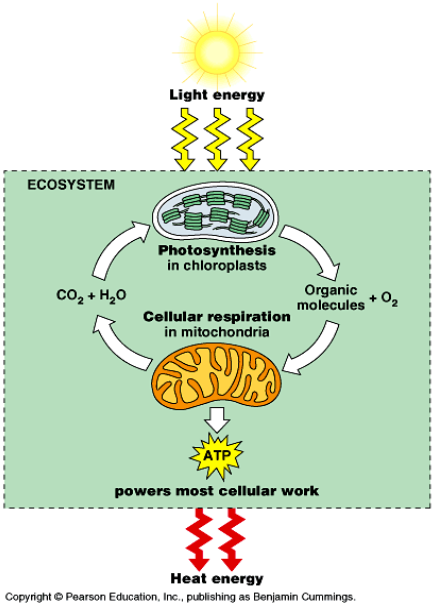
CHAPTER 9
CELLULAR RESPIRATION:
HARVESTING CHEMICAL ENERGY



Introduction

- Living is work.
- To perform their many tasks, cells require transfusions of energy from outside sources.
  - In most ecosystems, energy enters as sunlight.
  - Light energy trapped in organic molecules is available to both photosynthetic organisms and others that eat them.

A. The Principles of Energy Harvest

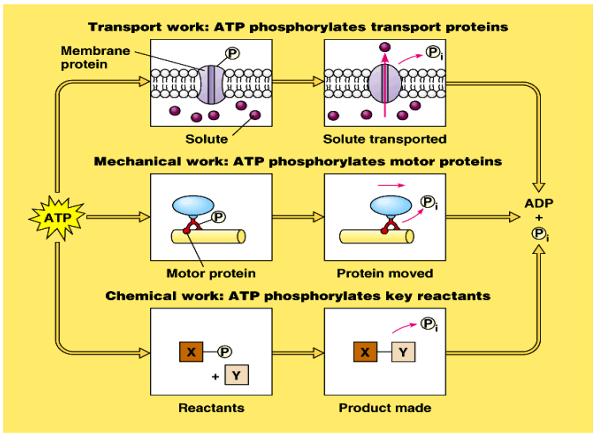
1. Cellular respiration and fermentation are catabolic, energy-yielding pathways

- Organic molecules store energy in their arrangement of atoms.
- Enzymes catalyze the systematic degradation of organic molecules that are rich in energy to simpler waste products with less energy.
- Some of the released energy is used to do work and the rest is dissipated as heat.
- Metabolic pathways that release the energy stored in complex organic molecules are catabolic.
- One type of catabolic process, **fermentation**, leads to the partial degradation of sugars in the absence of oxygen.
- A more efficient and widespread catabolic process, **cellular respiration**, uses oxygen as a reactant to complete the breakdown of a variety of organic molecules.
  - Most of the processes in cellular respiration occur in mitochondria.

- Cellular respiration is similar to the combustion of gasoline in an automobile engine.
- The overall process is:
  - Organic compounds + O_2 -> CO_2 + H_2O + Energy
- Carbohydrates, fats, and proteins can all be used as the fuel, but it is traditional to start learning with glucose.
  - $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ + Energy (ATP + heat)
- The catabolism of glucose is exergonic with a ΔG of - 686 kcal per mole of glucose.
  - Some of this energy is used to produce ATP that will perform cellular work.

2. Cells recycle the ATP they use for work

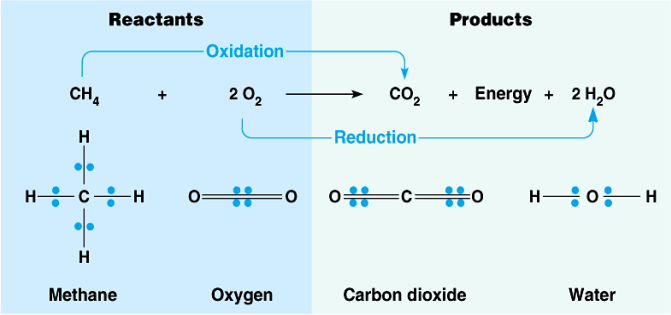
- ATP, adenosine triphosphate, is the pivotal molecule in cellular energetics.
- It is the chemical equivalent of a loaded spring.
  - The close packing of three negatively charged phosphate groups is an unstable, energy-storing arrangement.
  - Loss of the end phosphate group "relaxes" the "spring".
- The price of most cellular work is the conversion of ATP to ADP and inorganic phosphate (P_i).
- An animal cell regenerates ATP from ADP and P_i by the catabolism of organic molecules.
- The transfer of the terminal phosphate group from ATP to another molecule is phosphorylation.
  - This changes the shape of the receiving molecule, performing work (transport, mechanical, or chemical).
  - When the phosphate group leaves the molecule, the molecule returns to its alternate shape.



