Overview: Life Is Work

- To perform their many tasks, living cells require energy from outside sources.
- Energy enters most ecosystems as sunlight and leaves as heat.
- Photosynthesis generates oxygen and organic molecules that the mitochondria of eukaryotes use as fuel for cellular respiration.
- Cells harvest the chemical energy stored in organic molecules and use it to regenerate ATP, the molecule that drives most cellular work.
- Respiration has three key pathways: glycolysis, the citric acid cycle, and oxidative phosphorylation.

Concept 9.1 Catabolic pathways yield energy by oxidizing organic fuels

- The arrangement of atoms of organic molecules represents potential energy.
- 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; the rest is dissipated as heat.
- Catabolic metabolic pathways release the energy stored in complex organic molecules.
- 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, consumes oxygen as a reactant to complete the breakdown of a variety of organic molecules.
  - In eukaryotic cells, mitochondria are the site of most of the processes of cellular respiration.
- Cellular respiration is similar in broad principle to the combustion of gasoline in an automobile engine after oxygen is mixed with hydrocarbon fuel.
  - Food is the fuel for respiration. The exhaust is carbon dioxide and water.
The overall process is:

- Organic compounds $+ O_2 \rightarrow CO_2 + H_2O +$ energy (ATP + heat).
- Carbohydrates, fats, and proteins can all be used as the fuel, but it is most useful to consider glucose.
  - $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O +$ Energy (ATP + heat)
- The catabolism of glucose is exergonic with a $\Delta G$ of $-686$ kcal per mole of glucose.
  - Some of this energy is used to produce ATP, which can perform cellular work.

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

- Catabolic pathways transfer 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 transfer of electrons is not complete but involves 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 methane reacts with oxygen to form carbon dioxide, electrons end up farther away from the carbon atom and closer to their new covalent partners, the oxygen atoms, which are very electronegative.
  - In effect, the carbon atom has partially "lost" its shared electrons. Thus, methane has been oxidized.
- The two atoms of the oxygen molecule share their electrons equally. When oxygen reacts with the hydrogen from methane
to form water, the electrons of the covalent bonds are drawn closer to the oxygen.
- In effect, each oxygen atom has partially “gained” electrons, and so the oxygen molecule has been reduced.
- Oxygen is very electronegative, and is one of the most potent of all oxidizing agents.

- Energy must be added to pull an electron away from an atom.
- The more electronegative the atom, the more energy is required to take an electron away from it.
- An electron loses potential energy when it shifts from a less electronegative atom toward a more electronegative one.
- A redox reaction that relocates electrons closer to oxygen, such as the burning of methane, releases chemical energy that can do work.

**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 in a series of steps, each catalyzed by a specific enzyme.
  - At key steps, electrons are stripped from the glucose.
  - In many oxidation reactions, the electron is transferred with a proton, as a hydrogen atom.
- The hydrogen atoms are not transferred directly to oxygen but are passed first to a coenzyme called NAD⁺ (nicotinamide adenine dinucleotide).
- How does NAD⁺ trap electrons from glucose?
  - Dehydrogenase enzymes strip two hydrogen atoms from the fuel (e.g., glucose), oxidizing it.
  - The enzyme passes two electrons and one proton to NAD⁺.
  - The other proton is released as H⁺ to the surrounding solution.
- By receiving two electrons and only one proton, NAD⁺ has its charge neutralized when it is reduced to NADH.
  - NAD⁺ functions as the oxidizing agent in many of the redox steps during the catabolism of glucose.
- The electrons carried by NADH have lost very little of their potential energy in this process.
- Each NADH molecule formed during respiration represents stored energy. This energy is tapped to synthesize ATP as electrons “fall” from NADH to oxygen.
• How are electrons extracted from food and stored by NADH finally transferred to oxygen?
  ◦ Unlike the explosive release of heat energy that occurs when H₂ and O₂ are combined (with a spark for activation energy), cellular respiration uses an electron transport chain to break the fall of electrons to O₂ into several steps.
• The electron transport chain consists of several molecules (primarily proteins) built into the inner membrane of a mitochondrion.
• Electrons released from food are shuttled by NADH to the “top” higher-energy end of the chain.
• At the “bottom” lower-energy end, oxygen captures the electrons along with H⁺ to form water.
• Electron transfer from NADH to oxygen is an exergonic reaction with a free energy change of −53 kcal/mol.
• Electrons are passed to increasingly electronegative molecules in the chain until they reduce oxygen, the most electronegative receptor.
• In summary, during cellular respiration, most electrons travel the following “downhill” route: food → NADH → electron transport chain → oxygen.

