for alignment of reacting groups, formation of transient unstable charges, bond rearrangements, and other transformations required for the reaction to proceed in either direction. This is illustrated by the energy “hill” in Fig. 2.4 and Fig. 2.6. To undergo reaction, the molecules must overcome this barrier and therefore must be raised to a higher energy level. At the top of the energy hill is a point at which decay to the S or P state is equally probable (it is downhill either way). This is called the transition state. The transition state is not a chemical species with any significant stability and should not be confused with a reaction intermediate (such as ES or EP). It is simply a fleeting molecular moment in which events such as bond breakage, bond formation, and charge development have proceeded to the precise point at which decay to either substrate or product is equally likely. The difference between the energy levels of the ground state and the transition state is the activation energy, \( \Delta G \). The rate of a reaction reflects this activation energy: higher activation energy corresponds to a slower reaction. Reaction rates can be increased by raising the temperature and/or pressure, thereby increasing the number of molecules with sufficient energy to overcome the energy barrier. Alternatively, the activation energy can be lowered by adding a catalyst (Fig. 2.6). Catalysts enhance reaction rates by lowering activation energies.
Enzymes are no exception to the rule that catalysts do not affect reaction equilibria. The bidirectional arrows in equation 2.1 make this point: any enzyme that catalyses the reaction S → P also catalyses the reaction P → S. The role of enzymes is to accelerate the interconversion of S and P. The enzyme is not used up in the process, and the equilibrium point is unaffected. However, the reaction reaches equilibrium much faster when the appropriate enzyme is present, because the rate of the reaction is increased.

Any reaction may have several steps, involving the formation and decay of transient chemical species called reaction intermediates. When several steps occur in a reaction, the overall rate is determined by the step (or steps) with the highest activation energy. This is called the rate-limiting step. In a simple case, the rate-limiting step is the highest-energy point in the diagram for interconversion of S and P. In practice, the rate-limiting step can vary with reaction conditions, and for many enzymes several steps may have similar activation energies, which means they are all partially rate-limiting.

Activation energies are energy barriers to chemical reactions. These barriers are crucial to life itself. The rate at which a molecule undergoes a particular reaction decreases as the activation barrier for that reaction increases. Without such energy barriers, complex macromolecules would revert spontaneously to much simpler molecular forms, and the complex and highly ordered structures and metabolic processes of cells could not exist. Over the course of evolution, enzymes have developed to lower activation energies selectively for reactions that are needed for cell survival.

4.2. 2.4.2. Specificity of Enzymes

Enzymes are extraordinary catalysts. The rate enhancements they bring about are in the range of 5 to 17 orders of magnitude (Table 6–5). Enzymes are also very specific, readily discriminating between substrates with quite similar structures.

5. 2.5. Enzyme Kinetics

The oldest approach to understanding enzyme mechanisms, and the one that remains most important, is to determine the rate of a reaction and how it changes in response to changes in experimental parameters, a discipline known as enzyme kinetics.

A key factor affecting the rate of a reaction catalysed by an enzyme is the concentration of substrate, [S]. However, studying the effects of substrate concentration is complicated by the fact that [S] changes during the course of an in vitro reaction as substrate is converted to product. One simplifying approach in kinetics experiments is to measure the initial rate (or initial velocity), designated $V_0$. In a typical reaction, the enzyme
may be present in nanomolar quantities, whereas [S] may be five or six orders of magnitude higher. If only the beginning of the reaction is monitored (often the first 60 seconds or less), changes in [S] can be limited to a few percent and [S] can be regarded as constant. $V_0$ can then be explored as a function of [S], which is adjusted by the investigator. The effect on $V_0$ of varying [S] when the enzyme concentration is held constant is shown in Fig. 2.7. At relatively low concentrations of substrate, $V_0$ increases almost linearly with an increase in [S]. At higher substrate concentrations, $V_0$ increases by smaller and smaller amounts in response to increases in [S]. Finally, a point is reached beyond which increases in $V_0$ are vanishingly small as [S] increases. This plateau-like $V_0$ region is close to the maximum velocity, $V_{max}$.

**Figure 2.7. Enzyme kinetics**

http://www.ncbi.nlm.nih.gov/books/NBK26911/figure/A469/?report=objectonly

The curve expressing the relationship between [S] and $V_0$ (Fig. 2.7) has the same general shape for most enzymes (it approaches a rectangular hyperbola), which can be expressed algebraically by the Michaelis–Menten equation. Michaelis and Menten derived this equation starting from their basic hypothesis that the rate-limiting step in enzymatic reactions is the breakdown of the ES complex to product and free enzyme. The equation is

$$V_0 = \frac{V_{max} [S]}{K_m + [S]}$$

It is a statement of the quantitative relationship between the initial velocity $V_0$, the maximum velocity $V_{max}$, and the initial substrate concentration [S], all related through the Michaelis constant $K_m$. Note that $K_m$ has units of concentration. By the very useful, practical definition of $K_m$: $K_m$ is equivalent to the substrate concentration at which $V_0$ is one-half $V_{max}$.

### 6. 2.6. Enzymes are subject to reversible or irreversible inhibition

Enzyme inhibitors are molecules that interfere with catalysis, slowing or halting enzymatic reactions. Enzymes catalyse virtually all cellular processes, so it should not be surprising that enzyme inhibitors are among the most important pharmaceutical agents known. For example, aspirin (acetylsalicylate) inhibits the enzyme that catalyses the first step in the synthesis of prostaglandins, compounds involved in many processes, including some that produce pain. The study of enzyme inhibitors also has provided valuable information about enzyme mechanisms and has helped define some metabolic pathways. There are two broad classes of enzyme inhibitors: reversible and irreversible.
6.1. 2.6.1. Reversible inhibition

One common type of reversible inhibition is called competitive (Fig. 2.8, panel a). A competitive inhibitor competes with the substrate for the active site of an enzyme. While the inhibitor (I) occupies the active site it prevents binding of the substrate to the enzyme. Many competitive inhibitors are structurally similar to the substrate and combine with the enzyme to form an EI complex, but without leading to catalysis. Even fleeting combinations of this type will reduce the efficiency of the enzyme. Two other types of reversible inhibition, uncompetitive and mixed, though often defined in terms of one-substrate enzymes, are in practice observed only with enzymes having two or more substrates. An uncompetitive inhibitor (Fig. 2.8, panel b) binds at a site distinct from the substrate active site and, unlike a competitive inhibitor, binds only to the ES complex. A mixed inhibitor (Fig. 2.8, panel c) also binds at a site distinct from the substrate active site, but it binds to either E or ES.

Figure 2.8. Three types of reversible inhibition

6.2. 2.6.2. Irreversible Inhibition

The irreversible inhibitors bind covalently with or destroy a functional group on an enzyme that is essential for the enzyme’s activity, or form a particularly stable non-covalent association. Formation of a covalent link between an irreversible inhibitor and an enzyme is common.

Enzyme activity depends on pH: Enzymes have an optimum pH (or pH range) at which their activity is maximal at higher or lower pH, activity decreases.

7. 2.7. The regulation of enzyme activity

In cellular metabolism, groups of enzymes work together in sequential pathways to carry out a given metabolic process, such as the multireaction breakdown of glucose to lactate or the multireaction synthesis of an amino acid from simpler precursors. In such enzyme systems, the reaction product of one enzyme becomes the substrate of the next.
Most of the enzymes in each metabolic pathway follow the kinetic patterns we have already described. Each pathway, however, includes one or more enzymes that have a greater effect on the rate of the overall sequence. These regulatory enzymes exhibit increased or decreased catalytic activity in response to certain signals. Adjustments in the rate of reactions catalysed by regulatory enzymes, and therefore in the rate of entire metabolic sequences, allow the cell to meet changing needs for energy and for biomolecules required in growth and repair.
Chapter 3. Bioenergetics

All animals use the energy stored in the chemical bonds of organic molecules made by other organisms, which they take in as food. The molecules in food also provide the atoms that animals need to construct new living matter. Some animals obtain their food by eating other animals. But at the bottom of the animal food chain are animals that eat plants. The plants, in turn, trap energy directly from sunlight. As a result, all of the energy used by animal cells is derived ultimately from the sun.

Solar energy enters the living world through photosynthesis in plants and photosynthetic bacteria. Photosynthesis allows the electromagnetic energy in sunlight to be converted into chemical bond energy in the cell. Plants are able to obtain all the atoms they need from inorganic sources: carbon from atmospheric carbon dioxide, hydrogen and oxygen from water, nitrogen from ammonia and nitrates in the soil, and other elements needed in smaller amounts from inorganic salts in the soil. They use the energy they derive from sunlight to build these atoms into sugars, amino acids, nucleotides, and fatty acids. These small molecules in turn are converted into the proteins, nucleic acids, polysaccharides, and lipids that form the plant. All of these substances serve as food molecules for animals, if the plants are later eaten.

The net result of the entire process of photosynthesis, so far as the green plant is concerned, can be summarized simply in the equation:

\[ \text{light energy} + \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{sugars} + \text{O}_2 + \text{heat energy} \]

The sugars produced are then used both as a source of chemical bond energy and as a source of materials to make the many other small and large organic molecules that are essential to the plant cell.

1. 3.1. Cells obtain energy by the oxidation of organic molecules

All animal and plant cells are powered by energy stored in the chemical bonds of organic molecules, whether these be sugars that a plant has photosynthesized as food for itself or the mixture of large and small molecules that an animal has eaten. In order to use this energy to live, grow, and reproduce, organisms must extract it in a usable form. In both plants and animals, energy is extracted from food molecules by a process of gradual oxidation, or controlled burning.

The Earth's atmosphere contains a great deal of oxygen, and in the presence of oxygen the most energetically stable form of carbon is as CO\(_2\) and that of hydrogen is as H\(_2\)O. A cell is therefore able to obtain energy from sugars or other organic molecules by allowing their carbon and hydrogen atoms to combine with oxygen to produce CO\(_2\) and H\(_2\)O, respectively a process called respiration.

Photosynthesis and respiration are complementary processes (Fig.3.1). The oxygen released by photosynthesis is consumed in the combustion of organic molecules by nearly all organisms. And some of the CO\(_2\) molecules that are fixed today into organic molecules by photosynthesis in a green leaf were yesterday released into the atmosphere by the respiration of an animal or by that of a fungus or bacterium decomposing dead organic matter.

Figure 3.1. Photosynthesis and respiration as complementary processes in the living world

We therefore see that carbon utilization forms a huge cycle that involves the biosphere (all of the living organisms on Earth) as a whole, crossing boundaries between individual organisms (Fig. 3.2). Similarly, atoms of nitrogen, phosphorus, and sulphur move between the living and non-living worlds in cycles that involve plants, animals, fungi, and bacteria.

Figure 3.2. The carbon cycle - http://commons.wikimedia.org/wiki/File:Carbon-cycle-full.jpg

1.1. 3.1.1. Oxidation and Reduction Involve Electron Transfers

The cell does not oxidize organic molecules in one step, as occurs when organic material is burned in a fire. Through the use of enzyme catalysts, metabolism takes the molecules through a large number of reactions that only rarely involve the direct addition of oxygen. Oxidation, in the sense used above, does not mean only the addition of oxygen atoms. Rather, it applies more generally to any reaction in which electrons are transferred from one atom to another. Oxidation in this sense refers to the removal of electrons, and
reduction the converse of oxidation means the addition of electrons. Oxidation and reduction always occur simultaneously: that is, if one molecule gains an electron in a reaction (reduction), a second molecule loses the electron (oxidation). When a sugar molecule is oxidized to CO₂ and H₂O, for example, the O₂ molecules involved in forming H₂O gain electrons and thus are said to have been reduced.

When a molecule in a cell picks up an electron (e⁻), it often picks up a proton (H⁺) at the same time (protons being freely available in water). The net effect in this case is to add a hydrogen atom to the molecule:

\[ A + e^- + H^+ \rightarrow AH \]

Even though a proton plus an electron is involved (instead of just an electron) such hydrogenation reactions are reductions, and the reverse, dehydrogenation reactions, are oxidations. It is especially easy to tell whether an organic molecule is being oxidized or reduced: reduction is occurring if its number of C-H bonds increases, whereas oxidation is occurring if its number of C-H bonds decreases (Fig. 3.3).

Figure 3.3. Oxidation and reduction - http://www.ncbi.nlm.nih.gov/books/NBK26838/figure/A245/?report=objectonly

2.3.2. The free-energy change for a reaction determines whether it can occur

Although enzymes speed up reactions, they cannot by themselves force energetically unfavourable reactions to occur. In terms of a water analogy, enzymes by themselves cannot make water run uphill. Cells, however, must do just that in order to grow and divide: they must build highly ordered and energy-rich molecules from small and simple ones. We shall see that this is done through enzymes that directly couple energetically favourable reactions, which release energy and produce heat, to energetically unfavourable reactions, which produce biological order.

Before examining how such coupling is achieved, we must consider more carefully the term "energetically favourable." According to the second law of thermodynamics, a chemical reaction can proceed spontaneously only if it results in a net increase in the disorder of the universe. The criterion for an increase in disorder of the universe can be expressed most conveniently in terms of a quantity called the free energy, \( G \), of a system. The value of \( G \) is of interest only when a system undergoes a change, and the change in \( G \), denoted \( \Delta G \) (delta \( G \)), is critical. Suppose that the system being considered is a collection of molecules. Energetically favourable reactions, by definition, are those that decrease free energy, or, in other words, have a negative \( \Delta G \) and disorder the universe (Fig. 3.4).
Figure 3.4. The distinction between energetically favourable and energetically unfavourable reactions - http://www.ncbi.nlm.nih.gov/books/NBK26838/figure/A255/?report=objectonly

A familiar example of an energetically favourable reaction on a macroscopic scale is the "reaction" by which a compressed spring relaxes to an expanded state, releasing its stored elastic energy as heat to its surroundings or an example on a microscopic scale is the dissolving of salt in water. Conversely, energetically unfavourable reactions, with a positive ΔG such as those in which two amino acids are joined together to form a peptide bond by themselves create order in the universe. Therefore, these reactions can take place only if they are coupled to a second reaction with a negative ΔG so large that the ΔG of the entire process is negative (Fig.3.5).

Figure 3.5. How reaction coupling is used to drive energetically unfavourable reactions - http://www.ncbi.nlm.nih.gov/books/NBK26838/figure/A257/?report=objectonly
The concentration of reactants influences $\Delta G$. As we have just described, a reaction $A \leftrightarrow B$ will go in the direction $A \rightarrow B$ when the associated free-energy change, $\Delta G$, is negative, just as a tensed spring left to itself will relax and lose its stored energy to its surroundings as heat. For a chemical reaction, however, $\Delta G$ depends not only on the energy stored in each individual molecule, but also on the concentrations of the molecules in the reaction mixture. Remember that $\Delta G$ reflects the degree to which a reaction creates a more disordered, in other words, a more probable state of the universe. It is very likely that a coin will flip from a head to a tail orientation if a jiggling box contains 90 heads and 10 tails, but this is a less probable event if the box contains 10 heads and 90 tails. For exactly the same reason, for a reversible reaction $A \leftrightarrow B$, a large excess of $A$ over $B$ will tend to drive the reaction in the direction $A \rightarrow B$; that is, there will be a tendency for there to be more molecules making the transition $A \rightarrow B$ than there are molecules making the transition $B \rightarrow A$. Therefore, the $\Delta G$ becomes more negative for the transition $A \rightarrow B$ (and more positive for the transition $B \rightarrow A$) as the ratio of $A$ to $B$ increases.

How much of a concentration difference is needed to compensate for a given decrease in chemical bond energy (and accompanying heat release)? The answer is not intuitively obvious, but it can be determined from a thermodynamic analysis that makes it possible to separate the concentration-dependent and the concentration-independent parts of the free-energy change.

The $\Delta G$ for a given reaction can thereby be written as the sum of two parts: the first, called the standard free-energy change, $\Delta G^\circ$, depends on the intrinsic characters of the reacting molecules; the second depends on their concentrations. For the simple reaction $A \rightarrow B$ at 37°C, $\Delta G = \Delta G^\circ + 0.616 \ln \left[ \frac{[B]}{[A]} \right]$.

where $\Delta G$ is in kilocalories per mole, $[A]$ and $[B]$ denote the concentrations of $A$ and $B$, $\ln$ is the natural logarithm, and 0.616 is $RT$ the product of the gas constant, $R$, and the absolute temperature, $T$.

The overall free-energy change for a metabolic pathway is then simply the sum of the free-energy changes in each of its component steps.
3. 3.3. Activated carrier molecules: energy currencies

The energy released by the oxidation of food molecules must be stored temporarily before it can be channelled into the construction of other small organic molecules and of the larger and more complex molecules needed by the cell. In most cases, the energy is stored as chemical bond energy in a small set of activated "carrier molecules," which contain one or more energy-rich covalent bonds. These molecules diffuse rapidly throughout the cell and thereby carry their bond energy from sites of energy generation to the sites where energy is used for biosynthesis and other needed cell activities (Fig. 3.6).

Figure 3.6. Energy transfer and the role of activated carriers in metabolism - http://www.ncbi.nlm.nih.gov/books/NBK26838/figure/A265/?report=objectonly

The activated carriers store energy in an easily exchangeable form, either as a readily transferable chemical group or as high-energy electrons, and they can serve a dual role as a source of both energy and chemical groups in biosynthetic reactions. For historical reasons, these molecules are also sometimes referred to as coenzymes. The most important of the activated carrier molecules are ATP and two molecules that are closely related to each other, NADH and NADPH as we discuss in detail shortly. We shall see that cells use activated carrier molecules like money to pay for reactions that otherwise could not take place.

When a fuel molecule such as glucose is oxidized in a cell, enzyme-catalysed reactions ensure that a large part of the free energy that is released by oxidation is captured in achemically useful form, rather than being released wastefully as heat. This is achieved by means of a coupled reaction, in which an energetically favourable reaction is used to drive an energetically unfavourable one that produces an activated carrier molecule or some other useful energy store. Coupling mechanisms require enzymes and are fundamental to all the energy transactions of the cell.

3.1. 3.3.1. ATP is the most widely used activated carrier molecule

The most important and versatile of the activated carriers in cells is ATP (adenosine triphosphate). Just as the energy stored in the raised bucket of water in Fig. 3.7, panel B can be used to drive a wide variety of hydraulic machines, ATP serves as a convenient and versatile store, or currency, of energy to drive a variety of chemical reactions in cells.
ATP is synthesized in an energetically unfavourable phosphorylation reaction in which a phosphate group is added to ADP (adenosine diphosphate). When required, ATP gives up its energy packet through its energetically favourable hydrolysis to ADP and inorganic phosphate (Fig. 3.8). The regenerated ADP is then available to be used for another round of the phosphorylation reaction that forms ATP. The energetically favourable reaction of ATP hydrolysis is coupled to many otherwise unfavourable reactions through which other molecules are synthesized.

Figure 3.8. The hydrolysis of ATP to ADP and inorganic phosphate - http://www.ncbi.nlm.nih.gov/books/NBK26838/figure/A269/?report=objectonly
3.2. 3.3.2. FADH₂, NADH and NADPH are important electron carriers

Other important activated carrier molecules participate in oxidation-reduction reactions and are commonly part of coupled reactions in cells. These activated carriers are specialized to carry high-energy electrons and hydrogen atoms.

The most important of these electron carriers are NAD⁺ (nicotinamide adenine dinucleotide) and the closely related molecule NADP⁺ (nicotinamide adenine dinucleotide phosphate). Because the nicotinamide ring resembles pyridine, these compounds are sometimes called pyridine nucleotides. The vitamin niacin is the source of the nicotinamide moiety in nicotinamide nucleotides. NAD⁺ and NADP⁺ each pick up a “packet of energy” corresponding to two high-energy electrons plus a proton (H⁺) being converted to NADH (reduced nicotinamide adenine dinucleotide) and NADPH (reduced nicotinamide adenine dinucleotide phosphate), respectively. These molecules can therefore also be regarded as carriers of hydride ions (the H⁺ plus two electrons, or H) (Fig. 3.9). The difference of a single phosphate group has no effect on the electron-transfer properties of NADPH compared with NADH, but it gives to a molecule of NADPH a slightly different shape from that of NADH, and so NADPH and NADH bind as substrates to different sets of enzymes. Thus the two types of carriers are used to transfer electrons (or hydride ions) between different sets of molecules. NADPH operates chiefly with enzymes that catalyse anabolic reactions, supplying the high-energy electrons needed to synthesize energy-rich biological molecules. NADH, by contrast, has a special role as an intermediate in the catabolic system of reactions that generate ATP through the oxidation of food molecules.

Figure 3.9. NADPH, an important carrier of electrons - http://www.ncbi.nlm.nih.gov/books/NBK26838/figure/A274/?report=objectonly

The genesis of NADH from NAD⁺ and that of NADPH from NADP⁺ occur by different pathways and are independently regulated, so that the cell can independently adjust the supply of electrons for these two contrasting purposes. Inside the cell the ratio of NAD⁺ to NADH is kept high, whereas the ratio of NADP⁺ to...
NADPH is kept low. This provides plenty of NAD+ to act as an oxidizing agent and plenty of NADPH to act as a reducing agent as required for their special roles in catabolism and anabolism, respectively.

**FAD**

Flavoproteins are enzymes that catalyse oxidation-reduction reactions using either flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as coenzyme (Fig. 3.10).

*Figure 3.10. The structure of FADH$_2$ [Link](http://www.ncbi.nlm.nih.gov/books/NBK26882/figure/A306/?report=objectonly)*

These coenzymes, the flavin nucleotides, are derived from the vitamin riboflavin. The fused ring structure of flavin nucleotides (the *isoalloxazine ring*) undergoes **reversible reduction**, accepting either one or two **electrons** in the form of one or two hydrogen atoms (each atom an electron plus a proton) from a reduced substrate. The fully reduced forms are abbreviated FADH$_2$ and FMNH$_2$. When a fully oxidized flavin nucleotide accepts only one electron (one hydrogen atom), the semiquinone form of the isoalloxazine ring is produced, abbreviated FADH· and FMNH·. Because flavin nucleotides have a slightly different chemical specialty from that of the nicotinamide coenzymes, the ability to participate in either one- or two-electron transfers, flavoproteins are involved in a greater diversity of reactions than the NAD(P)-linked dehydrogenases.
3.3. 3.3.3. Other activated carriers

Other activated carriers also pick up and carry a chemical group in an easily transferred, high-energy linkage. For example, coenzyme A carries an acetyl group in a readily transferable linkage, and in this activated form is known as acetyl CoA (acetyl coenzyme A). The structure of acetyl CoA is illustrated in Fig. 3.11. It is used to add two carbon units in the biosynthesis of larger molecules.

Figure 3.11. The structure of the important activated carrier molecule acetyl CoA - http://www.ncbi.nlm.nih.gov/books/NBK26838/figure/A277/?report=objectonly
Chapter 4. Carbohydrate metabolism – glycolysis, gluconeogenesis

Carbohydrates are the single most abundant class of organic molecules found in nature. The name carbohydrate arises from the basic molecular formula (CH2O)n, which can be rewritten (C H2O)n to show that these substances are hydrates of carbon, where n=3 or more. Carbohydrates constitute a versatile class of molecules. Energy from the sun captured by green plants, algae, and some bacteria during photosynthesis is stored in the form of carbohydrates. In turn, carbohydrates are the metabolic precursors of virtually all other biomolecules. Breakdown of carbohydrates provides the energy that sustains animal life.

Carbohydrates are generally classified into three groups: monosaccharides (and their derivatives), oligosaccharides, and polysaccharides. The monosaccharides are also called simple sugars and have the formula (CH2O)n. Monosaccharides cannot be broken down into smaller sugars under mild conditions. Oligosaccharides derive their name from the Greek word oligo, meaning “few,” and consist of from two to ten simple sugar molecules. Disaccharides are common in nature, and trisaccharides also occur frequently. Four- to six-sugar-unit oligosaccharides are usually bound covalently to other molecules, including glycoproteins. As their name suggests, polysaccharides are polymers of the simple sugars and their derivatives. They may be either linear or branched polymers and may contain hundreds or even thousands of monosaccharide units. Their molecular weights range up to 1 million or more.

