ATP is the energy currency of the cell

Cells need to carry out many reactions that are energetically unfavorable. You have seen some examples of these non-spontaneous reactions in earlier chapters: the synthesis of nucleic acids and proteins from their corresponding nucleotide and amino acid building blocks and the transport of certain ions against concentration gradients across a membrane. In many cases, unfavorable reactions like these are coupled to the hydrolysis of ATP in order to make them energetically favorable under cellular conditions; we have learned that for these reactions the free energy released in breaking the phosphodiester bonds in ATP exceeds the energy consumed by the uphill reaction such that the sum of the free energy of the two reactions is negative (ΔG < 0). To perform these reactions, cells must then have a way of generating ATP efficiently so that a sufficient supply is always available. The amount of ATP used by a mammalian cell has been estimated to be on the order of 10^9 molecules per second. In other words, ATP is the principal energy currency of the cell.

How does the cell produce enough ATP to sustain life and what is the source of the energy required to drive the uphill reactions needed to form ATP? A biochemist by the name of Peter Mitchell considered these questions in the 1950s and 1960s and made the surprising discovery that membranes play a critical role (see Box 1). Specifically, this work showed, and as we shall see, that the energy released by transporting protons across the membranes of certain specialized organelles—chloroplasts and mitochondria—drives the synthesis of ATP from ADP and inorganic phosphate (P_i). Figure 1
In the 1950s, it was a mystery how the phosphorylation of ADP to ATP was mechanistically connected to the oxidation of glucose. Two main theories emerged—promoted by two men who were educated in the Biochemistry department at Cambridge in the years after World War II. Edward Charles Slater developed methods during the war to measure vitamin concentrations in food and later studied small molecule inhibitors of oxidative phosphorylation. These studies led him to suggest that the oxidation of glucose in some way led to the production of a high energy intermediate metabolite. This theory suggested that that intermediate was then used to convert ADP to ATP. Slater spent years trying to identify and isolate this proposed high energy intermediate without success.

Around the same time, Peter Mitchell began working on the same problem. During the war, Mitchell helped develop a treatment for skin lesions caused by a chemical warfare agent, Lewisite. The molecule they developed would not cross biological membranes and would scavenge the chemical agent from blood, allowing it to be excreted. After the war, Mitchell studied the mechanism of action of penicillin and the bilayer structure of bacterial membranes. His work on membranes and the transport of metabolites across them led him to propose the chemiosmotic theory of oxidative phosphorylation. This theory suggests that the energy in glucose is in some way stored in an electrochemical gradient across a biological membrane. The energy released upon movement of an ion down that gradient is then used to convert ADP and P to ATP. This was a radical idea at the time and Slater and Mitchell debated their theories in print for many years until Mitchell's gained widespread acceptance. He was awarded the Nobel Prize in Chemistry in 1978 for his work on the chemiosmotic theory.

Mitchell and his colleague, Jennifer Moyle, performed many of the definitive experiments on chemiosmosis at a research facility he built in a neglected mansion, Glynn House, in Cornwall, England. After stepping down from his position at Edinburgh University for health reasons, he spent several years restoring the mansion and building the laboratory, which was named the Glynn Research Institute. Mitchell continued to work there with a small group of research associates until 1987.

Box 1 Peter Mitchell and the Chemiosmotic Theory

In the 1950s, it was a mystery how the phosphorylation of ADP to ATP was mechanistically connected to the oxidation of glucose. Two main theories emerged—promoted by two men who were educated in the Biochemistry department at Cambridge in the years after World War II. Edward Charles Slater developed methods during the war to measure vitamin concentrations in food and later studied small molecule inhibitors of oxidative phosphorylation. These studies led him to suggest that the oxidation of glucose in some way led to the production of a high energy intermediate metabolite. This theory suggested that that intermediate was then used to convert ADP to ATP. Slater spent years trying to identify and isolate this proposed high energy intermediate without success.

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depicts the structure of these organelles and the following sections explain how these organelles capture energy from sunlight or energy from specific chemicals (e.g. carbohydrates) and convert it to the cell’s energy currency.

Chloroplasts harvest light energy to generate electrical energy

The ultimate source of chemical energy in the biosphere is the sun. Photosynthetic organisms capture the sun's energy and use it to generate chemical fuels (simple sugars), which are directly or indirectly consumed through the food chain. This conversion of sunlight into chemical energy takes place in three steps. First, photosynthetic organisms use light energy to generate an electrochemical gradient across a membrane, converting energy from light into electrical potential energy. A remarkable protein machine then converts this potential energy into chemical energy in the form of ATP. Finally, the ATP is used to drive the formation of carbohydrates by fixation.
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of carbon dioxide from the atmosphere. In what follows, we will consider these three phases of photosynthesis: the generation of an electrochemical gradient, the synthesis of ATP, and the conversion of carbon dioxide to carbohydrate.