3. Redox reactions release energy when electrons move closer to electronegative atoms

- Catabolic pathways relocate the electrons stored in food molecules, releasing energy that is used to synthesize ATP.
- Reactions that result in the transfer of one or more electrons from one reactant to another are oxidation-reduction reactions, or **redox reactions**.
  - The loss of electrons is called **oxidation**.
  - The addition of electrons is called **reduction**.
- The formation of table salt from sodium and chloride is a redox reaction.
  - $Na + Cl \rightarrow Na^+ + Cl^-$
  - Here sodium is oxidized and chlorine is reduced (its charge drops from 0 to -1).
- More generally: $Xe^- + Y \rightarrow X + Ye^-$
  - X, the electron donor, is the **reducing agent** and reduces Y.
  - Y, the electron recipient, is the **oxidizing agent** and oxidizes X.
- Redox reactions require both a donor and acceptor.
- Redox reactions also occur when the movement of electrons is not complete but involve a change in the degree of electron sharing in covalent bonds.

- In the combustion of methane to form water and carbon dioxide, the nonpolar covalent bonds of methane (C-H) and oxygen (O=O) are converted to polar covalent bonds (C=O and O-H).



- When these bonds shift from nonpolar to polar, the electrons move from positions equidistant between the two atoms for a closer position to oxygen, the more electronegative atom.
    - Oxygen is one of the most potent oxidizing agents.
- An electron loses energy as it shifts from a less electronegative atom to a more electronegative one.
- A redox reaction that relocates electrons closer to oxygen releases chemical energy that can do work.
- To reverse the process, energy must be added to pull an electron away from an atom.

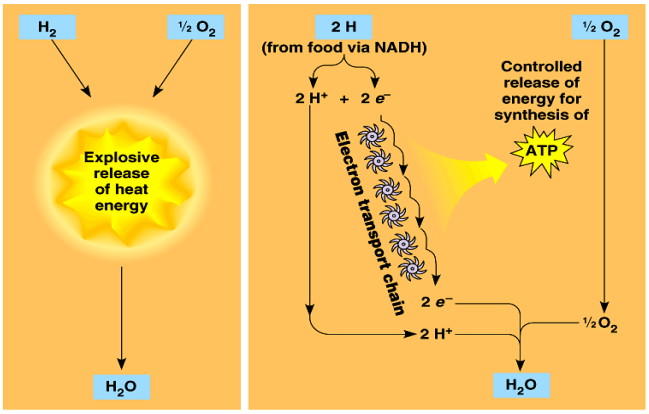
4. Electrons "fall" from organic molecules to oxygen during cellular respiration

- In cellular respiration, glucose and other fuel molecules are oxidized, releasing energy.
- In the summary equation of cellular respiration: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$
    - Glucose is oxidized, oxygen is reduced, and electrons lose potential energy.
- Molecules that have an abundance of hydrogen are excellent fuels because their bonds are a source of "hilltop" electrons that "fall" closer to oxygen.
- The cell has a rich reservoir of electrons associated with hydrogen, especially in carbohydrates and fats.
- However, these fuels do not spontaneously combine with O_2 because they lack the activation energy.
- Enzymes lower the barrier of activation energy, allowing these fuels to be oxidized slowly.

5. The "fall" of electrons during respiration is stepwise, via NAD^+ and an electron transport chain

- Cellular respiration does not oxidize glucose in a single step that transfers all the hydrogen in the fuel to oxygen at one time.
- Rather, glucose and other fuels are broken down gradually in a series of steps, each catalyzed by a specific enzyme.
- At key steps, hydrogen atoms are stripped from glucose and passed first to a coenzyme, like NAD^+ (nicotinamide adenine dinucleotide).
- Dehydrogenase enzymes strip two hydrogen atoms from the fuel (e.g., glucose), pass two electrons and one proton to NAD^+ and release H^+.
    - H-C-OH + NAD^+ -> C=O + NADH + H^+

- This changes the oxidized form, NAD^+, to the reduced form NADH.
  - NAD^+ functions as the oxidizing agent in many of the redox steps during the catabolism of glucose.
- The electrons carried by NADH lose very little of their potential energy in this process.
- This energy is tapped to synthesize ATP as electrons "fall" from NADH to oxygen.
- Unlike the explosive release of heat energy that would occur when H_2 and O_2 combine, cellular respiration uses an **electron transport chain** to break the fall of electrons to O_2 into several steps.
- The electron transport chain, consisting of several molecules (primarily proteins), is built into the inner membrane of a mitochondrion.
- NADH shuttles electrons from food to the "top" of the chain.
- At the "bottom," oxygen captures the electrons and H^+ to form water.
- The free energy change from "top" to "bottom" is -53 kcal/mole of NADH.

