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MBG304 Biochemistry II (Lecture PPTs)

Lecture 1: Bioenergetics: The Flow of Energy in the Cell
Lecture 2: Glycolysis and Fermentation
Lecture 3: Krebs Cycle
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MBG304 Biochemistry

Lecture 1- Bioenergetics: The Flow of Energy in the Cell

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- Broadly speaking, every cell has four essential needs
  - molecular building blocks
  - chemical catalysts called enzymes
  - information to guide all its activities
  - energy to drive the various reactions and processes that are essential to life and biological function.
- First semester we saw that cells need amino acids, nucleotides, sugars, and lipids in order to synthesize the macromolecules used to build cellular structures and organelles.

- Energy is needed:
  - to drive the chemical reactions involved in the formation of cellular components
  - to power the many activities that cells carry out.

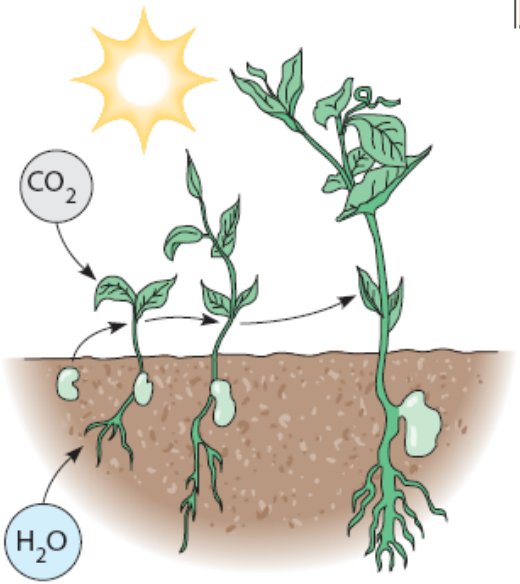
- The objective of this lecture:
  - learning the basics of bioenergetics
  - how the thermodynamic concept of free energy can allow us to predict whether specific chemical reactions can occur spontaneously in the cell.

The Importance of Energy

- All living systems require an ongoing supply of energy.
- Energy is usually defined as "*the capacity to do work*".
- A more useful definition: "*energy is the capacity to cause specific physical or chemical changes*".

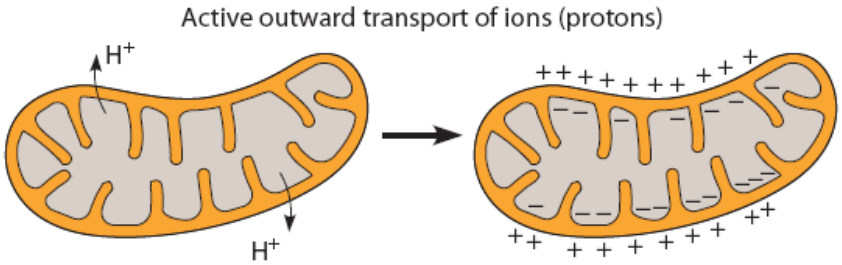
Cells Need Energy to Drive Six Different Kinds of Changes

(a) Synthetic work: the process of photosynthesis



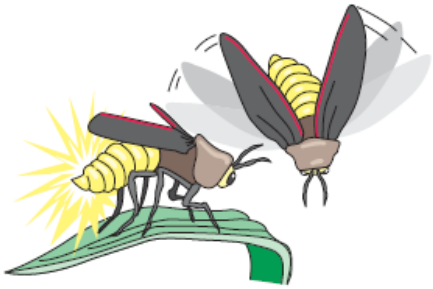
(b) Mechanical work: the contraction of a weight lifter's muscles

(d) Electrical work: the membrane potential of a mitochondrion

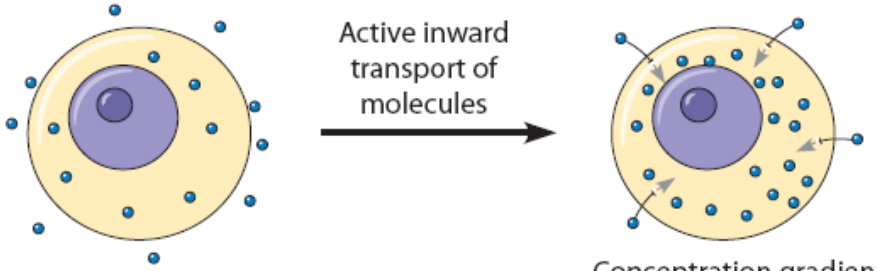


(e) Heat production: shivering in the cold

(f) Bioluminescence: the courtship of fireflies



(c) Concentration work: the accumulation of molecules in a cell



Organisms Obtain Energy Either from Sunlight or from the Oxidation of Chemical Compounds

- Nearly all life on Earth is sustained, directly or indirectly, by the sunlight.

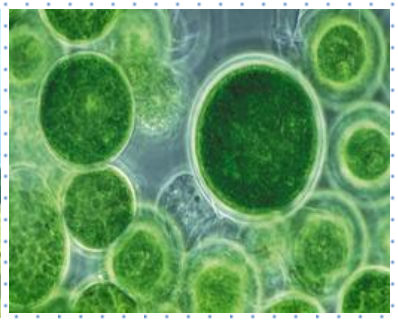
- In fact, based on their energy sources, organisms (and cells) can be classified as either phototrophs (“light-feeders”) or chemotrophs (“chemical-feeders”).

- Organisms can also be classified as autotrophs ("self-feeders") or heterotrophs ("other-feeders").

- Most organisms are either photoautotrophs or chemoheterotrophs.

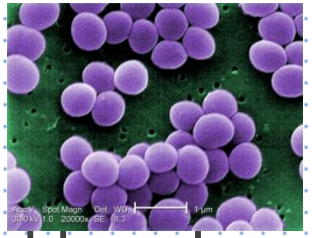
Phototrophs

- **Phototrophs** capture light energy from the sun using light-absorbing pigments and then transform this light energy into chemical energy, storing the energy in the form of ATP.
  - Photoautotrophs use solar energy to produce all their necessary carbon compounds from CO_2 during photosynthesis. Examples of photoautotrophs include
    - plants
    - Algae
    - Cyanobacteria
    - photosynthetic bacteria



  - Photoheterotrophs (some bacteria) harvest solar energy to power cellular activities, but they must rely on the intake of organic molecules for their carbon needs.

Chemotrophs



- **Chemotrophs** get energy by oxidizing chemical bonds in organic or inorganic molecules.
  - Chemoautotrophs (a few bacteria) oxidize inorganic compounds such as H_2S, H_2 gas, or inorganic ions for energy and synthesize all their organic compounds from CO_2. Examples of chemoheterotrophs:
    - all animals
    - Protozoa
    - Fungi
    - many bacteria

  - Chemoheterotrophs ingest and use chemical compounds such as carbohydrates, fats, and proteins to provide both energy and carbon for cellular needs. are chemoheterotrophs.

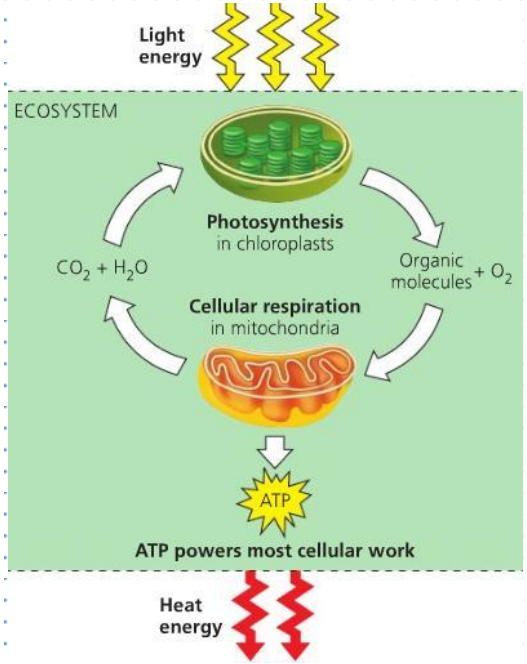
Keep in mind!!!

- Although phototrophs can utilize solar energy when it is available, they must function as chemotrophs whenever they are not illuminated.

- Most plants are really a mixture of phototrophic and chemotrophic cells. A plant root cell, for example, though part of an obviously phototrophic organism, usually cannot carry out photosynthesis and is every bit as chemotrophic as an animal cell (or a plant leaf cell in the dark).

Energy Flows Through the Biosphere Continuously

- Before we continue our discussion of energy flow, first let's review the chemical concepts of oxidation and reduction because they are critical to understanding energy flow in cells and organisms.



Oxidation

- Oxidation is the removal of electrons from a substance and, in biology, it usually involves the removal of hydrogen atoms (a hydrogen ion plus an electron) and the addition of oxygen atoms.

- Oxidation reactions release energy, as shown below when either glucose or methane is oxidized to carbon dioxide.

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

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O + \text{Energy}$$

- Note that each carbon atom in glucose or methane has lost hydrogen atoms and has gained oxygen atoms as carbon dioxide is formed and energy is released.

Reduction

- Reduction is the reverse reaction—the addition of electrons to a substance and usually the addition of hydrogen atoms (and a loss of oxygen atoms).

- Reduction reactions require an input of energy, as shown below when carbon dioxide is reduced to glucose during photosynthesis.

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

- The carbon atoms in six molecules of carbon dioxide have gained hydrogen atoms and lost oxygen atoms during this energy-requiring reduction to glucose.

- The flow of energy and matter through the biosphere is depicted in Figure 5-5, next slide.

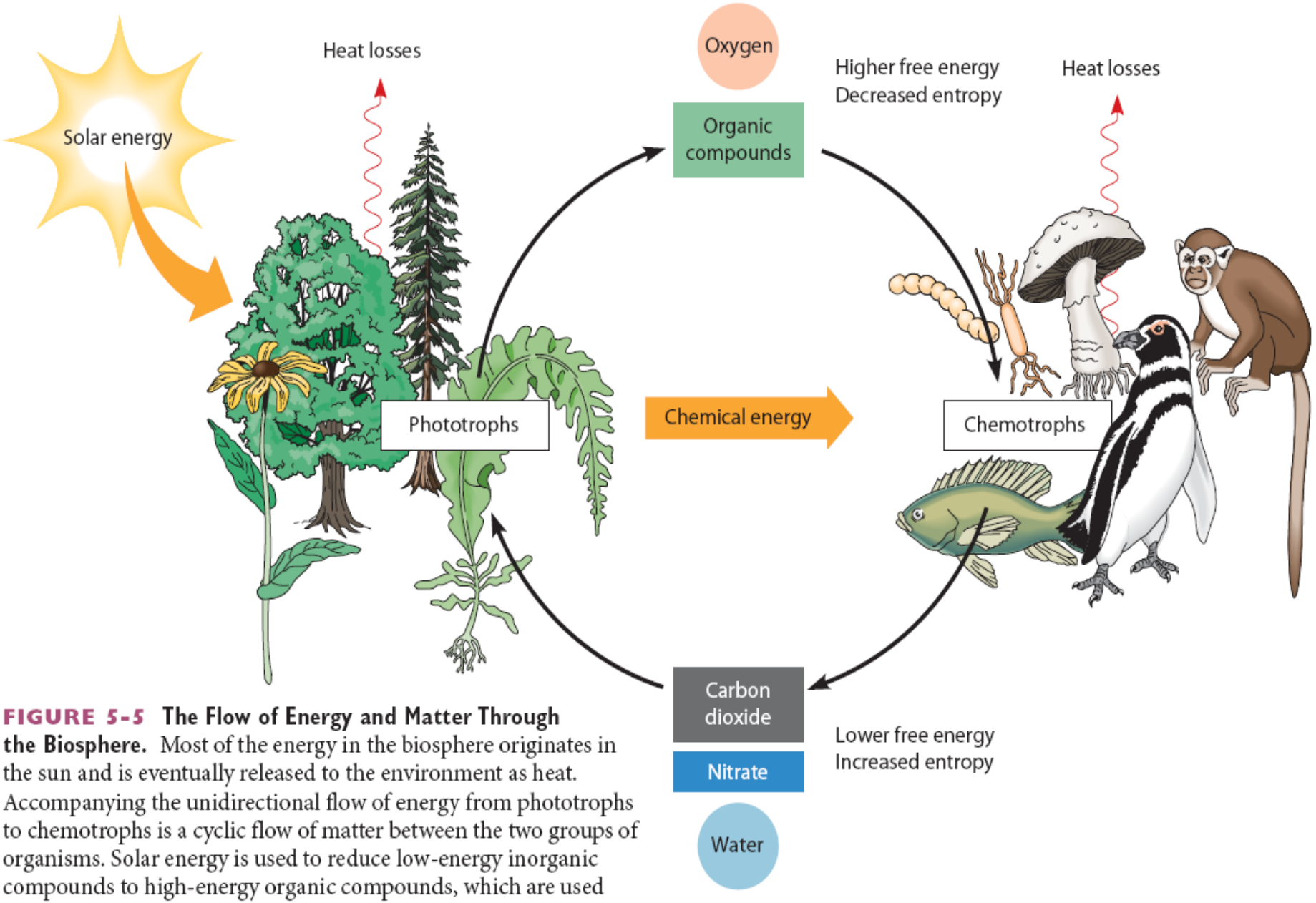


FIGURE 5-5 The Flow of Energy and Matter Through the Biosphere. Most of the energy in the biosphere originates in the sun and is eventually released to the environment as heat. Accompanying the unidirectional flow of energy from phototrophs to chemotrophs is a cyclic flow of matter between the two groups of organisms. Solar energy is used to reduce low-energy inorganic compounds to high-energy organic compounds, which are used by both phototrophs and chemotrophs.

- Both phototrophs and chemotrophs use energy to carry out the six kinds of work we have already discussed.

- An important principle of energy conversion:
  - no chemical or physical process occurs with 100% efficiency—some energy is released as heat.

- The heat liberated during cellular processes is utilized to maintain a constant body temperature. Some plants use metabolically generated heat to attract pollinators or to melt overlying snow (**Figure 5-6).**

- In general, however, the heat is simply dissipated into the environment, representing a loss of energy from the organism.

FIGURE 5-6 Skunk Cabbage, a Plant That Depends on Metabolically Generated Heat. The skunk cabbage plant (*Symplocarpus foetidus*) is one of the earliest-flowering plants in the eastern United States. The heat that it generates enables it to melt through overlying snow in late winter and begin growing when most other plants are still dormant.

The Flow of Energy Through the Biosphere Is Accompanied by a Flow of Matter

- Energy enters the biosphere as photons of light unaccompanied by matter and leaves the biosphere as heat similarly unaccompanied by matter.

- While it is passing through the biosphere, however, energy exists primarily in the form of chemical bond energies of oxidizable organic molecules in cells and organisms.

- Whereas energy flows unidirectionally from the sun through phototrophs to chemotrophs and to the environment, matter flows in a cyclic fashion between phototrophs and chemotrophs.

- Phototrophs use solar energy to create organic nutrients from inorganic starting materials such as carbon dioxide and water, releasing oxygen in the process.

- Phototrophs use some of these high-energy, reduced nutrients themselves, and some of them become available to chemotrophs that consume these phototrophs.

- Chemotrophs typically take in organic nutrients from their surroundings and use oxygen to oxidize them back to carbon dioxide and water, providing energy.

- Low-energy, oxidized molecules are returned to the environment and then become the raw materials that phototrophic organisms use to make new organic molecules, returning oxygen to the environment in the process and completing the cycle.

- In this transformation, there is also an accompanying cycle of nitrogen.

Nitrogen cycle

- Phototrophs obtain nitrogen from the environment in an oxidized, inorganic form (as nitrate from the soil or, in some cases, as from the atmosphere).
- They reduce it to ammonia a high-energy form of nitrogen used in the synthesis of amino acids, proteins, nucleotides, and nucleic acids.
- Eventually, these cellular molecules, like other components of phototrophic cells, are consumed by chemotrophs.
- The nitrogen in these molecules is then converted back into ammonia and eventually oxidized to nitrate, mostly by soil microorganisms.

nitrogen_cycle.swf

- Carbon, oxygen, nitrogen, and water thus cycle continuously between the phototrophic and chemotrophic worlds.

- They enter the chemotrophic sphere as reduced, energy-rich compounds and leave in an oxidized, energy-poor form.

- The two great groups of organisms can therefore be thought of as living in a symbiotic relationship with each other, with a cyclic flow of matter and a unidirectional flow of energy as components of that symbiosis.

carbon_cycle.swf

- When we deal with the overall macroscopic flux of energy and matter through living organisms, we find cellular biology interfacing with ecology.

- Our ultimate concern in cell biology is to know how the flux of energy and matter functions on a microscopic and molecular scale.

- For that we now turn to the topic of bioenergetics.

Bioenergetics

- The principles governing energy flow are the subject of an area of science known as **thermodynamics.**

- Specifically, thermodynamics concerns the laws governing the energy transactions that inevitably accompany most physical processes and all chemical reactions.

- **Bioenergetics,** is the application of thermodynamic principles to reactions and processes in the biological world.

To Understand Energy Flow, We Need to Understand Systems, Heat, and Work

- Energy exists in various forms, many of them of interest to biologists.

- Think, for example, of the energy represented by:
  - a ray of sunlight
  - a teaspoon of sugar
  - a moving flagellum
  - an excited electron
  - the concentration of ions or small molecules within a cell or an organelle.

- These phenomena are diverse, but they are all governed by certain basic principles of energetics.

Universe

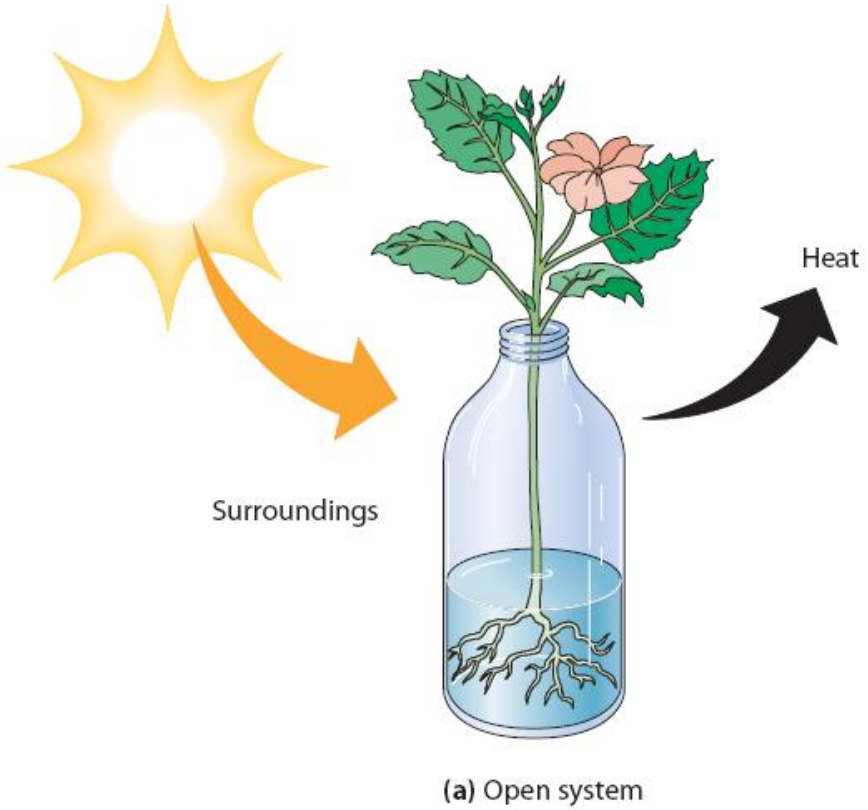


FIGURE 5-7 Open and Closed Systems. A system is that portion of the universe under consideration. The rest of the universe is called the surroundings of the system. (a) An open system can exchange energy with its surroundings, whereas (b) a closed system cannot. All living organisms are open systems, exchanging energy freely with their surroundings.

- The exchange of energy between a system and its surroundings occurs either as heat or as work.

Quantifying energy

- To quantify energy changes during chemical reactions or physical processes, we need units in which energy can be expressed.

- In biological chemistry, energy changes are usually expressed in terms of the **calorie (cal)**, which is defined as the amount of energy required to warm 1 gram of water 1 degree centigrade at a pressure of 1 atmosphere.

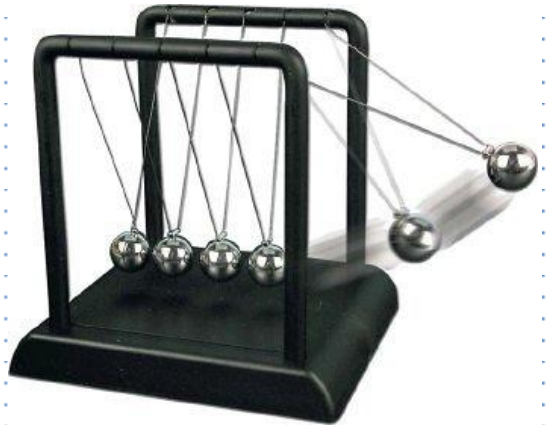
- One **kilocalorie (kcal)** equals 1000 calories. An alternative energy unit, the **joule (J)**, is preferred by physicists and is used in some biochemistry texts. Conversion is easy: 1 cal = 4.184 J, 1 J = 0.239 cal.

- Energy changes are often measured on a per-mole basis, and the most common forms of energy units in biological chemistry are calories or kilocalories per mole (cal/mol or kcal/mol).

- **Be careful!!!** The nutritional Calorie (i food labels) is represented with a capital C and indeed it a kilocalorie as defined here (i.e., 1 Calorie on the food label is equal to 1000 calorie)

The First Law of Thermodynamics Tells Us That Energy Is Conserved

- Much of what we understand about the principles governing energy flow can be summarized by the three laws of thermodynamics.
- For cell biologists, only the first and second laws are of particular relevance.
- The first law of thermodynamics is called ***the law of conservation of energy***. It states that in every physical or chemical change, the total amount of energy in the universe remains constant, although the form of the energy may change. Or, in other words, energy can be converted from one form to another but can never be created
- or destroyed.



- The total energy stored within a system is called the **internal energy of the system, represented by the symbol *E***.

- We are not usually concerned with the actual value of E for a system because that value cannot be measured directly. However, it is possible to measure the change in internal energy, ΔE, that occurs during a given process.

- ΔE is the difference in internal energy of the system before ($E1$) the process and after the process ($E2$):

  $\Delta E = E2 - E1$ (or $\Delta E = E_{products} - E_{reactants}$)

- In the case of biological reactions and processes, we are usually more interested in the change in **enthalpy**, or **heat** content. Enthalpy is represented by the symbol **H** (for heat) and is related to the internal energy E by a term that combines both pressure (P) and volume (V):

$$H = E + PV$$

- *H* is dependent on both *E* and *PV* because changes in heat content following a process or reaction can affect the total energy as well as the pressure and volume.

- Unlike many chemical reactions, biological reactions generally proceed with little or no change in either pressure or volume. So, for biological reactions, both ΔP and ΔV are usually zero, and we can write

$$\Delta H = \Delta E + \Delta(PV) = \Delta E$$

- Thus, biologists routinely determine changes in heat content for reactions of interest, confident that the values are valid estimates of ΔE.

- The enthalpy change that accompanies a specific reaction is simply the difference in the heat content between the reactants and the products of the reaction:

$$\Delta H = H_{products} - H_{reactants}$$

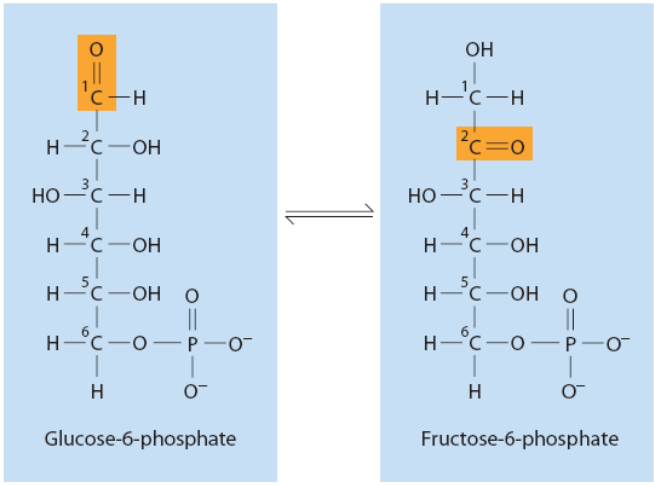
- The ΔH value for a specific reaction or process will be either negative or positive. If the heat content of the products is less than that of the reactants, heat is released, ΔH will be negative, and the reaction is said to be **exothermic.** For example, the burning (oxidation) of gasoline in your car is exothermic because the heat content of the products (CO_2 and H_2O) is less than the heat content of the reactants (gasoline).

- If the heat content of the products is greater than that of the reactants, ΔH will be positive, and the reaction is **endothermic.**

- In an endothermic reaction or process, heat energy is absorbed, as in the melting of an ice cube—the heat content of the resulting liquid water is greater than the heat content of the ice before melting. Thus, the value for any reaction is simply a measure of the heat that is either liberated from or taken up by that reaction as it occurs under conditions of constant temperature and pressure.

The Second Law of Thermodynamics Tells Us That Reactions Have Directionality

- Geuss the directionality of the reaction in right???
- Impossible!!!



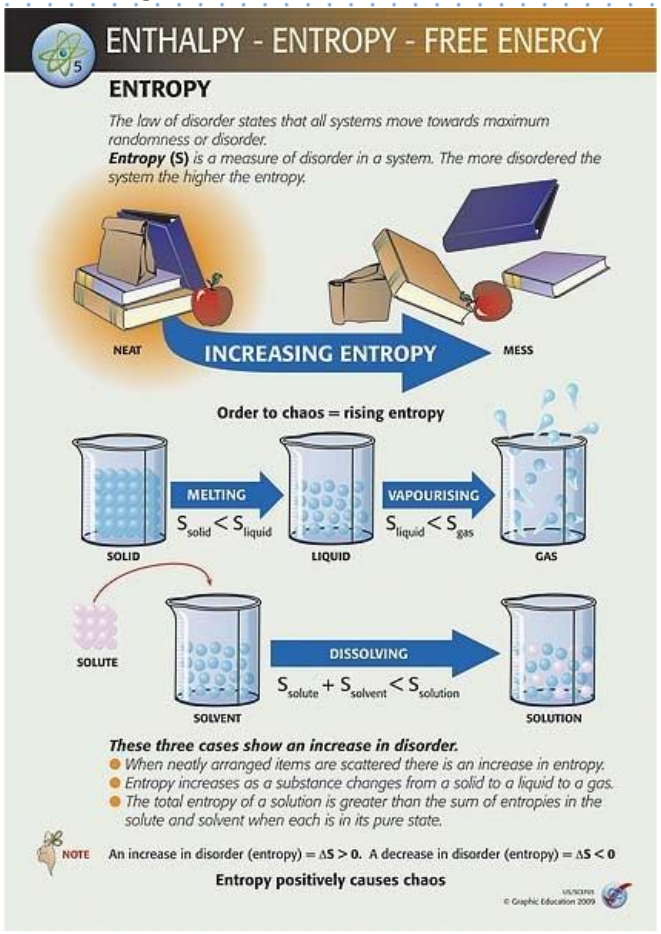
• We know what will happen with burning paper and melting ice, but we lack the familiarity and experience with phosphorylated sugars even to make an intelligent guess. Clearly, what we need is a reliable means of determining whether a given physical or chemical change can occur under specific conditions without having to rely on experience, intuition, or guesswork.

- Thermodynamics provides us with exactly such a measure of spontaneity in the **second law of thermodynamics,** or the law of thermodynamic spontaneity.

- This law tells us that ***in every physical or chemical change, the universe always tends toward greater disorder or randomness (entropy)***.

- The second law is useful for our purposes because it allows us to predict in what direction a reaction will proceed under specified conditions, how much energy the reaction will release as it proceeds, and how the energetics of the reaction will be affected by specific changes in the conditions.

- Thermodynamic spontaneity—whether a reaction can go—can be measured by changes in either of two parameters:



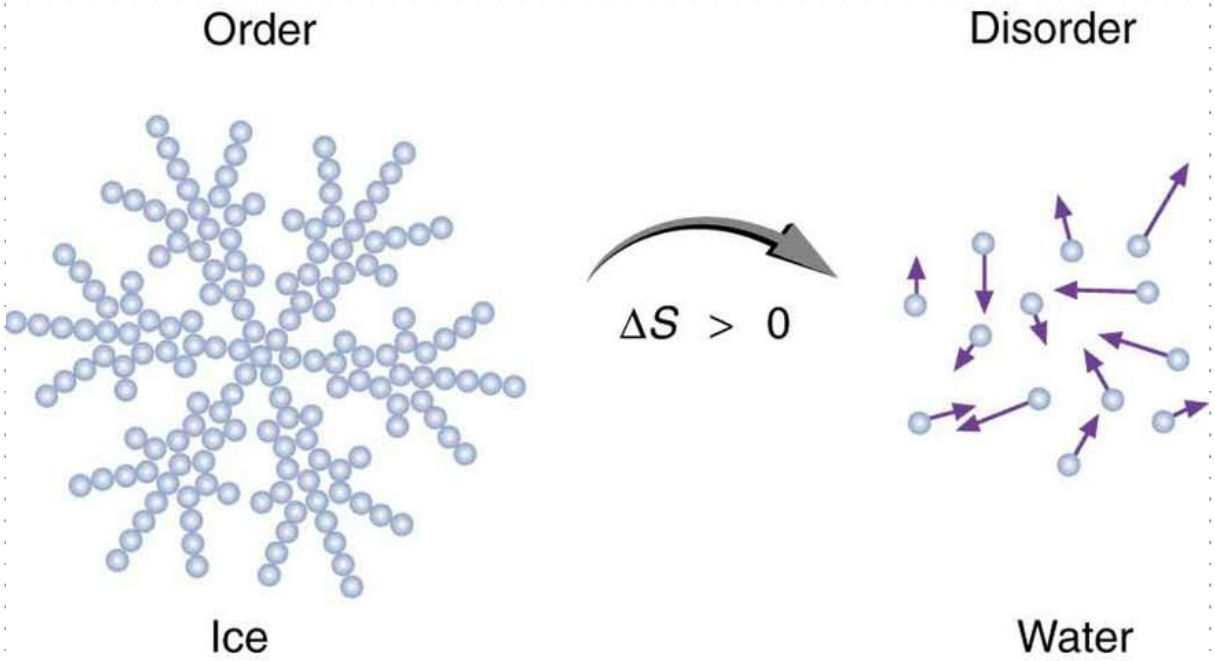
Entropy

- Although we cannot quantify entropy directly, we can get some feel for it by considering it to be a measure of randomness or disorder.

- Entropy is represented by the symbol ***S***. For any system, the change in entropy, represents a change in the degree of randomness or disorder of the components of the system.

- There is an important link between spontaneous events and entropy changes because *all processes or reactions that occur spontaneously result in an increase in the total entropy of the universe*.

- In other words, the value of $\Delta S_{universe}$ is positive for every spontaneous process or reaction.



Free Energy

- As you might guess, a measure of spontaneity for the system alone does in fact exist. It is called **free energy** and is represented by the symbol ***G*** (after Josiah Willard Gibbs, who first developed the concept).

- Because of its predictive value and its ease of calculation, the free energy function is one of the most useful thermodynamic concepts in biology.

- In fact, our entire discussion of thermodynamics so far has really been a way of getting us to this concept of free energy because it is here that the usefulness of thermodynamics for cell biologists becomes apparent.

- Like most other thermodynamic functions, free energy is defined in terms of mathematical relationships.

- For biological reactions at constant pressure, volume, and temperature, the **free energy change**,ΔG , is dependent on the free energies of the products and the reactants:

$$\Delta G = G_{products} - G_{reactants}$$

- This free energy change is related to the changes in enthalpy and entropy by the formula:

$$\Delta G = \Delta H - T\Delta S$$

where ΔG is the change in free energy, ΔH is the change in enthalpy, T is the temperature of the system in degrees Kelvin (K = C + 273, and ΔS is the change in entropy.

- *Free energy change, ΔG,* is therefore influenced by changes in both enthalpy and entropy.
- As we saw earlier, ΔH will be positive for endothermic reactions and negative for exothermic reactions. Similarly, ΔS for a specific reaction or process can be either positive (increase in entropy) or negative (decrease in entropy).
- Because of the minus sign, the term $-T\Delta S$ will be negative if entropy increases or positive if entropy decreases.
- Therefore, you can see that the change in free energy of a reaction, ΔG, will increase when the change in heat content, ΔH, increases or when the change in entropy (randomness), ΔS, decreases.

Free Energy Change as a Measure of Thermodynamic Spontaneity

- All processes or reactions that occur spontaneously result in a decrease in the free energy content of the system.
- In other words, the value of ΔG_{system} is negative for every spontaneous process or reaction.
- This occurs when the free energy of the products is less than the free energy of the reactants.
- Such processes or reactions are called **exergonic,** which means energy-yielding.

- In contrast, any process or reaction that would result in an increase in the free energy of the system is called **endergonic (energy-requiring)** and cannot proceed under the conditions for which ΔG was calculated.

- Because the values for ΔH and $-T\Delta S$ can each be either positive or negative, the value of ΔG for a given reaction will depend on both the signs and numerical values of the ΔH and $-T\Delta S$ terms.

- If both the ΔH and $-T\Delta S$ terms for a given reaction are positive (endothermic and a decrease in entropy), ΔG will be positive, and the reaction will be endergonic and not spontaneous.

- In contrast, a reaction that is exothermic (i.e., ΔH is negative) and results in an increase in entropy (i.e., ΔS is positive and $-T\Delta S$ is negative) has a ΔG value that is the sum of these two negative terms and is therefore exergonic and spontaneous.

- However, if the ΔH and $-T\Delta S$ terms differ in sign, the ΔG value can be either positive or negative, depending on the magnitudes of the ΔH and $-T\Delta S$ terms.

ENTHALPY - ENTROPY - FREE ENERGY

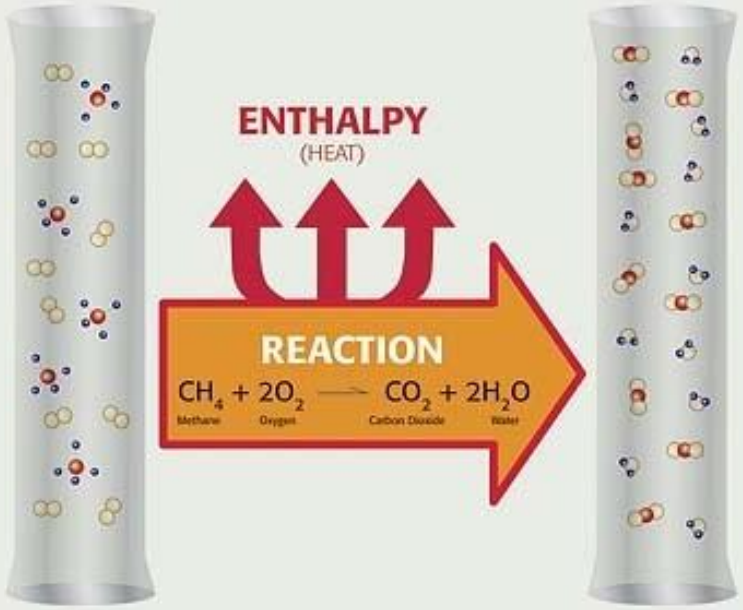
ENTHALPY

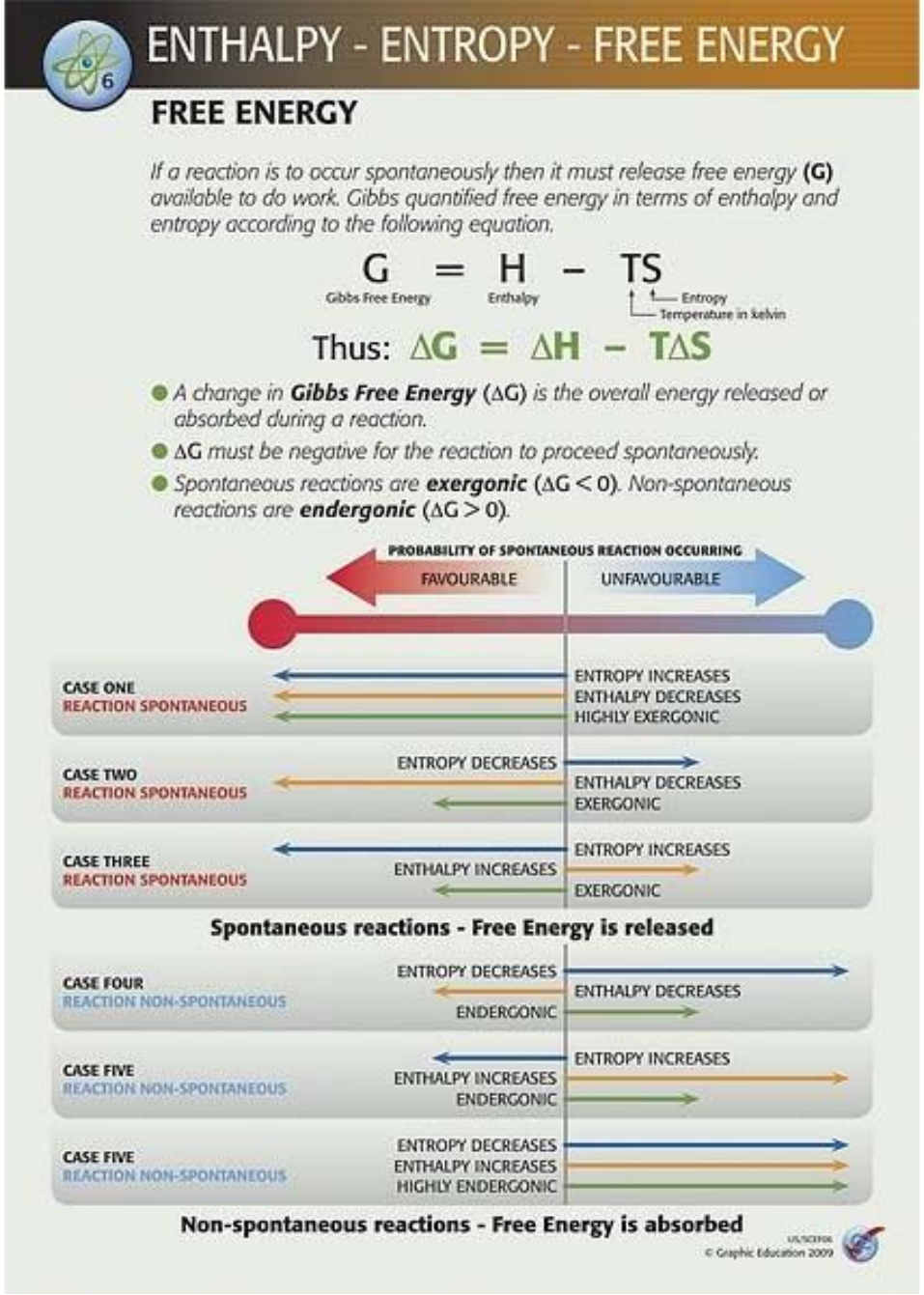
*Every system has **internal energy** due to the forces within and between atoms and molecules, as well as the motion of its particles. This stored energy (heat content) is called **enthalpy** (H).*

Measuring Enthalpy

Consider a gas jar filled with oxygen and methane. As the particles are in motion and have the potential to react violently, we know that the system contains a relatively large amount of enthalpy.

There is no way of directly measuring the total enthalpy of this system. However, if the methane and oxygen are allowed to react at a constant pressure the total heat released (change in enthalpy - ΔH) can be measured using a calorimeter.




ENTHALPY - ENTROPY - FREE ENERGY
FREE ENERGY
If a reaction is to occur spontaneously then it must release free energy (G) available to do work. Gibbs quantified free energy in terms of enthalpy and entropy according to the following equation.
G = H − TS
Gibbs Free Energy
Enthalpy
Entropy
Temperature in kelvin
Thus: ΔG = ΔH − TΔS
A change in Gibbs Free Energy (ΔG) is the overall energy released or absorbed during a reaction.
ΔG must be negative for the reaction to proceed spontaneously.
Spontaneous reactions are exergonic (ΔG < 0). Non-spontaneous reactions are endergonic (ΔG > 0).
PROBABILITY OF SPONTANEOUS REACTION OCCURRING
FAVOURABLE
UNFAVOURABLE
CASE ONE
REACTION SPONTANEOUS
ENTROPY INCREASES
ENTHALPY DECREASES
HIGHLY EXERGONIC
CASE TWO
REACTION SPONTANEOUS
ENTROPY DECREASES
ENTHALPY DECREASES
EXERGONIC
CASE THREE
REACTION SPONTANEOUS
ENTROPY INCREASES
ENTHALPY INCREASES
EXERGONIC
Spontaneous reactions - Free Energy is released
CASE FOUR
REACTION NON-SPONTANEOUS
ENTROPY DECREASES
ENTHALPY DECREASES
ENDERGONIC
CASE FIVE
REACTION NON-SPONTANEOUS
ENTROPY INCREASES
ENTHALPY INCREASES
ENDERGONIC
CASE FIVE
REACTION NON-SPONTANEOUS
ENTROPY DECREASES
ENTHALPY INCREASES
HIGHLY ENDERGONIC
Non-spontaneous reactions - Free Energy is absorbed
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A Biological Example

- For a biological example of an exergonic reaction, consider again the oxidation of glucose to carbon dioxide and water:

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

- You may recognize this as the summary equation for the process of aerobic respiration, whereby chemotrophs obtain energy from glucose. (Most of the cells in your body are carrying out this process right now.)

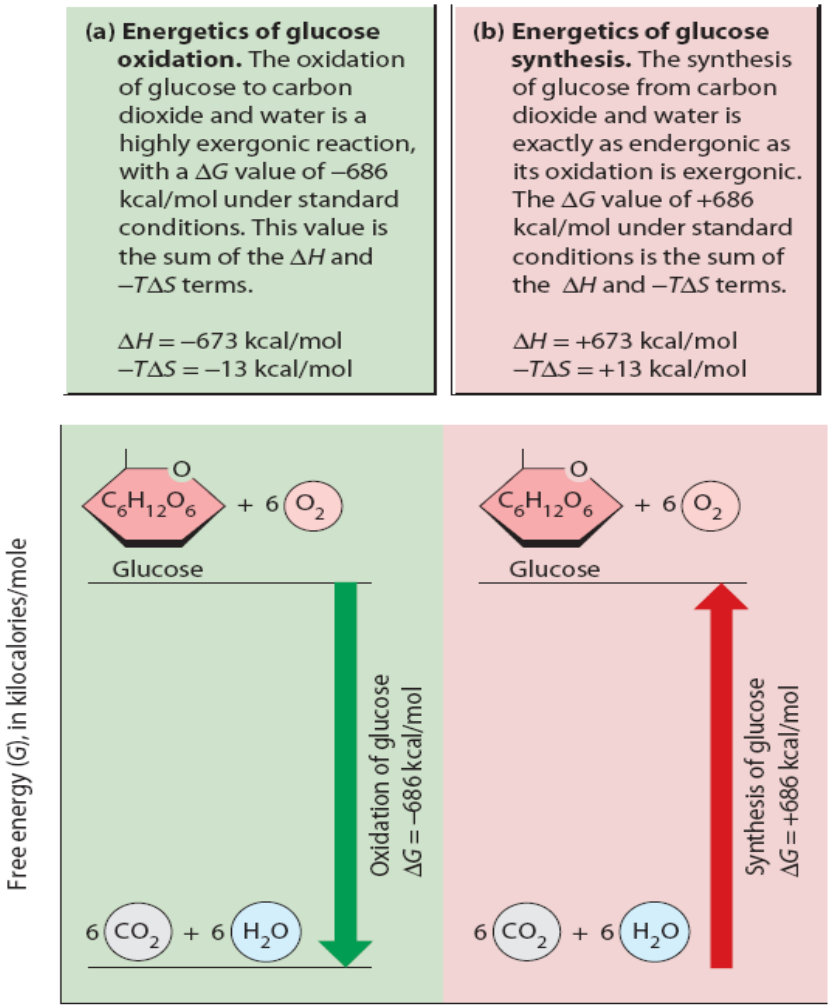


FIGURE 5-10 Changes in Free Energy for the Oxidation and Synthesis of Glucose The exergonic oxidation of glucose shown in (a) has a large negative ΔG that is exactly equal in magnitude but opposite in sign to the large positive ΔG for the endergonic synthesis of glucose shown in (b).

The Meaning of Spontaneity

- Before considering how we can actually calculate ΔG and use it as a measure of thermodynamic spontaneity, we need to look more closely at what is—and what is not—meant by the term spontaneous.

- As we noted earlier, spontaneity tells us only that a reaction can go; it says nothing at all about whether it will go.

- A reaction can have a negative ΔG value and yet not actually proceed to any measurable extent. The cellulose of paper obviously burns spontaneously once ignited, consistent with a highly negative ΔG value of 686 kcal/mol of glucose units. Yet in the absence of a match, paper is reasonably stable and might require hundreds of years to oxidize.

- Thus, ΔG can really tell us only whether a reaction or process is thermodynamically feasible—whether it has the potential for occurring. Whether an exergonic reaction will in fact proceed depends not only on its favorable (negative) ΔG but also on the availability of a mechanism or pathway to get from the initial state to the final state.

- Thermodynamic spontaneity is therefore a necessary but insufficient criterion for determining whether a reaction will actually occur.

Understanding ΔG

- Our final task in this lecture will be to understand how ΔG is calculated and how it can then be used to assess the thermodynamic feasibility of reactions under specified conditions.

- For that, let's go back to the reaction that converts glucose-6-phosphate into fructose-6-phosphate (go back to second law of thermodynamics) and ask what we can learn about the spontaneity of the conversion in the direction written (from left to right).

The Equilibrium Constant Is a Measure of Directionality

- To assess whether a reaction can proceed in a given direction under specified conditions, we must understand the **equilibrium constant, *Keq*,** which is the ratio of product concentrations to reactant concentrations at equilibrium.

- When a reversible reaction is at equilibrium, this means there is no net change in the concentrations of either products or reactants with time. For the general reaction in which A is converted reversibly into B, the equilibrium constant is simply the ratio of the equilibrium concentrations of A and B:

$$\mathrm{A} \rightleftharpoons \mathrm{B}$$

$$K_{eq} = \frac{[\mathrm{B}]_{eq}}{[\mathrm{A}]_{eq}}$$

where $[A]_{eq}$ and $[B]_{eq}$ are the concentrations of A and B, in moles per liter, when reaction is at equilibrium at 25 C.

- Given the equilibrium constant for a reaction, you can easily tell whether a specific mixture of products and reactants is at equilibrium. If it is not, it is easy to tell how far away the reaction is from equilibrium and the direction it must proceed to reach equilibrium.

- For example, the equilibrium constant for the reaction at 25 C is known to be 0.5. This means that, at equilibrium, there will be one-half as much fructose-6-phosphate as glucose-6-phosphate, regardless of the actual magnitudes of the concentrations:

$$K_{eq} = \frac{[\text{fructose-6-phosphate}]_{eq}}{[\text{glucose-6-phosphate}]_{eq}} = 0.5$$

- If the two compounds are present in any other concentration ratio, the reaction will not be at equilibrium and will move toward equilibrium.

- Thus, a concentration ratio less than *Keq* means that there is too little fructose-6-phosphate present, and the reaction will tend to proceed to the right to generate more fructose-6-phosphate at the expense of glucose-6-phosphate.

- Conversely, a concentration ratio greater than Keq indicates that the relative concentration of fructose-6-phosphate is too high, and the reaction will tend to proceed to the left.

- Figure 5-11 illustrates this concept for the inter-conversion of A and B, showing the relationship between the free energy of the reaction and how far the concentrations of A and B are from equilibrium. (Notice that Keq is assumed to be 1.0 in this illustration; for other values of Keq, the curve would be the same shape but still centered over Keq.)

- The point of Figure 5-11 is clear: The free energy is lowest at equilibrium and increases as the system is displaced from equilibrium in either direction.

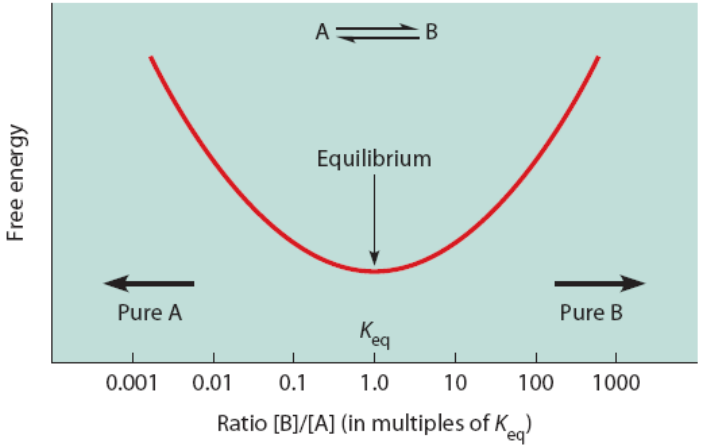


FIGURE 5-11 Free Energy and Chemical Equilibrium. The amount of free energy available from a chemical reaction depends on how far the components are from equilibrium. This principle is illustrated here for a reaction that interconverts A and B and has an equilibrium constant, K_{eq}, of 1.0. The free energy of the system increases as the [B]/[A] ratio changes on either side of the equilibrium point. For a reaction with a K_{eq} value other than 1.0, the graph would have the same shape but would be centered over the K_{eq} value for that reaction.

Table 5-1 The Meaning of $\Delta G^{\circ\prime}$ and $\Delta G'$

The Meaning of $\Delta G^{\circ\prime}$		
$\Delta G^{\circ\prime}$ Negative ($K'_{eq} > 1.0$)	**$\Delta G^{\circ\prime}$ Positive ($K'_{eq} < 1.0$)**	**$\Delta G^{\circ\prime} = 0$ ($K'_{eq} = 1.0$)**
Products predominate over reactants at equilibrium at standard temperature, pressure, and pH.	Reactants predominate over products at equilibrium at standard temperature, pressure, and pH.	Products and reactants are present equally at equilibrium at standard temperature, pressure, and pH.
Reaction goes spontaneously to the right under standard conditions.	Reaction goes spontaneously to the left under standard conditions.	Reaction is at equilibrium under standard conditions.
The Meaning of $\Delta G'$		
$\Delta G'$ Negative	**$\Delta G'$ Positive**	**$\Delta G' = 0$**
Reaction is thermodynamically feasible as written under the conditions for which $\Delta G'$ was calculated.	Reaction is not feasible as written under the conditions for which $\Delta G'$ was calculated.	Reaction is at equilibrium under the conditions for which $\Delta G'$ was calculated.
Work can be done by the reaction under the conditions for which $\Delta G'$ was calculated.	Energy must be supplied to drive the reaction under the conditions for which $\Delta G'$ was calculated.	No work can be done nor is energy required by the reaction under the conditions for which $\Delta G'$ was calculated.

- Thus, ***the tendency toward equilibrium provides the driving force for every chemical reaction.***

- A comparison of prevailing and equilibrium concentration ratios provides one measure of that tendency.

ΔG Can Be Calculated Readily

- Thus, ΔG is just a means of calculating how far from equilibrium a reaction lies under specified conditions and how much energy will be released as the reaction proceeds toward equilibrium.

- Both the equilibrium constant and the prevailing concentrations of reactants (A) and products (B) are needed to calculate ΔG.

- Thus, for the reaction of

the equation relating these variables is:

$$\Delta G = RT \ln \frac{[B]_{pr}}{[A]_{pr}} - RT \ln \frac{[B]_{eq}}{[A]_{eq}}$$

$$= RT \ln \frac{[B]_{pr}}{[A]_{pr}} - RT \ln K_{eq}$$

$$= -RT \ln K_{eq} + RT \ln \frac{[B]_{pr}}{[A]_{pr}}$$

- Where,
  - ΔG is the free energy change, in cal/mol, under the specified conditions
  - R is the gas constant (1.987 cal/mol-K)
  - T is the temperature in kelvins (use 25 C = 298 K)
  - [A]pr and [B]pr are the prevailing concentrations of A and B in moles per liter;
  - [A]eq and [B]eq are the equilibrium concentrations of A and B in moles per liter
  - Keq is the equilibrium constant at the standard temperature of 298 K (25 C)
  - *ln* stands for the natural logarithm of (i.e., the logarithm of a quantity to the base of the natural logarithm system, e, which equals approximately 2.718). Natural logarithms are used because they describe processes in which the rate of change of the process is directly related to the quantity of material undergoing the change (e.g., as in describing radioactive decay).

- More generally, for a reaction in which *a* molecules of reactant A combine with *b* molecules of reactant B to form *c* molecules of product C plus d molecules of product D:

$$aA + bB \rightleftharpoons cC + dD$$

- ΔG is calculated as

$$\Delta G = -RT \ln K_{eq} + RT \ln \frac{[C]_{pr}^{c} [D]_{pr}^{d}}{[A]_{pr}^{a} [B]_{pr}^{b}}$$

- Let's return to glucose-6-phosphate ⇔ fructose-6-phosphate reaction, assuming that the prevailing concentrations of glucose-6-phosphate and fructose-6-phosphate in a cell are 10 µM and 1 µM, respectively, at 25 C.

- Since the ratio of prevailing product concentrations to reactant concentrations is 0.1 and the equilibrium constant is 0.5, there is clearly too little fructose-6-phosphate present relative to glucose-6-phosphate for the reaction to be at equilibrium.

- The reaction should therefore tend toward the right in the direction of fructose-6-phosphate generation. In other words, the reaction is thermodynamically favorable in the direction written. This, in turn, means that ΔG must be negative under these conditions.

The actual value for ΔG is calculated as follows:

$$\Delta G = -(1.987 \text{ cal/mol-K})(298 \text{ K}) \ln(0.5) + (1.987 \text{ cal/mol-K})(298 \text{ K}) \ln \frac{1 \times 10^{-6} M}{10 \times 10^{-6} M}$$

$$= -(592 \text{ cal/mol}) \ln(0.5) + (592 \text{ cal/mol}) \ln(0.1)$$

$$= -(592 \text{ cal/mol})(-0.693) + (592 \text{ cal/mol})(-2.303)$$

$$= +410 \text{ cal/mol} - 1364 \text{ cal/mol}$$

$$= -954 \text{ cal/mol}$$

Notice that our expectation of a negative G is confirmed, and we now know exactly how much free energy is liberated upon the spontaneous conversion of 1 mole of glucose-6-phosphate into 1 mole of fructose-6-phosphate under the specified conditions. The free energy liberated in this or some other exergonic reaction can be either “harnessed” to do work, stored in the chemical bonds of ATP, or released as heat.

- Because it is a thermodynamic parameter, ΔG can tell us whether a reaction is thermodynamically possible as written, but it says nothing about the rate or the mechanism of the reaction.

- It simply says that if the reaction does occur, it will proceed to the right and will liberate 954 calories (0.954 kcal) of free energy for every mole of glucose-6-phosphate that is converted to fructose-6-phosphate, provided that the concentrations of both the reactant and the product are maintained at the initial values (10 and 1 mM, respectively) throughout the course of the reaction.

The Standard Free Energy Change Is ΔG Measured Under Standard Conditions

- As it is a thermodynamic parameter, ΔG is independent of the actual mechanism or pathway of a reaction, but it depends crucially on the conditions under which the reaction occurs.

- The melting of ice, for example, depends on temperature; it proceeds spontaneously above 0 C but goes in the opposite direction (freezing) below that temperature.

- It is therefore important to identify the conditions under which a given measurement of ΔG is made.

- By convention, biochemists have agreed on certain arbitrary conditions to define the **standard state of a system** for convenience in reporting, comparing, and tabulating free energy changes in chemical reactions.

- For systems consisting of dilute aqueous solutions, these are usually a standard temperature of 25 (298 K), a pressure of 1 atmosphere, and all products and reactants present at a concentration of 1 M.

- The only common exception to this standard concentration rule is water. The concentration of water in a dilute aqueous solution is approximately 55.5 M and does not change significantly during the course of reactions, even when water is itself a reactant or product.

- By convention, biochemists do not include the concentration of water in calculations of free energy changes, even though the reaction may indicate a net consumption or production of water.

- In addition to standard conditions of temperature, pressure, and concentration, biochemists also frequently specify a standard pH of 7.0 because most biological reactions occur at or near neutrality.

- The concentration of hydrogen ions (and of hydroxyl ions) is therefore 10-7 M, so the standard concentration of 1.0 M does not apply to H+ or OH- ions when a pH of 7.0 is specified.

- Values of Keq, ΔG , or other thermodynamic parameters determined or calculated at pH 7.0 are always written with a prime (as Keq', $\Delta G'$, and so on) to indicate this exception to standard conditions.

- The free energy change calculated under these conditions is called the **standard free energy change,** designated $\Delta G^{o\prime}$, where the superscript (o) refers to standard conditions of temperature, pressure, and concentration, and the prime (‘) emphasizes that the standard hydrogen ion concentration for biochemists is 10-7 M, not 1.0 M.

- It turns out that *ΔGo'* bears a simple linear relationship to the natural logarithm of the equilibrium constant *K'eq*.

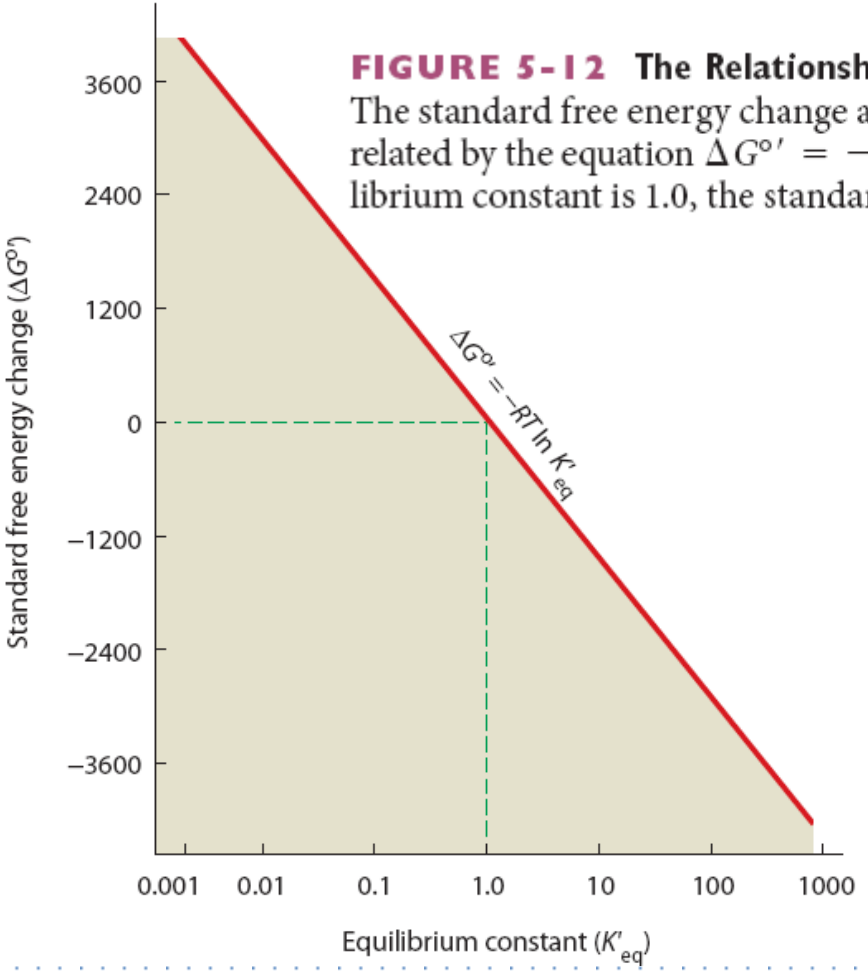


FIGURE 5-12 The Relationship Between $\Delta G^{\circ\prime}$ and K'_{eq}. The standard free energy change and the equilibrium constant are related by the equation $\Delta G^{\circ\prime} = -RT \ln K'_{eq}$. Note that if the equilibrium constant is 1.0, the standard free energy change is zero.

- This relationship can readily be seen by rewriting equation:

$$\Delta G = -RT \ln K_{eq} + RT \ln \frac{[C]_{pr}^{c} [D]_{pr}^{d}}{[A]_{pr}^{a} [B]_{pr}^{b}}$$

with primes and then assuming standard concentrations for all reactants and products.

- All concentration terms are now 1.0 and the natural logarithm of 1.0 is zero, so the second term in the general expression for *ΔGo'* is eliminated, and what remains is an equation for *ΔGo'*, the free energy change under standard conditions:

$$\begin{aligned} \Delta G^{\circ\prime} &= -RT \ln K'_{eq} + RT \ln 1 \\ &= -RT \ln K'_{eq} \end{aligned}$$

- In other words, *ΔGo'* can be calculated directly from the equilibrium constant, provided that the latter has also been determined under the same standard conditions of temperature, pressure, and pH.

- This, in turn, allows equation:

$$\Delta G = -RT \ln K_{eq} + RT \ln \frac{[C]^c_{pr}\,[D]^d_{pr}}{[A]^a_{pr}[B]^b_{pr}}$$

to be simplified as:

$$\Delta G' = \Delta G^{\circ\prime} + RT \ln \frac{[C]^c_{pr}\,[D]^d_{pr}}{[A]^a_{pr}\,[B]^b_{pr}}$$

- At the standard temperature of 25 C (298 K), the term RT becomes (1.987)(298) = 592 cal/mol, so Equations:

$$\Delta G^{\circ\prime} = -RT \ln K'_{eq} + RT \ln 1$$
$$= -RT \ln K'_{eq}$$

and

$$\Delta G' = \Delta G^{\circ\prime} + RT \ln \frac{[C]^c_{pr}\,[D]^d_{pr}}{[A]^a_{pr}\,[B]^b_{pr}}$$

can be rewritten as follows, in what are the most useful formulas for our purposes:

$$\Delta G^{\circ\prime} = -592 \ln K'_{eq}$$

$$\Delta G' = \Delta G^{\circ\prime} + 592 \ln \frac{[C]^c_{pr}\,[D]^d_{pr}}{[A]^a_{pr}\,[B]^b_{pr}}$$

$$\Delta G^{\circ\prime} = -592 \ln K'_{eq}$$

$$\Delta G' = \Delta G^{\circ\prime} + 592 \ln \frac{[C]^c_{pr}\,[D]^d_{pr}}{[A]^a_{pr}\,[B]^b_{pr}}$$

- Above equations represent the most important contribution of thermodynamics to biochemistry and cell biology—a means of assessing the feasibility of a chemical reaction based on the prevailing concentrations of products and reactants and a knowledge of the equilibrium constant.

•Equation;

$$\Delta G^{\circ\prime} = -592 \ln K'_{eq}$$

expresses the relationship between the standard free energy change *ΔGo'* and the equilibrium constant K'eq and enables us to calculate the free energy change that would be associated with any reaction of interest if all reactants and products were maintained at a standard concentration of 1.0 M.

- If *K'eq* is greater than 1.0, then ln Keq will be positive and $\Delta Go'$ will be negative, and the reaction can proceed to the right under standard conditions.

- This makes sense because if *K'eq* is greater than 1.0, products will predominate over reactants at equilibrium.

- Conversely, if *K'eq* is less than 1.0, then $\Delta Go'$ will be positive and the reaction cannot proceed to the right. Instead, it will tend toward the left because $\Delta Go'$ for the reverse reaction will have the same absolute value but will be opposite in sign. This is in keeping with the small value for *K'eq* , which specifies that reactants are favored over products (that is, the equilibrium lies to the left).

- The $\Delta G^{o\prime}$ values are convenient both because they can easily be determined from the equilibrium constant and because they provide a uniform convention for reporting free energy changes.

- But bear in mind that a $\Delta G^{o\prime}$ value is an arbitrary standard in that it refers to an arbitrary state specifying conditions of concentration that cannot be achieved with most biologically important compounds.

- $\Delta G^{o\prime}$ is therefore useful for standardized reporting, but it is not a valid measure of the thermodynamic spontaneity of reactions as they occur under real conditions.

- For real-life situations in cell biology, we will use $\Delta G'$, which provides a direct measure of how far from equilibrium a reaction is at the concentrations of reactants and products that actually prevail in the cell (i.e., below reaction).

$$\Delta G' = \Delta G^{\circ\prime} + 592 \ln \frac{[C]_{pr}^{c} [D]_{pr}^{d}}{[A]_{pr}^{a} [B]_{pr}^{b}}$$

Therefore, *$\Delta G'$ is the most useful measure of thermodynamic spontaneity*.

Free Energy Change: Sample Calculations

- To illustrate the calculation and utility of G and G , we return once more to the interconversion of glucose-6-phosphate and fructose-6-phosphate.
- We already know that the equilibrium constant for this reaction under standard conditions of temperature, pH, and pressure is 0.5.
- This means that if the enzyme that catalyzes this reaction in cells is added to a solution of glucose-6-phosphate at 25C, 1 atmosphere, and pH 7.0, and the solution is incubated until no further reaction occurs, fructose-6-phosphate and glucose-6-phosphate will be present in an equilibrium ratio of 0.5.
- The standard free energy change *ΔGo'* can be calculated from *K'eq* as follows:

$$\begin{aligned}\Delta G^{\circ\prime} &= -RT \ln K'_{eq} = -592 \ln K'_{eq} \\ &= -592 \ln 0.5 = -592(-0.693) \\ &= +410 \text{ cal/mol}\end{aligned}$$

- The positive value for $\Delta G^{o\prime}$ is therefore another way of expressing that the reactant (glucose-6-phosphate) is the predominant species at equilibrium. A positive $\Delta G^{o\prime}$ value also means that under standard conditions of concentration, the reaction is nonspontaneous (thermodynamically impossible) in the direction written.

- In other words, if we begin with both glucose-6-phosphate and fructose-6-phosphate present at concentrations of 1.0 M, no net conversion of glucose-6-phosphate to fructose-6-phosphate can occur.

- In a real cell, neither of these phosphorylated sugars would ever be present at a concentration even approaching 1.0 M. In fact, experimental values for the actual concentrations of these substances in human red blood cells are as follows:

$$[\text{glucose-6-phosphate}]: 83\ \mu M\ (83 \times 10^{-6}\ M)$$

$$[\text{fructose-6-phosphate}]: 14\ \mu M\ (14 \times 10^{-6}\ M)$$

Using these values, we can calculate the actual $\Delta G'$ for the interconversion of these sugars in red blood cells as follows:

$$\Delta G' = \Delta G^{\circ\prime} + 592 \ln \frac{[\text{fructose-6-phosphate}]_{pr}}{[\text{glucose-6-phosphate}]_{pr}}$$

$$= +410 + 592 \ln \frac{14 \times 10^{-6}}{83 \times 10^{-6}}$$

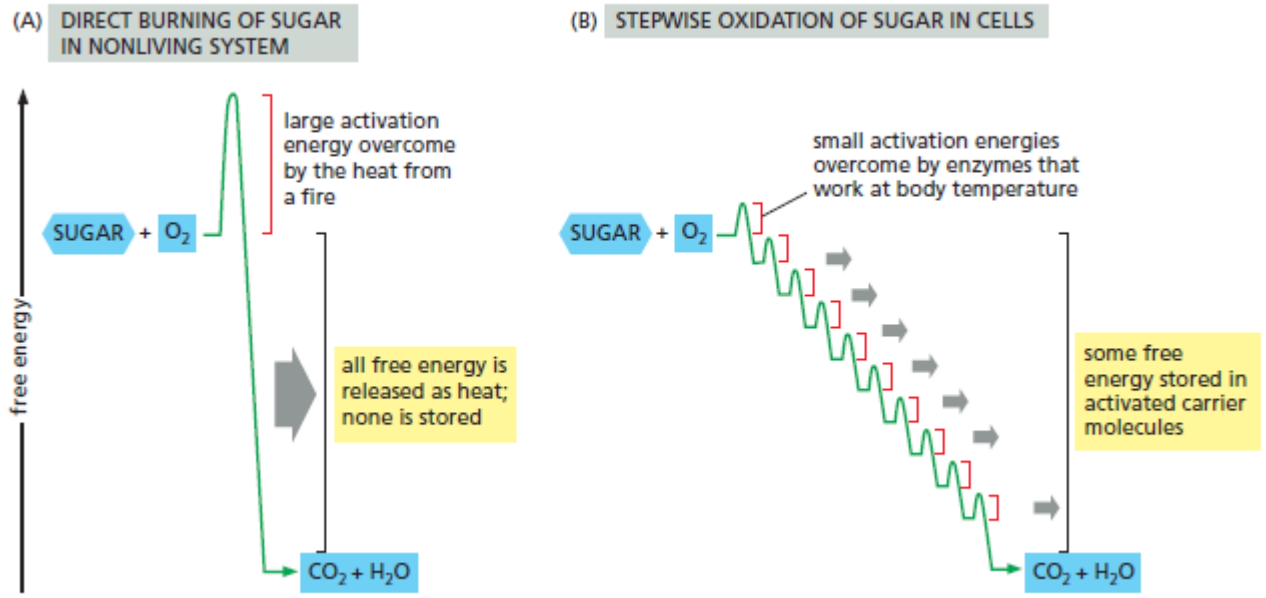
$$= +410 + 592 \ln 0.169$$

$$= +410 + 592(-1.78) = +410 - 1054$$

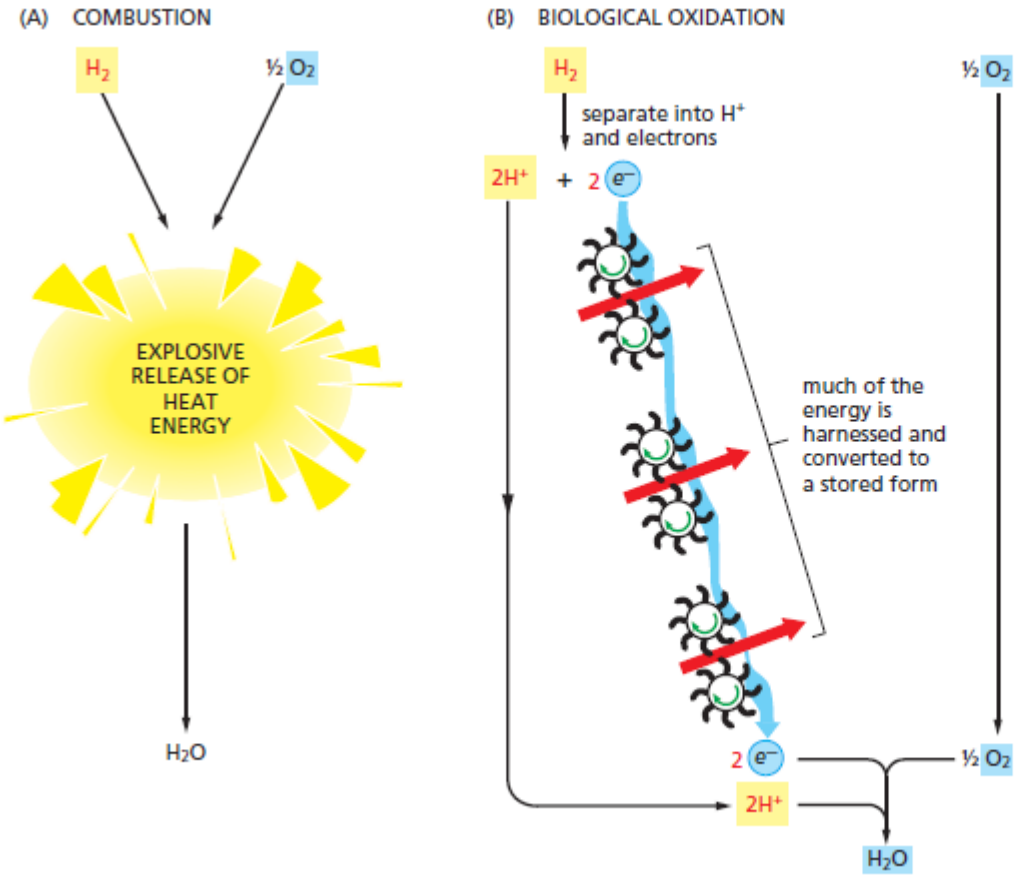
$$= -644 \text{ cal/mol}$$

- The negative value for $\Delta G'$ means that the conversion of glucose-6-phosphate into fructose-6-phosphate is thermodynamically possible under the actual conditions of concentration prevailing in red blood cells and that the reaction will yield 644 cal of free energy per mole of reactant converted to product.