Monosaccharides consist typically of three to seven carbon atoms and are described either as aldoses or ketoses, depending on whether the molecule contains an aldehyde function or a ketone group. The simplest aldose is glyceraldehyde, and the simplest ketose is dihydroxyacetone (Fig. 4.1). These two simple sugars are termed trioses because they each contain three carbon atoms.

Figure 4.1. Triose phosphates
http://www.ncbi.nlm.nih.gov/books/NBK26882/box/A293/?report=objectonly

Hexoses are the most abundant sugars in nature. Nevertheless, sugars from all these classes are important in metabolism. Monosaccharides, either aldoses or ketoses, are often given more detailed generic names to describe both the important functional groups and the total number of carbon atoms. Thus, one can refer to aldotetroses and ketotetroses, aldopentoses and ketopentoses, aldohexoses and ketohexoses, and so on. Sometimes the ketone-containing monosaccharides are named simply by inserting the letters -ul- into the simple generic terms, such as tetruloses, pentuloses, hexuloses, heptuloses, and so on. The simplest monosaccharides are water-soluble, and most taste sweet.

The simplest oligosaccharides are the disaccharides, which consist of two monosaccharide units linked by a glycosidic bond. In addition to the simple disaccharides, many other oligosaccharides are found in both prokaryotic and eukaryotic organisms, either as naturally occurring substances or as hydrolysis products of natural materials. Several are constituents of the sweet nectars or saps exuded or extracted from plants and trees.

By far the majority of carbohydrate material in nature occurs in the form of polysaccharides. Polysaccharides are storage materials, structural components, or protective substances. Thus, starch, glycogen, and other storage polysaccharides, as readily metabolizable food, provide energy reserves for cells. Chitin and cellulose...
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provide strong support for the skeletons of arthropods and green plants, respectively. Mucopolysaccharides, such as the hyaluronic acids, form protective coats on animal cells.

Storage polysaccharides are an important carbohydrate form in plants and animals. It seems likely that organisms store carbohydrates in the form of polysaccharides rather than as monosaccharides to lower the osmotic pressure of the sugar reserves. Because osmotic pressures depend only on numbers of molecules, the osmotic pressure is greatly reduced by formation of a few polysaccharide molecules out of thousands (or even millions) of monosaccharide units.

The polysaccharides (together with proteins, lipids) that make up most of the food we eat must be broken down into smaller molecules (monomers) before our cells can use them either as a source of energy or as building blocks for other molecules. The breakdown processes must act on food taken in from outside, but not on the macromolecules inside our own cells. Stage 1 in the enzymatic breakdown of food molecules is therefore digestion, which occurs either in our intestine outside cells, or in a specialized organelle within cells, the lysosome. (A membrane that surrounds the lysosome keeps its digestive enzymes separated from the cytosol.) In either case, the large polymeric molecules in food are broken down during digestion into their monomer subunits proteins into amino acids, polysaccharides into sugars, and fats into fatty acids and glycerol through the action of enzymes. After digestion, the small organic molecules derived from food enter the cytosol of the cell, where their gradual oxidation begins. As illustrated in Fig. 4.2, oxidation occurs in two further stages of cellular catabolism: stage 2 starts in the cytosol and ends in the major energy-converting organelle, the mitochondrion; stage 3 is entirely confined to the mitochondrion.

Figure 4.2. Simplified diagram of the three stages of cellular metabolism that lead from food to waste products in animal cells - http://www.ncbi.nlm.nih.gov/books/NBK26882/figure/A290/?report=objectonly
In stage 2 a chain of reactions called **glycolysis** converts each molecule of **glucose** into two smaller molecules of **pyruvate**. Sugars other than glucose are similarly converted to pyruvate after their conversion to one of the sugar intermediates in this glycolytic pathway. **During pyruvate formation**, two types of activated carrier molecules are produced ATP and NADH. The pyruvate then passes from the cytosol into mitochondria. There, each pyruvate molecule is converted into CO$_2$ plus a two-carbon acetyl group which becomes attached to coenzyme A (CoA), forming acetyl CoA, another activated carrier molecule (see Fig. 3.10). Large amounts of acetyl CoA are also produced by the stepwise breakdown and oxidation of fatty
acids derived from fats, which are carried in the bloodstream, imported into cells as fatty acids, and then moved into mitochondria for acetyl CoA production.

Stage 3 of the oxidative breakdown of food molecules takes place entirely in mitochondria. The acetyl group in acetyl CoA is linked to coenzyme A through a high-energy linkage, and it is therefore easily transferable to other molecules. After its transfer to the four-carbon molecule oxaloacetate, the acetyl group enters a series of reactions called the citric acid cycle. As we discuss shortly, the acetyl group is oxidized to CO₂ in these reactions, and large amounts of the electron carrier NADH are generated. Finally, the high-energy electrons from NADH are passed along an electron-transport chain within the mitochondrial inner membrane, where the energy released by their transfer is used to drive a process that produces ATP and consumes molecular oxygen (O₂). It is in these final steps that most of the energy released by oxidation is harnessed to produce most of the cell's ATP. Because the energy to drive ATP synthesis in mitochondria ultimately derives from the oxidative breakdown of food molecules, the phosphorylation of ADP to form ATP that is driven by electron transport in the mitochondrion is known as oxidative phosphorylation. The fascinating events that occur within the mitochondrial inner membrane during oxidative phosphorylation are the major focus of Chapter 7.

1. 4.1. Glycolysis

Glycolysis is one of the principle pathways for generating ATP in cells and is present in all cell types. The central role of glycolysis in fuel metabolism is related to its ability to generate ATP with, and without, oxygen (Fig. 4.3).

Figure 4.3. The outline of glycolysis - http://www.ncbi.nlm.nih.gov/books/NBK26882/figure/A292/?report=objectonly
Glucose is readily available from our diet, internal glycogen stores, and the blood. Carbohydrate provides 50% or more of the calories in most diets, and glucose is the major carbohydrate. Other dietary sugars, such as...
fructose and galactose, are oxidized by conversion to intermediates of glycolysis. Insulin and other hormones maintain blood glucose at a constant level (glucose homeostasis); thereby ensuring that glucose is always available to cells that depend on glycolysis for generation of ATP.

In addition to serving as an anaerobic and aerobic source of ATP, glycolysis is an anabolic pathway that provides biosynthetic precursors. For example, in liver and adipose tissue, this pathway generates pyruvate as a precursor for fatty acid biosynthesis. Glycolysis also provides precursors for the synthesis of compounds such as amino acids and ribose-5-phosphate, the precursor of nucleotides.

1.1. 4.1.1. The reactions of glycolysis

Glucose metabolism begins with transfer of a phosphate from ATP to glucose to form glucose-6-P (Fig. 4.4). Phosphorylation of glucose commits it to metabolism within the cell because glucose-6-P cannot be transported back across the plasma membrane. The phosphorylation reaction is irreversible under physiologic conditions because the reaction has a high negative ΔG. Phosphorylation does not, however, commit glucose to glycolysis. Glucose-6-P is a branchpoint in carbohydrate metabolism. It is a precursor for almost every pathway that uses glucose, including glycolysis, the pentose phosphate pathway, and glycogen synthesis. Hexokinases, the enzymes that catalyse the phosphorylation of glucose, are a family of tissue-specific isoenzymes that differ in their kinetic properties.

In the remainder of the preparative phase of glycolysis, glucose-6-P is isomerized to fructose 6-phosphate (fructose-6-P), again phosphorylated, and subsequently cleaved into two 3-carbon fragments (Fig. 4.4). The isomerization, which positions a keto group next to carbon 3, is essential for the subsequent cleavage of the bond between carbons 3 and 4.

Figure 4.4. Steps 1-5 of glycolysis - http://www.accessexcellence.org/RC/VL/GG/ecb/ten_steps_glycolysis.php
The next step of glycolysis, phosphorylation of fructose-6-P to fructose 1,6-bisphosphate (fructose-1,6-bisP) by phosphofructokinase-1 (PFK-1), is generally considered the first committed step of the pathway. This phosphorylation requires ATP and is thermodynamically and kinetically irreversible. Therefore, PFK-1 irrevocably commits glucose to the glycolytic pathway. PFK-1 is a regulated enzyme in cells, and its regulation controls the entry of glucose into glycolysis. Like hexokinase, it exists as tissue-specific isoenzymes whose regulatory properties match variations in the role of glycolysis in different tissues.

Fructose-1,6-bisP is cleaved into two phosphorylated 3-carbon compounds (triose phosphates) by aldolase (Fig. 4.4).
Dihydroxyacetone phosphate (DHAP) is isomerized to glyceraldehyde 3-phosphate (glyceraldehyde-3-P), which is a triose phosphate. Thus, for every mole of glucose entering glycolysis, 2 moles of glyceraldehyde-3-P continue through the pathway.

In the next part of the glycolytic pathway, glyceraldehyde-3-P is oxidized and phosphorylated so that subsequent intermediates of glycolysis can donate phosphate to ADP to generate ATP. The first reaction in this sequence, catalysed by glyceraldehyde-3-P dehydrogenase, is really the key to the pathway (Fig. 4.5).

Figure 4.5. Steps 6-10 of glycolysis - http://www.accessexcellence.org/RC/VL/GG/ecb/ten_steps_glycolysis_part2.php
This enzyme oxidizes the aldehyde group of glyceraldehyde-3-P to an enzyme-bound carboxyl group and transfers the electrons to NAD to form NADH. The oxidation step is dependent on a cysteine residue at the active site of the enzyme, which forms a high-energy thioester bond during the course of the reaction. The high-energy intermediate immediately accepts an inorganic phosphate to form the high-energy acyl phosphate bond in 1,3-bisphosphoglycerate, releasing the product from the cysteine residue on the enzyme. This high-energy phosphate bond is the start of substrate-level phosphorylation (the formation of a high-energy phosphate bond where none previously existed, without the utilization of oxygen).

In the next reaction, the phosphate in this bond is transferred to ADP to form ATP by 3-phosphoglycerate kinase. The energy of the acyl phosphate bond is high enough (~13 kcal/mole) so that transfer to ADP is an energetically favourable process. 3-phosphoglycerate is also a product of this reaction. To transfer the remaining low-energy phosphoester on 3-phosphoglycerate to ADP, it must be converted into a high-energy bond.

This conversion is accomplished by moving the phosphate to the second carbon (forming 2-phosphoglycerate) and then removing water to form phosphoenolpyruvate (PEP). The enolphosphate bond is a high-energy bond (its hydrolysis releases approximately 14 kcal/mole of energy), so the transfer of phosphate to ADP by pyruvate kinase is energetically favourable (see Fig. 4.5). This final reaction converts PEP to pyruvate.

The overall net reaction in the glycolytic pathway is:

\[ \text{Glucose} + 2\text{NAD}^+ + 2\text{Pi} + 2\text{ADP} \rightarrow 2\text{Pyruvate} + 2\text{NADH} + 4\text{H}^+ + 2\text{ATP} + 2\text{H}_2\text{O} \]

The pathway occurs with an overall negative \(\Delta G\) of approximately \(\sim 22\) kcal. Therefore, it cannot be reversed without the expenditure of energy.

**1.2. 4.1.2. Fates of pyruvate and NADH**

The NADH produced from glycolysis must be continuously reoxidized back to NAD\(^+\) to provide an electron acceptor for the glyceraldehyde-3-P dehydrogenase reaction and prevent product inhibition. Without oxidation of this NADH, glycolysis cannot continue. There are two alternate routes for oxidation of cytosolic NADH. One route is aerobic, involving shuttles that transfer reducing equivalents across the mitochondrial membrane and ultimately to the electron transport chain and oxygen (Fig. 4.6 and 4.7).

Figure 4.6. The malate-aspartate shuttle for transporting reducing equivalents from cytosolic NADH into the mitochondrial matrix - http://www.bioinfo.org.cn/book/biochemistry/chapt18/sim5.htm
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Figure 4.7. The glycerol-3-phosphate shuttle, an alternative means of moving reducing equivalents from the cytosol to the mitochondrial matrix - http://www.bioinfo.org.cn/book/biochemistry/chapt18/sim5.htm

The other route is anaerobic (without the use of oxygen). In anaerobic glycolysis, NADH is reoxidized in the cytosol by lactate dehydrogenase, which reduces pyruvate to lactate(Fig. 4.8). The fate of pyruvate depends on the route used for NADH oxidation. If NADH is reoxidized in a shuttle system, pyruvate can be used for other pathways, one of which is oxidation to acetyl-CoA and entry into the TCA cycle for complete oxidation. Alternatively, in anaerobic glycolysis, pyruvate is reduced to lactate and diverted away from other potential pathways. In some organisms that can grow anaerobically, such as yeasts, pyruvate is converted via acetaldehyde into carbon dioxide and ethanol (Fig. 4.8).

Figure 4.8. Two pathways for the anaerobic breakdown of pyruvate - http://www.ncbi.nlm.nih.gov/books/NBK26882/figure/A295/?report=objectonly
Thus, the use of the shuttle systems allows for more ATP to be generated than by anaerobic glycolysis by both oxidizing the cytoplasmically derived NADH in the electron transport chain and by allowing pyruvate to be oxidized completely to CO₂.

The reason that shuttles are required for the oxidation of cytosolic NADH by the electron transport chain is that the inner mitochondrial membrane is impermeable to NADH, and no transport protein exists that can directly translocate NADH across this membrane. Consequently, NADH is reoxidized to NAD⁺ in the cytosol by a reaction that transfers the electrons to DHAP in the glycerol 3-phosphate (glycerol-3-P) shuttle (Fig. 4.7) and oxaloacetate in the malate-aspartate shuttle (Fig. 4.6). The NAD⁺ that is formed in the cytosol returns to glycolysis while glycerol-3-P or malate carries the reducing equivalents that are ultimately transferred across the inner mitochondrial membrane. Thus, these shuttles transfer electrons and not NADH.

When the oxidative capacity of a cell is limited (e.g., the red blood cell, which has no mitochondria), the pyruvate and NADH produced from glycolysis cannot be oxidized aerobically. The NADH is therefore oxidized to NAD⁺ in the cytosol by reduction of pyruvate to lactate. This reaction is catalysed by lactate dehydrogenase (LDH) (Fig. 4.8). The net reaction for anaerobic glycolysis is:

\[
\text{Glucose} + 2\text{ADP} + 2\text{Pi} \rightarrow 2\text{Lactate} + 2\text{ATP} + 2\text{H₂O} + 2\text{H⁺}
\]

1.3. 4.1.3. Energy yield of aerobic versus anaerobic glycolysis

In both aerobic and anaerobic glycolysis, each mole of glucose generates 2 moles of ATP, 2 of NADH and 2 of pyruvate. The energy yield from anaerobic glycolysis (glucose to 2 lactate) is only 2 moles of ATP per mole of glucose, as the NADH is recycled to NAD by reducing pyruvate to lactate. Neither the NADH nor pyruvate produced is thus used for further energy generation. However, when oxygen is available, and cytosolic NADH can be oxidized via a shuttle system, pyruvate can also enter the mitochondria and be completely oxidized to CO₂ via PDH and the TCA cycle. The oxidation of pyruvate via this route generates roughly 12.5 moles of ATP per mole of pyruvate. If the cytosolic NADH is oxidized by the glycerol 3-P shuttle, approximately 1.5 moles of ATP are produced per NADH. If, instead, the NADH is oxidized by the malate-aspartate shuttle, approximately 2.5 moles are produced. Thus, the two NADH molecules produced during glycolysis can lead to 3 to 5 molecules of ATP being produced, depending on which shuttle system is used to transfer the reducing equivalents. Because each pyruvate produced can give rise to 12.5 molecules of ATP, altogether 30 to 32 molecules of ATP can be produced from one mole of glucose oxidized to carbon dioxide.

To produce the same amount of ATP per unit time from anaerobic glycolysis as from the complete aerobic oxidation of glucose to CO₂, anaerobic glycolysis must occur approximately 15 times faster, and use approximately 15 times more glucose. Cells achieve this high rate of glycolysis by expressing high levels of glycolytic enzymes. In certain skeletal muscles and in most cells during hypoxic crises, high rates of glycolysis are associated with rapid degradation of internal glycogen stores to supply the required glucose-6-P.

1.4. 4.1.4. Other functions of glycolysis
Glycolysis, in addition to providing ATP, generates precursors for biosynthetic pathways. Intermediates of the pathway can be converted to ribose 5-phosphate, the sugar incorporated into nucleotides such as ATP. Other sugars, such as UDP-glucose, mannose, and sialic acid, are also formed from intermediates of glycolysis. Serine is synthesized from 3-phosphoglycerate, and alanine from pyruvate. The backbone of triacylglycerols, glycerol 3-phosphate, is derived from dihydroxyacetone phosphate in the glycolytic pathway. The liver is the major site of biosynthetic reactions in the body. In addition to those pathways mentioned previously, the liver synthesizes fatty acids from the pyruvate generated by glycolysis. It also synthesizes glucose from lactate, glycerol 3-phosphate, and amino acids in the gluconeogenic pathway, which is principally a reversal of glycolysis. Consequently, in liver, many of the glycolytic enzymes exist as isoenzymes with properties suited for these functions.

1.5. 4.1.5. Regulation of glycolysis

One of the major functions of glycolysis is the generation of ATP, and, therefore, the pathway is regulated to maintain ATP homeostasis in all cells. Phosphofructokinase-1 (PFK-1) and pyruvate dehydrogenase (PDH), which links glycolysis and the TCA cycle, are both major regulatory sites that respond to feedback indicators of the rate of ATP utilization. The supply of glucose-6-P for glycolysis is tissue dependent and can be regulated at the steps of glucose transport into cells, glycogenolysis (the degradation of glycogen to form glucose), or the rate of glucose phosphorylation by hexokinase isoenzymes. Other regulatory mechanisms integrate the ATP-generating role of glycolysis with its anabolic roles.

All of the regulatory enzymes of glycolysis exist as tissue-specific isoenzymes, which alter the regulation of the pathway to match variations in conditions and needs in different tissues. For example, in the liver, an isoenzyme of pyruvate kinase introduces an additional regulatory site in glycolysis that contributes to the inhibition of glycolysis when the reverse pathway, gluconeogenesis, is activated.

2. 4.2. Gluconeogenesis

Gluconeogenesis, the process by which glucose is synthesized from non-carbohydrate precursors, occurs mainly in the liver under fasting conditions. Under the more extreme conditions of starvation, the kidney cortex also may produce glucose. For the most part, the glucose produced by the kidney cortex is used by the kidney medulla, but some may enter the bloodstream. Starting with pyruvate, most of the steps of gluconeogenesis (green) are simply reversals of those of glycolysis (red) (Fig. 4.9). In fact, these pathways differ at only three points. Enzymes involved in catalysing these steps are regulated so that either glycolysis or gluconeogenesis predominates, depending on physiologic conditions.

Most of the steps of gluconeogenesis use the same enzymes that catalyse the process of glycolysis. The flow of carbon, of course, is in the reverse direction. Three reaction sequences of gluconeogenesis differ from the corresponding steps of glycolysis. They involve the conversion of pyruvate to phosphoenolpyruvate (PEP) and the reactions that remove phosphate from fructose 1,6-bisphosphate to form fructose 6-phosphate and from glucose 6-phosphate to form glucose (Fig. 4.9).

Figure 4.9. The relationship of glycolysis and gluconeogenesis - http://qualityinvention.com/QILibraryPicture.php?picturename=Relationship between glycolysis and gluconeogenesis
Carbohydrate metabolism – glycolysis, gluconeogenesis

Glycolysis

- ATP
- Hexokinase
- ADP

Glyceraldehyde 3-phosphate

Fructose 1,6-bisphosphate

Fructose 6-phosphate

- ATP
- Hexokinase
- ADP

Dihydroxyacetone phosphate

- Dihydroxyacetone phosphate

Glyceraldehyde 3-phosphate

- (2) NAD⁺
- (2) NAD⁺

- (2) NADH + (2) H⁺
- (2) NADH + (2) H⁺

(2) 1,3-Bisphosphoglycerate

- (2) ADP
- (2) ATP

- (2) ATP
- (2) ADP

(2) 3-Phosphoglycerate

- (2) P₃
- (2) P₃

(2) 2-Phosphoglycerate

- (2) ADP
- (2) ATP

Phosphoenolpyruvate

- (2) GDP
- PEP carboxykinase

- (2) GTP
- PEP carboxykinase

- (2) Oxaloacetate

Pyruvate

- (2) ADP
- Pyruvate carboxylase

- (2) Pyruvate
The conversion of pyruvate to PEP is catalysed during gluconeogenesis by a series of enzymes instead of the single enzyme used for glycolysis. In glycolysis, PEP is converted to pyruvate by pyruvate kinase. In gluconeogenesis, a series of steps are required to accomplish the reversal of this reaction (Fig. 4.9). 

Pyruvate is carboxylated by pyruvate carboxylase to form oxaloacetate. Oxaloacetate is transported across the mitochondrial membrane as malate or aspartate depends on the need for reducing equivalents in the cytosol. Oxaloacetate, produced from malate or aspartate in the cytosol, is converted to PEP by the cytosolic PEP carboxykinase (Fig. 4.9).

The reactions that remove phosphate from fructose 1,6-bisphosphate and from glucose 6-phosphate each use single enzymes that differ from the corresponding enzymes of glycolysis. Although phosphate is added during glycolysis by kinases, which use adenosine triphosphate (ATP), it is removed during gluconeogenesis by phosphatases that release Pi via hydrolysis reactions.

The three major carbon sources for gluconeogenesis in humans are lactate, glycerol, and amino acids, particularly alanine. Lactate is produced by anaerobic glycolysis in tissues such as exercising muscle or red blood cells, as well as by adipocytes during the fed state. Glycerol is released from adipose stores of triacylglycerol, and amino acids come mainly from amino acid pools in muscle, where they may be obtained by degradation of muscle protein. Alanine, the major gluconeogenic amino acid, is produced in the muscle from other amino acids and from glucose.

Energy is required for the synthesis of glucose. During the gluconeogenic reactions, 6 moles of high-energy phosphate bonds are cleaved.
Chapter 5. Carbohydrate metabolism – pentose-phosphate pathway

The pentose phosphate pathway is essentially a scenic bypass route around the first stage of glycolysis that generates NADPH and ribose-5-P (as well as other pentose sugars). Glucose 6-phosphate is the common precursor for both pathways. The oxidative first stage of the pentose phosphate pathway generates two moles of NADPH per glucose 6-phosphate oxidized. The second stage of the pentose phosphate pathway generates ribose-5-P and converts unused intermediates to fructose-6-P and glyceraldehyde-3-P in the glycolytic pathway (Fig. 5.1). All cells require NADPH for reductive detoxification, and most cells require ribose-5-P for nucleotide synthesis. Consequently, the pathway is present in all cells. The enzymes reside in the cytosol, as do the enzymes of glycolysis.

1. 5.1. Oxidative phase of the pentose phosphate pathway

In the oxidative first phase of the pentose phosphate pathway, glucose 6-phosphate is oxidatively decarboxylated to a pentose sugar, ribulose 5-phosphate (Fig. 5.2). The first enzyme of this pathway, glucose 6-phosphate dehydrogenase, oxidizes the aldehyde at C1 and reduces NADP to NADPH. The gluconolactone that is formed is rapidly hydrolysed to 6-phosphogluconate, a sugar acid with a carboxylic acid group at C1. The next oxidation step releases this carboxyl group as CO₂, with the electrons being transferred to NADP. This reaction is mechanistically very similar to the one catalysed by isocitrate dehydrogenase in the TCA cycle. Thus, two moles of NADPH per mole of glucose 6-phosphate are formed from this portion of the pathway (Fig. 5.2).
To generate ribose 5-phosphate from the oxidative pathway, the ribulose 5-phosphate formed from the action of the two oxidative steps is isomerized to produce ribose 5-phosphate (a ketose-to-aldose conversion). The ribose 5-phosphate can then enter the pathway for nucleotide synthesis, if needed, or can be converted to glycolytic intermediates, as described below for the non-oxidative phase of the pentose phosphate pathway. The pathway through which the ribose 5-phosphate travels is determined by the needs of the cell at the time of its synthesis.