The molecules that absorb light in plants are found in the thylakoid membranes of their chloroplasts. Chloroplasts have three membrane bilayers: outer and inner membranes, which surround the organelle, and a thylakoid membrane, which folds back and forth upon itself into stacks of membranes called grana (Figure 1). Light-absorbing protein complexes called photosystems span the thylakoid bilayer and consist of several integral membrane proteins and hundreds of small pigment molecules. The most important of these pigments is chlorophyll—the molecule that gives plants their green color. Most of the chlorophyll molecules are bound to the proteins at the periphery of the photosystem in what are called antenna complexes, but two special chlorophyll molecules are also found in the middle of the photosystem in the reaction center (Figure 2). Photosystems convert light energy into electrical energy at this reaction center.

Chlorophyll molecules contain a chemical functional group, a porphyrin ring, that is capable of absorbing sunlight in the red region of the visible spectrum (Figure 2). When one of the chlorophyll molecules in the antenna complexes absorbs light, the electrons in its porphyrin ring move to a higher energy state. As the electrons return to their normal, lower energy state, they...
transfer their absorbed energy to a neighboring chlorophyll molecule. This second chlorophyll molecule, in turn, transfers its absorbed energy to yet another neighbor in a chain reaction that continues until the energy reaches the reaction center of the photosystem complex. The electrons in the pair of chlorophyll molecules in the reaction center also move to a higher state when they absorb the energy from the neighboring antenna chlorophyll, but they then transfer those high energy electrons to an acceptor molecule. In other words, the reaction center chlorophyll molecules transfer electrons with their absorbed energy rather than just transferring the energy (as the antenna chlorophyll molecules do). This electron transfer is the step in photosynthesis that converts light energy into electrical energy.

The transfer of electrons from the reaction center creates a charge separation in which the chlorophyll molecules now carry positive charges. This leaves us with two questions: How do the special chlorophyll molecules regain their electrons and what is the fate the electrons that had been ejected from the reaction center? We shall address the fate of the ejected electrons after we explain the answer to the first question.

The chlorophyll molecules regain their proper number of electrons by extracting them from water molecules in the thylakoid space. Reactions of this type, in which electrons are transferred from one molecule to another, are called redox reactions. The molecule that loses electrons is said to be “oxidized,” and the molecule that gains electrons is said to be “reduced.” As shown below, we can write the oxidation and reduction steps of a reaction as two separate “half reactions,” in which we explicitly show the lost or gained electrons as products or reactants, respectively.

**Half Reactions:**

\[
\text{Oxidation: } A \rightarrow A^+ + e^- \\
\text{Reduction: } B^+ + e^- \rightarrow B
\]

**Complete Redox Reaction:**

\[
A + B^+ \rightarrow A^+ + B
\]

In photosynthesis, the oxygen atoms in water molecules undergo oxidation, transferring some of their electrons to reduce the special chlorophyll molecules in the reaction center and in the process the water breaks down to
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Figure 3 The electron transport chain in the chloroplast thylakoid membrane.

Two photosystems harvest light energy and eject high energy electrons, which are then transferred to other electron carrier molecules in the chain. As the electrons move from water to NADPH, six protons are released into the thylakoid space, creating a proton gradient across the membrane. For a more detailed description of each of the electron transfers in this chain, see Box 2.
The electron transport chains of chloroplasts and mitochondria share several similar components despite the fact that they derive their high energy electrons from very different sources. A more detailed description of the electron transfers in these organelles illustrates these similarities. From the first photosystem in the chloroplast electron transport chain (photosystem II), high energy electrons are transferred to a small molecule, plastoquinone (pQ). Plastoquinone is soluble in the lipid bilayer and carries the electrons to the next protein complex in the thylakoid membrane, the cytochrome b$_{6}$f complex (yellow region in Figure 3). The cytochrome b$_{6}$f complex transfers the electrons from plastoquinone to plastocyanin (pC), a small water-soluble protein in the thylakoid space. As the cytochrome b$_{6}$f complex performs these electron transfers, it transports two protons across the thylakoid membrane. Plastocyanin then carries the electrons from the cytochrome b$_{6}$f complex to the second photosystem in the chain (Photosystem I). The electrons on plastocyanin are used to replace those that are ejected from the reaction center of Photosystem I. The high energy electrons from Photosystem I are transferred to ferredoxin (Fd), a protein on the stromal side of the membrane. The final protein complex in the chain is the ferredoxin-NADP$^{+}$ reductase, which transfers the electrons from ferredoxin to NADP$^{+}$, generating NADPH.