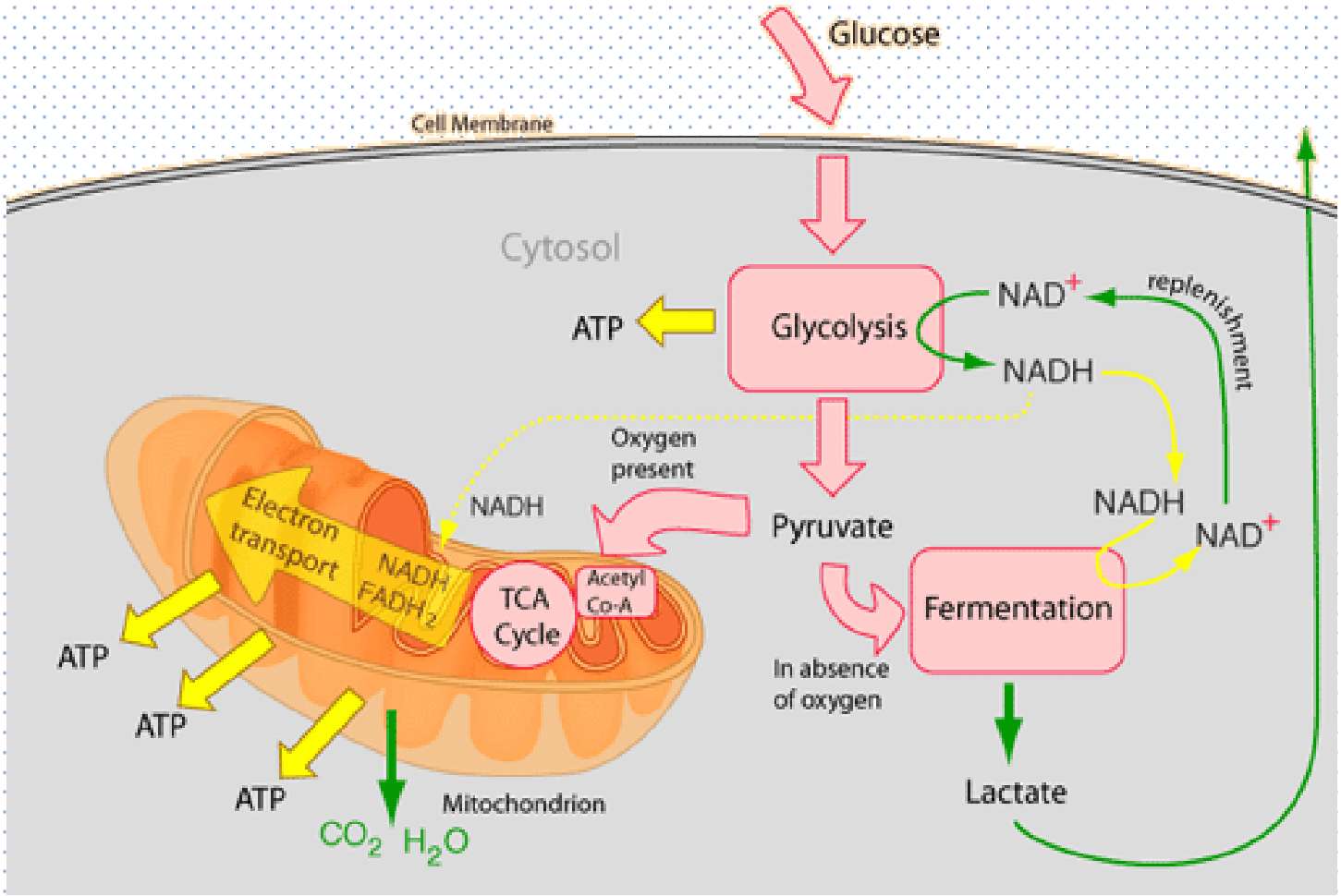
- Thus, life is possible only because living cells maintain themselves in a **steady state**, with most of their reactions far from thermodynamic equilibrium.

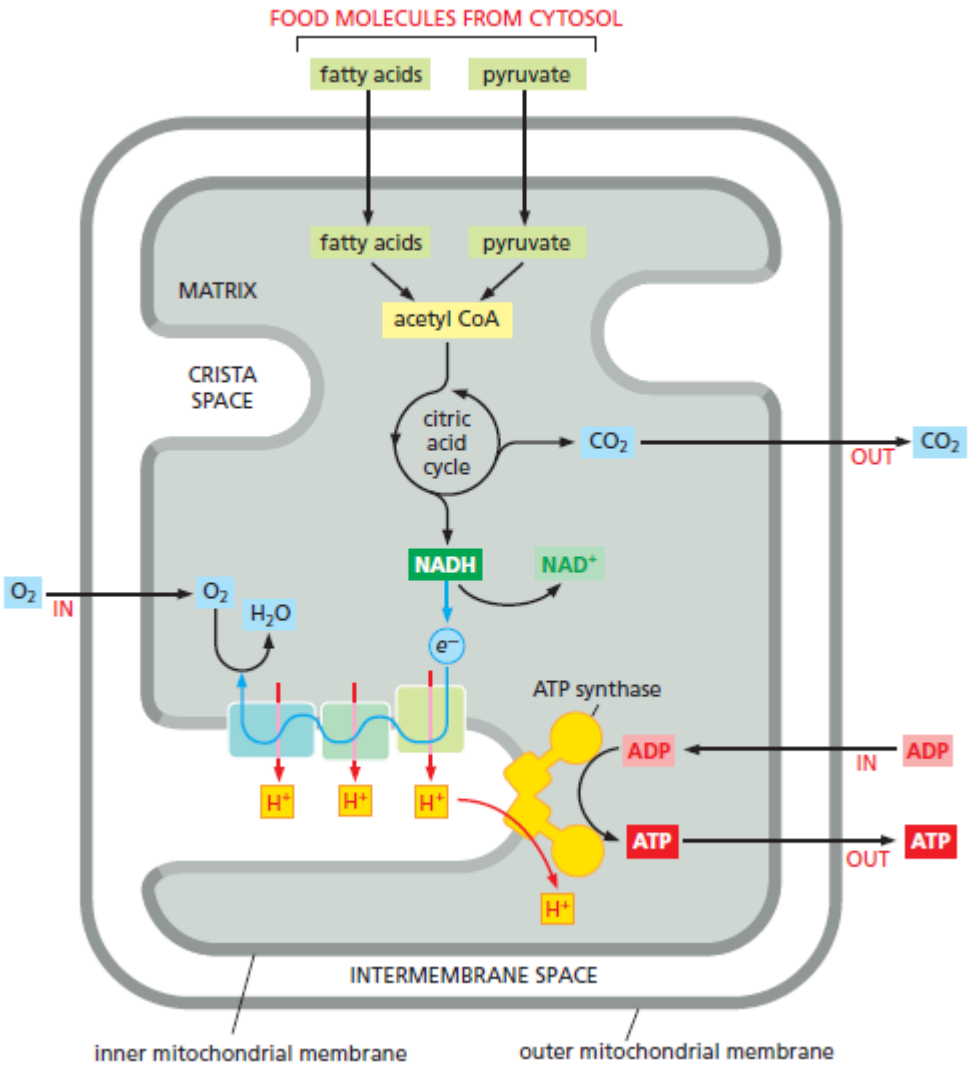


Schematic representation *of the controlled stepwise oxidation of sugar in a cell, compared with ordinary burning. (A) If the sugar were oxidized to CO2 and H2O in a single step, it would release an amount of energy much larger than could be captured for useful purposes. (B) In the cell, enzymes catalyze oxidation via a series of small steps in which free energy is transferred in conveniently sized packets to carrier molecules—most often ATP and NADH. At each step, an enzyme controls the reaction by reducing the activation-energy barrier that has to be surmounted before the specific reaction can occur. The total free energy released is exactly the same in (A) and (B).*



A comparison of biological oxidation with combustion. (A) If hydrogen were simply burned, nearly all of the energy would be released in the form of heat. (B) In biological oxidation reactions, about half of the released energy is stored in a form useful to the cell by means of the electron-transport chain (the respiratory chain) in the crista membrane of the mitochondrion. Only the rest of the energy is released as heat. In the cell, the protons and electrons shown here as being derived from H_2 are removed from hydrogen atoms that are covalently linked to NADH molecules.


Glucose
Cell Membrane
Cytosol
ATP
Glycolysis
NAD+
NADH
replenishment
Oxygen
present
NADH
Pyruvate
NADH
NAD+
Electron
transport
NADH
FADH2
TCA
Cycle
Acetyl
Co-A
Fermentation
In absence
of oxygen
ATP
ATP
ATP
Mitochondrion
CO2
H2O
Lactate


FOOD MOLECULES FROM CYTOSOL
fatty acids
pyruvate
fatty acids
pyruvate
MATRIX
acetyl CoA
CRISTA SPACE
citric acid cycle
CO_2
CO_2
OUT
NADH
NAD^+
O_2
IN
O_2
H_2O
e^-
ATP synthase
ADP
ADP
IN
H^+
H^+
H^+
ATP
ATP
OUT
H^+
INTERMEMBRANE SPACE
inner mitochondrial membrane
outer mitochondrial membrane

MBG304 Biochemistry

Lecture 2: Glycolysis and Fermentation

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- Cell cannot live without energy and a source of chemical “building blocks”.

- Building blocks are the small molecules from which macromolecules such as proteins, nucleic acids, and polysaccharides are synthesized.

- The desired energy and small molecules are both present in the food molecules that these organisms produce or ingest.

- In this lecture (*i.e.*, glycolysis) and the next (*i.e.*, Krebs Cycle and Electron Transport Chain), we will consider how chemotrophs obtain energy from the food they engulf or ingest.

- Then, we will discuss the process (*i.e.*, photsynthesis) by which phototrophs, such as green plants, algae, and some bacteria, tap the solar radiation that is the ultimate energy source for almost all living organisms.

- **Metabolism:** all the chemical reactions that occur within a cell

- The overall metabolism of a cell consists many specific **metabolic pathways,** each of which accomplishes a particular task.

Metabolic pathways

- Metabolic pathways are of two general types.

1. Pathways that synthesize cellular components are called **anabolic pathways**

2. Those involved in the breakdown of cellular constituents are called **catabolic pathways**.

Anabolic pathways

- Anabolic pathways usually involve a substantial increase in molecular order (decrease in entropy) and are *endergonic* (energy-requiring).
- Polymer synthesis and the biological reduction of carbon dioxide to sugar are examples of anabolic pathways.
- Certain steroid hormones, for example, are called anabolic steroids because they stimulate the synthesis of muscle proteins from amino acids.

Catabolic pathways

- Catabolic pathways, by contrast, are degradative pathways that typically involve a decrease in molecular order (increase in entropy) and are *exergonic* (energy- liberating).

- These reactions often involve hydrolysis of macromolecules or biological oxidations.

- Catabolic pathways play two roles in cells:

1. They release the free energy needed to drive cellular functions

2. They give rise to the small organic molecules, or *metabolites,* that are the building blocks for biosynthesis.

ATP: The Universal Energy Coupler

- The anabolic reactions of cells are responsible for: ***growth*** *and* ***repair processes***

- The catabolic reactions ***release the energy*** needed to drive the anabolic reactions and to carry out other kinds of cellular work.

- The efficient linking, or *coupling,* of energy-yielding processes to energy-requiring processes is therefore crucial to cell function.

- In virtually all cells, the molecule most commonly used as an energy intermediate is the phosphorylated compound **adenosine triphosphate (ATP).**

- ATP is, in other words, the primary energy "currency" of the biological world.

- Keep in mind, however, that ATP synthesis is not the only way that cells store chemical energy. Other high-energy molecules, such as GTP and creatine phosphate, store chemical energy that can be converted to ATP.

- In addition, chemical energy is stored as *reduced coenzymes* such as NADH that are a source of reducing power in cells.

ATP Contains Two Energy-Rich Phosphoanhydride Bonds

- ATP is a complex molecule containing the aromatic base adenine, the five-carbon sugar ribose, and a chain of three phosphate groups.

- The phosphate groups are linked to each other by **phosphoanhydride bonds** and to the ribose by a **phosphoester bond**.

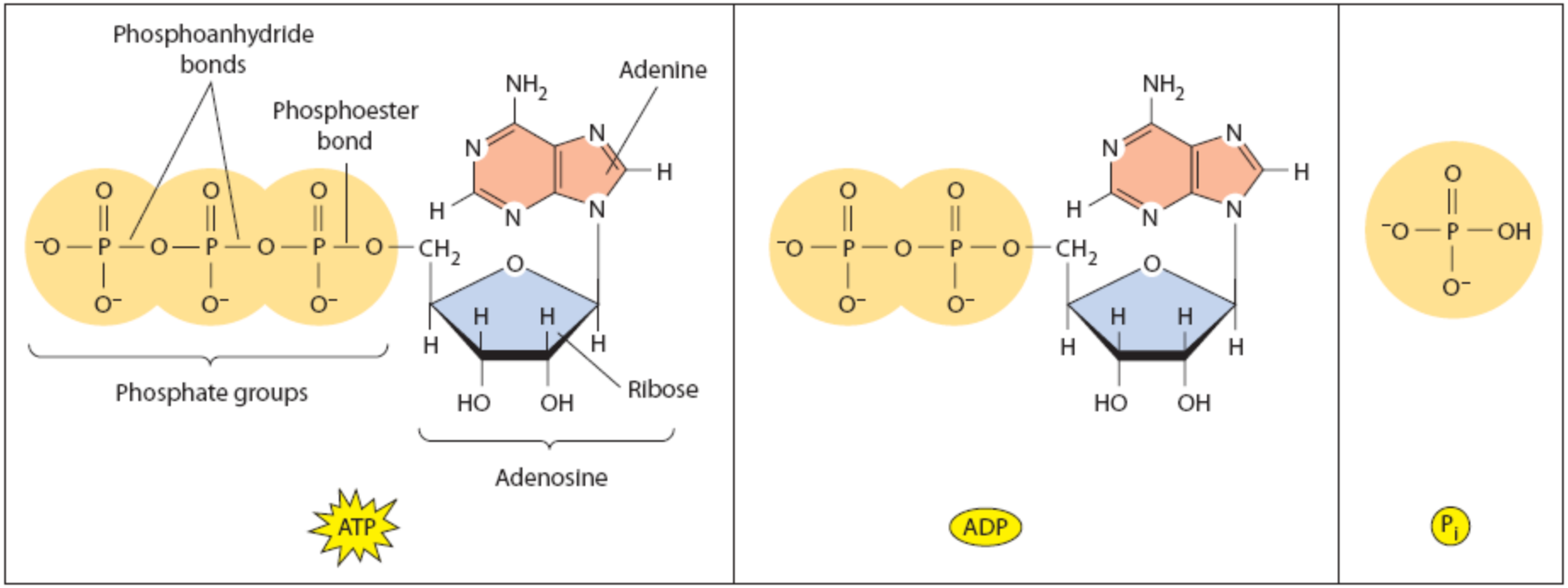
- The ATP molecule serves well as an intermediate in cellular energy metabolism because energy is released when ATP undergoes *hydrolysis.*

Why ATP?

- ATP) is perhaps the most important of the so-called energy-rich compounds in a cell. Its concentration in the cell varies from 0.5 to 2.5 mg/mL of cell fluid.

- The hydrolysis of ATP to form ADP and Pi is highly exergonic, with a standard free energy change ($\Delta G°'$) of -7.3 kcal/mol.

- The reverse reaction, whereby ATP is synthesized from ADP and Pi with the loss of a water molecule by condensation, is correspondingly endergonic, with a $\Delta G°'$ of +7.3 kcal/mol.



(a) Structures of ATP, ADP, and inorganic phosphate (at pH 7)

Reaction 1: Hydrolysis
($\Delta G^{\circ\prime} = -7.3$ kcal/mol)

$ATP^{4-} + H_2O \rightleftharpoons ADP^{3-} + P_i^{2-} + H^+$

Reaction 2: ATP synthesis
($\Delta G^{\circ\prime} = +7.3$ kcal/mol)

(b) Balanced chemical equation for ATP hydrolysis and synthesis

FIGURE 9-1 ATP Hydrolysis and Synthesis. (a) ATP consists of adenosine (adenine + ribose) plus three phosphate groups attached to carbon atom 5 of the ribose. (b) *Reaction 1:* ATP hydrolysis to ADP and inorganic phosphate (P_i) is highly exergonic, with a standard free energy change of −7.3 kcal/mol. *Reaction 2:* ATP synthesis by phosphorylation of ADP is highly endergonic, with a standard free energy change of +7.3 kcal/mol.

The energy released when ATP is hydrolyzed is approximately midway between those of the high-energy and the low-energy phosphate compounds.

This means that the hydrolysis of ATP can provide energy for the phosphorylation of the compounds below it in the table.

The hydrolysis of compounds, such as creatine phosphate, that appear *above* ATP in the table can provide the energy needed to re-synthesize ATP from ADP.

Standard Free Energies of Hydrolysis for Phosphorylated Compounds Involved in Energy Metabolism

Phosphorylated Compound and Its Hydrolysis Reaction	$\Delta G^{\circ\prime}$ (kcal/mol)
Phosphoenolpyruvate (PEP)	
$+ H_2O \longrightarrow$ pyruvate $+ P_i$	−14.8
1,3-bisphosphoglycerate	
$+ H_2O \longrightarrow$ 3-phosphoglycerate $+ P_i$	−11.8[1]
Phosphocreatine	
$+ H_2O \longrightarrow$ creatine $+ P_i$	−10.3
Adenosine triphosphate (ATP)	
$+ H_2O \longrightarrow$ adenosine diphosphate $+ P_i$	−7.3
Glucose-1-phosphate	
$+ H_2O \longrightarrow$ glucose $+ P_i$	−5.0
Glucose-6-phosphate	
$+ H_2O \longrightarrow$ glucose $+ P_i$	−3.3
Glycerol phosphate	
$+ H_2O \longrightarrow$ glycerol $+ P_i$	−2.2

$$\Delta G' = \Delta G^{\circ\prime} + RT \ln \frac{[\mathrm{ADP}][\mathrm{P_i}]}{[\mathrm{ATP}]}$$

- In most cells, the ATP/ADP ratio is significantly greater than 1:1, often in the range of about 5:1. As a result, the term ln([ADP][ ]/[ATP]) is negative, and ΔG' is therefore more negative than -7.3 kcal/mol usually in the range of -10 to -14 kcal/mol.

Biological Oxidations Usually Involve the Removal of Both Electrons and Protons and Are Highly Exergonic

To say that nutrients such as carbohydrates, fats, or proteins are sources of energy for cells means that these are oxidizable organic compounds and that their oxidation is highly exergonic. Recall from chemistry that **oxidation** is the removal of electrons. Thus, for example, a ferrous ion (Fe2+) is oxidizable because it readily gives up an electron as it is converted to a ferric ion (Fe3+):

$$Fe^{2+} \longrightarrow Fe^{3+} + e^-$$

The only difference in biological chemistry is that the oxidation of organic molecules frequently involves the removal not just of electrons but of hydrogen ions (protons) as well, so that the process is often also one of **dehydrogenation**. Consider, for example, the oxidation of ethanol to the corresponding aldehyde:

$$\underset{\text{Ethanol}}{CH_3{-}CH_2{-}OH} \xrightarrow{\text{oxidation}} \underset{\text{Acetaldehyde}}{CH_3{-}\overset{\overset{H}{|}}{C}{=}O} + 2e^- + 2H^+$$

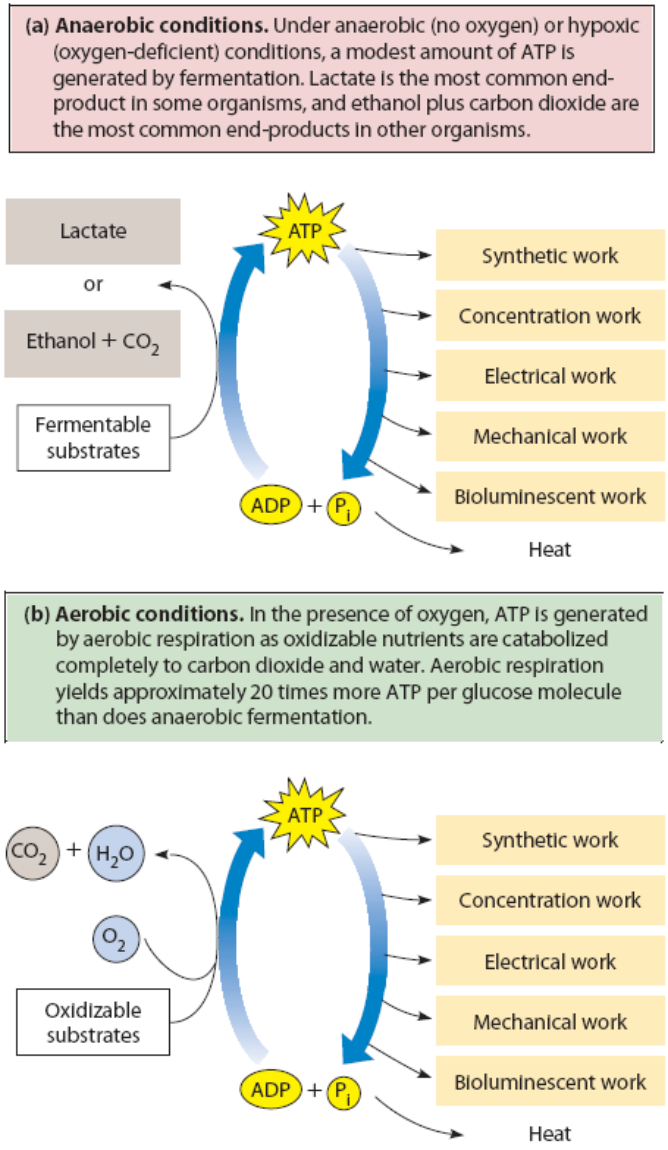
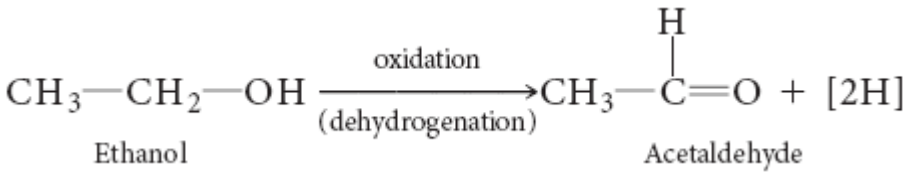


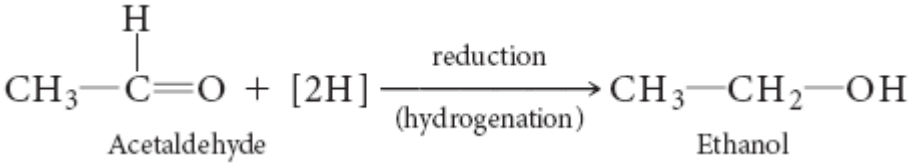
FIGURE 9-4 The ATP/ADP System as a Means of Conserving and Releasing Energy Within the Cell. ATP is generated during (a) anaerobic conditions or (b) aerobic conditions by the oxidative catabolism of nutrients (left side) and is used to do cellular work (right side).

- Electrons are removed, so this is clearly an oxidation. But protons are liberated as well, and an electron plus a proton is the equivalent of a hydrogen atom. Therefore what happens, in effect, is the removal of the equivalent of two hydrogen atoms:

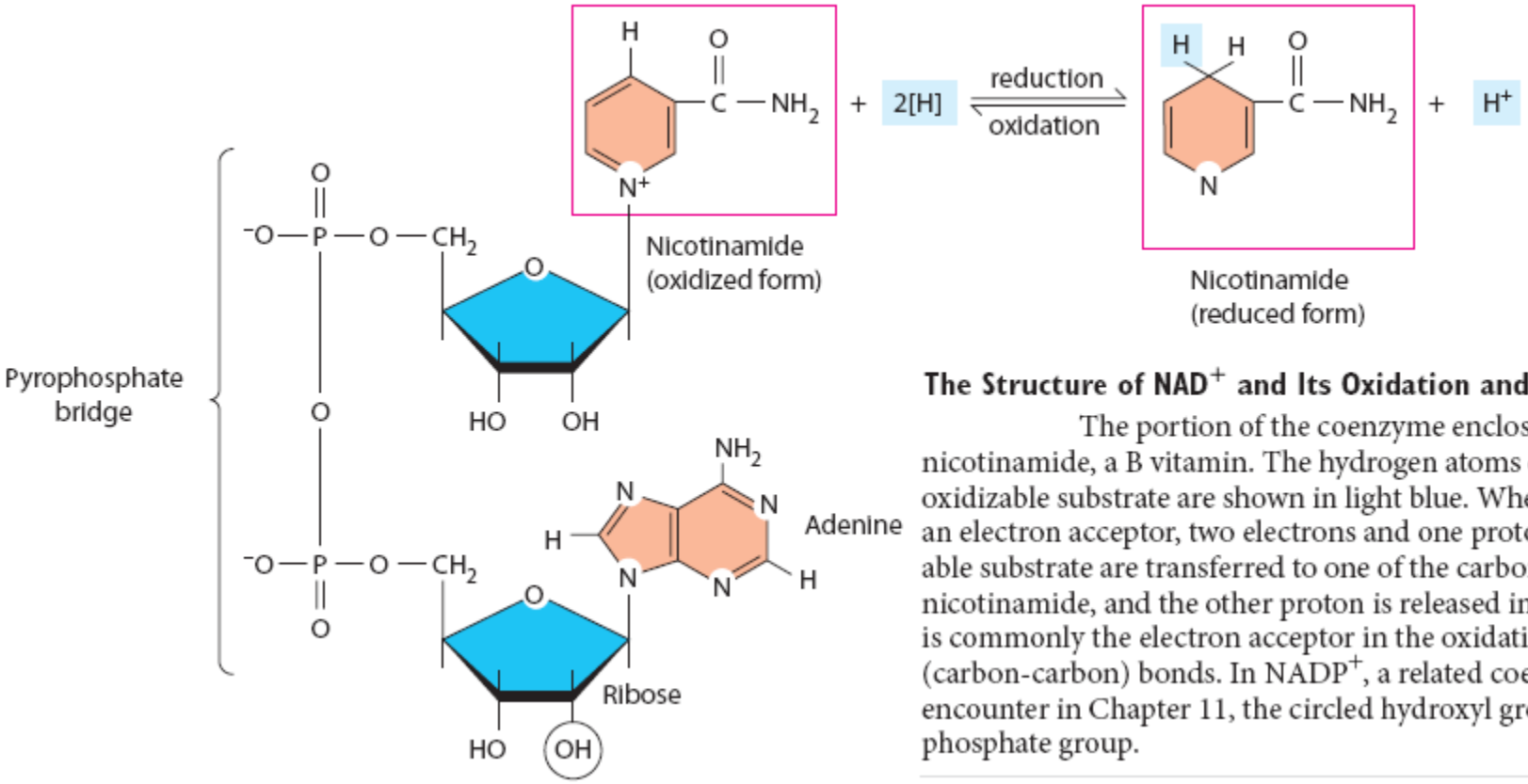


Thus, for cellular reactions involving organic molecules, oxidation is almost always manifested as a dehydrogenation reaction. Many of the enzymes that catalyze oxidative reactions in cells are in fact called dehydrogenases.

- None of the preceding oxidation reactions can take place in isolation, of course; the electrons must be transferred to another molecule, which is reduced in the process. **Reduction**, the opposite of oxidation, is defined as the addition of electrons and is an endergonic process. We will soon see that reduction of coenzymes is an important way that cells store chemical energy as reducing power. In biological reductions, as with oxidations, the electrons transferred are frequently accompanied by protons. The overall reaction is therefore a **hydrogenation:**



Thus, biological oxidation-reduction reactions almost always involve two-electron (and therefore two-proton) transfers.



The Structure of NAD^+ and Its Oxidation and Reduction

The portion of the coenzyme enclosed in the red box is nicotinamide, a B vitamin. The hydrogen atoms derived from an oxidizable substrate are shown in light blue. When NAD^+ is used as an electron acceptor, two electrons and one proton from the oxidizable substrate are transferred to one of the carbon atoms of nicotinamide, and the other proton is released into solution. NAD^+ is commonly the electron acceptor in the oxidation of C—C (carbon-carbon) bonds. In $NADP^+$, a related coenzyme we will encounter in Chapter 11, the circled hydroxyl group is replaced by a phosphate group.

Coenzymes Such as NAD+ Serve as Electron Acceptors in Biological Oxidations

- In most biological oxidations, electrons and hydrogens removed from the substrate being oxidized are transferred to one of several **coenzymes**.
- Coenzymes are small molecules that function along with enzymes, usually by serving as carriers of electrons or small functional groups.
- Coenzymes are not consumed but are recycled within the cell, so the relatively low intracellular concentration of a given coenzyme is adequate to meet the needs of the cell.
- The most common coenzyme involved in energy metabolism is **nicotinamide adenine dinucleotide NAD+.**
- **NAD+** serves as an electron acceptor by adding two electrons and one proton to its aromatic ring, thereby generating the reduced form, NADH, plus a proton:

- As a nutritional note, the nicotinamide of is a
- derivative of niacin, which we recognize as a B vitamin— one of a family of water-soluble compounds essential in the diet of humans and other vertebrates unable to synthesize these compounds for themselves.
- FAD+ and CoA, two other coenzymes, are also vitamin B derivatives.

Glucose Is One of the Most Important Oxidizable Substrates in Energy Metabolism

- Current guidelines recommend a diet of approximately 50% carbohydrate, 30% lipid, and 20% protein. Glucose is therefore an especially important molecule for you personally.

- Glucose is a good potential source of energy because its oxidation is a highly exergonic process, with a $\Delta G^{\circ\prime}$ of -686 kcal/mol for the complete conversion of glucose to carbon dioxide and water using oxygen as *the final electron acceptor*:

- As a thermodynamic parameter, $\Delta G^{\circ\prime}$ is unaffected by the route from substrates to products. Therefore, it will have the same value whether the oxidation is by direct combustion, with all of the energy released as heat, or by biological oxidation, with some of the energy conserved as ATP.

- Thus, oxidation of the sugar molecules in a marshmallow will release the same amount of free energy whether you burn the marshmallow over a campfire or eat it and catabolize the sugar molecules in your body. Biologically, however, the distinction is critical: *Uncontrolled combustion occurs at temperatures that are incompatible with life, and most of the free energy is lost as heat. Biological oxidations involve enzyme-catalyzed reactions that occur without significant temperature changes, and much of the free energy is conserved in chemical form as ATP.*

Glucose Catabolism Yields Much More Energy in the Presence of Oxygen than in Its Absence

- Access to the full 686 kcal/mol of free energy in glucose is possible only if glucose is completely oxidized to carbon dioxide and water.

- The complete oxidation of glucose (or other organic nutrients such as proteins and lipids) to carbon dioxide and water in the presence of oxygen is called aerobic respiration.

- Many organisms, typically bacteria, can carry out anaerobic respiration, using inorganic electron acceptors other than oxygen. Examples of alternative acceptors include elemental sulfur (S), protons (H+) , and ferric ions (Fe3+).

Based on Their Need for Oxygen, Organisms Are Aerobic, Anaerobic, or Facultative

- Organisms can be classified in terms of their need for and use of oxygen as an electron acceptor in energy metabolism.
- Most organisms have an absolute requirement for oxygen and are called **obligate aerobes.**
- On the other hand, some organisms, including many bacteria, cannot use oxygen as an electron acceptor and are called **obligate anaerobes**.
- Most strict anaerobes are bacteria, including organisms responsible for gangrene, food poisoning, and methane production.

- **Facultative** organisms can function under either aerobic or anaerobic conditions. Given the availability of oxygen, most facultative organisms carry out the full aerobic respiratory process. However, they can switch to anaerobic respiration or fermentation if oxygen is limiting or absent.

- Many bacteria and fungi are facultative organisms, as are most molluscs and annelids (worms).

- Some cells or tissues of otherwise aerobic organisms can function in the temporary absence or scarcity of oxygen if required to do so. Your skeletal muscle cells are an example; they normally function aerobically but switch to lactate fermentation whenever the oxygen supply becomes limiting—during periods of prolonged or strenuous exercise, for example.

Glycolysis and Fermentation: ATP Generation Without the Involvement of Oxygen

- The process of **glycolysis**, also called the glycolytic pathway, is a ten-step reaction sequence that converts one molecule of glucose into two molecules of pyruvate, a three-carbon compound.

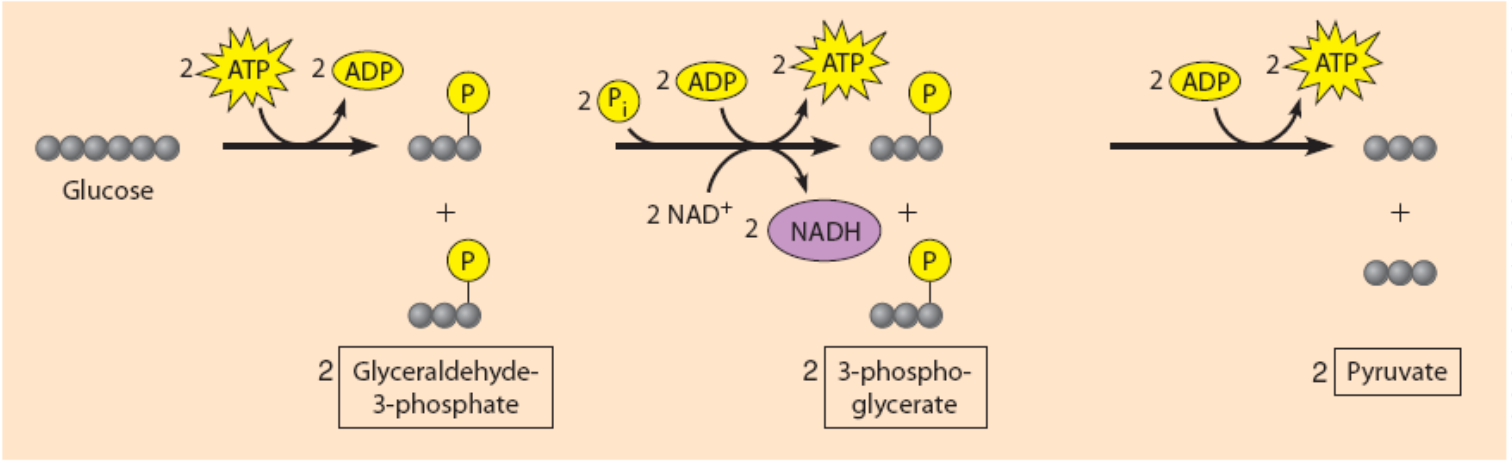
- During the partial oxidation of glucose to pyruvate in glycolysis, energy and reducing power are conserved in the form of ATP and NADH, respectively.

- Glycolysis is common to both aerobic and anaerobic glucose metabolism and is present in virtually all organisms. In most cells, these enzymes occur in the cytosol.

(a) Phase 1: Preparation and cleavage. The six-carbon glucose molecule is phosphorylated twice by ATP and split to form two molecules of glyceraldehyde-3-phosphate. This requires an input of two ATP per glucose.

(b) Phase 2: Oxidation and ATP generation. The two molecules of glyceraldehyde-3-phosphate are oxidized to 3-phosphoglycerate. Some of the energy from this oxidation is conserved as two ATP and two NADH molecules are produced.

(c) Phase 3: Pyruvate formation and ATP generation. The two 3-phosphoglycerate molecules are converted to pyruvate, with accompanying synthesis of two more ATP molecules.

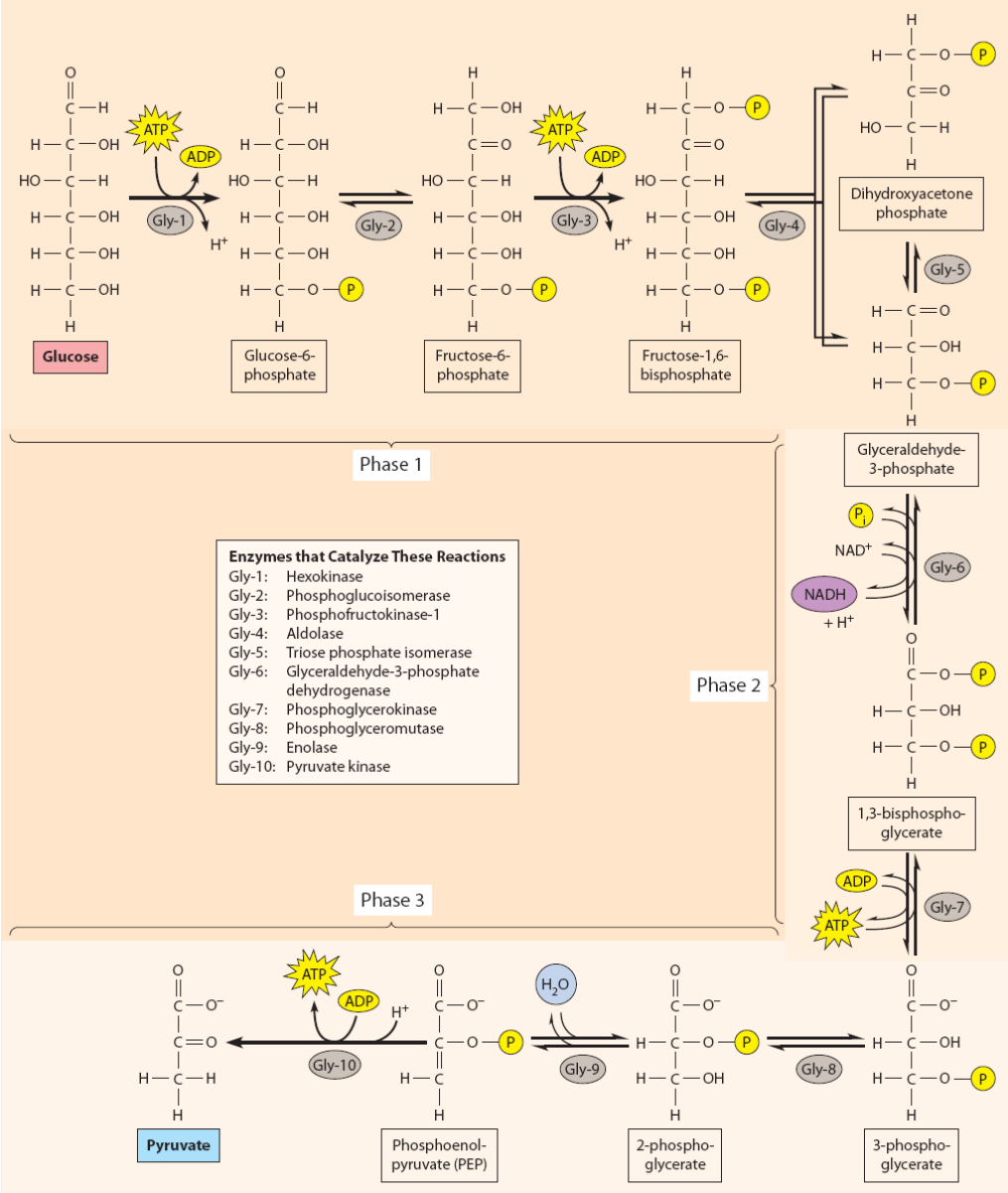


An Overview of the Glycolytic Pathway. During glycolysis, one molecule of glucose is split and partly oxidized, generating two molecules of pyruvate. In the process, energy is conserved as a net gain of two molecules of ATP and two molecules of NADH. This ten-step process occurs in three main phases (a–c), as shown above. Simplified structures show only carbon atoms (gray) and phosphate groups (yellow).

- The glycolytic pathway was the first major metabolic sequence to be elucidated.

- Most of the definitive work was done in the 1930s by the German biochemists Gustav Embden, Otto Meyerhof, and Otto Warburg.

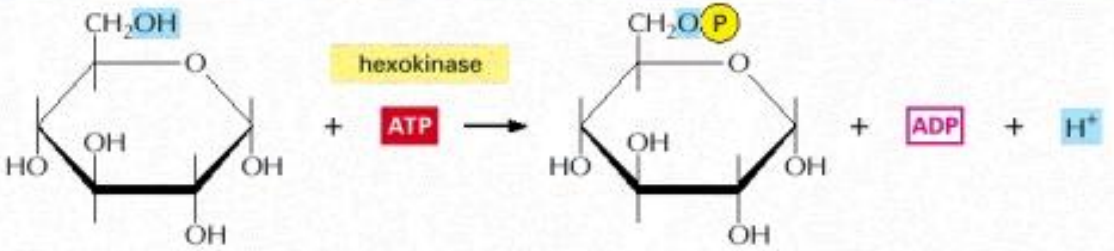
- In fact, an alternative name for the glycolytic pathway is the ***Embden–Meyerhof pathway***.



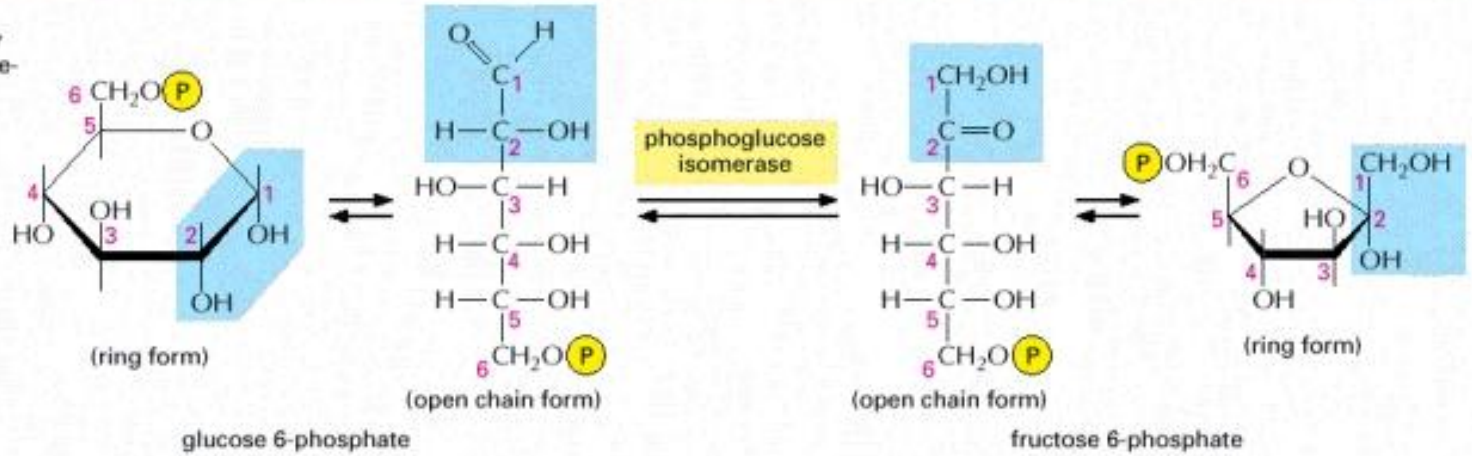
The Glycolytic Pathway from Glucose to Pyruvate. Glycolysis is a sequence of ten reactions in which glucose is catabolized to pyruvate, with a single oxidative reaction (Gly-6) and two ATP-generating steps (Gly-7 and Gly-10). The enzymes that catalyze these reactions are identified in the center box.

For each step, the part of the molecule that undergoes a change is shadowed in blue, and the name of the enzyme that catalyzes the reaction is in a yellow box.

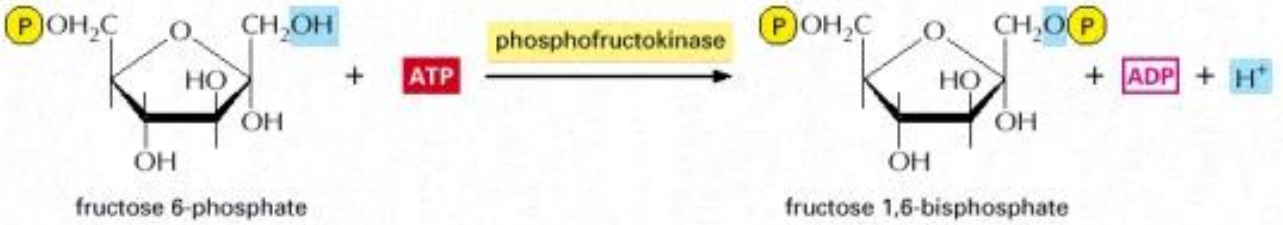
STEP 1 Glucose is phosphorylated by ATP to form a sugar phosphate. The negative charge of the phosphate prevents passage of the sugar phosphate through the plasma membrane, trapping glucose inside the cell.



STEP 2 A readily reversible rearrangement of the chemical structure (isomerization) moves the carbonyl oxygen from carbon 1 to carbon 2, forming a ketose from an aldose sugar. (See Panel 2–4.)



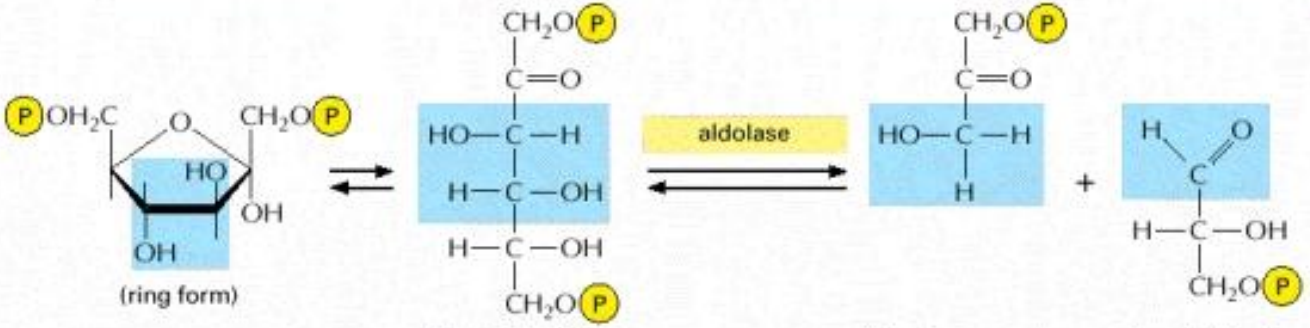
STEP 3 The new hydroxyl group on carbon 1 is phosphorylated by ATP, in preparation for the formation of two three-carbon sugar phosphates. The entry of sugars into glycolysis is controlled at this step, through regulation of the enzyme *phosphofructokinase*.



fructose 6-phosphate

fructose 1,6-bisphosphate

STEP 4 The six-carbon sugar is cleaved to produce two three-carbon molecules. Only the glyceraldehyde 3-phosphate can proceed immediately through glycolysis.



(ring form)

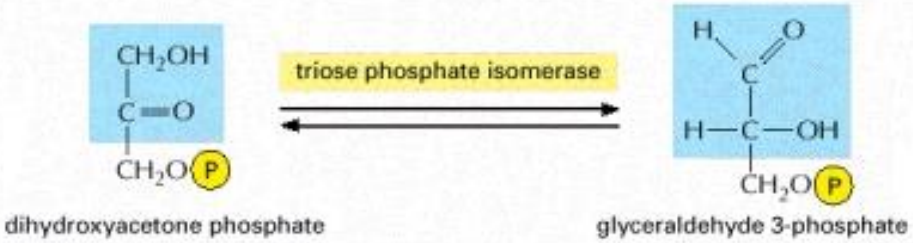
(open chain form)

fructose 1,6-bisphosphate

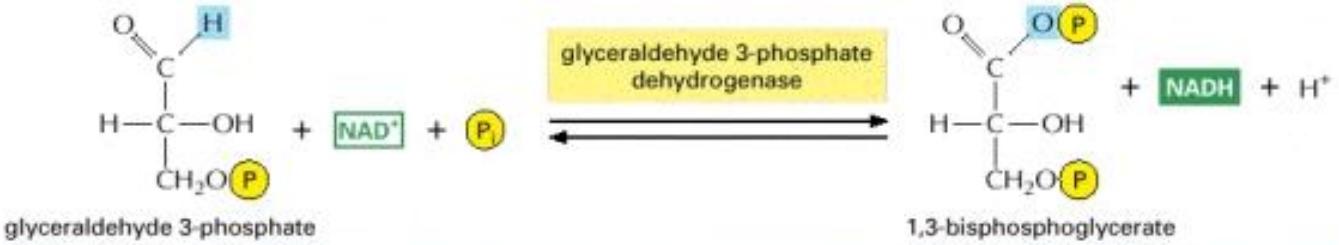
dihydroxyacetone phosphate

glyceraldehyde 3-phosphate

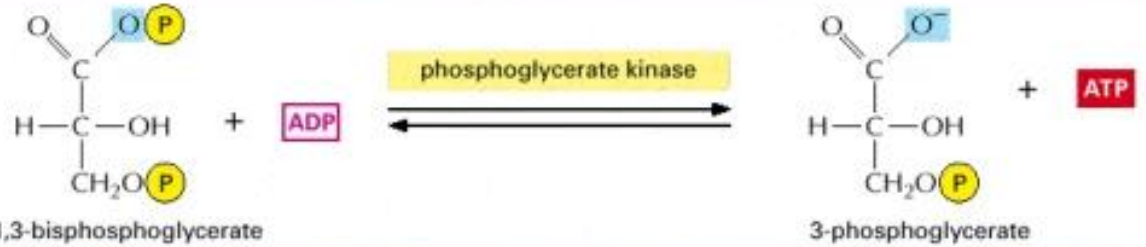
STEP 5 The other product of step 4, dihydroxyacetone phosphate, is isomerized to form glyceraldehyde 3-phosphate.



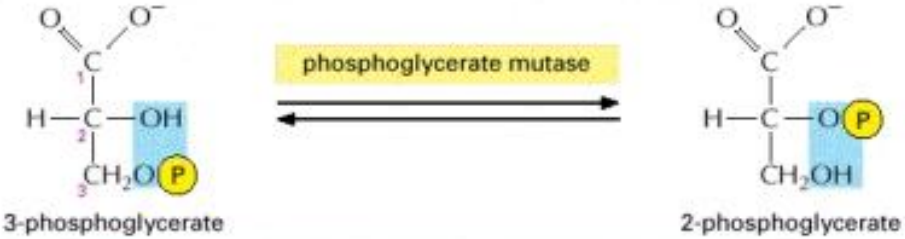
STEP 6 The two molecules of glyceraldehyde 3-phosphate are oxidized. The energy generation phase of glycolysis begins, as NADH and a new high-energy anhydride linkage to phosphate are formed (see Figure 2–73).



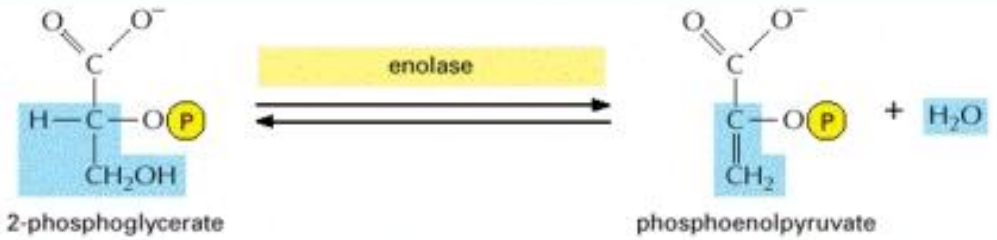
STEP 7 The transfer to ADP of the high-energy phosphate group that was generated in step 6 forms ATP.



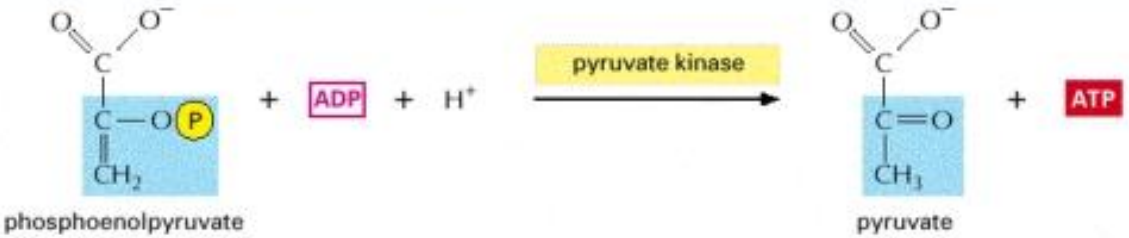
STEP 8 The remaining phosphate ester linkage in 3-phosphoglycerate, which has a relatively low free energy of hydrolysis, is moved from carbon 3 to carbon 2 to form 2-phosphoglycerate.

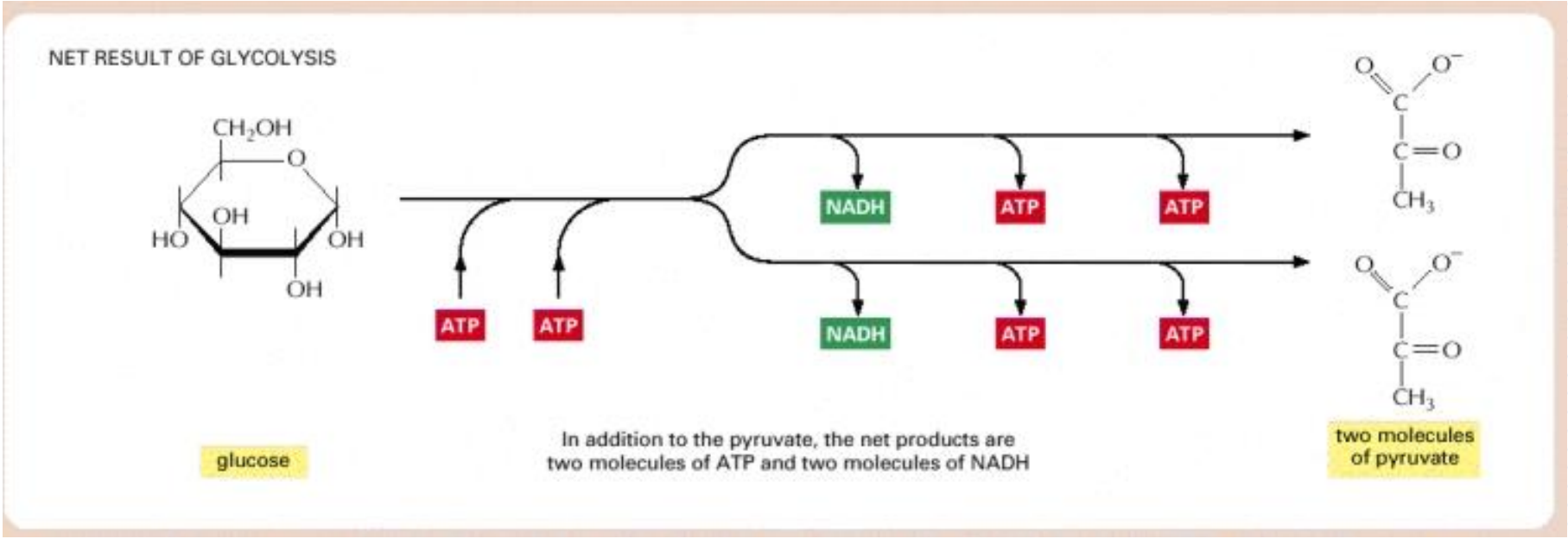


STEP 9 The removal of water from 2-phosphoglycerate creates a high-energy enol phosphate linkage.

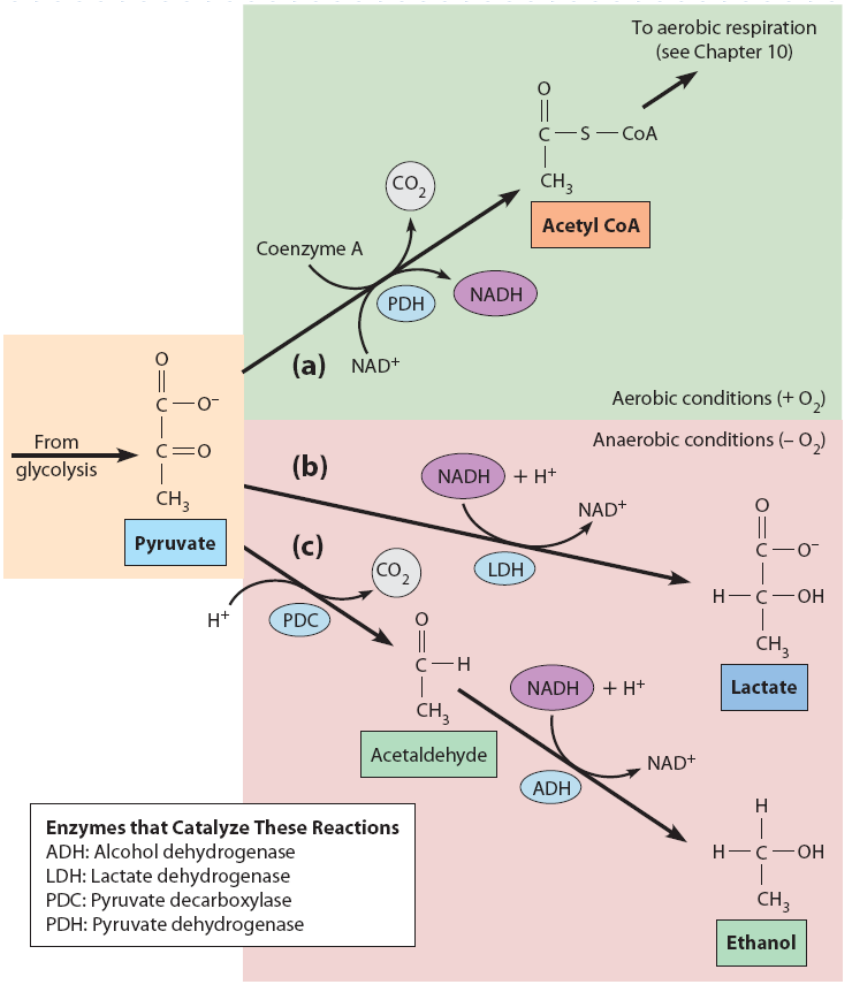


STEP 10 The transfer to ADP of the high-energy phosphate group that was generated in step 9 forms ATP, completing glycolysis.




NET RESULT OF GLYCOLYSIS
CH2OH
O
OH
HO
OH
OH
ATP
ATP
NADH
ATP
ATP
NADH
ATP
ATP
O
O⁻
C
C=O
CH3
glucose
In addition to the pyruvate, the net products are
two molecules of ATP and two molecules of NADH
two molecules
of pyruvate

- In the absence of oxygen, glycolysis leads to fermentation. In the presence of oxygen, glycolysis leads to aerobic respiration.



(a) Aerobic conditions. In the presence of oxygen, many organisms convert pyruvate to an activated form of acetate known as acetyl CoA. In this reaction, pyruvate is both oxidized (with NAD being reduced to NADH) and decarboxylated (liberation of a carbon atom as CO_2). Acetyl CoA then becomes the substrate for aerobic respiration, where NADH is oxidized back to NAD^+ by molecular oxygen (see Chapter 10).

(b) and (c) Anaerobic conditions. When oxygen is absent, pyruvate is reduced so that NADH can be oxidized to NAD, the form of this coenzyme required in Reaction Gly-6 of glycolysis. Common products of pyruvate reduction are **(b)** lactate (in most animal cells and many bacteria) or **(c)** ethanol and CO_2 (in many plant cells and in yeasts and other microorganisms).

The Fate of Pyruvate Under Aerobic and Anaerobic Conditions. The fate of pyruvate depends on the organism involved and on whether oxygen is available. The enzymes that catalyze these reactions are identified in the box.

- Glycolysis can be summurized in three phases:

  Phase 1: Preparation and Cleavage

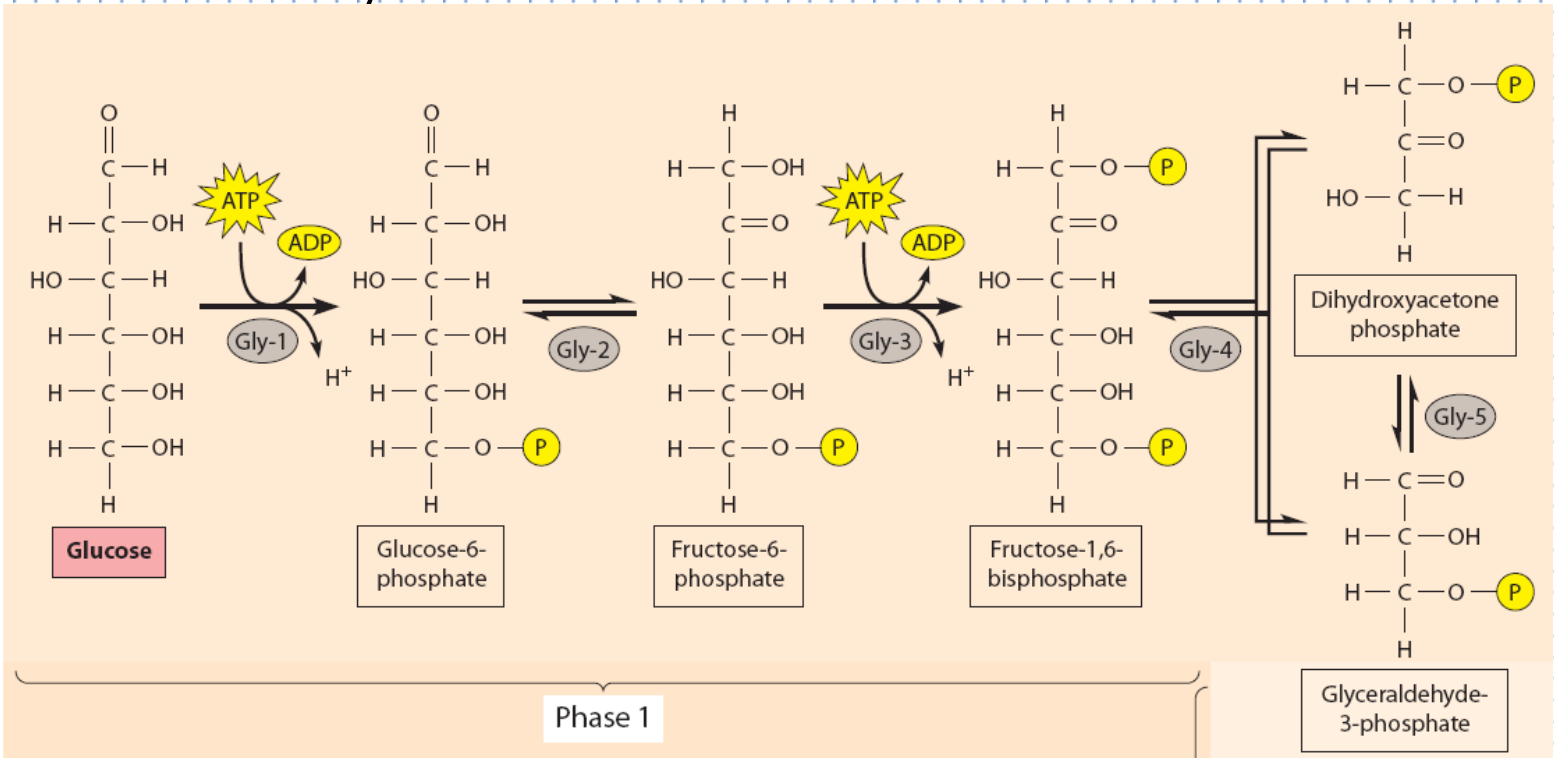
  Phase 2: Oxidation and ATP Generation

  Phase 3: Pyruvate Formation and ATP Generation

Phase 1: Preparation and Cleavage

- The net result of the first three reactions is to convert an unphosphorylated molecule (glucose) into a doubly phosphorylated molecule (*fructose-1,6-bisphosphate*).

- Reactions 1 and 3 are strongly exergonic (ΔG°' >= -4.0 kcal/mol), making them essentially irreversible.

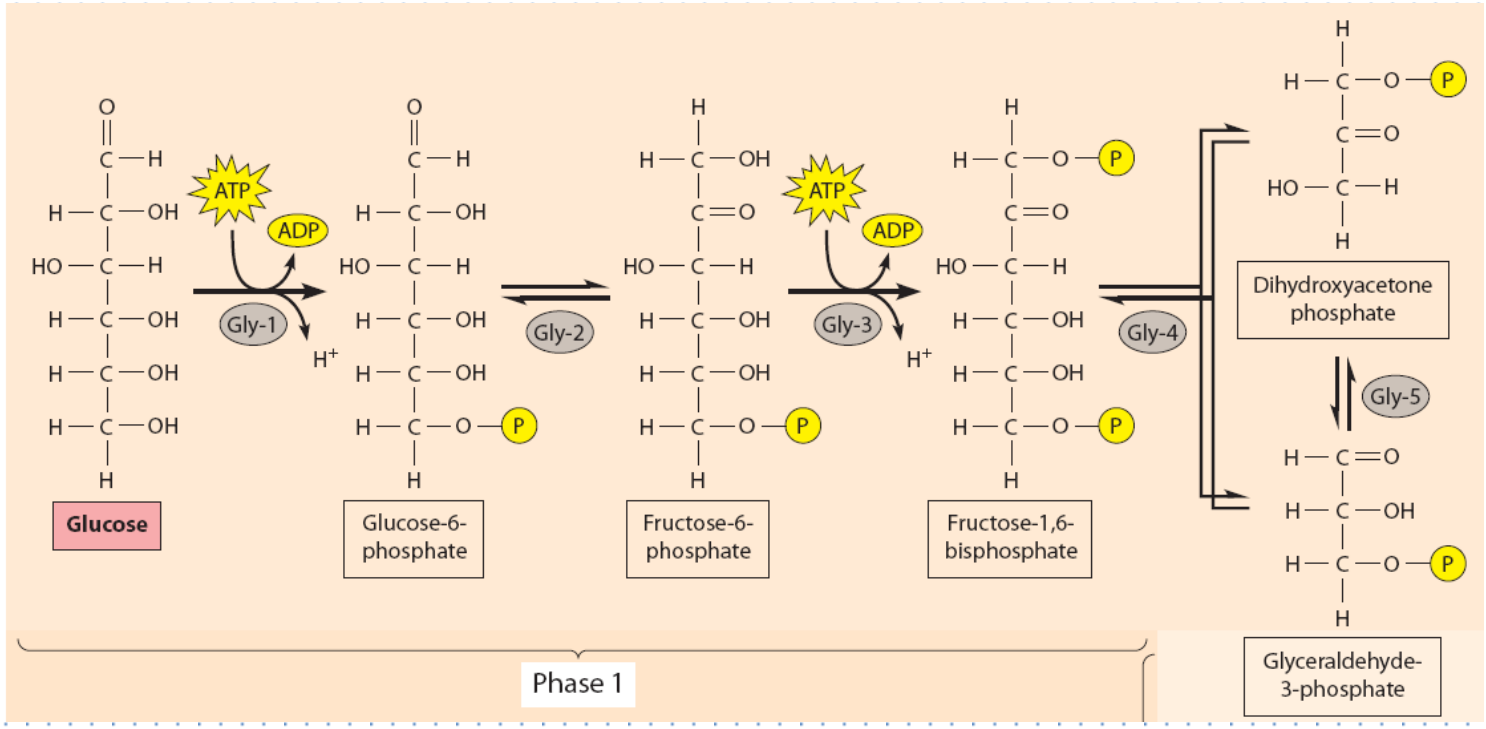


- This reaction 3 is catalyzed by phosphofructokinase-1 (PFK-1), an enzyme that is especially important in the regulation of glycolysis.

- Next, fructose-1,6-bisphosphate is split reversibly by the enzyme aldolase to yield two trioses (three-carbon sugars) called *dihydroxyacetone phosphate* and *glyceraldehyde-3-phosphate*.