These are the stages of cellular respiration: a preview.
• Respiration occurs in three metabolic stages: glycolysis, the citric acid 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 citric acid cycle occurs in the mitochondrial matrix.
  ◦ It completes the breakdown of glucose by oxidizing a derivative of pyruvate to carbon dioxide.
• Several steps in glycolysis and the citric acid cycle are redox reactions in which dehydrogenase enzymes 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 molecular oxygen and hydrogen ions to form water.
• As they are passed along the chain, the energy carried by these electrons is transformed in the mitochondrion into a form that can be used to synthesize ATP via oxidative phosphorylation.
• The inner membrane of the mitochondrion is the site of electron transport and chemiosmosis, processes that together constitute oxidative phosphorylation.
  ◦ Oxidative phosphorylation produces almost 90% of the ATP generated by respiration.
• Some ATP is also formed directly during glycolysis and the citric acid cycle by **substrate-level phosphorylation**.
  ◦ Here an enzyme transfers a phosphate group from an organic substrate to ADP, forming ATP.
• For each molecule of glucose degraded to carbon dioxide and water by respiration, the cell makes up to 38 ATP, each with 7.3 kcal/mol of free energy.
• 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.

**Concept 9.2 Glycolysis harvests chemical energy by oxidizing glucose to pyruvate**

• 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, the ionized form of pyruvic acid.
• 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, the cell invests ATP to provide 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 by electrons released by the oxidation of glucose.
• The net yield from glycolysis is 2 ATP and 2 NADH per glucose.
  ◦ No CO\(_2\) is produced during glycolysis.
• Glycolysis can occur whether O\(_2\) is present or not.

**Concept 9.3 The citric acid cycle completes the energy-yielding oxidation of organic molecules**
• More than three-quarters of the original energy in glucose is still present in the two molecules of pyruvate.
• If oxygen is present, pyruvate enters the mitochondrion where enzymes of the citric acid cycle complete the oxidation of the organic fuel to carbon dioxide.
• After pyruvate enters the mitochondrion via active transport, it is converted to a compound called acetyl coenzyme A or acetyl CoA.
• This step is accomplished by a multienzyme complex that catalyzes three reactions:
  1. A carboxyl group is removed as CO2.
  2. The remaining two-carbon fragment is oxidized to form acetate. An enzyme transfers the pair of electrons to NAD+ to form NADH.
  3. Acetate combines with coenzyme A to form the very reactive molecule acetyl CoA.
• Acetyl CoA is now ready to feed its acetyl group into the citric acid cycle for further oxidation.
• The citric acid cycle is also called the Krebs cycle in honor of Hans Krebs, who was largely responsible for elucidating its pathways in the 1930s.
• The citric acid cycle oxidizes organic fuel derived from pyruvate.
  ◦ The citric acid cycle has eight steps, each catalyzed by a specific enzyme.
  ◦ The acetyl group of acetyl CoA joins the cycle by combining with the compound oxaloacetate, forming citrate.
  ◦ The next seven steps decompose the citrate back to oxaloacetate. It is the regeneration of oxaloacetate that makes this process a cycle.
  ◦ Three CO2 molecules are released, including the one released during the conversion of pyruvate to acetyl CoA.
• The cycle generates one ATP per turn by substrate-level phosphorylation.
  ◦ A GTP molecule is formed by substrate-level phosphorylation.
  ◦ The GTP is then used to synthesize an ATP, the only ATP generated directly by the citric acid cycle.
• Most of the chemical energy is transferred to NAD+ and FAD during the redox reactions.
• The reduced coenzymes NADH and FADH2 then transfer high-energy electrons to the electron transport chain.
• Each cycle produces one ATP by substrate-level phosphorylation, three NADH, and one FADH2 per acetyl CoA.
**Concept 9.4 During oxidative phosphorylation, chemiosmosis couples electron transport to ATP synthesis**

*The inner mitochondrial membrane couples electron transport to ATP synthesis.*

- Only 4 of 38 ATP ultimately produced by respiration of glucose are produced by substrate-level phosphorylation.
  - Two are produced during glycolysis, and 2 are produced during the citric acid cycle.
- NADH and FADH$_2$ account for the vast majority of the energy extracted from the food.
  - These reduced coenzymes link glycolysis and the citric acid cycle to oxidative phosphorylation, which uses energy released by the electron transport chain to power ATP synthesis.
- The electron transport chain is a collection of molecules embedded in the cristae, the folded inner membrane of the mitochondrion.
  - The folding of the cristae increases its surface area, providing space for thousands of copies of the chain in each mitochondrion.
  - Most components of the chain are proteins bound to prosthetic groups, nonprotein components essential for catalysis.
- Electrons drop in free energy as they pass down the electron transport chain.
- During electron transport along the chain, electron carriers alternate between reduced and oxidized states as they accept and donate electrons.
  - Each component of the chain becomes reduced when it accepts electrons from its “uphill” neighbor, which is less electronegative.
  - It then returns to its oxidized form as it passes electrons to its more electronegative “downhill” neighbor.
- Electrons carried by NADH are transferred to the first molecule in the electron transport chain, a flavoprotein.
- The electrons continue along the chain that includes several cytochrome proteins and one lipid carrier.
  - The prosthetic group of each cytochrome is a heme group with an iron atom that accepts and donates electrons.
- The last cytochrome of the chain, cyt $a_3$, passes its electrons to oxygen, which is very electronegative.
  - Each oxygen atom also picks up a pair of hydrogen ions from the aqueous solution to form water.
For every two electron carriers (four electrons), one $O_2$ molecule is reduced to two molecules of water.