The mitochondrial electron transport chain starts at Complex I, which accepts electrons from NADH and transfers them to a hydrophobic small molecule, quinone, in the membrane bilayer. Quinone has similar properties to plastoquinone in chloroplasts; it is soluble in the membrane lipids and carries electrons through the membrane from one protein complex to the next. As complex I transfers electrons to quinone, it transports four protons across the membrane. Complex II also transfers electrons to quinone but it accepts them from a different substrate, succinate instead of NADH, and does not transport any protons. Quinone then carries the electrons it has accepted from Complex I or II to Complex III. Complex III pumps another four protons across the membrane as it transfers the electrons from quinone to cytochrome c. Cytochrome c is a soluble protein on the outer surface of the membrane (like plastocyanin in chloroplasts) that can move between complexes III and IV. When cytochrome c binds to complex IV, it transfers its electrons through complex IV to oxygen on the opposite side of the membrane. Oxygen is the ultimate electron acceptor in the electron transport chain. Complex IV reduces oxygen to water and transports another two protons across the membrane in this process.

There are thus electron transfers in both chains that involve a small lipid-soluble molecule, plastoquinone or quinone, and a soluble protein outside the membrane, plastocyanin or cytochrome c. These carrier molecules move the electrons from one membrane protein complex to the next in the chain. Both electron transport chains also clearly generate the proton gradients across their respective membranes that are critical for synthesizing ATP.
Box 3 Other Cellular Energy Currencies: NADPH, NADH, and FADH$_2$

Photosynthesis and aerobic glucose metabolism are redox processes in which electrons are transferred between carbon and oxygen. In photosynthesis, the carbon in carbon dioxide is reduced and the oxygen in water is oxidized; in aerobic glucose metabolism, the opposite occurs as the carbon in glucose is oxidized and oxygen is reduced to form water. The electrons in these processes, however, are not transferred directly between the carbon and oxygen atoms. The electrons are instead first transferred to or “captured” by carrier molecules, NADP$^+$, NAD$^+$, and FAD, which become NADPH, NADH, and FADH$_2$ upon reduction. These molecules are water-soluble dinucleotides that readily undergo reversible reduction and oxidation. The structures on the left show both the oxidized (left) and reduced (right) forms of these molecules and how the equilibrium mixture between these states is governed by whether they are receiving or donating electrons. The nicotinamide group (highlighted in yellow) of the nicotinamide adenine dinucleotides (NADP$^+$ and NAD$^+$) readily accepts and donates two electrons and a proton to other molecules in the cell. These two molecules differ only by the presence of a 2'-phosphate on NADP$^+$, but they are used in different processes in the cell. NADPH is used in biosynthetic processes like photosynthesis. By contrast, NAD$^+$ is used to oxidize substrates like glucose to make ATP. A similar molecule, FAD, is also used to metabolize substrates. The flavin group (also highlighted in yellow) carries two electrons, but it can transfer them one at a time to other molecules in the cell.

Note that because the oxidation of NADPH, NADH, and FADH$_2$ is energetically favorable, these molecules can be thought of as another energy currency in the cell. Their ability to donate electrons to other molecules can be harnessed to make other forms of chemical energy or an electrochemical gradient across a membrane.
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These electron transfers (Figure 4). The two photosystems each increase the energy of the electrons in the pathway; all of the other transfers are energetically downhill. Two inputs of light energy are required because photosystem II cannot generate an electron with high enough energy to be transferred to NADP⁺ and photosystem I cannot directly extract electrons from water. In tandem, however, the two photosystems are capable of transferring electrons uphill from H₂O to NADPH. The electrons on NADPH are then used to make a carbohydrate from carbon dioxide in the last phase of photosynthesis; however, this carbohydrate synthesis also requires ATP, which is made using the other product of the electron transport chain, the proton gradient across the thylakoid membrane.

For each NADPH molecule produced by the electron transport chain, six protons are released into the thylakoid space inside the membrane. Four of these come from the water splitting reaction at photosystem II and two are transported across the membrane using the energy of the subsequent downhill electron transfers. The resulting pH difference between the thylakoid space and the stroma is approximately 3 units (i.e., a 1,000-fold difference in [H⁺]). This proton gradient is a high energy, low entropy state, which effectively conserves some of the energy absorbed from the sun. Because protons are positively charged, this gradient has both chemical and electrical components (Figure 5). The chemical gradient is created by the difference in the concentration of the protons (a pH difference), and the electrical gradient is created by the charge separation (a potential difference, or voltage). Both of these gradients contribute to the proton motive force—i.e., a force that drives the movement of protons down these gradients from the thylakoid space into the stroma.

The ATP synthase is a molecular machine that couples proton transport to ATP synthesis through mechanical rotation

When protons flow down their electrochemical gradient across the thylakoid membrane, energy is released that can be used to produce ATP from ADP and inorganic phosphate (P). The ATP Synthase is a molecular

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**Figure 5 The electron transport chain generates an electrochemical gradient across the thylakoid membrane**

Both the chemical (pH) and electrical (voltage) components of the gradient favor the movement of protons into the stroma from the thylakoid space.
The ATP synthase couples proton transport to ATP synthesis through mechanical rotation.