- We can summarize this first phase of the glycolytic pathway (Gly-1 to Gly-5) as follows:

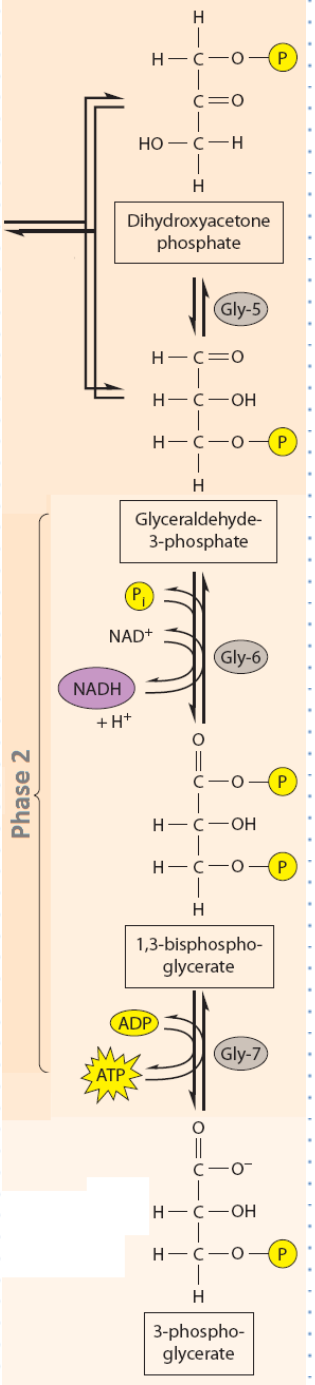
$$\text{glucose} + 2\text{ATP} \longrightarrow 2\ \text{glyceraldehyde-3-phosphate} + 2\text{ADP}$$



Phase 2: Oxidation and ATP Generation

- In this phase, ATP production is linked directly to an oxidative event.
- The oxidation of glyceraldehyde-3-phosphate 3-phosphoglycerate is highly exergonic and drive both the reduction of the coenzyme NAD+ (Gly-6) and the phosphorylation of ADP with inorganic phosphate (Gly-7).
- Historically, this was the first example of a reaction sequence in which the coupling of ATP generation to an oxidative event was understood.
- ATP generation by such direct Pi transfer is called **substrate-level phosphorylation.**
- Keep in mind that each reaction in the glycolytic pathway beyond glyceraldehyde-3-phosphate occurs twice per starting molecule of glucose
- We can summarize this second phase of the glycolytic pathway (Gly-6 to Gly-7) as follows:

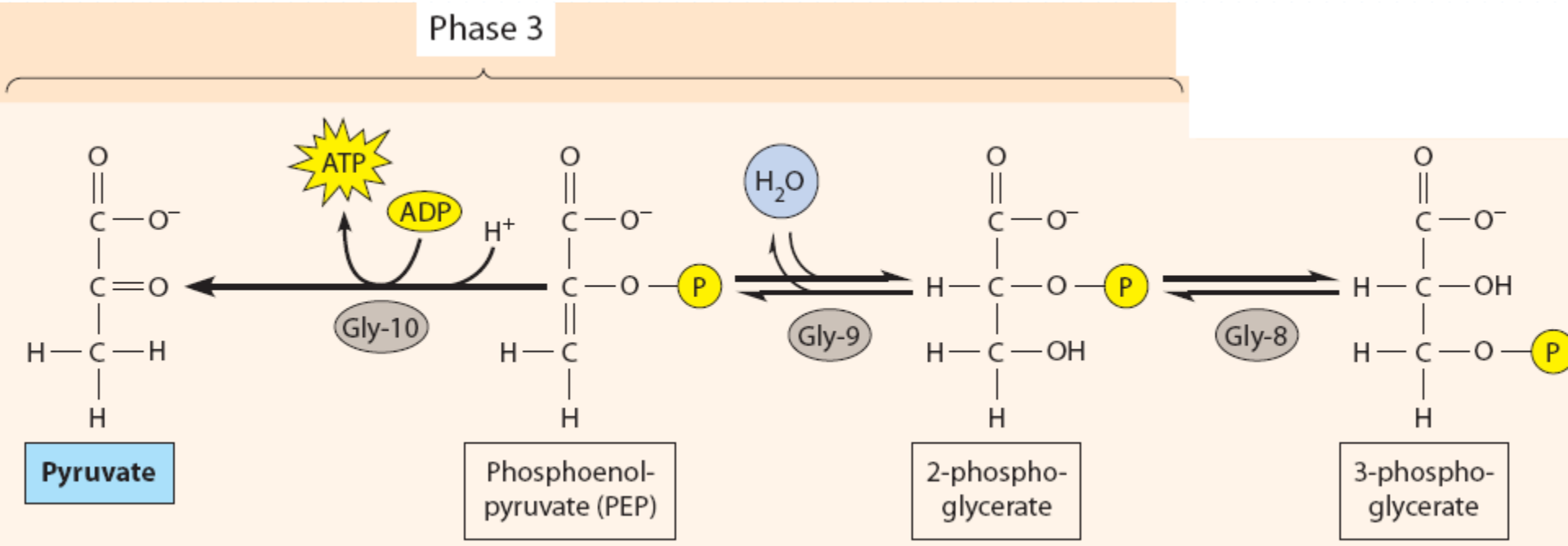
$$\text{glyceraldehyde-3-phosphate} + NAD^+ + ADP + P_i \longrightarrow \text{3-phosphoglycerate} + NADH + H^+ + ATP$$



Phase 3: Pyruvate Formation and ATP Generation

- PEP hydrolysis is exergonic enough to drive ATP synthesis in Reaction Gly-10 at substrate-level hosphorylation.
- This transfer, catalyzed by the enzyme pyruvate kinase, is highly exergonic ($\Delta G^{\circ\prime} = -7.5$ kcal/mol) and is therefore essentially irreversible.
- We can summarize the third phase of glycolysis as:

$$\text{3-phosphoglycerate} + \text{ADP} \longrightarrow \text{pyruvate} + \text{ATP}$$



Summary of Glycolysis

- Two molecules of ATP were initially invested and four were returned

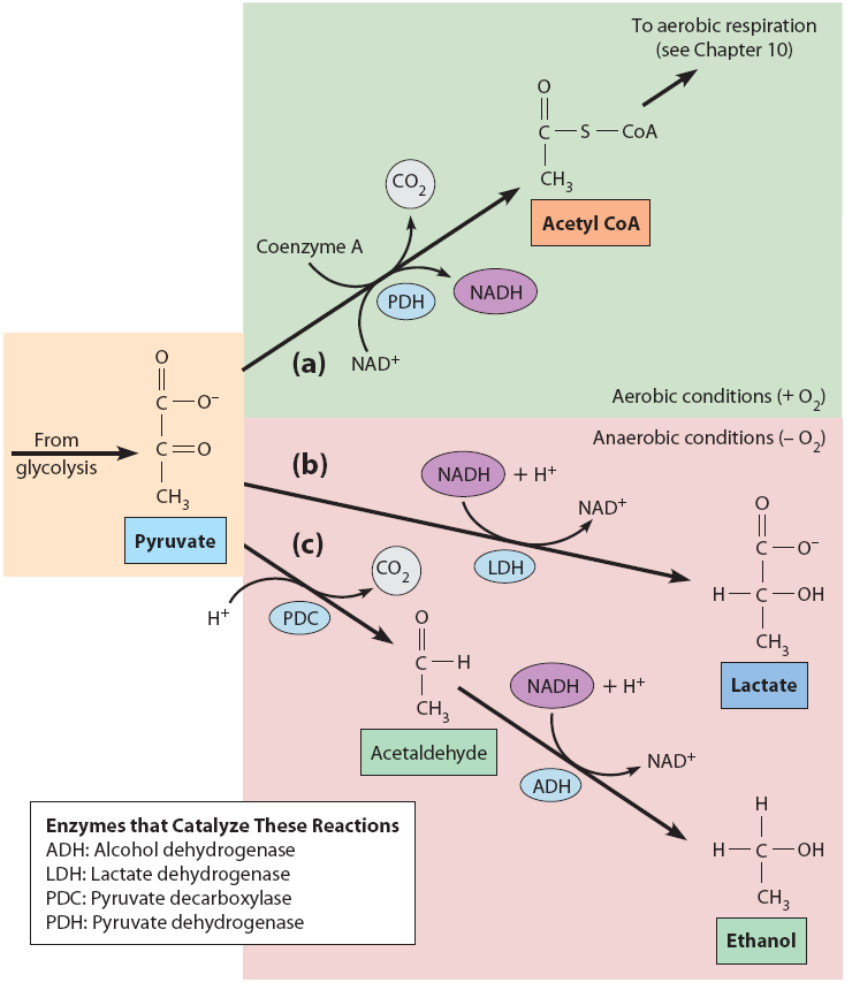
- The result is an overall expression for the pathway from glucose to pyruvate:

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

- This pathway is highly exergonic in the direction of pyruvate formation.

- Under typical intracellular conditions in your body, for example, ΔG' for the overall pathway from glucose to pyruvate with the generation of two molecules each of ATP and NADH is about -20 kcal/mol.

The Fate of Pyruvate Depends on Whether Oxygen Is Available



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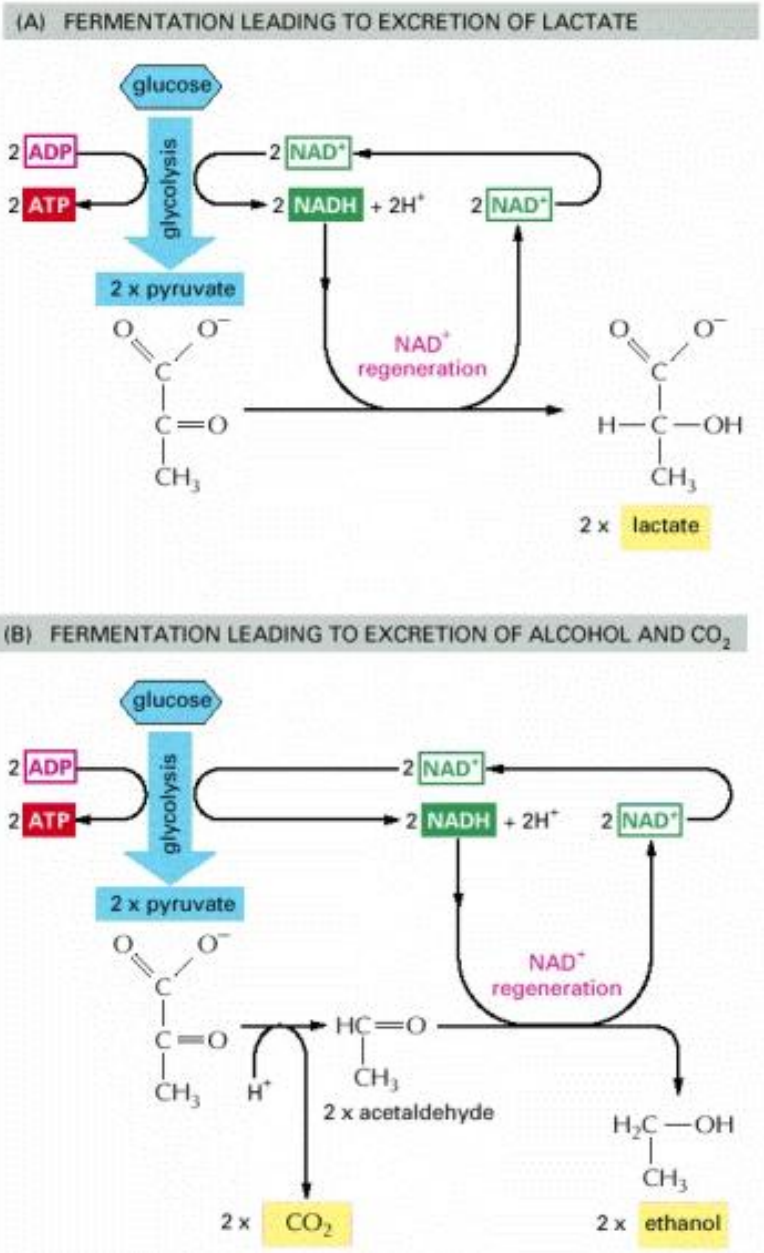
The Fate of Pyruvate Under Aerobic and Anaerobic Conditions. The fate of pyruvate depends on the organism involved and on whether oxygen is available. The enzymes that catalyze these reactions are identified in the box.

In the Absence of Oxygen, Pyruvate Undergoes Fermentation to Regenerate NAD

- An important feature of glycolysis is that it can also take place in the absence of oxygen.
- Under anaerobic conditions, no further oxidation of pyruvate occurs, no acetyl CoA is formed, and no additional ATP can be generated. Instead, the energy need of the cell is met by the modest ATP yield of two ATP per glucose in the glycolytic pathway.
- So, under anaerobic conditions cells must consume glucose much more rapidly in order to maintain steady-state cellular ATP levels.
- In anaerobic conditions, rather than being oxidized, pyruvate is reduced by accepting the electrons (and protons) that must be removed from NADH.

Fermentation

- The two most common pathways for fermentation use pyruvate as the electron acceptor, converting it either to ***lactate*** or to ***CO_2*** and ***ethanol***.


(A) FERMENTATION LEADING TO EXCRETION OF LACTATE
glucose
2 ADP
2 ATP
glycolysis
2 NAD+
2 NADH + 2H+
2 NAD+
2 x pyruvate
NAD+ regeneration
2 x lactate
(B) FERMENTATION LEADING TO EXCRETION OF ALCOHOL AND CO2
glucose
2 ADP
2 ATP
glycolysis
2 NAD+
2 NADH + 2H+
2 NAD+
2 x pyruvate
NAD+ regeneration
2 x acetaldehyde
2 x CO2
2 x ethanol

Lactate Fermentation

- The anaerobic process that terminates in lactate is called **lactate fermentation.**

- lactate is generated by the direct transfer of electrons from NADH to the carbonyl group of pyruvate, reducing it to the hydroxyl group of lactate.

- On a per-glucose basis, this reaction can be represented as:

$$2\ \text{pyruvate} + 2\text{NADH} + 2\text{H}^+ \rightleftharpoons 2\ \text{lactate} + 2\text{NAD}^+$$

- This reaction is readily reversible; in fact, the enzyme that catalyzes it is called *lactate dehydrogenase* because of its ability to catalyze the oxidation, or dehydrogenation, of lactate to pyruvate.

- Lactate fermentation is the major energy-yielding pathway in many anaerobic bacteria, as well as in animal cells operating under anaerobic or hypoxic conditions.

- Lactate fermentation is important to us commercially because the production of cheese, yogurt, and other dairy products depends on microbial fermentation of lactose, the main sugar found in milk.

- A more personal example of lactate fermentation involves your own muscles during periods of strenuous exertion.

- Whenever muscle cells use oxygen faster than it can be supplied by the circulatory system, the cells become temporarily hypoxic. Pyruvate is then reduced to lactate instead of being further oxidized, as it is under aerobic conditions.

- The lactate produced in this way is transported by the circulatory system from the muscle to the liver. There it is converted to glucose again by the process of *gluconeogenesis*.

Alcoholic Fermentation

- Under anaerobic conditions, plant cells can carry out **alcoholic fermentation** (in waterlogged roots, for example), as do yeasts and other microorganisms.
- In this process, pyruvate loses a carbon atom (as CO_2) to form the two-carbon compound acetaldehyde.
- Acetaldehyde reduction by NADH gives rise to ethanol, the alcohol for which the process is named.
- This reductive sequence is catalyzed by two enzymes, pyruvate decarboxylase and alcohol dehydrogenase.
- The overall reaction can be summarized as follows:

$$2\ \text{pyruvate} + 2\text{NADH} + 4\ \text{H}^+ \longrightarrow 2\ \text{ethanol} + 2\text{CO}_2 + 2\text{NAD}^+$$

- Alcoholic fermentation by yeast cells is a key process in the baking, brewing, and winemaking industries.

- The yeast cells in bread dough break down glucose anaerobically, generating both CO_2 and ethanol.

- Carbon dioxide is trapped in the dough, causing it to rise, and the alcohol is driven off during baking and becomes part of the pleasant aroma of baking bread.

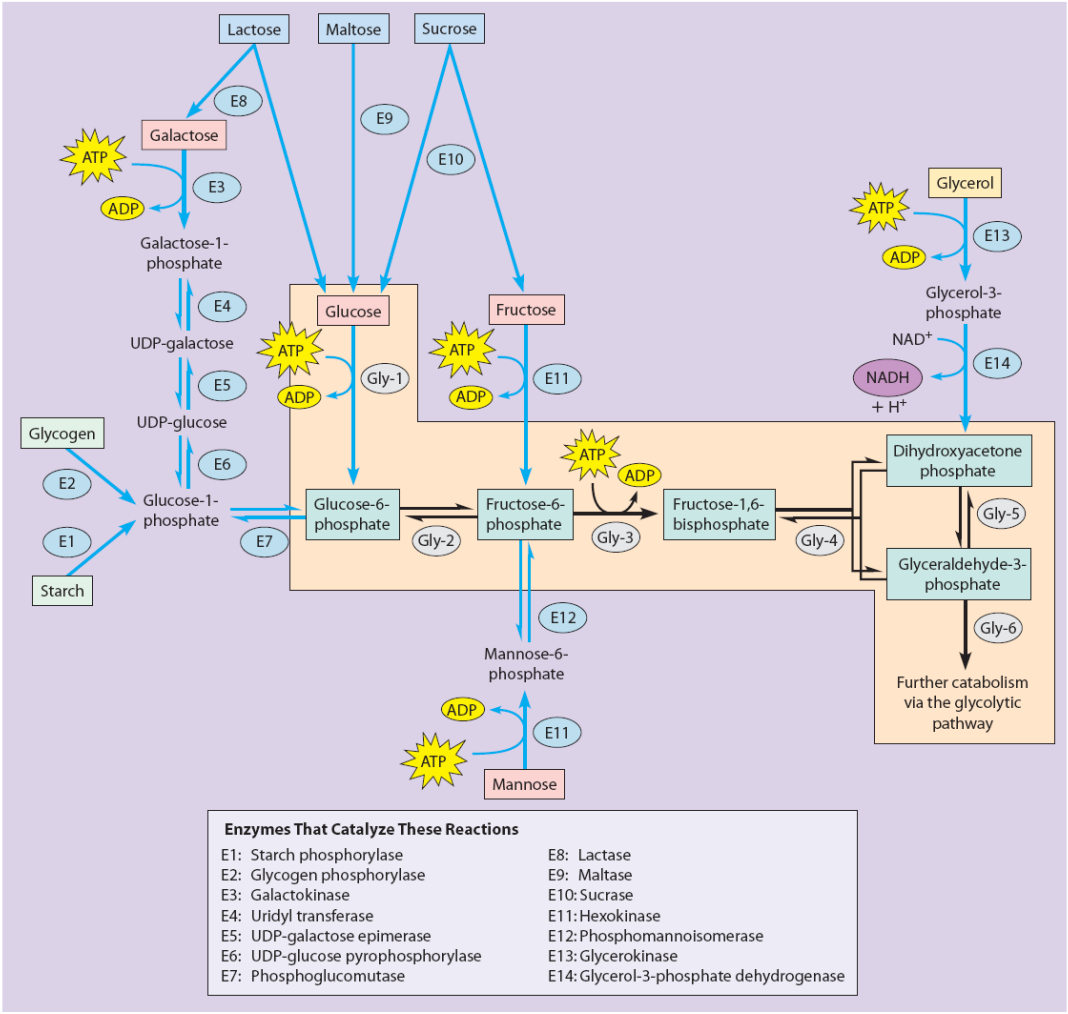
- For the brewer, both CO_2 and ethanol are essential; ethanol makes the product an alcoholic beverage, and CO_2 accounts for the carbonation.

Other Fermentation Pathways

- Many are only restricted to certain bacteria.

- Examples:
  - propionate fermentation
  - butylene glycol fermentation

Other Sugars and Glycerol Are Also Catabolized by the Glycolytic Pathway



Carbohydrate Catabolism by the Glycolytic Pathway. Carbohydrate substrates that can be metabolized by conversion to an intermediate in the glycolytic pathway are enclosed in colored boxes. These include the hexoses galactose, glucose, fructose, and mannose; the disaccharides lactose, maltose, and sucrose; the polysaccharides glycogen and starch; and the three-carbon compound glycerol. The conversion reactions are shown by blue arrows. The enzymes that catalyze these reactions are identified in the box at the bottom. The first six reactions of the glycolytic pathway are highlighted in tan; for the names of the enzymes that catalyze these reactions, see Figure 9-7. In some cases, other enzymes or reaction sequences may be involved, depending on the organism and tissue.

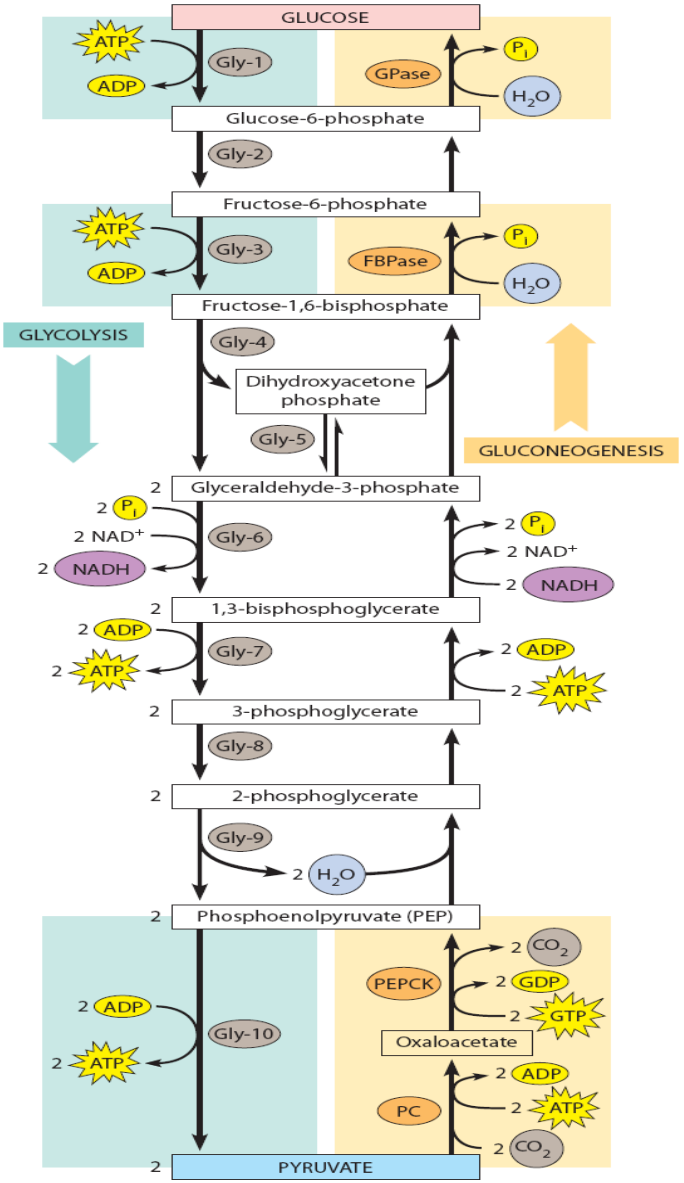
Gluconeogenesis

- Cells are able to catabolize glucose and other carbohydrates to meet their energy needs, and they can synthesize sugars and polysaccharides needed for other purposes.

- The process of glucose synthesis is called **gluconeogenesis**.

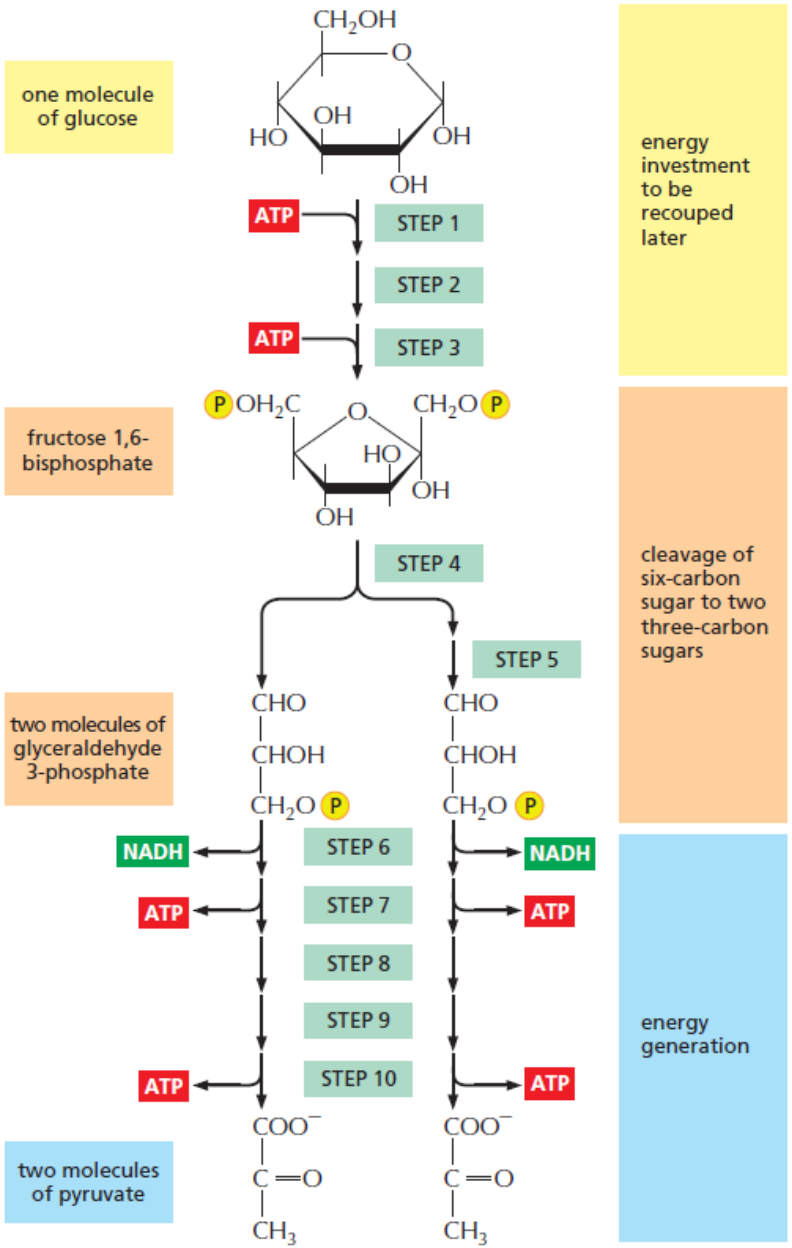
Pathways for Glycolysis and Gluconeogenesis Compared.

The pathways for glycolysis (left) and gluconeogenesis (right) have nine intermediates and seven enzyme-catalyzed reactions in common. The three essentially irreversible reactions of the glycolytic pathway (in green shading) are circumvented in gluconeogenesis by four bypass reactions (in yellow shading). Gluconeogenesis, on the other hand, is an anabolic pathway, requiring the coupled hydrolysis of six phosphoanhydride bonds (four from ATP, two from GTP) to drive it in the direction of glucose formation. The enzymes that catalyze the bypass reactions are shown in gold and are identified in the box. In animals, glycolysis occurs in muscle and various other tissues, whereas gluconeogenesis occurs mainly in the liver and to a lesser degree in the kidneys.

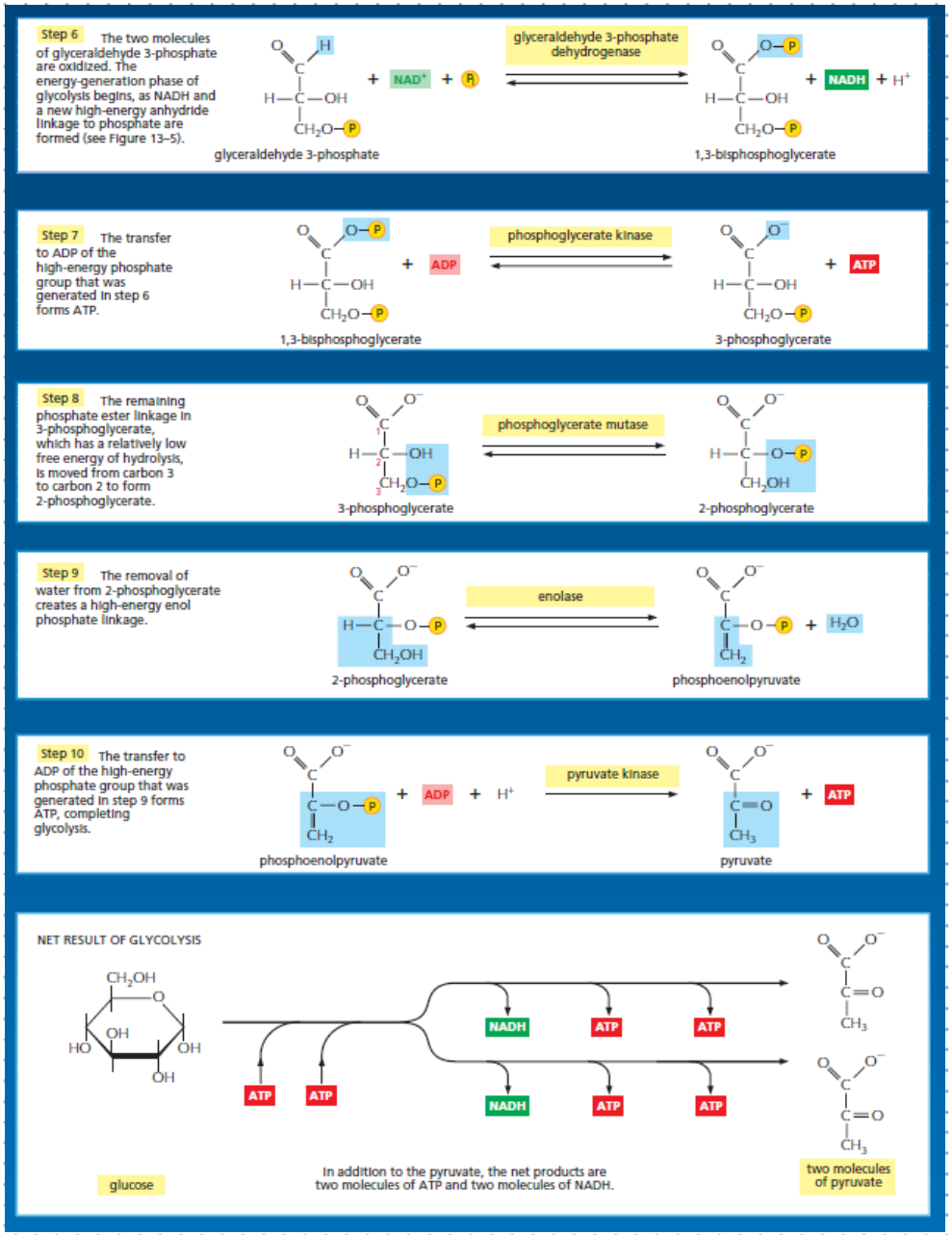


Enzymes That Catalyze the Bypass Reactions of Gluconeogenesis

PC:	Pyruvate carboxylase
PEPCK:	Phosphoenolpyruvate carboxykinase
FBPase:	Fructose-1,6-bisphosphatase
GPase:	Glucose-6-phosphatase

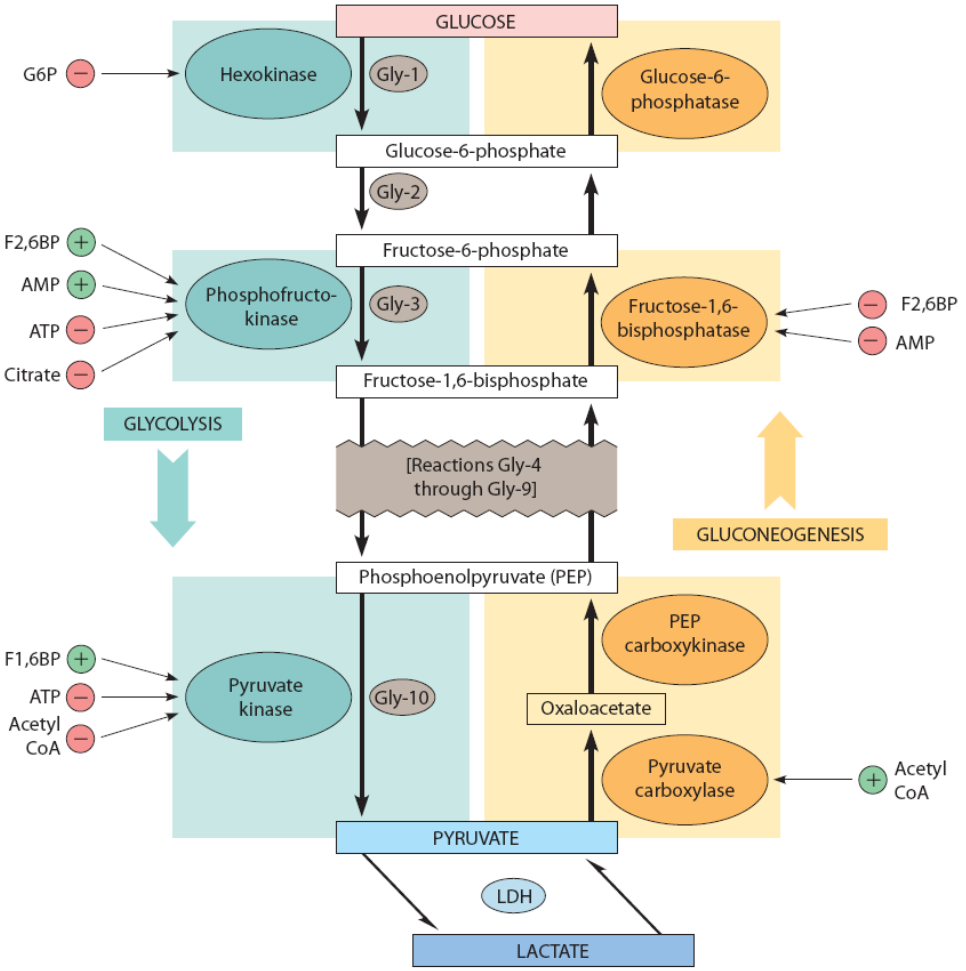


An outline of glycolysis. Each of the 10 steps shown is catalyzed by a different enzyme. *Note that step 4 cleaves a six-carbon sugar into two threecarbon sugars, so that the number of molecules at every stage after this doubles. As indicated, step 6 begins the energy generation phase of glycolysis. Because two molecules of ATP are hydrolyzed in the early, energy-investment phase, glycolysis results in the net synthesis of 2 ATP and 2 NADH molecules per molecule of glucose*



Glycolysis involves a sequence of 10 separate reactions, each producing a different sugar intermediate and each catalyzed by a different enzyme. Like most enzymes, these have names ending in ase—such as *isomerase* and *dehydrogenase*—to indicate the type of reaction they catalyze.Although no molecular oxygen is used in glycolysis, oxidation occurs, in that electrons are removed by NAD+ (producing NADH) from some of the carbons derived from the glucose molecule. The stepwise nature of the process releases the energy of oxidation in small packets, so that much of it can be stored in activated carrier molecules rather than all of it being released as heat. Thus, some of the energy released by oxidation drives the direct synthesis of ATP molecules from ADP and Pi, and some remains with the electrons in the electron carrier NADH. Two molecules of NADH are formed per molecule of glucose in the course of glycolysis. In aerobic organisms, these NADH molecules donate their electrons to the electron-transport chain, and the NAD+ formed from the NADH is used again for glycolysis.

The Regulation of Glycolysis and Gluconeogenesis



The Regulation of Glycolysis and Gluconeogenesis. Glycolysis and gluconeogenesis are regulated in a reciprocal manner. In both cases, regulation involves allosteric activation (+) or inhibition (−) of enzymes that catalyze reactions unique to the pathway. For glycolysis, the key regulatory enzymes are those that catalyze the three irreversible reactions unique to this pathway (green). For gluconeogenesis, two of the four bypass enzymes (gold) that are unique to this pathway are the main sites of allosteric regulation. Allosteric regulators include acetyl CoA, AMP, ATP, citrate, fructose-1,6-bisphosphate (F1,6BP), fructose-2,6-bisphosphate (F2,6BP), and glucose-6-phosphate (G6P). Acetyl CoA and citrate are intermediates in aerobic respiration. F2,6BP is synthesized by phosphofructokinase-2 (PFK-2).

MBG304 Biochemistry

Lecture 3: Krebs Cycle

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Aerobic respiration

- In the previous chapter, we learned that some cells meet their energy needs by anaerobic fermentation, either because they are strict anaerobes or because they are facultative cells functioning temporarily in the absence or scarcity of oxygen.

- However, we also noted that fermentation yields only modest amounts of energy due to the absence of an external electron acceptor. The electrons that are removed from glucose as it is partially oxidized during fermentation are transferred to pyruvate, and only two molecules of ATP can be generated per molecule of glucose.

- In addition, fermentation often results in the accumulation of waste products such as ethanol or lactate, which can be toxic to the cells if they accumulate.

Cellular Respiration: Maximizing ATP Yields

- With an *external* electron acceptor available, complete oxidation of substrates to CO_2 becomes possible, and ATP yields are much higher.

- *Cellular respiration is the flow of electrons, through or within a membrane, from reduced coenzymes to an external electron acceptor, usually accompanied by the generation of ATP.*

- For many organisms, including us, the terminal electron acceptor is *oxygen*, the reduced form of this terminal electron acceptor is *water*, and the overall process is called **aerobic respiration.**

- A variety of terminal electron acceptors other than molecular oxygen can be used by other organisms, especially bacteria and archaea.

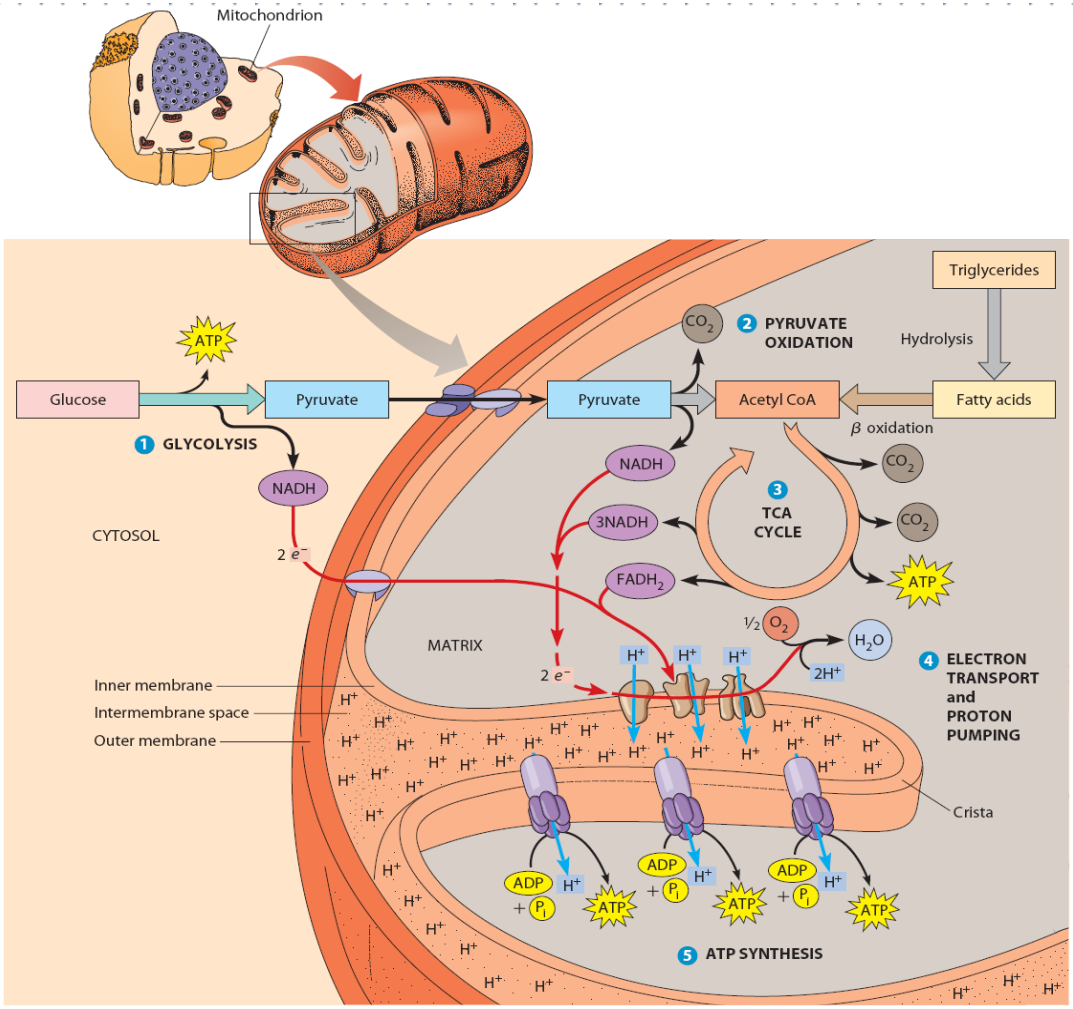
- Examples of alternative acceptors include sulfur , protons, and ferric ions .

- Respiratory processes that involve electron acceptors such as these require no molecular oxygen and are therefore examples of **anaerobic respiration.**

- We will focus much of our attention on the mitochondrion.

- Because, most aerobic ATP production in eukaryotic cells takes place within this organelle.

- With oxygen available as the terminal electron acceptor, pyruvate can be oxidized completely to CO_2 instead of being used to accept electrons from NADH.



The Role of the Mitochondrion in Aerobic Respiration. The mitochondrion plays a central role in aerobic respiration. Most respiratory ATP production in eukaryotic cells occurs in this organelle. Oxidation of glucose and other sugars begins in the cytosol with glycolysis (stage 1), producing pyruvate. Pyruvate is transported across the inner mitochondrial membrane and is oxidized within the matrix to acetyl CoA (stage 2), the primary substrate of the tricarboxylic acid (TCA) cycle (stage 3). Acetyl CoA can also be formed by β oxidation of fatty acids. Electron transport is coupled to proton pumping (stage 4), with the energy of electron transport conserved as an electrochemical proton gradient across the inner membrane of the mitochondrion (or across the plasma membrane, in the case of prokaryotes). The energy of the proton gradient is used in part to drive the synthesis of ATP from ADP and inorganic phosphate (stage 5).

Respiration Includes Glycolysis, Pyruvate Oxidation, the TCA Cycle, Electron Transport, and ATP Synthesis

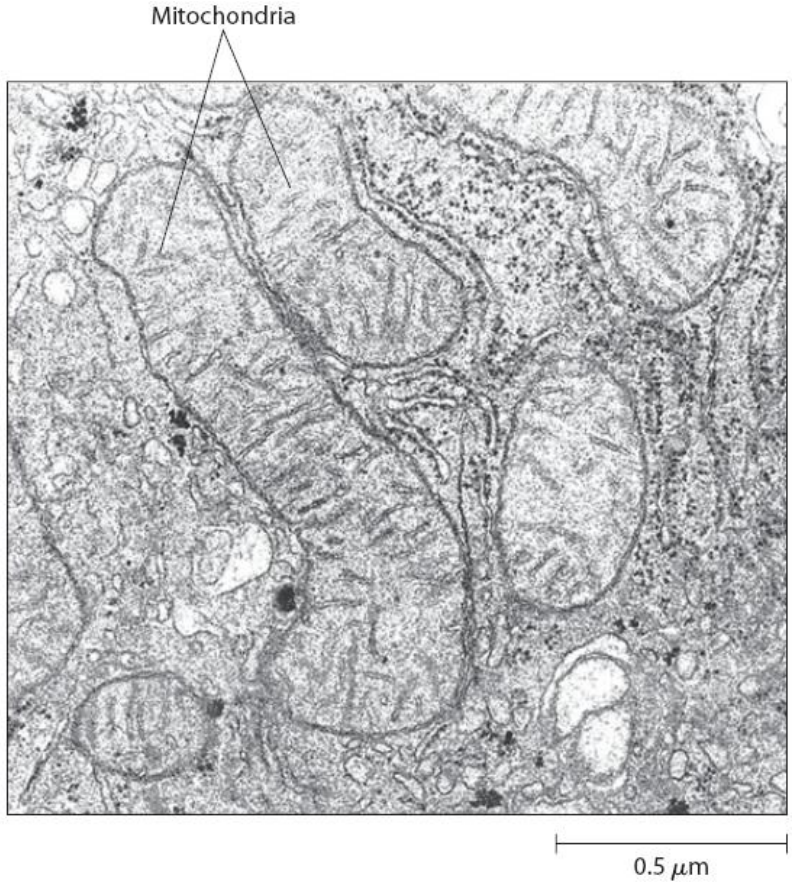
- We will consider aerobic respiration in five stages:
  1. Glycolytic pathway (glucose to pyruvate)
  2. Pyruvate dehydrogense reaction (pyruvate is oxidized to acetyl CoA)
  3. Krebs Cycle (acetyl CoA is oxidized to CO_2 and H_2O)
  4. Electron transport (NADH and $FADH_2$ formed in first 3 stages are channeled into electron transport chain)
  5. Oxidative phosphorylation (an electrochemical proton gradient is formed across the membrane during stage 4 and the energy of this proton gradient is used to drive ATP synthesis (i.e., oxidative phosphorylation).

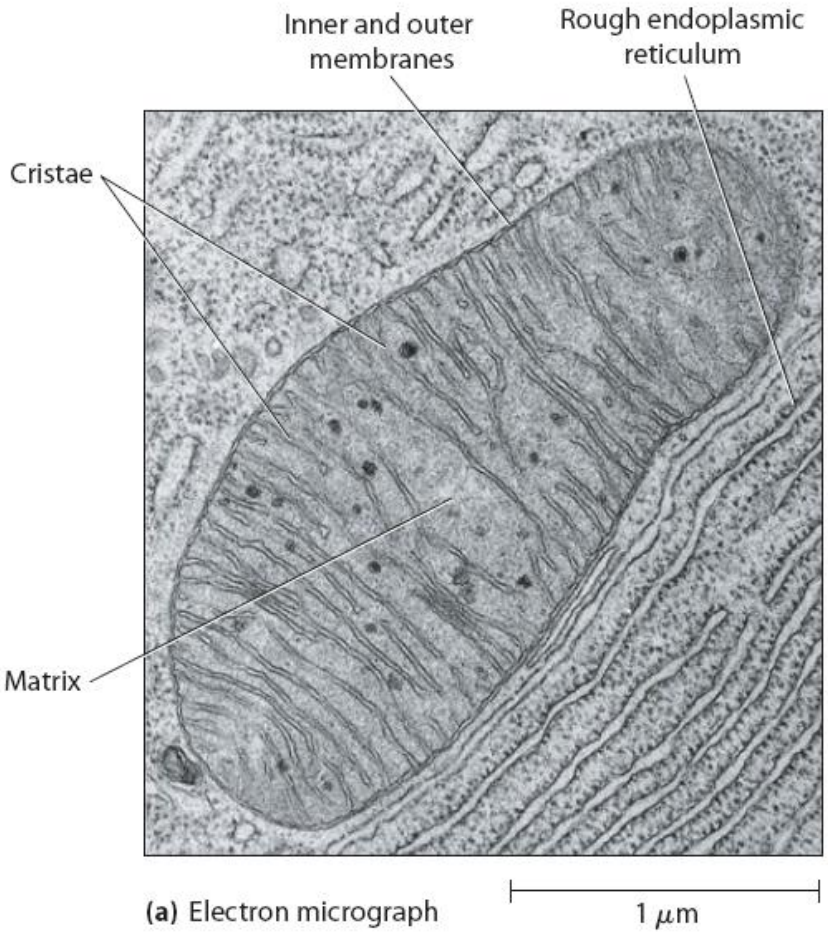
The Mitochondrion: Where the Action Takes Place

- The mitochondrion is often called the "*energy powerhouse*" of the eukaryotic cell.

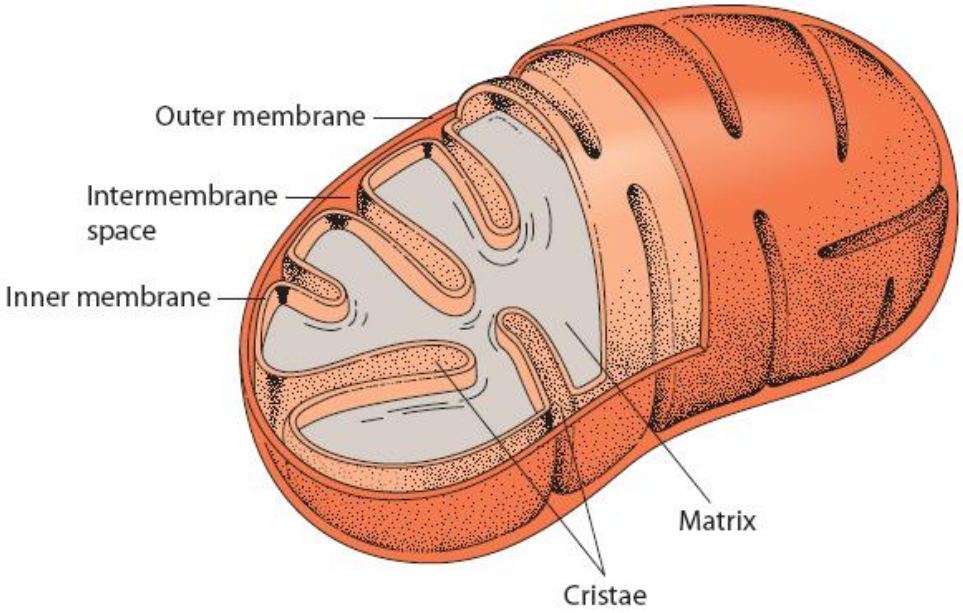
- The mitochondrion is capable of carrying out all the reactions of the TCAcycle, electron transport, and oxidative phosphorylation.

- Mitochondria are present in both chemotrophic and phototrophic cells.





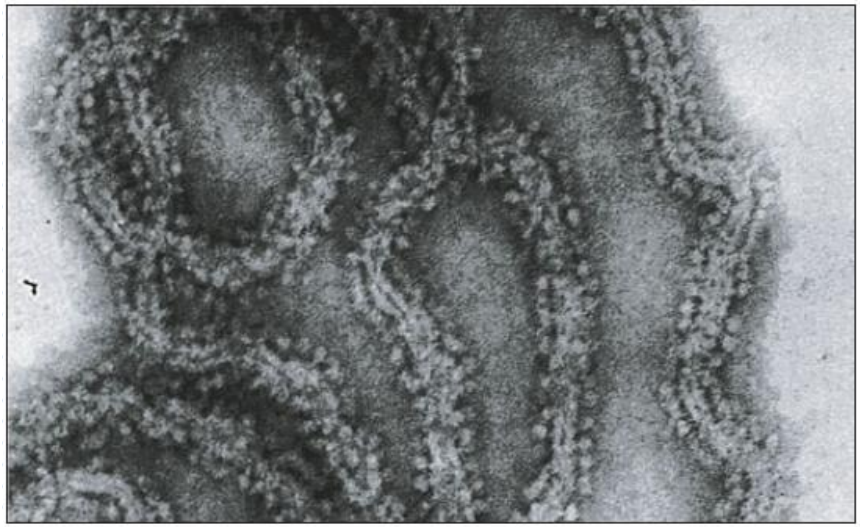
(a) Electron micrograph



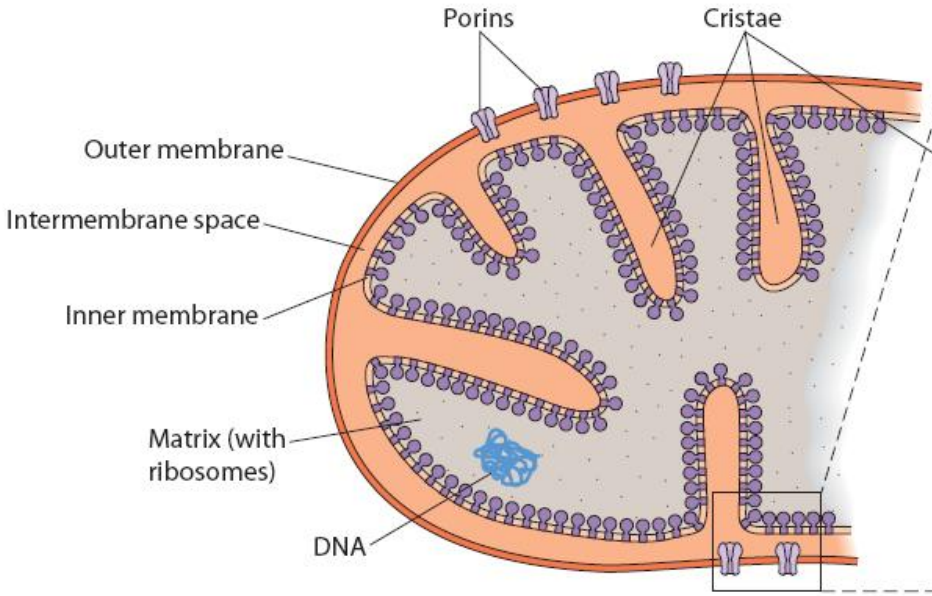
(b) Schematic diagram

Mitochondrial Structure. (a) A mitochondrion of a bat pancreas cell as seen by electron microscopy (TEM). The cristae are formed by infoldings of the inner membrane. (b) A mitochondrion is illustrated schematically in this cutaway view that shows the traditional "baffle" model of cristae structure. As noted in the text, however, this model is currently being reconsidered. Recent analysis using EM tomography suggests that the connections between the intracristal spaces and the intermembrane space are more limited tubular openings known as crista junctions.

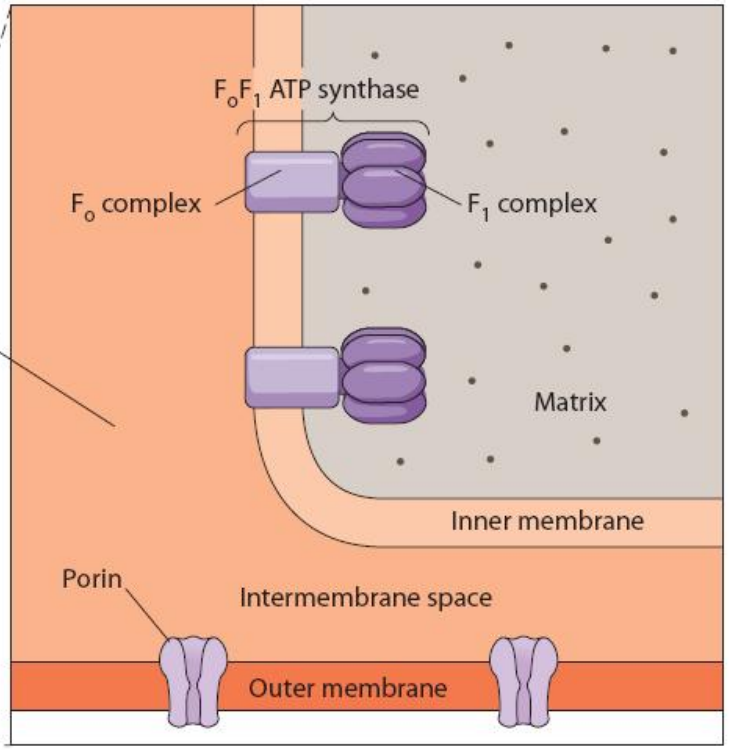
The F_1 and F_o Complexes of the Inner Mitochondrial Membrane. (a) This electron micrograph was prepared by negative staining to show the spherical F_1 complexes that line the matrix side of the inner membrane of a bovine heart mitochondrion (TEM). (b) Cross section of a mitochondrion, showing major structural features. (c) An enlargement of a small portion of a crista, showing the F_1 complexes that project from the inner membrane on the matrix side and the F_o complexes embedded in the inner membrane. Each F_1 complex is attached to an F_o complex by a short protein stalk. Together, an F_oF_1 pair constitutes a functional ATP synthase.



(a) Mitochondrial inner membrane



(b) Cross-sectional diagram of a mitochondrion



(c) Cross-sectional diagram of a portion of a crista showing F_oF_1 complexes

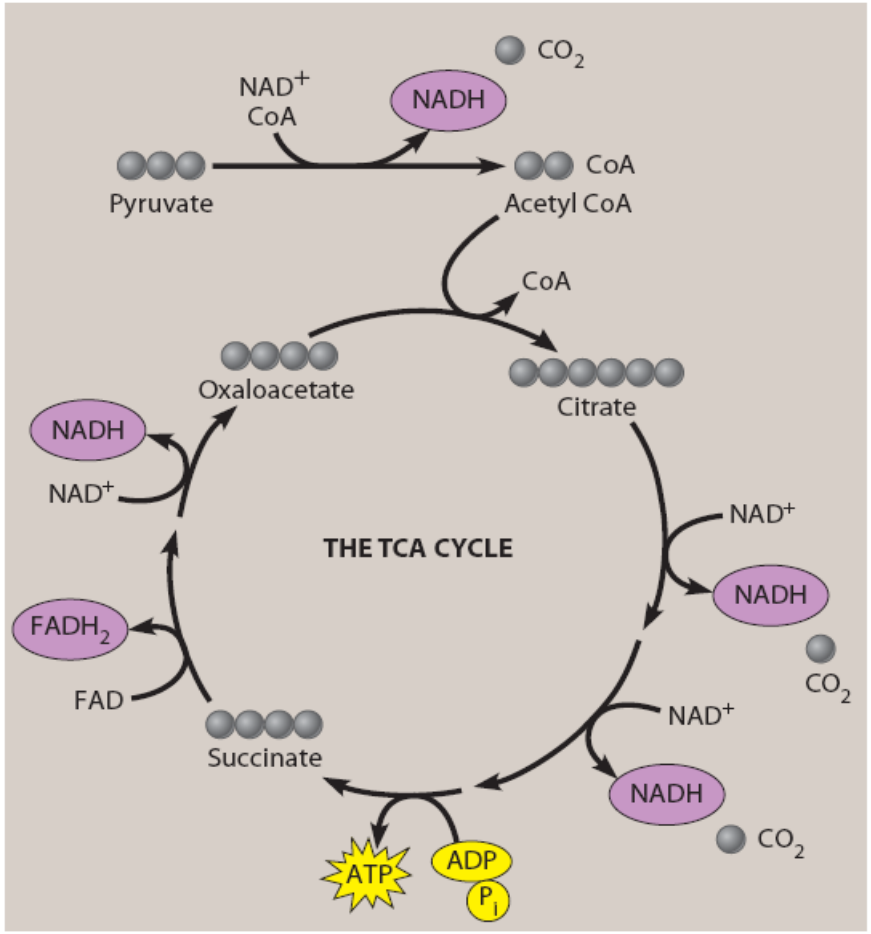
The Tricarboxylic Acid Cycle

- An important intermediate in this cyclic series of reactions is *citrate*, which has three carboxylic acid groups and is therefore a tricarboxylic acid. For this reason, this pathway is usually called the **tricarboxylic acid (TCA) cycle.**

- The TCA cycle metabolizes acetyl CoA, a compound produced from pyruvate decarboxylation.

- Acetyl CoA consists of a two-carbon acetate group from pyruvate linked to a carrier called coenzyme A.

- Acetyl CoA arises either by oxidative decarboxylation of pyruvate or by the stepwise oxidative breakdown of fatty acids.



Overview of the TCA Cycle. Pyruvate from glycolysis is oxidatively decarboxylated to acetyl CoA, generating NADH and CO_2. Acetyl CoA enters the TCA cycle by combining with oxaloacetate to form citrate. Two molecules of CO_2 are released and 2 NADH are formed as citrate is converted to succinate by two oxidative decarboxylation steps plus an ATP-generating step. Succinate is then oxidized and converted to oxaloacetate, generating $FADH_2$ and NADH. Overall, for each pyruvate metabolized to 3 CO_2, there are 4 NADH, 1 $FADH_2$, and 1 ATP generated.

Pyruvate Is Converted to Acetyl Coenzyme A by Oxidative Decarboxylation

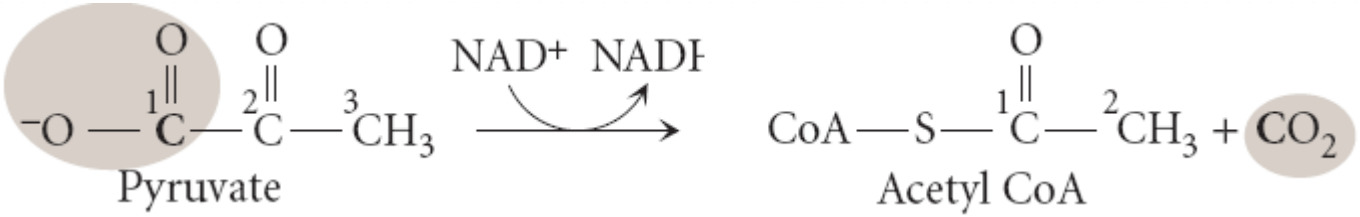
- Carbon enters the TCA cycle in theform of acetyl CoA
- The glycolytic pathway, however, ends with pyruvate, which is formed in the cytoplasm.
- So, how the acetyl CoA is generated?

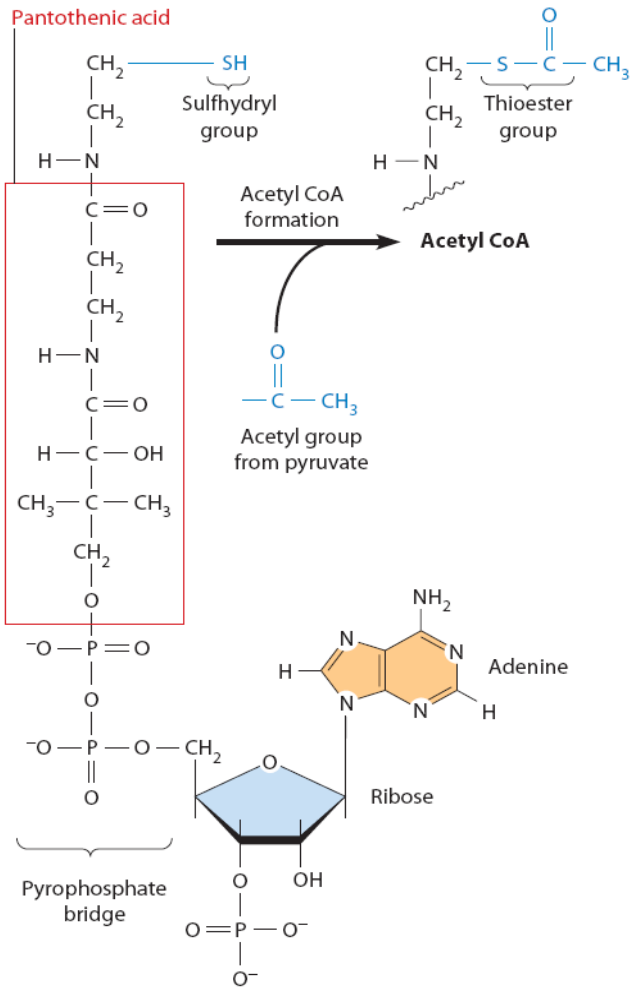
- The pyruvate molecule, being relatively small, passes through porins in the mitochondrial outer membrane into the intermembrane space.

- At the inner mitochondrial membrane, a specific pyruvate symporter transports pyruvate across the membrane into the matrix along with a proton.

- Once inside the mitochondrial matrix, pyruvate is converted to acetyl CoA by the ***pyruvate dehydrogenase complex (PDH),*** which consists of three different enzymes, five coenzymes, and two regulatory proteins.

- These components work together to catalyze the oxidative decarboxylation of pyruvate:



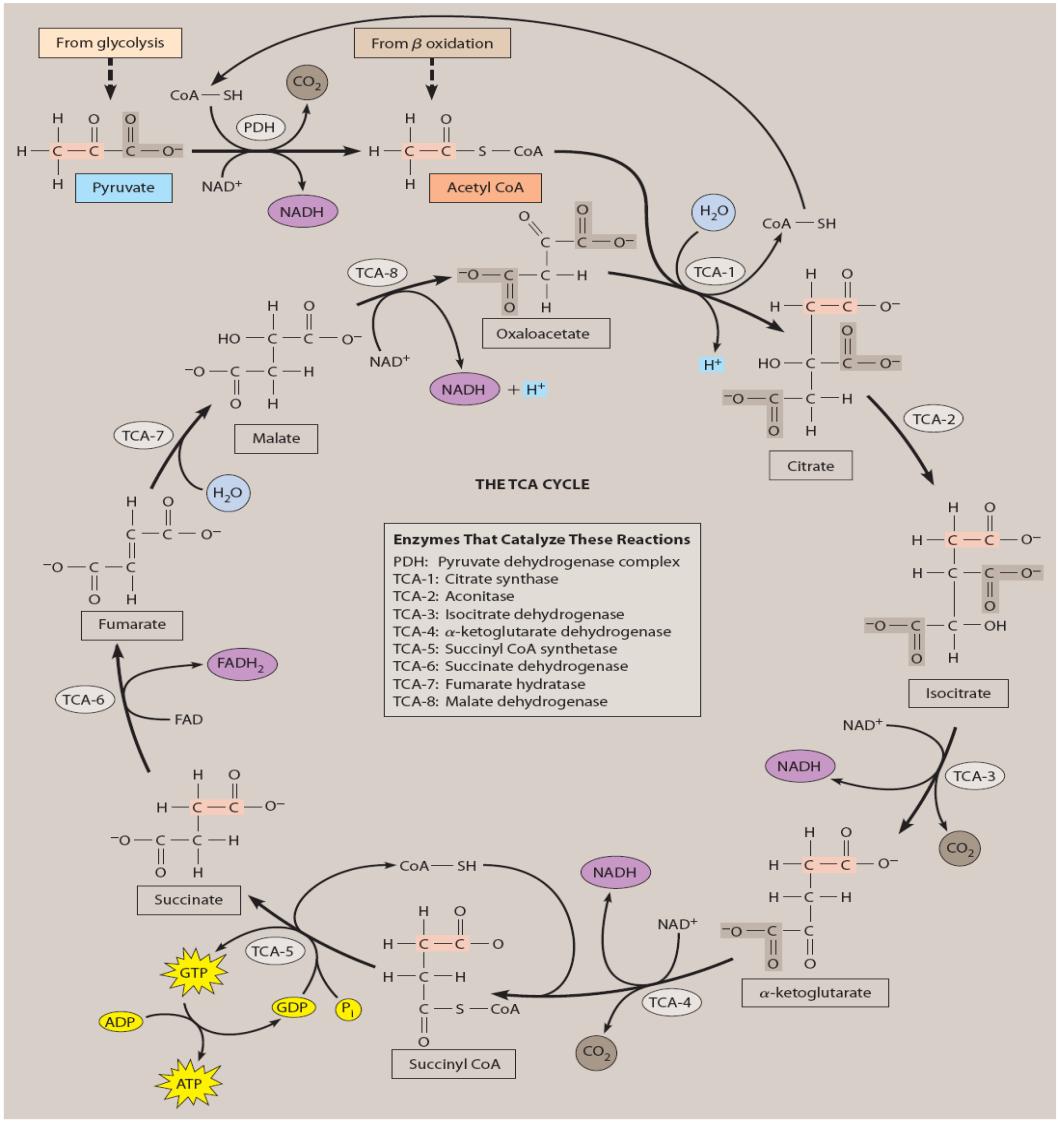


Structure of Coenzyme A and Acetyl CoA

Formation. The portion of the coenzyme enclosed in the red box is pantothenic acid, a B vitamin. Formation of a thioester bond between CoA and an acetyl group generates acetyl coenzyme A. The acetyl group is formed by the oxidative decarboxylation of pyruvate (Reaction PDH in Figure 10-8). It is transferred to CoA from one of the enzymes of the pyruvate dehydrogenase complex.

The TCA Cycle Begins with the Entry of Acetate as Acetyl CoA

- The TCA cycle begins with the entry of acetate in the form of acetyl CoA.
- With each round of TCA cycle activity, two carbon atoms enter in organic form (as acetate), and two carbon atoms leave in inorganic form (as carbon dioxide).
- In the first reaction (TCA-1), the two-carbon acetate group of acetyl CoA is added onto the four-carbon compound oxaloacetate to form citrate, a six-carbon molecule.



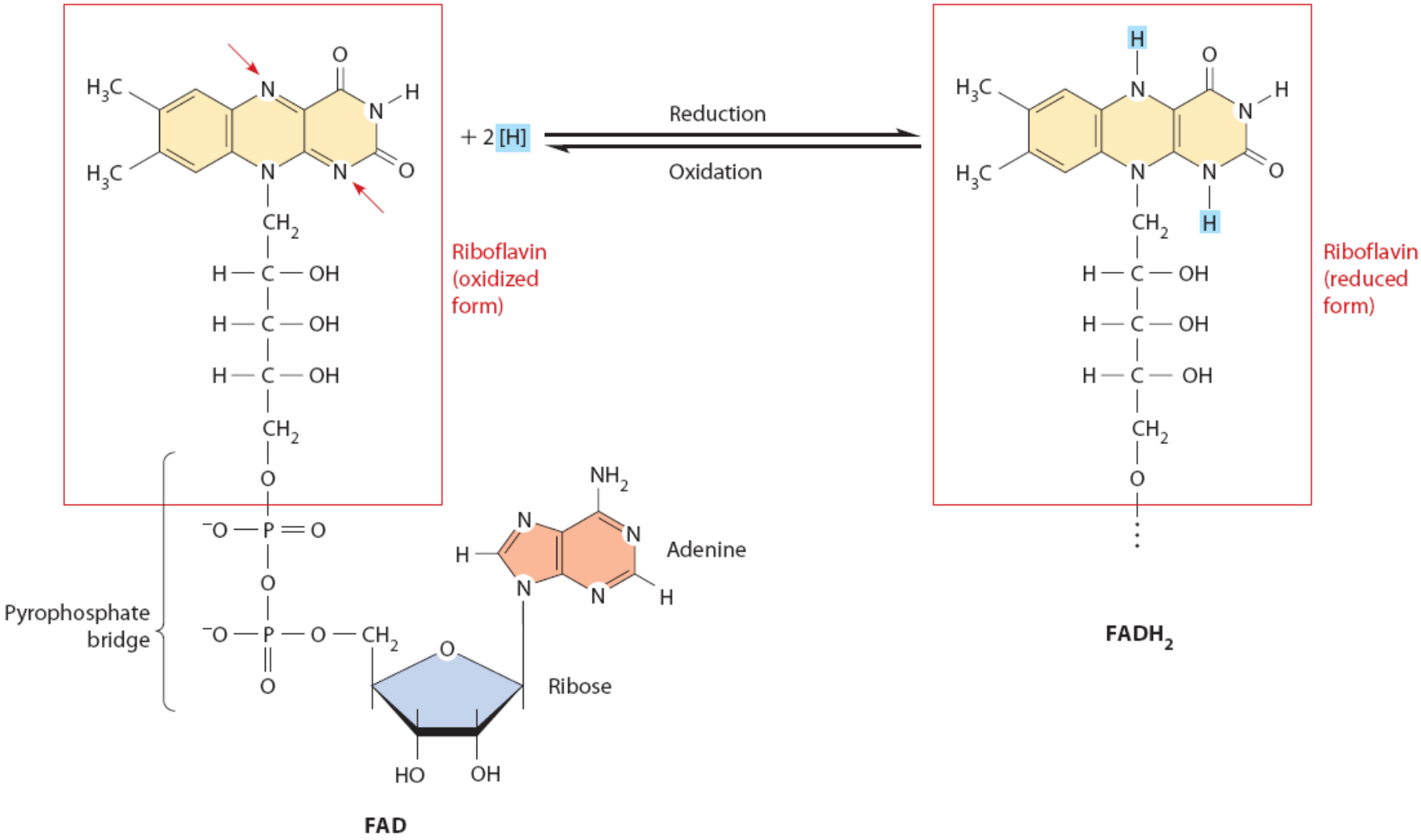
The Tricarboxylic Acid (TCA) Cycle. The two carbon atoms of pyruvate that enter the cycle via acetyl CoA are shown in pink in citrate and subsequent molecules until they are randomized by the symmetry of the fumarate molecule. The carbon atom of pyruvate that is lost as CO_2 is shown in gray, as are the two carboxyl groups of oxaloacetate that give rise to CO_2 in Reactions TCA-3 and TCA-4. Five of the reactions are oxidations, with NAD^+ as the electron acceptor in four reactions (PDH, TCA-3, TCA-4, and TCA-8) and FAD as the electron acceptor in one case (TCA-6). The reduced form of the coenzyme is shown in purple in each case. Note that when CO_2 is released, no H^+ is given off during NAD^+ reduction, thereby maintaining the charge balance of these reactions. The generation of GTP shown in Reaction TCA-5 is characteristic of animal mitochondria. In bacterial cells and plant mitochondria, ATP is formed directly.