- The electrons carried by FADH$_2$ have lower free energy and are added at a lower energy level than those carried by NADH.
- The electron transport chain provides about one-third less energy for ATP synthesis when the electron donor is FADH$_2$ rather than NADH.

- 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.
- How does the mitochondrion couple electron transport and energy release to ATP synthesis?
  - The answer is a mechanism called **chemiosmosis**.
- 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.
  - The 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.
- The chain is an energy converter that uses the exergonic flow of electrons to pump $H^+$ from the matrix into the intermembrane space.
- The protons pass back to the matrix through a channel in ATP synthase, using the exergonic flow of $H^+$ to drive the phosphorylation of ADP.
- Thus, the energy stored in a $H^+$ gradient across a membrane couples the redox reactions of the electron transport chain to ATP synthesis.
- From studying the structure of ATP synthase, scientists have learned how the flow of $H^+$ through this large enzyme powers ATP generation.
- ATP synthase is a multisubunit complex with four main parts, each made up of multiple polypeptides:
  1. A rotor in the inner mitochondrial membrane.
  2. A knob that protrudes into the mitochondrial matrix.
  3. An internal rod extending from the rotor into the knob.
  4. A stator, anchored next to the rotor, which holds the knob stationary.
- Protons flow down a narrow space between the stator and rotor, causing the rotor and its attached rod to rotate.
The spinning rod causes conformational changes in the stationary knob, activating three catalytic sites in the knob where ADP and inorganic phosphate combine to make ATP.

- How does the inner mitochondrial membrane generate and maintain the H⁺ gradient that drives ATP synthesis in the ATP synthase protein complex?
  - Creating the H⁺ gradient is the function of the electron transport chain.
  - The ETC is an energy converter that uses the exergonic flow of electrons to pump H⁺ across the membrane from the mitochondrial matrix to the intermembrane space.
  - The H⁺ has a tendency to diffuse down its gradient.

- The ATP synthase molecules are the only place that H⁺ can diffuse back to the matrix.
  - The 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.

- How does the electron transport chain pump protons?
  - Certain members of the electron transport chain accept and release H⁺ along with electrons.
  - At certain steps along the chain, electron transfers cause H⁺ to be taken up and released into the surrounding solution.

- The electron carriers are spatially arranged in the membrane in such a way that protons are accepted from the mitochondrial matrix and deposited in the intermembrane space.
  - The H⁺ gradient that results is the proton-motive force.
  - The gradient has the capacity to do work.

- 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 mitochondria, the energy for proton gradient formation comes from exergonic redox reactions, and ATP synthesis is the work performed.

- 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.

Here is an accounting of ATP production by cellular respiration.
During cellular respiration, most energy flows from glucose → NADH → electron transport chain → proton-motive force → ATP.

Let's consider the products generated when cellular respiration oxidizes a molecule of glucose to six CO₂ molecules.

Four ATP molecules are produced by substrate-level phosphorylation during glycolysis and the citric acid cycle.

Many more ATP molecules are generated by oxidative phosphorylation.

Each NADH from the citric acid cycle and the conversion of pyruvate contributes enough energy to the proton-motive force to generate a maximum of 3 ATP.

- The NADH from glycolysis may also yield 3 ATP.

Each FADH₂ from the citric acid cycle can be used to generate about 2 ATP.

Why is our accounting so inexact?

There are three reasons that we cannot state an exact number of ATP molecules generated by one molecule of glucose.

1. Phosphorylation and the redox reactions are not directly coupled to each other, so the ratio of number of NADH to number of ATP is not a whole number.
   - One NADH results in 10 H⁺ being transported across the inner mitochondrial membrane.
   - Between 3 and 4 H⁺ must reenter the mitochondrial matrix via ATP synthase to generate 1 ATP.
   - Therefore, 1 NADH generates enough proton-motive force for synthesis of 2.5 to 3.3 ATP.
   - We round off and say that 1 NADH generates 3 ATP.

2. The ATP yield varies slightly depending on the type of shuttle used to transport electrons from the cytosol into the mitochondrion.
   - The mitochondrial inner membrane is impermeable to NADH, so the two electrons of the NADH produced in glycolysis must be conveyed into the mitochondrion by one of several electron shuttle systems.
   - 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.