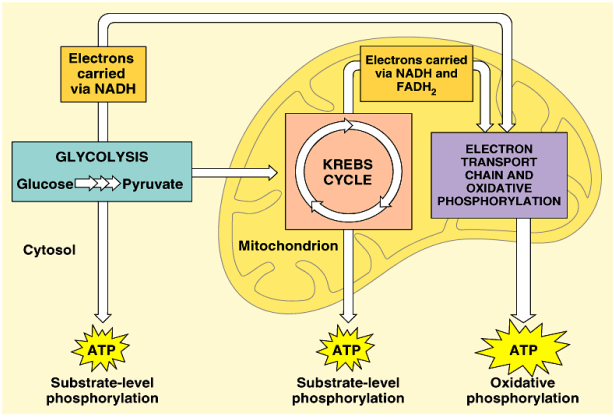


- Electrons are passed by increasingly electronegative molecules in the chain until they are caught by oxygen, the most electronegative.

B. The Process of Cellular Respiration

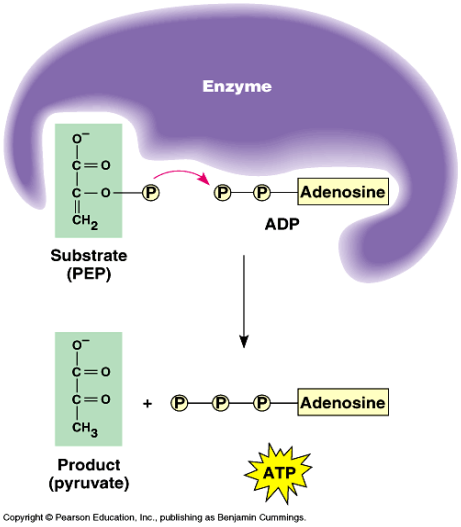
1. Respiration involves glycolysis, the Krebs cycle, and electron transport: *an overview*

- Respiration occurs in three metabolic stages: glycolysis, the Krebs cycle, and the electron transport chain and oxidative phosphorylation.
- **Glycolysis** occurs in the cytoplasm.
  - It begins catabolism by breaking glucose into two molecules of pyruvate.
- The **Krebs cycle** occurs in the mitochondrial matrix.
  - It degrades pyruvate to carbon dioxide.



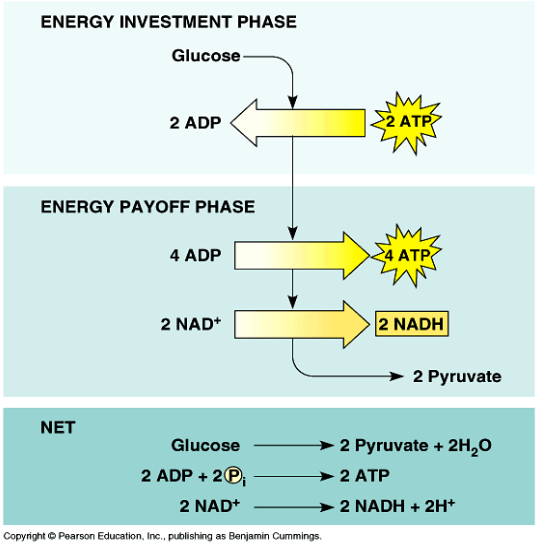
- Several steps in glycolysis and the Krebs cycle transfer electrons from substrates to NAD^+, forming NADH.
- NADH passes these electrons to the electron transport chain.
- In the electron transport chain, the electrons move from molecule to molecule until they combine with oxygen and hydrogen ions to form water.

- As they are passed along the chain, the energy carried by these electrons is stored in the mitochondrion in a form that can be used to synthesize ATP via **oxidative phosphorylation**.
  - Oxidative phosphorylation produces almost 90% of the ATP generated by respiration.
- Some ATP is also generated in glycolysis and the Krebs cycle by **substrate-level phosphorylation.**
  - Here an enzyme transfers a phosphate group from an organic molecule (the substrate) to ADP, forming ATP.
- Respiration uses the small steps in the respiratory pathway to break the large denomination of energy contained in glucose into the small change of ATP.
  - The quantity of energy in ATP is more appropriate for the level of work required in the cell.
- Ultimately 38 ATP are produced per mole of glucose that is degraded to carbon dioxide and water by respiration.



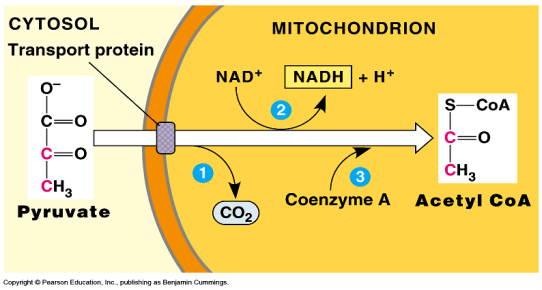
2. Glycolysis harvests chemical energy by oxidizing glucose to pyruvate: *a closer look*