- In the next reaction (TCA-2), citrate is converted to the related compound isocitrate.

- Isocitrate has a hydroxyl group that can be quite easily oxidized. This hydroxyl group of isocitrate is now the target of the first oxidation, or dehydrogenation, of the cycle (TCA-3).

- Four (TCA-3, TCA-4, TCA-6, and TCA-8) of the eight steps in the TCA cycle are oxidations.

- The first two of these reactions, TCA-3 and TCA-4, are also decarboxylation steps. One molecule of carbon dioxide is eliminated in each step, reducing the number of carbons from six to five to four.

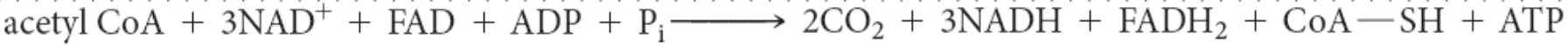


Structure of FAD and Its Oxidation and Reduction. The portion of the coenzyme enclosed in the red box is riboflavin, a B vitamin. The arrows point to the two nitrogen atoms of riboflavin that acquire one proton and one electron each when FAD is reduced to $FADH_2$. The half of the molecule that includes riboflavin and one phosphate group represents the structure of flavin mononucleotide (FMN), a closely related coenzyme.

Direct Generation of GTP (or ATP) Occurs at One Step in the TCA Cycle

- Succinyl CoA as an activated compound that, like acetyl CoA, has a high-energy thioester bond.
- The energy of this thioester bond is used to generate a molecule of either ATP (in bacterial cells and plant mitochondria) or GTP (in animal mitochondria).
- GTP and ATP are energetically equivalent because their terminal phosphoanhydride bonds have identical free energies of hydrolysis.

Summing Up: The Products of the TCA Cycle Are CO2, ATP,NADH,and FADH2



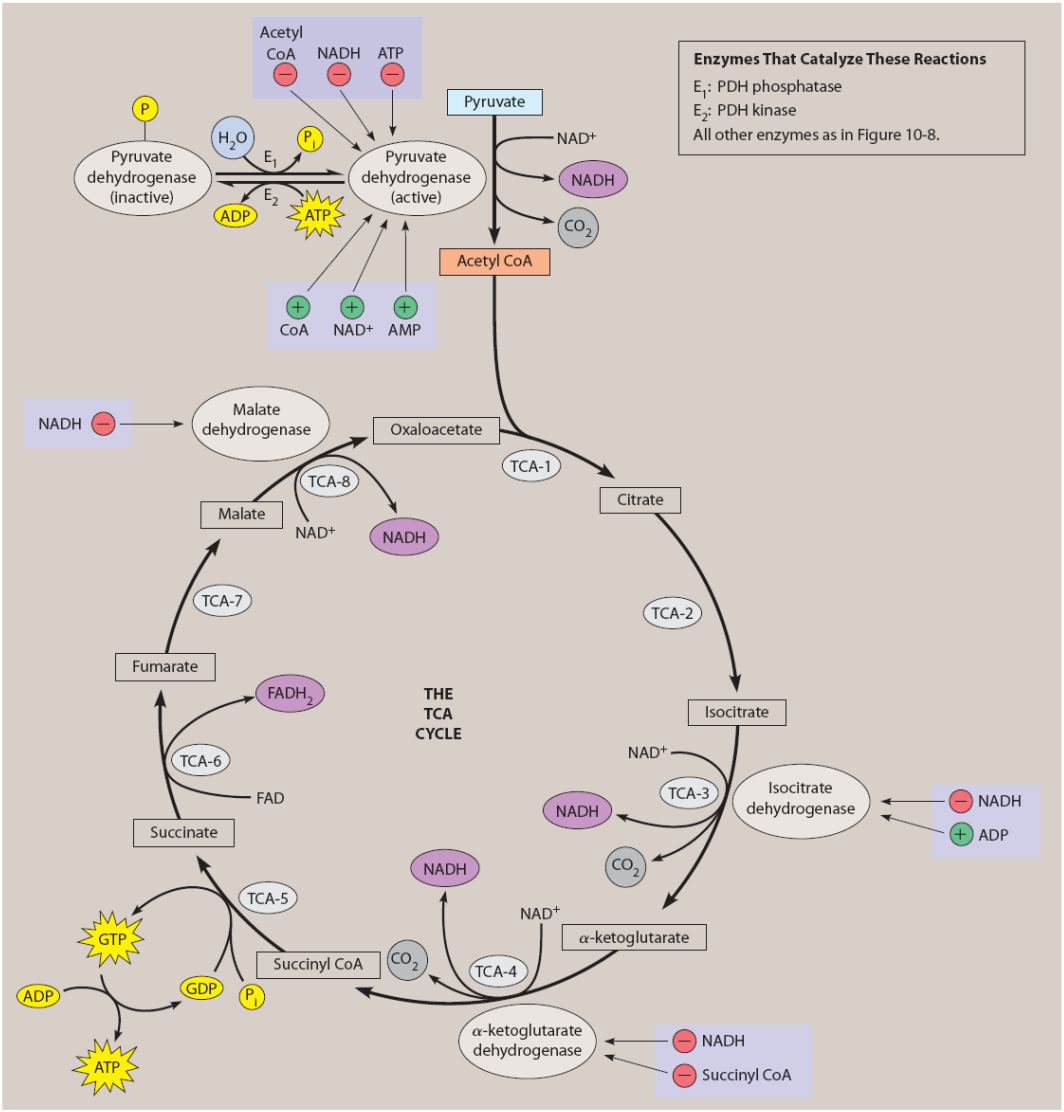
1. Two carbons enter the cycle as acetyl CoA, which is joined to the four-carbon acceptor molecule oxaloacetate to form citrate, a six-carbon compound.

2. Decarboxylation occurs at two steps in the cycle so that the input of two carbons as acetyl CoA is balanced by the loss of two carbons as carbon dioxide.

3. Oxidation occurs at four steps, with as the electron acceptor in three cases and FAD as the electron acceptor in one case.

4. ATP is generated at one point, with GTP as an intermediate in animal cells.

5. One turn of the cycle is completed upon regeneration of oxaloacetate, the original four-carbon acceptor.



Regulation of the TCA Cycle. The pyruvate dehydrogenase reaction and the TCA cycle are shown here in outline form, with full names given for regulatory enzymes. Major regulatory effects are indicated as either activation (+) or inhibition (−). Allosteric regulators include CoA, NAD^+, AMP, and ADP as activators and acetyl CoA, NADH, ATP, and succinyl CoA as inhibitors. In addition to its allosteric effect on pyruvate dehydrogenase activity, ATP activates PDH kinase (E_2), the enzyme that phosphorylates one component of the PDH complex, thereby converting it to an inactive form. The enzyme PDH phosphatase (E_1) removes the phosphate group, returning the enzyme to its active form.

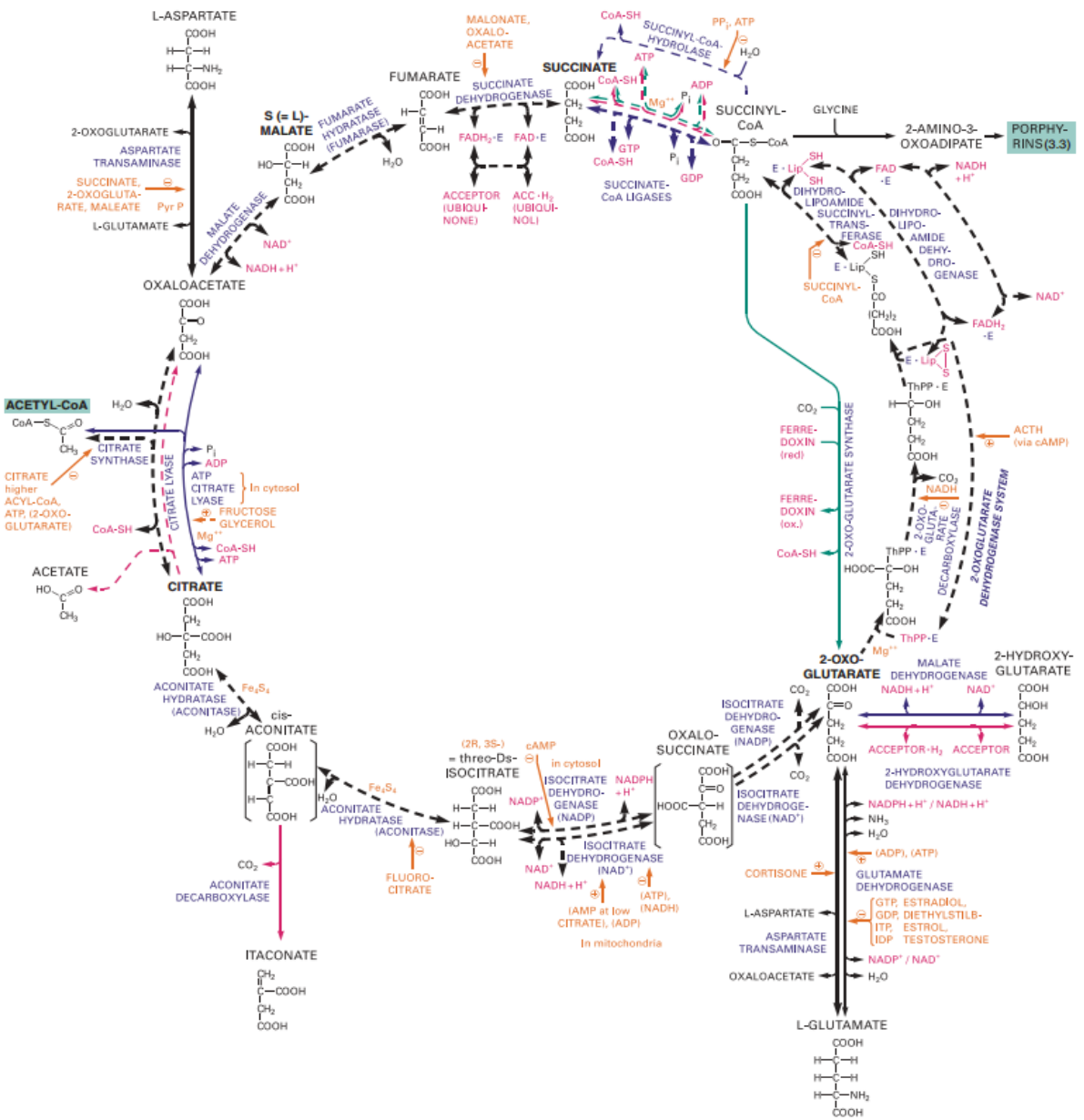
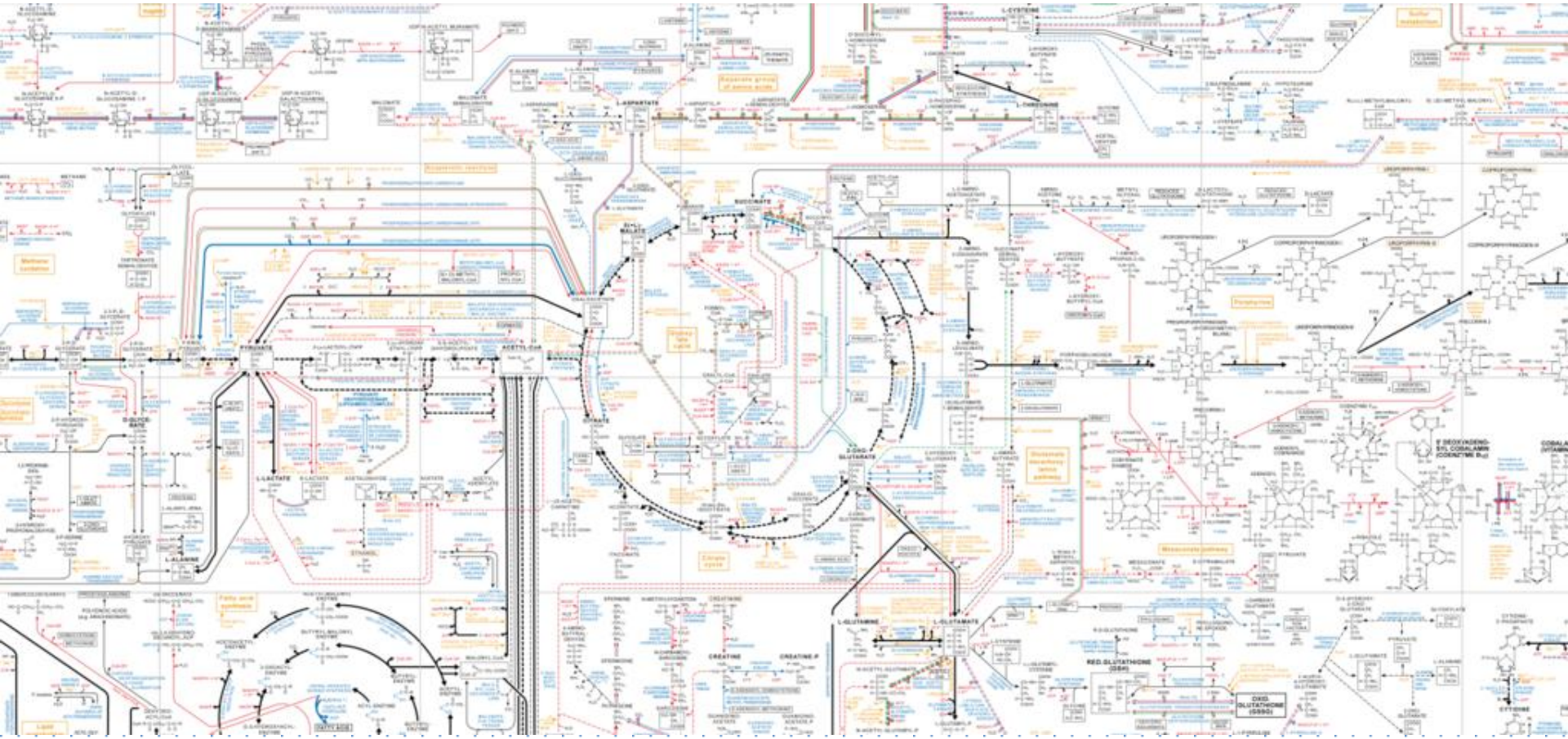


Figure 3.1.8-2. Citrate Cycle



MBG304 Biochemistry

Lecture 4: Electron Transport and Oxidative Phosphorylation

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Inonu University

Electron Transport: Electron Flow from Coenzymes to Oxygen

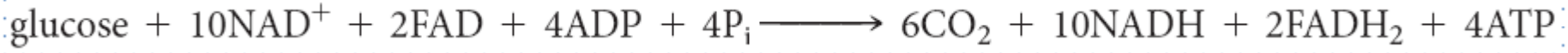
- Up to here we have seen the three stages of aerobic respiration:
  - Glycolysis
  - pyruvate oxidation
  - TCA (a.k.a. Krebs Cycle, Citric Acid Cycle) cycle

- Chemotrophic energy metabolism through these three steps accounts for the synthesis of four ATP molecules per glucose, two from glycolysis, and two from the TCA cycle (substrate-level phosphorylation) .

- Complete oxidation of glucose to CO_2 could yield 686 kcal/mol, but we have recovered less than 10% of that amount (only 4 ATP).

Where is the rest of the free energy?

- The rest is in below reaction (in reduced coenzymes NADH and $FADH_2$



- About 90% of the potential free energy present in a glucose molecule is conserved in the 12 molecules of NADH and $FADH_2$ that are formed when a molecule of glucose is oxidized to CO_2.

Electron transport from NADH and $FADH_2$

- The process of coenzyme reoxidation by the transfer of electrons to oxygen is called **electron transport.**

- *Electron transport* is the fourth stage of respiratory metabolism (after *glycolysis*, *pyruvate oxidation*, and *TCA*).

Electron Transport and Coenzyme Oxidation

- Electron transport involves the highly exergonic oxidation of NADH and $FADH_2$ with O_2 as the terminal electron acceptor.

- So we can write summary reactions as follows:

$$NADH + H^+ + \tfrac{1}{2}O_2 \longrightarrow NAD^+ + H_2O \qquad \Delta G^{\circ\prime} = -52.4 \text{ kcal/mol}$$

$$FADH_2 + \tfrac{1}{2}O_2 \longrightarrow FAD + H_2O \qquad \Delta G^{\circ\prime} = -45.9 \text{ kcal/mol}$$

The Electron Transport System

- Electron transfer is accomplished as a multistep process that involves an ordered series of reversibly oxidizable electron carriers functioning together in what is called the **electron transport system (ETS)**.

14.3-electron_transport.mov

- The ETS contains a number of integral membrane proteins that are found in the inner mitochondrial membrane of eukaryotes (or the plasma membrane of bacteria).

The Electron Transport System Consists of Five Kinds of Carriers

- The carriers that make up the ETS include:
  1. *flavoproteins*
  2. *iron-sulfur proteins*
  3. *Cytochromes*
  4. *copper-containing cytochromes*
  5. *quinone* known as *coenzyme Q*

- Except for *coenzyme Q*, all the carriers are proteins with specific prosthetic groups capable of being reversibly oxidized and reduced.

Electron Carriers in ETS

1. Flavoproteins

- participate in electron transport, using either FAD FMN as the prosthetic group.
- An example of a flavoprotein is NADH dehydrogenase, which is part of the protein complex that accepts pairs of electrons from NADH.
- Another example is the TCA cycle enzyme *succinate dehydrogenase*, which has FAD as its prosthetic group and is part of the membrane-bound respiratory complex that accepts pairs of electrons from succinate via FAD.

2. Iron-Sulfur Proteins

- are also called *nonheme iron proteins and* have an iron-sulfur (Fe-S) center complexed with cysteine groups of the protein.

- At least a dozen different Fe-S centers are known to be involved in the mitochondrial transport system.

- The iron atoms of these centers are the actual electron carriers (involved in transfer of only one electron and no protons).

3. Cytochromes

- Like the iron-sulfur proteins, cytochromes also contain iron but as part of a porphyrin prosthetic group called *heme*.
- Just like iron-sulfur proteins, cytochromes are also one-electron carriers that do not transfer protons.
- There are at least five different kinds of cytochromes in the electron transport system, designated as cytochromes *b, c, c_1, a,* and a_3.
- While cytochromes *b,* c_1 *, a,* and a_3 are integral membrane proteins, cytochrome c is a peripheral membrane protein.
- Moreover, cytochrome *c* is not a part of a large complex and can therefore diffuse much more rapidly, a key property in its role in transferring electrons between protein complexes.

4. Copper-Containing Cytochromes

- In addition to their Fe atoms, cytochromes *a* and a_3 also contain a single Cu atom bound to the *heme* group of the cytochrome (Fe-Cu center).

- Like iron atoms, copper ions can be reversibly converted from the oxidized (Cu2+) to the reduced (Cu+) form by accepting or donating single electrons.

- The iron-copper center helps the cytochrome oxidase complex bind an O_2 molecule until the O_2 molecule has picked up the requisite four electrons and four protons, at which point the oxygen atoms are released as two molecules of water.

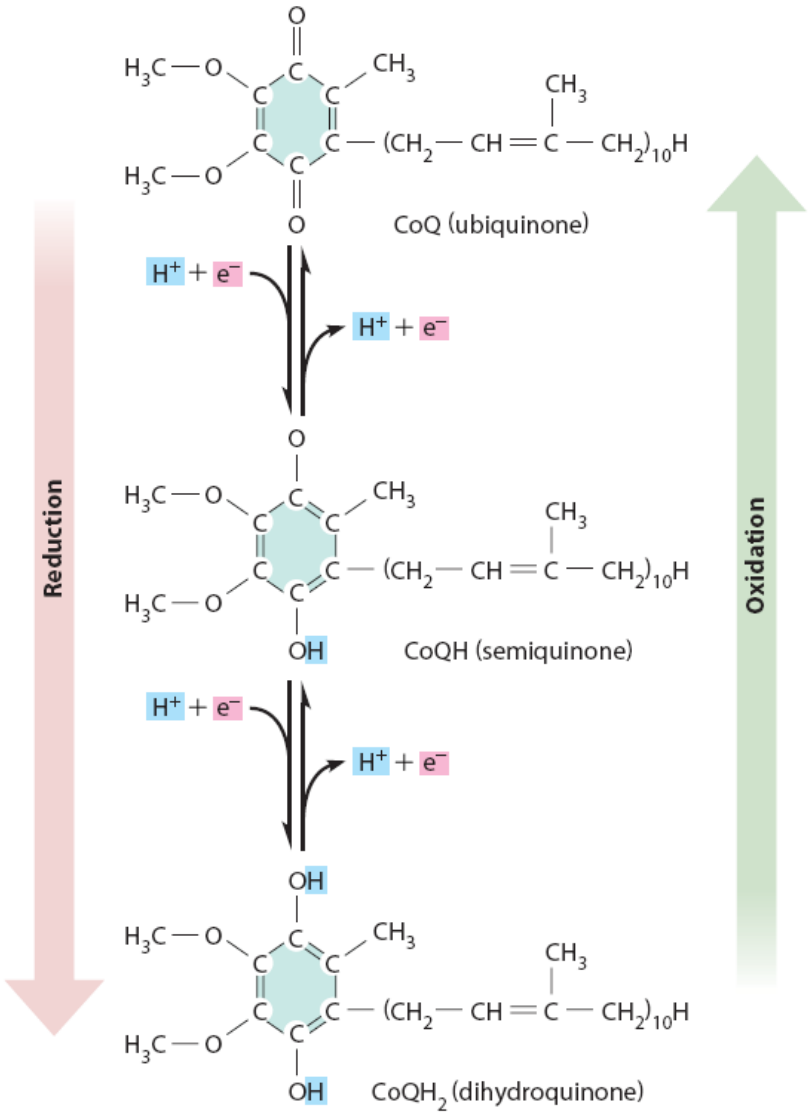
5. Coenzyme Q

- The only nonprotein component of the ETS is **coenzyme Q** (**CoQ**).

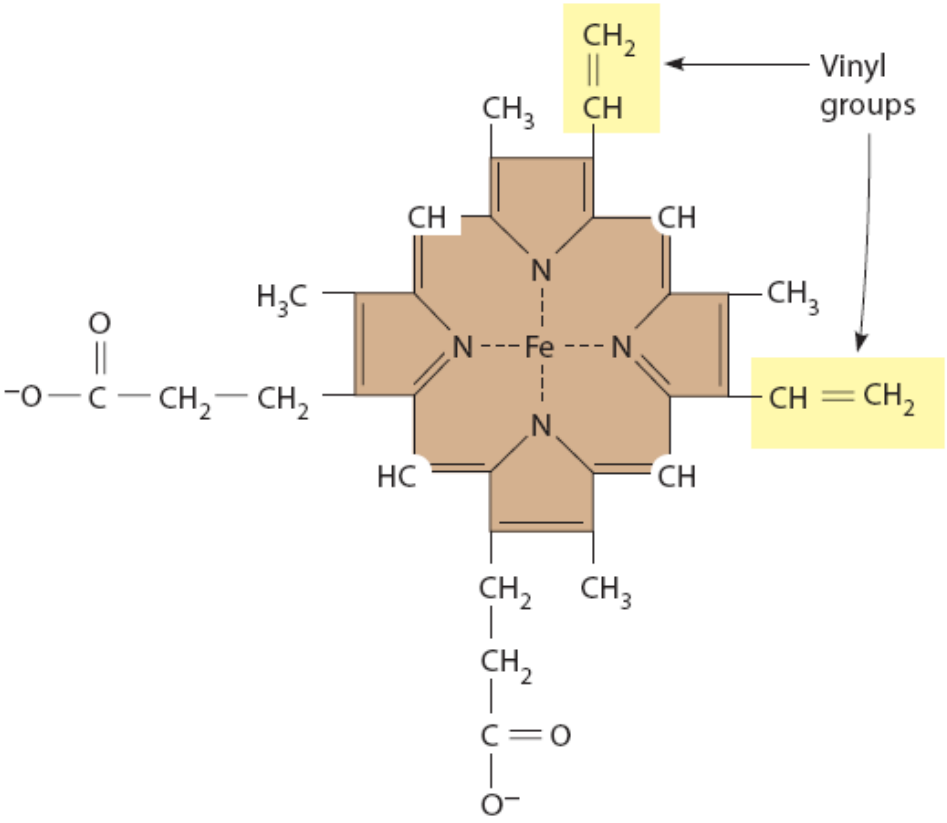
- Because of its ubiquitous occurrence in nature, coenzyme Q is also known as **ubiquinone**.

- CoQ molecules are the most abundant electron carriers in the membrane and occupy a central position in the ETS, serving as a collection point for electrons from the reduced prosthetic groups of FMN and FAD-linked dehydrogenases in the membrane.

- Coenzyme Q accepts electrons as well as protons when it is reduced and that it releases both electrons and protons when it is oxidized.



Oxidized and Reduced Forms of Coenzyme Q.
Coenzyme Q (also called ubiquinone) accepts both electrons and protons as it is reversibly reduced in two successive one-electron steps to form first CoQH (the semiquinone form) and then $CoQH_2$ (the dihydroquinone form).



HEME

The Structure of Heme. Heme, also called iron-protoporphyrin IX, is the prosthetic group in cytochromes *b*, *c*, and c_1. A similar molecule, called heme A, is present in cytochromes a_1 and a_3. The heme of cytochromes *c* and c_1 is covalently attached to the protein by thioether bonds between the sulfhydryl groups of two cysteines in the protein and the vinyl ($-CH=CH_2$) groups of the heme (highlighted in yellow). In other cytochromes, the heme prosthetic group is linked noncovalently to the protein.

The Electron Carriers Function in a Sequence Determined by Their Reduction Potentials

- The **standard reduction potential, E_0** which is a measure, in volts (*V*), of the affinity a compound has for electrons.
- It describes how easily a compound will gain electrons and become reduced.
- Reduction potentials are determined experimentally for a **redox (reduction-oxidation)** pair.
- Reduction-oxidation pair consists of two molecules or ions that are interconvertible by the loss or gain of electrons:

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

$$Fe^{3+} + e^- \longrightarrow Fe^{2+}$$

$$\tfrac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$$

- By convention, the $2H^+/H_2$ redox pair is used as a reference and is assigned the value 0.00 V

- For a redox pair to have a positive standard reduction potential means that, under standard conditions, the oxidized form of the pair has a higher affinity for electrons than H+ and will accept electrons from H_2.

- Conversely, a negative reduction potential means that the oxidized form of the pair has less affinity for electrons than H+, and its reduced form will donate electrons to H+ to form H_2.

- The redox pairs in the table: the most negative values (i.e., the best electron donors and hence the strongest reducing agents) at the top.
- The reduced form of any redox pair will spontaneously reduce the oxidized form of any pair below it on the table.
- Thus, NADH can reduce pyruvate to lactate but cannot reduce a-ketoglutarate to isocitrate.
- For example, $\Delta E0'$ for the transfer of electrons from NADH to O_2 is calculated as follows, with NADH as the donor and O_2 as the acceptor:

$$\Delta E_0' = E_{0,\text{ acceptor}}' - E_{0,\text{ donor}}'$$
$$= +0.816 - (-0.32) = +1.136\text{V}$$

Standard Reduction Potentials for Redox Pairs of Biological Relevance*

Redox Pair (oxidized form → reduced form)	No. of Electrons	E_0' (V)
Acetate → pyruvate	2	−0.70
Succinate → α-ketoglutarate	2	−0.67
Acetate → acetaldehyde	2	−0.60
3-phosphoglycerate → glyceraldehyde-3-P	2	−0.55
α-ketoglutarate → isocitrate	2	−0.38
$NAD^+ \rightarrow NADH$	2	−0.32
$FMN \rightarrow FMNH_2$	2	−0.30
1,3-bisphosphoglycerate → glyceraldehyde-3-P	2	−0.29
Acetaldehyde → ethanol	2	−0.20
Pyruvate → lactate	2	−0.19
$FAD \rightarrow FADH_2$	2	−0.18
Oxaloacetate → malate	2	−0.17
Fumarate → succinate	2	−0.03
$2H^+ \rightarrow H_2$	2	0.00**
$CoQ \rightarrow CoQH_2$	2	+0.04
Cytochrome *b* ($Fe^{3+} \rightarrow Fe^{2+}$)	1	+0.07
Cytochrome *c* ($Fe^{3+} \rightarrow Fe^{2+}$)	1	+0.25
Cytochrome *a* ($Fe^{3+} \rightarrow Fe^{2+}$)	1	+0.29
Cytochrome a_3 ($Fe^{3+} \rightarrow Fe^{2+}$)	1	+0.55
$Fe^{3+} \rightarrow Fe^{2+}$ (inorganic iron)	1	+0.77
$\frac{1}{2}O_2 \rightarrow H_2O$	2	+0.816

*Each $\Delta E_0'$ value is for the following half-reaction, where n is the number of electrons transferred:

$$\text{oxidized} + nH^+ + ne^- \rightarrow \text{reduced form.}$$

**By definition, this redox pair is the reference point for determining values of all other redox pairs. It requires that $[H^+] = 1.0\ M$ and therefore specifies pH 0.0. At pH 7.0, the value for the $2H^+/H_2$ pair is −0.42 V.

The Relationship Between $\Delta G'_0$ and $\Delta E'_0$.

- $\Delta E_0'$ is a measure of thermodynamic spontaneity for the redox reaction between any two redox pairs under standard conditions.

- The spontaneity of a redox reaction under standard conditions can therefore be expressed as either $\Delta G_0'$ or $\Delta E_0'$. The sign convention for $\Delta E_0'$ is the opposite of that for $\Delta G_0'$, so an *exergonic* reaction is one with a *negative* $\Delta G_0'$ and a *positive* $\Delta E_0'$.

- For any oxidation-reduction reaction, $\Delta G_0'$ is related to $\Delta E_0'$ by the equation:

$$\Delta G^{\circ\prime} = -nF\,\Delta E_0'$$

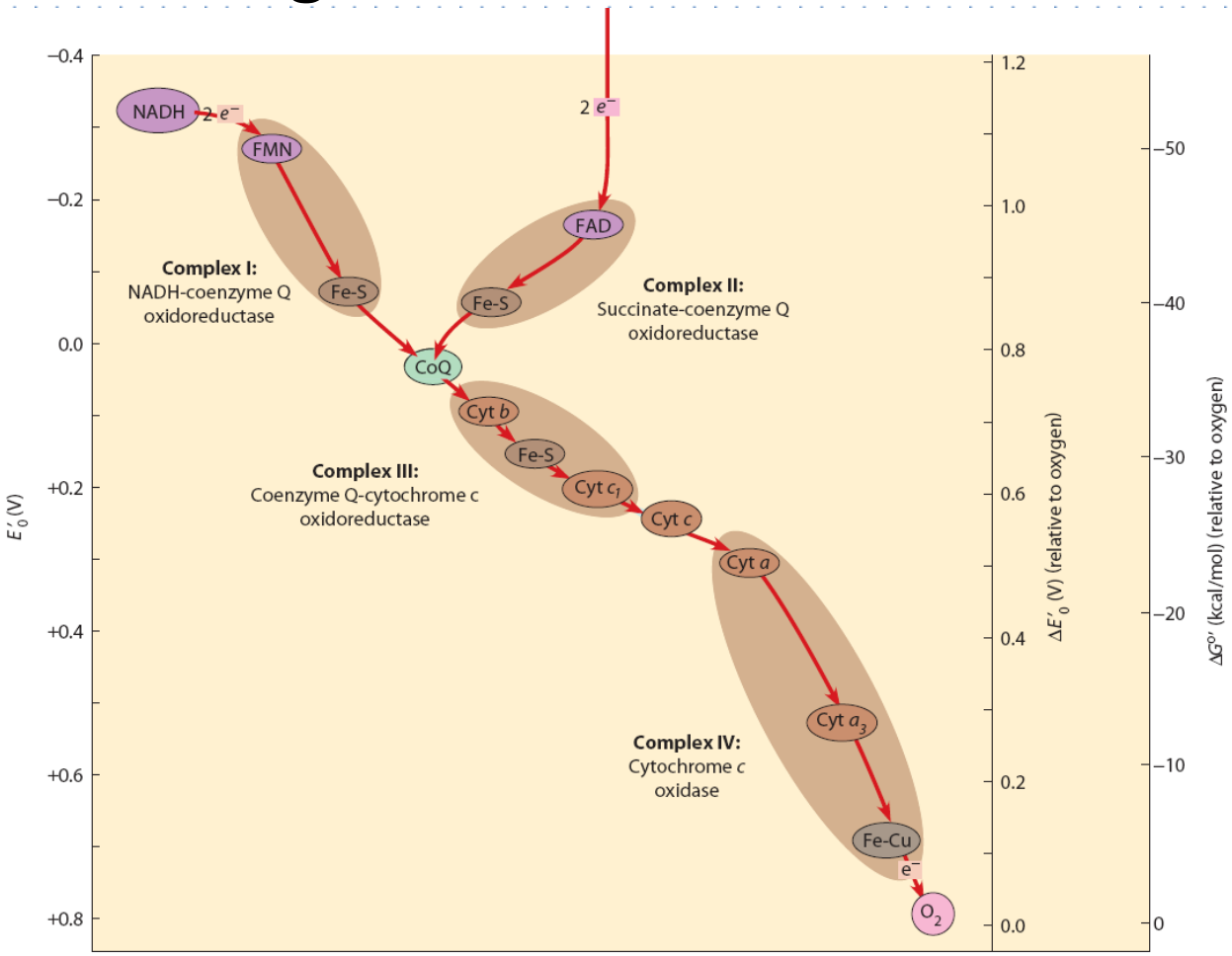
- where *n* is the number of electrons transferred, and *F* is the Faraday constant (23,062 cal/mol V). For example, the reaction of NADH with oxygen involves the transfer of two electrons, so $\Delta G_0'$ for the reaction can be calculated as

$$\begin{aligned}\Delta G^{\circ\prime} &= -2F\,\Delta E_0' = -2(23{,}062)(+1.136)\\ &= -52{,}400\ \text{cal/mol} = -52.4\ \text{kcal mol}\end{aligned}$$

- The $\Delta G_0'$ for this reaction is highly negative, so the transfer of electrons from NADH to O_2 is thermodynamically spontaneous under standard conditions.

- This difference in reduction potentials between the NAD+/NADH and O_2/H_2O redox pairs drives the ETS and creates a proton gradient whose electrochemical potential will drive ATP synthesis.

Ordering of the Electron Carriers



Major Components of the Respiratory Complexes and Their Energetics. Major intermediates in the transport of electrons from NADH (−0.32 V) and $FADH_2$ (−0.18 V) to oxygen (+0.816 V) are positioned vertically according to their energy levels, as measured by their standard reduction potentials (E_0', left axis). The four respiratory complexes are shown as large brown ovals, with the major electron carriers in each complex enclosed within inset ovals. Coenzyme Q and cytochrome *c* are small, mobile intermediates that transfer electrons between the several complexes. The red lines trace the exergonic flow of electrons through the system. On the right axes are the $\Delta E_0'$ and $\Delta G^{\circ\prime}$ values relative to oxygen (i.e., the changes in the standard reduction potential and the standard free energy for the transfer of two electrons to O_2).

Most of the Carriers Are Organized into Four Large Respiratory Complexes

- Most of the electron carriers in the ETS are thought to be organized within the inner mitochondrial membrane into four different kinds of **respiratory complexes.**

Properties of the Mitochondrial Respiratory Complexes

Respiratory Complex		Number of Polypeptides*	Prosthetic Groups	Electron Flow		Protons Translocated (per electron pair)
Number	Name			Accepted from	Passed to	
I	NADH–coenzyme Q oxidoreductase (NADH dehydrogenase)	43 (7)	1 FMN 6–9 Fe-S centers	NADH	Coenzyme Q	4
II	Succinate–coenzyme Q oxidoreductase (succinate dehydrogenase)	4 (0)	1 FAD 3 Fe-S centers	Succinate (via enzyme-bound FAD)	Coenzyme Q	0
III	Coenzyme Q–cytochrome *c* oxidoreductase (cytochrome b/c_1 complex)	11 (1)	2 cytochrome *b* 1 cytochrome c_1 1 Fe-S center	Coenzyme Q	Cytochrome *c*	4**
IV	Cytochrome *c* oxidase	13 (3)	1 cytochrome *a* 1 cytochrome a_3 2 Cu centers (as Fe-Cu centers with cytochrome a_3)	Cytochrome *c*	Oxygen (O_2)	2

*The number of polypeptides encoded by the mitochondrial genome is indicated in parentheses for each complex.

**The value for complex III includes two protons translocated by coenzyme Q.

- **Complex I** transfers electrons from NADH to coenzyme Q and is called the *NADH-coenzyme Q oxidoreductase* complex (or NADH dehydrogenase complex).

- **Complex II** transfers to CoQ the electrons derived from succinate This complex is called the *succinate–coenzyme Q oxidoreductase* complex, although it is often also referred to by its more common name, succinate dehydrogenase.

- **Complex III** is called the *coenzyme Q–cytochrome c oxidoreductase* complex because it accepts electrons from coenzyme Q and passes them to cytochrome *c*. This complex is also referred to as the cytochrome complex because those two cytochromes are its most prominent components.

- **Complex IV** transfers electrons from cytochrome *c* to oxygen and is called *cytochrome c oxidase*.

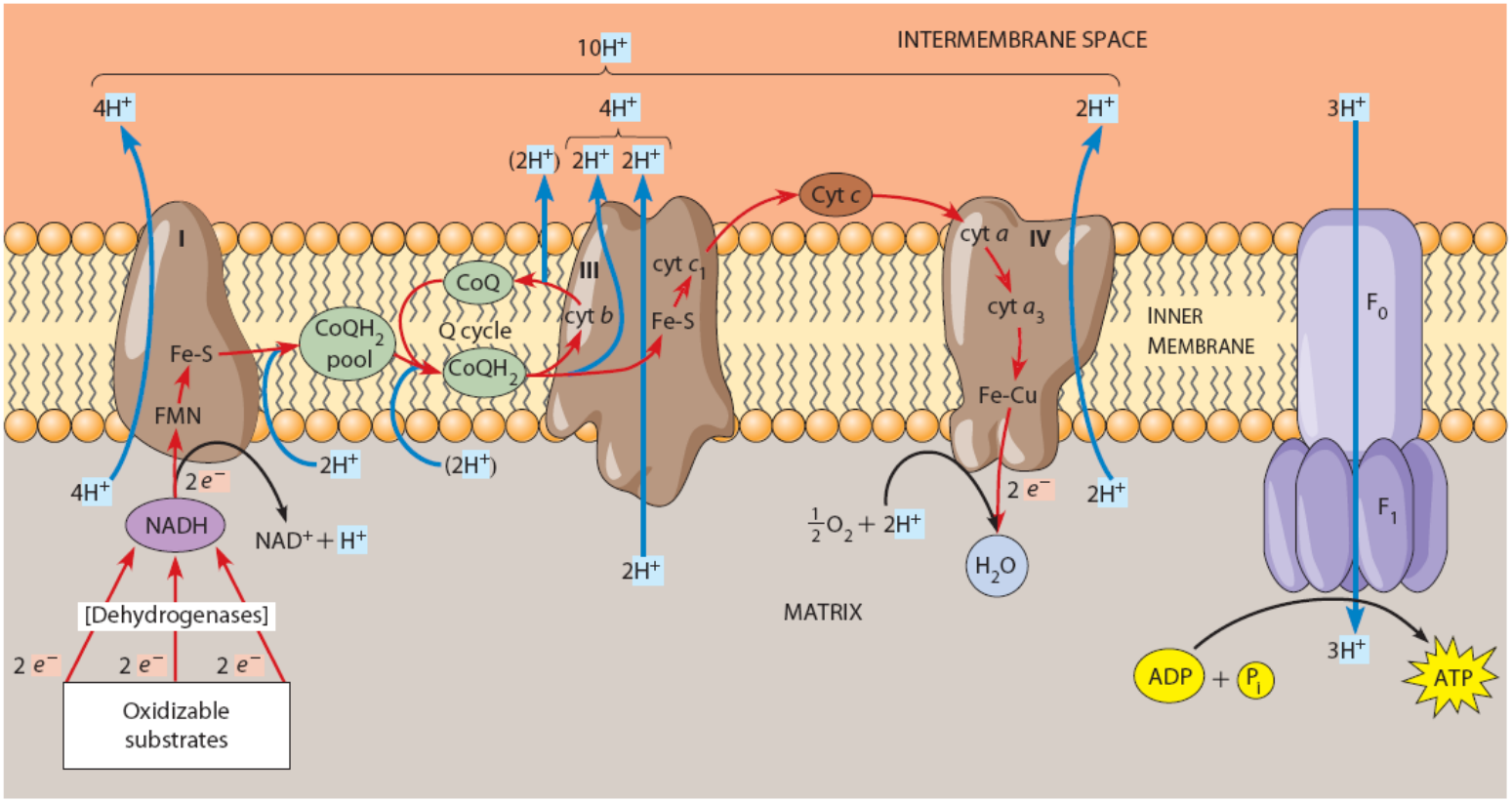
- For each pair of electrons transported through complexes I, III, and IV, 10 protons are pumped from the matrix into the intermembrane space.

(a) Complex I receives 2 electrons from NADH and passes them to CoQ via FMN and an Fe-S protein. During this process, 4 H^+ are pumped out of the matrix by complex I.

(b) Complex III passes electrons from $CoQH_2$ to cytochrome *c* via cytochromes *b* and c_1 and an Fe-S protein. $CoQH_2$ carries 2 H^+ across the inner membrane and 2 more H^+ are pumped out of the matrix.

(c) Complex IV receives electrons from cytochrome *c* and, via cytochrome *a* and a_3, passes them to molecular oxygen, which is reduced to water as 2 more H^+ are pumped from the matrix by complex IV.

(d) ATP synthase uses the energy from the proton gradient generated during electron transport to synthesize ATP from ADP and P_i.



The Flow of Electrons Through Respiratory Complexes I, III, and IV Causes Directional Proton Pumping. (a–c) Electrons derived from oxidizable substrates in the mitochondrial matrix flow exergonically from NADH to oxygen via respiratory complexes I, III, and IV. (**d**) During the transport of two electrons, 10 H^+ are pumped across the inner membrane, and 3 ATP are synthesized by the F_oF_1 ATP synthase. Two extra H^+ (shown in parentheses) are pumped when the Q cycle operates.

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The Role of Cytochrome *c Oxidase*

- Of the several respiratory complexes involved in aerobic respiration in humans, cytochrome *c* oxidase (complex IV) is the **terminal oxidase**, transferring electrons directly to oxygen.

- This complex is therefore the critical link between aerobic respiration and the oxygen that makes it all possible.

- Cyanide and azide ions are highly toxic to nearly all aerobic cells because they bind tightly to the Fe-Cu center of cytochrome *c* oxidase, thereby blocking electron transport.

- Transport of four electrons from cytochrome *c* to O_2 plus the addition of four protons results in the production of two molecules of H_2O.

- However, studies have shown that complexes I and III also can transfer electrons directly to O2, resulting in its incomplete reduction. This can generate toxic *superoxide anion* (*O_2*-) or *hydrogen peroxide* (H_2O_2), compounds that can contribute to cellular aging under both normal and pathological conditions.

The Electrochemical Proton Gradient: Key to Energy Coupling

- So far, we have learned that:
  - coenzymes are reduced during the oxidative events of glycolysis, pyruvate oxidation, and the TCA cycle, the first three stages of aerobic respiration
  - reduced coenzymes are reoxidized by the exergonic transfer of electrons to oxygen via a system of reversibly oxidizable intermediates located within the inner mitochondrial membrane
- Now we will consider:
  - how the free energy released during electron transport is used to generate an electrochemical proton gradient and how the energy of the gradient is then used to drive ATP synthesis

Oxidative phosphorylation

- This form of ATP synthesis involves phosphorylation events that are linked to oxygen-dependent electron transport, the process is called **oxidative phosphorylation (OP)**

ETS and OP.swf

- Thus, OP is different from substrate-level phosphorylation, which occurs as an integral part of a specific reaction in the glycolytic pathway and the TCA cycle.

Electron Transport and ATP Synthesis Are Coupled Events

- Mechanistically, oxidative phosphorylation is more complex than substrate-level phosphorylation.
- The crucial link between electron transport and ATP production is an **electrochemical proton gradient**, established by the directional pumping of protons across the membrane in which electron transport is occurring.
- As confirmed by using *uncouplers*, the ATP synthesis is strictly dependent on electron transport, but electron transport is not necessarily dependent on ATP synthesis.
- In brown adipose (fat) tissue in the bodies of newborn mammals, a similar uncoupling mechanism operates, using *exergonic* electron transport to generate heat rather than ATP.
- Oxidative phosphorylation is regulated by cellular ATP needs, called **respiratory control**. Electron transport and ATP generation will be favored when the ADP concentration is high and inhibited when the ADP concentration is low.

The Chemiosmotic Model: The use of a Proton Gradient.

- How can ATP synthesis, which is a dehydration reaction, is tightly coupled to electron transport

- According to Mitchell's **chemiosmotic coupling model**:
  - *the exergonic transfer of electrons through the respiratory complexes is accompanied by the unidirectional pumping of protons across the membrane.*
  - *the electrochemical proton gradient that is generated in this way represents potential energy that then provides the driving force for ATP synthesis.*

(a) Complex I receives 2 electrons from NADH and passes them to CoQ via FMN and an Fe-S protein. During this process, 4 H^+ are pumped out of the matrix by complex I.

(b) Complex III passes electrons from $CoQH_2$ to cytochrome *c* via cytochromes *b* and c_1 and an Fe-S protein. $CoQH_2$ carries 2 H^+ across the inner membrane and 2 more H^+ are pumped out of the matrix.

(c) Complex IV receives electrons from cytochrome *c* and, via cytochrome *a* and a_3, passes them to molecular oxygen, which is reduced to water as 2 more H^+ are pumped from the matrix by complex IV.

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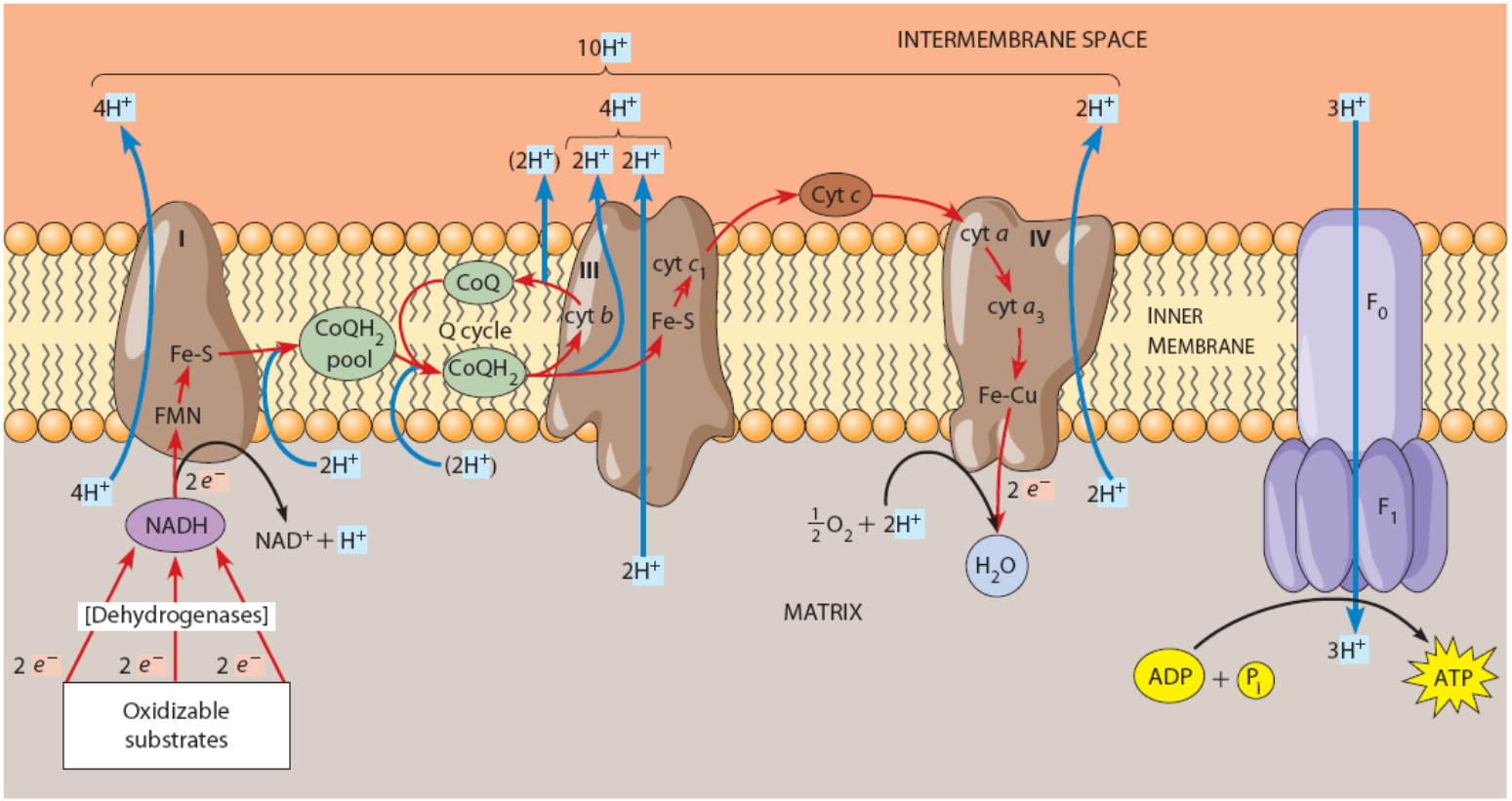

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- The essential feature of the chemiosmotic model is that the link between electron transport and ATP formation is an electrochemical potential across a membrane.

- Today the chemiosmotic coupling model is a well-verified concept that provides a unifying framework for understanding energy transformations not just in mitochondrial membranes but in chloroplast and bacterial membranes as well.

Coenzyme Oxidation Pumps Enough Protons to Form 3 ATP per NADH and 2 ATP per $FADH_2$

- These numbers (i.e., 3 ATP/NADH and 2 ATP/$FADH_2$ are ***estimates***.

- The disagreement arises partly due to a structural feature that varies in different ATP synthase complexes.

- Becuse, the number of protons required to produce 3 ATP is directly related to the number of *c* subunits in the F_o portion of the F_oF_1 ATP synthase, and this number can differ among organisms.

- As we have already seen, most of the dehydrogenases in the mitochondrial matrix transfer electrons from oxidizable substrates to NAD+, generating NADH.

- NADH, in turn, transfers electrons to the FMN component of complex I, thereby initiating the electron transport system.

- Transfer of two electrons from NADH down the respiratory chain to oxygen is accompanied by the transmembrane pumping of:
    - 4 protons by complex I
    - 4 protons by CoQ plus complex III
    - 2 protons by complex IV.

- This gives a total of 10 protons per NADH (12 protons per NADH if the Q cycle is operating).

- The number of protons required to drive the synthesis of one molecule of ATP by the ATP synthase is thought to be 3 or 4.

- If we assume that 10 protons are pumped per NADH oxidized and that 3 protons are required per ATP molecule, then we can conclude that oxidative phosphorylation yields about 3 molecules of ATP synthesized per molecule of NADH oxidized.

- These are reasonable numbers, considering that the for the oxidation of NADH by molecular oxygen is –52.4 kcal/mol.

- This is enough of a free energy change to produce 3 ATP (requiring about 10 kcal/mole each under cellular conditions), even assuming an efficiency of only 50%.

- A mitochondrion actively involved in aerobic respiration typically has a membrane potential of about 0.16 V (positive on the side that faces the intermembrane space) and a pH gradient of about 1.0 pH unit (higher on the matrix side).

- This electrochemical gradient exerts a **proton motive force (pmf**) that tends to drive protons back down their concentration gradient—back into the matrix of the mitochondrion, that is.

- The **pmf** can be calculated by summing the contributions of the membrane potential and the pH gradient using the following equation:

$$\text{pmf} = V_{\text{m}} + 2.303\, RT\, \Delta\text{pH}/F$$

where **pmf** is the proton motive force in volts, V_m is The membrane potential in volts, ΔpH is the Difference in pH across the membrane ($\Delta\text{pH} = \text{pH}_{\text{matrix}} - \text{pH}_{\text{cytosol}}$), R is the gas constant (1.987 cal/mol · K), T is the temperature in kelvins, and F is the Faraday constant (23,062 cal/mol · V).

- For a mitochondrion at 37 C with a V_m of 0.16 *V* and a pH gradient of 1.0 unit, the **pmf** can be calculated as follows:

$$\text{pmf} = 0.16 + \left(\frac{2.303(1.987)(37 + 273)(1.0)}{23{,}062}\right) = 0.16 + 0.06 = 0.22\ \text{V}$$

- Notice that the membrane potential accounts for more than 70% of the mitochondrial **pmf**.

- Like the redox potential, **pmf** is an electrical force in volts and can be used to calculate *ΔG°'*, the standard free energy change for the movement of protons across the membrane, using the following equation:

$$\Delta G^{\circ\prime} = -nF(\text{pmf}) = -(23.062)(0.22) = -5.1 \text{ kcal/mol}$$

- Thus, a proton motive force of 0.22 V across the inner mitochondrial membrane corresponds to a free energy change of about –5.1 kcal/mole of protons.

- This is the amount of energy that will be released as protons return to the matrix.

Is this enough to drive ATP synthesis?

- Not surprisingly, the answer is yes, though it depends on how many protons are required to drive the synthesis of one ATP molecule by the complex.

- Mitchell's original model assumed two protons per ATP, which would provide about 10.2 kcal/mol, barely enough to drive ATP formation, assuming the for phosphorylation of ADP to be about 10 to 14 kcal/mol under mitochondrial conditions.

- Differences of opinion still exist concerning the number of protons per ATP, but the real number is probably closer to ***three*** or ***four.***

- That number would provide about 15 to 20 kcal of energy per ATP, enough to ensure that the reaction is driven strongly in the direction of ATP formation.

ATP Synthesis: Putting It All Together

- This is the fifth, and final, stage of aerobic respiration: ATP synthesis.

- We have thus far seen that:
  1. some of the energy of glucose is transferred to reduced coenzymes during the oxidation reactions of glycolysis and the TCA cycle
  2. this energy is used to generate an electrochemical proton gradient across the inner membrane of the mitochondrion.

- Now we can ask how the **pmf** of that gradient is harnessed to drive ATP synthesis.

F_1 Particles Have ATP Synthase Activity

- When the F1 particles and the membranous vesicles were separated from each other by centrifugation, the membranous fraction could still carry out electron transport but could no longer synthesize ATP; the two functions had to be uncoupled!!.

- The isolated F1 particles of ATP synthase are not capable of either electron transport or ATP synthesis but had ATPase activity.

- The ATP-generating capability of the membranous fraction was restored by adding the F1 particles back to the membranes.

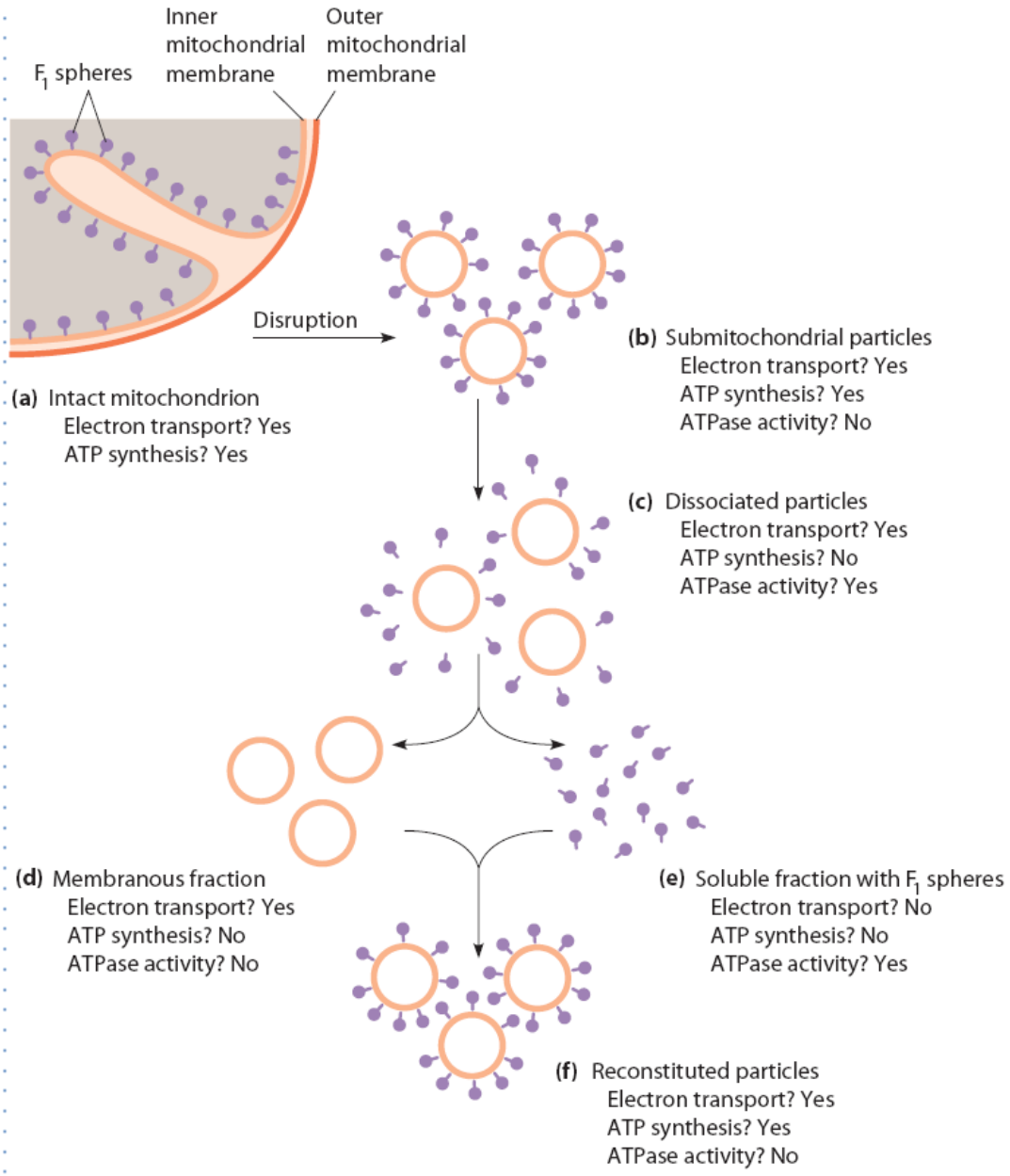
- Thus, these F1 particles were therefore referred to as coupling factors.

The FoF1 Complex: Proton Translocation Through Fo Drives ATP Synthesis by F1

- Although the F_1 portion of the F_oF_1 ATP synthase complex is not directly membrane bound, it is attached to the F_o complex that is embedded in the inner mitochondrial membrane.

- We now know that the F_o complex serves as the **proton translocator**, the channel through which protons flow across the mitochondrial inner membrane.

- Thus, the FoF1 complex is the complete, functional **ATP synthase.**

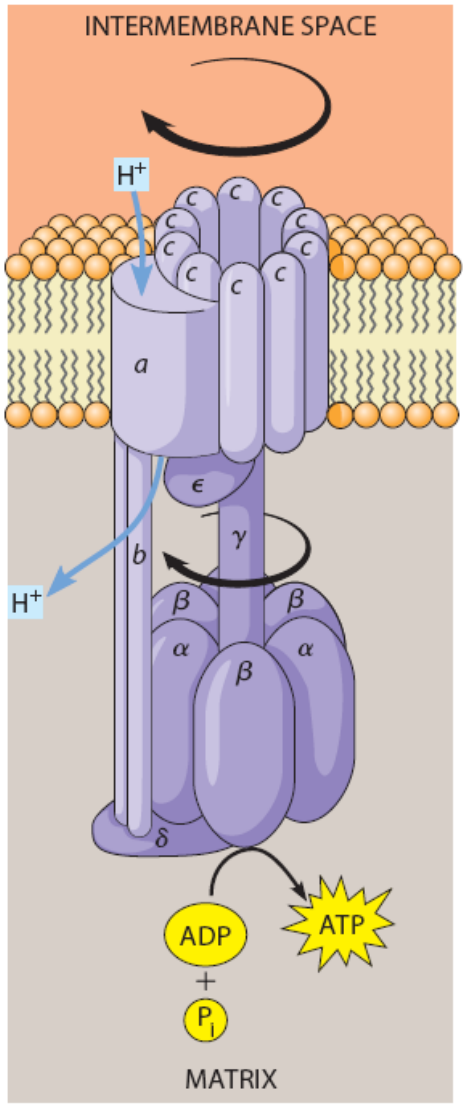


Dissociation and Reconstitution of the Mitochondrial ATP-Synthesizing System. (a) Intact mitochondria were disrupted so that fragments of the inner membrane formed (b) submitochondrial particles, capable of both electron transport and ATP synthesis. (c) When these particles were dissociated by mechanical agitation or enzyme treatment, the components could be separated into (d) a membranous fraction devoid of ATP-synthesizing capacity and (e) a soluble fraction with F_1 spheres having ATPase activity. (f) Mixing the two fractions reconstituted the structure and restored ATP synthase activity.

Polypeptide Composition of the *E. coli* F_oF_1 ATP Synthase (ATPase)*

Structure	Polypeptide	Molecular Weight	Number Present	Function
F_o	*a*	30,000	1	Proton channel
	b	17,000	2	Peripheral stator stalk connecting F_o and F_1
	c	8,000	10	Rotating ring that turns γ subunit of F_1
F_1	α	52,000	3	Promotes activity of β subunit
	β	55,000	3	Catalytic site for ATP synthesis
	δ	19,000	1	Anchors $\alpha_3\beta_3$ ring to stator stalk of F_o
	γ	31,000	1	Rotates to transmit energy from F_o to F_1
	ε	15,000	1	Anchors γ subunit to c_{10} ring of F_o

*Mitochondrial F_oF_1 complexes are similar to the bacterial complex but with somewhat different polypeptide compositions for F_o and F_1.



(a) **The F_o static component** consists of one *a* and two *b* subunits. The *a* subunit forms the proton channel and is immobilized in the membrane. The *b* subunits form the peripheral stalk and are attached both to the *a* subunit and to F_1.

(b) **The F_o mobile component** consists of a ring of 10 *c* subunits. Only one *c* subunit can form an ionic bond with the *a* subunit at a time. For each proton translocated, the ring rotates one-tenth of a turn as the adjacent *c* subunit in the ring bonds with the *a* subunit.

(c) **The F_1 static component** consists of the δ subunit plus a catalytic ring formed by a hexagon of alternating α and β subunits. The $\alpha_3\beta_3$ ring is the site of ATP synthesis and is immobilized by the δ subunit, which connects it to the b_2 peripheral stalk of F_o.

(d) **The F_1 mobile component** consists of the ϵ and γ subunits, which form the central stalk that is firmly attached to the c_{10} ring of F_o. As proton translocation turns the c_{10} ring, the γ subunit rotates inside the $\alpha_3\beta_3$ catalytic ring of F_1.

F_o and F_1 Components of the *E. coli* F_oF_1 ATP Synthase. This illustration shows the subunit composition of the static and mobile components of the F_o (**a**) and (**b**) and F_1 (**c**) and (**d**) complexes that comprise the functional F_oF_1 ATP synthase in *E. coli*. As 10 H^+ move through the F_o proton translocator, the ring of 10 *c* subunits in F_o rotates once, resulting in the synthesis of 3 ATP by the $\alpha_3\beta_3$ catalytic ring of F_1.

- The F_O complex is embedded in the bacterial membrane (or the inner mitochondrial membrane in eukaryotic cells) and consists of 1 *a* subunit, 2 *b* subunits, and 10 *c* subunits.

- The *a* and *b* subunits comprise the static component that is immobilized in the membrane.

- The ten *c* subunits form a ring that acts as a miniature gear that can rotate in the membrane relative to the *a* and *b* subunits.

- The *a* subunit functions as the proton channel, and the *b* subunits form the stator stalk that connects the surfaces of the F_O and F_1 complexes.

- The F_1 complex protrudes into the bacterial cytoplasm (or into the mitochondrial matrix in eukaryotic cells) and consists of 3 α subunits, 3 β subunits, plus 1 delta (δ), 1 gamma (γ), and 1 epsilon (ε) subunit.

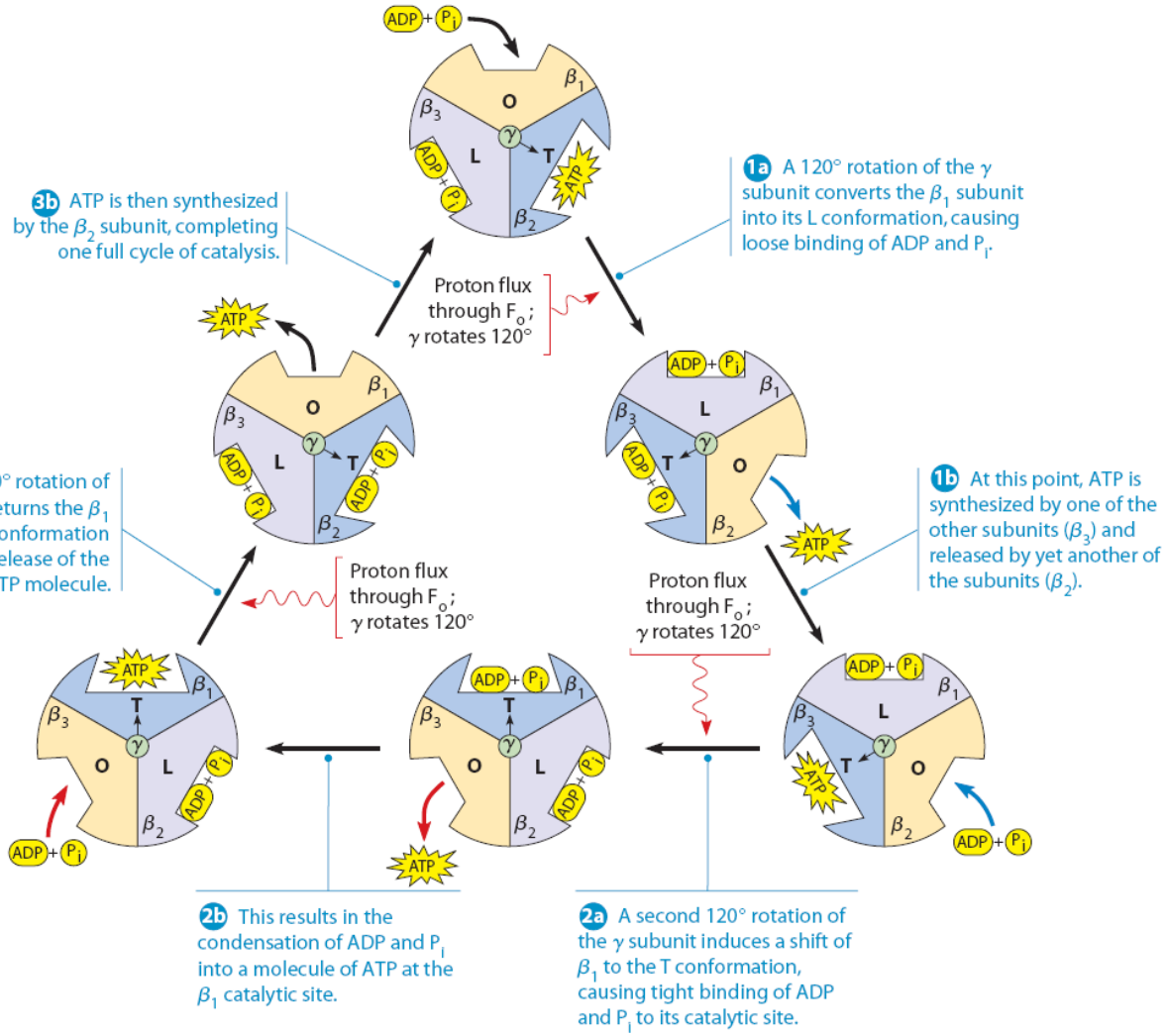
- ATP is synthesized by a catalytic ring of three $\alpha\beta$ complexes that form a hexagon of alternating subunits.

- The δ subunit of F1 anchors the $\alpha_3\beta_3$ catalytic ring to the b_2 stator stalk of F_o, immobilizing the catalytic ring.

- The mobile component of F_1 consists of the γ and ε subunits, which are attached to (and move with) the ring of *c* subunits in F_o.

- As protons move through the F_o proton channel, the $\boldsymbol{C}_{10}$ ring rotates, spinning the γ subunit within the $\alpha_3\beta_3$ catalytic ring.