Machine that directly couples the downhill transport of protons across the membrane to the uphill chemical reaction that synthesizes ATP. This remarkable machine is found in the membranes of chloroplasts, mitochondria, and bacteria where it performs the same, fundamental energy-converting reaction. The potential energy stored in the proton gradient is first converted to mechanical energy and then into chemical energy by a series of conformational changes that alter the catalytic sites of the ATP synthase as we will explain. The concept that an electrochemical gradient can be harnessed to generate chemical energy in the form of ATP came as a huge surprise and was the basis for a Nobel Prize to its advocate Peter Mitchell as discussed in Box 1.

The structure of the ATP Synthase reveals how the enzyme accomplishes these energy interconversions. The synthase has a lollipop structure with its base embedded in the membrane and its head protruding into the chloroplast stroma (or mitochondrial matrix or bacterial cytoplasm) (Figure 6). The membrane portion of the synthase contains the proton-transporting activity; protons passively flow down their gradient through a channel formed by the transmembrane protein subunits. The movement of protons across the membrane induces the transmembrane proteins to rotate in the plane of the membrane, which in turn makes the axle of the ATP synthase rotate as well. The axle consists of a set of extended proteins which link the transmembrane proteins to the enzymatic, ATP-generating part of the ATP synthase. These axle proteins sit inside the central cavity of the ATP generator (the head of the lollipop). The ATP generator contains 6 subunits (3 α and 3 β), which are arranged like segments of an orange around the axle. Each β subunit contains a catalytic site for synthesizing ATP. The ATP generator does not rotate with the axle; it is held in place by the stator protein, which is anchored in the membrane. When the axle rotates inside the ATP generator, it interacts differently with the three individual β subunits; these changing interactions drive conformational changes in the β subunits that facilitate ATP synthesis.
Figure 7 ATP synthesis is catalyzed by a binding change mechanism that dramatically stabilizes ATP in the enzyme active site

(A) The subunits of the ATP generator exist in different conformations (indicated by the different shapes) depending on their interactions with the axle proteins at their center. The subunits of the ATP generator do not rotate, but each 120° rotation of the axle induces their conformations to interconvert such that the affinity of the β subunits for ADP and ATP changes. The text describes the reaction cycle from the perspective of the black β subunit, but notice that all three of the β subunits are engaged in synthesizing ATP. (B) The energetic changes associated with each step of the ATP synthesis reaction are shown. Each step corresponds to a 120° rotation of the axle proteins. The initial association of the substrates, ADP and Pi, with the enzyme is spontaneous. Upon rotation of the axle, the active site converts to a conformation that binds ATP tightly and the new bond between ADP and Pi accordingly forms. The final rotation of the axle provides the energy to release the newly formed ATP from the active site.

The rotation of the axle proteins induce conformational changes in the structure of the β subunit active sites (Figure 7A). These changes cause the affinities for ATP and ADP and Pi to vary as the axel rotates (Figure 7B. Paul Boyer described this “binding change mechanism” using biochemical experiments, and John Walker verified it by determining the structure of the ATP synthase; they received the Nobel Prize in Chemistry in 1997 for their work. This mechanism dictates that at any given time, the three β subunits are each in a different state: one has ADP and Pi bound, the second has ATP bound tightly; and the third is empty. The rotation of the axle induces the subunit states to sequentially interconvert (Figure 7A). If we consider the reaction cycle from the perspective of a single β subunit that starts in the conformation that binds ADP and Pi tightly, it will spontaneously pick up these substrates from the chloroplast stroma as the binding equilibrium is favorable ($\Delta G < 0$). The first rotation of the axle then induces this subunit to adopt the conformation that binds ATP tightly. This conformation binds ATP so tightly that the free energies of the ADP- and ATP-bound states
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Overview of photosynthesis

The oxygen in water is oxidized and the carbon in carbon dioxide is reduced in two separate steps of photosynthesis: the light and dark reactions, respectively. NADPH acts as an intermediary electron carrier between these two reactions. The NADPH and ATP produced by the light-dependent oxidation of water is used to fix carbon dioxide into a water-soluble carbohydrate building block.

Light reactions

$H_2O \rightarrow O_2$

NADP$^+$

ADP + $P_i$ \rightarrow NADPH

ATP

Dark reactions

carbohydrate \rightarrow CO$_2$

The critical carbon-fixing reaction of the Calvin cycle is carried out by an enzyme named ribulose 1,5-bisphosphate carboxylase/oxygenase, or rubisco for short. Rubisco attaches CO$_2$ to a 5-carbon molecule, ribulose 1,5-bisphosphate. This molecule is a derivative of the sugar ribulose in which two hydroxyls (OH groups) have been replaced by phosphates.