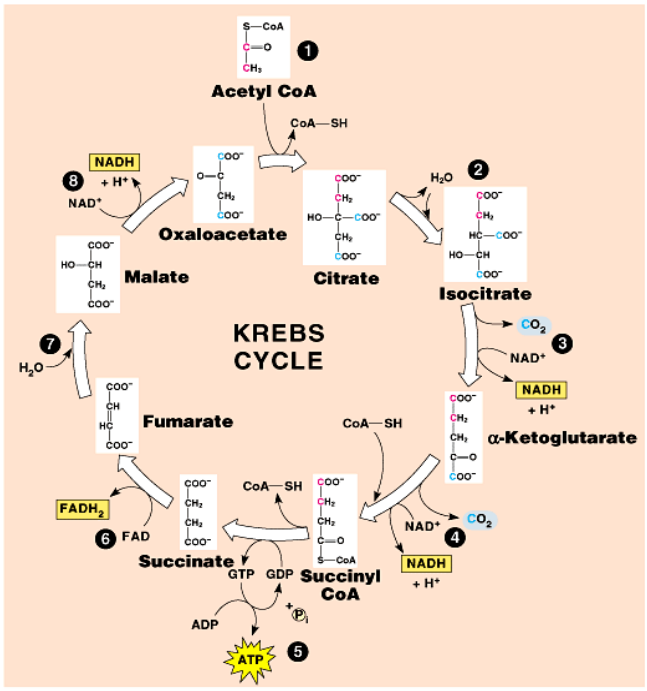
- During glycolysis, glucose, a six carbon-sugar, is split into two three-carbon sugars.
- These smaller sugars are oxidized and rearranged to form two molecules of pyruvate.
- Each of the ten steps in glycolysis is catalyzed by a specific enzyme.
- These steps can be divided into two phases: an energy investment phase and an energy payoff phase.
- In the energy investment phase, ATP provides activation energy by phosphorylating glucose.
  - This requires 2 ATP per glucose.
- In the energy payoff phase, ATP is produced by substrate-level phosphorylation and NAD^+ is reduced to NADH.
- 4 ATP (net) and 2 NADH are produced per glucose.
- The net yield from glycolysis is 2 ATP and 2 NADH per glucose.
  - No CO_2 is produced during glycolysis.
- Glycolysis occurs whether O_2 is present or not.
  - If O_2 is present, pyruvate moves to the Krebs cycle and the energy stored in NADH can be converted to ATP by the electron transport system and oxidative phosphorylation.

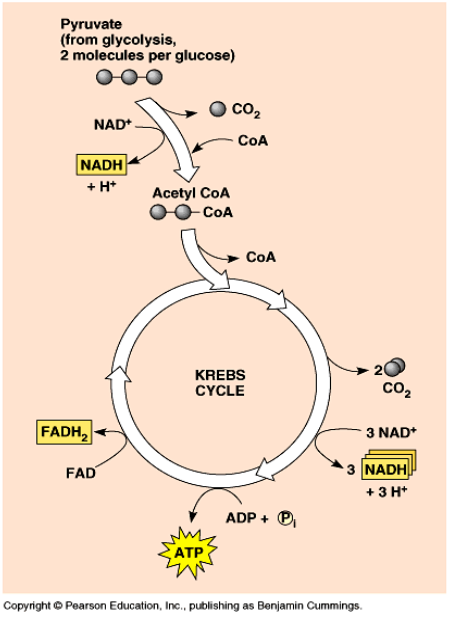


3. The Krebs cycle completes the energy-yielding oxidation of organic molecules: *a closer look*

- More than three quarters of the original energy in glucose is still present in two molecules of pyruvate.
- If oxygen is present, pyruvate enters the mitochondrion where enzymes of the Krebs cycle complete the oxidation of the organic fuel to carbon dioxide.
- As pyruvate enters the mitochondrion, a multienzyme complex modifies pyruvate to **acetyl CoA** which enters the Krebs cycle in the matrix.
  - A carboxyl group is removed as CO_2.
  - A pair of electrons is transferred from the remaining two-carbon fragment to NAD^+ to form NADH.
  - The oxidized fragment, acetate, combines with coenzyme A to form acetyl CoA.
- The Krebs cycle is named after Hans Krebs who was largely responsible for elucidating its pathways in the 1930s.
  - This cycle begins when acetate from acetyl CoA combines with oxaloacetate to form citrate.





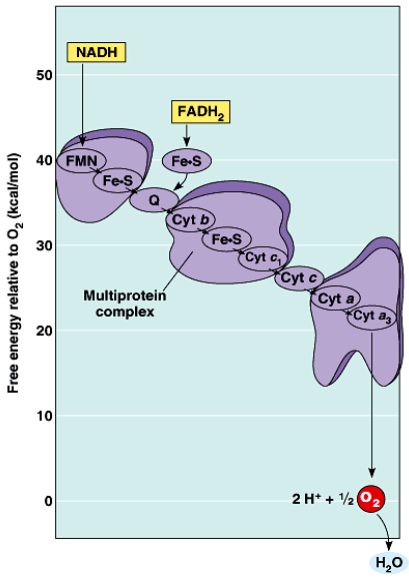


- Ultimately, the oxaloacetate is recycled and the acetate is broken down to CO_2.
- Each cycle produces one ATP by substrate-level phosphorylation, three NADH, and one $FADH_2$ (another electron carrier) per acetyl CoA.
- The Krebs cycle consists of eight steps.
- The conversion of pyruvate and the Krebs cycle produces large quantities of electron carriers.