- This results in ATP synthesis by the catalytic ring.



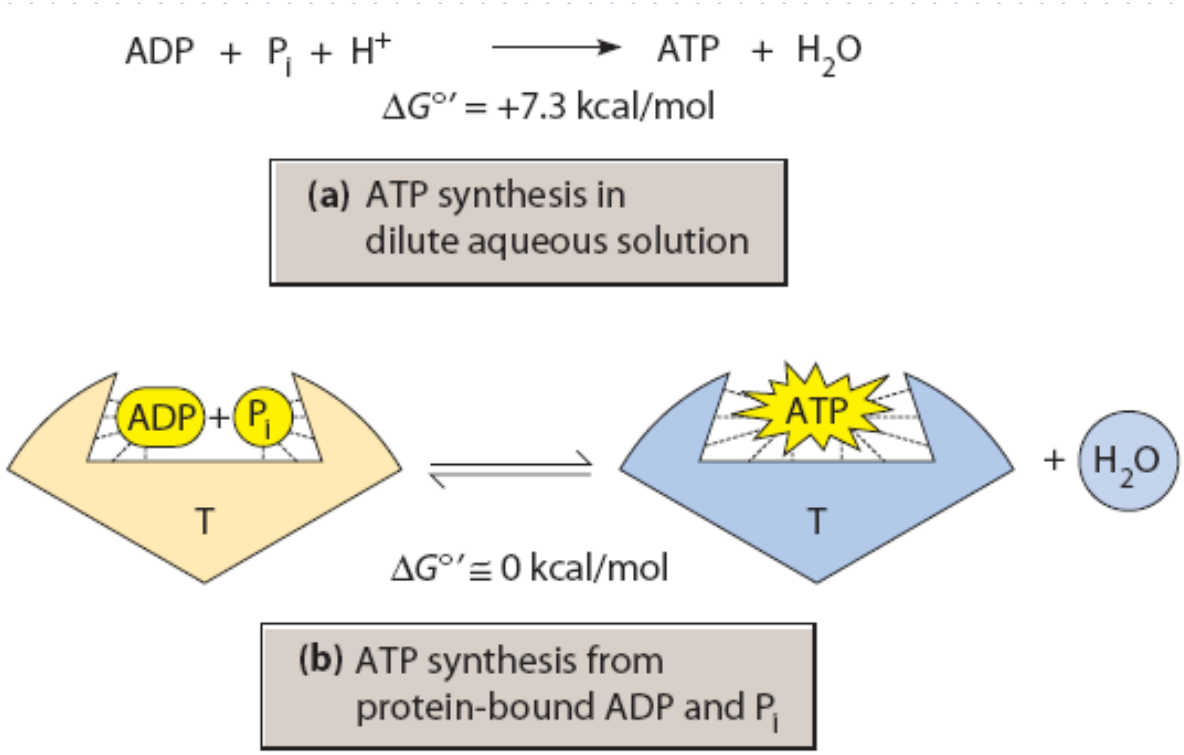
The Binding Change Model for ATP synthesis by the β Subunits of the F_oF_1 Complex. According to this model, each of the β subunits of the F_1 complex is in a different conformation at any instant. Each undergoes a sequence of conformational changes from the O (open) conformation through the L (loose) conformation to the T (tight) conformation. These conformational changes are driven by the rotation of the γ subunit. The process of ATP synthesis begins with one of the β subunits (arbitrarily identified as β_1) in its O conformation and involves the six steps shown, involving three 120° rotations and synthesis of 3 ATP per full 360° rotation. Notice that the same sequence of events occurs at each of the other sites, but it is offset temporally.

ATP Synthesis by FoF1 Involves Physical Rotation of the Gamma Subunit

- Once the link between electron transport within the membrane and proton pumping across the membrane had been established, the next piece of the puzzle was almost as daunting:
  - How does the exergonic flux of protons throughdrive the otherwise endergonic synthesis of ATP by the three b subunits of ?
- A novel answer to this question was suggested in 1979 by Paul Boyer, who proposed the **binding change model** (see the figure in the previous slide).

- Boyer's model envisioned the catalytic site on each of the three β subunits of the F_1 complex as progressing through three distinctly different conformations with quite different affinities for the substrates (ADP and) and the product (ATP).

- Boyer identified these as the L (for loose) conformation, which binds ADP and loosely; the T (for tight) conformation, which binds ADP and tightly and catalyzes their condensation into ATP; and the O (for open) conformation, which has a very low affinity for substrates or product and is unoccupied most of the time.



Comparative Energetics of ATP Synthesis. The energy requirement for ATP synthesis differs greatly, depending on the environment. (a) In dilute aqueous solution, ATP synthesis from soluble ADP and P_i is highly endergonic, with a $\Delta G^{\circ\prime}$ of +7.3 kcal/mol. (b) At the catalytic site of a β subunit in its T conformation, however, the environment is drastically different and the reaction has a $\Delta G^{\circ\prime}$ close to 0 (i.e., the K_{eq} is close to 1) and can therefore proceed spontaneously with no immediate energy requirement.

Summing it all up:

The Maximum ATP Yield of Aerobic Respiration Is 38 ATPs per Glucose

$$10NADH + 10H^+ + 5O_2 + 30ADP + 30P_i \longrightarrow 10NAD^+ + 10H_2O + 30ATP$$

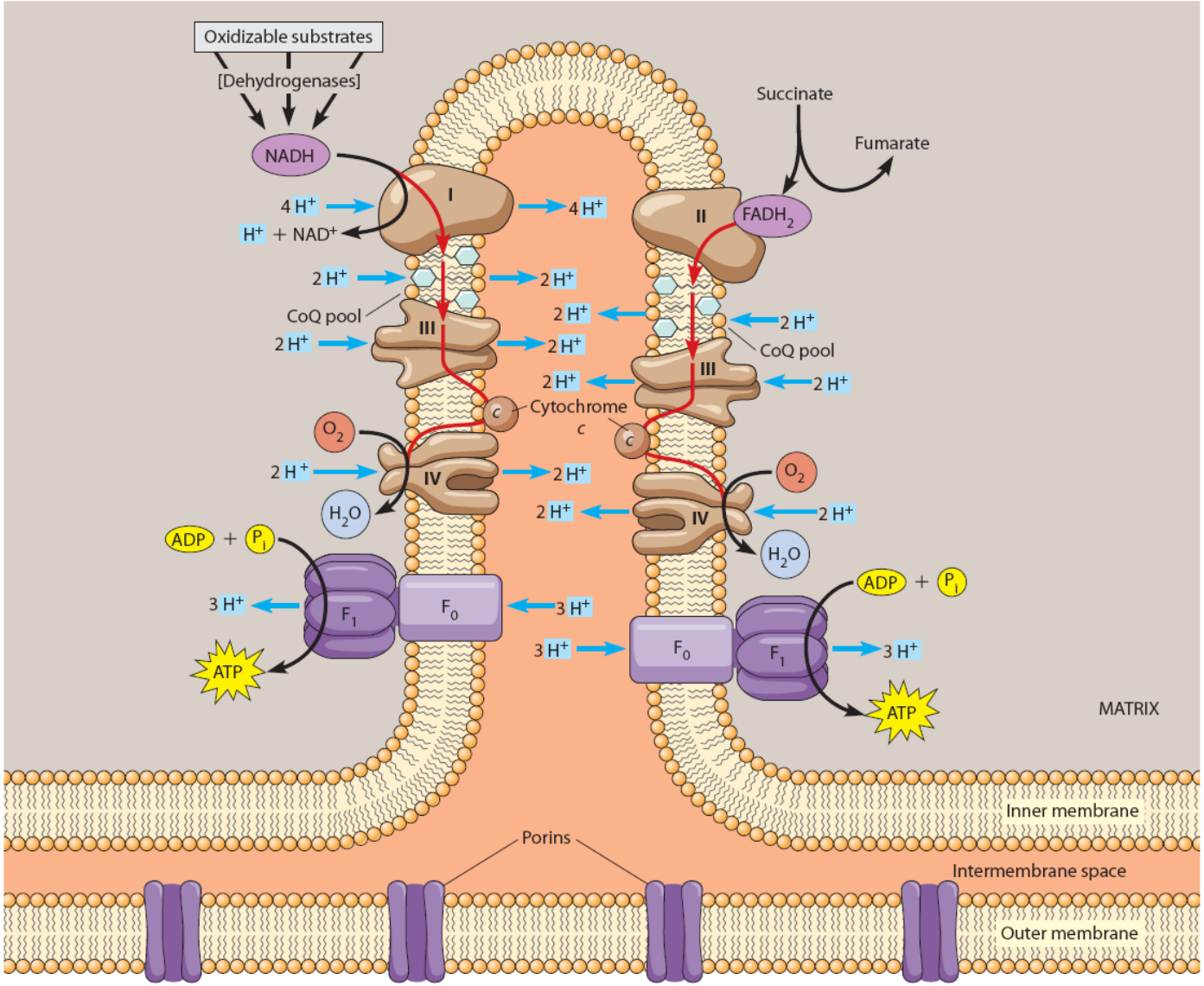
$$2FADH_2 + O_2 + 4ADP + 4P_i \longrightarrow 2FAD + 2H_2O + 4ATP$$

Summing these reactions gives us an overall reaction for electron transport and ATP synthesis:

$$10NADH + 10H^+ + 2FADH_2 + 6O_2 + 34ADP + 34P_i \longrightarrow 10NAD^+ + 2FAD + 12H_2O + 34ATP$$

Addition of ATP synthesized by glycolysis and the TCA cycle through "substrate level-phosphorylation" leads to the following overall expression for the maximum theoretical ATP yield obtainable by the complete aerobic respiration of glucose or other hexoses:

$$C_6H_{12}O_6 + 6O_2 \xrightarrow{38ADP + 38P_i \;\; 38ATP} 6CO_2 + 6H_2O$$



Dynamics of the Electrochemical Proton Gradient. Respiratory complexes I through IV are integral components of the inner mitochondrial membrane. Complexes I, III, and IV (but not complex II) couple the exergonic flow of electrons (red lines) through the complexes with the outward pumping of protons (blue) across the membrane. The proton motive force of the resulting electrochemical proton gradient drives ATP synthesis by F_1 as protons are translocated back across the membrane by the F_o complex, which is also embedded in the inner membrane.

Why Does the Maximum ATP Yield in Eukaryotic Cells Vary Between 36 and 38 ATPs Per Glucose?

- When glucose is catabolized aerobically in a eukaryotic cell, glycolysis gives rise to two molecules of NADH per glucose in the cytosol, while the catabolism of pyruvate generates another eight molecules of NADH in the matrix of the mitochondrion.

- This spatial distinction is important because the inner membrane of the mitochondrion does not have a carrier protein for NADH or NAD+ , so NADH generated in the cytosol cannot enter the mitochondrion to deliver its electrons to complex I of the ETS.

- Instead, the electrons and H+ ions are passed inward by one of several electron shuttle systems that differ in the number of ATP molecules formed per NADH molecule oxidized. An **electron shuttle system** consists of one or more electron carriers that can be reversibly reduced, with transport proteins present in the membrane for both the oxidized and the reduced forms of the carrier.

- In liver, kidney, and heart cells, electrons from cytosolic NADH are transferred into the mitochondrion by means of the *malate-aspartate shuttle*.

- After NADH reduces oxaloacetate in the cytosol to malate, the malate is transported into the mitochondrial matrix, where it reduces NAD+ to NADH.

- Thus, electrons derived from cytosolic NADH pass through all three proton-pumping complexes of the mitochondrial ETS and generate three molecules of ATP.

- Aspartate is transported back to the cytosol for production of more oxaloacetate to accept electrons from NADH.

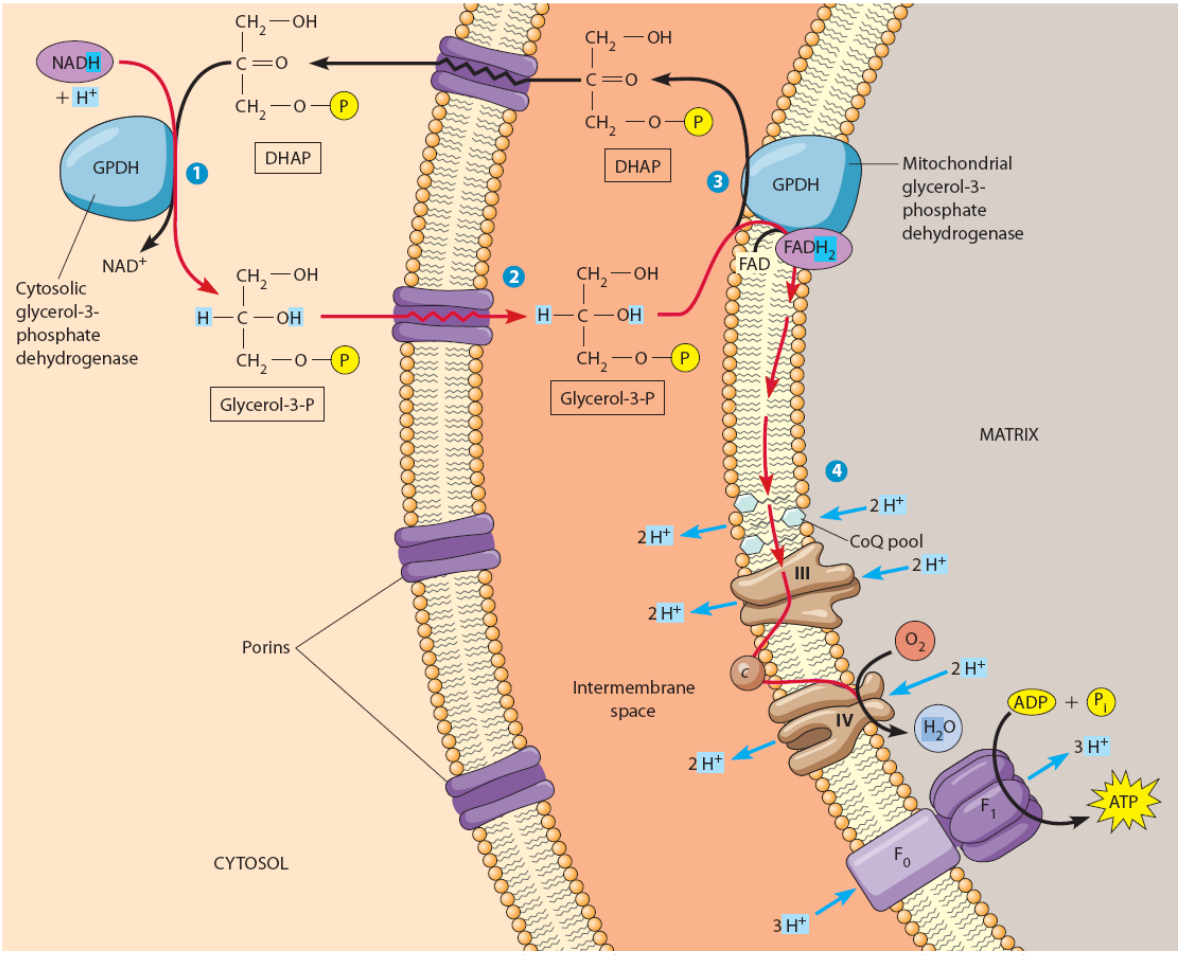
- In contrast, in skeletal muscle, brain, and other tissues, electrons are delivered from cytosolic NADH to the mitochondrial respiratory complexes by **glycerol phosphate shuttle** in which FAD rather than NAD+ is used as the mitochondrial electron acceptor.

- NADH in the cytosol reduces dihydroxyacetone phosphate (DHAP) to glycerol-3-phosphate, which is transported into the mitochondrion.

- There, glycerol-3-phosphate is reoxidized to DHAP by an enzyme that uses FAD instead of NAD+ as its electron acceptor.

- As a result, these electrons bypass complex I of the ETS, generating only two molecules of ATP instead of three.

- This reduces the maximum theoretical yield by one ATP per cytosolic NADH and therefore by two ATP molecules per molecule of glucose (from 38 to 36).



The Glycerol Phosphate Shuttle. The inner mitochondrial membrane is impermeable to NADH, so electrons from cytosolic NADH are moved into the mitochondrion by one of two shuttle mechanisms. Cells of skeletal muscle, brain, and other tissues use the glycerol phosphate shuttle for this purpose. The red line traces the path of electrons from cytosolic NADH to oxygen. ❶ The cytosolic enzyme glycerol-3-phosphate dehydrogenase (GPDH) uses electrons (and protons) from NADH to reduce dihydroxyacetone phosphate (DHAP) to glycerol-3-phosphate (glycerol-3-P), which ❷ can cross the outer mitochondrial membrane by diffusing through a porin channel. ❸ Glycerol-3-P is then reoxidized to DHAP by an FAD-linked GPDH in the inner membrane, with concomitant reduction of FAD to $FADH_2$. Because NADH is a more energy-rich coenzyme than $FADH_2$, the inward transport of electrons is exergonic, driven by the difference in the reduction potentials of the two coenzymes. The cost of this inward transport is a decreased ATP yield because ❹ electrons from the $FADH_2$ generated by the mitochondrial GPDH bypass complex I, thereby reducing the number of protons pumped across the inner membrane.

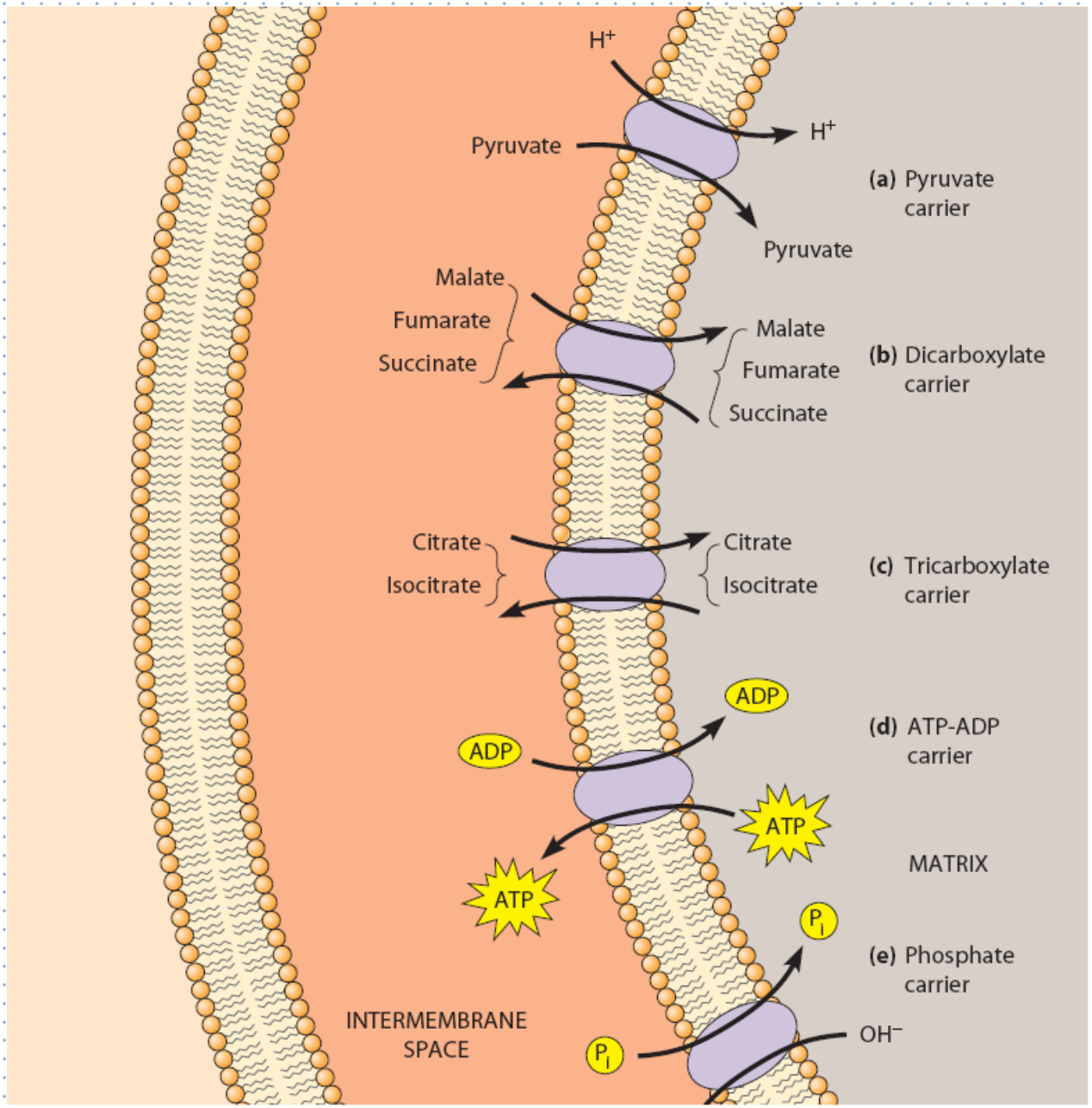
- This is because the pmf of the proton gradient provides the driving force not only for ATP synthesis, but for other energy-requiring reactions and processes.

- For example, some of the energy of the proton gradient is used to drive the transport of various metabolites and ions across the membrane and to drive the import of proteins into mitochondria.

- Depending on the relative concentrations of pyruvate, fatty acids, amino acids, and TCA-cycle intermediates in the cytosol and in the mitochondrial matrix, variable amounts of energy may be needed to ensure that the mitochondrion has adequate supplies of oxidizable substrates and TCA-cycle intermediates.

- Moreover, the inward transport of phosphate ions needed for ATP synthesis is accompanied by the concomitant outward movement of hydroxyl ions, which are neutralized by protons in the intermembrane space, thereby also diminishing the proton gradient.

- In some cells, the phosphate transporter can act as a symporter, transporting a phosphate ion and a proton inward simultaneously.



Major Transport Systems of the Inner Mitochondrial Membrane. The major transport proteins localized in the inner mitochondrial membrane are shown here. (**a**) The pyruvate carrier cotransports pyruvate and protons inward, driven by the pmf of the electrochemical proton gradient. The (**b**) dicarboxylate and (**c**) tricarboxylate carriers exchange organic acids across the membrane, with the direction of transport depending on the relative concentrations of dicarboxylic and tricarboxylic acids on the inside and outside of the inner membrane, respectively. (**d**) The ATP-ADP carrier exchanges ATP outward for ADP inward, and (**e**) the phosphate carrier couples the inward movement of phosphate with the outward movement of hydroxyl ions, which are neutralized by protons in the intermembrane space.

- The $\Delta Go'$ value for the complete oxidation of glucose to CO_2 and H_2O is –686 kcal/mol.

- ATP hydrolysis has a standard $\Delta Go'$ of about –7.3 kcal/mol, but the actual $\Delta G'$ under cellular conditions is typically in the range of –10 to –14 kcal/mol.

- Assuming a value of 10 kcal/mol, the 36–38 moles of ATP generated by aerobic respiration of 1 mole of glucose in an aerobic cell correspond to about 360–380 kcal of energy conserved per mole of glucose oxidized.

- This is an efficiency of about 52–55%, well above that obtainable with the most efficient machines we are capable of creating.

SUMMARY

Cellular Respiration: Maximizing ATP Yields

- Compared with fermentation, aerobic respiration gives the cell access to much more of the free energy that is available from organic substrates such as sugars, fats, and proteins by using molecular oxygen as a terminal electron acceptor.

- The complete catabolism of glucose derived from carbohydrates begins with glycolysis in the cytosol, forming pyruvate. Pyruvate enters the mitochondrion, where it is oxidatively decarboxylated to acetyl CoA, releasing CO_2. Acetyl CoA is then oxidized fully to CO_2 by enzymes of the TCA cycle.

SUMMARY

The Mitochondrion:Where the Action Takes Place

- Mitochondria are the site of respiratory metabolism in eukaryotic cells and are prominent organelles, especially in cells requiring a large amount of ATP. They may form large, interconnected networks in some cell types but are typically regarded as discrete organelles.

- A mitochondrion is surrounded by two membranes. The outer membrane is freely permeable to ions and small molecules due to the presence of porins. The inner membrane is a significant permeability barrier that contains specific carriers for the inward transport of pyruvate, fatty acids, and other organic molecules into the mitochondrial matrix.

- The inner membrane has many infoldings called cristae, which greatly increase the surface area of the membrane and hence its ability to accommodate the numerous respiratory complexes, F1Fo complexes, and transport proteins needed for respiratory function. Spaces within the cristae provide a region where protons pumped across the membrane during electron transport are concentrated in order to drive subsequent ATP synthesis.

SUMMARY
The Tricarboxylic Acid Cycle: Oxidation in the Round

- Pyruvate from glycolysis is oxidatively decarboxylated to acetyl CoA, generating NADH and CO_2. Acetyl CoA enters the TCA cycle by combining with oxaloacetate to form citrate.
- As citrate is converted to succinate, two molecules of CO_2 are released, and 2 NADH are formed. This involves two oxidative decarboxylation steps plus an ATP-generating step. Succinate is then oxidized and converted to oxaloacetate, generating $FADH_2$ and NADH.
- Several of the TCA cycle enzymes are subject to allosteric regulation. Three of the products of pyruvate catabolism (NADH, ATP, and acetyl CoA) act as inhibitors. The substrates ,NAD+, AMP, ADP, and CoA act as activators.
- The TCA cycle is also important in the catabolism of fats and proteins for energy. Fatty acid catabolism via b oxidation occurs in the mitochondrial matrix, generating acetyl CoA, which then enters the TCA cycle. Proteins are degraded to amino acids, which are converted to intermediates of either the glycolytic pathway or the TCA cycle.
- In addition, several intermediates of the TCA cycle serve as a source of precursors for anabolic pathways such as amino acid and heme synthesis. In certain plant seeds, acetyl CoA from stored fat can be converted to carbohydrates.

SUMMARY
Electron Transport: Electron Flow from Coenzymes to Oxygen

- Most of the energy yield from aerobic catabolism of glucose is obtained as the reduced coenzymes NADH and $FADH_2$ are reoxidized by an electron transport system. This system consists of several distinct respiratory complexes—large multiprotein assemblies embedded in the inner mitochondrial membrane.

- The respiratory complexes are free to move laterally within the membrane and assemble into larger supercomplexes known as respirasomes.

- Key intermediates in the electron transport system are coenzyme Q and cytochrome c, which transfer electrons between the complexes. In aerobic organisms, oxygen is the ultimate electron acceptor and water is the product of oxygen reduction.

SUMMARY
The Electrochemical Proton Gradient and ATP Synthesis

- Of the four main respiratory complexes, three (complexes I, III, and IV) couple the transfer of electrons to the outward pumping of protons. This establishes an electrochemical proton gradient that is the driving force for ATP generation.

- The ATP-synthesizing system consists of a proton translocator, Fo, embedded in the membrane and an ATP synthase, F_1, that projects from the inner membrane on the matrix side.

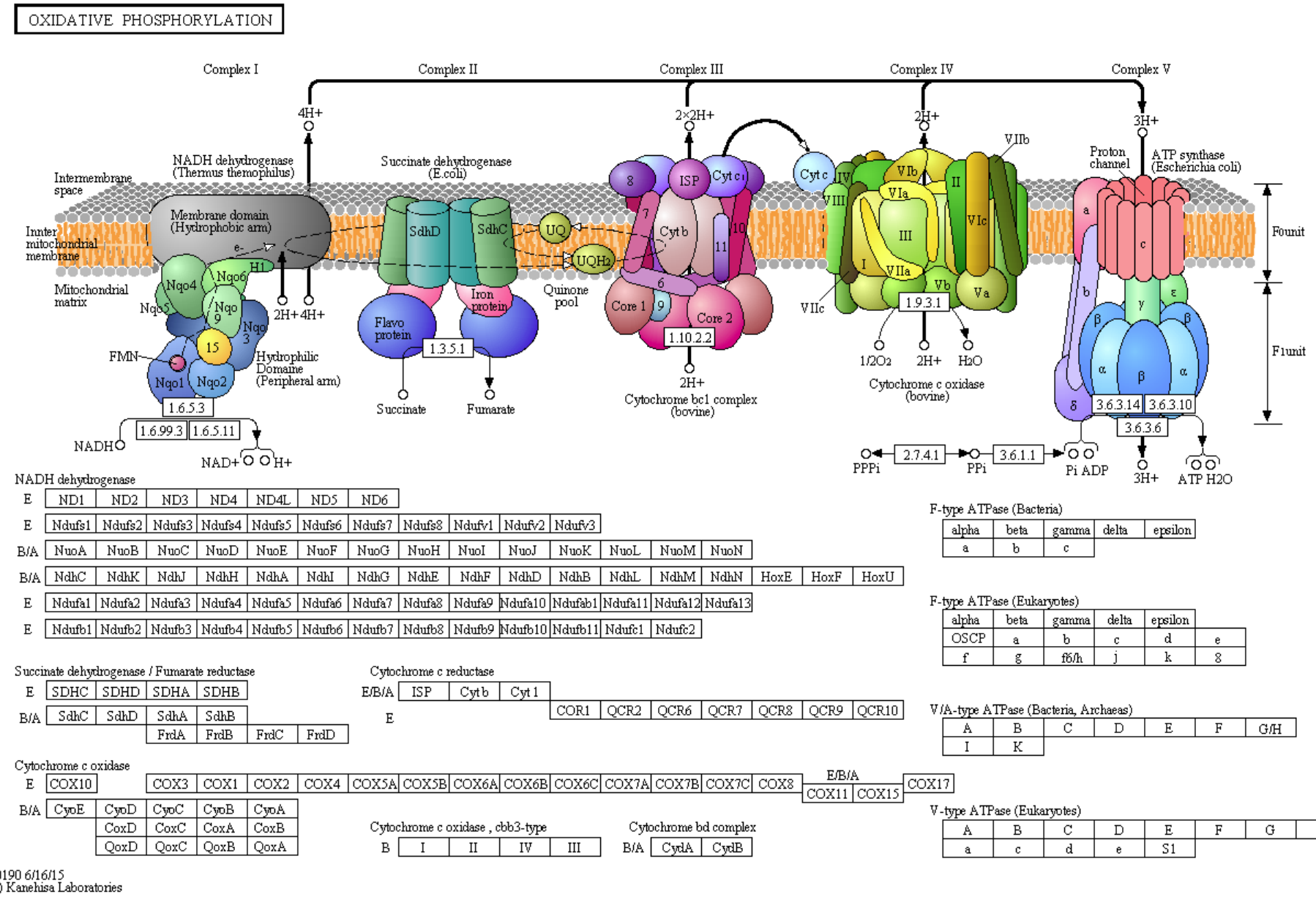
- ATP is synthesized by F1 as the proton gradient powers the movement of protons through Fo. Thus, the electrochemical proton gradient and ATP are, in effect, interconvertible forms of stored energy.

SUMMARY

Aerobic Respiration: Summing It All Up

- Complete oxidation of glucose to six molecules of CO_2 yields a total of 10 NADH, 2 $FADH_2$, and 4 ATP. Each NADH oxidized in the electron transport system results in synthesis of 3 ATP, and each $FADH_2$ oxidized gives 2 ATP.

- Therefore, the maximum ATP yield of aerobic respiration is 38 ATP per molecule of glucose. However, in some eukaryotic cells, the ATP yield is reduced to 36 as NADH generated by glycolysis in the cytosol passes its electrons to mitochondrial via the g FADH2 lycerol phosphate shuttle.


OXIDATIVE PHOSPHORYLATION
Complex I
Complex II
Complex III
Complex IV
Complex V
4H+
2×2H+
2H+
3H+
NADH dehydrogenase (Thermus thermophilus)
Succinate dehydrogenase (E.coli)
Intermembrane space
Inner mitochondrial membrane
Mitochondrial matrix
Membrane domain (Hydrophobic arm)
Hydrophilic Domaine (Peripheral arm)
Nqo1
Nqo2
Nqo3
Nqo4
Nqo5
Nqo6
Nqo9
FMN
15
H1
2H+ 4H+
1.6.5.3
1.6.99.3
1.6.5.11
NADH
NAD+ H+
SdhD
SdhC
UQ
UQH2
Quinone pool
Flavo protein
Iron protein
1.3.5.1
Succinate
Fumarate
8
ISP
Cyt c1
7
Cyt b
10
11
6
Core 1
9
Core 2
1.10.2.2
2H+
Cytochrome bc1 complex (bovine)
Cyt c
IV
VIIb
VIIIb
VIIa
II
VIIc
III
I
VIIIa
VIIIc
Vb
Va
1.9.3.1
1/2O2
2H+
H2O
Cytochrome c oxidase (bovine)
Proton channel
ATP synthase (Escherichia coli)
a
c
b
γ
ε
β
α
δ
3.6.3.14
3.6.3.10
3.6.3.6
Founit
F1unit
PPPi
2.7.4.1
PPi
3.6.1.1
Pi ADP
3H+
ATP H2O
NADH dehydrogenase
E ND1 ND2 ND3 ND4 ND4L ND5 ND6
E Ndufs1 Ndufs2 Ndufs3 Ndufs4 Ndufs5 Ndufs6 Ndufs7 Ndufs8 Ndufv1 Ndufv2 Ndufv3
B/A NuoA NuoB NuoC NuoD NuoE NuoF NuoG NuoH NuoI NuoJ NuoK NuoL NuoM NuoN
B/A NdhC NdhK NdhJ NdhH NdhA NdhI NdhG NdhE NdhF NdhD NdhB NdhL NdhM NdhN HoxE HoxF HoxU
E Ndufa1 Ndufa2 Ndufa3 Ndufa4 Ndufa5 Ndufa6 Ndufa7 Ndufa8 Ndufa9 Ndufa10 Ndufab1 Ndufa11 Ndufa12 Ndufa13
E Ndufb1 Ndufb2 Ndufb3 Ndufb4 Ndufb5 Ndufb6 Ndufb7 Ndufb8 Ndufb9 Ndufb10 Ndufb11 Ndufc1 Ndufc2
Succinate dehydrogenase / Fumarate reductase
E SDHC SDHD SDHA SDHB
B/A SdhC SdhD SdhA SdhB
FrdA FrdB FrdC FrdD
Cytochrome c reductase
E/B/A ISP Cyt b Cyt 1
E COR1 QCR2 QCR6 QCR7 QCR8 QCR9 QCR10
Cytochrome c oxidase
E COX10 COX3 COX1 COX2 COX4 COX5A COX5B COX6A COX6B COX6C COX7A COX7B COX7C COX8
E/B/A COX11 COX15 COX17
B/A CyoE CyoD CyoC CyoB CyoA
CoxD CoxC CoxA CoxB
QoxD QoxC QoxB QoxA
Cytochrome c oxidase , cbb3-type
B I II IV III
Cytochrome bd complex
B/A CydA CydB
F-type ATPase (Bacteria)
alpha beta gamma delta epsilon
a b c
F-type ATPase (Eukaryotes)
alpha beta gamma delta epsilon
OSCP a b c d e
f g f6/h j k 8
V/A-type ATPase (Bacteria, Archaeas)
A B C D E F G/H
I K
V-type ATPase (Eukaryotes)
A B C D E F G H
a c d e S1
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(c) Kanehisa Laboratories

Qs

Calculation of the Maximum ATP Yield from Aerobic Oxidation of Glucose

Stage of Respiration	Glycolysis (glucose → 2 pyruvate)	Pyruvate Oxidation (2 pyruvate → 2 acetyl CoA)	TCA Cycle (2 turns)
Yield of CO_2			
Yield of NADH			
ATP per NADH			
Yield of $FADH_2$			
ATP per $FADH_2$			
ATP from substrate-level phosphorylation			
ATP from oxidative phosphorylation			
Maximum ATP yield			

MBG304 Biochemistry

Lecture 5: Photosynthesis (ETC and Photophosphorylation)

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Aerobic respiration

- Up to here we have seen the five stages of aerobic respiration (in non-photosyntehtic cells):
  1. Glycolysis
  2. Pyruvate oxidation
  3. TCA (a.k.a. Krebs Cycle, Citric Acid Cycle) cycle
  4. ETS
  5. OP

- In plants (i.e., photosyntehtic organisms)
  - Same 5 stages of aerobic respiration are in operation (remember, plant cells have mitochpndria as well as chloroplast, the photosynthetic machinery for energy generation and synthesis of organic molecules such as glucose)
  - However, plants carry out also a “Photosynthetic Electron Transport and ATP Synthesis”

Phototrophic Energy Metabolism: Photosynthesis

- In this lecture, we will learn:
  - how photosynthetic organisms produce the chemical energy and organic carbon that are drained from the biosphere by chemotrophs.
  - How these photosynthetic organisms use solar energy to drive the reduction of CO_2 to produce carbohydrates, fats, and proteins—the reduced forms of carbon that all chemotrophs depend upon.

- The use of solar energy to drive the anabolic pathways that produce these building blocks of life is named **photosynthesis**—the conversion of light energy to chemical energy and its subsequent use in synthesizing organic molecules.

- **Phototrophs** are organisms that convert solar energy to chemical energy in the form of ATP and the reduced coenzyme NADPH.

- NADPH is the electron and hydrogen carrier for a large number of anabolic pathways and is closely related to NADH.

Phototrophs

- Phototrophs are two main type:

1. **Photoautotrophs:** plants, algae, and most photosynthetic bacteria. They use solar energy to synthesize energy-rich organic molecules from simple inorganic starting materials such as carbon dioxide and water.

2. **Photoheterotrophs,** acquire energy from sunlight but depend on organic sources of reduced carbon (e.g., halobacteria)

- Many photoautotrophs release molecular oxygen as a by-product of photosynthesis.

- Thus, phototrophs not only replenish reduced carbon in the biosphere but also provide the oxygen in the atmosphere used by aerobic organisms to oxidize these reduced compounds for energy.

Photosynthetic Electron Transport and ATP Synthesis .swf

Photosynthesis: An Overview

- Photosynthesis involves two major biochemical processes:

1. Energy transduction

   light energy is captured by chlorophyll molecules and converted to chemical energy in the form of ATP and NADPH.

2. Carbon assimilation

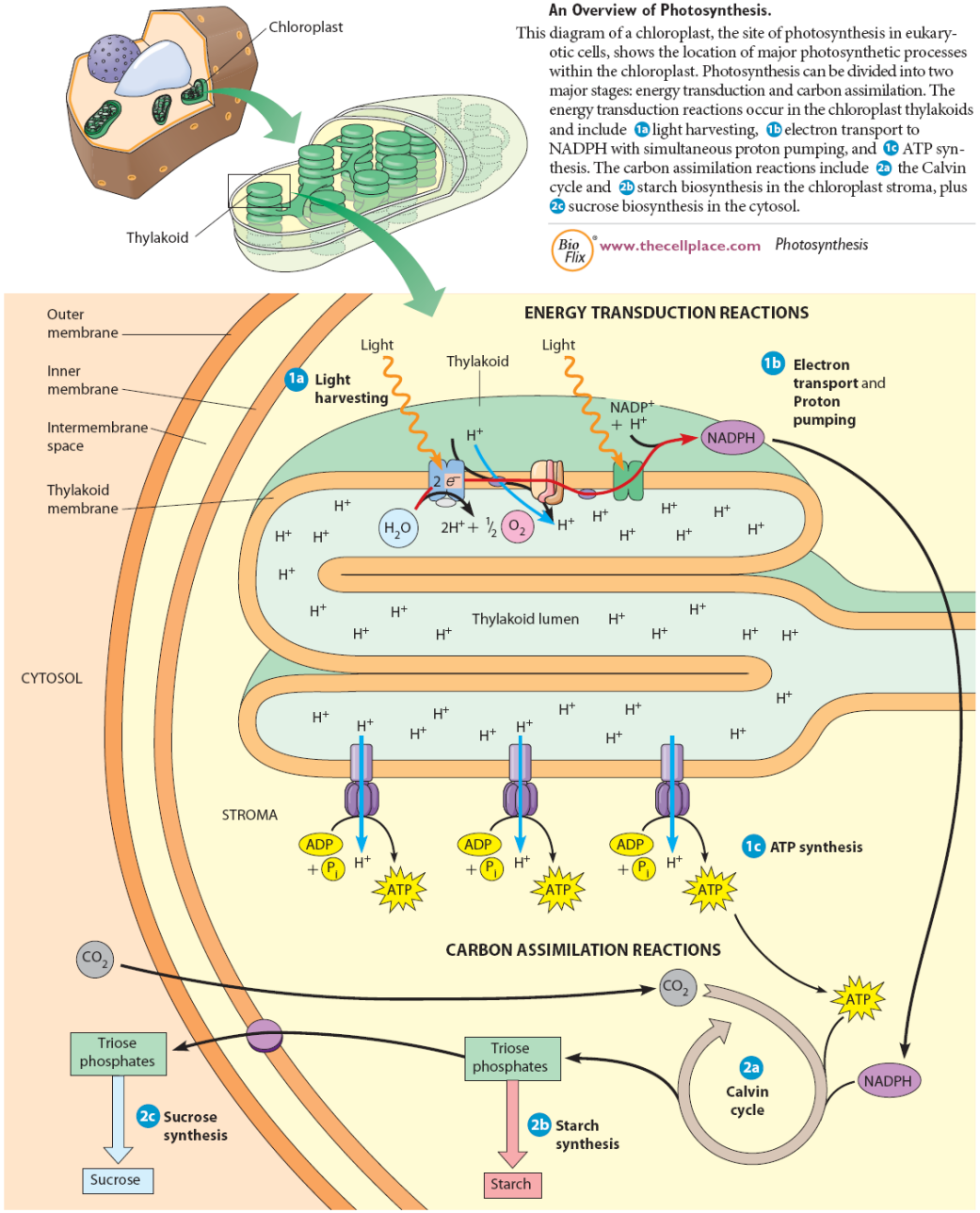
   The ATP and NADPH provide energy and reducing power, respectively, for the **carbon assimilation reactions, a.k.a.** carbon fixation reactions (Calvin cycle).

- Both processes take place in the chloroplast of eukaryotic phototrophs or in specialized membrane systems of photosynthetic bacteria.

Converting Solar Energy to Chemical Energy

- Light energy from the sun is captured by a variety of green pigment molecules called chlorophylls, which are present in the green leaves of plants and in the cells of algae and photosynthetic bacteria.

- Light absorption by a *chlorophyll* molecule excites one of its electrons, which is then ejected from the molecule and flows energetically downhill through an electron transport system (ETS) much like the ETS we saw previously in mitochondria.



An Overview of Photosynthesis.

This diagram of a chloroplast, the site of photosynthesis in eukaryotic cells, shows the location of major photosynthetic processes within the chloroplast. Photosynthesis can be divided into two major stages: energy transduction and carbon assimilation. The energy transduction reactions occur in the chloroplast thylakoids and include 1a light harvesting, 1b electron transport to NADPH with simultaneous proton pumping, and 1c ATP synthesis. The carbon assimilation reactions include 2a the Calvin cycle and 2b starch biosynthesis in the chloroplast stroma, plus 2c sucrose biosynthesis in the cytosol.

Bio Flix® www.thecellplace.com *Photosynthesis*

- As in mitochondria, this flow of electrons in chloroplast is coupled to unidirectional proton pumping, which first stores energy in an electrochemical proton gradient that subsequently drives an ATP synthase.

- While in mitochondria this process is known as ***oxidative phosphorylation***—ATP synthesis driven by energy derived from the oxidation of organic compounds, in photosynthetic organisms, ATP synthesis driven by energy derived from the sun is called ***photophosphorylation***.

- To incorporate fully oxidized carbon atoms from carbon dioxide into organic molecules, these carbon atoms must be reduced.

- Therefore, photoautotrophs need not only energy in the form of ATP but also reducing power, which is provided by NADPH, a coenzyme related to NADH.

- In **oxygenic *phototrophs***—*plants, algae,* and *cyanobacteria*—"water" is the electron donor, and light energy absorbed by chlorophyll powers the movement of two electrons from water to $NADP^+$, which is reduced to NADPH.

- Molecular oxygen is released, as water is oxidized.

- In **anoxygenic phototrophs**—*green* and *purple photosynthetic bacteria*—compounds such as sulfide S^{2-}, thiosulfate $S_2O_3^{-2}$, or succinate serve as electron donors, and oxidized forms of these compounds are released.

- In both **oxygenic** and **anoxygenic** phototrophs, the light-dependent generation of NADPH is called *photoreduction*.

The Carbon Assimilation

- Most of the energy accumulated within photosynthetic cells by the light-dependent generation of ATP and NADPH is used for carbon dioxide fixation and reduction.

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

- The immediate product of photosynthetic carbon fixation is a three-carbon sugar (a triose), not a hexose as shown above.

- These trioses are then used for a variety of biosynthetic pathways, including the biosynthesis of glucose, sucrose, and starch.

- Sucrose is the major transport carbohydrate in most plant species. It conveys energy and reduced carbon through the plant from photosynthetic cells to nonphotosynthetic cells.

The Chloroplast: Photosynthetic Organelle

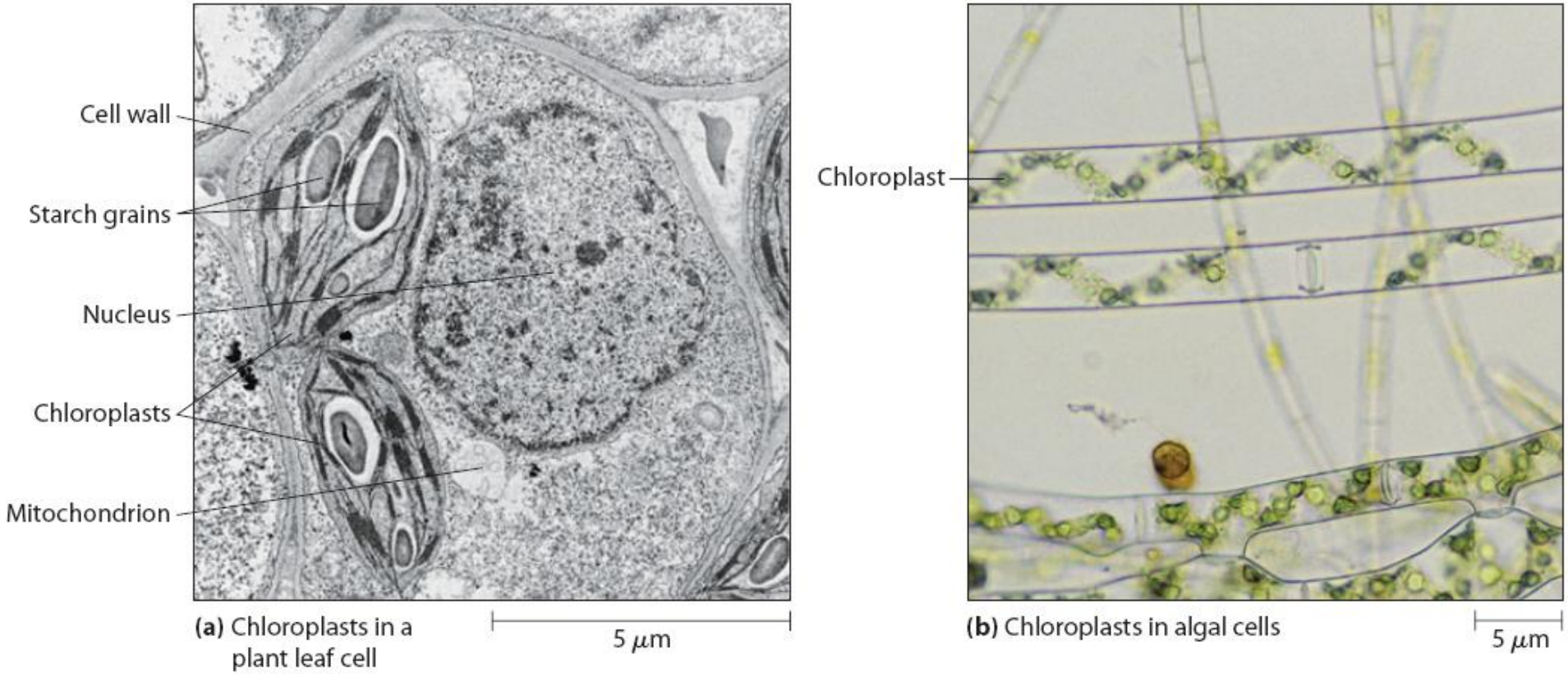
- A plant leaf cell usually contains 20–100 chloroplasts, whereas an algal cell typically contains only one or a few chloroplasts.

- Chloroplasts are composed of three membrane systems. Like a mitochondrion, it has both an **outer membrane** and an **inner membrane**, often separated by a narrow **intermembrane space**.

- *The inner membrane* encloses the **stroma**, a gel-like matrix filled with enzymes for carbon, nitrogen, and sulfur reduction and assimilation.

- *The outer membrane* contains transmembrane proteins called **porins** that are similar to those found in the outer membrane of mitochondria.

- In addition to these two membranes, chloroplasts have **thylakoids**, a third membrane system inside the chloroplast that creates an internal compartment.



(a) Chloroplasts in a plant leaf cell

(b) Chloroplasts in algal cells

Chloroplasts. (a) The prominence of chloroplasts in a leaf cell of a plant is demonstrated by this electron micrograph of a parenchyma cell from a *Coleus* leaf. The cell contains many chloroplasts, three of which are seen in this particular cross section. The presence of large starch granules in the chloroplasts indicates that the cell was photosynthetically active just prior to fixation for electron microscopy (TEM). (b) This light micrograph reveals the unusual ribbon-shaped chloroplasts in cells of the filamentous green alga *Spirogyra*.

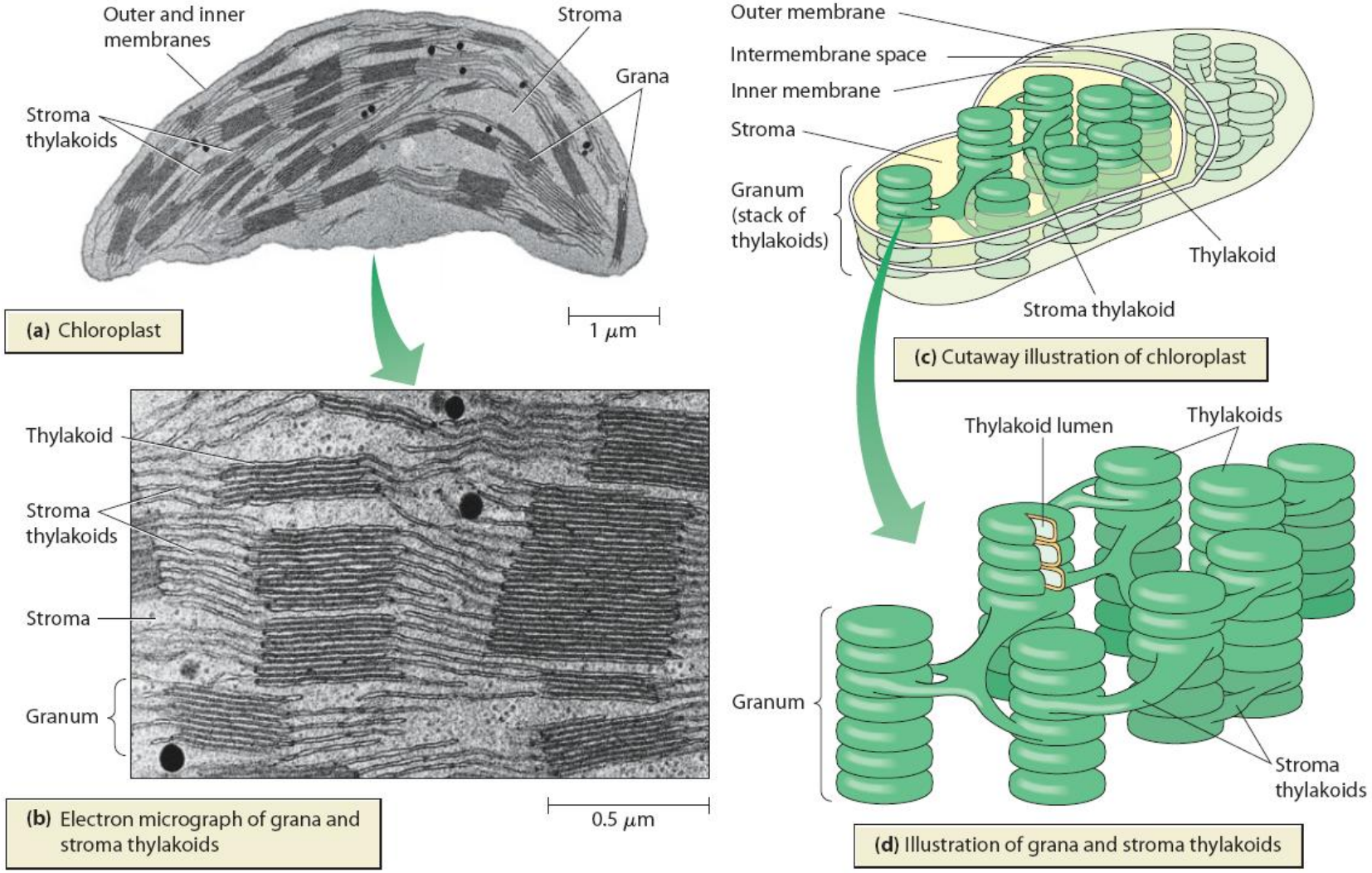
- Because porins permit the passage of solutes with molecular weights up to about 5000, the outer membrane is freely permeable to most small organic molecules and ions.

- However, the inner membrane forms a significant permeability barrier. Transport proteins in the inner membrane control the flow of most metabolites between the intermembrane space and the stroma.

- Three important metabolites that are able to diffuse freely across both the outer and the inner membranes are:
  1. Water
  2. carbon dioxide
  3. oxygen

- Thylakoids are flat, saclike structures suspended in the stroma and they are usually arranged in stacks called **grana** (singular: **granum**).

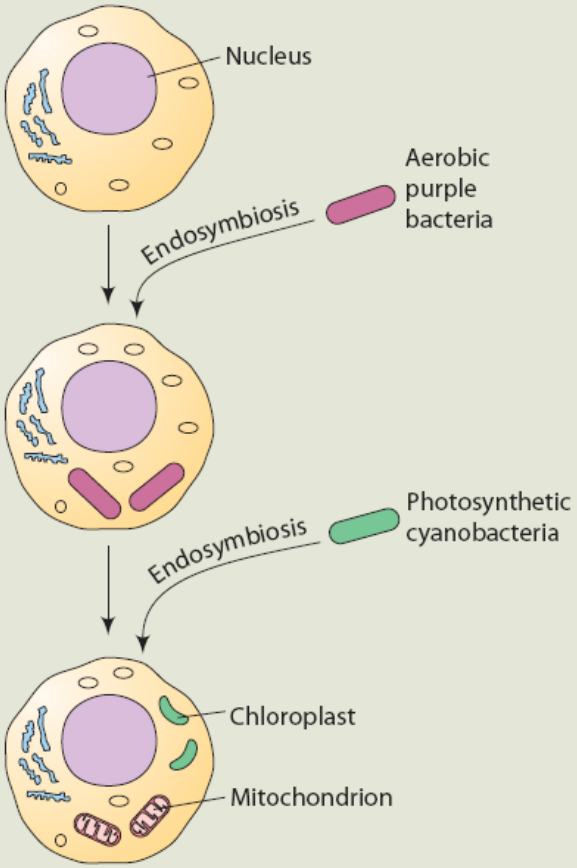
- Essentially all of the photosynthetic components, such as pigments, enzymes, and electron carriers, are localized on or in these thylakoid membranes.



Structural Features of a Chloroplast. (a) An electron micrograph of a chloroplast from a leaf of timothy grass *(Phleum pratense)* (TEM). (b) A more magnified electron micrograph of the chloroplast in part a shows the arrangement of grana and stroma thylakoids (TEM). (c) An illustration showing the three-dimensional structure of a typical chloroplast. (d) An illustration depicting the continuity of the thylakoid membranes, the arrangement of thylakoids into stacks called grana, and the stroma thylakoids that interconnect the grana. The thylakoid membranes enclose a separate compartment called the thylakoid lumen.

- Photosynthetic bacteria do not have chloroplasts. Cyanobacteria appear to be free-living chloroplasts.

- Similarities among mitochondria, chloroplasts, and bacterial cells have led biologists to formulate the *endosymbiont theory*, which suggests mitochondria and chloroplasts evolved from bacteria that were engulfed by primitive cells 1 to 2 billion years ago.



Major Events That Might Have Occurred During the Evolution of Eukaryotic Cells. Considerable evidence exists for an endosymbiotic origin for mitochondria and chloroplasts. Most biologists agree that the primitive cells that were ingested by protoeukaryotes and then evolved into mitochondria and chloroplasts were, respectively, purple bacteria and cyanobacteria. This is based on similarities in size and membrane lipid composition, comparisons of rRNA base sequences, the presence of circular DNA molecules, and the ability to reproduce autonomously.

Photosynthetic Energy Transduction I: Light Harvesting

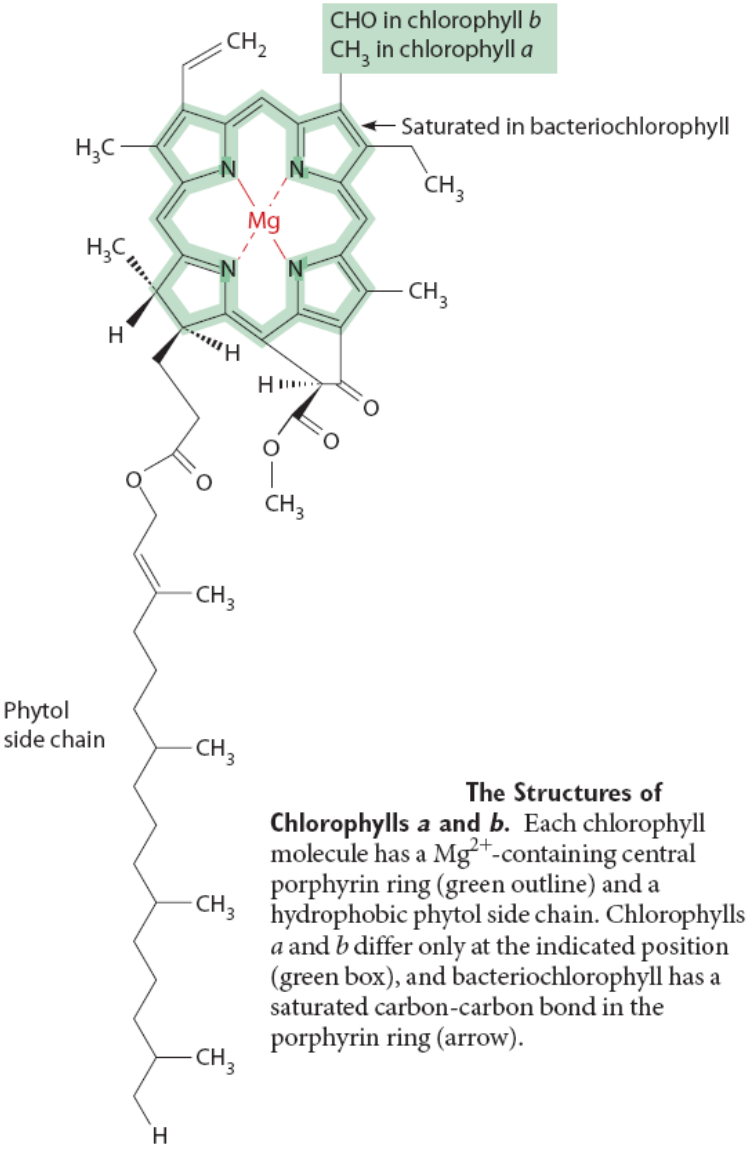
- The first stage of photosynthetic energy transduction is the capture of light energy from the sun.

- When a photon is absorbed by a **pigment** (lightabsorbing molecule), such as chlorophyll, the energy of the photon is transferred to an electron, which is energized.

- This event, called **photoexcitation**, is the first step in photosynthesis.

Chlorophyll Is Life's Primary Link to Sunlight

- **Chlorophyll** is the primary energy-transduction pigment that channels solar energy into the biosphere.
- The structures of chlorophylls *a* and *b* are shown in figure in left.
- The skeleton of each molecule consists of a central **porphyrin ring** and a strongly hydrophobic phytol side chain which interacts with lipids of the thylakoid or cyanobacterial membranes, anchoring the light-absorbing molecules in these membranes.



The Structures of Chlorophylls *a* and *b*. Each chlorophyll molecule has a Mg^{2+}-containing central porphyrin ring (green outline) and a hydrophobic phytol side chain. Chlorophylls *a* and *b* differ only at the indicated position (green box), and bacteriochlorophyll has a saturated carbon-carbon bond in the porphyrin ring (arrow).

- The magnesium ion (Mg^{2+}) found in chlorophylls ***a*** and ***b*** affects the electron distribution in the porphyrin ring.

- As a result, several specific wavelengths of light can be absorbed. Chlorophyll *a*, for example, has a broad absorption spectrum, with maxima at about 420 and 660 nm.

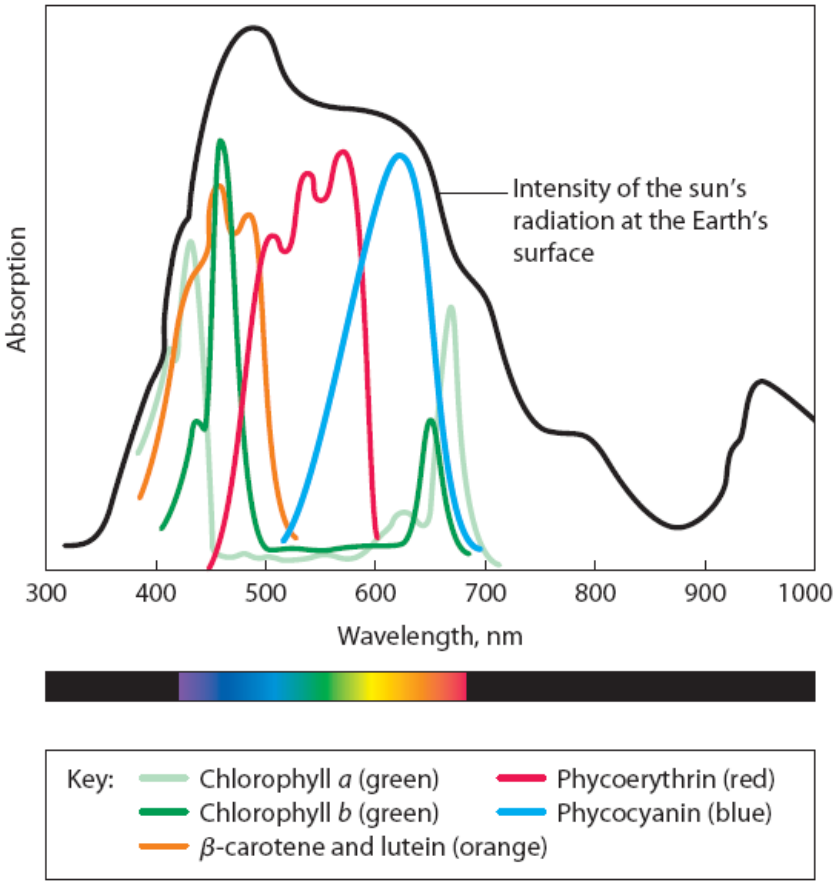
- Chlorophyll *b* is distinguished from chlorophyll *a* by the presence of a formyl (—CHO) group in place of one of the methyl ($—CH_3$) groups on the porphyrin ring.

- This minor structural alteration shifts the absorption maxima toward the center of the visible spectrum.

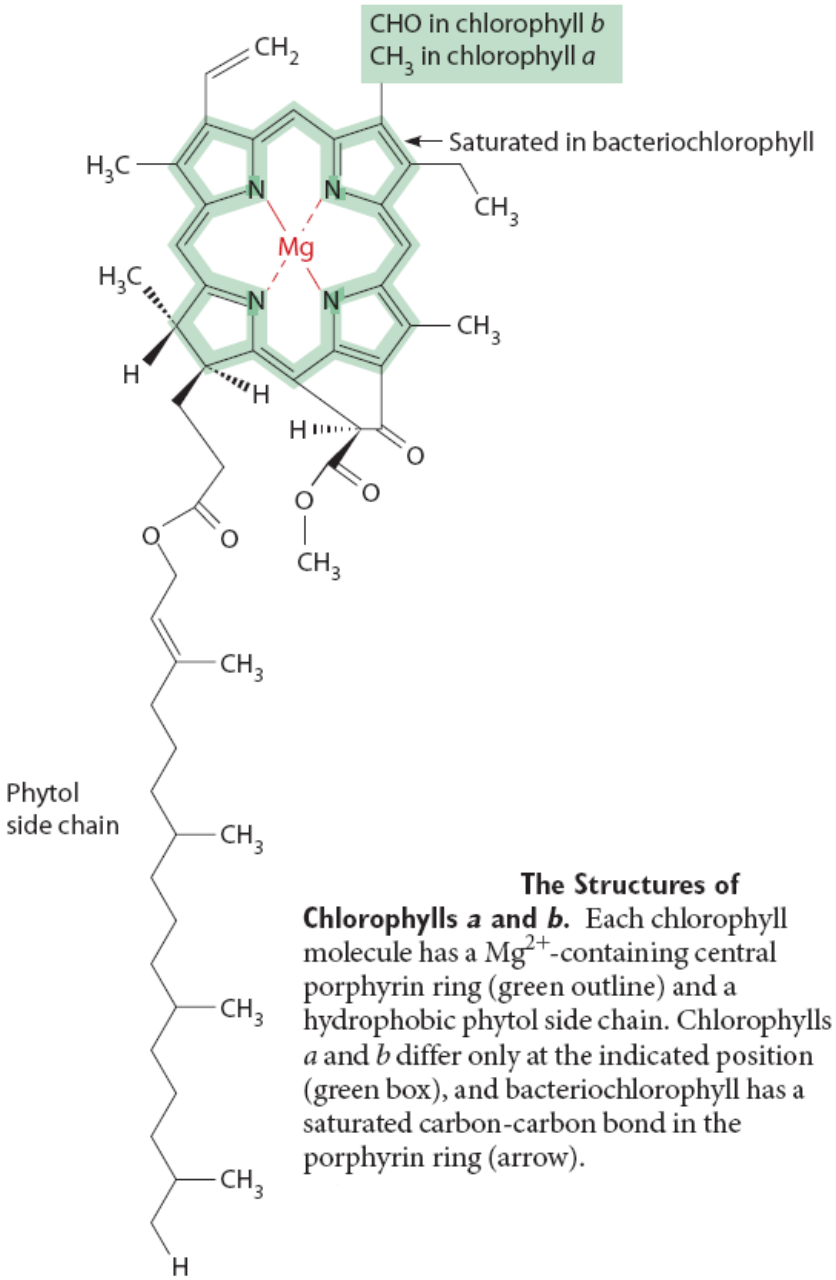
- All plants and green algae contain both chlorophyll *a* and *b*.

- Because chlorophylls absorb mainly **blue** and **red** wavelengths of light, they appear **green**.

- Other oxygenic photosynthetic organisms supplement chlorophyll *a* with either chlorophyll *c* (brown algae, diatoms, and dinoflagellates), chlorophyll *d* (red algae), or phycobilin (red algae and cyanobacteria).



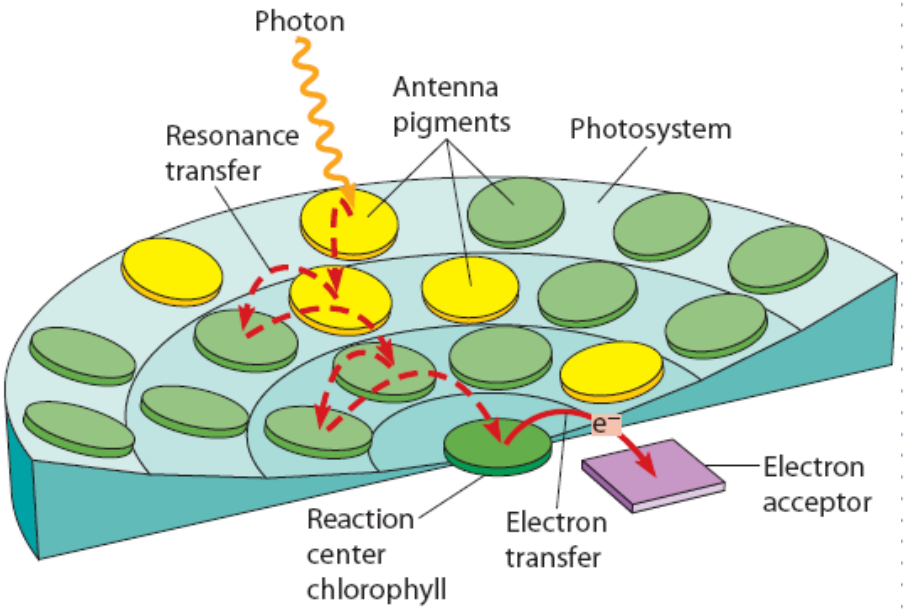
The Absorption Spectra of Common Photosynthetic Pigments. The graph shows absorption spectra of various chlorophylls and accessory pigments (colored lines) and compares them with the spectral distribution of solar energy reaching the Earth's surface (black line). The small reference strip below the graph shows the color that each wavelength appears to the human eye.



The Structures of Chlorophylls *a* and *b*. Each chlorophyll molecule has a Mg^{2+}-containing central porphyrin ring (green outline) and a hydrophobic phytol side chain. Chlorophylls *a* and *b* differ only at the indicated position (green box), and bacteriochlorophyll has a saturated carbon-carbon bond in the porphyrin ring (arrow).

- Most pigments of a photosystem serve as light-gathering **antenna pigments**, which collect light energy much like a radio antenna collects radio waves.