3. The proton-motive force generated by the redox reactions of respiration may drive other kinds of work, such as mitochondrial uptake of pyruvate from the cytosol.
   - If all the proton-motive force generated by the electron transport chain were used to drive ATP synthesis, one glucose molecule could generate a maximum of 34 ATP by oxidative phosphorylation plus 4 ATP (net) from substrate-
level phosphorylation to give a total yield of 36–38 ATP (depending on the efficiency of the shuttle).

- How efficient is respiration in generating ATP?
  - Complete oxidation of glucose releases 686 kcal/mol.
  - Phosphorylation of ADP to form ATP requires at least 7.3 kcal/mol.
  - Efficiency of respiration is 7.3 kcal/mol times 38 ATP/glucose divided by 686 kcal/mol glucose, which equals 0.4 or 40%.
  - Approximately 60% of the energy from glucose is lost as heat.
    - Some of that heat is used to maintain our high body temperature (37°C).
- Cellular respiration is remarkably efficient in energy conversion.

**Concept 9.5 Fermentation enables some cells to produce ATP without the use of oxygen**

- Without electronegative oxygen to pull electrons down the transport chain, oxidative phosphorylation ceases.
- However, fermentation provides a mechanism by which some cells can oxidize organic fuel and generate ATP without the use of oxygen.
  - In glycolysis, glucose is oxidized to two pyruvate molecules with NAD⁺ as the oxidizing agent.
  - Glycolysis is exergonic and 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 transport 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.
First, pyruvate is converted to a two-carbon compound, acetaldehyde, by the removal of CO₂.

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 (the ionized form of lactic acid) without release of CO₂.

Lactic acid fermentation by some fungi and bacteria is used to make cheese and yogurt.

Human muscle cells switch from aerobic respiration to lactic acid fermentation to generate ATP when O₂ 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 oxidizing agent to accept electrons from food during glycolysis.

The two processes differ in their mechanism for oxidizing NADH to NAD⁺.

- In fermentation, the electrons of NADH are passed to an organic molecule to regenerate NAD⁺.
- In respiration, the electrons of NADH are ultimately passed to O₂, generating ATP by oxidative phosphorylation.

More ATP is generated from the oxidation of pyruvate in the citric acid 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.

Yeast and many bacteria are facultative anaerobes that can survive using either fermentation or respiration.

- At a cellular level, human muscle cells can behave as facultative anaerobes.

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

- Under aerobic conditions, pyruvate is converted to acetyl CoA and oxidation continues in the citric acid cycle.
- Under anaerobic conditions, pyruvate serves as an electron acceptor to recycle NAD⁺.
• The oldest bacterial fossils are more than 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 a ubiquitous metabolic pathway and occurs in the cytosol without membrane-enclosed organelles suggests that glycolysis evolved early in the history of life.

**Concept 9.6 Glycolysis and the citric acid cycle connect to many other metabolic pathways**
• Glycolysis can accept a wide range of carbohydrates for catabolism.
  ◦ Polysaccharides like starch or glycogen can be hydrolyzed to glucose monomers that enter glycolysis.
  ◦ Other hexose sugars, such as galactose and fructose, can also be modified to undergo glycolysis.
• The other two major fuels, proteins and fats, can also enter the respiratory pathways 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 citric acid cycle, depending on their structure.
• Catabolism can also harvest energy stored in fats.
• 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 citric acid cycle as acetyl CoA.
• A gram of fat oxides by respiration generates twice as much ATP as a gram of carbohydrate.
• The metabolic pathways of respiration also play a role in anabolic pathways of the cell.
• Intermediaries in glycolysis and the citric acid 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 citric acid cycle.
Glucose can be synthesized from pyruvate; fatty acids can be synthesized from acetyl CoA.

- Glycolysis and the citric acid 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 citric acid cycle.

- Metabolism is remarkably versatile and adaptable.

   **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 intermediary molecules from the citric acid 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 catalyzes the earliest step that irreversibly commits the substrate to glycolysis.
  - Phosphofructokinase is an allosteric enzyme with receptor sites for specific inhibitors and activators.
  - It is inhibited by ATP and stimulated by AMP (derived from ADP).
    - When ATP levels are high, inhibition of this enzyme slows glycolysis.
    - As ATP levels drop and ADP and AMP levels rise, the enzyme becomes active again and glycolysis speeds up.
- Citrate, the first product of the citric acid cycle, is also an inhibitor of phosphofructokinase.
  - This synchronizes the rate of glycolysis and the citric acid cycle.
- If intermediaries from the citric acid 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 citric acid cycle.
• Cells are thrifty, expedient, and responsive in their metabolism.