2. 5.2. The non-oxidative phase of the pentose phosphate pathway

The non-oxidative reactions of this pathway are reversible reactions that allow intermediates of glycolysis (specifically glyceraldehyde-3-P and fructose-6-P) to be converted to five-carbon sugars (such as ribose-5-P), and vice versa. The needs of the cell will determine in which direction this pathway proceeds. If the cell has produced ribose-5-P, but does not need to synthesize nucleotides, then the ribose-5-P will be converted to glycolytic intermediates. If the cell still requires NADPH, the ribose-5-P will be converted back into glucose-6-P using non-oxidative reactions (see below). And finally, if the cell already has a high level of NADPH, but needs to produce nucleotides, the oxidative reactions of the pentose phosphate pathway will be inhibited, and the glycolytic intermediates fructose-6-P and glyceraldehyde-3-P will be used to produce the five carbon sugars using exclusively the non-oxidative phase of the pentose phosphate pathway.

The non-oxidative portion of the pentose phosphate pathway consists of a series of rearrangement and transfer reactions that first convert ribulose 5-phosphate to ribose 5-phosphate and xylulose 5-phosphate, and then the ribose 5-phosphate and xylulose 5-phosphate are converted to intermediates of the glycolytic pathway. The enzymes involved are an epimerase, an isomerase, transketolase, and transaldolase.

The epimerase and isomerase convert ribulose 5-phosphate to two other 5-carbon sugars (Fig. 5.3). The isomerase converts ribulose 5-phosphate to ribose 5-phosphate. The epimerase changes the stereochemical position of one hydroxyl group (at carbon 3), converting ribulose 5-phosphate to xylulose 5-phosphate.

**Figure 5.3. Conversion of ribulose 5-phosphate to ribose 5-phosphate and xylulose 5-phosphate**
Transketolase transfers 2-carbon fragments of keto sugars (sugars with a keto group at C2) to other sugars. Transketolase picks up a 2-carbon fragment from xylulose 5-phosphate by cleaving the carbon–carbon bond between the keto group and the adjacent carbon, thereby releasing glyceraldehyde 3-phosphate (Fig. 5.4). Two reactions in the pentose phosphate pathway use transketolase; in the first, the 2-carbon keto fragment from xylulose 5-phosphate is transferred to ribose 5-phosphate to form sedoheptulose 7-phosphate, and in the other, a 2-carbon keto fragment (usually derived from xylulose 5-phosphate) is transferred to erythrose 4-phosphate to form fructose 6-phosphate. Transaldolase transfers a 3-carbon keto fragment from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate to form erythrose 4-phosphate and fructose 6-phosphate (Fig. 5.4). The aldol cleavage occurs between the two hydroxyl carbons adjacent to the keto group (on carbons 3 and 4 of the sugar). This reaction is similar to the aldolase reaction in glycolysis, and the enzyme uses an active amino group, from the side chain of lysine, to catalyse the reaction.

The net result of the metabolism of 3 moles of ribulose 5-phosphate in the pentose phosphate pathway is the formation of 2 moles of fructose 6-phosphate and 1 mole of glyceraldehyde 3-phosphate, which then continue through the glycolytic pathway with the production of NADH, ATP, and pyruvate. Because the pentose phosphate pathway begins with glucose 6-phosphate, and feeds back into the glycolytic pathway, it is sometimes called the hexose monophosphate shunt (a shunt or a pathway for glucose 6-phosphate).

Figure 5.4. The non-oxidative phase of the pentose phosphate pathway
The reactions catalysed by the epimerase, isomerase, transketolase, and transaldolase are all reversible reactions under physiologic conditions. Thus, ribose-5-phosphate required for purine and pyrimidine synthesis can be generated from intermediates of the glycolytic pathway, as well as from the oxidative phase of the pentose phosphate pathway.
Chapter 6. Pyruvate dehydrogenase enzyme complex – TCA cycle

1. 6.1. Pyruvate Dehydrogenase Complex

The pyruvate dehydrogenase complex (PDC) oxidizes pyruvate to acetyl CoA, thus linking glycolysis and the TCA cycle. In the brain, which is dependent on the oxidation of glucose to CO₂ to fulfil its ATP needs, regulation of the PDC is a life and death matter.

1.1. 6.1.1. Structure of PDC

PDC belongs to the α-ketoacid dehydrogenase complex family and, thus, shares structural and catalytic features with the α-ketoglutarate dehydrogenase complex and the branched chain α-ketoacid dehydrogenase complex. It contains the same three basic types of catalytic subunits: (1) pyruvate decarboxylase subunits that bind thiamine-pyrophosphate (TPP) (A); (2) transacetylase subunits that bind lipoate (B), and (3) dihydrolipoyl dehydrogenase subunits that bind FAD (C) (Fig. 6.1).

Figure 6.1. The oxidation of pyruvate to acetyl CoA and CO₂ - http://worldofbiochemistry.blogspot.hu/2011/08/oxidation-of-pyruvate.html

Although the A and B enzymes in PDC are relatively specific for pyruvate, the same dihydrolipoyl dehydrogenase participates in all of the α-ketoacid dehydrogenase complexes.

1.2. 6.1.2. Regulation of PDC

PDC activity is controlled principally through phosphorylation by pyruvate dehydrogenase kinase, which inhibits the enzyme, and dephosphorylation by pyruvate dehydrogenase phosphatase, which activates it. PDC kinase transfers a phosphate from ATP to specific serine hydroxyl (ser-OH) groups on pyruvate decarboxylase (A). PDC phosphatase removes these phosphate groups by hydrolysis. Phosphorylation of just one serine on the PDC Aα subunit can decrease its activity by over 99%. PDC kinase is, itself, inhibited by
ADP and pyruvate. Thus, when rapid ATP utilization results in an increase of ADP, or when activation of glycolysis increases pyruvate levels, PDC kinase is inhibited, and PDC remains in an active, non-phosphorylated form. PDC phosphatase requires Ca\(^{2+}\) for full activity. In the heart, increased intramitochondrial Ca\(^{2+}\) during rapid contraction activates the phosphatase, thereby increasing the amount of active, non-phosphorylated PDC.

PDC is also regulated through inhibition by its products, acetyl CoA and NADH.

This inhibition is stronger than regular product inhibition because their binding to PDC stimulates its phosphorylation to the inactive form. The substrates of the enzyme, CoASH and NAD\(^{+}\), antagonize this product inhibition. Thus, when an ample supply of acetyl CoA for the tricarboxylic acid (TCA) cycle is already available from fatty acid oxidation, acetyl CoA and NADH build up and dramatically decrease their own further synthesis by PDC. The rate of other fuel oxidation pathways that feed into the TCA cycle is also increased when ATP utilization increases.

2. 6.2. The TCA cycle

The initial steps for the discovery of the reactions of TCA cycle was taken by the Hungarian Nobel laureate biochemist Albert Szent-Györgyi. Building on his earlier work in the biochemistry of plant respiration, Szent-Györgyi had begun to investigate respiration in muscle tissue, using minced pigeon breast muscle. Pigeon breast was an ideal material, a powerful muscle that burned energy at a high rate to sustain flight, and also readily available. It was already known that fumaric, malic, and succinic acids (collectively called dicarboxylic acids) played some role in respiration, but scientists assumed that they were consumed in the process. When Szent-Györgyi added small amounts of these to his minced pigeon muscle, he found that far more oxygen was consumed than would be needed to oxidize them. The acids were not consumed as fuels, he realized, but served as catalysts, i.e., they maintained the combustion reaction without being changed themselves. Each of them stimulated the oxidation of a carbohydrate present in the tissue cells. This was an important new idea. Szent-Györgyi proposed that the hydrogen from a substance in the cell (e.g., a carbohydrate) reduced a first dicarboxylic acid, the oxaloacetic acid; the resulting malic acid reduced fumaric acid; the succinic acid thus produced in turn transferred its hydrogen to cytochromes. By 1937, Szent-Györgyi had identified the process as a cycle and was close to elaborating all of the steps that generate adenosine triphosphate (ATP), the energy-carrying molecule in all living cells. As it turned out, Szent-Györgyi's focus on malate and oxaloacetate was an error, and Hans Krebs soon found that the key link was citric acid. Thus "Szent-Györgyi's cycle" became the citric acid cycle or Krebs cycle. Krebs, who won a Nobel prize in 1953 for the work, later called it the tricarboxylic acid cycle. The most common name for this pathway, the tricarboxylic acid or TCA cycle, denotes the involvement of the tricarboxylates citrate and isocitrate.

The pathways for oxidation of fatty acids, glucose, amino acids, acetate, and ketone bodies all generate acetyl CoA, which is the substrate for the TCA cycle. As the activated 2-carbon acetyl group is oxidized to two molecules of CO\(_2\), energy is conserved as NADH, FADH\(_2\), and GTP (Fig. 6.2). NADH and FADH\(_2\) subsequently donate electrons to O\(_2\) via the electron transport chain, with the generation of ATP from oxidative phosphorylation. Thus, the TCA cycle is central to energy generation from cellular respiration.

2.1. 6.2.1. Reactions of the TCA cycle

In the TCA cycle, the 2-carbon acetyl group of acetyl CoA is oxidized to 2 CO\(_2\) molecules (Fig. 6.2). The function of the cycle is to conserve the energy from this oxidation, which it accomplishes principally by transferring electrons from intermediates of the cycle to NAD\(^{+}\) and FAD. The eight electrons donated by the acetyl group eventually end up in three molecules of NADH and one of FADH\(_2\) (Fig. 6.2). As a consequence, ATP can be generated from oxidative phosphorylation when NADH and FADH\(_2\) donate these electrons to O\(_2\) via the electron transport chain.

Figure 6.2. The complete citric acid cycle  
2.1.1. 6.2.1.1. Formation and oxidation of isocitrate

The TCA cycle begins with condensation of the activated acetyl group and oxaloacetate to form the 6-carbon intermediate citrate, a reaction catalysed by the enzyme citrate synthase (Fig. 6.2 and 6.3). Because oxaloacetate is regenerated with each turn of the cycle, it is not really considered a substrate of the cycle, or a source of electrons or carbon.

Figure 6.3. Condensation of the activated acetyl group and oxaloacetate to citrate - http://www.ncbi.nlm.nih.gov/books/NBK26882/box/A307/?report=objectonly

In the next step of the TCA cycle, the hydroxyl (alcohol) group of citrate is moved to an adjacent carbon so that it can be oxidized to form a keto group. The isomerization of citrate to isocitrate is catalysed by the enzyme aconitase, which is named for an intermediate of the reaction (Fig. 6.4).

Figure 6.4. Isomerization of citrate to isocitrate by aconitase - http://www.ncbi.nlm.nih.gov/books/NBK26882/box/A307/?report=objectonly
The enzyme isocitrate dehydrogenase catalyses the oxidation of the alcohol group and the subsequent cleavage of the carboxyl group to release CO2 (an oxidative decarboxylation).

Figure 6.5. Oxidative decarboxylation of isocitrate - http://www.ncbi.nlm.nih.gov/books/NBK26882/box/A307/?report=objectonly

The next step of the TCA cycle is the oxidative decarboxylation of α-ketoglutarate to succinyl CoA, catalysed by the α-ketoglutarate dehydrogenase complex (Fig. 6.6). The dehydrogenase complex contains the coenzymes thiamine pyrophosphate, lipoic acid, and FAD.

Figure 6.6. Oxidative decarboxylation of α-ketoglutarate to succinyl CoA - http://www.ncbi.nlm.nih.gov/books/NBK26882/box/A307/?report=objectonly

In this reaction, one of the carboxyl groups of α-ketoglutarate is released as CO2, and the adjacent keto group is oxidized to the level of an acid, which then combines with CoASH to form succinyl CoA (Fig. 6.6). Energy from the reaction is conserved principally in the reduction state of NADH, with a smaller amount present in the high-energy thioester bond of succinyl CoA.

Energy from the succinyl CoA thioester bond is used to generate GTP from GDP and Pi in the reaction catalysed by succinate thiokinase (Fig. 6.7). This reaction is an example of substrate level phosphorylation. By definition, substrate level phosphorylation is the formation of a high-energy phosphate bond where none previously existed without the use of molecular O2 (in other words, NOT oxidative phosphorylation). The high-energy phosphate bond of GTP is energetically equivalent to that of ATP, and can be used directly for energy-requiring reactions like protein synthesis.

Figure 6.7. Formation of GTP from GDP and Pion the expense of the energy of the succinyl CoA thioester bond - http://www.ncbi.nlm.nih.gov/books/NBK26882/box/A307/?report=objectonly
Up to this stage of the TCA cycle, two carbons have been stripped of their available electrons and released as CO₂. Two pairs of these electrons have been transferred to 2 NAD⁺, and one GTP has been generated. However, two additional pairs of electrons arising from acetyl CoA still remain in the TCA cycle as part of succinate. The remaining steps of the TCA cycle transfer these two pairs of electrons to FAD and NAD⁺ and add H₂O, thereby regenerating oxaloacetate.

The sequence of reactions converting succinate to oxaloacetate begins with the oxidation of succinate to fumarate (Fig. 6.8). Single electrons are transferred from the two adjacent - CH2 - methylene groups of succinate to an FAD bound to succinate dehydrogenase, thereby forming the double bond of fumarate. From the reduced enzyme-bound FAD, the electrons are passed into the electron transport chain.

An OH group and a proton from water add to the double bond of fumarate, converting it to malate (Fig. 6.9).

In the last reaction of the TCA cycle, the alcohol group of malate is oxidized to a keto group through the donation of electrons to NAD⁺. With regeneration of oxaloacetate, the TCA cycle is complete (Fig. 6.10.). The chemical bond energy, carbon, and electrons donated by the acetyl group have been converted to CO₂, NADH, FADH₂, GTP, and heat.

2.2. 6.2.2. Energetics of the TCA cycle
Like all metabolic pathways, the TCA cycle operates with an overall net negative $\Delta G$. The conversion of substrates to products is, therefore, energetically favourable. However, some of the reactions, such as the malate dehydrogenase reaction, have a positive value.

Three reactions in the TCA cycle have large negative values for $\Delta G$ that strongly favour the forward direction: the reactions catalysed by citrate synthase, isocitrate dehydrogenase, and $\alpha$-ketoglutarate dehydrogenase (Fig. 6.2, 6.3, 6.5, 6.6). Within the TCA cycle, these reactions are physiologically irreversible for two reasons: the products do not rise to high enough concentrations under physiological conditions to overcome the large negative $\Delta G$ values, and the enzymes involved catalyse the reverse reaction very slowly. These reactions make the major contribution to the overall negative $\Delta G$ for the TCA cycle, and keep it going in the forward direction.

In contrast to these irreversible reactions, the reactions catalysed by aconitase and malate dehydrogenase have a positive $\Delta G$ for the forward direction, and are thermodynamically and kinetically reversible. Because aconitase is rapid in both directions, equilibrium values for the concentration ratio of products to substrates are maintained, and the concentration of citrate is about 20 times that of isocitrate. The accumulation of citrate instead of isocitrate facilitates transport of excess citrate to the cytosol, where it can provide a source of acetyl CoA for pathways like fatty acid and cholesterol synthesis. It also allows citrate to serve as an inhibitor of citrate synthase when flux through isocitrate dehydrogenase is decreased. Likewise, the equilibrium constant of the malate dehydrogenase reaction favours the accumulation of malate over oxaloacetate, resulting in a low oxaloacetate concentration that is influenced by the NADH/NAD$^+$ ratio. Thus, there is a net flux of oxaloacetate towards malate in the liver during fasting (due to fatty acid oxidation, which raises the NADH/NAD$^+$ ratio), and malate can then be transported out of the mitochondria to provide a substrate for gluconeogenesis.

2.3. 6.2.3. Regulation of the TCA cycle

The oxidation of acetyl CoA in the TCA cycle and the conservation of this energy as NADH and FADH$_2$ is essential for generation of ATP in almost all tissues in the body. In spite of changes in the supply of fuels, type of fuels in the blood, or rate of ATP utilization, cells maintain ATP homeostasis (a constant level of ATP). The rate of the TCA cycle, like that of all fuel oxidation pathways, is principally regulated to correspond to the rate of the electron transport chain, which is regulated by the ATP/ADP ratio and the rate of ATP utilization.

Two major messengers feed information on the rate of ATP utilization back to the TCA cycle: (a) the phosphorylation state of ATP, as reflected in ATP and ADP levels, and (b) the reduction state of NAD, as reflected in the ratio of NADH/NAD$^+$. Within the cell, even within the mitochondrion, the total adenine nucleotide pool (AMP, ADP, plus ATP) and the total NAD pool (NAD$^+$ plus NADH) are relatively constant. Thus, an increased rate of ATP utilization results in a small decrease of ATP concentration and an increase of ADP. Likewise, increased NADH oxidation to NAD$^+$ by the electron transport chain increases the rate of pathways producing NADH. Under normal physiological conditions, the TCA cycle and other oxidatiow paths respond so rapidly to increased ATP demand that the ATP concentration does not significantly change.

2.4. 6.2.4. TCA cycle in biosynthetic pathways and anaplerotic reactions

2.4.1. 6.2.4.1. TCA cycle intermediates are precursors for biosynthetic pathways

The intermediates of the TCA cycle serve as precursors for a variety of different pathways present in different cell types (Fig. 6.11). This is particularly important in the central metabolic role of the liver. After a high carbohydrate meal, citrate efflux and cleavage to acetyl CoA provides acetyl units for cytosolic fatty acid synthesis. During fasting, gluconeogenic precursors are converted to malate, which leaves the mitochondria for cytosolic gluconeogenesis. The liver also uses TCA cycle intermediates to synthesize carbon skeletons of amino acids. Succinyl CoA may be removed from the TCA cycle to form heme in cells of the liver and bone marrow.
In the brain, α-ketoglutarate is converted to glutamate and then to γ-aminobutyric acid (GABA), a neurotransmitter. In skeletal muscle, α-ketoglutarate is converted to glutamine, which is transported through the blood to other tissues.

2.4.2. 6.2.4.2. Anaplerotic Reactions

Removal of any of the intermediates from the TCA cycle removes the 4 carbons that are used to regenerate oxaloacetate during each turn of the cycle. With depletion of oxaloacetate, it is impossible to continue oxidizing acetyl CoA. To enable the TCA cycle to keep running, cells have to supply enough four-carbon intermediates from degradation of carbohydrate or certain amino acids to compensate for the rate of removal. Pathways or reactions that replenish the intermediates of the TCA cycle are referred to as anaplerotic (“filling up”).

Pyruvate carboxylase is one of the major anaplerotic enzymes in the cell. It catalyses the addition of CO₂ to pyruvate to form oxaloacetate. The activated CO₂ is then transferred to pyruvate to form the carboxyl group of oxaloacetate. Pyruvate carboxylase is found in many tissues, such as liver, brain, adipocytes, and fibroblasts, where its function is anaplerotic. Its concentration is high in liver and kidney cortex, where there is a continuous removal of oxaloacetate and malate from the TCA cycle to enter the gluconeogenic pathway. Pyruvate carboxylase is activated by acetyl CoA and inhibited by high concentrations of many acyl CoA derivatives. As the concentration of oxaloacetate is depleted through the efflux of TCA cycle intermediates, the rate of the citrate synthase reaction decreases and acetyl CoA concentration rises. The acetyl CoA then activates pyruvate carboxylase to synthesize more oxaloacetate.

The pathways for oxidation of many amino acids convert their carbon skeletons into 5- and 4-carbon intermediates of the TCA cycle that can regenerate oxaloacetate. Alanine and serine carbons can enter through pyruvate carboxylase. In all tissues with mitochondria (except for, surprisingly, the liver), oxidation of the two branched chain amino acids isoleucine and valine to succinyl CoA forms a major anaplerotic route. In the liver, other compounds forming propionyl CoA (e.g., methionine, thymine and odd-chain length or branched fatty acids) also enter the TCA cycle as succinyl CoA. In most tissues, glutamine is taken up from the blood, converted to glutamate, and then oxidized to α-ketoglutarate, forming another major anaplerotic route. However, the TCA cycle cannot be resupplied with intermediates by even chain length fatty acid oxidation, or ketone body oxidation, which forms only acetyl CoA. In the TCA cycle, two carbons are lost from citrate before succinyl CoA is formed, and, therefore, there is no net conversion of acetyl carbon to oxaloacetate.
3. 6.3. The glyoxylate cycle

Vertebrates cannot convert fatty acids, or the acetate derived from them, to carbohydrates. Conversion of phosphoenolpyruvate to pyruvate and of pyruvate to acetyl-CoA is so exergonic as to be essentially irreversible. If a cell cannot convert acetate into phosphoenolpyruvate, acetate cannot serve as the starting material for the gluconeogenic pathway, which leads from phosphoenolpyruvate to glucose. Without this capacity, then, a cell or organism is unable to convert fuels or metabolites that are degraded to acetate (fatty acids and certain amino acids) into carbohydrates.

In plants, certain invertebrates, and some microorganisms (including E. coli and yeast) acetate can serve both as an energy-rich fuel and as a source of phosphoenolpyruvate for carbohydrate synthesis. In these organisms, enzymes of the glyoxylate cycle catalyse the net conversion of acetate to succinate or other four-carbon intermediates of the citric acid cycle:

In the glyoxylate cycle, acetyl-CoA condenses with oxaloacetate to form citrate, and citrate is converted to isocitrate, exactly as in the citric acid cycle. The next step, however, is not the breakdown of isocitrate by isocitrate dehydrogenase but the cleavage of isocitrate by isocitralase, forming succinate and glyoxylate. The glyoxylate then condenses with a second molecule of acetyl-CoA to yield malate, in a reaction catalysed by malate synthase. The malate is subsequently oxidized to oxaloacetate, which can condense with another molecule of acetyl-CoA to start another turn of the cycle (Fig. 6.12). Each turn of the glyoxylate cycle consumes two molecules of acetyl-CoA and produces one molecule of succinate, which is then available for biosynthetic purposes. The succinate may be converted through fumarate and malate into oxaloacetate, which can then be converted to phosphoenolpyruvate by PEP carboxykinase, and thus to glucose by gluconeogenesis. Vertebrates do not have the enzymes specific to the glyoxylate cycle (isocitralase and malate synthase) and therefore cannot bring about the net synthesis of glucose from lipids.

Figure 6.12. The glyoxylate cycle - http://www.ncbi.nlm.nih.gov/books/NBK22383/figure/A2423/?report=objectonly
In plants, the enzymes of the glyoxylate cycle are sequestered in membrane-bounded organelles called **glyoxysomes**, which are specialized peroxisomes. Those enzymes common to the citric acid and glyoxylate cycles have two isozymes, one specific to mitochondria, the other to glyoxysomes. **Glyoxysomes are not present in all plant tissues at all times.** They develop in lipid-rich seeds **during germination**, before the developing plant acquires the ability to make glucose by photosynthesis. In addition to glyoxylate cycle enzymes, glyoxysomes contain all the enzymes needed for the degradation of the fatty acids stored in seed oils. Acetyl-CoA formed from lipid breakdown is converted to succinate via the glyoxylate cycle, and the succinate is exported to mitochondria, where citric acid cycle enzymes transform it to malate. A cytosolic isozyme of malate dehydrogenase oxidizes malate to oxaloacetate, a precursor for gluconeogenesis. Germinating seeds can therefore convert the carbon of stored lipids into glucose.
Chapter 7. Terminal oxidation – oxidative phosphorylation, ATP synthesis in the mitochondria

Aerobic organisms are able to capture a far greater proportion of the available free energy of respiratory substrates than anaerobic organisms. Most of this takes place inside mitochondria, which have been termed the “powerhouses” of the cell. Mitochondria have an outer membrane that is permeable to most metabolites, an inner membrane that is selectively permeable, and a matrix within (Fig. 7.1). The phospholipid cardiolipin is concentrated in the inner membrane together with the enzymes of the respiratory chain. Generation of ATP from oxidative phosphorylation requires an electron donor (NADH or FADH$_2$), an electron acceptor (O$_2$), an intact inner mitochondrial membrane that is impermeable to protons, all the components of the electron transport chain, and ATP synthase. It is regulated by the rate of ATP utilization.