This generates an unstable 6-carbon intermediate, which breaks down into two 3-carbon molecules of 3-phosphoglycerate (Figure 9). This phase consists of a cycle of reactions that are collectively termed the dark reactions of photosynthesis or, alternatively, the Calvin cycle. These reactions utilize the NADPH and ATP produced by the electron transport chain and the ATP synthase. In addition to producing carbohydrates, the dark reactions also regenerate NADP$^+$ and ADP so that photosynthesis can continue (Figure 8).

The dark reactions of photosynthesis generate a sugar from carbon dioxide

Carbon dioxide is fixed, that is, removed from the atmosphere and incorporated into a small, water-soluble carbohydrate in the last phase of photosynthesis. (Carbohydrates are molecules that have the general formula C$_n$(H$_2$O)$_n$ in which each carbon is hydrated with water. They can consist of a single sugar such as glucose [C$_6$H$_{12}$O$_6$] or ribulose [see below] or a polymer of multiple sugars as in starch and cellulose.) This last phase consists of a cycle of reactions that are collectively termed the dark reactions of photosynthesis or, alternatively, the Calvin cycle. These reactions utilize the NADPH and ATP produced by the electron transport chain and the ATP synthase. In addition to producing carbohydrates, the dark reactions also regenerate NADP$^+$ and ADP so that photosynthesis can continue (Figure 8).

The oxygen in water is oxidized and the carbon in carbon dioxide is reduced in two separate steps of photosynthesis: the light and dark reactions, respectively. NADPH acts as an intermediary electron carrier between these two reactions. The NADPH and ATP produced by the light-dependent oxidation of water is used to fix carbon dioxide into a water-soluble carbohydrate building block.
Figure 9 Rubisco catalyzes the carbon-fixing reaction of photosynthesis by attaching a carbon dioxide molecule to ribulose 1,5-bisphosphate, but the enzyme must contend with a competing oxygenation reaction.

This oxygenation reaction (A) creates a product, phosphoglycolate, which cannot be easily recycled to make ribulose 1,5-bisphosphate. The enzyme has consequently evolved to suppress the oxygenation reaction but with detrimental effects on the efficiency of carboxylation reaction (B).

Box 4 Carbon Fixation by the Calvin Cycle

Melvin Calvin received the Nobel Prize in Chemistry in 1961 for describing how plants assimilate carbon dioxide. This process is accordingly named the Calvin cycle and it consists of three steps. The first step is catalyzed by rubisco and results in the incorporation of carbon dioxide molecules into ribulose 1,5-bisphosphate to produce 3-phosphoglycerate. In the second step, 3-phosphoglycerate is phosphorylated and reduced to make glyceraldehyde 3-phosphate using the ATP and NADPH produced in the light reactions of photosynthesis. This molecule, glyceraldehyde 3-phosphate, is the product of photosynthesis; it is a simple sugar that can be used as a biosynthetic building block for larger sugars, fatty acids, and amino acids. In step three of the Calvin cycle, however, some of the glyceraldehyde 3-phosphate molecules produced by step two are recombined and phosphorylated to regenerate ribulose 1,5-bisphosphate. This step also requires ATP and is necessary to keep the Calvin cycle running in the forward direction. If the rubisco substrate, ribulose 1,5-bisphosphate, was depleted, CO₂ could no longer be fixed.
barrier to its reaction increases and the reaction rate decreases.

Rubisco thus illustrates the tradeoff between substrate specificity and catalytic activity. Enzymes must bind their substrates with enough affinity to allow those substrates to outcompete other similar non-substrate molecules that might fit in the active site; however, enzymes cannot bind their substrates too tightly or they will make them too stable to react. The balance of these two competing factors forces rubisco to a low catalytic efficiency, but plants make lots of this protein to compensate. Rubisco accounts for 50% of the protein weight of chloroplasts and is consequently one of the most abundant enzymes on the planet.

The remaining reactions in the Calvin cycle convert the 3-phosphoglycerate made by rubisco to glyceraldehyde 3-phosphate and regenerate the rubisco substrate, ribulose 1,5-bisphosphate (see Box 4 for details). The glyceraldehyde 3-phosphate is used as a substrate to generate the sugar glucose and, in turn, the polymer of glucose, starch. Plants (and animals) generally store chemical energy that is not immediately required to perform cellular reactions in the form of sugar or starch rather than as ATP because sugars are more soluble in water than ATP. More sugar can be dissolved in the cell and, therefore, more energy can be stored in the cell in this form.