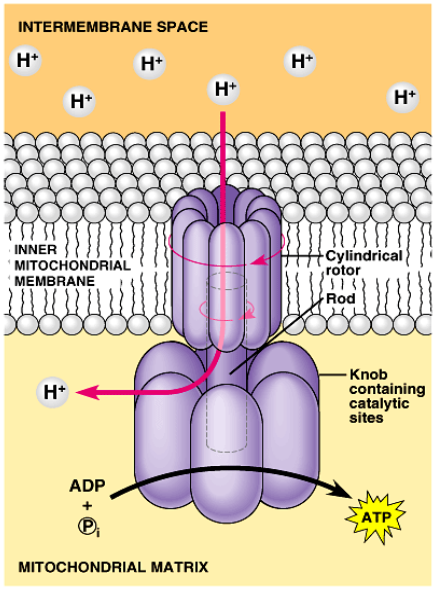
4. The inner mitochondrial membrane couples electron transport to ATP synthesis: *a closer look*

- Only 4 of 38 ATP ultimately produced by respiration of glucose are derived from substrate-level phosphorylation.

- The vast majority of the ATP comes from the energy in the electrons carried by NADH (and $FADH_2$).
- The energy in these electrons is used in the electron transport system to power ATP synthesis.
- Thousands of copies of the electron transport chain are found in the extensive surface of the cristae, the inner membrane of the mitochondrion.
    - Most components of the chain are proteins that are bound with prosthetic groups that can alternate between reduced and oxidized states as they accept and donate electrons.
- Electrons drop in free energy as they pass down the electron transport chain.
- Electrons carried by NADH are transferred to the first molecule in the electron transport chain, flavoprotein.
- The electrons continue along the chain that includes several **cytochrome** proteins and one lipid carrier.
- The electrons carried by $FADH_2$ have lower free energy and are added to a later point in the chain.



- Electrons from NADH or $FADH_2$ ultimately pass to oxygen.
    - For every two electron carriers (four electrons), one O_2 molecule is reduced to two molecules of water.
- The electron transport chain generates no ATP directly.
- Its function is to break the large free energy drop from food to oxygen into a series of smaller steps that release energy in manageable amounts.
- The movement of electrons along the electron transport chain does contribute to chemiosmosis and ATP synthesis.
- A protein complex, **ATP synthase**, in the cristae actually makes ATP from ADP and P_i.
- ATP uses the energy of an existing proton gradient to power ATP synthesis.
    - This proton gradient develops between the intermembrane space and the matrix.



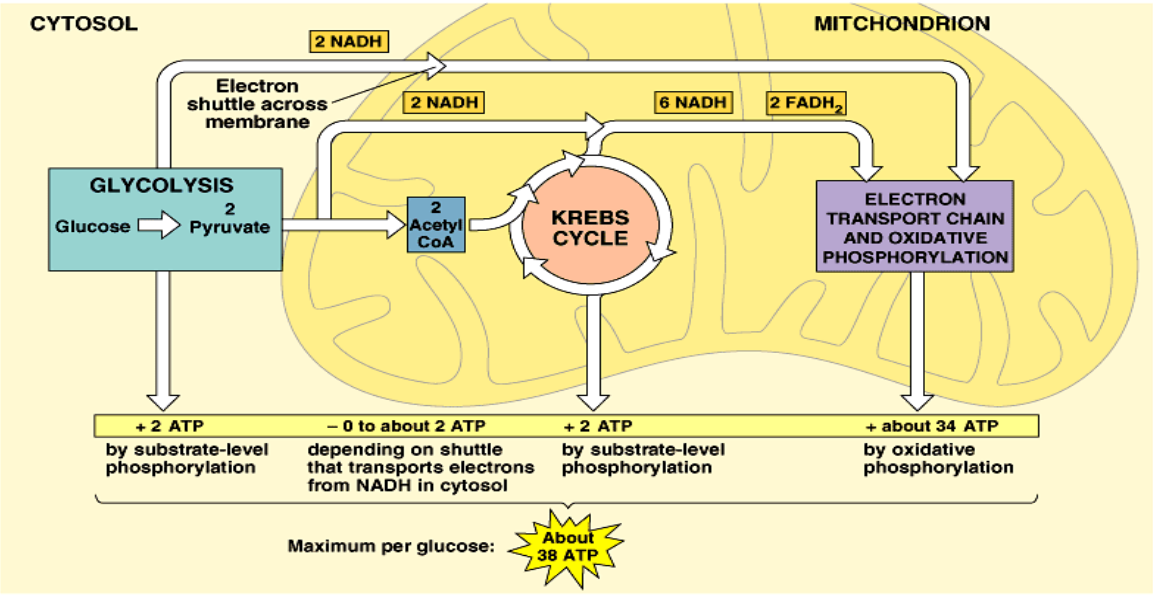
- The proton gradient is produced by the movement of electrons along the electron transport chain.
- Several chain molecules can use the exergonic flow of electrons to pump H^+ from the matrix to the intermembrane space.
    - This concentration of H^+ is the **proton-motive force**.
- The ATP synthase molecules are the only place that will allow H^+ to diffuse back to the matrix.