Figure 7.1. The general organization of a mitochondrion - http://bio1151.nicerweb.com/Locked/media/ch06/mitochondrion.html

Our understanding of oxidative phosphorylation is based on the chemiosmotic hypothesis, which proposes that the energy for ATP synthesis is provided by an electrochemical gradient across the inner mitochondrial membrane. This electrochemical gradient is generated by the components of the electron transport chain, which pump protons across the inner mitochondrial membrane as they sequentially accept and donate electrons (Fig. 7.2). The final acceptor is O$_2$, which is reduced to H$_2$O.

1. 7.1. Overview of terminal oxidation and oxidative phosphorylation

In the electron transport chain, electrons donated by NADH or FADH$_2$ are passed sequentially through a series of electron carriers embedded in the inner mitochondrial membrane. Electron transport to O$_2$ occurs via a series of oxidation–reduction steps in which each successive component of the chain is reduced as it accepts electrons and oxidized as it passes electrons to the next component of the chain (Fig. 7.2). The oxidation–reduction components of the chain include flavin mononucleotide (FMN), Fe-S centres, CoQ, and Fe
Terminal oxidation – oxidative phosphorylation, ATP synthesis in the mitochondria

in the cytochromes b, c1, c, a, and a3. Cu is also a component of cytochromes a and a3. With the exception of CoQ, all of these electron acceptors are tightly bound to the protein subunits of the carriers.

**Figure 7.2. Overview of terminal oxidation and oxidative phosphorylation**

![Diagram of terminal oxidation and oxidative phosphorylation]

The reduction potential of each complex of the chain is at a lower energy level than the previous complex, so that energy is released as electrons pass through each complex. This energy is used to move protons against their concentration gradient, so that they become concentrated on the cytosolic side of the inner membrane (Fig. 7.2).

2. **7.2. Electron transfer from NADH to O₂**

From NADH, electrons are transferred sequentially through NADH dehydrogenase (complex I), CoQ (Q), the cytochrome b-c1 complex (complex III), cytochrome c, and finally cytochrome c oxidase (complex IV).

NADH dehydrogenase, the cytochrome b-c1 complex and cytochrome c oxidase are each multisubunit protein complexes that span the inner mitochondrial membrane. CoQ is a lipid soluble quinone that is not protein-bound and is free to diffuse in the lipid membrane. It transports electrons from complex I to complex III and is an intrinsic part of the proton pumps for each of these complexes. Cytochrome c is a small protein in the inner membrane space that transfers electrons from the b–c1complex to cytochrome oxidase. The terminal complex, cytochrome c oxidase, contains the binding site for O₂. As O₂ accepts electrons from the chain, it is reduced to H₂O (Fig. 7.3).

**Figure 7.3. The electrochemical potential gradient**
In addition to NADH dehydrogenase, succinic dehydrogenase and other flavoproteins in the inner mitochondrial membrane also pass electrons to CoQ (Fig. 7.3). Succinate dehydrogenase is part of the TCA cycle. ETF-CoQoxidoreductase accepts electrons from ETF (electron transferring flavoprotein), which acquires them from fatty acid oxidation and other pathways. The free energy drop between NADH and CoQ of approximately -13 to -14 kcal is able to support movement of four protons. However, the FAD in succinate dehydrogenase (as well as ETF-CoQoxidoreductase and α-glycerophosphate dehydrogenase) is at roughly the same energy level as CoQ, and there is no energy released as they transfer electrons to CoQ. These proteins do not span the membrane and consequently do not have a proton pumping mechanism.

3. 7.3. The electrotechnical potential gradient

At each of the three large membrane-spanning complexes in the chain, electron transfer is accompanied by proton pumping across the membrane (Fig. 7.3). There is an energy drop of approximately 16 kcal in reduction potential as electrons pass through each of these complexes, which provides the energy required to move protons against a concentration gradient. The membrane is impermeable to protons, so they cannot diffuse through the lipid bilayer back into the matrix. Thus, in actively respiring mitochondria, the intermembrane space and cytosol may be approximately 0.75 pH units lower than the matrix.

The transmembrane movement of protons generates an electrochemical gradient with two components: the membrane potential (the external face of the membrane is charged positive relative to the matrix side) and the proton gradient (the inter membrane space has a higher proton concentration and is therefore more acidic than the matrix) (Fig. 7.2 and 7.3). The electrochemical gradient is sometimes called the proton motive force because it is the energy pushing the protons to re-enter the matrix to equilibrate on both sides of the membrane. The protons are attracted to the more negatively charged matrix side of the membrane, where the pH is more alkaline.

4. 7.4. ATP Synthase

ATP synthase (F0F1ATPase), the enzyme that generates ATP, is a multisubunit enzyme containing an inner membrane portion (F0, deep pink on Fig. 7.3) and a stalk and headpiece (F1, light pink on Fig. 7.3) that
Terminal oxidation – oxidative phosphorylation, ATP synthesis in the mitochondria

...project into the matrix. The subunits in the membrane form a rotor (Fig. 7.3). The influx of protons through the proton channel turns the rotor. Energy from the electrochemical gradient is used to change the conformation of the ATP synthase subunits so that the newly synthesized ATP is released. It takes 12 protons to complete one turn of the rotor and synthesize three ATP.

Most of the newly synthesized ATP that is released into the mitochondrial matrix must be transported out of the mitochondria, where it is used for energy-requiring processes such as active ion transport, muscle contraction, or biosynthetic reactions. Likewise, ADP, phosphate, pyruvate, and other metabolites must be transported into the matrix. This requires transport of compounds through both the inner and outer mitochondrial membranes.

5. 7.5. Energy yield from the electron transport chain

The overall free energy release from oxidation of NADH by O₂ is approximately -53 kcal, and from FADH₂, it is approximately -41 kcal. This ΔG is so negative that the chain is never reversible. We never synthesize oxygen from H₂O. It is so negative that it drives NADH and FADH₂ formation from the pathways of fuel oxidation, such as the TCA cycle and glycolysis, to completion.

Overall, each NADH donates two electrons, equivalent to the reduction of 1/2 of an O₂ molecule. A generally (but not universally) accepted estimate of the stoichiometry of ATP synthesis is that four protons are pumped at complex I, four protons at complex III, and two at complex IV. With four protons translocated for each ATP synthesized, an estimated 2.5 ATPs are formed for each NADH oxidized and 1.5 ATPs for each of the other FADH₂-containing flavoproteins that donate electrons to CoQ. (This calculation neglects proton requirements for the transport of phosphate and substrates from the cytosol, as well as the basal proton leak.) Thus, only approximately 30% of the energy available from NADH and FADH₂ oxidation by O₂ is used for ATP synthesis. Some of the remaining energy in the electrochemical potential is used for the transport of anions and Ca²⁺ into the mitochondrion.

The remainder of the energy is released as heat. Consequently, the electron transport chain is also our major source of heat.

6. 7.6. Respiratory chain inhibition and sequential transfer

In the cell, electron flow in the electron transport chain must be sequential from NADH or a flavoprotein all the way to O₂ to generate ATP (Fig. 7.3). In the absence of O₂, there is no ATP generated from oxidative phosphorylation because electrons back up in the chain. Even complex I cannot pump protons to generate the electrochemical gradient, because every molecule of CoQ already has electrons that it cannot pass down the chain without an O₂ to accept them at the end.

The action of the respiratory chain inhibitor cyanide, which binds to cytochrome oxidase, is similar to that of anoxia; it prevents proton pumping by all three complexes. Complete inhibition of the b-c₁ complex prevents pumping at cytochrome oxidase because there is no donor of electrons; it prevents pumping at complex I because there is no electron acceptor. Although complete inhibition of any one complex inhibits proton pumping at all of the complexes, partial inhibition of proton pumping can occur when only a fraction of the molecules of a complex contain bound inhibitor. The partial inhibition results in a partial decrease of the maximal rate of ATP synthesis.

7. 7.7. Coupling of electron transport and ATP synthesis

The electrochemical gradient couples the rate of the electron transport chain to the rate of ATP synthesis. Because electron flow requires proton pumping, electron flow cannot occur faster than protons are used for ATP synthesis (coupled oxidative phosphorylation) or returned to the matrix by a mechanism that short circuits the ATP synthase pore (uncoupling).

7.1. 7.7.1. Regulation through Coupling
As ATP chemical bond energy is used by energy-requiring reactions, ADP and Pi concentrations increase. The more ADP present to bind to the ATP synthase, the greater will be proton flow through the ATP synthase pore, from the intermembrane space to the matrix. Thus, as ADP levels rise, proton influx increases, and the electrochemical gradient decreases (Fig. 7.2 and 7.3). The proton pumps of the electron transport chain respond with increased proton pumping and electron flow to maintain the electrochemical gradient. The result is increased O$_2$ consumption. The increased oxidation of NADH in the electron transport chain and the increased concentration of ADP stimulate the pathways of fuel oxidation, such as the TCA cycle, to supply more NADH and FADH$_2$ to the electron transport chain. For example, during exercise, we use more ATP for muscle contraction, consume more oxygen, oxidize more fuel (which means burn more calories), and generate more heat from the electron transport chain. If we rest, and the rate of ATP utilization decreases, proton influx decreases, the electrochemical gradient increases, and proton “back-pressure” decreases the rate of the electron transport chain. NADH and FADH$_2$ cannot be oxidized as rapidly in the electron transport chain, and consequently, their build-up inhibits the enzymes that generate them. The electron transport chain has a very high capacity and can respond very rapidly to any increase in ATP utilization.

7.2. 7.7.2. Uncoupling ATP synthesis from electron transport

When protons leak back into the matrix without going through the ATP synthase pore, they dissipate the electrochemical gradient across the membrane without generating ATP. This phenomenon is called “uncoupling” oxidative phosphorylation. It occurs with chemical compounds, known as uncouplers, and it occurs physiologically with uncoupling proteins that form proton conductance channels through the membrane. Uncoupling of oxidative phosphorylation results in increased oxygen consumption and heat production as electron flow and proton pumping attempt to maintain the electrochemical gradient.
Chapter 8. Photosynthesis – Calvin cycle

The capture of solar energy by photosynthetic organisms and its conversion to the chemical energy of reduced organic compounds is the ultimate source of nearly all biological energy. Photosynthetic and heterotrophic organisms live in a balanced steady state in the biosphere. Photosynthetic organisms trap solar energy and form ATP and NADPH, which they use as energy sources to make carbohydrates and other organic compounds from CO₂ and H₂O; simultaneously, they release O₂ into the atmosphere. Aerobic heterotrophs (humans, for example, as well as plants during dark periods) use the O₂ so formed to degrade the energy-rich organic products of photosynthesis to CO₂ and H₂O, generating ATP. The CO₂ returns to the atmosphere, to be used again by photosynthetic organisms. Solar energy thus provides the driving force for the continuous cycling of CO₂ and O₂ through the biosphere and provides the reduced substrates - fuels, such as glucose - on which non-photosynthetic organisms depend.

Photosynthesis occurs in a variety of bacteria and in unicellular eukaryotes (algae) as well as in vascular plants. Although the process in these organisms differs in detail, the underlying mechanisms are remarkably similar, and much of our understanding of photosynthesis in vascular plants is derived from studies of simpler organisms. The overall equation for photosynthesis in vascular plants describes an oxidation-reduction reaction in which H₂O donates electrons (as hydrogen) for the reduction of CO₂ to carbohydrate (CH₂O):

\[ \text{H}_2\text{O} \rightarrow \frac{3}{2} \text{O}_2 + \text{CH}_2\text{O} \]

1. 8.1. General features of photophosphorylation

Unlike NADH (the major electron donor in oxidative phosphorylation), H₂O is a poor donor of electrons. Its standard reduction potential is +0.816 V, compared with -0.320 V for NADH. Photophosphorylation differs from oxidative phosphorylation in requiring the input of energy in the form of light to create a good electron donor and a good electron acceptor. In photophosphorylation, electrons flow through a series of membrane bound carriers including cytochromes, quinones, and iron-sulphur proteins, while protons are pumped across a membrane to create an electrochemical potential. Electron transfer and proton pumping are catalysed by membrane complexes homologous in structure and function to Complex III of mitochondria. The electrochemical potential they produce is the driving force for ATP synthesis from ADP and Pi, catalysed by a membrane-bound ATP synthase complex closely similar to that of oxidative phosphorylation.

Photosynthesis in plants encompasses two processes: the light-dependent reactions, or light reactions, which occur only when plants are illuminated, and the carbon-assimilation reactions (or carbon-fixation reactions), sometimes misleadingly called the dark reactions, which are driven by products of the light reactions (Fig. 8.1). In the light reactions, chlorophyll and other pigments of photosynthetic cells absorb light energy and conserve it as ATP and NADPH; simultaneously, O₂ is evolved. In the carbon-assimilation reactions, ATP and NADPH are used to reduce CO₂ to form triose phosphates, starch, and sucrose, and other products derived from them (Fig. 8.1).

Figure 8.1. The reactions of photosynthesis in a chloroplast - http://mriclassroom.weebly.com/photosynthesis.html
In photosynthetic eukaryotic cells, both the light-dependent and the carbon-assimilation reactions take place in the chloroplasts (Fig. 8.2), intracellular organelles that are variable in shape and generally a few micrometres in diameter. Like mitochondria, they are surrounded by two membranes, an outer membrane that is permeable to small molecules and ions, and an inner membrane that encloses the internal compartment. This compartment contains many flattened, membrane-surrounded vesicles or sacs, the thylakoids, usually arranged in stacks called grana (Fig. 8.2).

Figure 8.2. A mitochondrion and chloroplast compared - http://www.ncbi.nlm.nih.gov/books/NBK26819/figure/A2565/?report=objectonly
Embedded in the thylakoid membranes (commonly called lamellae) are the photosynthetic pigments and the enzyme complexes that carry out the light reactions and ATP synthesis. The stroma (the aqueous phase enclosed by the inner membrane) contains most of the enzymes required for the carbon-assimilation reactions.

2. 8.2. Light absorption

Visible light is electromagnetic radiation of wavelengths 400 to 700 nm, a small part of the electromagnetic spectrum, ranging from violet to red. The energy of a single photon is greater at the violet end of the spectrum than at the red end. Shorter wavelength (and higher frequency) corresponds to higher energy. The energy of a photon of visible light ranges from 150 kJ/einstein for red light to 300 kJ/einstein for violet light. When a photon is absorbed, an electron in the absorbing molecule (chromophore) is lifted to a higher energy level (Fig. 8.3). This is an all-or-nothing event; to be absorbed, the photon must contain a quantity of energy (a quantum) that exactly matches the energy of the electronic transition. A molecule that has absorbed a photon is in an excited state, which is generally unstable. An electron lifted into a higher-energy orbital usually returns rapidly to its lower-energy orbital. The excited molecule decays to the stable ground state, giving up the absorbed quantum as light or heat or using it to do chemical work. Just as the photon is a quantum of light energy, so the exciton is a quantum of energy passed from an excited molecule to another molecule in a process called exciton transfer (Fig. 8.3).

Figure 8.3. How light energy is harvested by a reaction centre chlorophyll molecule - http://biomooocnews.blogspot.hu/2012/10/daily-newsletter-october-8-2012.html

2.1. 8.2.1. Chlorophylls Absorb Light Energy for Photosynthesis

The most important light-absorbing pigments in the thylakoid membranes are the chlorophylls, green pigments with polycyclic, planar structures resembling the protoporphyrin of haemoglobin, except that Mg²⁺, not Fe²⁺, occupies the central position (Fig. 8.4). All chlorophylls have a long phytol side chain, esterified to a carboxyl-group substituent in ring IV, and chlorophylls also have a fifth five-membered ring not present in heme.

Chlorophyll is always associated with specific binding proteins, forming light-harvesting complexes (LHCs) in which chlorophyll molecules are fixed in relation to each other, to other protein complexes, and to the
membrane (Fig. 8.5). In addition to chlorophylls, thylakoid membranes contain secondary light-absorbing pigments, or accessory pigments, called carotenoids.

Figure 8.4. The structure of chlorophyll - http://www.ncbi.nlm.nih.gov/books/NBK26819/figure/A2577/?report=objectonly
hydrophobic tail region
The light-absorbing pigments of thylakoid or bacterial membranes are arranged in functional arrays called **photosystems** (Fig. 8.5). In spinach chloroplasts, for example, each photosystem contains about 200 chlorophyll and 50 carotenoid molecules. All the pigment molecules in a photosystem can absorb photons, but only a few chlorophyll molecules associated with the **photochemical reaction centre** are specialized to **transduce light into chemical energy**. The other pigment molecules in a photosystem are called light-harvesting or antenna molecules. They absorb light energy and transmit it rapidly and efficiently to the reaction centre (Fig. 8.5).

The excited antenna chlorophyll – in light-harvesting complexes – transfers energy directly to a neighbouring chlorophyll molecule, which becomes excited as the first molecule returns to its ground state (step 2). This transfer of energy, excitation transfer, extends to a third, fourth, or subsequent neighbour, until one of a special pair of chlorophyll a molecules at the photochemical reaction centre is excited (step 3). In this excited chlorophyll molecule, an electron is promoted to a higher-energy orbital. This electron then passes to a nearby electron acceptor that is part of the electron-transfer chain, leaving the reaction-centre chlorophyll with a missing electron (an "electron hole,") (step 4).

**Figure 8.5. The antenna complex and photochemical reaction centre in a photosystem** - http://www.ncbi.nlm.nih.gov/books/NBK26819/figure/A2583/?report=objectonly

The electron acceptor acquires a negative charge in this transaction. The electron lost by the reaction-centre chlorophyll is replaced by an electron from a neighbouring electron-donor molecule (step 5), which thereby becomes positively charged. In this way, excitation by light causes electric charge separation and initiates an oxidation-reduction chain (Fig. 8.5).

### 3.8.3. Light-Driven Electron Flow

Light-driven electron transfer in plant chloroplasts during photosynthesis is accomplished by **multienzyme systems in the thylakoid membrane**.

The photosynthetic apparatus of modern cyanobacteria, algae, and vascular plants is complex. **The thylakoid membranes of chloroplasts have two different kinds of photosystems (PS II and PS I), each with its own type of photochemical reaction centre and set of antenna molecules.** The two systems have distinct and complementary functions (Fig. 8.6). These **two reaction centres in plants act in tandem** to catalyse the light-driven movement of electrons from $\text{H}_2\text{O}$ to $\text{NADP}^+$ (Fig. 8.6 and 8.7). Electrons are carried between the two photosystems by the soluble protein **plastocyanin**, a one-electron carrier functionally similar to cytochrome c of mitochondria.
To replace the electrons that move from PSII through PSI to NADP⁺, cyanobacteria and plants oxidize H₂O producing O₂ (Fig. 8.6 and 8.7, bottom left). All O₂-evolving photosynthetic cells - those of plants, algae, and cyanobacteria - contain both PSI and PSII. Organisms with only one photosystem do not evolve O₂.

The diagram in Fig. 8.7, often called the Z scheme because of its overall form, outlines the pathway of electron flow between the two photosystems and the energy relationships in the light reactions. The Z scheme thus describes the complete route by which electrons flow from H₂O to NADP⁺, according to the equation

\[
2\text{H}_2\text{O} + 2\text{NADP}^+ + 8 \text{photons} \rightarrow \text{O}_2 + 2\text{NADPH} + 2\text{H}^+
\]

For every two photons absorbed (one by each photosystem), one electron is transferred from H₂O to NADP⁺. To form one molecule of O₂, which requires transfer of four electrons from two H₂O to two NADP⁺, a total of eight photons must be absorbed, four by each photosystem.

**Figure 8.7. The transfer of electrons during photosynthesis, the Z-scheme**
Excitation of the reaction centre P680 in PSII produces P680*, an excellent electron donor that, within picoseconds, transfers an electron to pheophytin, giving it a negative charge (Pheo-). With the loss of its electron, P680* is transformed into a radical cation, P680+. Pheo- very rapidly passes its extra electron to a protein-bound plastoquinone, PQA (or QA), which in turn passes its electron to another, more loosely bound plastoquinone, PQB (or QB). When PQB has acquired two electrons from such transfers from PQA and two protons from the solvent water, it is in its fully reduced quinol form, PQBH2. The overall reaction initiated by light in PSII is

$$4 \text{P680}^+ + 4\text{H}^+ + 2\text{PQB} + 4\text{photons} \rightarrow 4\text{P680}^+ + 2\text{PQBH}_2$$

Eventually, the electrons in PQBH2 pass through the cytochrome b6 f complex (Fig. 8.6 and 8.7). The electron initially removed from P680 is replaced with an electron obtained from the oxidation of water, as described below.

The **binding site for plastoquinone** is the point of action of many commercial herbicides that kill plants by blocking electron transfer through the cytochrome b6 f complex and preventing photosynthetic ATP production.

The photochemical events that follow excitation of PSI at the reaction-centre P700 are formally similar to those in PSII. The excited reaction-centre P700* loses an electron to an acceptor, A0 (believed to be a special form of chlorophyll, functionally homologous to the pheophytin of PSII), creating A0− and P700+. Again, excitation results in charge separation at the photochemical reaction centre. **P700** is a strong oxidizing agent, which quickly acquires an electron from plastocyanin, a soluble Cu-containing electron-transfer protein. A0− is an exceptionally strong reducing agent that passes its electron through a chain of carriers that leads to NADP+. First, phylloquinone accepts an electron and passes it to an iron-sulphur protein (through three Fe-S centres in PSI). From here, the electron moves to ferredoxin (Fd), another iron-sulphur protein loosely associated with the thylakoid membrane (Fig. 8.6 and 8.7). The fourth electron carrier in the chain is the flavoproteinferredoxin:NADP+ oxidoreductase, which transfers electrons from reduced ferredoxin (Fdred) to NADP+:

$$2\text{Fd}_{\text{red}} + 2\text{H}^+ + \text{NADP}^+ \rightarrow 2\text{Fd}_{\text{ox}} + \text{NADPH} + \text{H}^+$$
3.1.8.3.1. The cytochrome b6f complex links photosystems II and I

Electrons temporarily stored in plastoquinol as a result of the excitation of P680 in PSII are carried to P700 of PSI via the cytochrome b6 f complex and the soluble protein plastocyanin (Fig. 8.7, centre). Like Complex III of mitochondria, the cytochrome b6 f complex contains a b-type cytochrome with two heme groups, a Rieske iron-sulphur protein, and cytochrome f (named for the Latin frons, “leaf”). Electrons flow through the cytochrome b6 f complex from PQBH2 to cytochrome f, then to plastocyanin, and finally to P700, thereby reducing it.

Like Complex III of mitochondria, cytochrome b6 f conveys electrons from a reduced quinone - a mobile, lipid-soluble carrier of two electrons (Q in mitochondria, PQB in chloroplasts) - to a water-soluble protein that carries one electron (cytochrome c in mitochondria, plastocyanin in chloroplasts). As in mitochondria, the function of this complex involves a Q cycle in which electrons pass, one at a time, from PQBH2 to cytochrome b6. This cycle results in the pumping of protons across the membrane. In chloroplasts, the direction of proton movement is from the stromal compartment to the thylakoid lumen, up to four protons moving for each pair of electrons. The result is production of a proton gradient across the thylakoid membrane as electrons pass from PSII to PSI. Because the volume of the flattened thylakoid lumen is small, the influx of a small number of protons has a relatively large effect on luminal pH. The measured difference in pH between the stroma (pH 8) and the thylakoid lumen (pH 5) represents a 1,000-fold difference in proton concentration, a powerful driving force for ATP synthesis.