In summary, through the light and dark reactions of photosynthesis, plants convert light energy first to potential energy in the form an electrochemical gradient, then to chemical energy in the form of ATP and the reducing potential of NADPH, and finally to chemical energy in the form of a simple sugar. Glyceraldehyde 3-phosphate molecules can be combined to make sugar molecules like glucose and then assembled into larger starch molecules for storage. Starch can then be broken down to make ATP according to the cell's needs. The next sections address how organisms break down sugars to meet their energy demands.

**Non-photosynthetic organisms generate ATP by metabolizing glucose and other carbon-containing molecules**

Many organisms are not photosynthetic and hence are unable to capture the sun's energy to make ATP. Instead, they consume carbon-containing compounds, such as carbohydrates, lipids and proteins produced by other organisms and metabolize them to generate ATP. Ultimately, this food chain originates from glucose generated by photosynthetic organisms. (Even photosynthetic cells in plants rely on glucose generated by their own chloroplasts to generate ATP and many plant cells, such as those of the roots and stem, are not photosynthetic.)

Here we will focus on generating ATP from glucose. This can be achieved in two ways: one requires oxygen ($O_2$) and the other does not. The **anaerobic** (oxygen-independent) process is called fermentation and is much simpler than the **aerobic** (oxygen-dependent) process.

Fermentation begins with a series of ten reactions that are collectively called **glycolysis**. Glucose, a sugar (“glyco”) molecule containing 6-carbons, is split (“lysis”) into two 3-carbon molecules. The first five reactions are the preparatory phase of glycolysis, in which two ATP molecules are expended
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Figure 10 The ten steps of glycolysis

(A) Two ATP molecules have to be invested in the preparatory phase of glycolysis to start the glucose metabolism process. Four molecules of ATP, however, are produced in the payoff phase by generating 1,3-bisphosphoglycerate (step 6) and phosphoenolpyruvate (steps 8 and 9). These molecules contain high energy phosphate bonds, which enable them to directly phosphorylate ADP (steps 7 and 10). (B) Shown are chemical structures depicting the molecules involved in steps 6 through 10. (Note that steps 6 and 7 reverse the reactions of the second step of the Calvin cycle as seen in Box 4.) Regions of molecules highlighted in yellow depict unstable phosphate bonds. ADP reacts with these reactive phosphate groups to make ATP.

to phosphorylate the sugar substrate and enable its cleavage into two 3-carbon molecules. The second five reactions constitute the payoff phase, as molecules containing high energy phosphates are generated and then used to directly phosphorylate ADP. In total, these ten reactions produce two molecules of ATP and two molecules of NADH (Figure 10A and Box 3).

The sixth and seventh steps of glycolysis are the key reactions that generate the first molecule containing a high energy phosphate, 1,3-bisphosphoglycerate (Figure 10B). Importantly, the phosphate bond in 1,3-bisphosphoglycerate (highlighted in yellow) is at a higher energy state than ATP. This molecule can, therefore, spontaneously transfer a phosphate to ADP to make ATP. The remaining steps in glycolysis generate another molecule with a similarly unstable phosphate bond, phosphoenolpyruvate, which also directly phosphorylates ADP.
Glycolysis produces a net yield of 2 molecules of ATP, which can be used to carry out cellular reactions; however, and as we have seen, glycolysis also consumes 2 molecules of NAD$^+$ (Figure 10, step 6). These NAD$^+$ molecules must be regenerated so that glycolysis can continue breaking down more molecules of glucose. In cells that are oxygen-deprived, the NAD$^+$ is regenerated by reducing the pyruvate from glycolysis to either lactate or ethanol using NADH. This process is ancient; early cellular organisms evolved in an atmosphere that contained very little oxygen and needed to generate energy anaerobically, but many contemporary organisms have retained the ability to ferment glucose in this way. For example, when muscle cells are being strenuously exerted, they become depleted of oxygen and start to convert pyruvate to lactate (Figure 11). The build-up of the acidic lactate molecule in these cells causes muscle soreness. Many microorganisms, including brewer’s and baker’s yeast, perform a different type of fermentation in which pyruvate is converted into ethanol and carbon dioxide. The carbon dioxide produced by these organisms makes bread rise and champagne bubble, but the critical product for sustaining life is NAD+. The entire fermentation process thus produces a net yield of 2 molecules of ATP without consuming oxygen or changing the concentrations of NAD$^+$ and NADH in the cell.

**Respiration extracts much more additional energy from glucose**

Fermentation does not extract all of the chemical energy stored in glucose. Much more ATP can be produced if pyruvate is further metabolized and NADH is re-oxidized using oxygen. Instead of fermenting pyruvate to lactate or ethanol, pyruvate can be completely converted into carbon dioxide through the action of the enzyme pyruvate dehydrogenase and a series of reactions called the citric acid cycle (Figure 12). These phases of the glucose metabolism process produce two more molecules of ATP and several more molecules of NADH and FADH$_2$ (another electron carrier with similar properties to NADH; see Box 3). These NADH molecules carry much of the energy that was originally contained in the chemical bonds of glucose in the form of high energy electrons.