- This exergonic flow of H^+ is used by the enzyme to generate ATP.
- This coupling of the redox reactions of the electron transport chain to ATP synthesis is called **chemiosmosis**.
- The mechanism of ATP generation by ATP synthase is still an area of active investigation.
    - As hydrogen ions flow down their gradient, they cause the cylinder portion and attached rod of ATP synthase to rotate.
    - The spinning rod causes a conformational change in the knob region, activating catalytic sites where ADP and inorganic phosphate combine to make ATP.
- Chemiosmosis is an energy-coupling mechanism that uses energy stored in the form of an H^+ gradient across a membrane to drive cellular work.
    - In the mitochondrion, chemiosmosis generates ATP.
    - Chemiosmosis in chloroplasts also generates ATP, but light drives the electron flow down an electron transport chain and H^+ gradient formation.
    - Prokaryotes generate H^+ gradients across their plasma membrane.
        - They can use this proton-motive force not only to generate ATP but also to pump nutrients and waste products across the membrane and to rotate their flagella.

5. Cellular respiration generates many ATP molecules for each sugar molecule it oxidizes: *a review*

- During respiration, most energy flows from glucose -> NADH -> electron transport chain -> proton-motive force -> ATP.
- Considering the fate of carbon, one six-carbon glucose molecule is oxidized to six CO_2 molecules.
- Some ATP is produced by substrate-level phosphorylation during glycolysis and the Krebs cycle, but most comes from oxidative phosphorylation.
- Each NADH from the Krebs cycle and the conversion of pyruvate contributes enough energy to generate a maximum of 3 ATP (rounding up).
    - The NADH from glycolysis may also yield 3 ATP.
- Each $FADH_2$ from the Krebs cycle can be used to generate about 2 ATP.
- In some eukaryotic cells, NADH produced in the cytosol by glycolysis may be worth only 2 ATP.
    - The electrons must be shuttled to the mitochondrion.
    - In some shuttle systems, the electrons are passed to NAD^+, which generates 3 ATP. In others, the electrons are passed to FAD, which generates only 2 ATP.
- A maximum yield of 34 ATP is produced by oxidative phosphorylation.
- This plus the 4 ATP from substrate-level phosphorylation gives a bottom line of 38 ATP.

- This maximum figure does not consider other uses of the proton-motive force.



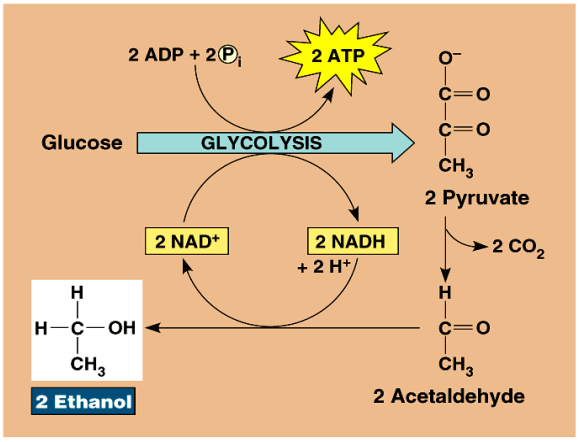
- How efficient is respiration in generating ATP?
  - Complete oxidation of glucose releases 686 kcal per mole.
  - Formation of each ATP requires at least 7.3 kcal/mole.
  - Efficiency of respiration is 7.3 kcal/mole x 38 ATP/glucose/686 kcal/mole glucose = 40%.
  - The other approximately 60% is lost as heat.
- Cellular respiration is remarkably efficient in energy conversion.

C. Related Metabolic Processes

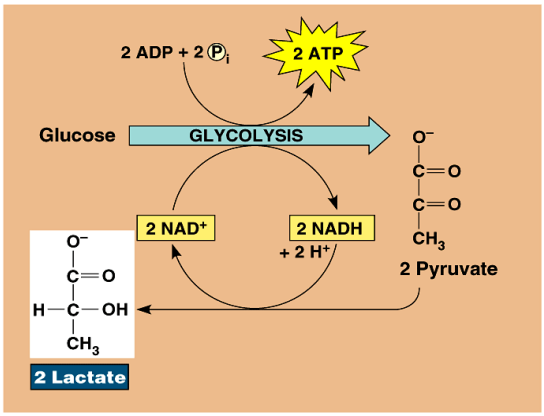
1. Fermentation enables some cells to produce ATP without the help of oxygen

- Oxidation refers to the loss of electrons to any electron acceptor, not just to oxygen.
  - In glycolysis, glucose is oxidized to two pyruvate molecules with NAD^+ as the oxidizing agent, not O_2.
  - Some energy from this oxidation produces 2 ATP (net).
  - If oxygen is present, additional ATP can be generated when NADH delivers its electrons to the electron transport chain.
- Glycolysis generates 2 ATP whether oxygen is present **(aerobic)** or not **(anaerobic)**.
- Anaerobic catabolism of sugars can occur by fermentation.
- Fermentation can generate ATP from glucose by substrate-level phosphorylation as long as there is a supply of NAD^+ to accept electrons.
  - If the NAD^+ pool is exhausted, glycolysis shuts down.
  - Under aerobic conditions, NADH transfers its electrons to the electron transfer chain, recycling NAD^+.