3.2.8.3.2. Cyclic electron flow between PSI and the cytochrome b6f complex increases the production of ATP relative to NADPH

Electron flow from PSII through the cytochrome b6 f complex, then through PSI to NADP+, is sometimes called noncyclic electron flow, to distinguish it from cyclic electron flow, which occurs to varying degrees depending primarily on the light conditions. The noncyclic path produces a proton gradient, which is used to drive ATP synthesis, and NADPH, which is used in reductive biosynthetic processes. Cyclic electron flow involves only PSI, not PSII. Electrons passing from P700 to ferredoxin do not continue to NADP+, but move back through the cytochrome b6 f complex to plastocyanin. Plastocyanin then donates electrons to P700, which transfers them to ferredoxin. In this way, electrons are repeatedly recycled through the cytochrome b6 f complex and the reaction centre of PSI, each electron propelled around the cycle by the energy of one photon. Cyclic electron flow is not accompanied by net formation of NADPH or evolution of O2. However, it is accompanied by proton pumping by the cytochrome b6 f complex and by phosphorylation of ADP to ATP, referred to as cyclic photophosphorylation.

By regulating the partitioning of electrons between NADP+ reduction and cyclic photophosphorylation, a plant adjusts the ratio of ATP to NADPH produced in the light-dependent reactions to match its needs for these products in the carbon-assimilation reactions and other biosynthetic processes. The carbon-assimilation reactions require ATP and NADPH in the ratio 3:2. This regulation of electron-transfer pathways is part of a short-term adaptation to changes in light colour (wave-length) and quantity (intensity).

4.8.4. Water is split by the oxygen-evolving complex

The ultimate source of the electrons passed to NADPH in plant (oxygenic) photosynthesis is water. Having given up an electron to pheophytin, P680+ (of PSII) must acquire an electron to return to its ground state in preparation for capture of another photon. In principle, the required electron might come from any number of organic or inorganic compounds. Photosynthetic bacteria use a variety of electron donors for this purpose - acetate, succinate, malate, or sulphide - depending on what is available in a particular ecological niche. About 3 billion years ago, evolution of primitive photosynthetic bacteria (the progenitors of the modern cyanobacteria) produced a photosystem capable of taking electrons from a donor that is always available: water (Fig. 8.6 and 8.7). Two water molecules are split, yielding four electrons, four protons, and molecular oxygen:

\[ 2H_2O \rightarrow 4H^+ + 4e^- + O_2 \]

A single photon of visible light does not have enough energy to break the bonds in water. Four photons are required in this photolytic cleavage reaction.
5. 8.5. ATP synthesis by photophosphorylation

The combined activities of the two plant photosystems move electrons from water to NADP⁺, conserving some of the energy of absorbed light as NADPH (Fig. 8.6 and 8.7). Simultaneously, protons are pumped across the thylakoid membrane and energy is conserved as an electrochemical potential.

During photosynthetic electron transfer ATP is generated from ADP and Pi in illuminated spinach chloroplasts. This process is called photophosphorylation, to distinguish it from oxidative phosphorylation in respiring mitochondria.

Several properties of photosynthetic electron transfer and photophosphorylation in chloroplasts indicate that a proton gradient plays the same role as in mitochondrial oxidative phosphorylation. (1) The reaction centres, electron carriers, and ATP-forming enzymes are located in a proton-impermeable membrane - the thylakoid membrane - which must be intact to support photophosphorylation. (2) Photophosphorylation can be uncoupled from electron flow by reagents that promote the passage of protons through the thylakoid membrane. (3) Photophosphorylation can be blocked by venturicidin and similar agents that inhibit the formation of ATP from ADP and Pi by the mitochondrial ATP synthase. (4) ATP synthesis is catalysed by F,F₁, complexes, located on the outer surface of the thylakoid membranes that are very similar in structure and function to the FoF₁ complexes of mitochondria.

Electron-transferring molecules in the chain of carriers connecting PSII and PSI are oriented asymmetrically in the thylakoid membrane, so photoinduced electron flow results in the net movement of protons across the membrane, from the stromal side to the thylakoid lumen (Fig. 8.6). The pH gradient across the thylakoid membrane (alkaline outside) could furnish the driving force to generate ATP.

The buffer slowly penetrated into the inner compartment of the thylakoids of the chloroplasts incubated in the dark in a pH 4 buffer, lowering their internal pH. The addition of ADP and Pi to the dark suspension of chloroplasts then suddenly raised the pH of the outer medium to 8, momentarily creating a large pH gradient across the membrane. As protons moved out of the thylakoids into the medium, ATP was generated from ADP and Pi (Fig. 8.8). Because the formation of ATP occurred in the dark (with no input of energy from light), this experiment showed that a pH gradient across the membrane is a high-energy state that, as in mitochondrial oxidative phosphorylation, can mediate the transduction of energy from electron transfer into the chemical energy of ATP (Fig. 8.8).

Figure 8.8. Photophosphorylation is chemiosmotic - http://www.biologie.uni-hamburg.de/b-online/library/bio201/jagendorf.html

5.1. 8.5.1. The ATP synthase of chloroplasts is like that of mitochondria

The enzyme responsible for ATP synthesis in chloroplasts is a large complex with two functional components, CF₀ and CF₁ (C denoting its location in chloroplasts). CF₀ is a transmembrane proton pore composed of
several integral membrane proteins and is homologous to mitochondrial F$_0$. CF$_1$ is a peripheral membrane protein complex very similar in subunit composition, structure, and function to mitochondrial F1. The orientation of the ATP synthase and the direction of proton pumping is the same in chloroplasts and mitochondria. In both cases, the F$_0$ portion of ATP synthase is located on the more alkaline side of the membrane through which protons flow down their concentration gradient (Fig. 8.9). The direction of proton flow relative to F$_1$ is the same in both cases. The mechanism of chloroplast ATP synthase is also believed to be essentially identical to that of its mitochondrial analogue. ADP and Pi readily condense to form ATP on the enzyme surface, and the release of this enzyme-bound ATP requires a proton-motive force. Rotational catalysis sequentially engages each of the three β subunits of the ATP synthase in ATP synthesis, ATP release, and ADP + Pi binding.

Figure 8.9. Comparison of the flow of H$^+$ and the orientation of the ATP synthase in mitochondria and chloroplasts - http://www.ncbi.nlm.nih.gov/books/NBK26819/figure/A2595/?report=objectonly
6. 8.6. Carbohydrate biosynthesis in plants

The synthesis of carbohydrates in animal cells always employs precursors having at least three carbons, all of which are less oxidized than the carbon in CO\textsubscript{2} (see chapter on gluconeogenesis). Plants and photosynthetic microorganisms, by contrast, can synthesize carbohydrates from CO\textsubscript{2} and water, reducing CO\textsubscript{2} at the expense of the energy and reducing power furnished by the ATP and NADPH that are generated by the light-dependent reactions of photosynthesis (Fig. 8.6).

Plants (and other autotrophs) can use CO\textsubscript{2} as the sole source of the carbon atoms required for the biosynthesis of cellulose and starch, lipids and proteins, and the many other organic components of plant cells. By contrast, heterotrophs cannot bring about the net reduction of CO\textsubscript{2} to achieve a net synthesis of glucose.
Green plants contain in their chloroplasts unique enzymatic machinery that catalyses the conversion of CO$_2$ to simple (reduced) organic compounds, a process called CO$_2$ assimilation.

Carbon dioxide is assimilated via a cyclic pathway, its key intermediates constantly regenerated. The pathway was elucidated in the early 1950s by Melvin Calvin, Andrew Benson, and James A. Bassham, and is often called the Calvin cycle or, more descriptively, the photosynthetic carbon reduction cycle.

6.1. 8.6.1. Carbon Dioxide assimilation occurs in three stages

The first stage in the assimilation of CO$_2$ into biomolecules (Fig. 8.10) is the carbon-fixation reaction: condensation of CO$_2$ with a five-carbon acceptor, ribulose 1,5-bisphosphate, to form two molecules of 3-phosphoglycerate. In the second stage, the 3-phosphoglycerate is reduced to triose phosphates.

Figure 8.10. The initial reaction in carbon fixation - http://www.ncbi.nlm.nih.gov/books/NBK26819/figure/A2571/?report=objectonly

Overall, three molecules of CO$_2$ are fixed to three molecules of ribulose 1,5-bisphosphate to form six molecules of glyceraldehyde 3-phosphate (18 carbons) in equilibrium with dihydroxyacetone phosphate. In the third stage, five of the six molecules of triose phosphate (15 carbons) are used to regenerate three molecules of ribulose 1,5-bisphosphate (15 carbons), the starting material. The sixth molecule of triose phosphate, the net product of photosynthesis, can be used to make hexoses for fuel and building materials, sucrose for transport to non-photosynthetic tissues, or starch for storage (Fig. 8.11). Thus the overall process is cyclical, with the continuous conversion of CO$_2$ to triose and hexose phosphates.

Figure 8.11. The carbon-fixation cycle, which forms organic molecules from CO$_2$ and H$_2$O - http://www.ncbi.nlm.nih.gov/books/NBK26819/figure/A2573/?report=objectonly
Photosynthesis – Calvin cycle

Fructose 6-phosphate is a key intermediate in stage 3 of CO₂ assimilation. It stands at a branch point, leading either to regeneration of ribulose 1,5-bisphosphate or to synthesis of starch. The pathway from **hexose phosphate to pentose bisphosphate** involves many of the same reactions used in animal cells for the conversion of pentose phosphates to hexose phosphates during the non-oxidative phase of the **pentose phosphate pathway** (Fig. 5.4). In the photosynthetic assimilation of CO₂, essentially the same set of reactions operates in the other direction, converting **hexose phosphates to pentose phosphates**. **This reductive pentose phosphate cycle uses the same enzymes as the oxidative pathway, and several more enzymes that make the reductive cycle irreversible. All 13 enzymes of the pathway are in the chloroplast stroma.**

The enzyme that catalyses incorporation of CO₂ into an organic form is ribulose 1,5-bisphosphate carboxylase/oxygenase, a name mercifully shortened to **rubisco**. As a carboxylase, rubisco catalyses the covalent attachment of CO₂ to the five-carbon sugar ribulose 1,5-bisphosphate and cleavage of the unstable six-carbon intermediate to form two molecules of 3-phosphoglycerate, one of which bears the carbon introduced as CO₂ in its carboxyl group (Fig. 8.10). The plant enzyme has an exceptionally low turnover number. Only three molecules of CO₂ are fixed per second per molecule of rubisco at 25 °C. To achieve high rates of CO₂ fixation, plants therefore need **large amounts** of this enzyme. In fact, rubisco makes up almost 50% of soluble protein in chloroplasts and is probably **one of the most abundant enzymes in the biosphere**.
The 3-phosphoglycerate formed in stage 1 is converted to glyceraldehyde-3-phosphate in two steps that are essentially the reversal of the corresponding steps in glycolysis, with one exception: the nucleotide cofactor for the reduction of 1,3-bisphosphoglycerate is NADPH rather than NADH (Fig. 8.11). The chloroplast stroma contains all the glycolytic enzymes except phosphoglycerate mutase.

In the first step of stage 2, the stromal 3-phospho-glycerate kinase catalyses the transfer of a phosphoryl group from ATP to 3-phosphoglycerate, yielding 1,3-bisphosphoglycerate. Next, NADPH donates electrons in a reduction catalysed by the chloroplast-specific isozyme of glyceraldehyde 3-phosphate dehydrogenase, producing glyceraldehyde 3-phosphate and Pi. Triose phosphate isomerase then interconverts glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (Fig. 8.11). Most of the triose phosphate thus produced is used to regenerate ribulose 1,5-bisphosphate; the rest is either converted to starch in the chloroplast and stored for later use or immediately exported to the cytosol and converted to sucrose for transport to growing regions of the plant. In developing leaves, a significant portion of the triose phosphate may be degraded by glycolysis to provide energy.

The first reaction in the assimilation of CO₂ into triose phosphates consumes ribulose 1,5-bisphosphate and, for continuous flow of CO₂ into carbohydrate, ribulose 1,5-bisphosphate must be constantly regenerated. This is accomplished in a series of reactions that, together with stages 1 and 2, constitute the cyclic pathway shown in Fig. 8.11. The product of the first assimilation reaction (3-phosphoglycerate) thus undergoes transformations that regenerate ribulose 1,5-bisphosphate. The intermediates in this pathway include three-, four-, five-, six-, and seven-carbon sugars.

Steps 1 and 4 are catalysed by the same enzyme, aldolase. It first catalyses the reversible condensation of glyceraldehyde 3-phosphate with dihydroxyacetone phosphate, yielding fructose 1,6-bisphosphate (step 1); this is cleaved to fructose 6-phosphate and Pi by fructose 1,6-bisphosphatase in step 2. The reaction is strongly exergonic and essentially irreversible. Step 3 is catalysed by transketolase. Transketolase catalyses the reversible transfer of a 2-carbon ketol group (CH₂OH-CO₂) from a ketose phosphate donor, fructose 6-phosphate, to an aldose phosphate acceptor, glyceraldehyde 3-phosphate, forming the pentose xylulose 5-phosphate and the tetrose erythrose 4-phosphate. In step 4, aldolase acts again, combining erythrose 4-phosphate with dihydroxyacetone phosphate to form the seven-carbon sedoheptulose 1,7-bisphosphate. An enzyme unique to plastids, sedoheptulose 1,7-bisphosphatase, converts the bisphosphate to sedoheptulose 7-phosphate (step 5); this is the second irreversible reaction in the pathway. Transketolase now acts again, converting sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate to two pentose phosphates in step 6. The pentose phosphates formed in the transketolase reactions - ribose 5-phosphate and xylulose 5-phosphate - are converted to ribulose 5-phosphate (steps 7 and 8), which in the final step (9) of the cycle is phosphorylated to ribulose 1,5-bisphosphate by ribulose 5-phosphate kinase. This is the third very exergonic reaction of the pathway, as the phosphate anhydride bond in ATP is swapped for a phosphate ester in ribulose 1,5-bisphosphate.

Synthesis of each triose phosphate from CO₂ requires six NADPH and nine ATP. The net result of three turns of the Calvin cycle is the conversion of three molecules of CO₂ and one molecule of phosphate to a molecule of triose phosphate. Three molecules of ribulose 1,5-bisphosphate (a total of 15 carbons) condense with three CO₂ (3 carbons) to form six molecules of 3-phosphoglycerate (18 carbons). These six molecules of 3-phosphoglycerate are reduced to six molecules of glyceraldehyde 3-phosphate (which is in equilibrium with dihydroxyacetone phosphate), with the expenditure of six ATP (in the synthesis of 1,3-bisphosphoglycerate) and six NADPH (in the reduction of 1,3-bisphosphoglycerate to glyceraldehyde 3-phosphate). One molecule of glyceraldehyde 3-phosphate is the net product of the carbon assimilation pathway. The other five triose phosphate molecules (15 carbons) are rearranged to form three molecules of ribulose 1,5-bisphosphate (15 carbons). The last step in this conversion requires one ATP per ribulose 1,5-bisphosphate, or a total of three ATP.

Thus, in summary, for every molecule of triose phosphate produced by photosynthetic CO₂ assimilation, six NADPH and nine ATP are required. NADPH and ATP are produced in the light-dependent reactions of photosynthesis in about the same ratio (2:3) as they are consumed in the Calvin cycle.

6.2. 8.6.2. Photorespiration and the C4 and CAM pathways

In the dark, plants also carry out mitochondrial respiration, the oxidation of substrates to CO₂ and the conversion of O₂ to H₂O. And there is another process in plants that, like mitochondrial respiration, consumes O₂ and produces CO₂ and, like photosynthesis, is driven by light. This process, photorespiration, is a costly side reaction of photosynthesis, a result of the lack of specificity of the enzyme rubisco. Rubisco is not absolutely specific for CO₂ as a substrate.
Molecular oxygen (O₂) competes with CO₂ at the active site, and about once in every three or four turnovers, rubisco catalyses the condensation of O₂ with ribulose1,5-bisphosphate to form 3-phosphoglycerate and 2-phosphoglycolate (Fig. 8.12), a metabolically useless product. This is the oxygenase activity referred to in the full name of the enzyme: ribulose 1,5-bisphosphate carboxylase/oxygenase. The reaction with O₂ results in no fixation of carbon and seems to be a net liability to the cell. Salvaging the carbons from 2-phosphoglycolate consumes significant amounts of cellular energy and releases some previously fixed CO₂ (Fig. 8.12). Given that the reaction with oxygen is deleterious to the organism, why did the evolution of rubisco produce an active site unable to discriminate well between CO₂ and O₂? Perhaps much of this evolution occurred before the time, about 2.5 billion years ago, when production of O₂ by photosynthetic organisms started to raise the oxygen content of the atmosphere.

Before that time, there was no selective pressure for rubisco to discriminate between CO₂ and O₂. The Kₘ for CO₂ is about 9 μM, and that for O₂ is about 350 μM. The modern atmosphere contains about 20% O₂ and only 0.04% CO₂, so an aqueous solution in equilibrium with air at room temperature contains about 250 μM O₂ and 11 μM CO₂, concentrations that allow significant O₂ “fixation” by rubisco and thus a significant waste of energy. The temperature dependence of the solubilities of O₂ and CO₂ is such that at higher temperatures, the ratio of O₂ to CO₂ in solution increases. In addition, the affinity of rubisco for CO₂ decreases with increasing temperature, exacerbating its tendency to catalyse the wasteful oxygenase reaction.

6.2.1. 8.6.2.1. The salvage of phosphoglycolate

The glycolate pathway converts two molecules of 2-phosphoglycolate to a molecule of serine (three carbons) and a molecule of CO₂ (Fig. 8.12). In the chloroplast, a phosphatase converts 2-phosphoglycolate to glycolate, which is exported to the peroxisome. There, glycolate is oxidized by molecular oxygen, and the resulting aldehyde (glyoxylate) undergoes transamination to glycine. The hydrogen peroxide formed as a side product of glycolate oxidation is rendered harmless by peroxidases in the peroxisome. Glycine passes from the peroxisome to the mitochondrial matrix, where it undergoes oxidative decarboxylation by the glycine
decarboxylase (GDC) complex, an enzyme similar in structure and mechanism to two mitochondrial complexes we have already encountered: the pyruvate dehydrogenase complex and the α-ketoglutarate dehydrogenase complex. The glycine decarboxylase complex oxidizes glycine to CO₂ and NH₃, with the concomitant reduction of NAD⁺ to NADH and transfer of the remaining carbon from glycine to a second glycine producing serine. The serine is converted to hydroxypruvinate, to glycerate, and finally to 3-phosphoglycercrate, which is used to regenerate ribulose 1,5-bisphosphate, completing the long, expensive cycle (Fig. 8.12).

In bright sunlight, the flux through the glycolate salvage pathway can be very high, producing about five times more CO₂ than is typically produced by all the oxidations of the citric acid cycle. To generate this large flux, mitochondria contain prodigious amounts of the glycine decarboxylase complex: the four proteins of the complex make up half of all the protein in the mitochondrial matrix in the leaves of pea and spinach plants! The combined activity of the rubisco oxygenase and the glycolate salvage pathway consumes O₂ and produces CO₂, hence the name photorespiration.

6.2.2. 8.6.2.2. In C₄ plants, CO₂ fixation and rubisco activity are spatially separated

In many plants that grow in the tropics (and in temperate-zone crop plants native to the tropics, such as maize, sugarcane, and sorghum) a mechanism has evolved to circumvent the problem of wasteful photorespiration.

The step in which CO₂ is fixed into a three-carbon product, 3-phosphoglycerate, is preceded by several steps, one of which is temporary fixation of CO₂ into a four-carbon compound. Plants that use this process are referred to as C₄ plants, and the assimilation process as C₄ metabolism or the C₄ pathway. Plants that use the carbon-assimilation method we have described thus far, in which the first step is reaction of CO₂ with ribulose 1,5-bisphosphate to form 3-phosphoglycerate, are called C₃ plants.

The C₄ plants, which typically grow at high light intensity and high temperatures, have several important characteristics: high photosynthetic rates, high growth rates, low photorespiration rates, low rates of water loss, and a specialized leaf structure. Photosynthesis in the leaves of C₄ plants involves two cell types: mesophyll and bundle-sheath cells (Fig. 8.13).

Figure 8.13. Comparative leaf anatomy in a C₃ plant and a C₄ plant - http://www.ncbi.nlm.nih.gov/books/NBK26819/figure/A2575/?report=objectonly
In plants of tropical origin, the first intermediate is oxaloacetate, a four-carbon compound. This reaction, which occurs in the cytosol of leaf mesophyll cells, is catalysed by phosphoenolpyruvate carboxylase, for which the substrate is \( \text{HCO}_3^- \), not \( \text{CO}_2 \). The oxaloacetate thus formed is either reduced to malate at the expense of NADPH (Fig. 8.14) or converted to aspartate by transamination: The malate or aspartate formed in the mesophyll cells then passes into neighbouring bundle-sheath cells. In the bundle-sheath cells, malate is oxidized and decarboxylated to yield pyruvate and \( \text{CO}_2 \) by the action of malic enzyme, reducing NADP+. In plants that use aspartate as the \( \text{CO}_2 \) carrier, aspartate arriving in bundle-sheath cells is transaminated to form oxaloacetate and reduced to malate, then the \( \text{CO}_2 \) is released by malic enzyme or PEP carboxykinase. The free \( \text{CO}_2 \) released in the bundle-sheath cells is the same \( \text{CO}_2 \) molecule originally fixed into oxaloacetate in the mesophyll cells. This \( \text{CO}_2 \) is now fixed again, this time by rubisco, in exactly the same reaction that occurs in C3 plants: incorporation of \( \text{CO}_2 \) into C-1 of 3-phosphoglycerate. The pyruvate formed by decarboxylation of malate in bundle-sheath cells is transferred back to the mesophyll cells, where it is converted to PEP by an unusual enzymatic reaction catalysed by pyruvate phosphate dikinase (Fig. 8.14).

**Figure 8.14. CO\textsubscript{2} assimilation pathway in C4 plants** - http://plantcellbiology.masters.grkraj.org/html/Plant_Cell_Biochemistry_And_Metabolism6-Plant_Cell_Energy_transductions2-Photosynthesis.htm

This enzyme is called a dikinase because two different molecules are simultaneously phosphorylated by one molecule of ATP: pyruvate to PEP, and phosphate to pyrophosphate. The pyrophosphate is subsequently hydrolysed to phosphate, so two high-energy phosphate groups of ATP are used in regenerating PEP. The PEP is now ready to receive another molecule of \( \text{CO}_2 \) in the mesophyll cell.

**The PEP carboxylase of mesophyll cells has a high affinity for HCO\textsubscript{3}^-** (which is favoured relative to \( \text{CO}_2 \) in aqueous solution and can fix \( \text{CO}_2 \) more efficiently than can rubisco). Unlike rubisco, it does not use \( \text{O}_2 \) as an alternative substrate, so there is no competition between \( \text{CO}_2 \) and \( \text{O}_2 \). The PEP carboxylase reaction, then, serves to fix and concentrate \( \text{CO}_2 \) in the form of malate. Release of \( \text{CO}_2 \) from malate in the bundle-sheath cells yields a sufficiently high local concentration of \( \text{CO}_2 \) for rubisco to function near its maximal rate, and for suppression of the enzyme’s oxygenase activity.

Once \( \text{CO}_2 \) is fixed into 3-phosphoglycerate in the bundle-sheath cells, the other reactions of the Calvin cycle take place exactly as described earlier. Thus in C4 plants, mesophyll cells carry out \( \text{CO}_2 \) assimilation by the C4 pathway and bundle-sheath cells synthesize starch and sucrose by the C3 pathway.
Three enzymes of the C4 pathway are regulated by light, becoming more active in daylight: malate dehydrogenase, PEP carboxylase and pyruvate phosphate dikinase.