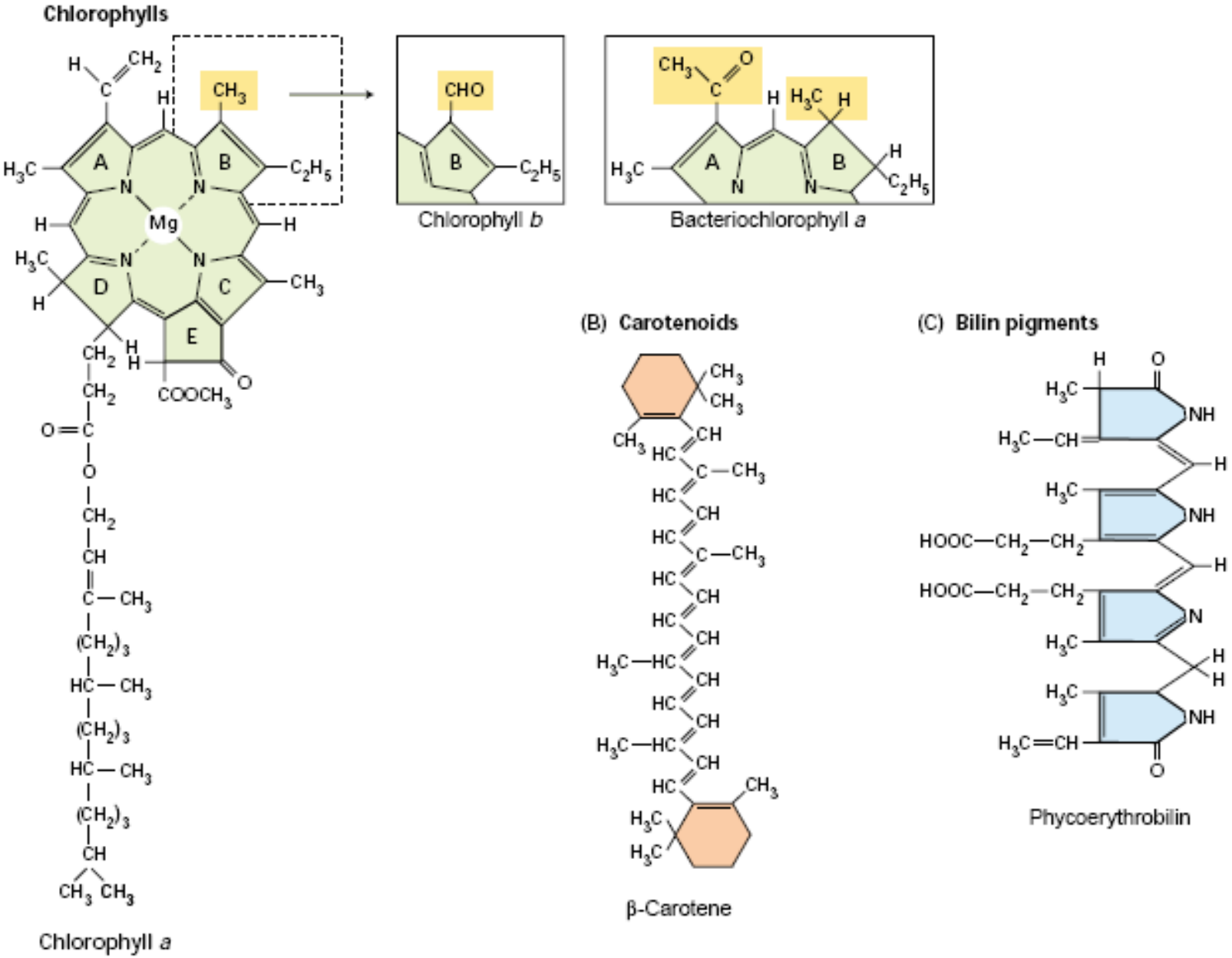
- These antenna pigments absorb photons and pass the energy to a neighboring chlorophyll molecule or accessory pigment by resonance energy transfer.



The Transfer of Energy to the Reaction Center of a Photosystem. Light energy absorbed by antenna pigments is passed along by resonance energy transfer until it reaches a specific chlorophyll *a* molecule at the reaction center of a photosystem. The energy is conserved as an excited electron that is ejected from the chlorophyll and passed along to an organic acceptor molecule.

- Most photosynthetic organisms also contain **accessory pigments**, which absorb photons that cannot be captured by chlorophyll.

- Two prominent types of accessory pigments are **carotenoids** (e.g., β-*carotene* and *lutein*) found in plants and **phycobilins** (e.g., *phycoerythrin* and *phycocyanin*) only found in red algae and cyanobacteria.


Chlorophylls
Chlorophyll b
Bacteriochlorophyll a
Chlorophyll a
(B) Carotenoids
β-Carotene
(C) Bilin pigments
Phycoerythrobilin

- Chlorophyll molecules, accessory pigments, and associated proteins are organized into functional units called **photosystems**, which are localized to thylakoid or photosynthetic bacterial membranes.

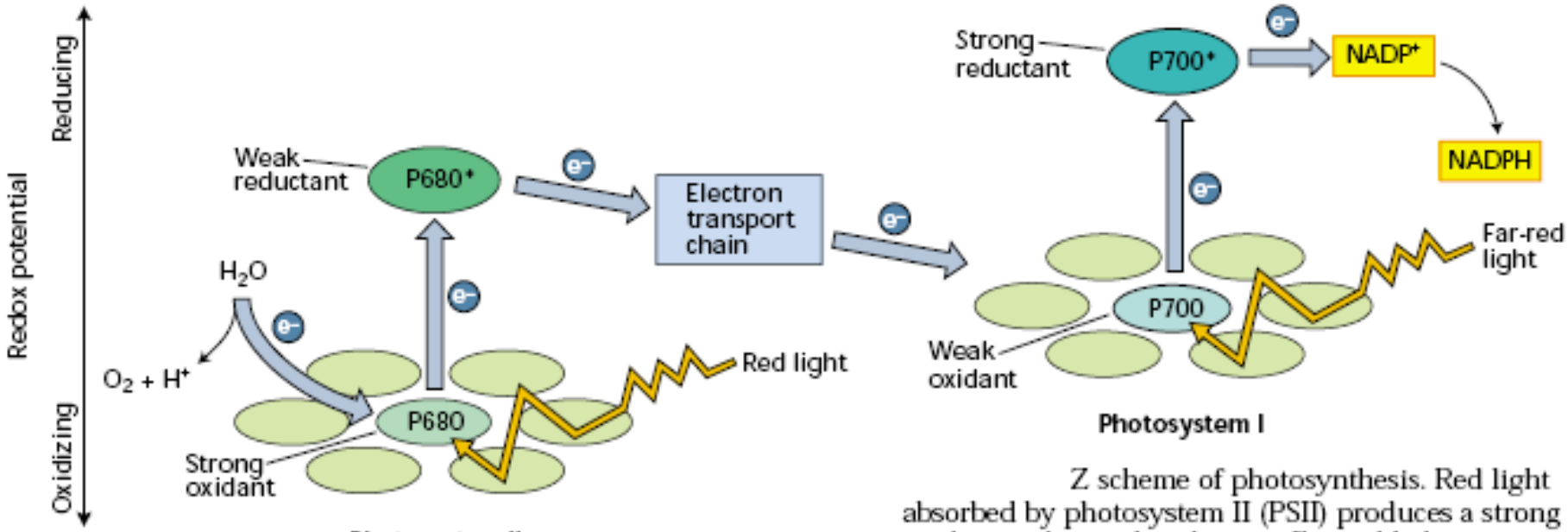
- The chlorophyll molecules are anchored to the membranes by the long hydrophobic phytol side chains.

Oxygenic Phototrophs Have Two Types of Photosystems

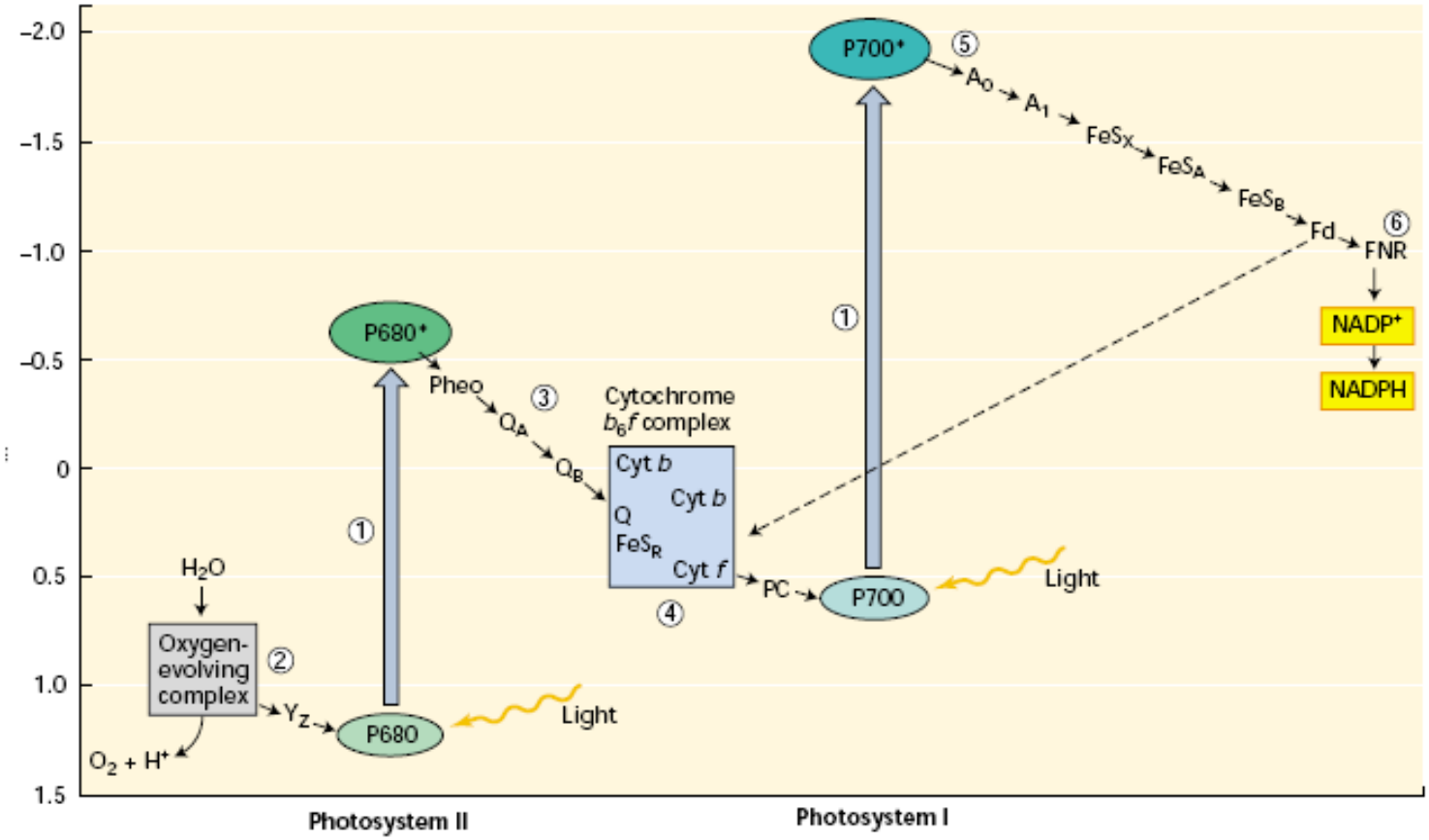
- **Photosystem I (PSI)** has an absorption maximum of 700 nm, whereas **photosystem II (PSII)** has an absorption maximum of 680 nm.

- Each electron that passes from water to $NADP^+$ must be photoexcited once by each photosystem.

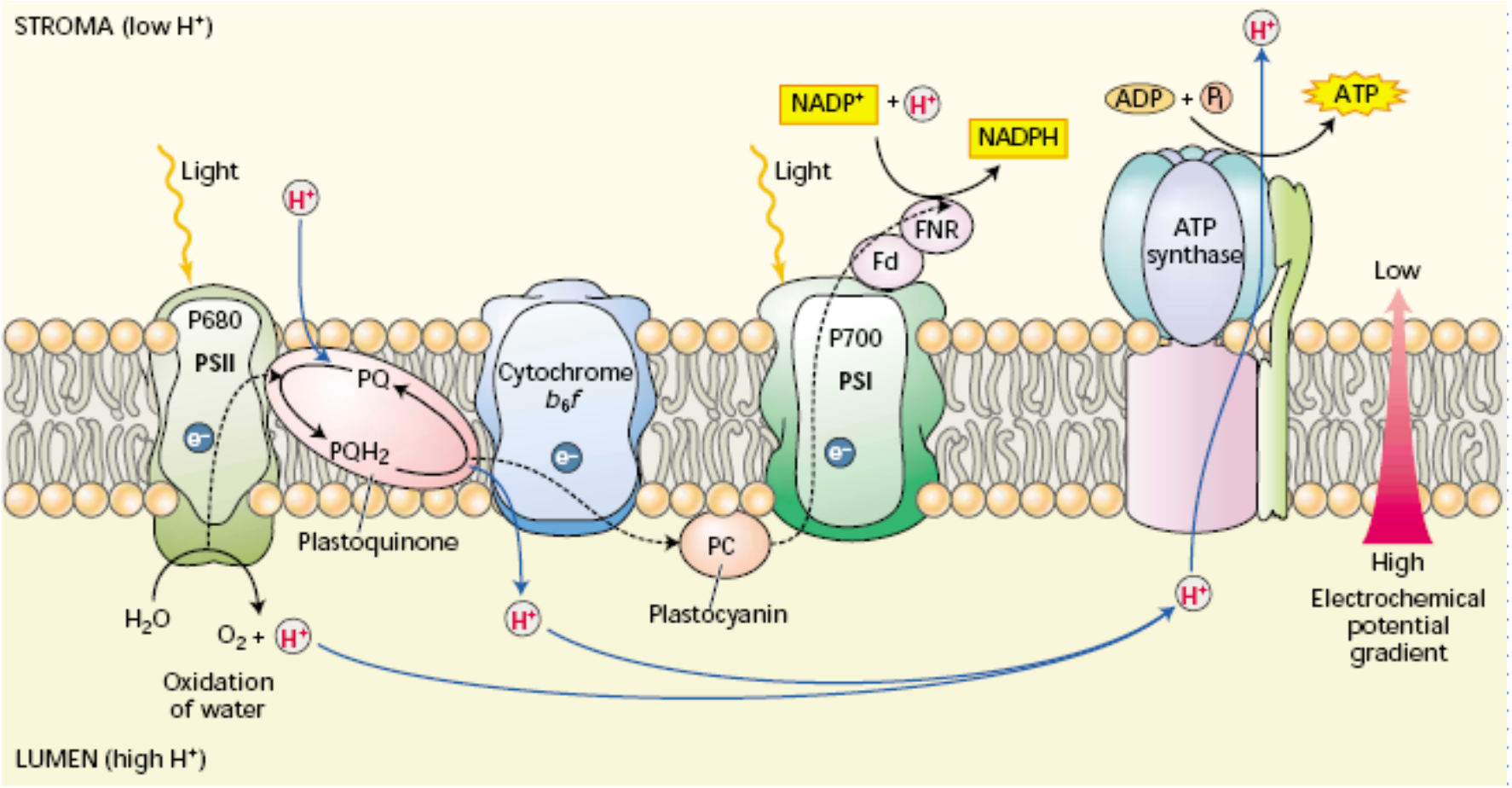
- Each electron is first excited by PSII and then by PSI.



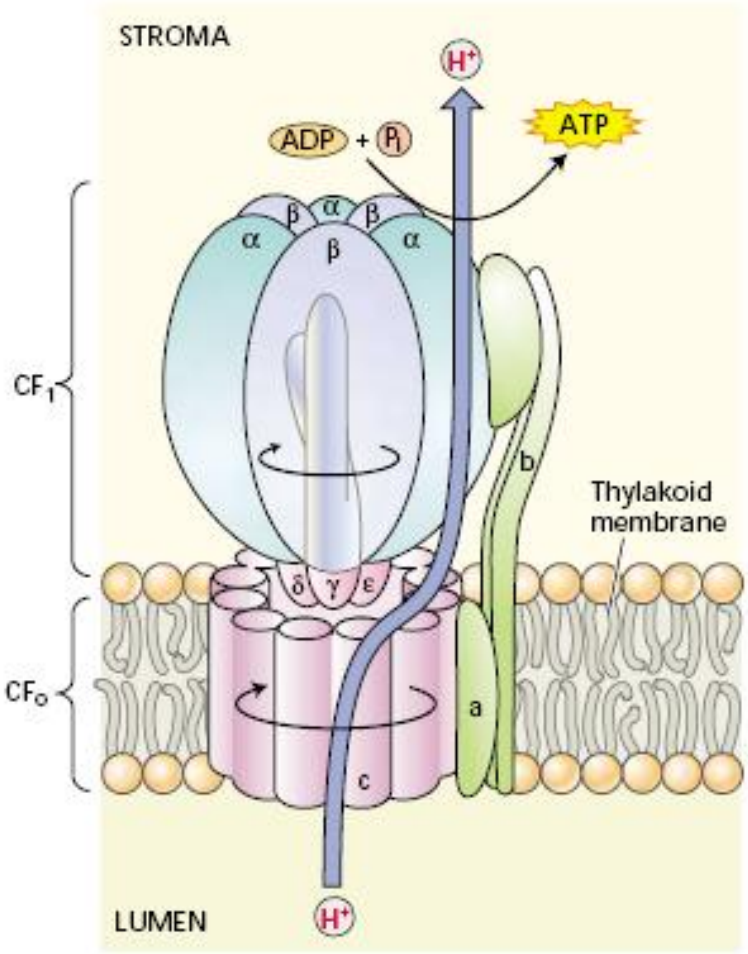
Z scheme of photosynthesis. Red light absorbed by photosystem II (PSII) produces a strong oxidant and a weak reductant. Far-red light absorbed by photosystem I (PSI) produces a weak oxidant and a strong reductant. The strong oxidant generated by PSII oxidizes water, while the strong reductant produced by PSI reduces $NADP^+$. This scheme is basic to an understanding of photosynthetic electron transport. P680 and P700 refer to the wavelengths of maximum absorption of the reaction center chlorophylls in PSII and PSI, respectively.



Detailed Z scheme for O_2-evolving photosynthetic organisms. The redox carriers are placed at their midpoint redox potentials (at pH 7). (1) The vertical arrows represent photon absorption by the reaction center chlorophylls: P680 for photosystem II (PSII) and P700 for photosystem I (PSI). The excited PSII reaction center chlorophyll, P680*, transfers an electron to pheophytin (Pheo). (2) On the oxidizing side of PSII (to the left of the arrow joining P680 with P680*), P680 oxidized by light is re-reduced by Y_z, that has received electrons from oxidation of water. (3) On the reducing side of PSII (to the right of the arrow joining P680 with P680*), pheophytin transfers electrons to the acceptors Q_A and Q_B, which are plastoquinones. (4) The cytochrome b_6f complex transfers electrons to plastocyanin (PC), a soluble protein, which in turn reduces P700* (oxidized P700). (5) The acceptor of electrons from P700* (A_0) is thought to be a chlorophyll, and the next acceptor (A_1) is a quinone. A series of membrane-bound iron–sulfur proteins (FeS_X, FeS_A, and FeS_B) transfers electrons to soluble ferredoxin (Fd). (6) The soluble flavoprotein ferredoxin–NADP reductase (FNR) reduces $NADP^+$ to NADPH, which is used in the Calvin cycle to reduce CO_2 (see Chapter 8). The dashed line indicates cyclic electron flow around PSI.

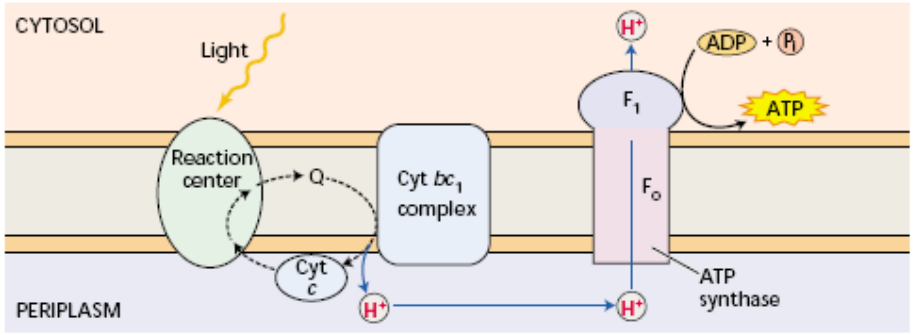


The transfer of electrons and protons in the thylakoid membrane is carried out vectorially by four protein complexes. Water is oxidized and protons are released in the lumen by PSII. PSI reduces $NADP^+$ to NADPH in the stroma, via the action of ferredoxin (Fd) and the flavoprotein ferredoxin–NADP reductase (FNR). Protons are also transported into the lumen by the action of the cytochrome b_6f complex and contribute to the electrochemical proton gradient. These protons must then diffuse to the ATP synthase enzyme, where their diffusion down the electrochemical potential gradient is used to synthesize ATP in the stroma. Reduced plastoquinone (PQH_2) and plastocyanin transfer electrons to cytochrome b_6f and to PSI, respectively. Dashed lines represent electron transfer; solid lines represent proton movement.

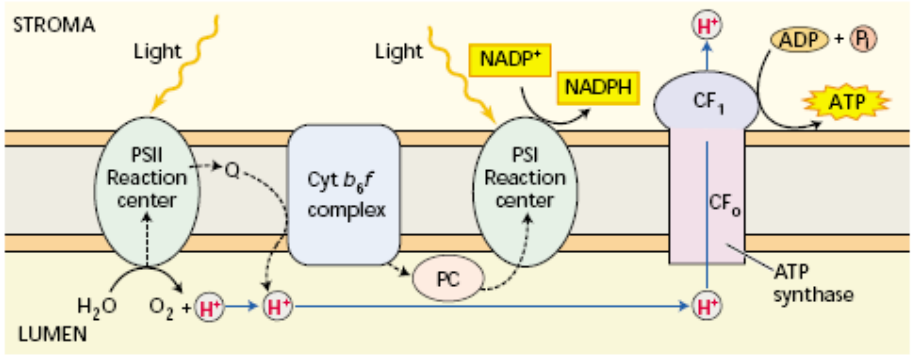


Structure of ATP synthase. This enzyme consists of a large multisubunit complex, CF_1, attached on the stromal side of the membrane to an integral membrane portion, known as CF_o. CF_1 consists of five different polypeptides, with a stoichiometry of α_3, β_3, γ, δ, ε. CF_o contains probably four different polypeptides, with a stoichiometry of a, b, b′, c_{12}.

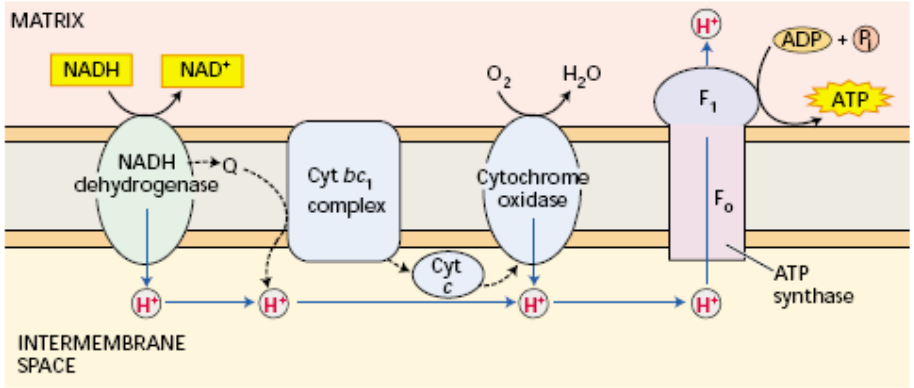
(A) **Purple bacteria**



(B) **Chloroplasts**



(C) **Mitochondria**

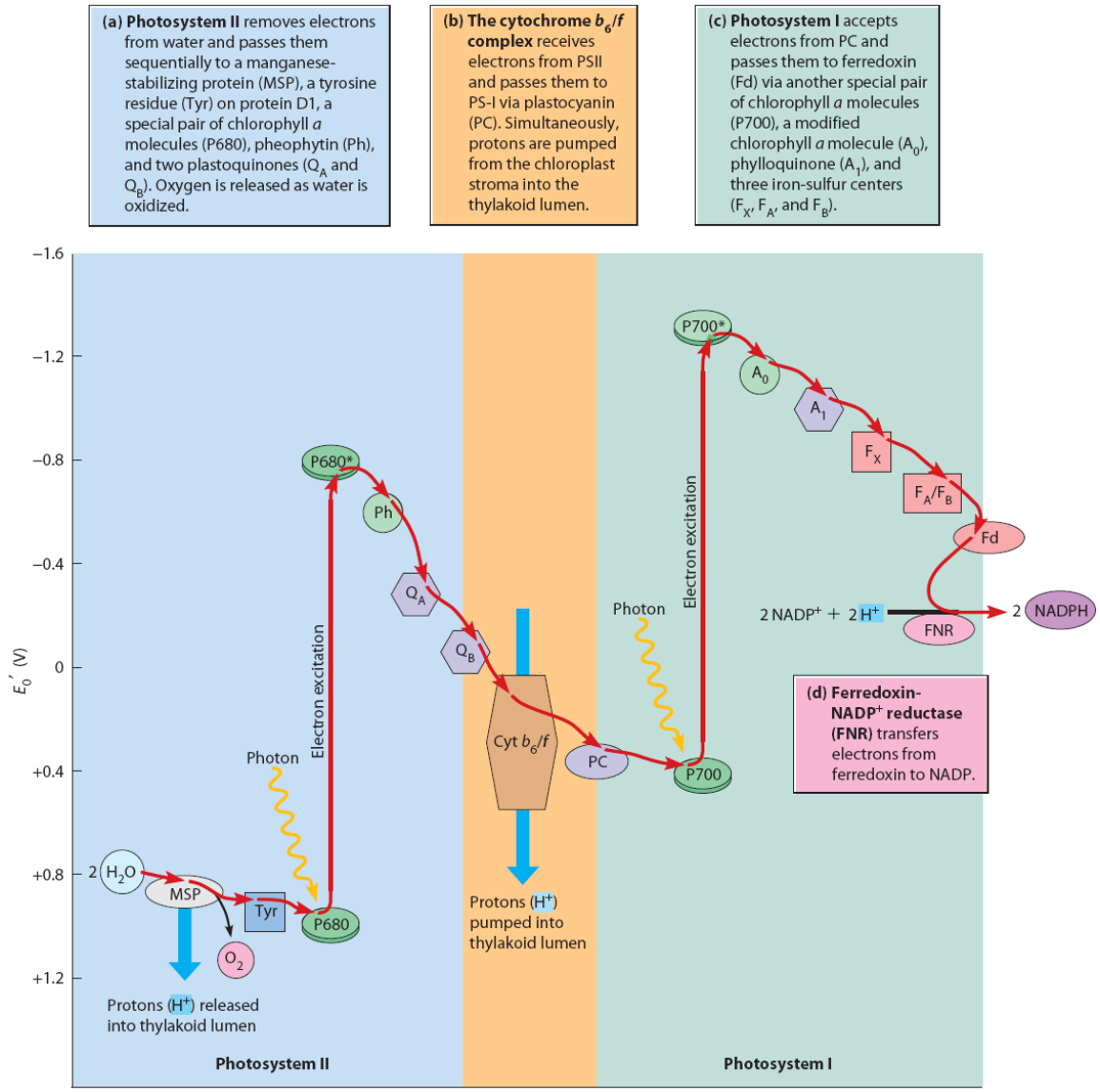


Photosynthetic EnergyTransduction II: NADPH Synthesis

- In the the second stage of photosynthetic energy transduction NADPH is formed.

- The process is known as **photoreduction** and involves a chloroplast electron transport system (ETS) that resembles the mitochondrial ETS.

- $NADP^+$ is the coenzyme of choice for a large number of anabolic pathways, whereas NAD^+ is usually involved in catabolic pathways.



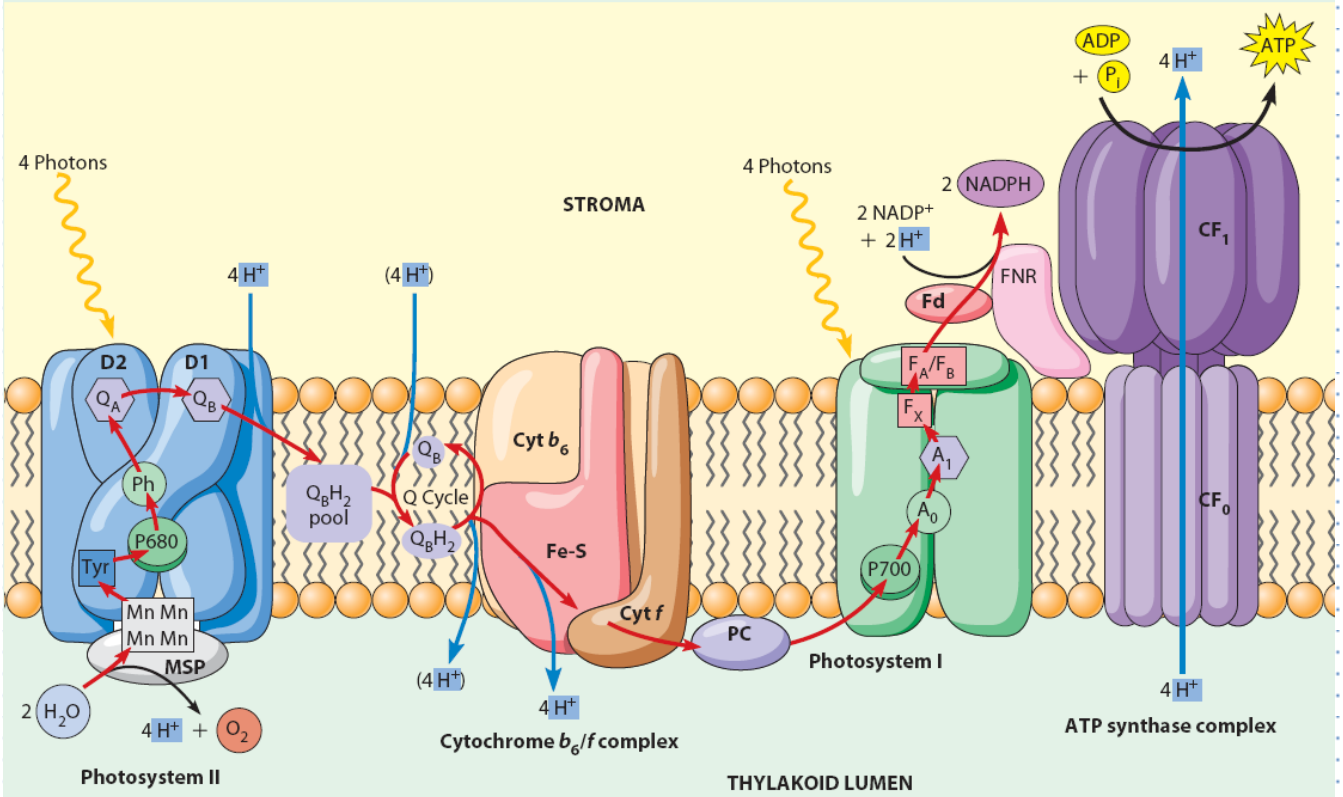
Noncyclic Electron Flow in Oxygenic Phototrophs. The flow of electrons from water to NADPH (red arrows) involves a linear pathway through (**a**) photosystem II (PSII), (**b**) the cytochrome b_6/f complex, (**c**) photosystem I (PSI), and (**d**) ferredoxin-$NADP^+$ reductase (FNR). Note how absorption of photons by chlorophylls P680 and P700 causes a large decrease in their reduction potentials (vertical axis), enabling them to donate excited electrons to an acceptor with a highly negative reduction potential. Figure 11-9 shows the orientation of these components plus the ATP synthase complex within a thylakoid membrane.

(a) **PSII** includes 25 polypeptides, two of which (D1 and D2) bind chlorophyll P680 and components of the electron transport system. The cluster of four manganese atoms aids in the oxidation of water.

(b) **The cytochrome b_6/f complex** contains seven polypeptides and includes two cytochromes (b_6 and f) and an iron-sulfur protein (Fe-S).

(c) **PSI** contains more than ten polypeptides and binds chlorophyll P700 and several additional electron carriers. Electrons are transferred to NADPH via soluble ferredoxin (Fd) using ferredoxin-$NADP^+$ reductase (FNR).

(d) **The ATP synthase complex** uses the proton gradient generated by electron transport to synthesize ATP.



A Model of the Orientation of the Major Energy Transduction Complexes Within the Thylakoid Membrane. This diagram shows the predicted arrangement of (a) photosystem II (PSII), (b) the cytochrome b_6/f complex, (c) photosystem I (PSI), and (d) the CF_oCF_1-ATP synthase complex within the thylakoid membrane. The stoichiometry shown is for the transport of four electrons (red arrows) from H_2O to $NADP^+$ as four photons are absorbed by each photosystem. This removes 6 H^+ from the stroma and adds 8 H^+ to the lumen. Operation of the Q cycle can transfer an additional four protons from the stroma to the lumen per four electrons transported, as shown in parentheses.

- Photosystem II uses electrons from water to reduce downhill components .

- PSII is generally associated with **light-harvesting complex II (LHCII)**, which contains about 250 chlorophyll and numerous carotenoid molecules.

- Energy captured by antenna pigments of PSII or LHCII is funneled to the reaction center by resonance energy transfer.

- When the energy reaches the reaction center, it lowers the reduction potential of a P680 chlorophyll *a* molecule to about -0.80 V, making it a better electron donor.

- The protons accumulating in the lumen contribute to an electrochemical proton gradient across the thylakoid membrane, and the oxygen molecule diffuses out of the chloroplast.

- Photosystem I transfers photoexcited electrons from reduced plastocyanin to a protein known as ferredoxin, the immediate electron donor to $NADP^+$.

- PSI in plants and green algae is associated with **lightharvesting complex I (LHCI)**, which contains fewer antennae molecules than LHCII does—between 80 and 120 chlorophyll and a few carotenoid molecules.

- As in the PSII complex, energy in PSI is funneled to a reaction center containing a special pair of chlorophyll a molecules.

- The energy absorbed by PSI lowers the reduction potential of its special pair of chlorophylls, designated P700, to about -1.30 V.

- Electrons flow exergonically through the ETS to *ferredoxin*, the final electron acceptor for PSI.

Photosynthetic Energy Transduction III: ATP Synthesis

- Given that the energy used to phosphorylate ADP is originated from the sun, this process is known as **photophosphorylation.**

- The total proton motive force (pmf) across the thylakoid membrane can be calculated by summing a proton concentration (pH) term and a membrane potential (V_m) term.

- This can be used to determine the standard free energy change ($\Delta G^{o'}$) for the movement of protons from the lumen to the stroma.

- In mitochondria, the pH difference (ΔpH) across the inner membrane is only about 1 unit, contributing about 0.06 V (30%) of the total pmf of 0.22 V.

- The other 70% of the pmf comes from the membrane potential difference of about 0.16 V.

- In chloroplasts, however, ΔpH is more important than ΔV_m and contributes about 80% of the total pmf.

- Light-induced proton pumping into the thylakoid lumen causes its internal pH to drop to about 6 and a rise in stromal pH to about 8.

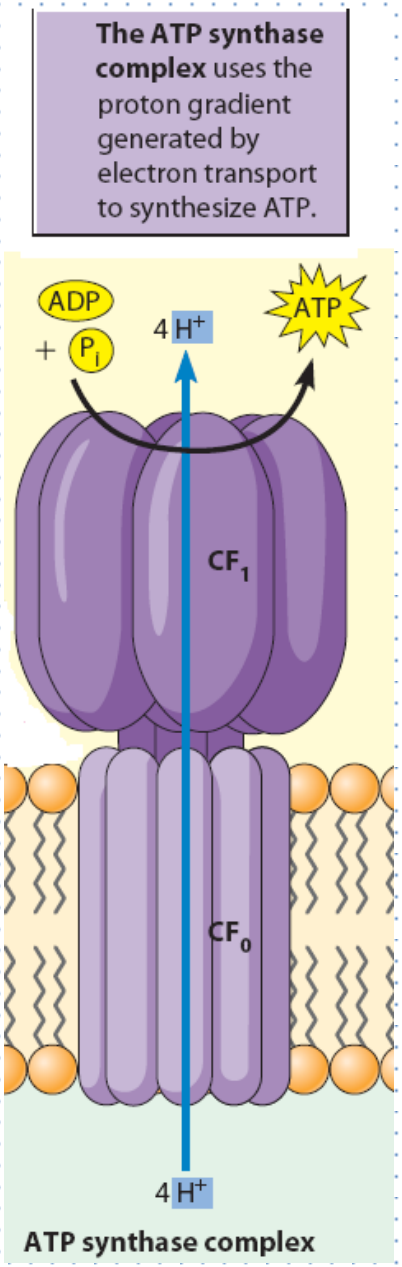
- This 2 units pH difference (i.e., 100 fold H^+ difference) generates a pmf of about 0.12 V.

- Together with a membrane potential difference (ΔV_m) of approximately 0.03 V, this gives a total pmf of 0.15 V, representing a of about 3.5 kcal/mol of protons moving across the thylakoid membrane.

The ATP Synthase Complex Couples Transport of Protons Across the Thylakoid Membrane to ATP Synthesis

- In chloroplasts as in mitochondria and bacteria, the movement of protons across a membrane from high to low concentration drives the synthesis of ATP by an **ATP synthase complex**.

- The ATP synthase complex found in chloroplasts, designated the **CF_oCF_1 complex**, is remarkably similar to the **F_oF_1** complexes of mitochondria and bacteria.



- Assuming a H^+/ATP ratio of four, the reaction catalyzed by the ATP synthase complex can be summarized as

$$4H^+_{lumen} + ADP + P_i \longrightarrow 4H^+_{stroma} + ATP$$

- The $\Delta G'$ value for the synthesis of ATP within the chloroplast stroma is usually 10–14 kcal/mol, whereas the energy available is about 14 kilocalories per 4 moles of protons passing through the ATP synthase complex (3.5 kcal/mol x 4 mol = 14 kcal).

- Thus, the flow of four electrons through the noncyclic pathway (without the Q cycle) not only generates two NADPH molecules but also leads to the synthesis of two ATP molecules (4 electrons x 2 protons/electron x 1 ATP/4 protons = 2 ATP molecules).

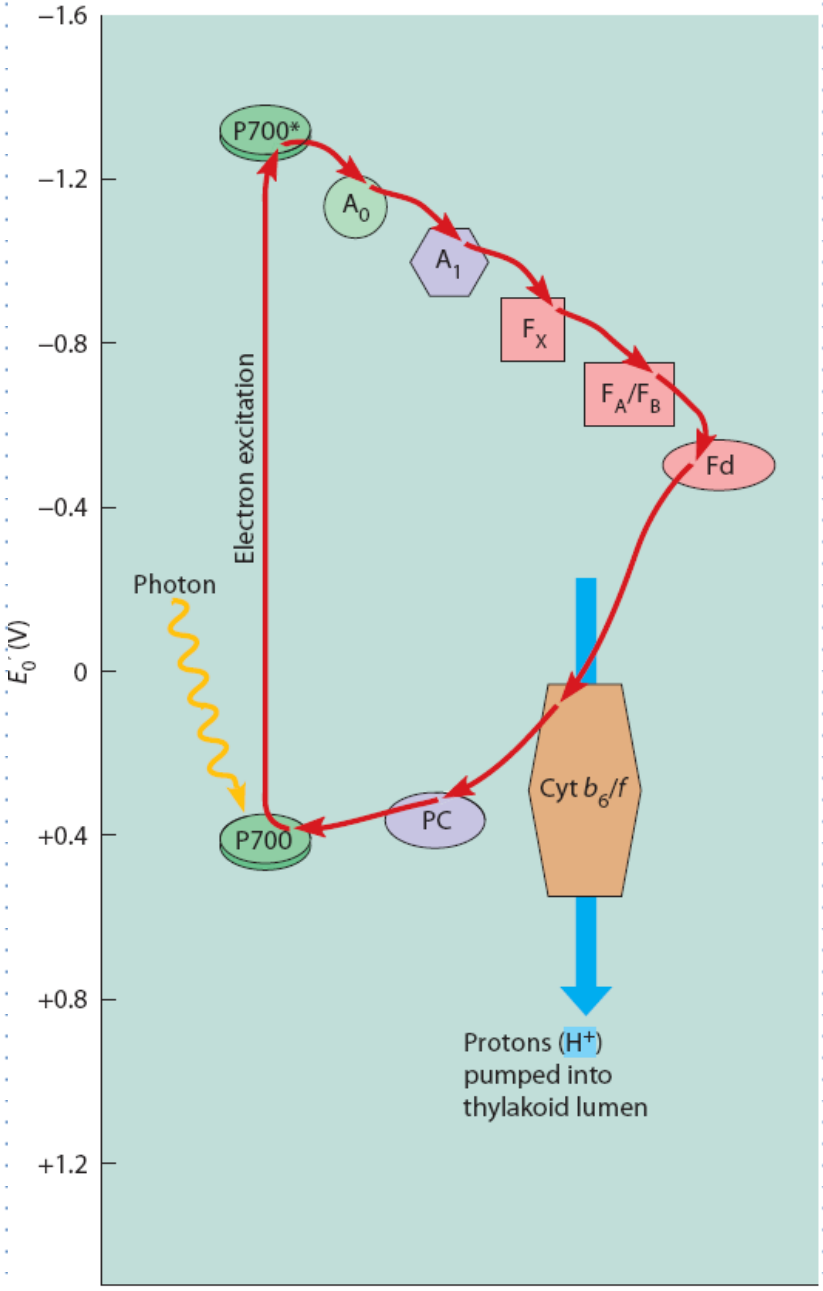
- Noncyclic electron flow leads to the generation of two ATP for every two NADPH molecules.

- Typically, cells will require more ATP than NADPH because many cellular activities, such as active transport across membranes, require ATP but not NADPH.

- When NADPH consumption is low and/or when additional ATP is needed, an optional process known as **cyclic electron flow** can divert the reducing power generated at PSI into ATP synthesis rather than $NADP^+$ reduction.

- The excess ATP synthesis resulting from this cyclic electron flow is called *cyclic photophosphorylation*.

- No water is oxidized and no oxygen is released since the flow of electrons from PSII is not involved.



Cyclic Electron Flow

•Cyclic electron flow through PSI enables oxygenic phototrophs to increase the ratio of ATP/NADPH production within photosynthetic cells.

•When the concentration of $NADP^+$ is low (i.e., when the concentration of NADPH is high), ferredoxin (Fd) donates electrons to the cytochrome b_6/f complex.

•Electrons then return to P700 via plastocyanin (PC). Because this cyclic electron flow is coupled to unidirectional proton pumping across the thylakoid membrane, excess reducing power is channeled into ATP synthesis.

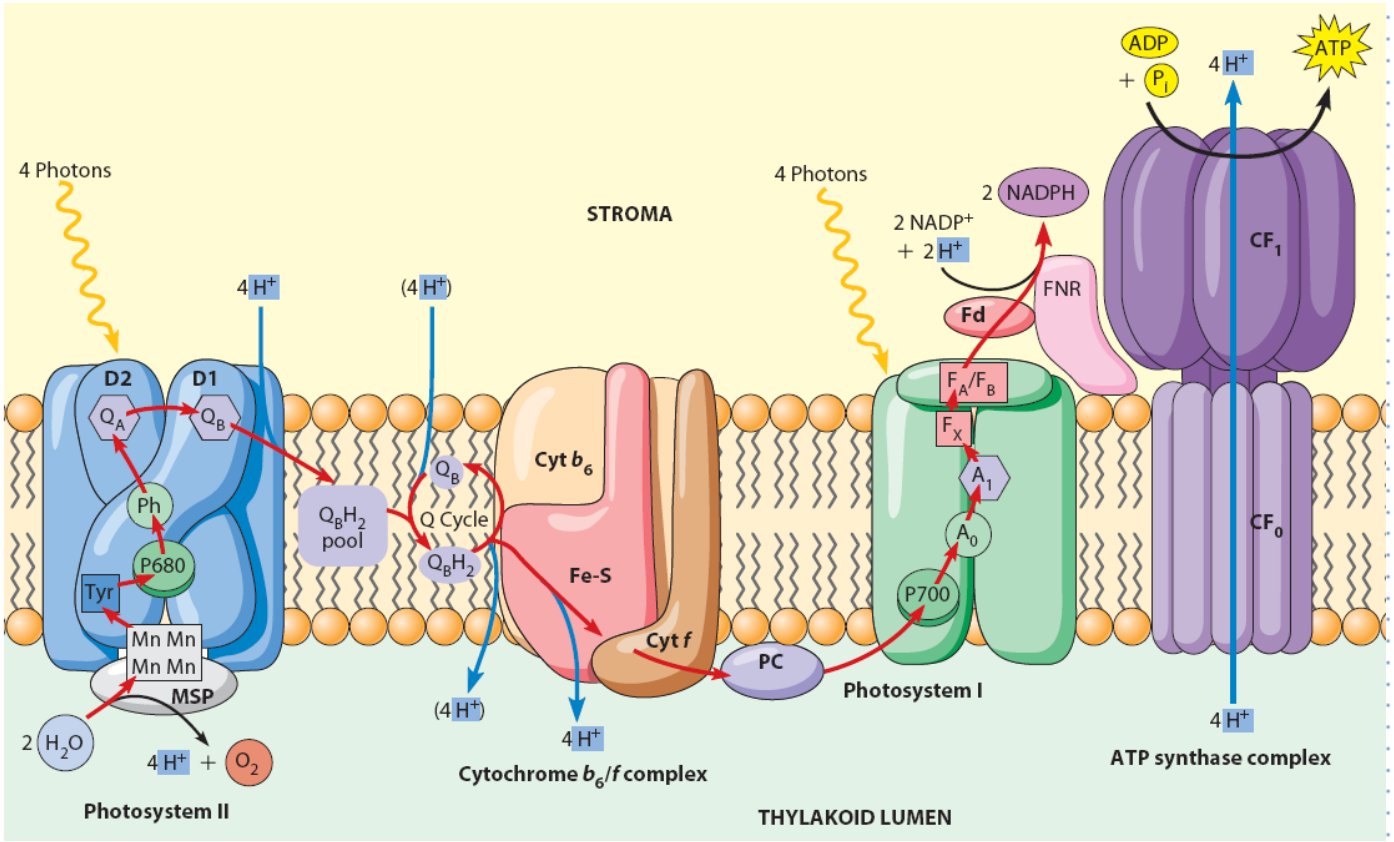
A Summary of the Complete Energy Transduction System

(a) **PSII** includes 25 polypeptides, two of which (D1 and D2) bind chlorophyll P680 and components of the electron transport system. The cluster of four manganese atoms aids in the oxidation of water.

(b) **The cytochrome b_6/f complex** contains seven polypeptides and includes two cytochromes (b_6 and f) and an iron-sulfur protein (Fe-S).

(c) **PSI** contains more than ten polypeptides and binds chlorophyll P700 and several additional electron carriers. Electrons are transferred to NADPH via soluble ferredoxin (Fd) using ferredoxin-NADP$^+$ reductase (FNR).

(d) **The ATP synthase complex** uses the proton gradient generated by electron transport to synthesize ATP.



The model shown in left represents the entire photosynthetic energy transduction system within a thylakoid membrane.

The essential features of the complete system for the transfer of electrons from water to and the resulting ATP synthesis can be summarized in terms of the following component parts:

1. **Photosystem II complex**

- An assembly of chlorophyll molecules, accessory pigments, and proteins that contains the P680 reaction center chlorophyll.

- Water is oxidized and split by the oxygen-evolving complex, and electrons flow from water to P680.

- Following photon absorption, photoexcited P680 molecules donate electrons to plastoquinone, reducing it to plastoquinol, a mobile electron carrier.

2. Cytochrome b_6/f complex

- An electron transport system that transfers electrons from plastoquinol to plastocyanin, thereby linking PSII with PSI.

- Electron flow through this complex is coupled to unidirectional proton pumping across the thylakoid membrane, establishing an electrochemical proton gradient that drives ATP synthesis.

- Optional cyclic electron flow allows additional ATP synthesis.

3. Photosystem I complex

- An assembly of chlorophyll molecules, accessory pigments, and proteins that contains the P700 reaction center chlorophyll, which accepts electrons from plastocyanin.

- Following photoexcitation, P700 donates electrons to ferredoxin, a stromal protein.

4. Ferredoxin- $NADP^+$ reductase

- An enzyme on the stromal side of the thylakoid membrane that catalyzes the transfer of electrons from two reduced ferredoxin proteins along with a proton to a single $NADP^+$ molecule.

- The NADPH generated in the stroma is an essential reducing agent in many anabolic pathways.

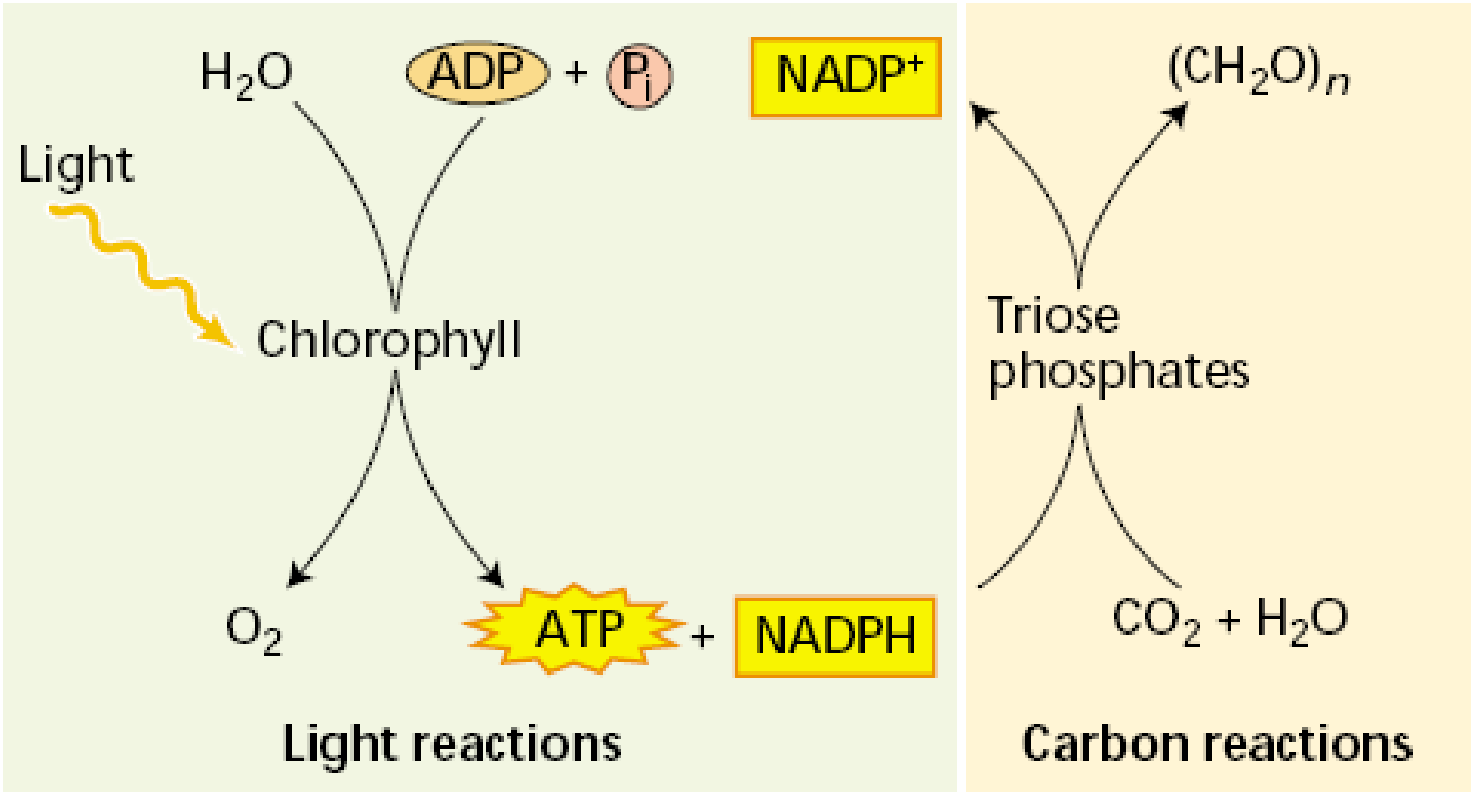
5. ATP synthase complex

- A proton channel and ATP synthase that couples the exergonic flow of protons from the thylakoid lumen to the stroma with the synthesis of ATP.

- Like NADPH, the ATP accumulates in the stroma, where it provides energy for carbon assimilation.

- Within a chloroplast, both *noncyclic* and *cyclic* pathways of electron flow operate (with or without the Q cycle), thereby providing flexibility in the relative amounts of ATP and NADPH generated.

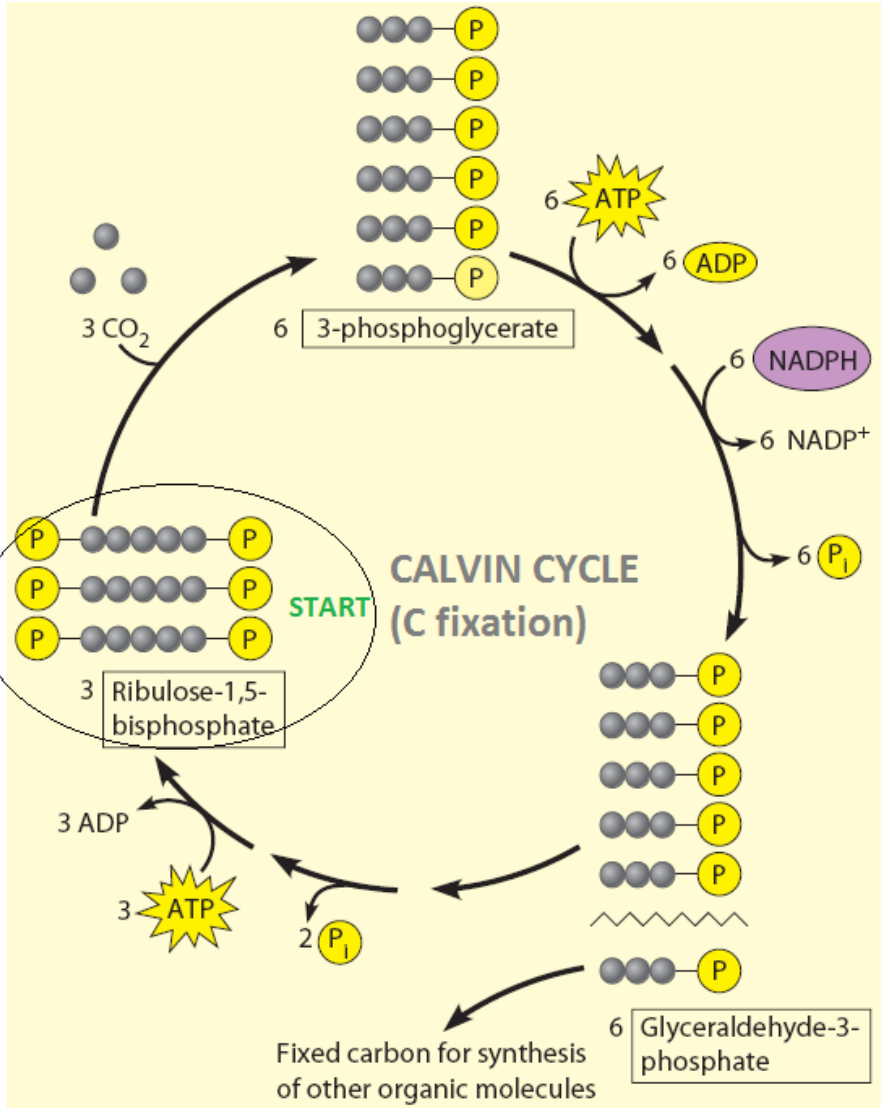
- ATP can be produced on a close to equimolar basis with respect to NADPH if the noncyclic pathway is operating alone, or ATP can be generated in excess by using either the Q cycle or the cyclic pathway of PSI.

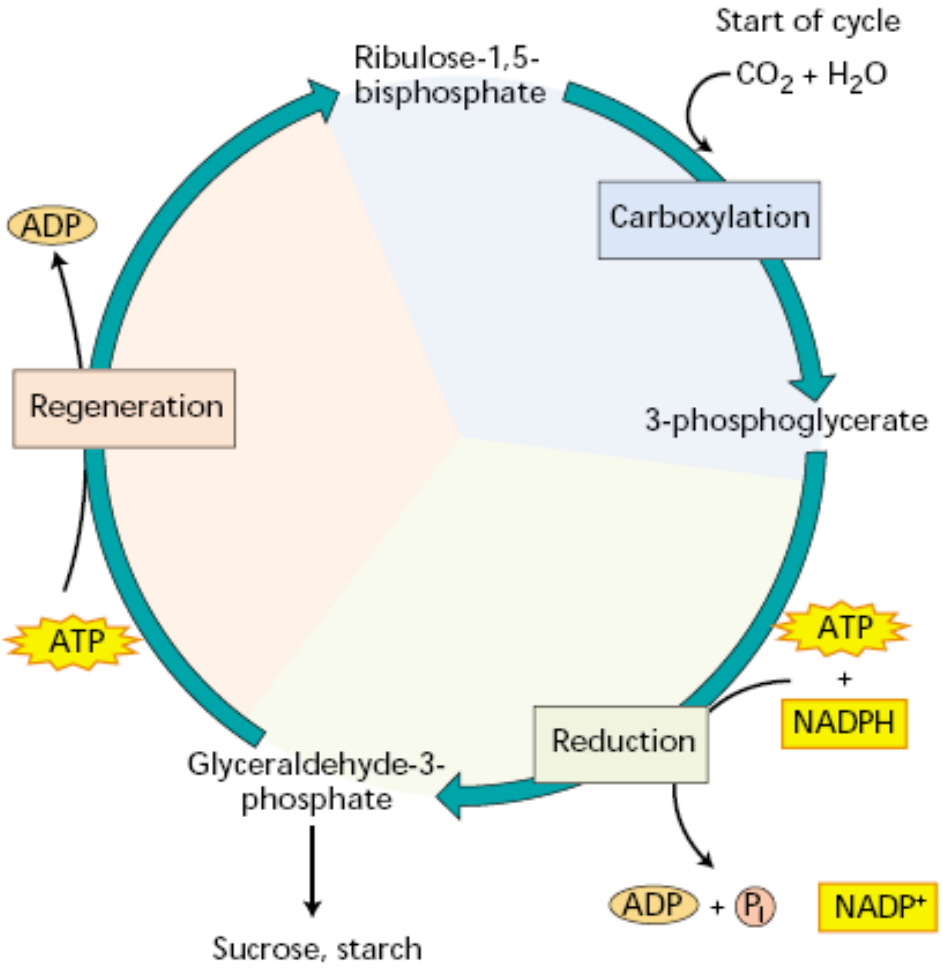


The light and carbon reactions of photosynthesis. Light is required for the generation of ATP and NADPH. The ATP and NADPH are consumed by the carbon reactions, which reduce CO_2 to carbohydrate (triose phosphates).

Photosynthetic Carbon Assimilation I: The Calvin Cycle

- The fundamental pathway for the movement of inorganic carbon into the biosphere is the **Calvin cycle (**named after its discoverer Melvin Calvin), which is found in all oxygenic and most anoxygenic phototrophs.





The Calvin cycle proceeds in three stages: (1) carboxylation, during which CO_2 is covalently linked to a carbon skeleton; (2) reduction, during which carbohydrate is formed at the expense of the photochemically derived ATP and reducing equivalents in the form of NADPH; and (3) regeneration, during which the CO_2 acceptor ribulose-1,5-bisphosphate re-forms.

- In plants and algae, the Calvin cycle is confined to the chloroplast stroma, where the ATP and NADPH generated by photosynthetic energy transduction reactions accumulate.

- In plants, carbon dioxide generally enters a leaf through special pores called **stomata** (singular: **stoma**).

- Once inside the leaf, carbon dioxide molecules diffuse into **mesophyll cells** into the chloroplast stroma, the site of carbon fixation.

- For convenience, the Calvin cycle can be divided into three stages:

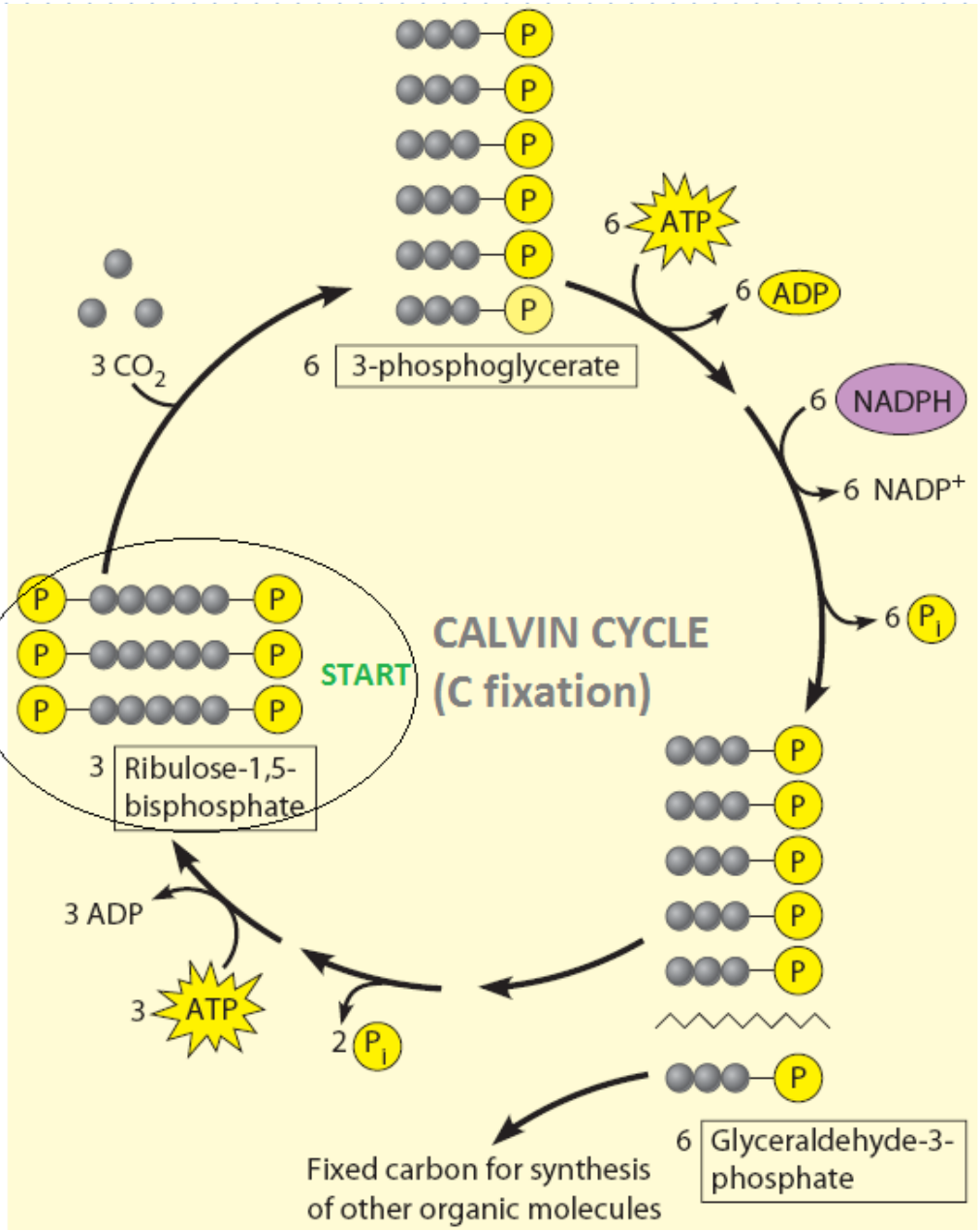
1. The carboxylation of acceptor molecule *ribulose-1,5-bisphosphate* and its immediate hydrolysis to generate two molecules of *3-phosphoglycerate*.

2. The reduction of *3-phosphoglycerate* to form the *triose phosphate glyceraldehyde-3-phosphate*.

3. The regeneration of acceptor molecule *ribulose-1,5-bisphosphate* to allow continued carbon assimilation.

CO_2 Enters the Calvin Cycle by Carboxylation of Ribulose-1, 5-Bisphosphate

- The first stage of the Calvin cycle begins with the covalent attachment of carbon dioxide to the carbonyl carbon of *ribulose-1,5-bisphosphate*, which is immediately hydrolyzed to generate two molecules of *3-phosphoglycerate*.



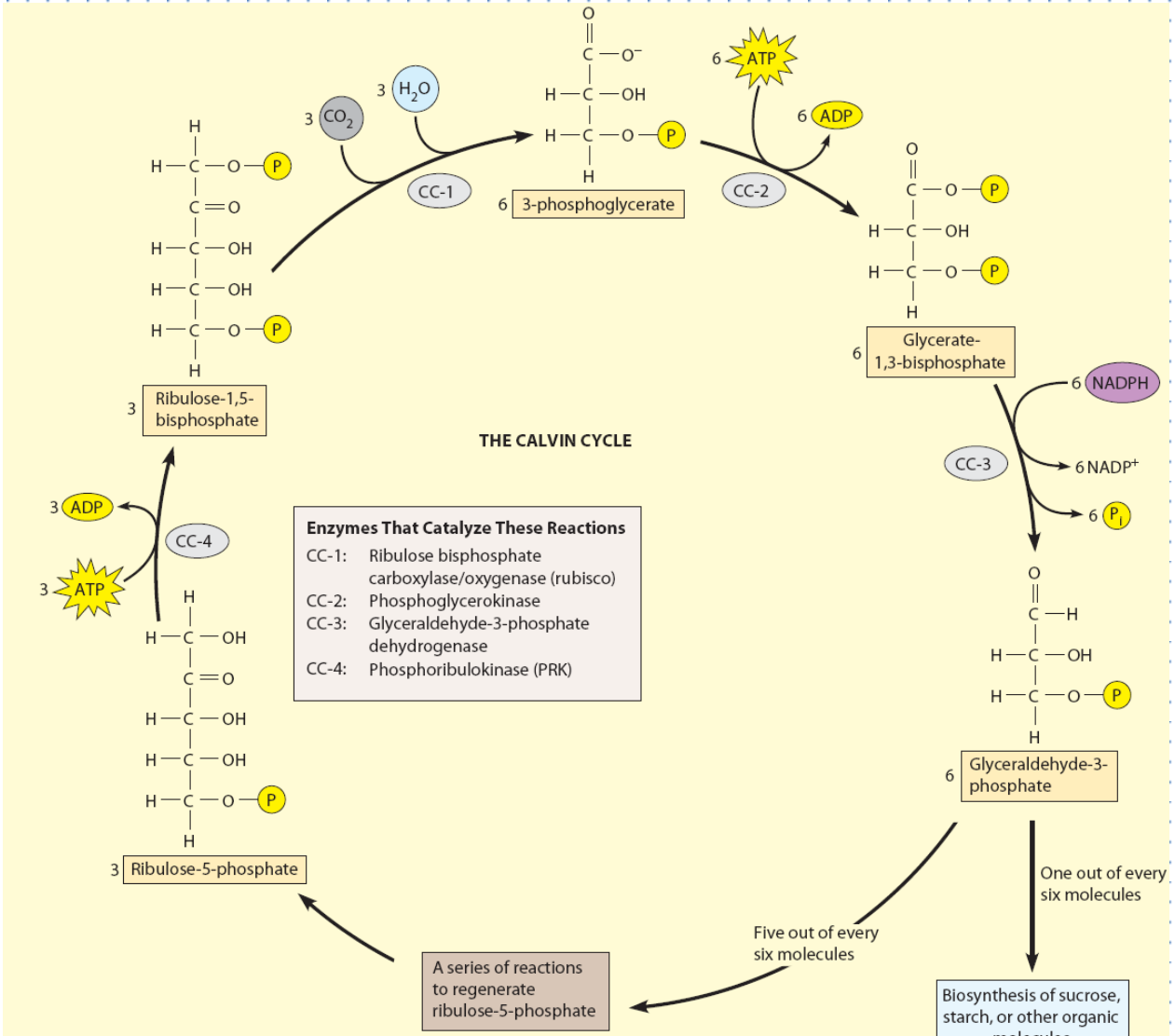
Overview of the Calvin Cycle

•Starting with three molecules of ribulose-1,5-bisphosphate, three molecules of CO_2 are fixed and the products are rearranged to form six molecules of 3-phosphoglycerate.

•Using ATP and NADPH, these six molecules of 3-phosphoglycerate are reduced to six molecules of glyceraldehyde-3-phosphate.

•More ATP is used as five of these glyceraldehyde-3-phosphate molecules are rearranged to regenerate three molecules of Ru-P_2, which will accept three more CO_2.

•The sixth glyceraldehyde-3-phosphate, representing the three CO_2 that were fixed, leaves the cycle for synthesis of other organic compounds in the cell.



The Calvin Cycle for Photosynthetic Carbon Assimilation. During one turn of the Calvin cycle (CC), 3 CO_2 molecules are fixed, forming one molecule of triose phosphate. CO_2 is fixed in Reaction CC-1, forming 3-phosphoglycerate. Reactions CC-2 and CC-3 use ATP and NADPH to reduce 3-phosphoglycerate to glyceraldehyde-3-phosphate. One out of every six glyceraldehyde-3-phosphate molecules is used for the biosynthesis of sucrose, starch, or other organic molecules. The other five glyceraldehyde-3-phosphate molecules are used to form three molecules of ribulose-5-phosphate. The ribulose-5-phosphate is then phosphorylated using ATP in Reaction CC-4 to regenerate ribulose-1, 5-bisphosphate, the acceptor molecule for Reaction CC-1, thus completing one turn of the cycle.

- The enzyme that catalyzes the capture of the carbon dioxide and the formation of 3-phosphoglycerate is called **ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco).**

- This relatively large enzyme (a molecular weight of about 560,000) is unique to phototrophs and is found in all photosynthetic organisms except for a few photosynthetic bacteria.

- Rubisco is thought to be the most abundant protein on the planet. About 10–25% of soluble leaf protein is rubisco, and one estimate puts the total amount of rubisco on the Earth at 40 million tons, or almost 7 kg for each living person.

- For every carbon dioxide molecule that is fixed by rubisco, two 3-phosphoglycerate molecules are generated.

- The reduction of both of these molecules to glyceraldehyde-3-phosphate requires the hydrolysis of two ATP molecules and the oxidation of two NADPH molecules—all for a net gain of only one carbon atom.