- Under anaerobic conditions, various fermentation pathways generate ATP by glycolysis and recycle NAD^+ by transferring electrons from NADH to pyruvate or derivatives of pyruvate.
- In **alcohol fermentation**, pyruvate is converted to ethanol in two steps.

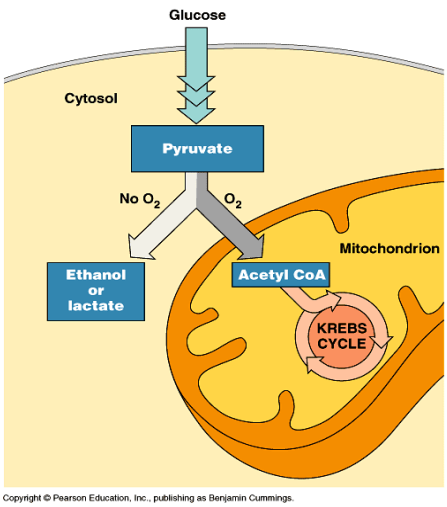


(a) Alcohol fermentation



(b) Lactic acid fermentation

- First, pyruvate is converted to a two-carbon compound, acetaldehyde by the removal of CO_2.
- Second, acetaldehyde is reduced by NADH to ethanol.
- Alcohol fermentation by yeast is used in brewing and winemaking.
- During **lactic acid fermentation**, pyruvate is reduced directly by NADH to form lactate (ionized form of lactic acid).
- Lactic acid fermentation by some fungi and bacteria is used to make cheese and yogurt.
- Muscle cells switch from aerobic respiration to lactic acid fermentation to generate ATP when O_2 is scarce.
- The waste product, lactate, may cause muscle fatigue, but ultimately it is converted back to pyruvate in the liver.
- Fermentation and cellular respiration are anaerobic and aerobic alternatives, respectively, for producing ATP from sugars.
- Both use glycolysis to oxidize sugars to pyruvate with a net production of 2 ATP by substrate-level phosphorylation.
  - Both use NAD^+ as an electron acceptor.
- In fermentation, the electrons of NADH are passed to an organic molecule, regenerating NAD^+.
- In respiration, the electrons of NADH are ultimately passed to O_2, generating ATP by oxidative phosphorylation.
- In addition, even more ATP is generated from the oxidation of pyruvate in the Krebs cycle.
- Without oxygen, the energy still stored in pyruvate is unavailable to the cell.
- Under aerobic respiration, a molecule of glucose yields 38 ATP, but the same molecule of glucose yields only 2 ATP under anaerobic respiration.
- Some organisms (**facultative anaerobes**), including yeast and many bacteria, can survive using either fermentation or respiration.
- At a cellular level, human muscle cells can behave as facultative anaerobes, but nerve cells cannot.

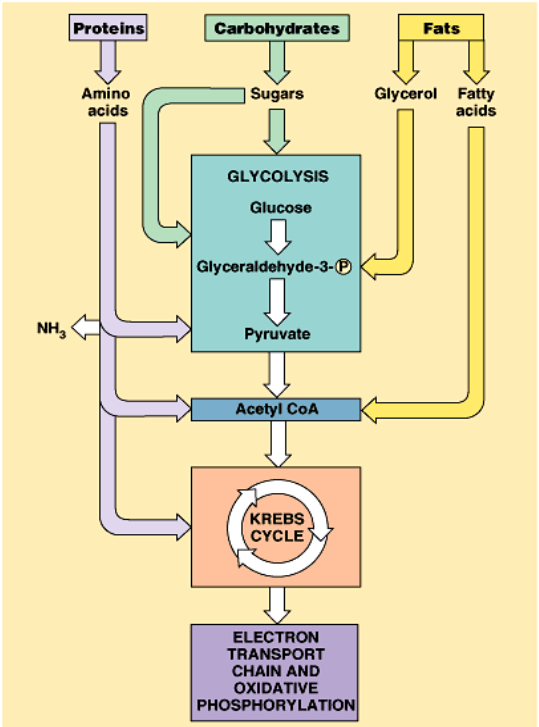


- For facultative anaerobes, pyruvate is a fork in the metabolic road that leads to two alternative routes.

- The oldest bacterial fossils are over 3.5 billion years old, appearing long before appreciable quantities of O_2 accumulated in the atmosphere.
- Therefore, the first prokaryotes may have generated ATP exclusively from glycolysis.
- The fact that glycolysis is also the most widespread metabolic pathway and occurs in the cytosol without membrane-enclosed organelles, suggests that glycolysis evolved early in the history of life.