**The pathway of CO₂ assimilation has a greater energy cost in C4 plants than in C3 plants.** For each molecule of CO₂ assimilated in the C4 pathway, a molecule of PEP must be regenerated at the expense of two high-energy phosphate groups of ATP. **Thus C4 plants need five ATP molecules to assimilate one molecule of CO₂, whereas C3 plants need only three (nine per triose phosphate).** As the temperature increases (and the affinity of rubisco for CO₂ decreases, as noted above), a point is reached (at about 28 to 30 °C) at which the gain in efficiency from the elimination of photorespiration more than compensates for this energetic cost. C4 plants (crabgrass, for example) outgrow most C3 plants during the summer, as any experienced gardener can attest.

6.2.3. 8.6.2.3. **In CAM plants, CO₂ capture and rubisco action are temporally separated**

Succulent plants such as cactus and pineapple, which are native to very hot, very dry environments, have another variation on photosynthetic CO₂ fixation, which reduces loss of water vapour through the pores (stomata) by which CO₂ and O₂ must enter leaf tissue. Instead of separating the initial trapping of CO₂ and its fixation by rubisco across space (as do the C4 plants), they separate these two events over time. **At night, when the air is cooler and moister, the stomata open to allow entry of CO₂, which is then fixed into oxaloacetate by PEP carboxylase.** The oxaloacetate is reduced to malate and stored in the vacuoles, to protect cytosolic and plastid enzymes from the low pH produced by malic acid dissociation. During the day the stomata close, preventing the water loss that would result from high daytime temperatures, and the CO₂ trapped overnight in malate is released as CO₂ by the NADP-linked malic enzyme. **This CO₂ is now assimilated by the action of rubisco and the Calvin cycle enzymes.** Because this method of CO₂ fixation was first discovered in stonecrops, perennial flowering plants of the family Crassulaceae, it is called crassulacean acid metabolism, and the plants are called **CAM plants.**
Chapter 9. Lipid metabolism – Fatty acid oxidation

Lipids are poorly soluble in the aqueous interior of cells and in extracellular fluids. Cells use lipids for storing energy, building membranes, signalling within and between cells, sensing the environment, covalently modifying proteins, forming specialized permeability barriers (e.g., in skin), and protecting cells from highly reactive chemicals. Fatty acids, which are oxidized in mitochondria to release energy for cellular functions, are stored and transported primarily in the form of triglycerides. Fatty acids are also precursors of phospholipids, the structural backbone of cellular membranes. Cholesterol, another important membrane component, is a precursor for steroid hormones and other biologically active lipids that function in cell-cell signalling.

1. 9.1. Lipid transport

Fat absorbed from the diet and lipids synthesized by the liver and adipose tissue must be transported between the various tissues and organs for utilization and storage. Since lipids are insoluble in water, the problem of how to transport them in the aqueous blood plasma is solved by associating nonpolar lipids (triacylglycerol and cholesteryl esters) with amphipathic lipids (phospholipids and cholesterol) and proteins to make water-miscible lipoproteins.

Lipoproteins transport lipids from the intestines as chylomicrons - and from the liver as very low density lipoproteins (VLDL) - to most tissues for oxidation and to adipose tissue for storage. Lipid is mobilized from adipose tissue as free fatty acids (FFA) attached to serum albumin. Abnormalities of lipoprotein metabolism cause various hypo- or hyperlipoproteinemias.

Four major groups of lipoproteins have been identified that are important physiologically and in clinical diagnosis.

These are (1) chylomicrons, derived from intestinal absorption of triacylglycerol and other lipids; (2) very low density lipoproteins (VLDL, or pre-β-lipoproteins), derived from the liver for the export of triacylglycerol; (3) low-density lipoproteins (LDL, or β-lipoproteins), representing a final stage in the catabolism of VLDL; and (4) high-density lipoproteins (HDL, or α-lipoproteins), involved in VLDL and chylomicron metabolism and also in cholesterol transport. Triacyl-glycerol is the predominant lipid in chylomicrons and VLDL, whereas cholesterol and phospholipid are the predominant lipids in LDL and HDL, respectively (Fig. 9.1).

Figure 9.1. The primary pathways for the metabolism of human plasma lipoproteins - http://www.nature.com/ng/journal/v40/n2/fig_tab/ng0208-129_F1.html
2.9.2. Mitochondrial oxidation of fatty acids

Fatty acids are stored as triacylglycerols, primarily as droplets in adipose (fat-storing) cells. In response to hormones such as adrenaline, triacylglycerols are hydrolysed in the cytosol to free fatty acids and glycerol: Fatty acids released into the blood are taken up and oxidized by most other cells, constituting the major energy source for many tissues, particularly heart muscle. In humans, the oxidation of fats is quantitatively more important than the oxidation of glucose as a source of ATP. The oxidation of 1 g of triacylglycerol to $\text{CO}_2$ generates about six times as much ATP as does the oxidation of 1 g of hydrated glycogen, the polymeric storage form of glucose in muscle and liver. Triglycerides are more efficient for storage of energy because they are stored in anhydrous form and are much more reduced (have more hydrogen) than carbohydrates, and therefore yield more energy when oxidized. In the cytosol, free fatty acids are esterified to coenzyme A to form a fatty acyl-CoA in an exergonic reaction coupled to the hydrolysis of ATP to AMP and PP$_i$ (inorganic pyrophosphate):

Subsequent hydrolysis of PP$_i$, to two molecules of phosphate (P$_i$), drives this reaction to completion. Then the fatty acyl group is transferred to carnitine and moved across the inner mitochondrial membrane by an acylcarnitine transporter protein (Fig. 9.2); on the matrix side, the fatty acyl group is released from carnitine and reattached to another CoA molecule.

Figure 9.2. The transport of fatty acyl group into the mitochondrial matrix - http://www.ncbi.nlm.nih.gov/books/NBK28177/figure/A2964/

Abbreviations: LCAT: lecithin-cholesterol acyltransferase, CETP: Cholesteryl ester transfer protein
Each molecule of a fatty acyl CoA in the mitochondrion is oxidized in a cyclical sequence of four reactions in which all the carbon atoms are converted to acetyl CoA with generation of NADH and FADH$_2$ (Fig. 9.3). For example, mitochondrial oxidation of each molecule of the 18-carbon stearic acid, CH$_3$(CH$_2$)$_{16}$COOH, yields nine molecules of acetyl CoA and eight molecules each of NADH and FADH$_2$. In β-oxidation (Fig. 9.3), two carbons at a time are cleaved from acyl-CoA molecules, starting at the carboxyl end. The chain is broken between the α(2)- and β(3)-carbon atoms, hence the name β-oxidation. The two-carbon units formed are acetyl-CoA; thus, palmitoyl-CoA forms eight acetyl-CoA molecules.

The first step is the removal of two hydrogen atoms from the 2(α)- and 3(β)-carbon atoms, catalysed by acyl-CoA dehydrogenase and requiring FAD. This results in the formation of Δ2-trans-enoyl-CoA and FADH$_2$. The reoxidation of FADH$_2$ by the respiratory chain requires the mediation of another flavoprotein, termed electron-transferring flavoprotein. Water is added to saturate the double bond and form 3-hydroxyacyl-CoA, catalysed by Δ2-enoyl-CoA hydratase. The 3-hydroxy derivative undergoes further dehydrogenation on the 3-carbon catalysed by L(+)-3-hydroxyacyl-CoA dehydrogenase to form the corresponding 3-ketoacyl-CoA compound. In this case, NAD$_+$ is the coenzyme involved. Finally, 3-ketoacyl-CoA is split at the 2,3-position by thiolase (3-keto-acyl-CoA-thiolase), forming acetyl-CoA and a new acyl-CoA two carbons shorter than the original acyl-CoA molecule. The acyl-CoA formed in the cleavage reaction reenters the oxidative pathway at reaction 2 (Fig. 9.3). In this way, a long-chain fatty acid may be degraded completely to acetyl-CoA (C2 units).
Since acetyl-CoA can be oxidized to CO₂ and water via the citric acid cycle (which is also found within the mitochondria), the complete oxidation of fatty acids is achieved.

Electrons from the reduced coenzymes produced in the oxidation of fatty acyl CoA to acetyl CoA and in the subsequent oxidation of acetyl CoA in the citric acid cycle move via the respiratory chain to O₂. This electron movement is coupled to generation of a proton-motive force that is used to power ATP synthesis as described previously for the oxidation of pyruvate.

A modified form of β-oxidation is found in peroxisomes and leads to the formation of acetyl-CoA and H₂O₂ (from the flavoprotein-linked dehydrogenase step), which is broken down by catalase. Thus, this
dehydrogenation in peroxisomes is not linked directly to phosphorylation and the generation of ATP. The system facilitates the oxidation of very long chain fatty acids (C20, C22). The enzymes in peroxisomes do not attack shorter chain fatty acids; the β-oxidation sequence ends at octanoyl-CoA. Octanoyl and acetyl groups are both further oxidized in mitochondria. Another role of peroxisomal β-oxidation is to shorten the side chain of cholesterol in bile acid formation.

2.1. 9.2.1. Oxidation of a fatty acid with an odd number of carbon atoms

Fatty acids with an odd number of carbon atoms are oxidized by the pathway of β-oxidation, producing acetyl-CoA, until a three-carbon (propionyl-CoA) residue remains. This compound is converted to succinyl-CoA, a constituent of the citric acid cycle. Hence, the propionyl residue from an odd-chain fatty acid is the only part of a fatty acid that is glucogenic.

2.2. 9.2.2. Oxidation of unsaturated fatty acids

The CoA esters of these acids are degraded by the enzymes normally responsible for β-oxidation until either a Δ3-cis-acyl-CoA compound or a Δ4-cis-acyl-CoA compound is formed, depending upon the position of the double bonds. The former compound is isomerized to the corresponding Δ2-trans-CoA stage of β-oxidation for subsequent hydration and oxidation. Any Δ4-cis-acyl-CoA either remaining, as in the case of linoleic acid, or entering the pathway at this point after conversion by acyl-CoA dehydrogenase to Δ2-trans-Δ4-cis-dienoyl-CoA, is then metabolized as indicated in.

3. 9.3. Generation of ketone bodies

Under metabolic conditions associated with a high rate of fatty acid oxidation, the liver produces considerable quantities of acetoacetate and β-hydroxybutyrate. Acetoacetate continually undergoes spontaneous decarboxylation to yield acetone.

These three substances are collectively known as the ketone bodies. Acetoacetate and 3-hydroxybutyrate are interconverted by the mitochondrial enzyme β-hydroxybutyrate dehydrogenase; the equilibrium is controlled by the mitochondrial [NAD+] /[NADH] ratio, i.e., the redox state (Fig. 9.4).

Figure 9.4. Formation of ketone bodies - http://www.ncbi.nlm.nih.gov/books/NBK22387/figure/A3076/

The concentration of total ketone bodies in the blood of well-fed mammals does not normally exceed 0.2 mmol/L, except in ruminants, where 3-hydroxybutyrate is formed continuously from butyric acid (a product of ruminal fermentation) in the rumen wall. In vivo, the liver appears to be the only organ in non-ruminants to add significant quantities of ketone bodies to the blood. Extrahepatic tissues utilize them as respiratory substrates. The net flow of ketone bodies from the liver to the extrahepatic tissues results from active hepatic synthesis coupled with very low utilization. The reverse situation occurs in extrahepatic tissues. Enzymes responsible for ketone body formation are associated mainly with the mitochondria. Two acetyl-CoA molecules formed in β-oxidation condense with one another to form acetoacetyl-CoA by a reversal of the thiolase reaction. Acetoacetyl-CoA, which is the starting material for ketogenesis, also arises directly from the terminal four carbons of a fatty acid during β-oxidation. Condensation of acetoacetyl-CoA with another molecule of acetyl-CoA by 3-hydroxy-
3-methylglutaryl-CoA synthase forms HMG-CoA. 3-Hydroxy-3-methylglutaryl-CoA lyase then causes acetyl-CoA to split off from the HMG-CoA, leaving free acetoacetate (Fig. 9.4). The carbon atoms split off in the acetyl-CoA molecule are derived from the original acetoacetyl-CoA molecule. Both enzymes must be present in mitochondria for ketogenesis to take place. This occurs solely in liver and rumen epithelium. D-3-Hydroxybutyrate is quantitatively the predominant ketone body present in the blood and urine in ketosis.

In most cases, ketonemia is due to increased production of ketone bodies by the liver rather than to a deficiency in their utilization by extrahepatic tissues.

4. 9.4. Biosynthesis of fatty acids

Fatty acids are synthesized by an extramitochondrial system, which is responsible for the complete synthesis of palmitate from acetyl-CoA in the cytosol. This system is present in many tissues, including liver, kidney, brain, lung, mammary gland, and adipose tissue. Its cofactor requirements include NADPH, ATP, Mn²⁺, biotin, and HCO₃⁻. **Acetyl-CoA is the immediate substrate and free palmitate is the end product.**

Bicarbonate as a source of CO₂ is required in the initial reaction for the carboxylation of acetyl-CoA to malonyl-CoA in the presence of ATP and acetyl-CoA carboxylase. In bacteria and plants, the individual enzymes of the fatty acid synthase system are separate, and the acyl radicals are found in combination with a protein called the acyl carrier protein (ACP). However, in yeast, mammals, and birds, the synthase system is a **multienzyme polypeptide complex** that incorporates ACP, which takes over the role of CoA.
In mammals, the fatty acid synthase complex is a dimer comprising two identical monomers, each containing all seven enzyme activities of fatty acid synthase on one polypeptide chain. Initially, a priming molecule of acetyl-CoA combines with a cysteine -SH group catalysed by acetyl transacylase (Fig. 9.5). Malonyl-CoA combines with the adjacent -SH on the 4′-phosphopantetheine of ACP of the other monomer, catalysed by malonyltransacylase (reaction 2b), to form acetyl (acyl)-malonyl enzyme. The acetyl group attacks the methylene group of the malonyl residue, catalysed by 3-ketoacyl synthase, and liberates CO₂, forming 3-ketoacyl enzyme (acetoacetyl enzyme) (reaction 3), freeing the cysteine -SH group. Decarboxylation allows the reaction to go to completion, pulling the whole sequence of reactions in the forward direction. The 3-ketoacyl group is reduced, dehydrated, and reduced again (reactions 4, 5, 6) to form the corresponding saturated acyl-enzyme. A new malonyl-CoA molecule combines with the -SH of 4′-phosphopantetheine, displacing the saturated acyl residue onto the free cysteine -SH group.

The sequence of reactions is repeated six more times until a saturated 16-carbon acyl radical (palmityl) has been assembled. It is liberated from the enzyme complex by the activity of a seventh enzyme in the complex, thioesterase (deacylase). The free palmitate must be activated to acyl-CoA before it can proceed via any other metabolic pathway. Its usual fate is esterification into acylglycerols, chain elongation or desaturation, or esterification to cholesteryl ester.

The equation for the overall synthesis of palmitate from acetyl-CoA and malonyl-CoA is:

\[ \text{CH}_3\text{CO}-\text{S-CoA} + 7 \text{HOOC-CH}_2\text{CO-S-CoA} + 14 \text{NADPH} + 14 \text{H}^+ \rightarrow \text{CH}_3(\text{CH}_2)_14\text{COOH} + 7\text{CO}_2 + 6\text{H}_2\text{O} + 8 \text{CoA-SH} + 14\text{NADP}^+ \]
The acetyl-CoA used as a primer forms carbon atoms 15 and 16 of palmitate. The addition of all the subsequent C2 units is via malonyl-CoA. Propionyl-CoA acts as primer for the synthesis of long-chain fatty acids having an odd number of carbon atoms, found particularly in ruminant fat and milk.

The main source of NADPH for lipogenesis is the pentose phosphate pathway.

Citrate, formed after condensation of acetyl-CoA with oxaloacetate in the citric acid cycle within mitochondria, is translocated into the extramitochondrial compartment via the tricarboxylate transporter, where in the presence of CoA and ATP it undergoes cleavage to acetyl-CoA and oxaloacetate catalysed by ATP-citrate lyase, which increases in activity in the well-fed state. The acetyl-CoA is then available for malonyl-CoA formation and synthesis to palmitate.

Elongation of fatty acid chains occurs in the endoplasmic reticulum.

Lipogenesis converts surplus glucose and intermediates such as pyruvate, lactate, and acetyl-CoA to fat, assisting the anabolic phase of this feeding cycle. The nutritional state of the organism is the main factor regulating the rate of lipogenesis.

5. 9.5. Cholesterol

Cholesterol is present in tissues and in plasma either as free cholesterol or as a storage form, combined with a long-chain fatty acid as cholesteryl ester. In plasma, both forms are transported in lipoproteins (Chapter 9.1). Cholesterol is an amphipathic lipid and as such is an essential structural component of membranes and of the outer layer of plasma lipoproteins. It is synthesized in many tissues from acetyl-CoA and is the precursor of all other steroids in the body such as corticosteroids, sex hormones, bile acids, and vitamin D. As a typical product of animal metabolism, cholesterol occurs in foods of animal origin such as egg yolk, meat, liver, and brain. Plasma low-density lipoprotein (LDL) is the vehicle of uptake of cholesterol and cholesteryl ester into many tissues. Free cholesterol is removed from tissues by plasma high-density lipoprotein (HDL) and transported to the liver, where it is eliminated from the body either unchanged or after conversion to bile acids in the process known as reverse cholesterol transport. Cholesterol is a major constituent of gallstones. However, its chief role in pathologic processes is as a factor in the genesis of atherosclerosis of vital arteries, causing cerebrovascular, coronary, and peripheral vascular disease.

At this point we should mention a class of drugs the statins. These drugs can lower blood cholesterol by blocking the HMG-CoA-reductase enzyme (Fig. 9.6).

**Figure 9.6. Cholesterol biosynthesis**

http://circ.ahajournals.org/content/123/18/1925/F1.large.jpg
Chapter 10. Protein, amino acid metabolism

All 20 of the amino acids present in proteins are essential for health. Humans can synthesize 12 of the 20 common amino acids from the amphibolic intermediates of glycolysis and of the citric acid cycle (Fig. 10.1). While nutritionally nonessential, these 12 amino acids are not “nonessential.”

All 20 amino acids are biologically essential. Of the 12 nutritionally nonessential amino acids, nine are formed from amphibolic intermediates and three (cysteine, tyrosine and hydroxylysine) from nutritionally essential amino acids.

1. 10.1. Nutritionally nonessential amino acids have short biosynthetic pathways

The enzymes glutamate dehydrogenase, glutamine synthetase, and aminotransferases occupy central positions in amino acid biosynthesis. The combined effect of those three enzymes is to transform ammonium ion into the α-amino nitrogen of various amino acids.

Glutamate and Glutamine: Reductive amination of α-ketoglutarate is catalysed by glutamate dehydrogenase (Fig. 10.1). Amination of glutamate to glutamine is catalysed by glutamine synthetase (Fig. 10.1).

Alanine: Transamination of pyruvate forms alanine (Fig. 10.1).

Aspartate and Asparagine: Transamination of oxaloacetate forms aspartate. The conversion of aspartate to asparagine is catalysed by asparagine synthetase (Fig. 10.1), which resembles glutamine synthetase (Fig. 10.1) except that glutamine, not ammonium ion, provides the nitrogen. Bacterial asparagine synthetases can, however, also use ammonium ion. Coupled hydrolysis of PP_i to P_i by pyrophosphatase ensures that the reaction is strongly favoured.

Serine: Oxidation of the α-hydroxyl group of the glycolytic intermediate 3-phosphoglycerate converts it to an oxo acid, whose subsequent transamination and dephosphorylation leads to serine (Fig. 10.1).

Glycine: Glycine aminotransferases can catalyse the synthesis of glycine from glyoxylate and glutamate or alanine. Unlike most aminotransferase reactions, these strongly favour glycine synthesis. Additional important mammalian routes for glycine formation are from choline and from serine.

Proline: Proline is formed from glutamate by reversal of the reactions of proline catabolism (Fig. 10.1).

Cysteine: Cysteine, while not nutritionally essential, is formed from methionine, which is nutritionally essential. Following conversion of methionine to homocysteine, homocysteine and serine form cysteine and homoserine.

Tyrosine: Phenylalanine hydroxylase converts phenylalanine to tyrosine. Provided that the diet contains adequate nutritionally essential phenylalanine, tyrosine is nutritionally nonessential.

Valine, Leucine, and Isoleucine: While leucine, valine, and isoleucine are all nutritionally essential amino acids, tissue aminotransferases reversibly interconvert all three amino acids and their corresponding α-keto acids. These α-keto acids thus can replace their amino acids in the diet.

Figure 10.1. Synthesis of non-essential Amino Acids - http://www.personal.kent.edu/~cearley/PChem/amo/nonessential.htm
2. 10.2. Catabolism of proteins and of amino acid nitrogen

While ammonia, derived mainly from the α-amino nitrogen of amino acids, is highly toxic, tissues convert ammonia to the amide nitrogen of nontoxic glutamine. Subsequent deamination of glutamine in the liver releases ammonia, which is then converted to nontoxic urea.

Urea biosynthesis occurs in four stages: (1) transamination, (2) oxidative deamination of glutamate, (3) ammonia transport, and (4) reactions of the urea cycle (Fig. 10.2).

2.1. 10.2.1. Transamination

Transamination interconverts pairs of α-amino acids and α-keto acids (Fig. 10.2). All the protein amino acids except lysine, threonine, proline, and hydroxyproline participate in transamination. Transamination is readily reversible, and aminotransferases also function in amino acid biosynthesis. All the amino nitrogen from amino acids that undergo transamination can be concentrated in glutamate. This is important because L-glutamate is the only amino acid that undergoes oxidative deamination at an appreciable rate in mammalian tissues.

2.2. 10.2.2. Oxidative deamination of glutamate
The formation of ammonia from α-amino groups thus occurs mainly via the α-amino nitrogen of L-glutamate. Transfer of amino nitrogen to α-ketoglutarate forms L-glutamate. Release of this nitrogen as ammonia is then catalysed by hepatic L-glutamate dehydrogenase (GDH), which can use either NAD⁺ or NADP⁺. Conversion of α-amino nitrogen to ammonia by the concerted action of glutamate aminotransferase and GDH is often termed “transdeamination.”

The ammonia produced by enteric bacteria and absorbed into portal venous blood and the ammonia produced by tissues are rapidly removed from circulation by the liver and converted to urea. Only traces (10–20 μg/dL) thus normally are present in peripheral blood. This is essential, since ammonia is toxic to the central nervous system.

2.3. 10.2.3. Ammonia transport

Glutamine synthase fixes ammonia as glutamine. Formation of glutamine is catalysed by mitochondrial glutamine synthase (Fig. 10.2). Since amide bond synthesis is coupled to the hydrolysis of ATP to ADP and Pₐ, the reaction strongly favours glutamine synthesis. One function of glutamine is to sequester ammonia in a nontoxic form.

Hydrolytic release of the amide nitrogen of glutamine as ammonia, catalysed by glutaminase (Fig. 10.2), strongly favours glutamate formation. The concerted action of glutamine synthase and glutaminase thus catalyses the interconversion of free ammonium ion and glutamine. An analogous reaction is catalysed by L-asparaginase.

2.4. 10.2.4. Reactions of the urea cycle

Synthesis of 1 mol of urea requires 3 mol of ATP plus 1 mol each of ammonium ion and of the α-amino nitrogen of aspartate. Five enzymes catalyse the numbered reactions of Fig. 10.2. Urea synthesis is a cyclic process. Since the ornithine consumed in reaction 2 is regenerated in reaction 5, there is no net loss or gain of ornithine, citrulline, argininosuccinate, or arginine. Ammonium ion, CO₂, ATP, and aspartate are, however, consumed. Some reactions of urea synthesis occur in the matrix of the mitochondrion, other reactions in the cytosol (Fig. 10.2).