NADH then acts as the starting material for a chain of electron transfer reactions that end with the electrons being transferred to molecular oxygen (O$_2$) to produce water (H$_2$O). These reactions are catalyzed by...
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a set of protein complexes in the inner membrane of the mitochondria. The electrons are progressively stabilized as they are transferred down the chain, and the released energy is used to transport protons from the most internal aqueous compartment in the mitochondria, the matrix, to the space between the two membranes, the intermembrane space (Figure 13). The protein complexes of the mitochondrial electron transport chain thus have a similar function to those in the chloroplasts of photosynthetic organisms; these protein complexes create a proton gradient that is harnessed by an ATP synthase to make ATP. The production of ATP in the mitochondria is, however, coupled to the consumption of oxygen in what is referred to as respiration.

Interestingly, in certain cells the proton gradient across the mitochondrial membrane also serves as an energy source for generating heat (thermal energy) instead of ATP (chemical energy). This alternative process is used by babies and hibernating animals to stay warm (see Box 5), and it illustrates the generality of the electrochemical gradient as an energy reservoir.

The mitochondrial and chloroplastic electron transport chains affect the energy of the electrons they transport in opposite ways. In chloroplasts, the net reaction destabilizes the electrons; they start on \( \text{H}_2\text{O} \) and end on \( \text{NADPH} \). In mitochondria, the net reaction stabilizes the electrons; they start on \( \text{NADH} \) and end on \( \text{H}_2\text{O} \). The key, however, is that both electron transport chains produce an electrochemical gradient across their respective membranes. This gradient conserves some of the energy derived from sunlight or the chemical bonds of glucose. The source of the high energy electrons that are used to make the gradient then becomes immaterial and ATP is made by a common machine that is found in all organisms.

Glucose is oxidized to carbon dioxide in the first three stages of the pathway. The electrons that are lost in this oxidation process are transferred to \( \text{NAD}^+ \) and \( \text{FAD} \) to produce \( \text{NADH} \) and \( \text{FADH}_2 \). In the last stage of the pathway, the electrons are transferred from these molecules to oxygen, reducing \( \text{O}_2 \) to water.

(Note that the stoichiometry of ATP production by the electron transport chain and the ATP synthase is shown per NADH molecule. \( \text{FADH}_2 \) is used by the electron transport chain in a similar manner.)

Figure 12  Overview of respiration

Glucose is oxidized to carbon dioxide in the first three stages of the pathway. The electrons that are lost in this oxidation process are transferred to \( \text{NAD}^+ \) and \( \text{FAD} \) to produce \( \text{NADH} \) and \( \text{FADH}_2 \). In the last stage of the pathway, the electrons are transferred from these molecules to oxygen, reducing \( \text{O}_2 \) to water.

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a set of protein complexes in the inner membrane of the mitochondria. The electrons are progressively stabilized as they are transferred down the chain, and the released energy is used to transport protons from the most internal aqueous compartment in the mitochondria, the matrix, to the space between the two membranes, the intermembrane space (Figure 13). The protein complexes of the mitochondrial electron transport chain thus have a similar function to those in the chloroplasts of photosynthetic organisms; these protein complexes create a proton gradient that is harnessed by an ATP synthase to make ATP. The production of ATP in the mitochondria is, however, coupled to the consumption of oxygen in what is referred to as respiration.

Interestingly, in certain cells the proton gradient across the mitochondrial membrane also serves as an energy source for generating heat (thermal energy) instead of ATP (chemical energy). This alternative process is used by babies and hibernating animals to stay warm (see Box 5), and it illustrates the generality of the electrochemical gradient as an energy reservoir.

The mitochondrial and chloroplastic electron transport chains affect the energy of the electrons they transport in opposite ways. In chloroplasts, the net reaction destabilizes the electrons; they start on \( \text{H}_2\text{O} \) and end on \( \text{NADPH} \). In mitochondria, the net reaction stabilizes the electrons; they start on \( \text{NADH} \) and end on \( \text{H}_2\text{O} \). The key, however, is that both electron transport chains produce an electrochemical gradient across their respective membranes. This gradient conserves some of the energy derived from sunlight or the chemical bonds of glucose. The source of the high energy electrons that are used to make the gradient then becomes immaterial and ATP is made by a common machine that is found in all organisms.

Glucose is oxidized to carbon dioxide in the first three stages of the pathway. The electrons that are lost in this oxidation process are transferred to \( \text{NAD}^+ \) and \( \text{FAD} \) to produce \( \text{NADH} \) and \( \text{FADH}_2 \). In the last stage of the pathway, the electrons are transferred from these molecules to oxygen, reducing \( \text{O}_2 \) to water.