2. Glycolysis and the Krebs cycle connect to many other metabolic pathways

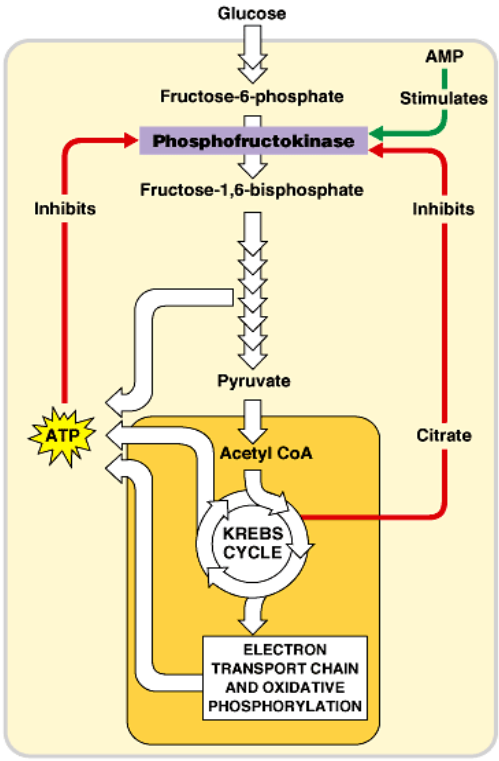
- Glycolysis can accept a wide range of carbohydrates.
  - Polysaccharides, like starch or glycogen, can be hydrolyzed to glucose monomers that enter glycolysis.
  - Other hexose sugars, like galactose and fructose, can also be modified to undergo glycolysis.
- The other two major fuels, proteins and fats, can also enter the respiratory pathways, including glycolysis and the Krebs cycle, used by carbohydrates.
- Proteins must first be digested to individual amino acids.
- Amino acids that will be catabolized must have their amino groups removed via deamination.
  - The nitrogenous waste is excreted as ammonia, urea, or another waste product.
- The carbon skeletons are modified by enzymes and enter as intermediaries into glycolysis or the Krebs cycle depending on their structure.
- The energy of fats can also be accessed via catabolic pathways.
- Fats must be digested to glycerol and fatty acids.
  - Glycerol can be converted to glyceraldehyde phosphate, an intermediate of glycolysis.
  - The rich energy of fatty acids is accessed as fatty acids are split into two-carbon fragments via **beta oxidation**.
  - These molecules enter the Krebs cycle as acetyl CoA.
- In fact, a gram of fat will generate twice as much ATP as a gram of carbohydrate via aerobic respiration.
- Carbohydrates, fats, and proteins can all be catabolized through the same pathways.
- The metabolic pathways of respiration also play a role in anabolic pathways of the cell.



- Not all the organic molecules of food are completely oxidized to make ATP.
- Intermediaries in glycolysis and the Krebs cycle can be diverted to anabolic pathways.
  - For example, a human cell can synthesize about half the 20 different amino acids by modifying compounds from the Krebs cycle.
  - Glucose can be synthesized from pyruvate and fatty acids from acetyl CoA.
- Glycolysis and the Krebs cycle function as metabolic interchanges that enable cells to convert one kind of molecule to another as needed.
  - For example, excess carbohydrates and proteins can be converted to fats through intermediaries of glycolysis and the Krebs cycle.
- Metabolism is remarkably versatile and adaptable.

3. Feedback mechanisms control cellular respiration

- Basic principles of supply and demand regulate the metabolic economy.
  - If a cell has an excess of a certain amino acid, it typically uses feedback inhibition to prevent the diversion of more intermediary molecules from the Krebs cycle to the synthesis pathway of that amino acid.
- The rate of catabolism is also regulated, typically by the level of ATP in the cell.
  - If ATP levels drop, catabolism speeds up to produce more ATP.
- Control of catabolism is based mainly on regulating the activity of enzymes at strategic points in the catabolic pathway.
- One strategic point occurs in the third step of glycolysis, catalyzed by phosphofructokinase.



- Allosteric regulation of phosphofructokinase sets the pace of respiration.
  - This enzyme is inhibited by ATP and stimulated by AMP (derived from ADP).
  - It responds to shifts in balance between production and degradation of ATP: ATP <-> ADP + P_i <-> AMP + P_i.
    - Thus, when ATP levels are high, inhibition of this enzyme slows glycolysis.
  - When ATP levels drop and ADP and AMP levels rise, the enzyme is active again and glycolysis speeds up.
- Citrate, the first product of the Krebs cycle, is also an inhibitor of phosphofructokinase.
  - This synchronizes the rate of glycolysis and the Krebs cycle.

    - Also, if intermediaries from the Krebs cycle are diverted to other uses (e.g., amino acid synthesis), glycolysis speeds up to replace these molecules.
- Metabolic balance is augmented by the control of other enzymes at other key locations in glycolysis and the Krebs cycle.
- Cells are thrifty, expedient, and responsive in their metabolism.