1. Condensation of CO₂, ammonia, and ATP to form carbamoyl phosphate is catalysed by mitochondrial carbamoyl phosphate synthase I (Fig. 10.2). Carbamoyl phosphate synthase I, the rate-limiting enzyme of the urea cycle, is active only in the presence of its allosteric activator N-acetylglutamate.

2. L-Ornithine transcarbamoylase catalyses transfer of the carbamoyl group of carbamoyl phosphate to ornithine, forming citrulline and orthophosphate (Fig. 10.2). While the reaction occurs in the mitochondrial matrix, both the formation of ornithine and the subsequent metabolism of citrulline take place in the cytosol. Entry of ornithine into mitochondrial and export of citrulline from mitochondria therefore involve mitochondrial inner membrane transport systems (Fig. 10.2).

3. Argininosuccinate synthase links aspartate and citrulline via the amino group of aspartate (Fig. 10.2) and provides the second nitrogen of urea.

4. Cleavage of argininosuccinate, catalysed by argininosuccinase, proceeds with retention of nitrogen in arginine and release of the aspartate skeleton as fumarate (Fig. 10.2). Addition of water to fumarate forms L-malate, and subsequent NAD⁺ dependent oxidation of malate forms oxaloacetate. Transamination of oxaloacetate by glutamate aminotransferase then reforms aspartate. The carbon skeleton of aspartate-fumarate thus acts as a carrier of the nitrogen of glutamate into a precursor of urea.

5. Hydrolytic cleavage of the guanidino group of arginine, catalysed by liver arginase, releases urea (Fig. 10.2). The other product, ornithine, reenters liver mitochondria for additional rounds of urea synthesis.

Figure 10.2. Catabolism of amino acid nitrogen

Abbreviations of enzymes: OTC, ornithine transcarbamoylase; ASS, argininosuccinate synthase; ASL, argininosuccinatelyase; ARG, arginase; CPS, carbamoyl phosphate synthetase; GOGAT, glutamate synthase; GS, glutamine synthetase; GLU, glutaminase; GDH, glutamate dehydrogenase; AAT, amino-acid N-acetyltransferase; AGK, acetylglutamate kinase; AGPR, N-acetyl-γ-glutamylphosphateductase; AOT, acetylornithine transaminase; AO, acetylornithinedeacetylase; FH, fumaratehydratase; MDH, malate dehydrogenase; AST, asparate aminotransferase; CS, citrate (Si)-synthase; AH, aconitahdratase; IDH, isocitrate dehydrogenase; OGDH, oxoglutarate dehydrogenase; DST, dihydrolipoxygenase-residue succinyltranseferase; E3, dihydrolipoyl dehydrogenase; SCS, succinyl-CoA synthetase; SDH, succinate dehydrogenase

2.5. 10.2.5. Catabolism of the carbon skeletons of amino acids

Removal of α-amino nitrogen by transamination (Fig. 10.2) is the first catabolic reaction of amino acids except in the case of proline, hydroxyproline, threonine, and lysine. The residual hydrocarbon skeleton is then degraded to amphibolic intermediates.
Chapter 11. Nucleotides

Nucleotides - the monomer units or building blocks of nucleic acids (Fig.11.1) - serve multiple additional functions.

Figure 11.1. Structure of nucleotides - http://www.ncbi.nlm.nih.gov/books/NBK26883/box/A217/?report=objectonly
They form a part of many coenzymes and serve as donors of phosphoryl groups (e.g., ATP or GTP), of sugars (e.g., UDP- or GDP-sugars), or of lipid (e.g., CDP-acylglycerol). Regulatory nucleotides include the second messengers: cAMP and cGMP, the control by ADP of oxidative phosphorylation, and allosteric regulation of enzyme activity by ATP, AMP, and CTP (Fig. 11.2). Purines and pyrimidines are nitrogen-containing heterocycles, cyclic compounds whose rings contain both carbon and other elements (hetero atoms).
Figure 11.2. Functions of nucleotides - http://www.ncbi.nlm.nih.gov/books/NBK26883/box/A217/?report=objectonly

Nucleotides have many other functions.

- Mical energy in their easily hydrolyzed phospho
- Example: ATP (or ADP)
- With other groups to form coenzymes.
  - Example: coenzyme A (CoA)
- As specific signaling molecules in the cell.
  - cAMP (cAMP)
Nucleosides are derivatives of purines and pyrimidines (Fig. 11.3) that have a sugar linked to a ring nitrogen (Fig. 11.4). Numerals with a prime (e.g., 2' or 3') distinguish atoms of the sugar from those of the heterocyclic base.

**Figure 11.3.** Nucleosides are derivatives of purines and pyrimidines - [http://www.ncbi.nlm.nih.gov/books/NBK26883/box/A217/?report=objectonly](http://www.ncbi.nlm.nih.gov/books/NBK26883/box/A217/?report=objectonly)

The sugar in ribonucleosides is D-ribose, and in deoxyribonucleosides it is 2-deoxy-D-ribose (Fig. 11.5).

**Figure 11.5.** Sugars in (deoxy)ribonucleosides - [http://www.ncbi.nlm.nih.gov/books/NBK26883/box/A217/?report=objectonly](http://www.ncbi.nlm.nih.gov/books/NBK26883/box/A217/?report=objectonly)
The sugar is linked to the heterocyclic base via a \(-N\)-glycosidic bond, almost always to N-1 of a pyrimidine or to N-9 of a purine (Fig. 11.3 and 11.4). Mononucleotides are nucleosides with a phosphoryl group esterified to a hydroxyl group of the sugar. The 3’- and 5’-nucleotides are nucleosides with a phosphoryl group on the 3’- or 5’- hydroxyl group of the sugar, respectively. Since most nucleotides are 5’, the prefix “5’-” is usually omitted when naming them.

1. 11.1. Metabolism of purine and pyrimidine nucleotides

The biosynthesis of purines and pyrimidines is stringently regulated and coordinated by feedback mechanisms that ensure their production in quantities and at times appropriate to varying physiologic demand.

1.1. 11.1.1. Purines and pyrimidines are dietarily nonessential

Human tissues can synthesize purines and pyrimidines from amphibolic intermediates. Ingested nucleic acids and nucleotides, which therefore are dietarily nonessential, are degraded in the intestinal tract to mononucleotides, which may be absorbed or converted to purine and pyrimidine bases. The purine bases are then oxidized to uric acid, which may be absorbed and excreted in the urine.

1.2. 11.1.2. Biosynthesis of purine nucleotides

Purine and pyrimidine nucleotides are synthesized in vivo at rates consistent with physiologic need. Intracellular mechanisms sense and regulate the pool sizes of nucleotide triphosphates (NTPs), which rise during growth or tissue regeneration when cells are rapidly dividing. Early investigations of nucleotide biosynthesis employed birds, and later ones used Escherichia coli.

Three processes contribute to purine nucleotide biosynthesis. These are, in order of decreasing importance: (1) synthesis from amphibolic intermediates (synthesis de novo), (2) phosphoribosylation of purines, and (3) phosphorylation of purine nucleosides.

1.3. 11.1.3. Biosynthesis of pyrimidinenucleotides

The catalyst for the initial reaction is cytosolic carbamoyl phosphate synthase II, a different enzyme from the mitochondrial carbamoyl phosphate synthase I of urea synthesis (Fig. 10.2). Compartmentation thus provides two independent pools of carbamoyl phosphate. PRPP, an early participant in purine nucleotide synthesis, is a much later participant in pyrimidine biosynthesis.
Chapter 12. DNA replication

The ability of cells to maintain a high degree of order in a chaotic universe depends upon the accurate duplication of vast quantities of genetic information carried in chemical form as DNA. This process, called DNA replication, must occur before a cell can produce two genetically identical daughter cells. Maintaining order also requires the continued surveillance and repair of this genetic information because DNA inside cells is repeatedly damaged by chemicals and radiation from the environment, as well as by thermal accidents and reactive molecules.

1. 12.1. Replication is semiconservative

DNA replication is a highly coordinated process in which the parent strands are simultaneously unwound and replicated. Replication is semiconservative: Each DNA strand serves as a template for the synthesis of a new strand, producing two new DNA molecules, each with one new strand and one old strand (Fig. 12.1).

Figure 12.1. The semiconservative nature of DNA replication - http://www.ncbi.nlm.nih.gov/books/NBK26850/figure/A760/?report=objectonly
Base-pairing between an incoming deoxyribonucleoside triphosphate and an existing strand of DNA (the template strand) guides the formation of the new strand of DNA and causes it to have a complementary nucleotide sequence. The hydrogen bonds between complementary bases and the common geometry of the standard $A=T$ and $G=C$ base pairs provide the correct pairing (Fig. 12.2).

DNA polymerization has two requirements. First, all DNA polymerases require a template. The polymerization reaction is guided by a template DNA strand according to the base-pairing rules ($A=T$ and $G=C$) (Fig. 12.2). Second, the polymerases require a primer. A primer is a strand segment (complementary to the template) with a free 3′-hydroxyl group (Fig. 12.2). So part of the new strand must already be in place: all DNA polymerases can only add nucleotides to a preexisting strand. Many primers are oligonucleotides of RNA rather than DNA, and synthesized by specialized enzymes. After adding a nucleotide to a growing DNA strand, a DNA
DNA replication

polymerase either dissociates or moves along the template and adds another nucleotide. The average number of nucleotides added before a polymerase dissociates defines its processivity.

The replication is bidirectional (Fig. 12.3). It means that both ends of the loop have active replication forks (Where parent DNA is being unwound and the separated strands replicated is called a replication fork because of its Y-shaped structure). The replication loops always initiate at a unique point, which was termed an origin.

A new strand of DNA is always synthesized in the 5' → 3' direction (Fig. 12.3). Because the two DNA strands are antiparallel (the polarity of one strand is oriented in the opposite direction to that of the other), the strand serving as the template is read from its 3' end toward its 5' end. If both strands were synthesized continuously while the replication fork moved, one strand would have to undergo 3' → 5' synthesis (Fig. 12.3).

One strand is synthesized continuously and the other discontinuously. The leading strand is continuously synthesized in the direction taken by the replication fork. The other strand, the lagging strand, is synthesized discontinuously in short pieces (Okazaki fragments; in bacteria 1000-2000; in eukaryotic cells 150-200 nucleotides long) in a direction opposite to that in which the replication fork moves (Fig. 12.3).

Figure 12.3. The structure of a DNA replication fork - http://www.ncbi.nlm.nih.gov/books/NBK26850/figure/A763/?report=objectonly

E. coli has at least 5 DNA polymerases. The main function of DNA polymerase I is repair. It is not the primary enzyme of replication. It has both 3' → 5' and 5' → 3' exonuclease activity beyond its polymerizing activity. (Exonucleases degrade nucleic acids from one end of the molecule, while endonucleases degrade at specific internal sites in a nucleic acid strand, reducing it to smaller fragments.) DNA polymerase II is an enzyme involved in one type of DNA repair. DNA polymerase III is the principal replication enzyme in E. coli. DNA polymerases IV and V, identified in 1999, are involved in an unusual form of DNA repair.

Replication in E. coli requires not just a single DNA polymerase but 20 or more different enzymes and proteins, each performing a specific task. The entire complex is called replisome.

For DNA synthesis to proceed, the DNA double helix must be opened up ahead of the replication fork so that the incoming deoxyribonucleoside triphosphates can form base pairs with the template strand. However, the DNA double helix is very stable under normal conditions; the base pairs are locked in place so strongly that temperatures approaching that of boiling water are required to separate the two strands in a test tube. For this reason, DNA polymerases and DNA primases can copy a DNA double helix only when the template strand has already been exposed by separating it from its complementary strand. Additional replication proteins are needed to help in opening the double helix and thus provide the appropriate single-stranded DNA template for the DNA polymerase to copy. Two types of protein contribute to this process DNA helicases and single-strand DNA-binding proteins.

DNA helicases were first isolated as proteins that hydrolyse ATP when they are bound to single strands of DNA. ATP can change the shape of a protein molecule in a cyclical manner that allows the protein to perform mechanical work. DNA helicases use this principle to propel themselves rapidly along a DNA single strand. When they encounter a region of double helix, they continue to move along their strand, thereby prying apart the helix at rates of up to 1000 nucleotide pairs per second (Fig. 12.4).

Figure 12.4. The action of DNA helicase - http://www.ncbi.nlm.nih.gov/books/NBK26850/figure/A775/?report=objectonly
The unwinding of the template DNA helix at a replication fork could in principle be catalysed by two DNA helicases acting in concert one running along the leading strand template and one along the lagging strand template.

Since the two strands have opposite polarities, these helicases would need to move in opposite directions along a DNA single strand and therefore would be different enzymes. Both types of DNA helicase exist.

**Single-strand DNA-binding (SSB) proteins**, also called helix-destabilizing proteins, bind tightly and cooperatively to exposed single-stranded DNA strands without covering the bases, which therefore remain available for templating. These proteins are unable to open a long DNA helix directly, but they **aid helicases by stabilizing the unwound, single-stranded conformation**. In addition, their cooperative binding coats and straightens out the regions of single-stranded DNA on the lagging-strand template, thereby preventing the formation of the short hairpin helices that readily form in single-strand DNA (Fig. 12.5). These hairpin helices can impede the DNA synthesis catalysed by DNA polymerase.

**Figure 12.5. Single-strand DNA-binding proteins**

http://www.ncbi.nlm.nih.gov/books/NBK26850/figure/A777/?report=objectonly
Leading strand synthesis begins with the synthesis of a short RNA primer at the replication origin by primase. Primase interacts with helicase, and the primer is synthesized in the direction opposite to that in which the helicase is moving. In effect, the helicase moves along the strand that becomes the lagging strand in DNA synthesis. Deoxyribonucleotides are added to this primer by a DNA polymerase III complex linked to the helicase. Leading strand synthesis then proceeds continuously, keeping step with the unwinding of DNA at the replication fork.

Lagging strand synthesis is accomplished in short Okazaki fragments. First, an RNA primer is synthesized by primase and, as in leading strand synthesis, DNA polymerase III binds to the RNA primer and adds deoxyribonucleotides. Helicase and primase constitute a functional unit, the primosome. DNA polymerase III uses one set of its core subunits to synthesize the leading strand continuously, while the other set moves from one Okazaki fragment to the next on the lagging strand. Helicase, bound in front of DNA polymerase III, unwinds the DNA at the replication fork as it migrates along the lagging strand template in the 5’ → 3’ direction. The replisome promotes rapid DNA synthesis, adding 1000 nucleotides to each strand (leading and lagging) (Fig. 12.6).

Figure 12.6. The proteins at a bacterial DNA replication fork - http://www.ncbi.nlm.nih.gov/books/NBK26850/figure/A783/?report=objectonly
Once an Okazaki fragment has been completed, its RNA primer is removed and replaced with DNA by DNA polymerase I, and the remaining nick is sealed by DNA ligase. DNA ligase catalyses the formation of a phosphodiester bond between a 3’ hydroxyl at the end of one DNA strand and a 5’ phosphate at the end of another strand (Fig. 12.7).

Figure 12.7. The synthesis of one of the many DNA fragments on the lagging strand - http://www.ncbi.nlm.nih.gov/books/NBK26850/figure/A772/?report=objectonly
DNA replication

RNA primer

new RNA primer

synthesis by primase

3’ ← 5’

DNA polymerase adds RNA primer to start new Okazaki fragment

5’ 3’ ← 5’

DNA polymerase finish DNA fragment

5’

old RNA primer erased and replaced by DNA

5’

nick sealing by DNA lig joins new Okazaki frag to the growing chain

5’
Eventually, the two replication forks of the circular E. coli chromosome meet at a terminus region. The separated chromosomes then segregate into daughter cells at cell division.

**Replication is very accurate.** In E. coli, a mistake is made only once for every $10^9$ to $10^{10}$ nucleotides added. Incorrect bases can be rejected before the phosphodiester bond is formed. DNA polymerases insert one incorrect nucleotide for every $10^4$ to $10^5$ correct ones. If the polymerase has added wrong nucleotide, translocation of the enzyme to the next position where the next nucleotide is to be added is inhibited. This kinetic pause provides the opportunity for a correction. The $3' \rightarrow 5'$ exonuclease activity removes the mispaired nucleotide, and the polymerase begins again. This is **proofreading activity**. Proofreading improves the accuracy of the polymerization reaction $10^2$- to $10^3$-fold. When base selection and proofreading are combined, DNA polymerase makes one mistake for every $10^6$ to $10^8$ bases added. Yet the measured accuracy of replication is still higher. The additional accuracy is provided by a separate enzyme system that repairs the mismatched base pairs after replication.

**Differences between the prokaryotic (bacterial) and eukaryotic replication**

Much of what we know about DNA replication was first derived from studies of purified bacterial and bacteriophage multienzyme systems capable of DNA replication in vitro. It is clear that the fundamental features of DNA replication - including replication fork geometry and the use of a multiprotein replication machine - have been conserved during the long evolutionary process that separates bacteria and eukaryotes. There are more protein components in eukaryotic replication machines than there are in the bacterial analogues, even though the basic functions are the same. Thus, for example, the eukaryotic single-strand binding (SSB) protein is formed from three subunits, whereas only a single subunit is found in bacteria. Similarly, the DNA primase is incorporated into a multisubunit enzyme called DNA polymerase α. The polymerase α begins each Okazaki fragment on the lagging strand with RNA and then extends the RNA primer with a short length of DNA. Before passing the 3' end of this primer to a second enzyme, DNA polymerase δ. This second DNA polymerase then synthesizes the remainder of each Okazaki fragment with the help of a clamp protein. Although both forks use the same basic components, the mammalian fork differs in at least two important respects. First, it uses two different DNA polymerases on the lagging strand. Second, the mammalian DNA primase is a subunit of one of the lagging-strand DNA polymerases, DNA polymerase α, while that of bacteria is associated with a DNA helicase in the primosome. The polymerase α (with its associated primase) begins chains with RNA, extends them with DNA, and then hands the chains over to the second polymerase (β), which elongates them. It is not known why eukaryotic DNA replication requires two different polymerases on the lagging strand. The eukaryotic replication machinery has the added complication of having to replicate through nucleosomes, the repeating structural unit of chromosomes. Nucleosomes are spaced at intervals of about 200 nucleotide pairs along the DNA, which may explain why new Okazaki fragments are synthesized on the lagging strand at intervals of 100–200 nucleotides in eukaryotes, instead of 1000–2000 nucleotides as in bacteria. Nucleosomes may also act as barriers that slow down the movement of DNA polymerase molecules, which may be why eukaryotic replication forks move only one-tenth as fast as bacterial replication forks.
Chapter 13. Transcription

Expression of the information in a gene generally involves production of an RNA molecule transcribed from a DNA template. Unlike DNA, most RNAs are single-stranded. RNA is the only macromolecule known to have a role both in the storage and transfer of information and in catalysis. Three major kinds of RNA are produced. Messenger RNAs (mRNAs) encode the amino acid sequence of one or more polypeptides specified by a gene or set of genes. Transfer RNAs (tRNAs) read the information encoded in the mRNA and transfer the appropriate amino acid to a growing polypeptide chain during protein synthesis. Ribosomal RNAs (rRNAs) are constituents of ribosomes (Fig. 13.1).

Figure 13.1. The RNAs in action - http://www.ncbi.nlm.nih.gov/books/NBK26864/figure/A19/?report=objectonly
During transcription, an enzyme system converts the genetic information in a segment of double-stranded DNA into an RNA strand with a base sequence complementary to one of the DNA strands. Transcription is selective. **Only particular genes or groups of genes are transcribed**, and some parts of the DNA genome are never transcribed. Specific regulatory sequences mark the beginning and end of the DNA segments to be transcribed and designate which strand in duplex DNA is to be used as the template.

Transcription resembles replication in its fundamental chemical mechanism (Fig. 13.2), direction of synthesis, and its use of a template. Transcription has **three phases, initiation, elongation, and termination**. Transcription differs from replication in that it **does not require a primer** and involves only limited segments of a DNA molecule. Additionally, within transcribed segments only one DNA strand serves as a template for a particular RNA molecule.

*Figure 13.2. The chemical structure of RNA* - http://www.ncbi.nlm.nih.gov/books/NBK26887/figure/A978/?report=objectonly
DNA-dependent RNA polymerase requires a DNA template and all four NTPs of the nucleotide units of RNA. The chemistry and mechanism of RNA synthesis is similar to that of DNA. RNA polymerase elongates an RNA strand by adding ribonucleotide units to the 3'-hydroxyl end, building RNA in the 5' → 3' direction. The 3'-hydroxyl group acts as a nucleophile, attacking the α phosphate of the incoming NTP and releasing pyrophosphate.

RNA polymerase requires DNA for activity and is most active when bound to a double-stranded DNA. Only one of the two DNA strands serves as a template. The template DNA strand is copied in the 3' → 5' direction (antiparallel to the new RNA strand), just as in DNA replication (Fig. 13.3).
Each nucleotide in the newly formed RNA is selected by base-pairing interactions; U=A, G≡C. Unlike DNA polymerase, RNA polymerase does not require a primer to initiate synthesis. Initiation occurs when RNA polymerase binds at specific DNA sequences called promoters (Fig. 13.4). The 5'-triphosphate group of the first residue in a newly formed RNA molecule is not cleaved to release PP\textsubscript{i}, but instead remains intact throughout the transcription process. The strand that serves as template for RNA synthesis is called the template strand. The DNA strand complementary to the template, called coding strand, is identical in base sequence to the RNA transcribed from the gene, with U in the RNA in place of T in the DNA. By convention, the regulatory sequences that control transcription are designated by the sequences in the coding strand.

The DNA duplex must unwind over a short distance, forming a transcription bubble. RNA polymerase generally keeps about 17 bp unwound. During the elongation phase of transcription, the growing end of the RNA strand forms an 8 bp long hybrid RNA-DNA double helix with the DNA template (Fig. 13.3). The 8 bp RNA-DNA hybrid occurs in this unwound region. The RNA leaves the hybrid duplex shortly after its formation, and the DNA duplex reforms. Elongation of a transcript proceeds at a rate of 50 to 90 nucleotides/s. Because DNA is a helix, movement of a transcription bubble requires strand rotation. DNA strand rotation is limited in most DNAs by DNA-binding proteins and other structural barriers. As a result, a moving RNA polymerase generates waves of positive supercoils ahead of the transcription bubble and negative supercoils behind. The topological problems caused by transcription are eased by topoisomerases.

The DNA-dependent RNA polymerase of E. coli is a large, complex enzyme with five core subunits (α\textsubscript{2}ββ′ω; Mr 390,000) and a sixth subunit, σ, with variants designated by size. So the holoenzyme exists in several forms, depending on the type of σ subunit. The most common subunit is σ\textsubscript{70} (Mr 70,000). The subunit binds transiently to the core and directs the enzyme to specific binding sites on the DNA (Fig. 13.4).

Figure 13.4. The transcription cycle of bacterial RNA polymerase - http://www.ncbi.nlm.nih.gov/books/NBK26887/figure/A988/?report=objectonly
RNA polymerases lack a proofreading 3' → 5' exonuclease activity. The error rate for transcription is higher than that for DNA replication - one error occurs for every $10^4$ to $10^5$ ribonucleotides. Because many copies of an RNA are generally produced from a single gene and all RNAs are eventually degraded and replaced, a mistake in an RNA molecule is of less consequence to the cell than a mistake in the permanent information stored in DNA. Many RNA polymerases do pause when a mispaired base is added, and they can remove mismatched nucleotides from the 3' end by direct reversal of the polymerase reaction. It is not known whether this activity is a true proofreading function.