- The net synthesis of one triose phosphate molecule requires the fixation and reduction of three carbon dioxide molecules to maintain carbon balance and will therefore consume six ATP and six NADPH:

$$3\ \text{ribulose-1,5-bisphosphate} + 3CO_2 + 3H_2O + 6ATP + 6NADPH \longrightarrow 6G3P + 6ADP + 6P_i + 6NADP^+$$

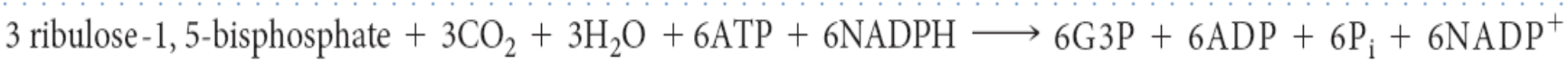
- One out of every six triose phosphate molecules—the net gain following three carboxylations in the Calvin cycle—is used for the biosynthesis of sucrose, starch, or other organic molecules.

- The five remaining triose phosphates are required in the third and final stage of the Calvin cycle for regeneration of three molecules of the acceptor pentose ribulose-1,5-bisphosphate.

- Regeneration of ribulose-1,5-bisphosphate from glyceraldehyde-3-phosphate consumes three more ATP molecules. The series of events may be summarized by the following net reaction:

$$5\text{G3P} + 3\text{ATP} + 2\text{H}_2\text{O} \longrightarrow 3 \text{ ribulose-1,5-bisphosphate} + 3\text{ADP} + 2\text{P}_\text{i}$$

- Primary carbon assimilation by the Calvin cycle may be summarized by combining reactions:



and

- The resulting net reaction is:

- The Calvin cycle consumes nine ATP molecules and six NADPH molecules for every three-carbon carbohydrate synthesized, which is three ATP and two NADPH for each carbon dioxide molecule fixed.

- This demand can be met by the flow of 12 electrons through the noncyclic pathway, which requires 24 photons to be absorbed by the two photosystems in series.

- This provides all six of the NADPH molecules and eight of the nine ATP molecules.

- The flow of two electrons through the cyclic pathway of PSI will require two more photons and will provide one more ATP molecule.

- Thus, the net reaction for energy transduction is:

$$26\ \text{photons} + 9\text{ADP} + 9\text{P}_\text{i} + 6\text{NADP}^+ \longrightarrow \text{O}_2 + 9\text{ATP} + 6\text{NADPH} + 3\text{H}_2\text{O}$$

- The absorption of 26 photons is accounted for by 12 photoexcitation events at PSI and 12 at PSII during noncyclic electron flow and 2 photoexcitation events at PSI during cyclic electron flow.

- By adding the

$$3CO_2 + 9ATP + 6NADPH + 5H_2O \longrightarrow G3P + 9ADP + 6NADP^+ + 8P_i$$

to

$$26\ photons + 9ADP + 9P_i + 6NADP^+ \longrightarrow O_2 + 9ATP + 6NADPH + 3H_2O$$

We get:

$$26\ photons + 3CO_2 + 5H_2O + P_i \longrightarrow G3P + 3O_2 + 3H_2O$$

Photosynthetic Carbon Assimilation II: Carbohydrate Synthesis

- Because triose phosphates generated by the Calvin cycle are consumed by metabolic pathways in the cytosol as well as the chloroplast stroma, there must be a mechanism for transporting them across the chloroplast inner membrane.

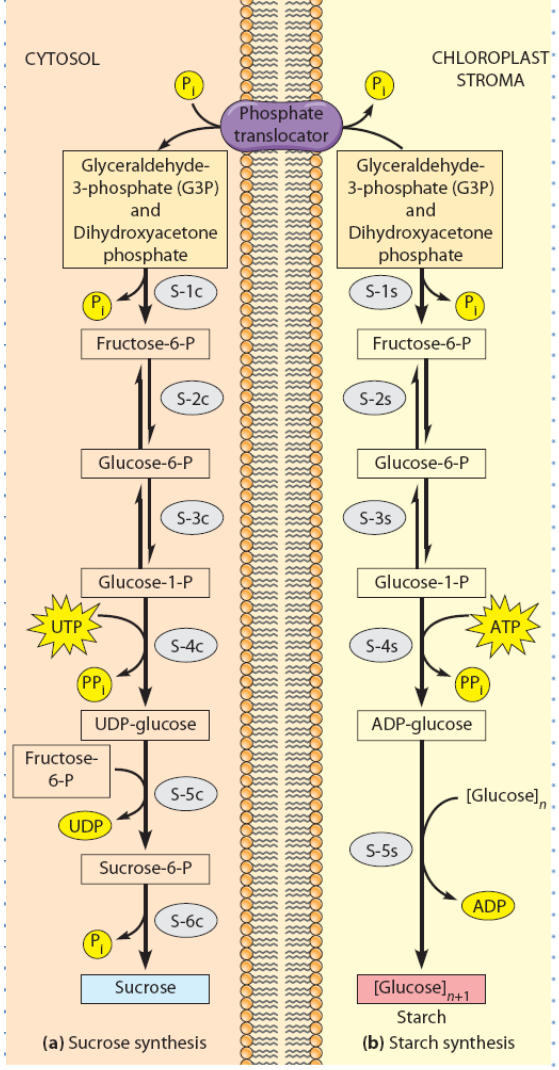
- The most abundant protein in the chloroplast inner membrane is a phosphate translocator that catalyzes the exchange of dihydroxyacetone phosphate, glyceraldehyde-3-phosphate, or 3-phosphoglycerate in the stroma for in the cytosol.

- This antiport system ensures that triose phosphates will not be exported unless P_i—which is required for synthesizing new triose phosphates—returns to the stroma.

The Biosynthesis of Sucrose and Starch from Products of the Calvin Cycle

- The triose phosphates glyceraldehyde-3-phosphate and dihydroxyacetone phosphate from the chloroplast stroma are exchanged for inorganic phosphate from the cytosol via a phosphate translocator. **(a) Sucrose** synthesis is confined to the cytosol, whereas **(b) starch synthesis** occurs only in the chloroplast stroma.

- The enzymes and isoenzymes that catalyze these reactions are restricted to either the cytosol (c) or the stroma (s).



Enzymes That Catalyze These Reactions

- S-1: Aldolase and fructose-1,6-bisphosphatase
- S-2: Phosphoglucoisomerase
- S-3: Phosphoglucomutase
- S-4c: UDP-glucose pyrophosphorylase
- S-5c: Sucrose phosphate synthase
- S-6c: Sucrose phosphatase
- S-4s: ADP-glucose pyrophosphorylase
- S-5s: Starch synthase

Photosynthesis Also Produces Reduced Nitrogen and Sulfur Compounds

- Photosynthesis encompasses more than carbon dioxide fixation and carbohydrate synthesis. In plants and algae, the ATP and NADPH generated by photosynthetic energy transduction reactions are consumed by a variety of other anabolic pathways found in chloroplasts.

- Several key steps of nitrogen and sulfur assimilation are localized in chloroplasts.

- The reduction of nitrite (NO_2^-) to ammonia (NH_3) is catalyzed by a reductase enzyme in the chloroplast stroma, with reduced ferredoxin serving as an electron donor.

- The ammonia is then channeled into amino acid and nucleotide synthesis, portions of which also occur in chloroplasts.

- Furthermore, much of the reduction of sulfate (SO_4^{2-})to sulfide (S^{2-}) is catalyzed by enzymes in the chloroplast stroma.

- The sulfide, like ammonia, may then be used for amino acid synthesis.

Rubisco's Oxygenase Activity Decreases Photosynthetic Efficiency

- The primary reaction catalyzed by rubisco—acting as a carboxylase—is the addition of carbon dioxide and water to ribulose-1,5-bisphosphate, forming two molecules of 3-phosphoglycerate.

- However, rubisco can also function as an *oxygenase*. Through this activity, rubisco catalyzes the addition of molecular oxygen, rather than carbon dioxide, to ribulose-1,5-bisphosphate and the products are ***phosphoglycolate*** and ***3-phosphoglycerate.***

- Because phosphoglycolate cannot be used in the next step of the Calvin cycle, it appears to be a wasteful diversion of material from carbon assimilation.

- No alternative functions for rubisco's oxygenase activity have been clearly demonstrated.

- Why then does rubisco have this detrimental oxygenase activity?

- According to one theory, the oxygenase activity is an evolutionary relic from a time when oxygen did not make up a large part of the Earth's atmosphere.

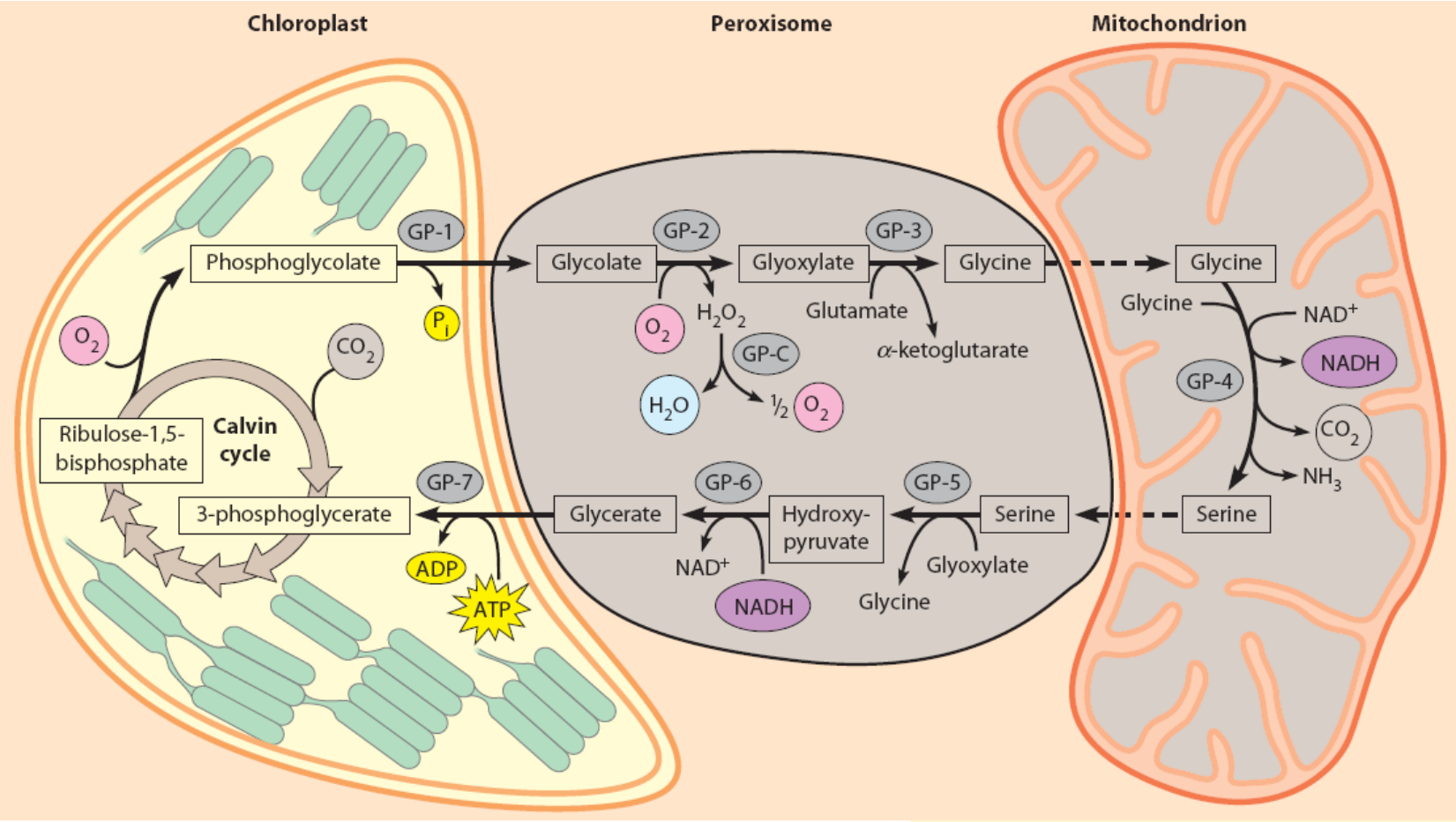
- Thus it cannot be eliminated without seriously compromising the carboxylase function of these enzyme.

Glycolate pathway

- In all photosynthetic plant cells, phosphoglycolate generated by rubisco's oxygenase activity is channeled into the **glycolate pathway**.

- This pathway disposes of phosphoglycolate and returns about 75% of the reduced carbon present in phosphoglycolate to the Calvin cycle as 3-phosphoglycerate, with the other 25% released as CO_2.

- Because the glycolate pathway is characterized by light-dependent uptake of oxygen and evolution of carbon dioxide, it is also referred to as **photorespiration.**



The Glycolate Pathway. Glycolate arises as a result of the oxygenase activity of rubisco. The immediate product is phosphoglycolate, which is converted to free glycolate by a phosphatase localized in the chloroplast membrane (Reaction GP-1). Free glycolate diffuses out of the chloroplast stroma and is metabolized by a five-step pathway (Reactions GP-2 through GP-6) that occurs partially in the peroxisome and partially in the mitochondrion. Glycerate then diffuses into the chloroplast and is phosphorylated to form 3-phosphoglycerate (Reaction GP-7), which enters the Calvin cycle. The oxygen uptake and carbon dioxide evolution characteristic of photorespiration occur in the peroxisome (Reaction GP-2) and mitochondrion (Reaction GP-4), respectively. Note that two molecules of glycolate are required to form one molecule of 3-phosphoglycerate.

Enzymes That Catalyze These Reactions

- GP-1: Phosphoglycolate phosphatase
- GP-2: Glycolate oxidase
- GP-3: Glutamate: glyoxylate aminotransferase
- GP-4: Glycine decarboxylase and serine hydroxymethyl transferase
- GP-5: Serine: glyoxylate aminotransferase
- GP-6: Hydroxypyruvate reductase
- GP-7: Glycerate kinase
- GP-C: Catalase

- Several steps of the glycolate pathway are localized in a specific type of peroxisome called a **leaf peroxisome**.

- Peroxisomes are organelles containing oxidase enzymes that generate hydrogen peroxide (H_2O_2).

- The potentially destructive hydrogen peroxide is then eliminated by another peroxisomal enzyme, *catalase*, which degrades it to water and oxygen.

- Because of their essential role in the glycolate pathway, peroxisomes are found in all photosynthetic plant tissues.

C4 Plants Minimize Photorespiration by Confining Rubisco to Cells Containing High Concentrations of CO2

- Plants in hot, arid environments under intense illumination are particularly affected by rubisco's oxygenase activity.
- As the temperature increases, the solubility of carbon dioxide declines more rapidly than the solubility of oxygen, thereby lowering the CO_2:O_2 ratio in solution.
- Another problem occurs when plants respond to drought by closing their stomata during the day to reduce water loss.
- When the stomata are closed, carbon dioxide cannot enter the leaf, and the concentration of carbon dioxide in leaf cells may decline.
- Moreover, water photolysis continues to generate oxygen, which accumulates because it cannot diffuse out of the leaf when the stomata are closed.

- In some cases, the potential for energy and carbon drain through photorespiration is so overwhelming that plants must depend on adaptive strategies for solving the problem.

- One general approach is to confine rubisco to cells that contain a high concentration of carbon dioxide, thereby minimizing the enzyme's inherent oxygenase activity.

- Plants containing this pathway are referred to as **C_4 plants** because the immediate product of carbon dioxide fixation is the four-carbon organic acid *oxaloacetate*.

- This term distinguishes such plants from **C_3 plants**, in which the first detectable product of carbon dioxide fixation is the three-carbon compound *3-phosphoglycerate*.

- C4 plants, unlike C3 plants, have in their leaves two distinct types of photosynthetic cells— *mesophyll cells* and *bundle sheath cells*—that differ in their enzyme composition and hence their metabolic activities.

- The carbon dioxide fixation step within a C4 plant is accomplished by an enzyme other than rubisco in mesophyll cells, which are exposed to the carbon dioxide and oxygen that enter a leaf through its stomata.

- The carbon dioxide that is fixed in mesophyll cells is subsequently released in **bundle sheath cells**, which are relatively isolated from the atmosphere.

- The entire Calvin cycle, including rubisco, is confined to chloroplasts in the bundle sheath cells.

- The carbon dioxide concentration in bundle sheath cells may be as much as ten times the level in the atmosphere, strongly favoring rubisco's carboxylase activity and minimizing its oxygenase activity.

- When temperatures exceed about 30^{o}C, the photosynthetic efficiency of a plant exposed to intense sunlight may be twice that of a plant.

- While the higher efficiency of a plant is largely due to reduced photorespiration.

- Certain plant species that live in deserts, salt marshes, and other environments where access to water is severely limited contain a preliminary carbon dioxide fixation pathway

- These plants segregate the carboxylation and decarboxylation reactions by time rather than by space.

- Because the pathway was first recognized in the family of succulent plants known as the Crassulaceae, it is called **crassulacean acid metabolism (CAM**), and plants that take advantage of CAM photosynthesis are called **CAM plants**.

- CAM photosynthesis has been found in about 4% of plant species investigated, including many succulents, cacti, orchids, and pineapple.

- CAM plants, unlike most C3 and C4 plants, generally open their stomata only at night, when the atmosphere is relatively cool and moist.

- As carbon dioxide diffuses into mesophyll cells, it is assimilated by the first two steps of a pathway to malate.

- Instead of being exported from mesophyll cells, however, the malate is stored in large vacuoles, which become very acidic.

- The process of moving malate into vacuoles consumes ATP but is necessary to protect cytosolic enzymes from a large drop in pH at night.

- During the day, CAM plants close their stomata to conserve water. The malate then diffuses from vacuoles to the cytosol.

- Carbon dioxide released by decarboxylation of malate diffuses into the chloroplast stroma, where it is refixed and reduced by the Calvin cycle. The high carbon dioxide and low oxygen concentrations established when light is available for generating ATP and NADPH strongly favor rubisco's carboxylase activity and minimize the loss of carbon through photorespiration.

- With their remarkable ability to conserve water, CAM plants may assimilate over 25 times as much carbon as a plant does for each unit of water transpired.

- Moreover, some CAM plants display a process called CAM idling, whereby the plant keeps its stomata closed night and day.

- Carbon dioxide is simply recycled between photosynthesis and respiration, with virtually no loss of water. Such plants will not, of course, display a net gain of carbohydrate and will not show much if any growth. This ability, however, can enable them to survive droughts lasting up to several months and may contribute to their long life spans, which can exceed 100 years.

SUMMARY OF KEY POINTS

- Photosynthesis is the single most vital metabolic process for virtually all forms of life on Earth because all of us, whatever our immediate sources of energy, ultimately depend on the energy radiating from the sun.

- The energy transduction reactions of photosynthesis convert solar energy into chemical energy in the form of NADPH and ATP. The carbon assimilation reactions use this chemical energy to fix and reduce carbon dioxide to carbohydrates.

- In eukaryotic phototrophs, photosynthesis occurs in the chloroplasts, which contain an internal membrane system known as the thylakoids that contains many of the required components.

- Photons of light are absorbed by chlorophyll or accessory pigment molecules within the thylakoid or photosynthetic bacterial membranes. Their energy is rapidly passed to a special pair of chlorophyll molecules at the reaction center of a photosystem.

- At the reaction center, the energy is used to excite and eject an electron from chlorophyll and induce charge separation. In oxygenic phototrophs, this electron is replaced by an electron obtained from a water molecule, generating oxygen.

- Electron transfer from water to $NADP^+$ relies on two photosystems acting in series, with photosystem II responsible for the oxidation of water and photosystem I responsible for the reduction of $NADP^+$ to NADPH in the stroma.

- Electron flow between the two photosystems passes through a cytochrome b_6/f complex, which pumps protons into the thylakoid lumen. The resulting proton gradient represents the stored energy of sunlight.

- The proton motive force across the thylakoid membrane is used to drive ATP synthesis by the CF_oCF_1 complex embedded in the membrane.

- As protons flow back from the lumen to the stroma through the CF_o proton channel in the membrane, ATP is synthesized by the portion of the CF_1 complex that extends into the stroma.

- In the stroma, ATP and NADPH are used for the fixation and reduction of carbon dioxide into organic form by enzymes of the Calvin cycle.

- The Calvin cycle involves three main stages: fixation of carbon dioxide by rubisco to form 3-phosphoglycerate, reduction of 3-phosphoglycerate to glyceraldehyde-3-phosphate, and regeneration of ribulose-1,5-bisphosphate, the initial carbon dioxide acceptor.

- The net synthesis of one triose phosphate molecule requires the fixation of three CO_2 molecules and uses nine ATP and six NADPH molecules. For each three CO_2 fixed, one molecule of glyceraldehyde-3-phosphate leaves the cycle to be used for further carbohydrate synthesis.

- The initial product of carbon dioxide fixation is glyceraldehyde-3-phosphate, which can be converted to a second triose phosphate called dihydroxyacetone phosphate. Some of these triose phosphate molecules are used for the biosynthesis of more complex carbohydrates, such as glucose, sucrose, starch, or glycogen. Others are used as sources of energy or carbon skeletons for other metabolic pathways.

- In addition, ATP produced in the chloroplast is used for fatty acid and chlorophyll synthesis and for nitrogen and sulfur reduction and assimilation.

- Rubisco can use oxygen as well as CO_2, resulting in the production of phosphoglycolate, which cannot be used in the Calvin cycle. The glycolate pathway converts two molecules of phosphoglycolate into one molecule of 3-phosphoglycerate, which can enter the Calvin cycle.

- The glycolate pathway involves the chloroplast, the peroxisome, and the mitochondrion. Because CO_2 is released and oxygen is consumed in a light-dependent manner, this process is also called photorespiration.

- In C4 and CAM plants, carbon dioxide is fixed by a preliminary carboxylation that does not involve rubisco. Then it is decarboxylated under conditions of low oxygen and concentrated CO_2—either in a different cell type or at a different time of day—conditions that favor rubisco's carboxylase activity.

MBG304 Biochemistry

Lecture 6: Pentose Phosphate Pathway

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Inonu University

Glucose flux

Glycolysis

Pentose Phosphate Pathway

Pentose Phosphate Pathway (PPP)

Other names:

- The Pentose Phosphate Shunt
- Phosphogluconate Pathway
- Hexose Monophosphate Shunt

PPP as a metabolic pathway

- Tissues most heavily involved in fatty acid and cholesterol biosynthesis (liver, mammary gland, adipose tissue, and adrenal cortex) are rich in pentose phosphate pathway enzymes.

- 30% of the glucose oxidation in liver occurs via the pentose phosphate pathway.

What is it for?

1. **To produce 3, 4, 5, 6 and 7 carbon sugars.**
   - ribose 5-phosphate (R5P) is used in the synthesis of nucleotides and nucleic acids.
   - erythrose 4-phosphate (E4P) used in the synthesis of aromatic amino acids.

2. **To produce NADPH**
   - NADPH is used in reductive biosynthesis reactions (e.g. fatty acid synthesis).

- The principal products of the pentose phosphate pathway are R5P and NADPH.

- The transaldolase and transketolase reac- tions serve to convert excess R5P to glycolytic intermediates (e.g., GAP and F6P) when the metabolic need for NADPH exceeds that of R5P in nucleotide biosynthesis.

- The resulting GAP and F6P can be consumed through glycolysis and oxidative phosphorylation or recycled by gluconeogenesis to form G6P.

Reducing power: NADPH

- ATP is the cell's "energy currency"; its exergonic hydrolysis is coupled to many otherwise endergonic cell functions.

- *Cells have a second currency, reducing power.*

- Many endergonic reactions, notably the reductive biosynthesis of fatty acids and cholesterol, as well as photosynthesis, require NADPH in addition to ATP.

NADPH is different from NADH

- Despite their close chemical resemblance, *NADPH and NADH are not metabolically interchangeable*
- NADH participates in utilizing the free energy of metabolite oxidation to synthesize ATP (oxidative phospho- rylation)
- *NADPH is involved in utilizing the free energy of metabolite oxidation for otherwise endergonic reductive biosynthesis.*
- Cells normally maintain their $[NAD^+]/[NADH]$ ratio near 1000, which favors metabolite oxidation.
- Cells keep their $[NADP^+]/[NADPH]$ ratio near 0.01, which favors metabolite reduction.

Pathways requiring NADPH

Synthesis

Fatty acid biosynthesis

Cholesterol biosynthesis

Neurotransmitter biosynthesis

Nucleotide biosynthesis

Detoxification

Reduction of oxidized glutathione

Cytochrome P450 monooxygenases

NADPH and Cytochrome P450 system

- Monooxygenases (mixed function oxidases) incorporate one atom from molecular oxygen into a substrate (creating a hydroxyl group),with the other atom being reduced to water.

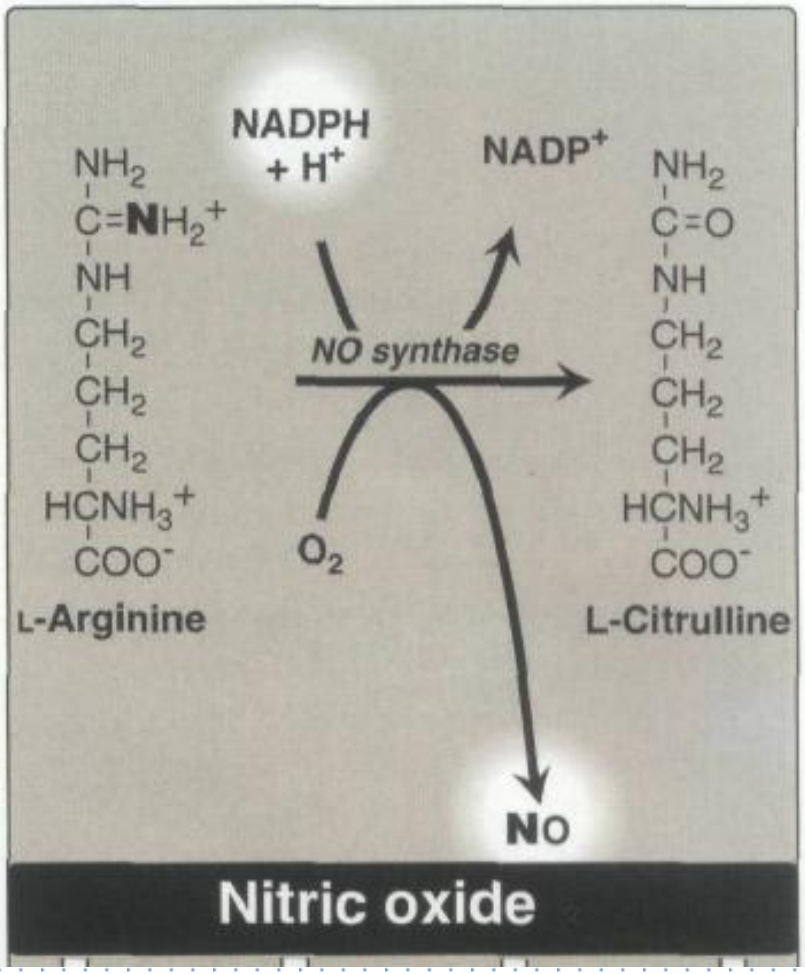
- In the cytochrome P450 monooxygenase system, NADPH provides the reducing equivalentsrequired by this series of reactions.

- The function of the *mitochondrial cytochrome P450 monooxygenase* system is to participate in the hydroxylation of steroids, a process that makes these hydrophobic compounds more water soluble.

- The function of *the microsomal cytochrome P450 monooxygenase* system found associated with the membranes of the smooth endoplasmic reticulum is the detoxification of foreign compounds (xenobiotics).

NADPH and the synthesis of nitric oxide (NO)

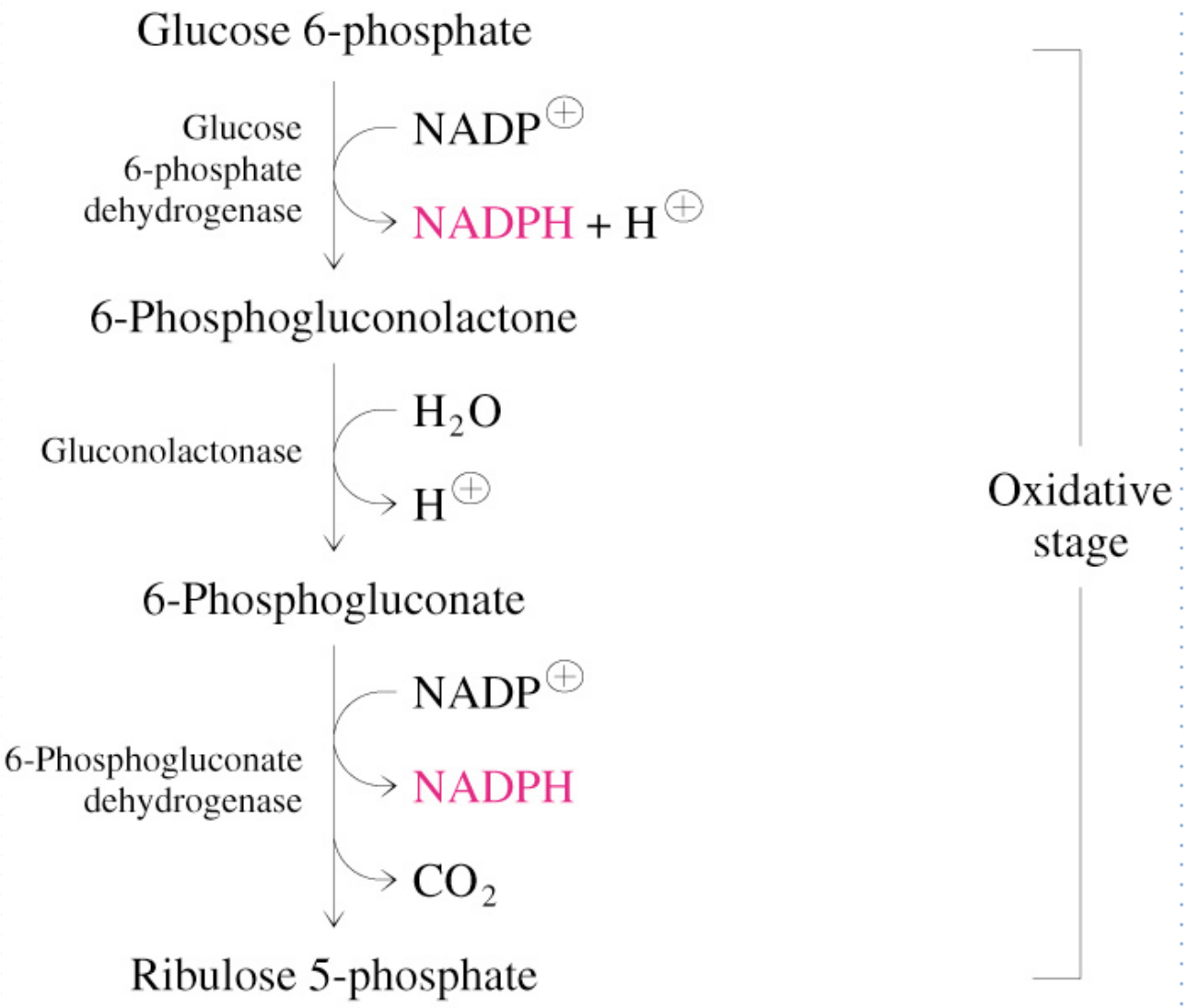
- Nitric oxide (NO) is recognized as a mediator in a broad array of biologic systems.
- NO is the endothelium-derived relaxing factor, which causes vasodilation by relaxing vascular smooth muscle.
- NO also acts as a neurotransmitter, prevents platelet aggregation, and plays an essential role in macrophage function.
- Arginine, O_2, and NADPH are substrates for cytosolic *NO synthase*.
- NO and citrulline are products of the reaction.



PPP

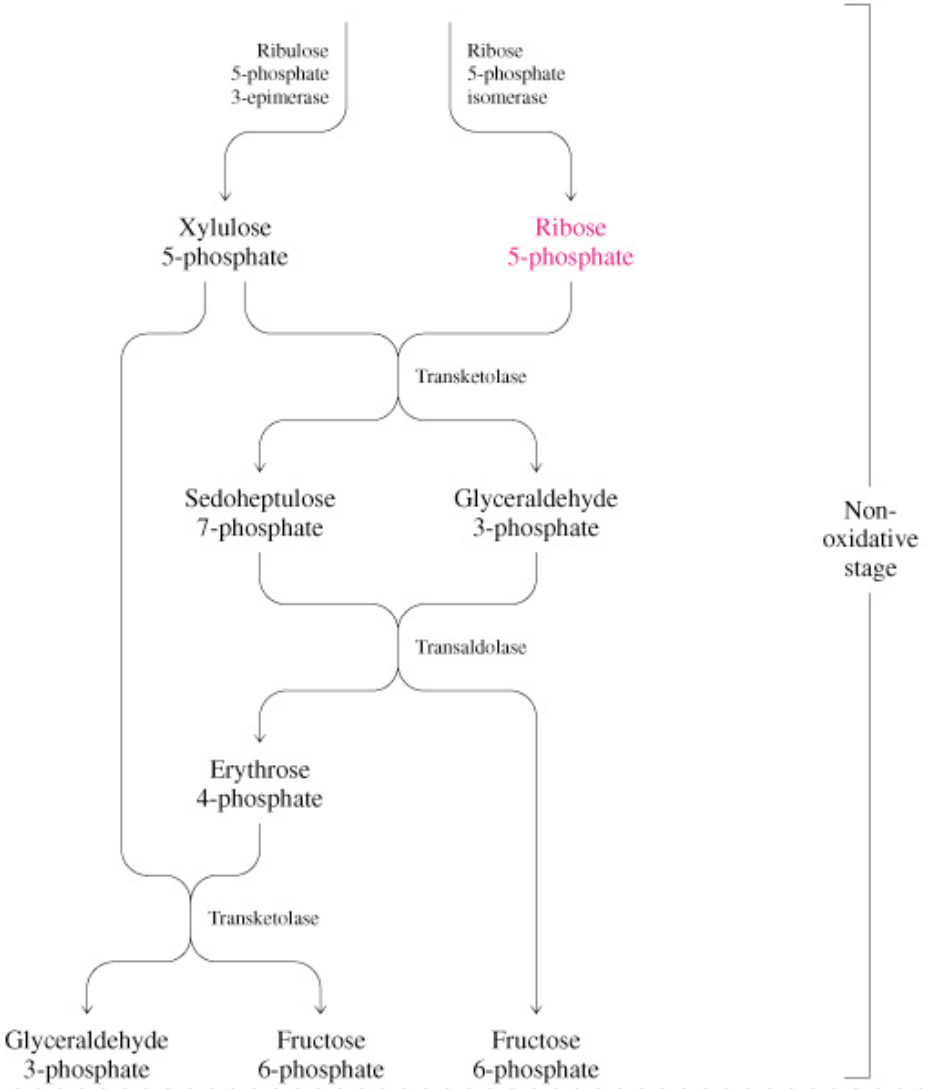
- Two distinct phases in the pathway:
  1. Oxidative phase: NADPH is generated
  2. Non-oxidative phase: 5-carbon sugars are generated

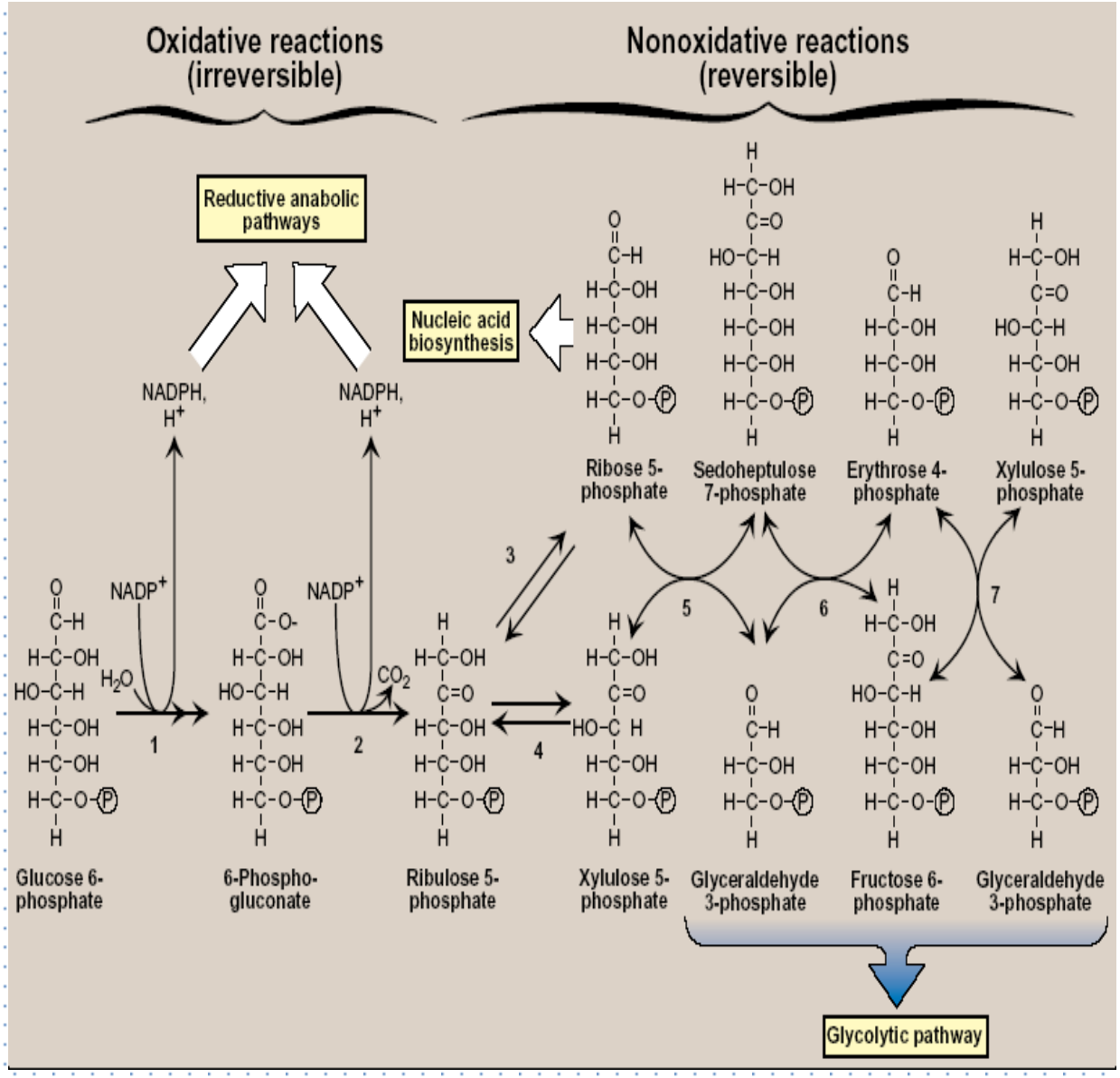
Oxidative phase of pentose phosphate cycle



- *Flux through the oxidative pentose phosphate pathway and thus the rate of NADPH production is controlled by the rate of the* ***glucose-6-phosphate dehydrogenase*** *reaction, the first reaction in PPP pathway.*

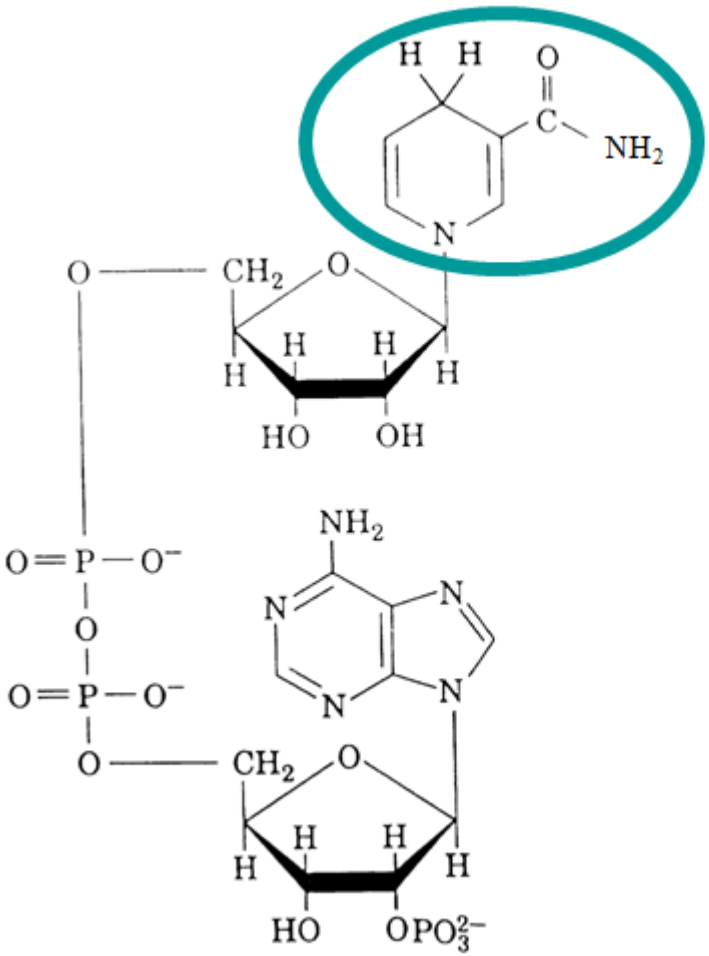
Nonoxidative phase of pentose phosphate cycle





The PPP operates in the cytosol and oxidation of glucose produces 3,4,5,6,7 C sugars, NADPH and CO2 but not ATP.

NADPH



derived from niacin (vitamin B3)

Deficiency of Niacin causes *pellegra (a.k.a. rough skin)* which is a disease characterized by the "three Ds" - dematitis, diarrhea, and dementia.

PPP carries most common genetic enzymopathy:
Glucose 6-Phosphate Dehydrogenase (G6PD) Deficiency

- It is a recessive sex-linked mutation on X-chromosome
- Rare in females (two X-chromosomes)
- It causes hemolytic anemia which is caused by the inability to detoxify oxidizing agents
- 400 million people affected worldwide
- Over 400 genetic variants of the G6PD protein are known
- The result of high rate of hemolysis: low RBC count and low hemoglobin

- Diminished G6PD activity impairs the ability of the cell to form NADPH that is essential for the maintenance of reduced glutathione (GSH) pool.

- GSH is a cysteine-containing tripeptide and its major function of is to eliminate H_2O_2 and organic hydroperoxides in the erythrocyte. Peroxides are eliminated through the action of *glutathione peroxidase*.

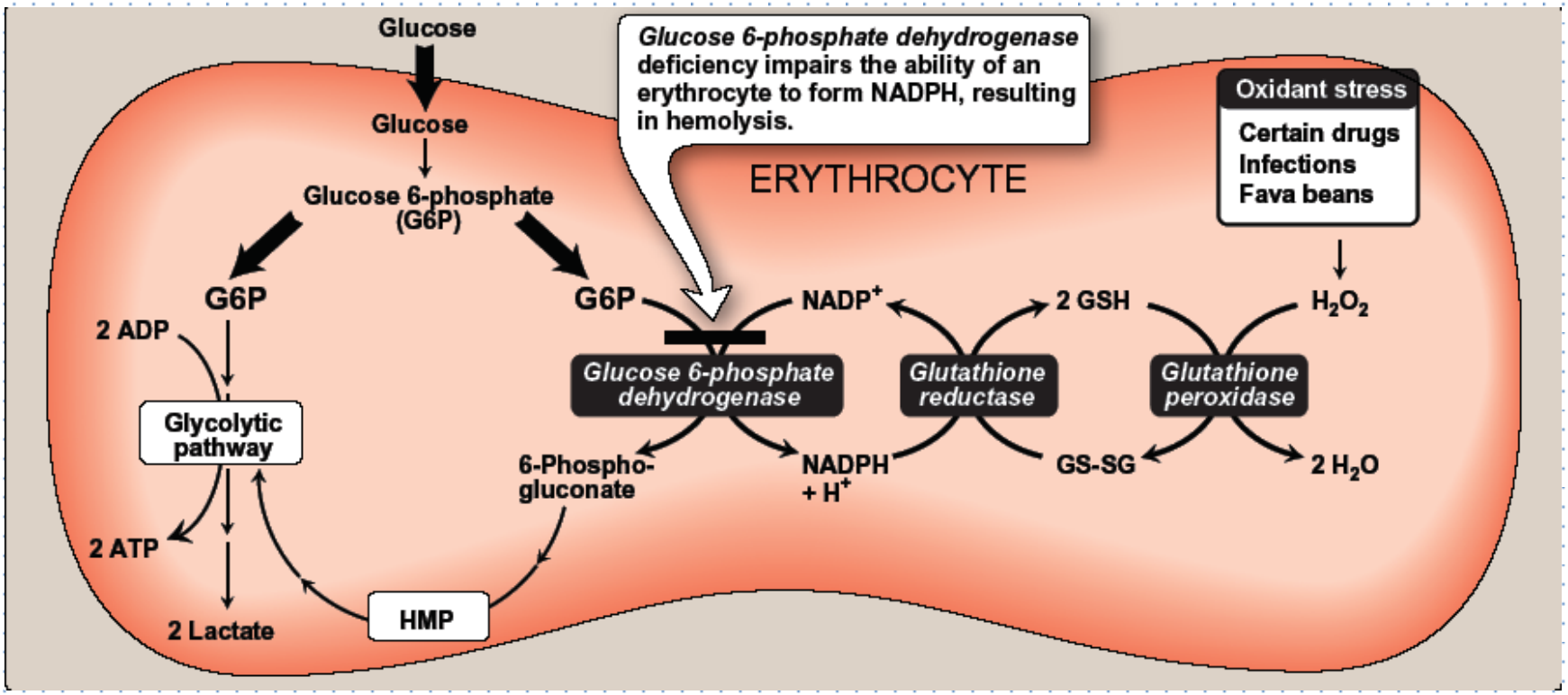
- Howevre, if H2O2 accumulate it reacts with double bonds in the fatty acid residues of the erythrocyte cell membrane to form organic hydroperoxides.

- These, in turn, react to cleave fatty acid C¬C bonds, thereby damaging the membrane, causing premature cell lysis.

- GSH also helps maintain the reduced states of sulfhydryl groups in proteins, including Hb.

- Oxidation of those sulfhydryl groups leads to formation of denatured proteins that form insoluble masses (called Heinz bodies) that attach to the red cell membranes.

- Oxidation of membrane proteins causes the red cells to be rigid and so they are removed from the circulation by macrophages.



Pathways of G-6-P metabolism in the erythrocyte

Erythrocytes and the PPP

- The predominant pathways of carbohydrate metabolism in the red blood cell (RBC) are:
  - Glycolysis
  - the PPP
  - 2,3-bisphosphoglycerate (2,3-BPG) metabolism

- Glycolysis provides ATP for membrane ion pumps and NADH for re-oxidation of methemoglobin.

- The PPP supplies the RBC with NADPH to maintain the reduced state of glutathione which blocks the accumulation of peroxides (predominantly H_2O_2). Increased peroxide accumulation causes the weakening of the cell membrane and concomitant hemolysis.

- 2,3-BPG derived from the glycolytic intermediate 1,3-bisphosphoglycerate, is a potent allosteric effector on the oxygen binding properties of hemoglobin.

- The synthesis of 2,3-BPG in erythrocytes is critical for controlling hemoglobin affinity for oxygen.

- In the deoxygenated T conformation, a cavity capable of binding 2,3-BPG forms in the center of the hemoglobin tetramer. A single molecule of 2,3-BPG can occupy this cavity which thereby, stabilizes the T state. Conversely, when 2,3-BPG is not available, or not bound in the central cavity, Hb can bind oxygen (forming HbO_2) more readily.

- When glucose is oxidized by this pathway the erythrocytes have a net energy yield of “zero” ATPs in glycolytic pathway.

- This is beacuse 2 moles of ATP from glycolytic oxidation of 1,3-BPG to 3-phosphoglycerate via the phosphoglycerate kinase reaction are not produced in the 2,3-BPG pathway.

- red blood cells are particularly affected because they lack mitochondria.

- NADPH is important for maintaining adequate levels of reduced glutathione (GSH) in the cell.

- The GSH is critical for destroying hydrogen peroxide and maintaining the cysteine residues in hemoglobin and other rbc proteins in the reduced state as well as the iron in hemoglobin in the ferrous state.

- Although G6PD deficiency occurs in all cells of the affected individual, it is most severe in erythrocytes, where the PPP provides the only means of generating NADPH

- Other tissues have alternative sources for NADPH production (e.g., $NADP^+$-dependent malate dehydrogenase) that keep glutathione reduced

- The erythrocyte has no nucleus or ribosomes and thus cannot renew its supply of the enzymes.

- Individuals with G6PD deficiency must not eat Fava beans (Favism)

- Interesting!!!
  - The growth Plasmodium falciparum (malaria parasite) fails in G6PD deficient individuals.
  - Thus people with G6PD deficiency are resistant to malaria

- Babies with G6PD deficiency may experience neonatal jaundice appearing 1-4 days after birth.

- The jaundice results from impaired hepatic catabolism of heme or increased production of bilirubin (a.k.a., haematoidin).

- Bilirubin is the yellow breakdown product of normal *heme* catabolism, caused by the clearance of aged red blood cells which contain hemoglobin.

- Heme is a cofactor consisting of an Fe^{2+} (ferrous) ion contained in the centre of a large heterocyclic organic ring called a porphyrin.

- Hemes are most commonly recognized as components of hemoglobin, but are also found in a number of other biologically important hemoproteins such as myoglobin, cytochrome, catalase, and nitric oxide synthase.

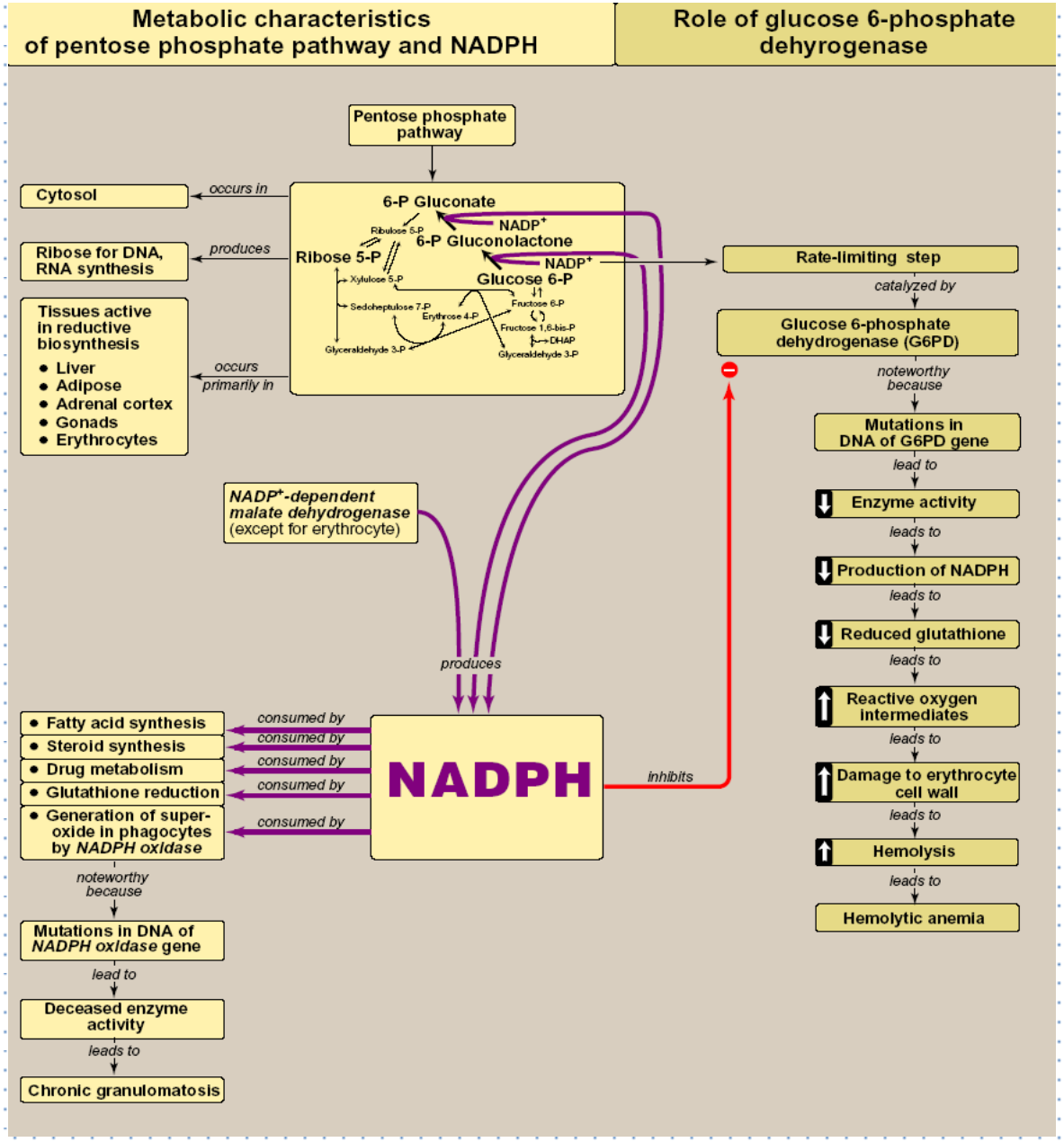
- Bilirubin is excreted in bile and urine, and elevated levels may indicate certain diseases.

- It is responsible for the yellow color of bruises and the yellow discoloration in jaundice.

- It is also responsible for the brown color of feces.

- People with the G6PD disorder are not normally anemic and display no evidence of the disease until the red cells are exposed to an oxidant or stress.

- **Drugs that can precipitate this reaction:**
  - antimalarial drugs
  - sulfonamides (antibiotic)
  - aspirin


Metabolic characteristics of pentose phosphate pathway and NADPH
Role of glucose 6-phosphate dehyrogenase
Pentose phosphate pathway
occurs in
Cytosol
produces
Ribose for DNA, RNA synthesis
occurs primarily in
Tissues active in reductive biosynthesis
Liver
Adipose
Adrenal cortex
Gonads
Erythrocytes
6-P Gluconate
NADP+
Ribulose 5-P
6-P Gluconolactone
Ribose 5-P
NADP+
Glucose 6-P
Xylulose 5-P
Fructose 6-P
Sedoheptulose 7-P
Erythrose 4-P
Fructose 1,6-bis-P
DHAP
Glyceraldehyde 3-P
Glyceraldehyde 3-P
NADP+-dependent malate dehydrogenase (except for erythrocyte)
produces
NADPH
consumed by
Fatty acid synthesis
consumed by
Steroid synthesis
consumed by
Drug metabolism
consumed by
Glutathione reduction
consumed by
Generation of super-oxide in phagocytes by NADPH oxidase
noteworthy because
Mutations in DNA of NADPH oxidase gene
lead to
Deceased enzyme activity
leads to
Chronic granulomatosis
inhibits
Rate-limiting step
catalyzed by
Glucose 6-phosphate dehydrogenase (G6PD)
noteworthy because
Mutations in DNA of G6PD gene
lead to
Enzyme activity
leads to
Production of NADPH
leads to
Reduced glutathione
leads to
Reactive oxygen intermediates
leads to
Damage to erythrocyte cell wall
leads to
Hemolysis
leads to
Hemolytic anemia

What you have learned

1. How many ATPs are produced by the oxidation of 1 glucose molecule in the pentose phosphate pathway?

None. The pathway is not designed to produce ATP

2. How many ATPs are required to produce the final products of the pathway?

None. The pathway does not require ATP

3. How many NADPHs are produced by oxidation of 3 glucose molecules in the pentose phosphate pathway?

Six. Two for each glucose oxidized.

4. Is the pentose phosphate pathway considered anaerobic or aerobic? Anabolic or catabolic? Explain.

Anaerobic and Anabolic. The pathway does not require oxygen. Therefore, it is an anaerobic pathway. Since it results in the synthesis of pentoses and NADPH, it must be considered primarily anabolic.

MBG304 Biochemistry

Lecture 7: Gluconeogenesis

Hikmet Geçkil, Professor

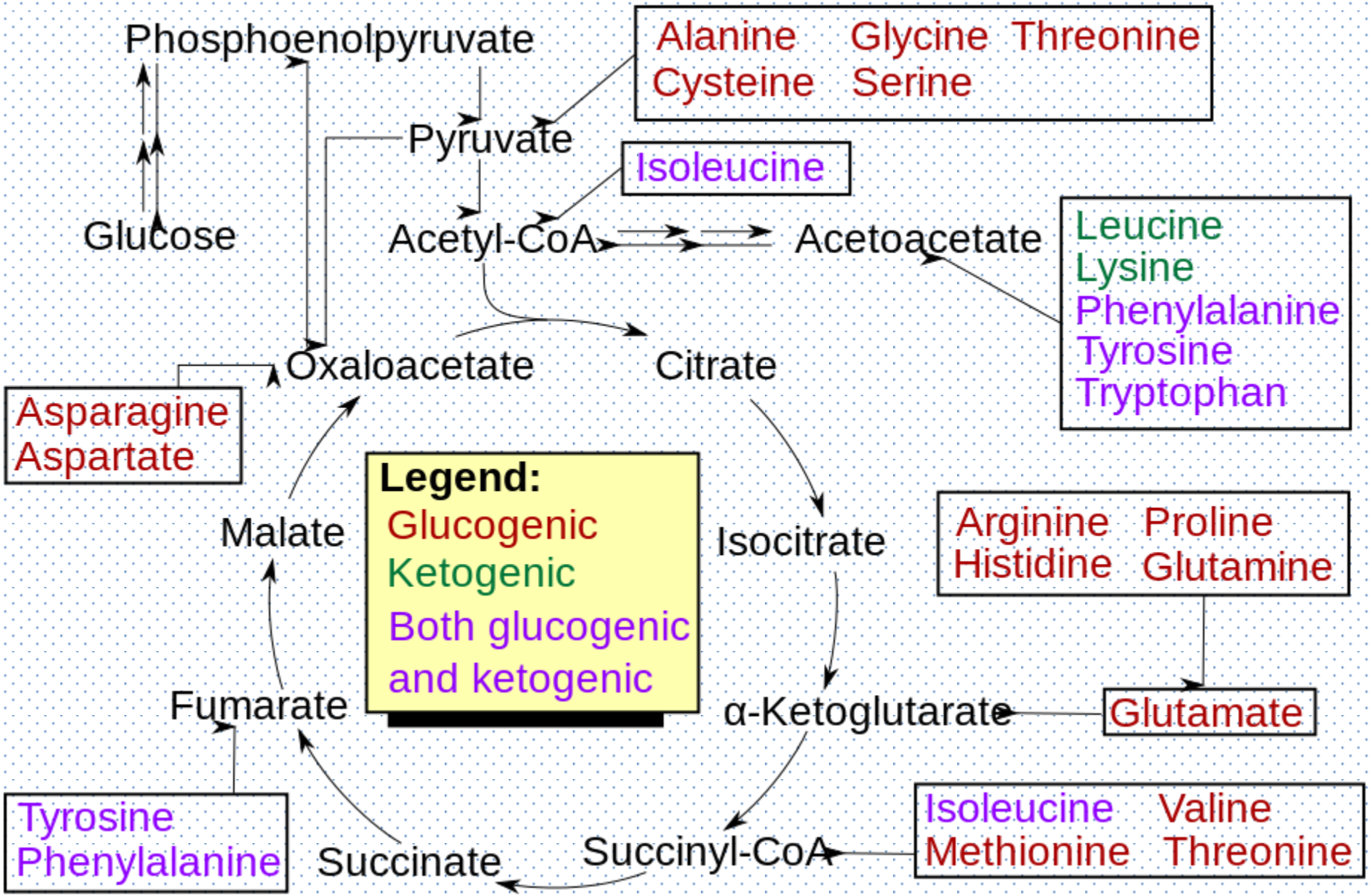
Department of Molecular Biology and Genetics

Inonu University

- Gluconeogenesis is the name for metabolic pathways that result in the generation of glucose from non-carbohydrate substrates such as pyruvate, lactate, glycerol, and glucogenic amino acids.

- Gluconeogenesis is one of the two main mechanisms used by humans and many other animals to maintain blood glucose levels, avoiding low blood glucose level (hypoglycemia).

- The other means of maintaining blood glucose levels is through the degradation of glycogen (glycogenolysis).


Phosphoenolpyruvate
Alanine Glycine Threonine
Cysteine Serine
Pyruvate
Isoleucine
Glucose
Acetyl-CoA
Acetoacetate
Leucine
Lysine
Phenylalanine
Tyrosine
Tryptophan
Oxaloacetate
Citrate
Asparagine
Aspartate
Legend:
Glucogenic
Ketogenic
Both glucogenic and ketogenic
Malate
Isocitrate
Arginine Proline
Histidine Glutamine
Fumarate
α-Ketoglutarate
Glutamate
Tyrosine
Phenylalanine
Succinate
Succinyl-CoA
Isoleucine Valine
Methionine Threonine

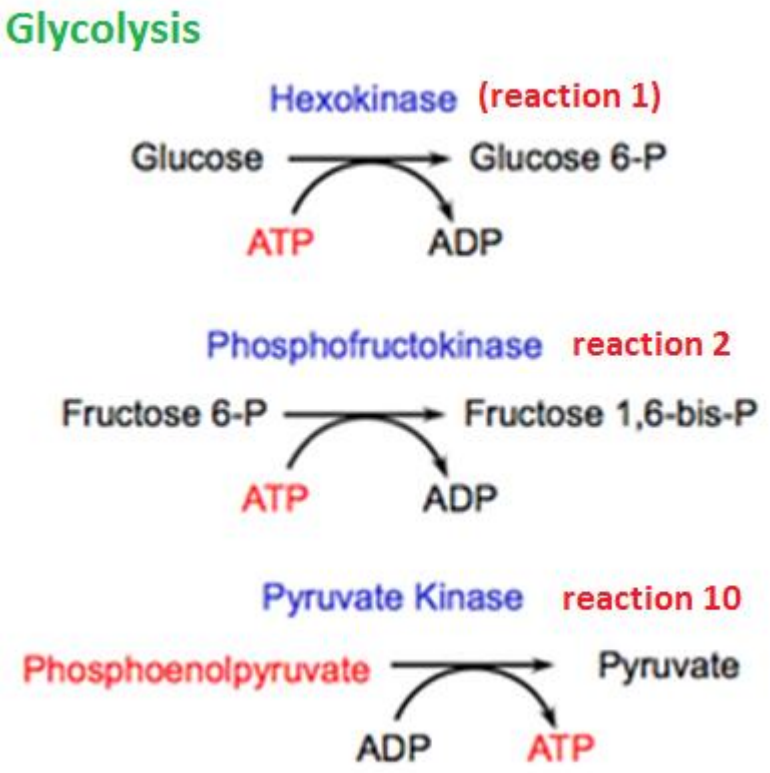
- In humans the main gluconeogenic precursors are:
  - Lactate
  - Glycerol
  - Alanine
  - Glutamine

  - They account for over 90% of the overall gluconeogenesis.

- Lactate is transported back to the liver where it is converted into pyruvate by the Cori cycle using the enzyme lactate dehydrogenase.
- Pyruvate can then be used to generate glucose.
- Transamination or deamination of amino acids facilitates entering of their carbon skeleton into the cycle directly (as pyruvate or oxaloacetate), or indirectly via the citric acid cycle.

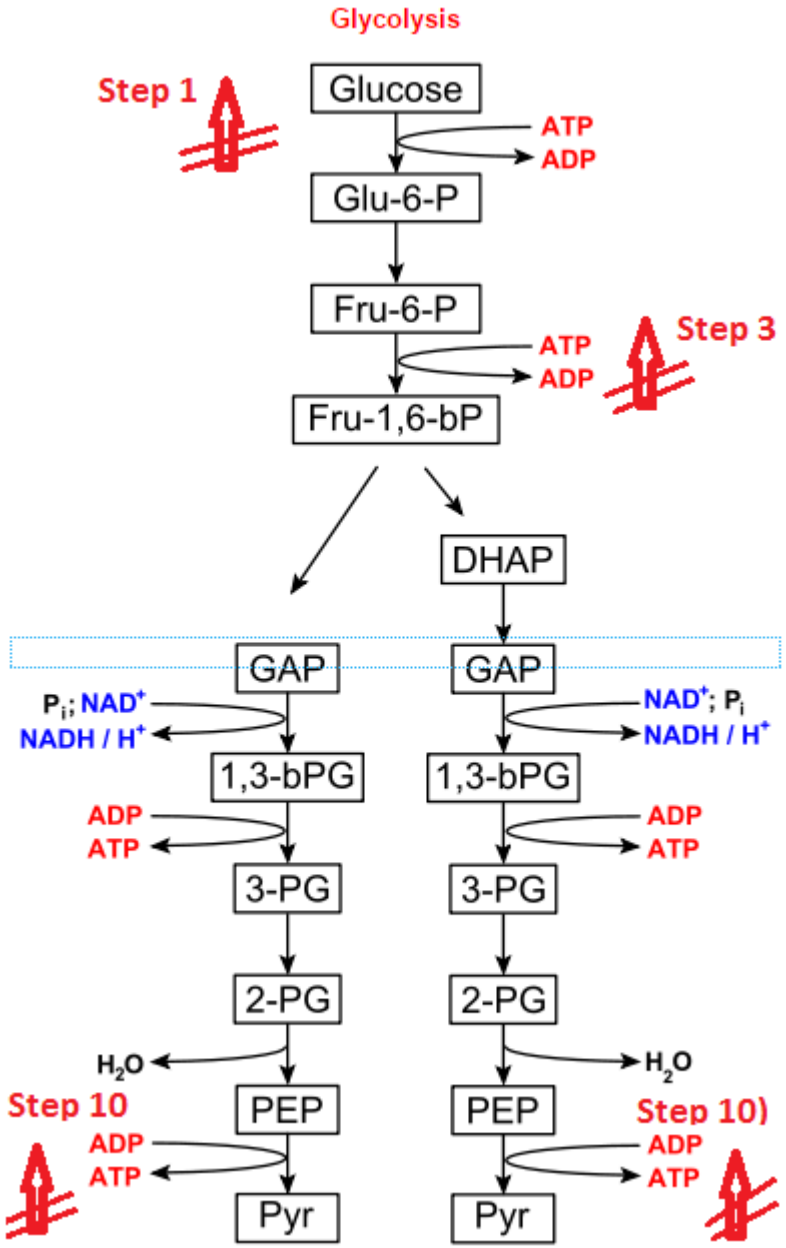
- Many of the reactions gluconeogenesis are the reversible steps found in glycolysis.

- In glycolysis there are three highly exergonic steps (steps 1,3,10) and these are also regulatory steps which include the enzymes *hexokinase*, *phosphofructokinase*, and *pyruvate kinase*.

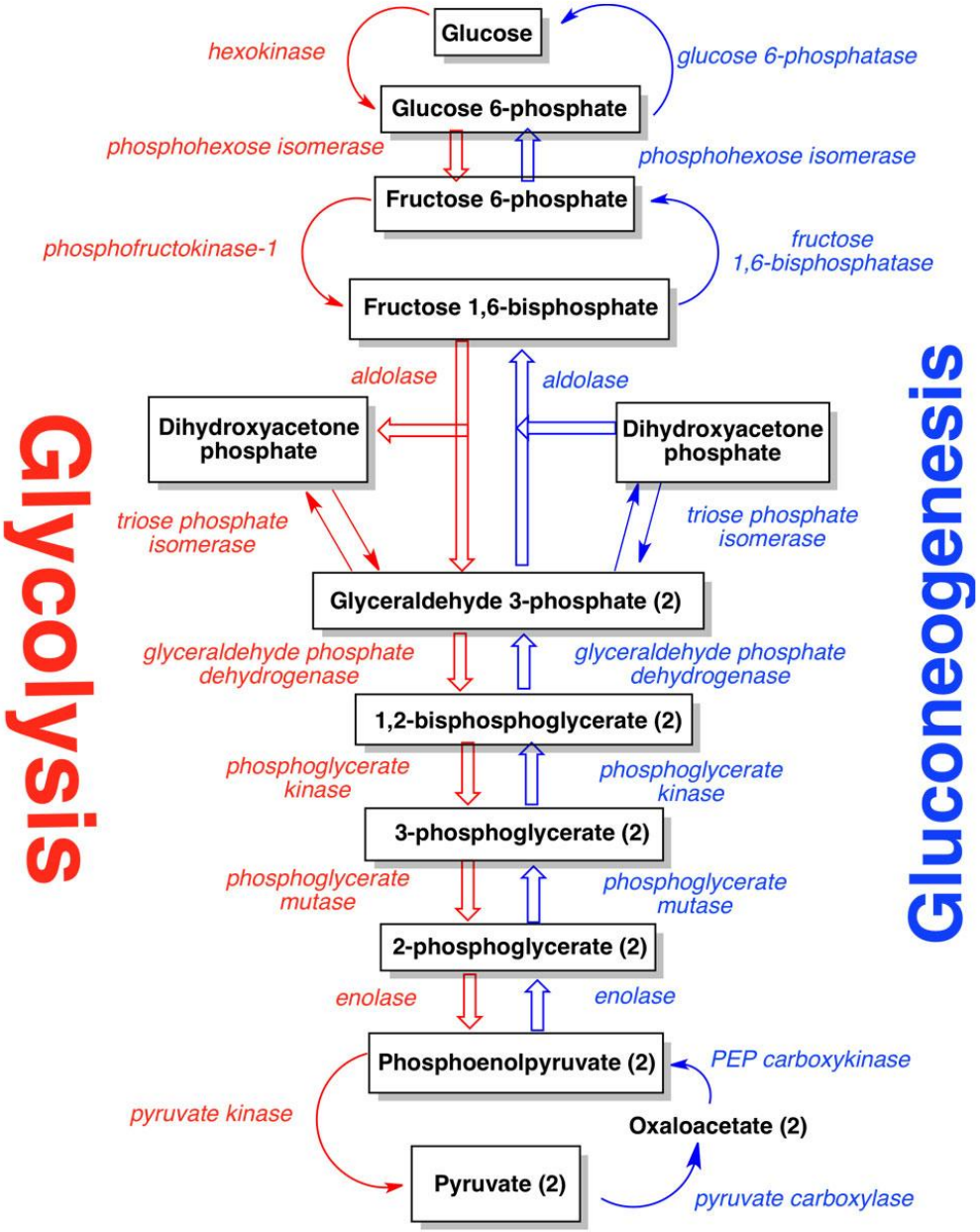


Irreversible steps

- Glucose 6-phosphatase (reaction 1)
- Fructose 1,6-biphosphatase (rection3)
- Pyruvate carboxylase (pyruvate → oxaloacetate) and then Phosphoenol pyruvate carboxykinase (oxaloacetate → phosphosenol pyruvate) (reaction 10)



- It begins in the mitochondria with the formation of oxaloacetate by the carboxylation of pyruvate in a reaction that requires one molecule of ATP, and is catalyzed by *pyruvate carboxylase*.
- Oxaloacetate is reduced to malate using NADH, a step required for its transportation out of the mitochondria.
- Malate is oxidized to oxaloacetate using NAD^+ in the cytosol, where the remaining steps of gluconeogenesis take place.
- Oxaloacetate is decarboxylated and then phosphorylated to form phosphoenolpyruvate.
- The next steps in the reaction are the same as reversed glycolysis.