(Note that the stoichiometry of ATP production by the electron transport chain and the ATP synthase is shown per NADH molecule. \( \text{FADH}_2 \) is used by the electron transport chain in a similar manner.)
Photosynthesis and Respiration

Chapter 18

Free energy of electrons

reaction coordinate

NADH

H2O

Complex I

Complex III

Complex IV

4 H+ 4 H+ 2 H+

1/2 O2 + 2 H+

NADH NAD+ + H+

H2O

INTERMEMBRANE SPACE

MATRIX

A

B

Figure 13 The mitochondrial electron transport chain uses the energy from the electrons in NADH to transport 10 protons across the mitochondrial inner membrane from the matrix into the intermembrane space

(A) Electrons are transferred from NADH to H2O through a series of protein complexes in the membrane and other carrier molecules. (The carrier molecules and one additional protein complex are not shown in this simplified depiction of the electron transport chain; see Box 2 for a more detailed description.) Each of the transmembrane protein complexes transports protons across the membrane as it transfers electrons. Complex IV, the last complex in the chain, carries out the critical oxygen-consuming reaction; this one complex consumes approximately 90% of the oxygen taken up by the cell. (B) All of the electron transfers in the mitochondrial electron transport chain are energetically favorable.

Box 5 Brown Fat, Hibernation, and the Uncoupling Proteins

The efficiency of the ATP synthase depends on the tight coupling of proton transport to ATP synthesis; in other words, protons do not cross (or “leak” through) the membrane without passing through the ATP synthase and generating ATP. In certain circumstances, however, it is advantageous to decouple proton transport across the inner mitochondrial membrane from ATP synthesis. Some fat cells produce a protein that creates a pore in the inner mitochondrial membrane. This protein is called an uncoupling protein because it allows protons to flow freely down the gradient created by the electron transport chain without producing ATP. The uncoupling protein effectively dissipates the electrochemical gradient and releases the potential energy stored in the membrane as heat. The fat cells that produce this protein have a higher than average number of mitochondria to enable this heat production, and they are named brown fat cells because the many iron-containing proteins in their mitochondria give them a brown appearance. Newborn babies and hibernating animals have more brown fat than (alert) adults because they are more susceptible to temperature changes and less able to regulate their temperature via other means (e.g., shivering, moving to a different location, putting on clothes). Different cell types can thus harness the energy stored in the electrochemical gradient across the mitochondrial membrane for different purposes - for generating ATP to perform chemical reactions or for generating heat to maintain the organism’s homeostasis.
Summary

Living systems must maintain conditions that are far from equilibrium. This requires a constant input of energy. The energy that drives living systems ultimately derives from the sun. Energy from sunlight is captured by photosynthetic organisms, which convert light energy into electrical energy using special complexes in the membranes of their chloroplasts. These photosystem complexes produce high energy electrons that are then used to pump protons across the thylakoid membrane. The electrons are derived from water, which is split (oxidized) to generate oxygen, protons and electrons, which become a source of reducing power in the form of NADPH. Next, a rotary motor, ATP synthase, converts the potential energy stored in the proton gradient to chemical energy in the form of ATP through a mechanical process that changes the nucleotide binding affinities of its active sites. The ATP and NADPH from these first two phases of photosynthesis are then used to reduce (“fix”) carbon dioxide to generate chemical energy in the form of three-carbon sugar phosphate molecules by a series of chemical reactions known as the Calvin cycle or the dark reactions of photosynthesis, which are catalyzed by the enzyme rubisco. The three-carbon sugar phosphates can be used to create more complex biological molecules or stored in the form of carbohydrates and later metabolized to generate ATP again.

Non-photosynthetic organisms metabolize the carbohydrates produced by photosynthetic organisms to generate ATP. They can do so anaerobically in a process called fermentation. Fermentation produces ATP by extracting some of the chemical energy stored in glucose. The carbons in glucose are partially oxidized to produce molecules containing high energy phosphates that can be transferred to ADP. The carbons are subsequently re-reduced so that there is no net oxidation in fermentation. Aerobic metabolism or respiration, on the other hand, extracts much more energy from each glucose molecule by fully oxidizing each carbon atom to generate carbon dioxide. The energy in the chemical bonds of glucose is captured as high energy electrons in the form of NADH and related molecules that can be fed into an electron transport chain in the mitochondrial membrane. As in photosynthesis, the energy carried by those electrons is used to generate a proton gradient across the membrane, which is, in turn, converted into ATP by the same kind of ATP synthase machine as found in chloroplasts. Thus, life is powered by the excitation of electrons, which drive oxidation and reduction reactions that, in turn, generate chemical energy in the form of ATP and sugars.