RNA polymerase binds to specific sequences in the DNA called promoters, which direct the transcription of adjacent segments of DNA (Fig. 13.4). The sequences where RNA polymerases bind can be quite variable. By convention, the DNA base pairs that correspond to the beginning of an RNA molecule are given positive numbers, and those preceding the RNA start site are given negative numbers. The promoter region thus extends between positions -70 and +30. Analyses have revealed similarities in two short sequences centred about positions -10 and -35. These sequences are important interaction sites for the $\sigma^{70}$ subunit. Although the sequences are not identical for all promoters, certain nucleotides that are common at each position form a consensus sequence. The consensus sequence at the -10 region is (5')TATAAT(3'); the consensus sequence at
the -35 region is (5′)TTGACA(3′). A third AT-rich region occurs between positions -40 and -60 in the promoters of certain highly expressed genes called the UP (upstream promoter) element. This is bound by the α subunit of RNA polymerase. Variations in the consensus sequence affect the efficiency of RNA polymerase binding and transcription initiation.

The initiation divided into two phases, binding and initiation. First, the polymerase binds to the promoter. A closed complex (in which the bound DNA is intact) and an open complex (in which the bound DNA is intact and partially unwound near the -10 sequence) form in succession (Fig. 13.4). Second, transcription is initiated within the complex, leading to a conformational change that converts the complex to the elongation form. The σ subunit dissociates as the polymerase enters the elongation phase of transcription (Fig. 13.4). The protein NusA binds to the elongating RNA polymerase, competitively with the σ subunit. When RNA polymerase reaches a terminator sequence, RNA synthesis halts, NusA dissociates from the polymerase, and the RNA polymerase dissociates from the DNA. A σ factor can now bind to the enzyme to initiate transcription, in a cycle sometimes called the σ cycle.

RNA polymerase has high processivity - necessarily, because if an RNA transcript is released prematurely, it could not resume synthesis of the same RNA but instead would have to start again.

E. coli has two classes of termination signals: one class relies on a protein factor called ρ and the other is ρ-independent. Most ρ-independent terminators have two distinguishing features. The first is a region that produces an RNA transcript with self-complementary sequences, allowing the formation of a hairpin structure before the projected end of the RNA strand (Fig. 13.4). The second feature is a highly conserved string of three A residues in the template strand that are transcribed into U residues near the 3′ end of the hairpin. When a polymerase arrives at a termination site with this structure, it pauses. Formation of the hairpin structure in the RNA disrupts several AUU base pairs in the RNA-DNA hybrid and may disrupt important interactions between RNA and the RNA polymerase, promoting dissociation of the transcript. The ρ-dependent terminators include a CA-rich sequence called a rho utilization element. The protein associates with the RNA and migrates in the 5′ → 3′ direction until it reaches the transcription complex. Here it cooperates to release of the RNA transcript. The ρ protein has an ATP-dependent RNA-DNA helicase activity that promotes translocation of the protein along the RNA, and ATP is hydrolysed by protein during the termination process.

The transcription in a eukaryotic cell is much more complex than that in bacteria. Eukaryotes have three RNA polymerases, designated I, II, and III, which are distinct complexes but have certain subunits in common. Each polymerase has a specific function and binds to a specific promoter sequence.

RNA polymerase I is responsible for the synthesis of pre-ribosomal RNA. Pol I promoters vary greatly in sequence from one species to another. The principal function of RNA polymerase II is synthesis of mRNAs and some specialized RNAs. This enzyme can recognize thousands of promoters that vary greatly in sequence. Many Pol II promoters have a few sequence features in common, including a TATA box (eukaryotic consensus sequence TATAAA) near base pair -30 and an Inr sequence (initiator) near base pair +1. The formation of a closed complex begins when the TATA-binding protein (TBP) binds to the TATA box. RNA polymerase III makes tRNAs, and some other small specialized RNAs. The promoters recognized by Pol III are well characterized.

RNA polymerase II is central to eukaryotic gene expression. Pol II is a huge enzyme with 12 subunits. The largest subunit (RBP1) exhibits a high degree of homology to the β subunit of bacterial RNA polymerase. Another subunit (RBP2) is structurally similar to the bacterial β subunit, and two others (RBP3 and RBP11) show some structural homology to the two bacterial α subunits. The largest subunit of Pol II also has an unusual feature, a long carboxyl-terminal tail consisting of many repeats of an amino acid sequence. This carboxyl-terminal domain (CTD) has many important roles in Pol II function. RNA polymerase II requires an array of other proteins, called transcription factors (designated TFII), in order to form the active transcription complex. The process of transcription by Pol II has several phases - assembly, initiation, elongation and termination - each associated with characteristic proteins.

Many of the RNA molecules participate in a postsynthetic processing. A newly synthesized RNA molecule is called primary transcript. The primary transcript for a eukaryotic mRNA typically contains two types of sequences: noncoding segments that break up the coding region are called introns, and the coding segments are called exons. In a process called splicing, the introns are removed from the primary transcript and the exons are joined to form a continuous sequence that defines a functional polypeptide (Fig. 13.5).
In eukaryotic mRNAs, most exons are less than 1000 nucleotides long. Introns vary in size from 50 to 20,000 nucleotides. There are self-splicing introns - no protein enzymes are involved, spliceosomal introns - catalysed by a spliceosome and the splicing occurs within this (Fig. 13.6). The spliceosome contains specialized RNA-protein complexes and small nuclear ribonucleoproteins (snRNP). And the introns of certain tRNAs requires ATP and endonuclease to the splicing. The splicing endonuclease cleaves the phosphodiester bonds at both ends of the intron, and the two exons are joined by a mechanism similar to the DNA ligase reaction.
Chapter 14. Translation

Proteins are essential for every cell in all living systems. A typical cell requires thousands of different proteins to catalyse metabolic reactions, transport molecules or replicate DNA. During the protein synthesis, almost 300 different macromolecules cooperate. This process uses up to 90% of the chemical energy used by a cell for all biosynthetic reactions. More than 35% of a typical bacterial cell’s dry weight is the macromolecules related to the synthesis. Despite the great complexity of protein synthesis, proteins are made at exceedingly high rates. A polypeptide of 100 residues is synthesized in an Escherichia coli cell at 37 °C in about 5 seconds.


Three developments helped to identify the major stages of protein synthesis. The first was Zamecnik’s experiment in the early 1950s. He injected radioactive amino acids into rats and at different time intervals after the injection, examined the subcellular fractions for the presence of radioactive proteins. When hours had elapsed after the injection, all subcellular fractions contained labelled proteins. However, when only minutes had elapsed, radioactive protein appeared only in a fraction containing small ribonucleoprotein particles. These particles were identified as the site of protein synthesis, and later were named ribosomes. The second discovery was that amino acids attach to a special RNA type, called transfer RNA (tRNA), to form aminoacyl-tRNAs. Aminoacyl-tRNAsynthetase is the enzyme, which catalyse this process. Francis Crick achieved the third advance: the genetic information is encoded in four-letter language of nucleic acids and it could be translated into the 20-letter language of proteins. The nucleotide sequence of an mRNA is translated into amino acid sequence of a polypeptide with help of tRNAs. The overall process of protein synthesis is called translation.


It was obvious that four nucleotide bases in groups of two can yield only 4²=16 different combinations, which is not enough for the 20 amino acids. Groups of three yield 4³=64 combinations, so a triplet of nucleotides (codon) codes an amino acid. The genetic code in all living systems is known to be non-overlapping which means that codons do not share nucleotids. There is no punctuation between the codons, so the mRNA is read without pauses. A specific first codon in the sequence establishes the reading frame, in which a new codon begins every three nucleotide residues. Therefore an mRNA has three possible reading frames, but only one is likely to encode a given protein (Fig. 14.1).

Figure 14.1. Reading frames in protein synthesis -
http://www.uic.edu/classes/phar/phar331/lecture3/
In the 1960s, many experiments started to match the amino acids with their codons. Nirenberg and Matthaei added synthetic polyuridylate (polyU), GTP, ATP and mixture of the 20 amino acids to an E. coli extract. They made 20 different samples, and in each sample there was a labelled amino acid. A labelled polypeptide was found in only one sample, which contained labelled phenylalanine. Thus the codon UUU encodes phenylalanine. Using the same method, they revealed that CCC encodes proline and AAA encodes lysine. PolyG did not generate any polypeptide because it spontaneously forms tetraplexes that cannot be bound by ribosomes. Later scientists could produce mRNAs with defined proportion of different nucleotide bases. For example the produced mRNA in which about five-sixth of the residues were adenine and one-sixth were cytosine. Soon experiments revealed the base composition of the triplets coding for almost all the amino acids. But they usually could not reveal the sequence of the bases. Nirenberg and Leder found that even trinucleotides could promote the binding of the appropriate tRNA to the ribosome. Therefore they prepared every triplet and determined which aminoacyl-tRNA bound to the codon. 54 of the 64 possible codon-amino acid pairs were identified by this method. For some codons, either no aminoacyl-tRNA or more than one would bind. Khorana developed chemical methods to synthesize polyribonucleotides with defined repeated sequences of two to four bases. This approach permitted the assignment of 61 of the 64 possible codons. The remaining three codons do not encode any amino acid, these are termination codons. In the presence of these codons the synthesizing process would stop. By 1966 meanings for all the triplet codons were established and verified (Fig. 14.2).

Figure 14.2. The genetic code -
http://www.ncbi.nlm.nih.gov/books/NBK26829/figure/A1054/?report=objectonly

In the dictionary there are four special codons. AUG is the initiation codon which is responsible for the beginning of the translation. It also encodes methionine residues in internal positions. UAA, UAG and UGA are the termination codons. The genetic code is nearly universal: with few minor variations, amino acid codons are identical in all species examined so far. This suggests that all life forms have a common evolutionary ancestor. Several exceptions to the original dictionary can be found in different living systems. These exceptions mostly occur in mitochondrial RNA because mitochondria have their own tRNAs so their code variations do not affect the whole cell. In some species the normal termination codon UGA specifies tryptophan. Thus tRNA of tryptophan recognizes either UGA or the normal Trp codon UGG. There are few exceptions because to alter the code, changes must occur in the genes encoding tRNAs also.

An interesting feature of the genetic code is that an amino acid may be encoded by more than one codon, so the code is described as degenerate. Although an amino acid may have two or more codons, each codon specifies only one amino acid. The degeneracy of the code is not uniform, for example methionine has a single codon, isoleucine has three and arginine has six codons.

1.2. 14.1.2. Wobble Hypothesis

When several different codons specify one amino acid the difference between them usually lies at third base position. The first two letters of each codon are the primary determinants of specificity. A three-base sequence on a specific location of the tRNA, called anticodon, pairs with mRNA codons. The first base of the codon in mRNA (read in the 5′-3′ direction) pairs with the third base of the anticodon (Fig.14.3) If the anticodon triplet of tRNA recognize only one codon triplet through Watson-Crick base pairing (G-C, A-U) at all three positions, cells would have a different tRNA for each amino acid codon. This is not the case because the anticodons in some tRNAs contain the nucleotide inosinate (I) which can form weak hydrogen bonds with three different nucleotides (U, C and A). For example in yeast the tRNA of Arg has the anticodon (5′)ICG which recognizes three Arg codons: (5′)CGA, (5′)CGU and (5′)CGC. The first two bases (CG) forms strong hydrogen bonds with the anticodon (GC) and the third base (U, A or C) forms weak hydrogen bonds with the I residue. Crick examined numerous codon anticodon relations and found that the third base of most codons forms weak bonds with the corresponding anticodon so he called these bases wobbles. He set up the wobble hypothesis which contains four relationships:
1. **The first two bases of an mRNA codon always form strong** Watson-Crick base pairs with the anticodon and are responsible for most of the coding specificity.

2. The first base of the anticodon (reading in the 5’-3’ direction) determines the number of codons recognized by tRNA. When this base is C or A, only one codon is recognized by that tRNA. When it is U or G, two different codons may be read. When I is the first nucleotide, three different codons can be recognized (A, U or C).

3. When an amino acid is specified by different codons, the codons that differ in either of the first two bases require different tRNAs.

4. A minimum of 32 tRNAs are required to translate all 61 codons.

**Figure 14.3. Wobble base-pairing between codons and anticodons** - http://www.ncbi.nlm.nih.gov/books/NBK26829/figure/A1058/?report=objectonly
tRNA

anticodon

5'
wobble position

3' codon

mRNA

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Codon-anticodon interactions balance the requirements for accuracy and speed. In one hand the wobble base decreases specificity, on the other if all three bases of a codon engaged in strong Watson-Crick pairing, tRNAs would dissociate too slowly so this would limit the rate of the protein synthesis. Therefore the wobble base increases the speed of translation.

### 1.3. 14.1.3. Translational Frameshifting and RNA Editing

We discussed earlier that from the three possible reading frames usually one contains useful information. But a few genes are structured so that ribosomes change the reading frame at a certain point. This mechanism allow either two or more related proteins to be produced from a single mRNA or regulating the synthesis of a protein.

Some RNAs are edited before translation. RNA editing can involve the addition, deletion or alteration of nucleotides, which affects the meaning of the code. Addition and deletion have been commonly observed in RNAs from the mitochondrial and chloroplast genomes. These processes require special guide RNAs which act as templates for the editing. RNA editing by alteration of nucleotides most commonly involves the enzymatic deamination of adenosine or cytidine residues forming inosine or uridine. For example the apoB-100 protein and the apoB-48 protein are encoded by an mRNA produced from the gene apoB-100. In the human intestine there is an enzyme which converts a C to a U at a specific position of the mRNA. Therefore CAA (Gln) codon is transformed to UAA (Stop) and the resulting protein will be the smaller, apoB-48.

### 2. 14.2. The process of protein synthesis

Protein synthesis follows the same pattern as DNA or RNA synthesis; the main stages are initiation, elongation and termination. Before these processes the activation of precursors is needed and after the termination the folding and enzymatic processing is required. First of all we discuss the structure and role of the two key components: the ribosome and tRNA.

#### 2.1. 14.2.1. The ribosome

The ribosome is a complex supramolecular unit which contain about 65% rRNA and 35% protein. Bacterial ribosomes are composed of two unequal subunits with sedimentation coefficients of 30S (Svedberg unit) and 50S and a combined sedimentation coefficient of 70S. Nomura found that both subunits can be broken down into their RNA and protein components then reconstituted in vitro. Multiple high-resolution images of the ribosome and its subunits helped the recognition of the structure of the ribosomes. Each subunit has a huge RNA core and proteins are secondary elements decorating the surface. It is also important that there is no protein within 18 Å of the active site for peptide formation. This suggests that the synthesizing of proteins is a ribozyme-catalysed process, and not a protein-catalysed one. The two subunits fit together to form a cleft through which mRNA passes during translation. The function of the proteins is not entirely clarified but a structural role seems evident for many of them. The ribosomes of eukaryotic cells are larger and more complex than bacterial ones with 80S total sedimentation coefficient. They also have two subunits (60S and 40S) and the function is similar (Fig. 14.4).

Figure 14.4. A comparison of the structures of prokaryotic and eukaryotic ribosomes - http://www.ncbi.nlm.nih.gov/books/NBK26829/figure/A1073/?report=objectonly
2.2. 14.2.2. Transfer RNAs

During the translation, tRNAs serve as adaptors in translating the language of nucleic acids into the language of proteins. They consist of a single strand of RNA with 73 to 93 nucleotide residues. In every cell there is at least one kind of tRNA for each amino acid. However minimum 32 tRNAs are required for reading every codon. A typical tRNA contains 8 or more modified bases and sugars usually methylated derivatives. When drawn into 2D the tRNA forms a cloverleaf structure with four arms, the longer tRNAs have a short extra arm (Fig. 14.5), where the dots symbolize nucleotide bases and the blue lines represent hydrogen bonds between them. The amino acid arm can carry a specific amino acid esterified by its carboxyl group the 2’- or 3’- hydroxyl group of the A at the 3’ and of the tRNA. The anticodon arm contains the anticodons. The other two main arms are responsible for the folding of tRNA and they interact with the rRNA. In 3D, a tRNA forms a twisted L (Fig. 14.5).

Figure 14.5. The 2D and 3D structure of tRNA - http://chemistry.umeche.maine.edu/CHY431/Nucleic7.html
2.3. 14.2.3. Stages of the translation process

2.3.1. 14.2.3.1. Activation of amino acids

The first stage takes place in the cytosol where the aminoacyl-tRNAsynthetases esterify the 20 amino acids to their corresponding tRNAs. Most organisms have one aminoacyl-tRNAsynthetase for each amino acid. Each enzyme is specific for one amino acid and one or more corresponding tRNAs (Fig. 14.6). Regarding the structure of the enzymes, researchers divided them into two classes. The reaction catalysed by an aminoacyl-tRNAsynthetase is:

$$K_i \quad \text{aminoacid} + \text{tRNA} + \text{ATP} \rightarrow \text{aminoacyl\text{-}tRNA} + \text{AMP} + PP_i$$

This reaction occurs in two steps. In the first step, an enzyme-bound intermediate, aminoacyl-AMP is formed. In the second step the aminoacyl group is transferred to its corresponding tRNA. In the case of class I enzymes, the aminoacyl group is first transferred to 2’ position of the A of the tRNA and then with a transesterification reaction, it moves to the 3’ position. While in the case of class II enzymes, the aminoacyl group is transferred directly to the 3’ position. The hydrolysis of two phosphate group provides the necessary energy for the reaction and makes it irreversible (Fig. 14.6). The resulting ester linkage between the amino acid and the tRNA has a highly negative standard free energy of hydrolysis (-29 kJ/mol). So the first step of translation activates the amino acid for peptide bound formation and ensures that the amino acid is bound to its corresponding tRNA. This later is essential because the identity of the amino acid attached to a tRNA is not checked on the ribosome. The required specificity of aminoacyl-tRNAsynthetases is ensured by at least two step filtering. The first filter is the binding of amino acid to the enzyme. The second filter is the binding of any incorrect aminoacyl-AMP (formed in the first step of the reaction) to a separate active site on the enzyme where the substrate is hydrolysed. In addition most aminoacyl-tRNAsynthetases can hydrolyse the ester linkage between amino acids and incorrect tRNAs. Thus the overall error rate of protein synthesis is one mistake per nearly 10^4 amino acids.

The interaction between an aminoacyl-tRNAsynthetase and a tRNA is often referred to as the second genetic code. This underlines that an aminoacyl-tRNAsynthetase must be specific not only for a single amino acid but for certain tRNAs as well (Fig. 14.6). Researchers identified nucleotide positions of tRNAs that are involved in discrimination by the aminoacyl-tRNAsynthetases. Usually ten or more nucleotides are involved in the recognition. These are mostly positioned in the amino acid arm and the anticodon arm.
Figure 14.6. The genetic code is translated by means of two adaptors that act one after another.
http://www.ncbi.nlm.nih.gov/books/NBK26829/figure/A1065/?report=objectonly

2.3.2. 14.2.3.2. Initiation

Protein synthesis begins at the amino-terminal end with the codon of AUG. The formation of the initiation complex in bacteria requires the 30S ribosomal subunit, the mRNA, the initiating fMet-tRNA, 3 initiation factors (IF-1, IF-2, IF-3), GTP, the 50S subunit and Mg²⁺. At first, the 30S subunit binds IF-1 and IF-3 (Fig.14.7). Then the initiating AUG codon is guided to its correct position by the Shine–Dalgarno sequence in the mRNA. It is a sequence near the 5’ end of the mRNA which helps distinguishing the initiation AUG codon from other AUG codons. Every ribosome has three sites that bind tRNA: the aminoacyl site (A), the peptidyl site (P) and an exit site (E) (Fig.14.7). In the next step the fMet-tRNA and IF-2-GTP (Met-tRNA and eIF-2-GTP in eukaryotes) bind to the P site of the 30S subunit. In the last step the complex combines with the 50S subunit during the hydrolysis of GTP to GDP+P, and the three initiation factors depart from the ribosome. The correct binding of the tRNA in the initiation complex is assured by the codon-anticodon interaction, the interaction between the Shine–Dalgarno sequence and the rRNA and at last the interaction between ribosomal P site and tRNA. The translation is generally similar in eukaryotic cells but the initiating AUG is detected by a scanning process of the mRNA instead of the use of Shine–Dalgarno sequence. Another difference is that there are at least nine initiation factors in eukaryotic cells and both end of the mRNA strand is bound to the ribosome (Fig. 14.7).

Figure 14.7. The initiation phase of protein synthesis in eukaryotes.
http://www.ncbi.nlm.nih.gov/books/NBK26829/figure/A1084/?report=objectonly
2.3.3. 14.2.3.3. Elongation

Elongation in bacterial cells requires the initiation complex, aminoacyl-tRNAs, three elongation factors and GTP. Three steps are repeated for adding each amino acid residue: At first the appropriate incoming aminoacyl-tRNA binds to the A site of the ribosome with the help of elongation factors and the energy of the GTP hydrolysis. The regeneration of GTP needs several milliseconds and the ribosome uses this time to check the codon-anticodon pairing. However this proofreading does not verify if the proper amino acid is attached to the tRNA. That selectivity must be ensured by the aminoacyl-tRNAsynthetase. The second step is the peptide bond formation when the α-amino group of the amino acid in the A site displace the tRNA in the P site to form a peptide bond (Fig.14.8). This reaction is catalysed by the rRNA. The last step is translocation when the ribosome moves one codon toward the 3’ end of the mRNA. This shifts the anticodon of the peptidyl-tRNA to the P site. The uncharged tRNA (which lost its amino acid) moves from the P site to the E site. This movement requires GTP and an elongation factor. Therefore for adding each amino acid, the hydrolysis of two GTP is required. The eukaryotic elongation stage is similar; the only difference is that eukaryotic ribosomes do not have an E site, uncharged tRNAs leave directly from the P site.
2.3.4. 14.2.3.4. Termination

Termination is signalled by one of the three termination codons (UAA, UAG, UGA). In bacteria when a termination codon arrives at the A site three termination factors hydrolyse the terminal peptidyl-tRNA bond, release the free polypeptide and the last tRNA from the P site and make the ribosome dissociate into the two subunits (Fig. 14.9).

Figure 14.9. The final phase of protein synthesis - http://www.ncbi.nlm.nih.gov/books/NBK26829/figure/A1087/?report=objectonly
Translation

1. BOUNDING OF RELEASE FACTOR TO THE A-SITE
2. TERMINATION
Formation of each aminoacyl-tRNA requires **two high energy phosphate groups**; elongation needs **two GTP→GDP+P hydrolysis**. An additional ATP is consumed each time an incorrect amino acid is hydrolysed by an aminoacyl-tRNA synthetase. This is at least 122 kJ/mol (4x30.5 kJ/mol) of phosphodiester bond energy to generate a peptide bond of 21 kJ/mol. The surplus energy permits very high fidelity during the translation because the goal of the process is not only to form a peptide bond between amino acids but to form a peptide bond between two specified amino acid.

Large clusters of 10 to 100 ribosomes can be observed in cells which are called polysomes. These are ribosomes which read the same mRNA and allow the rapid production of many copies of the same protein (Fig.14.10). The ribosomes begin the synthesis at the 5’ end of the mRNA, so those nearer the 3’ end have larger polypeptide chains. In this way a lot of copies can be produced from one mRNA.

**Figure 14.10. A polyribosome**


2.3.5. 14.2.3.5. Folding and Posttranslational Processing

In this final stage, the polypeptide chain is folded and processed into its biologically active form. The proteins have various three dimensional forms created by the formation of hydrogen bonds, van der Waals, ionic and hydrophobic interactions. Thus the 1D genetic messages are converted into 3D structures. It is called the **folding** process. Some proteins require additional **posttranslational modifications**. The observed modifications are:

- amino-terminal and carboxyl-terminal modifications
- loss of signal sequences
- modification of individual amino acids
- attachment of carbohydrate side chains
• addition of isoprenyl groups
• addition of prosthetic groups
• proteolytic processing
• formation of disulfide cross-links

Protein synthesis is an important function of a cell, so it is one of the primary targets of many antibiotics and toxins. These antibiotics usually inhibit protein synthesis in bacteria and are relatively harmless to eukaryotic cells because they exploit the differences between eukaryotic and bacterial translation.
Chapter 15. References

The links for figure references were downloaded on 13th of December 2012 for Chapters 1-4, on 14th of December 2012 for Chapters 5-10 and on 24th of December 2012 for Chapters 11-14.

List of references


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