Glycolysis
Gluconeogenesis
Glucose
Glucose 6-phosphate
Fructose 6-phosphate
Fructose 1,6-bisphosphate
Dihydroxyacetone phosphate
Dihydroxyacetone phosphate
Glyceraldehyde 3-phosphate (2)
1,2-bisphosphoglycerate (2)
3-phosphoglycerate (2)
2-phosphoglycerate (2)
Phosphoenolpyruvate (2)
Pyruvate (2)
Oxaloacetate (2)
hexokinase
glucose 6-phosphatase
phosphohexose isomerase
phosphohexose isomerase
phosphofructokinase-1
fructose 1,6-bisphosphatase
aldolase
aldolase
triose phosphate isomerase
triose phosphate isomerase
glyceraldehyde phosphate dehydrogenase
glyceraldehyde phosphate dehydrogenase
phosphoglycerate kinase
phosphoglycerate kinase
phosphoglycerate mutase
phosphoglycerate mutase
enolase
enolase
PEP carboxykinase
pyruvate kinase
pyruvate carboxylase

Gluconeogenesis- ANIMATED

gluconeogenesis.swf

MBG304 Biochemistry

Lecture 8: Lipid metabolism

Hikmet Geçkil, Professor

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- In the past few lectures we have seen how energy can be released by the catabolic breakdown of carbohydrates (mainly glucose) in aerobic and anaerobic processes.

- In this lecture, we will learn how the metabolic oxidation of lipids releases large quantities of energy through production of acetyl-CoA, NADH, and $FADH_2$.

- Lipids include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids, and others (we have seen all these in first semester).

- The main biological functions of lipids:
  - storing energy
  - signaling
  - acting as structural components of cell membranes

Lipids*

- Three main group of lipids:
  1. **Triacylglycerols** (a.k.a., triacylglycerids)
     - the main storage form of the chemical energy of lipids
  2. **Phospholipids** (a.k.a., phosphoacylglycerols)
     - important components of biological membranes
  1. **Sterols**
     - not catabolized as a source of energy but are excreted (e.g., cholesterol)

*The topic in first semester

Triacylglycerols (a.k.a., triglycerides, triacylglycerides)

- They are the esters derived from glycerol and three fatty acids

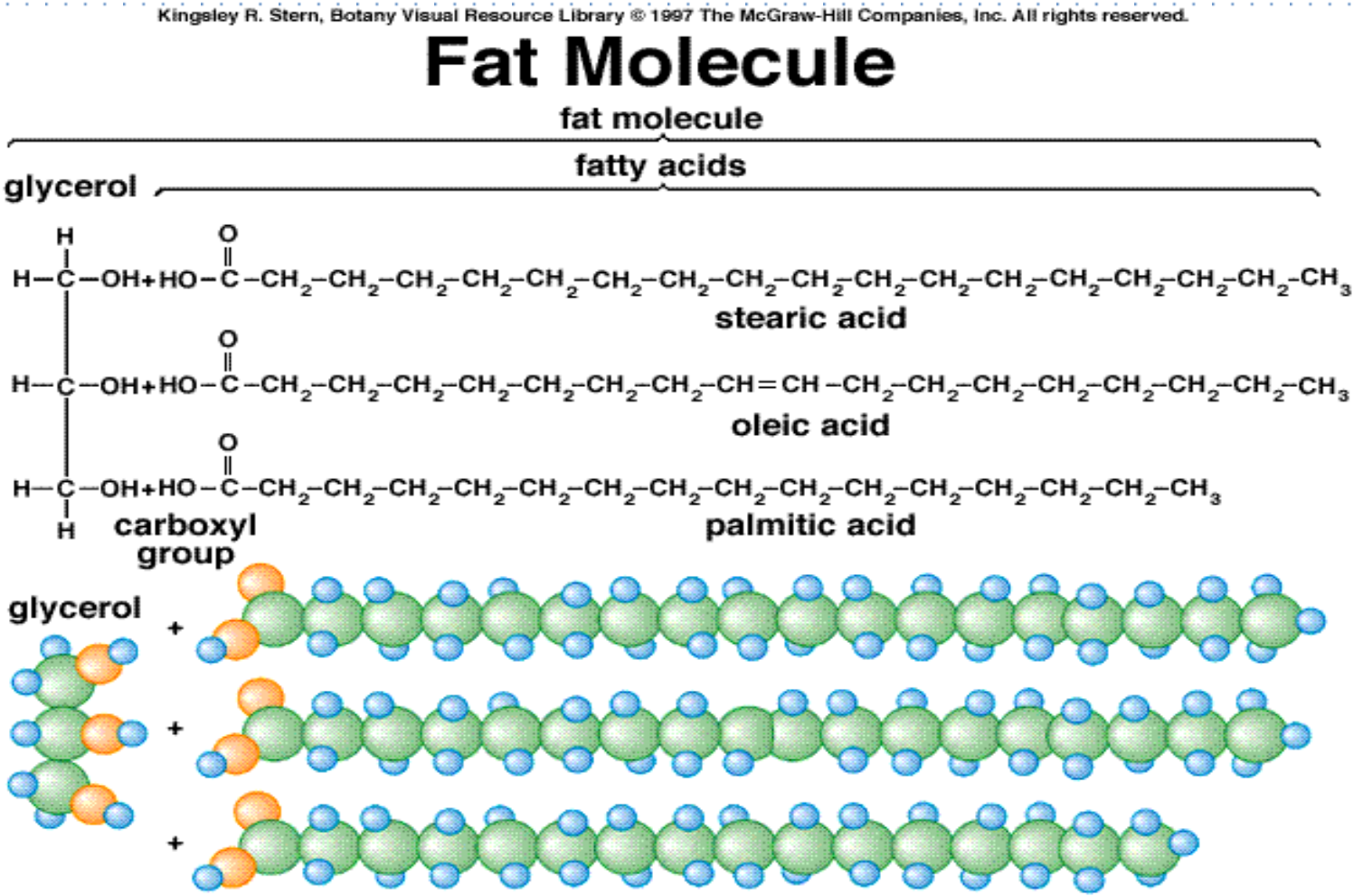
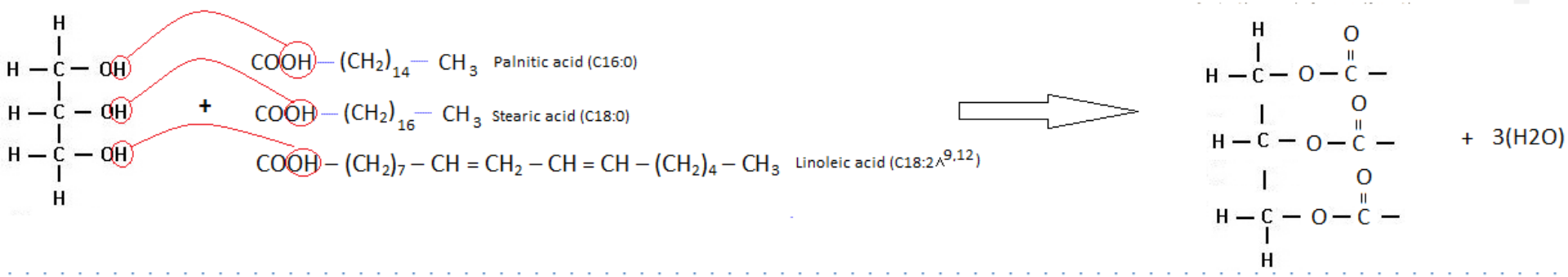


TABLE Stored Metabolic Fuel in a 70-kg Person

Constituent	Energy (kJ/g dry weight)	Dry Weight (g)	Available Energy (kJ)
Fat (adipose tissue)	37	15,000	555,000
Protein (muscle)	17	6,000	102,000
Glycogen (muscle)	16	120	1,920
Glycogen (liver)	16	70	1,120
Glucose (extracellular fluid)	16	20	320
Total			660,360

- Fat constitutes approximately 40% of the calories in the average diet. Of this, more than 95% of the calories are present as triacylglycerols

- Triglycerides are formed by combining glycerol with three fatty acid molecules.



- Triacylglycerols are the most important energy reserves

- They are mostly stored in insoluble form in the cells of adipose tissue—the *adipocytes*—where they are constantly being synthesized and broken down again.

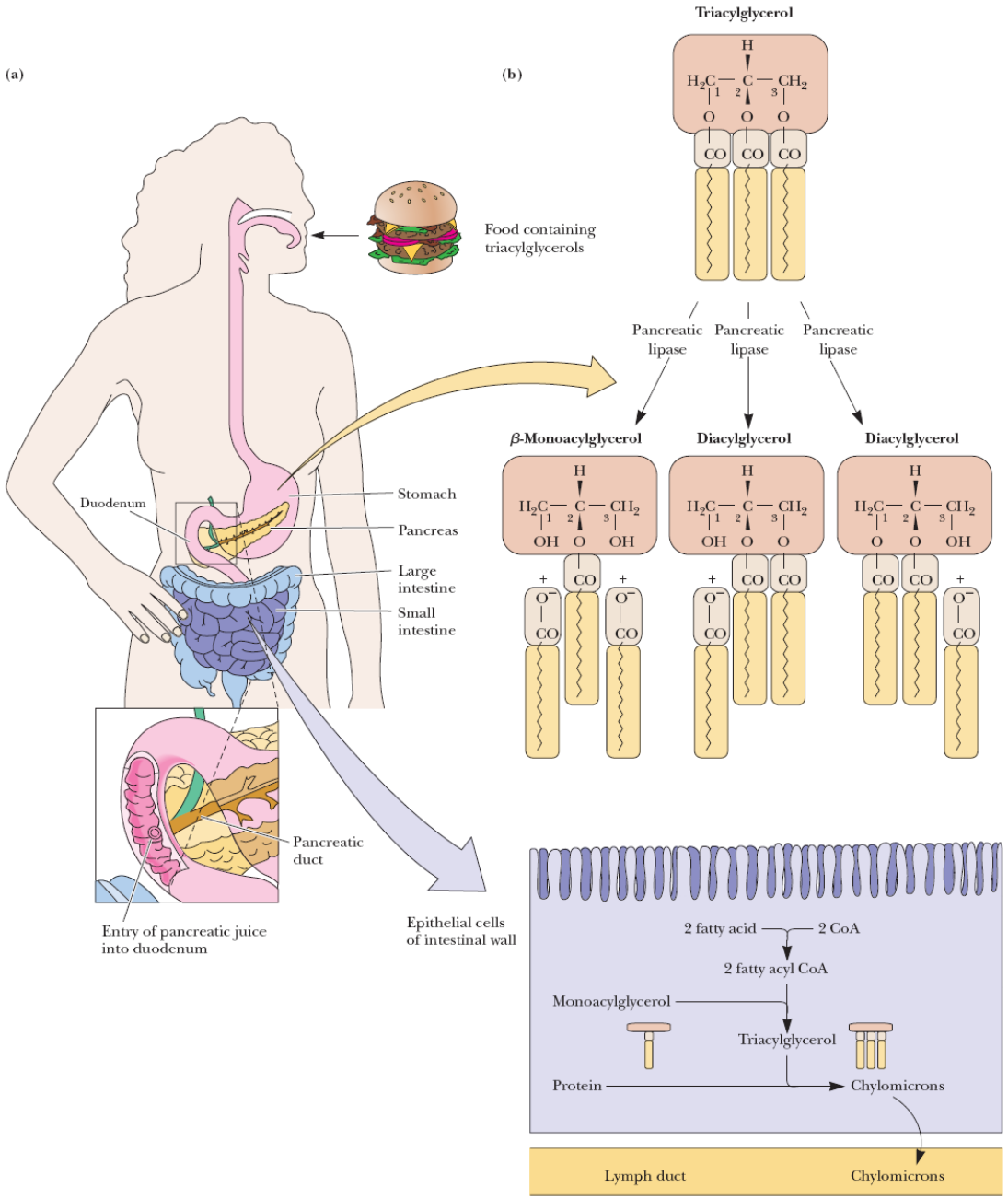
- The metabolism of fatty acids is particularly intensive in the hepatocytes in the liver.

Scanning electron micrograph of an adipose cell (fat cell). Globules of triacylglycerols occupy most of the volume of such cells.

(a) The pancreatic duct secretes digestive fluids into the duodenum, the first portion of the small intestine.

(b) Hydrolysis of triacylglycerols by pancreatic and intestinal lipases. Pancreatic lipases cleave fatty acids at the C-1 and C-3 positions. Resulting monoacylglycerols with fatty acids at C-2 are hydrolyzed by intestinal lipases.

Fatty acids and monoacylglycerols are absorbed through the intestinal wall and assembled into lipoprotein aggregates termed chylomicrons.



- Fatty acids are both oxidized to acetyl-CoA and synthesized from acetyl-CoA.

- But, fatty acid oxidation is not the simple reverse of fatty acid biosynthesis.

- They are entirely different process: fatty acid oxidation takes place in mitochondria while biosynthesis is in the cytosol

Typical Naturally Occurring Saturated Fatty Acids

Acid	Number of Carbon Atoms	Formula	Melting Point (°C)
Lauric	12	$CH_3(CH_2)_{10}CO_2H$	44
Myristic	14	$CH_3(CH_2)_{12}CO_2H$	58
Palmitic	16	$CH_3(CH_2)_{14}CO_2H$	63
Stearic	18	$CH_3(CH_2)_{16}CO_2H$	71
Arachidic	20	$CH_3(CH_2)_{18}CO_2H$	77

Typical Naturally Occurring Unsaturated Fatty Acids

Acid	Number of Carbon Atoms	Degree of Unsaturation*	Formula	Melting Point (°C)
Palmitoleic	16	16:1—Δ^9	$CH_3(CH_2)_5CH{=}CH(CH_2)_7CO_2H$	−0.5
Oleic	18	18:1—Δ^9	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2H$	16
Linoleic	18	18:2—$\Delta^{9,12}$	$CH_3(CH_2)_4CH{=}CH(CH_2)CH{=}CH(CH_2)_7CO_2H$	−5
Linolenic	18	18:3—$\Delta^{9,12,15}$	$CH_3(CH_2CH{=}CH)_3(CH_2)_7CO_2H$	−11
Arachidonic	20	20:4—$\Delta^{5,8,11,14}$	$CH_3(CH_2)_4CH{=}CH(CH_2)_4(CH_2)_2CO_2H$	−50

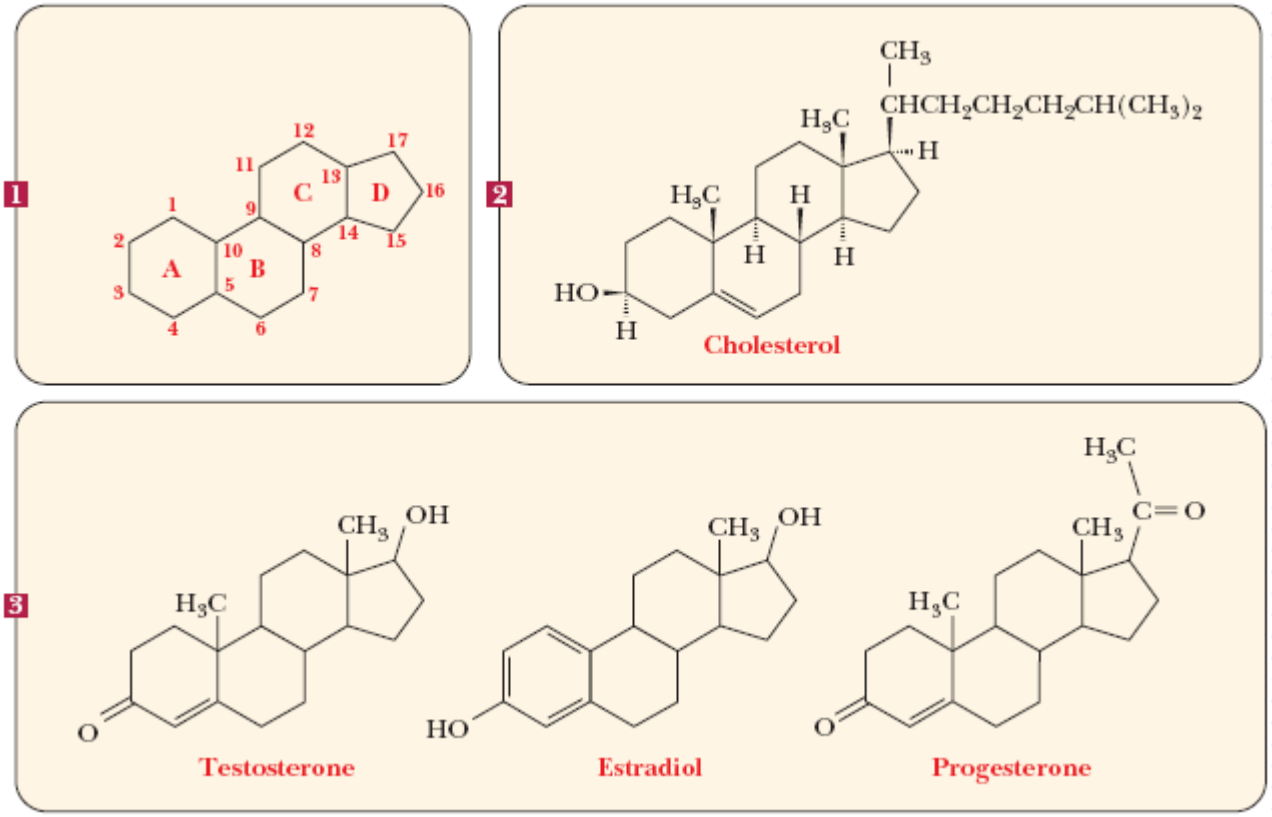
- In both **triacylglycerols** and **phospholipids**, the bond between the fatty acid and the rest of the molecule can be hydrolyzed with the reaction catalyzed by *lipases* and *phospholipases*, respectively.

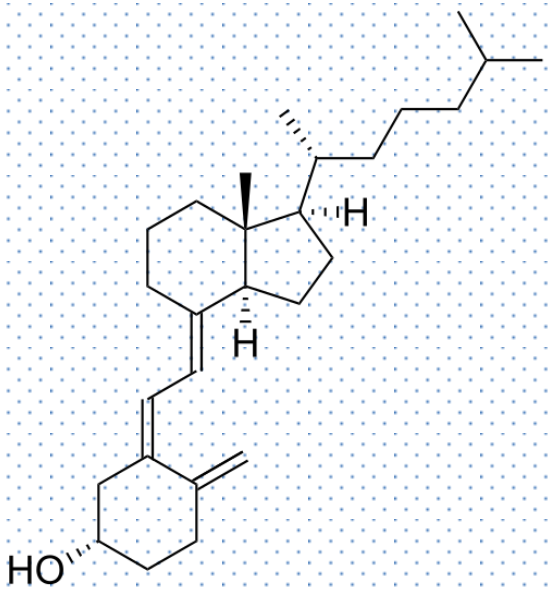
- Fatty acids enter cells both by a saturable transport process and by diffusion through the lipid plasma membrane. A fatty acid binding protein in the plasma membrane facilitates transport.

- An additional fatty acid binding protein binds the fatty acid intracellularly and may facilitate its transport to the mitochondrion. The free fatty acid concentration in cells is, therefore, extremely low.

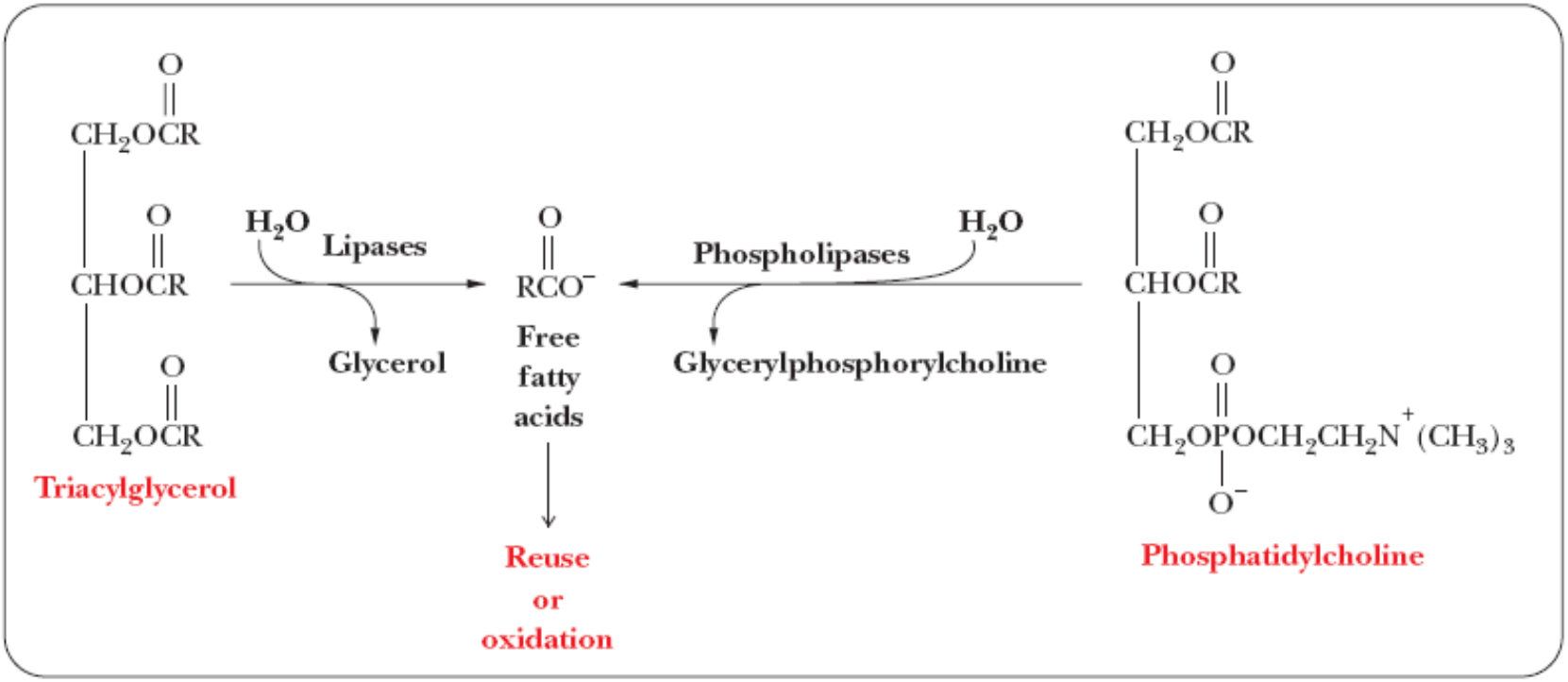
- Phospholipases are involved in hydrolysis of phospholipids and they also occur in venom and are responsible for the tissue damage. The lipid products of hydrolysis lyse red blood cells, preventing clot formation. Thus, snakebite victims bleed to death in this situation.

Structures of some steroids





cholecalciferol, aka vitamin D3



The release of fatty acids for future use. The source of fatty acids can be a triacylglycerol *(left) or a phospholipid such as phosphatidylcholine (right).*

- The release of fatty acids from triacylglycerols in adipocytes is controlled by hormones.

- A hormone (insulin counterregulatory hormones such as glucagon, epinephrine*) binds to a receptor on the plasma membrane and activates adenylate cyclase.

- Protein kinase phosphorylates triacylglycerol lipase, which cleaves the fatty acids from the glycerol backbone.

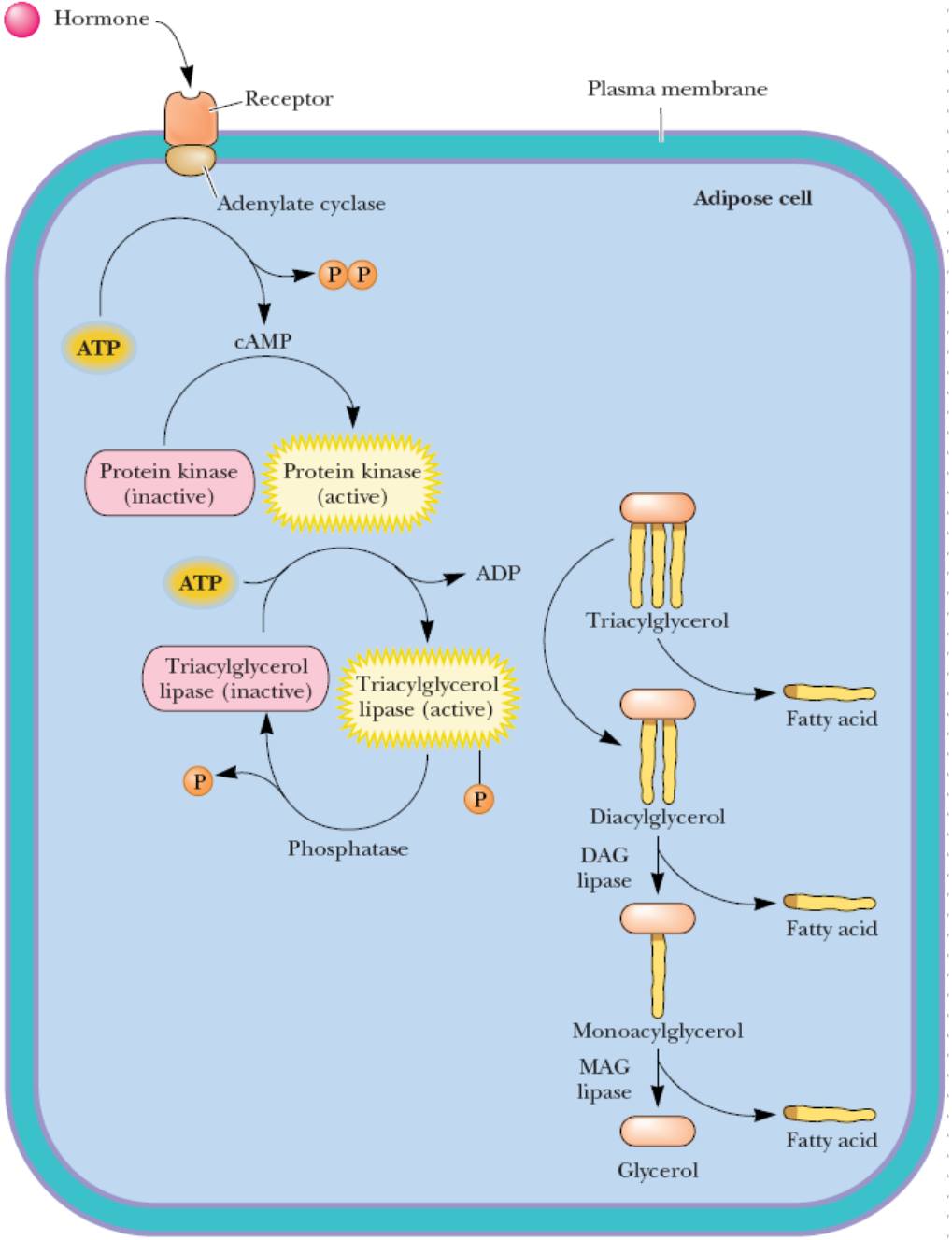
*Caffeine mimics the effect epinephrine and glucagon. Distance runners often drink caffeine the morning to burn fat more efficiently and to spare their carbohydrate stores for the later stages of the race.

•Liberation of fatty acids from triacylglycerols in adipose tissue is hormone dependent.

•The hormones (e.g., glucagon, epinefrin, ACTH) bind to receptors on the plasma membrane of adipose cells and lead to the activation of adenylyl cyclase, which forms cyclic AMP from ATP.

•cAMP activates protein kinase A, which phosphorylates and activates a **triacylglycerol lipase.**

•Lipase hydrolyzes triacylglycerols yielding fatty acids and glycerol. Serum albumin transports free fatty acids to sites of utilization.



Fatty-acid oxidation

- Fatty-acid oxidation begins with **activation of the molecule**.

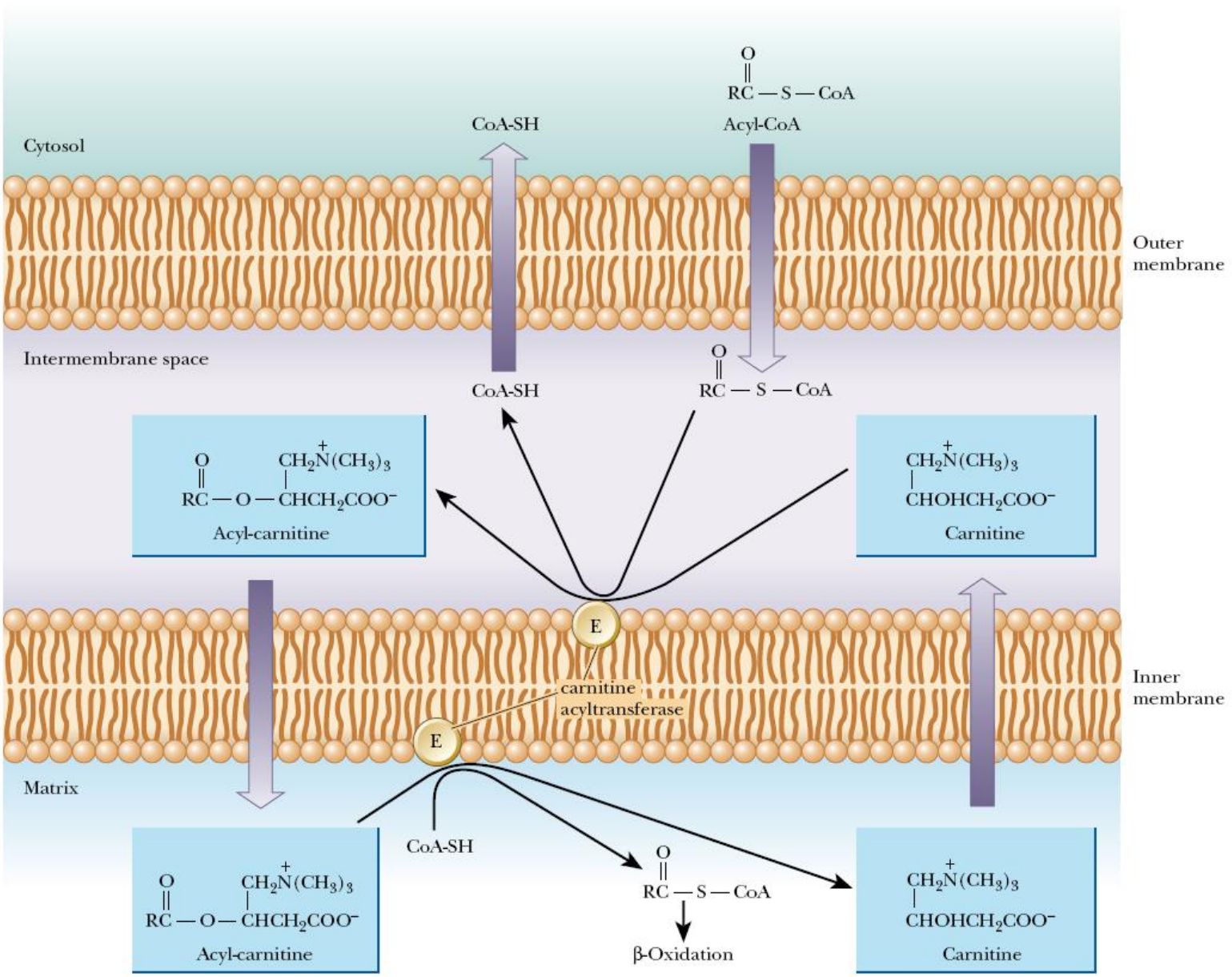
- The process of activation involves an **acyl CoA synthetase** (also called a thiokinase) that uses ATP energy to form the fatty acyl CoA thioester bond. The activated form of the fatty acid is an acyl-CoA.

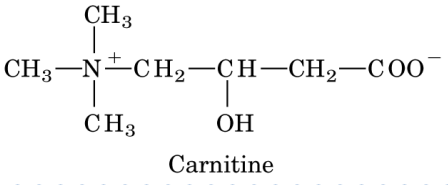
- The acyl CoA synthetase that activates long-chain fatty acids, 12 to 20 carbons in length, is present in three locations in the cell: the *endoplasmic reticulum*, *outer mitochondrial membranes*, and *peroxisomal membranes*.

- The acyl-CoA can cross the outer mitochondrial membrane but not the inner membrane

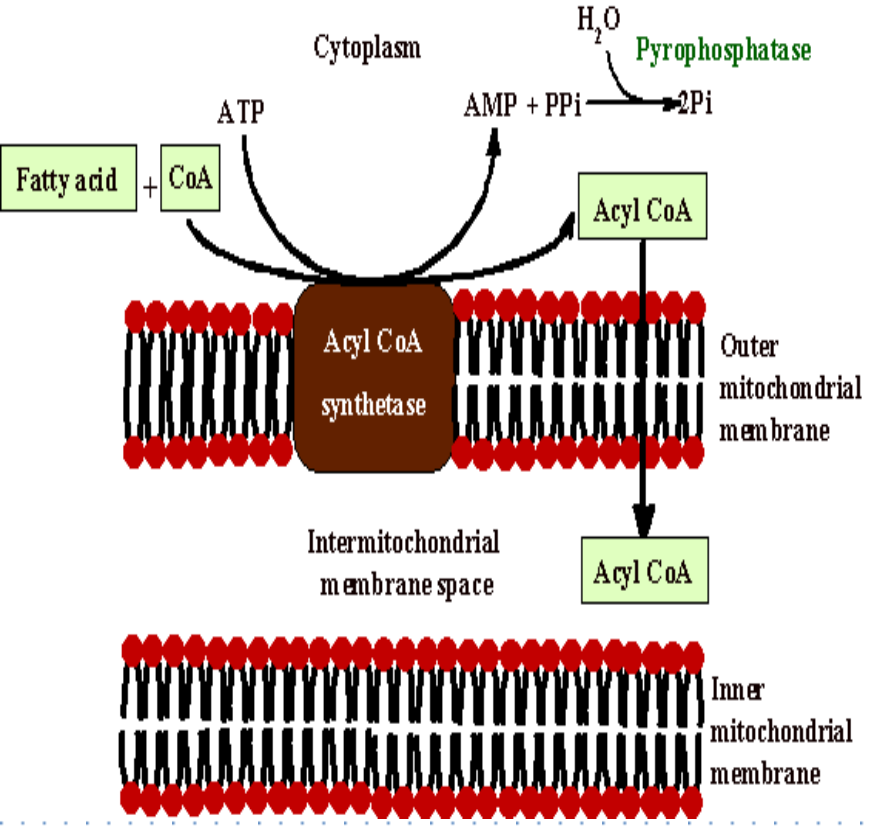
- In the intermembrane space, the acyl group is transferred to **carnitine** to form acyl-carnitine
- Acyl-carnitine can cross the inner mitochondrial membrane via *carnitine translocase*.
- Once in the matrix, the acyl group is transferred from carnitine to mitochondrial CoA-SH.
- In the matrix, a repeated sequence of reactions successively cleaves two-carbon units from the fatty acid, starting from the carboxyl end.
- This process is called **beta-oxidation**, since the oxidative cleavage takes place at the **beta-carbon** of the acyl group.

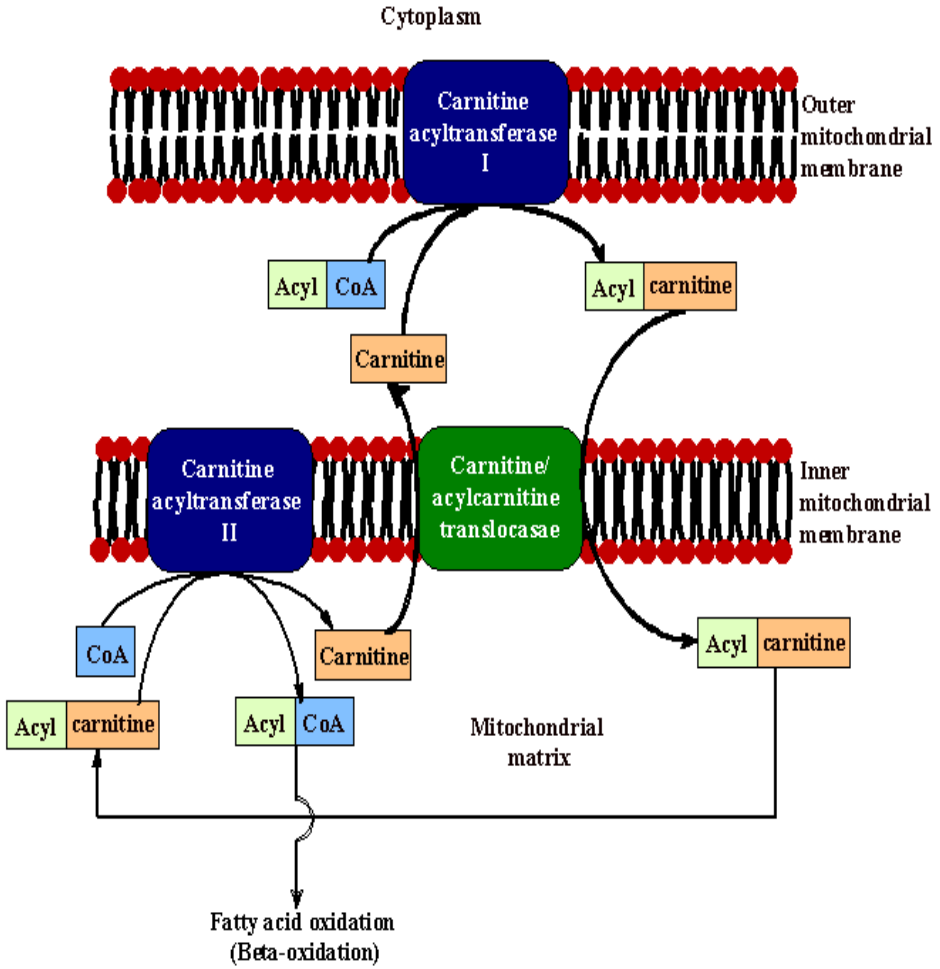
The role of carnitine in the transfer of acyl groups to the mitochondrial matrix





Role of carnitine in the transport of long-chain fatty acids through the inner mitochondrial membrane. Long-chain acyl-CoA cannot pass through the inner mitochondrial membrane, but its metabolic product, acylcarnitine, can.





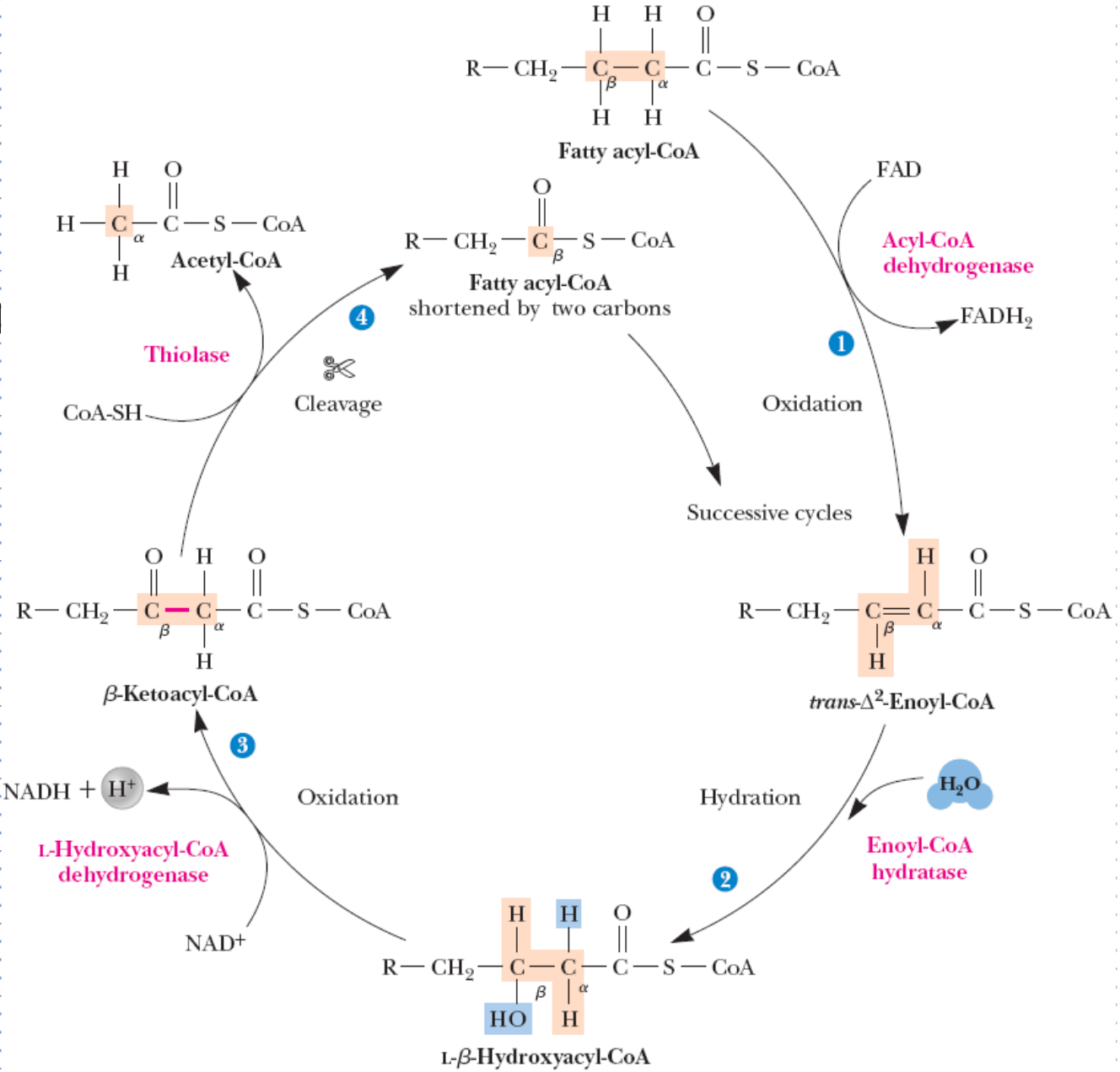
Beta-oxidation

- β-oxidation is the catabolic process by which fatty acid molecules are broken down in the mitochondria to generate acetyl-CoA.

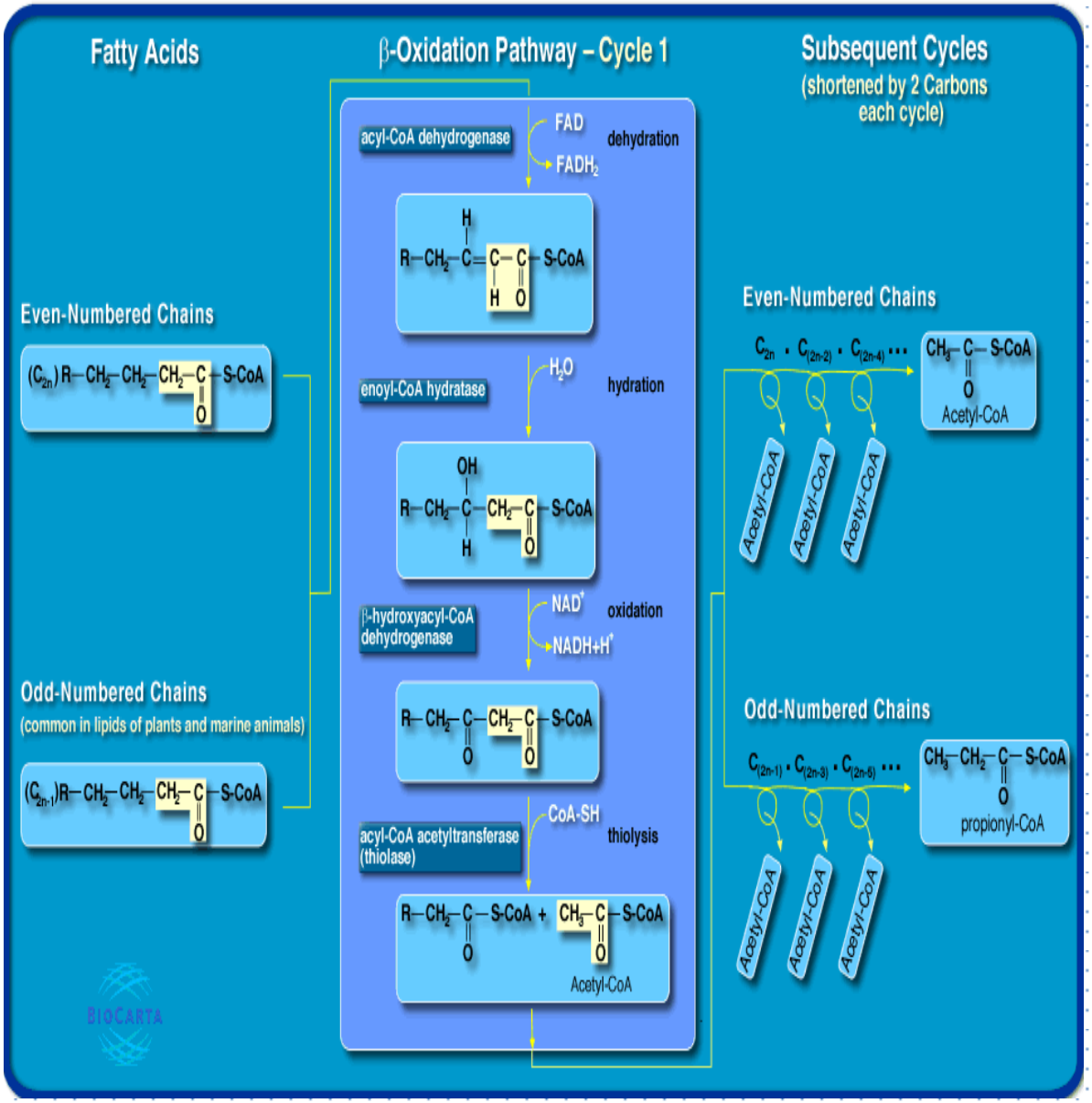
- Acetyl-CoA enters the citric acid cycle (a.k.a., Krebs Cycle)

- In a previous whole lecture we have seen how acetyl-CoA is oxidized through Krebs Cyle resulting ATP (or GTP and also generating NADH and FADH2, which are co-enzymes used in the electron transport chain.

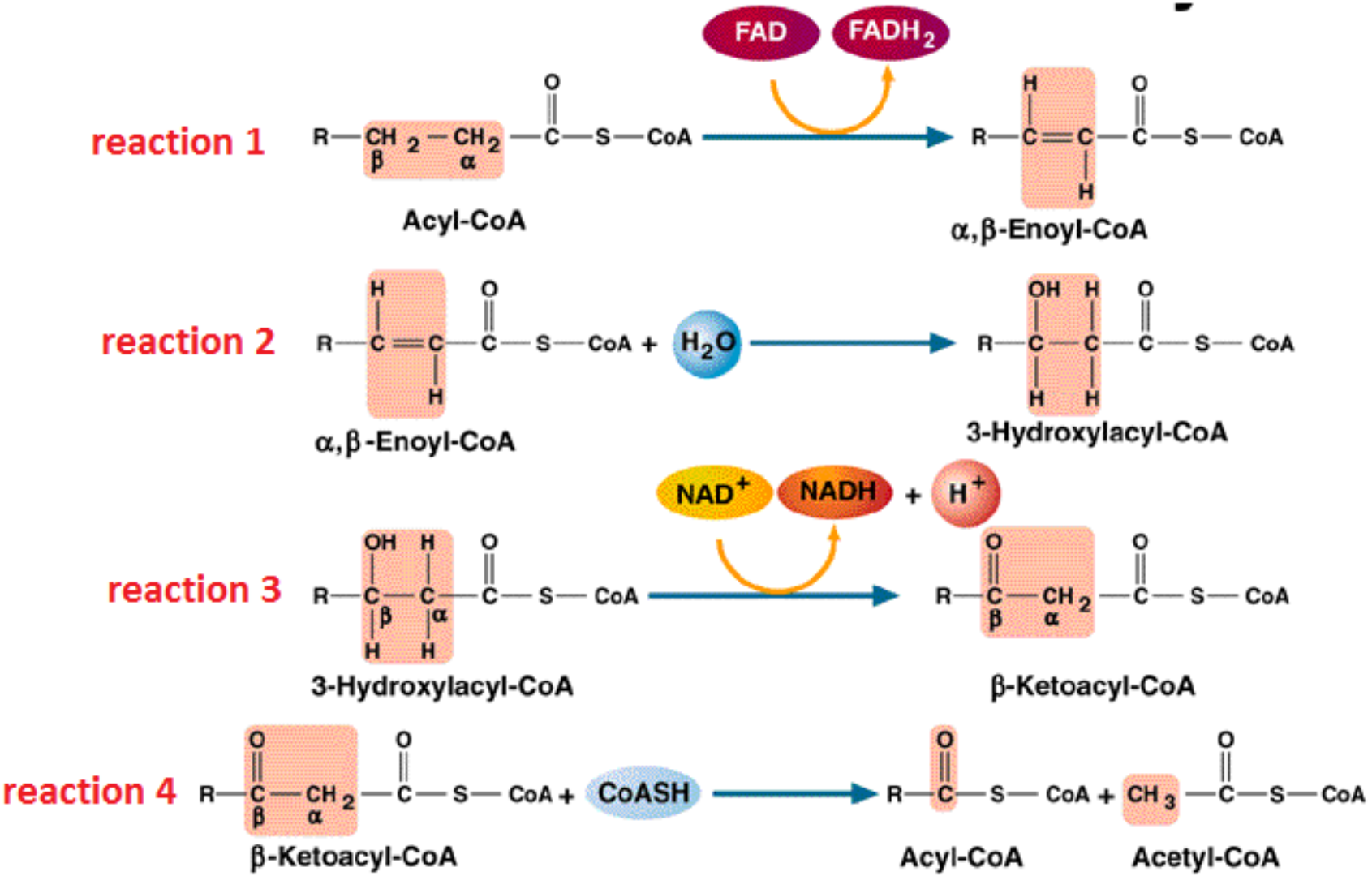
The oxidation of saturated fatty acids involves a cycle of four enzyme-catalyzed reactions. Each cycle produces one FADH2 and one NADH, and it liberates acetyl-CoA, resulting in a fatty acid that is two carbons shorter. The symbol represents a double bond, and the number associated with it is the location of the double bond (based on counting the carbonyl group as carbon one).



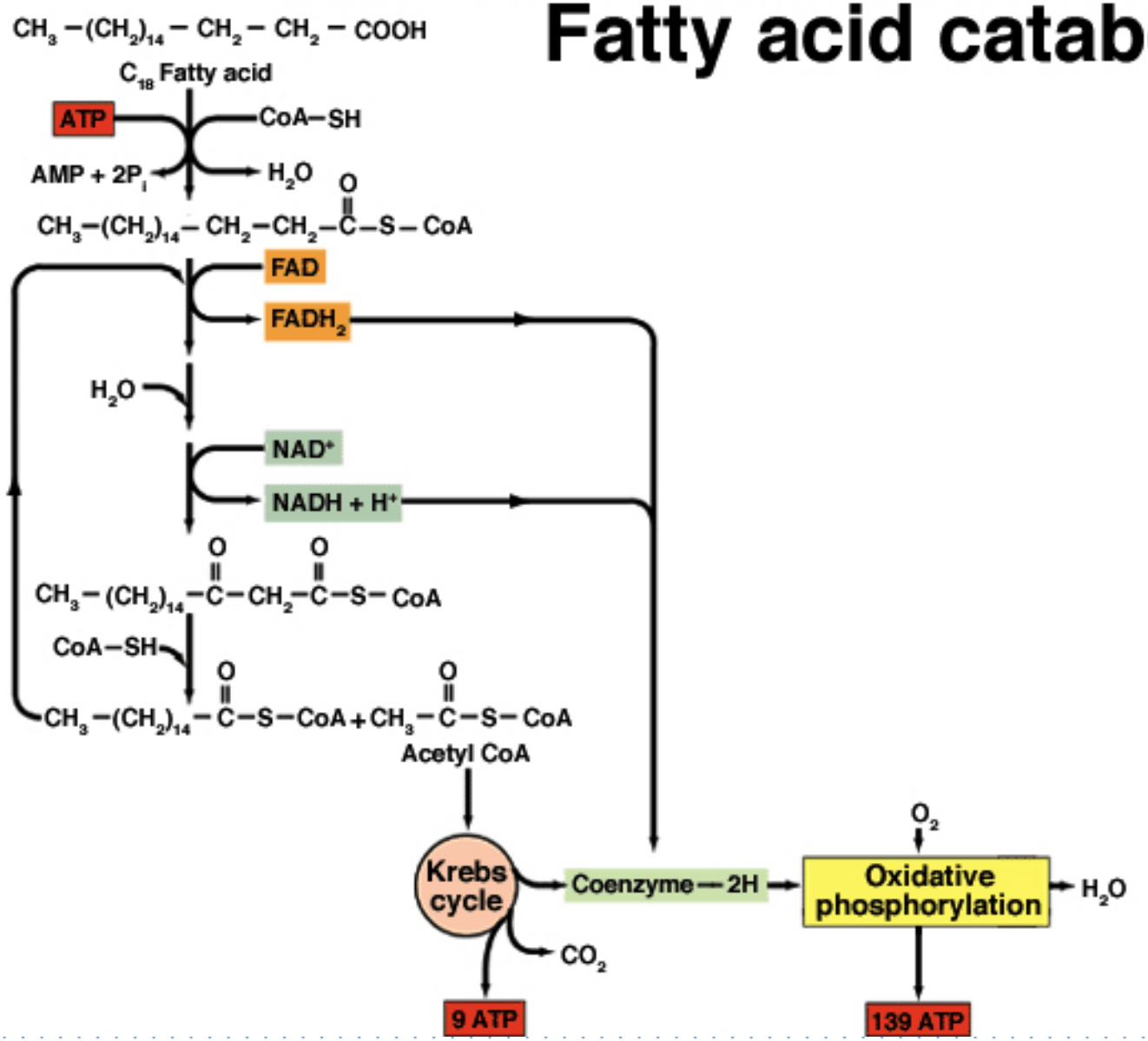
- The main steps of beta-oxydation of fatty acid in mitochondria.
- An activated fatty acid (acyl-CoA) is shortaned by two carbons in each round of 4 step oxydation.



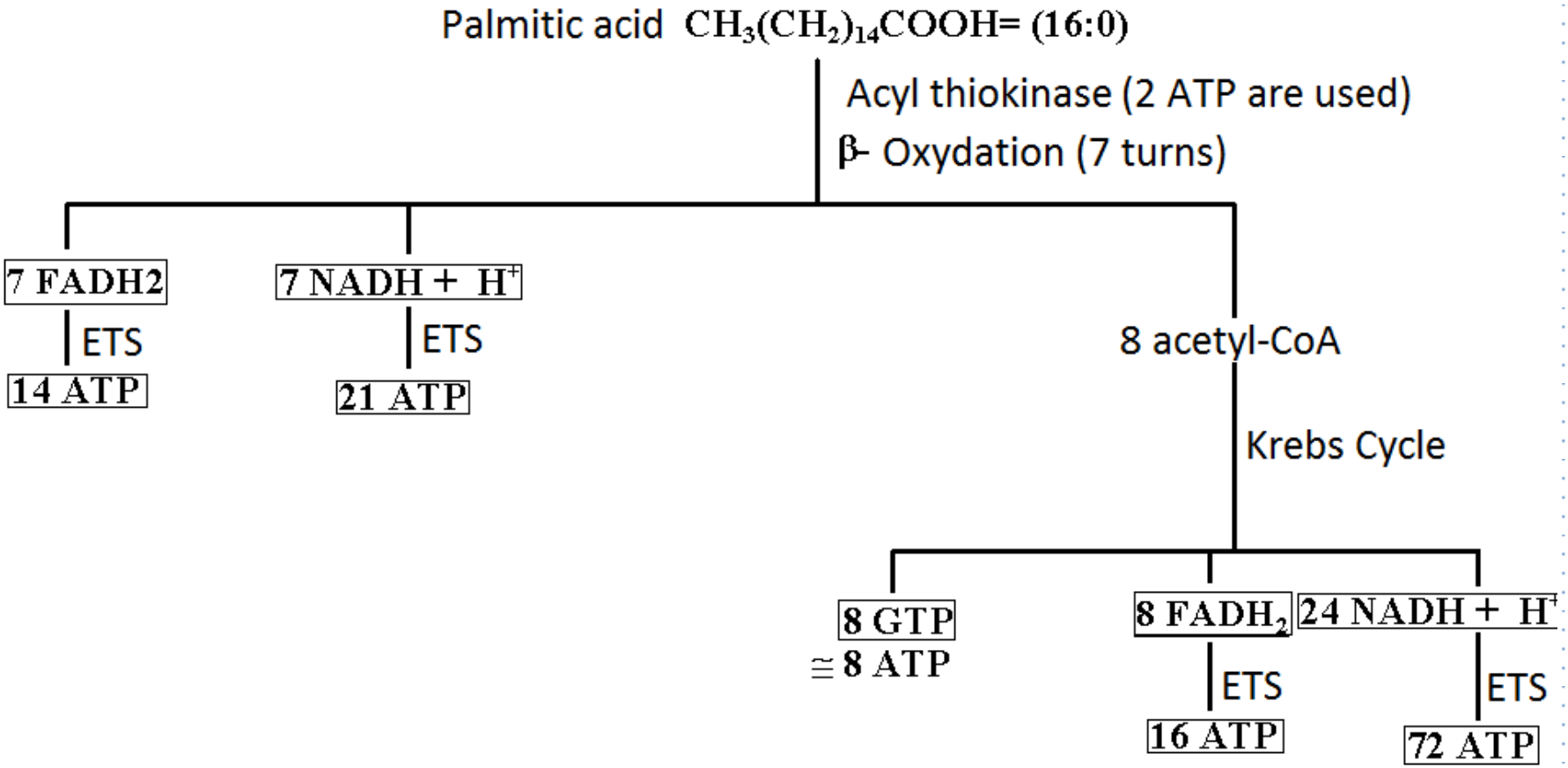
β-Oxidation of Saturated Fatty Acids



Fatty acid catabolism



How rich are the fatty acids for energy (ATP) production?



Net ATP yield: 131-2 = 129 ATP/palmitic acid

Beta-oxidation and ATP generation

Example: Palmitic acid (16:0)

(CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CO-CoA)

Palmitoil CoA + FAD + NAD + CoA + H_2O

1 round:

1) CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CO-CoA + CH_3-CO-CoA

2) CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CO-CoA + CH_3-CO-CoA +$FADH_2$ + NADH

3) CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CO-CoA + CH_3-CO-CoA +$FADH_2$ + NADH

4) CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CO-CoA + CH_3-CO-CoA +$FADH_2$ + NADH

5) CH_3-CH_2-CH_2-CH_2-CH_2-CO-CoA + CH_3-CO-CoA +$FADH_2$ + NADH

6) CH_3-CH_2-CH_2-CO-CoA + CH_3-CO-CoA +$FADH_2$ + NADH

7) CH_3-CO-CoA + CH_3-CO-CoA +$FADH_2$ + NADH

7 round:

8 Acetyl CoA + 7 $FADH_2$ + 7 NADH + 7 H^+ oluşur

Stearic acid (18:0)

answer: 146 ATP

Oleic acid	**(C18:1) = 146 – 2 = 144 ATP**
Linoleic acid	**(C18:2) = 146 – 4 = 142 ATP**
Arachidonic acid	**(C20:4) = 163 – 8 = 155 ATP**

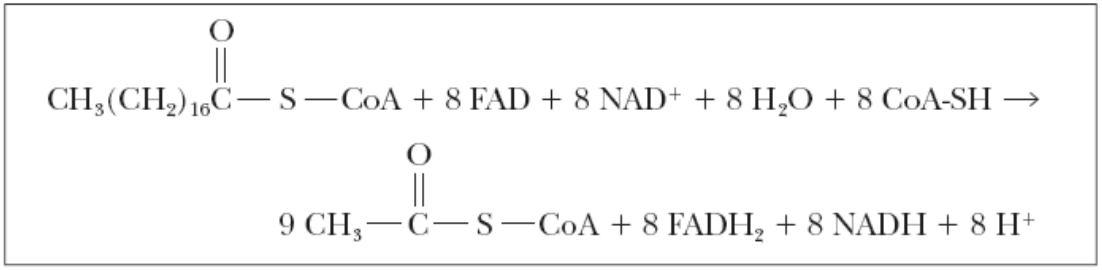
Breakage of -C=C- bonds releases no $FADH_2$. Thus, 2 ATP less.

The Energy Yield from the Oxidation of Fatty Acids

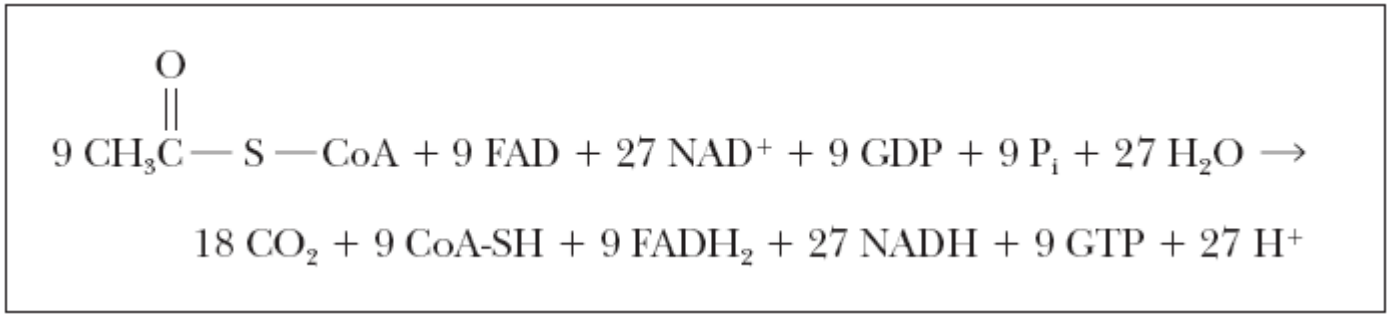
- There are two sources of ATP in beta-oxidation:
  1. Reoxidation of the NADH and FADH2 produced by the beta-oxidation of the fatty acid to acetyl-CoA.
  2. ATP production from the processing of the acetyl-CoA through the citric acid cycle and oxidative phosphorylation.

Example: the oxidation of stearic acid (18:0)

- Eight cycles of β-oxidation convert 1 mole of stearic acid to 9 moles of acetyl-CoA; in the process 8 moles of $FADH_2$ and 8 moles of NADH, and 9 moles of acetyl-CoA are formed.



- The 9 moles of acetyl-CoA produced enter the Krebs cycle and energy yield per acetyl-CoA is as usual (i.e., 1 mole $FADH_2$, 3 moles NADH and 1 mole GTP)



- The $FADH_2$ and NADH produced by beta oxidation and by the citric acid cycle enter the electron transport chain, and ATP is produced by oxidative phosphorylation.

- In our example, there are 17 moles of $FADH_2$ (8 from β -oxidation and 9 from the citric acid cycle); there are also 35 moles of NADH (8 from β-oxidation and 27 from the citric acid cycle).

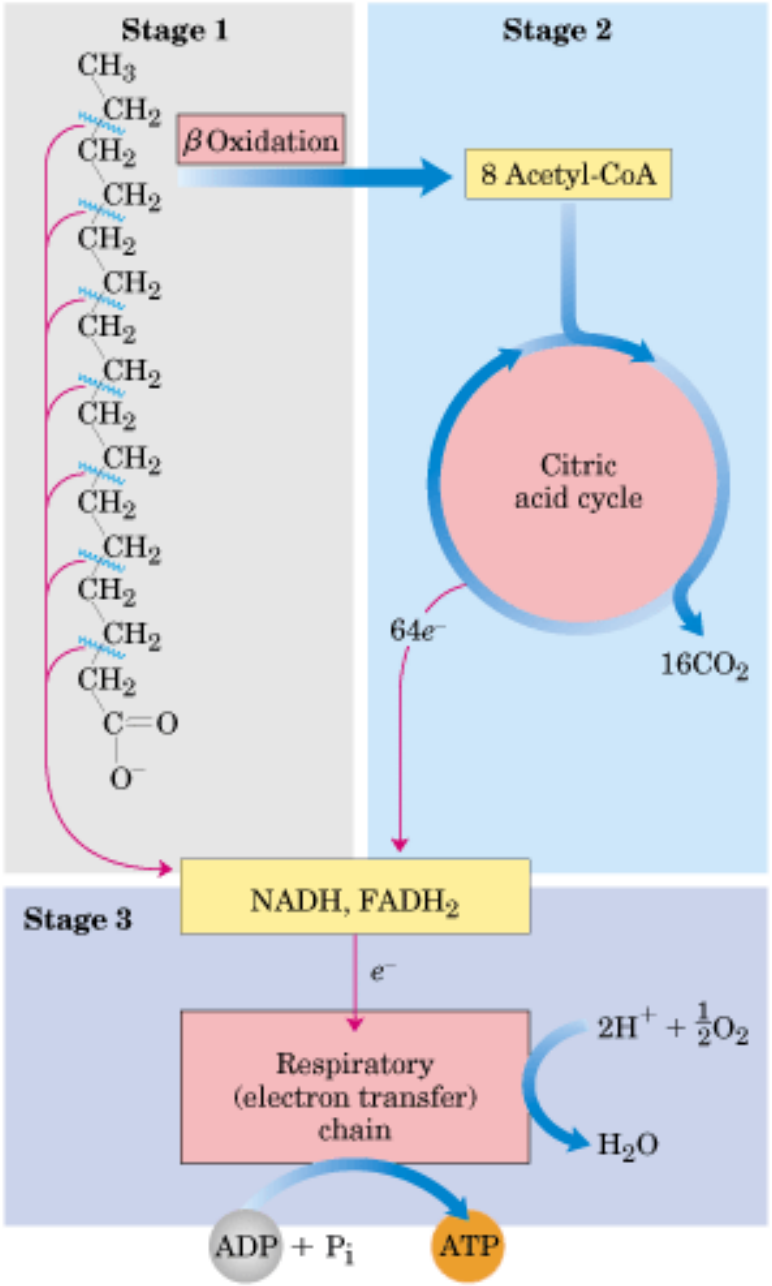
- Recall that about 3 moles of ATP are produced for each mole of NADH that enters the electron transport chain, and about 2 moles of ATP result from each mole of $FADH_2$.

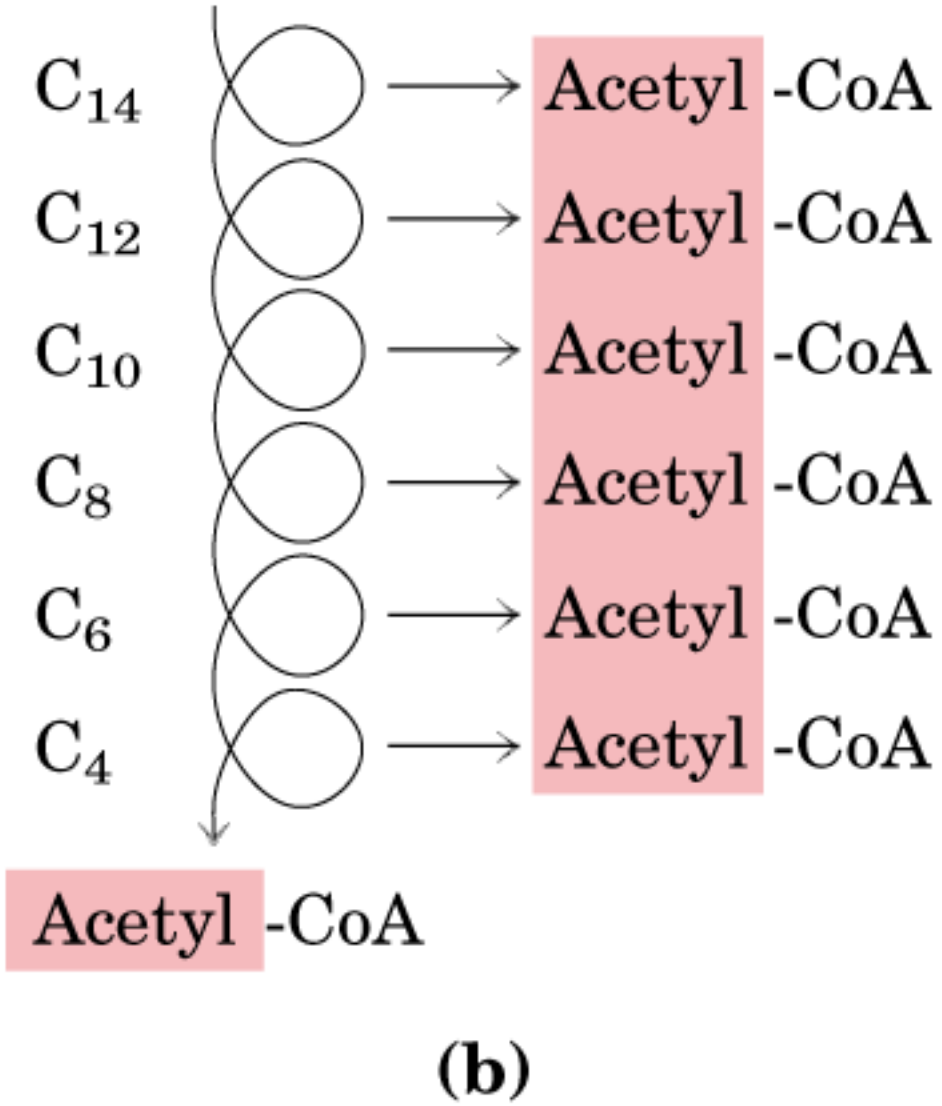
- Thus, 17 x 2 = 34 (from FADH2) and 35 x 3 = 105 (from NADH) and 9 GTP (from substrat level phosphorylation at Krebs cyle) at total 148 ATP

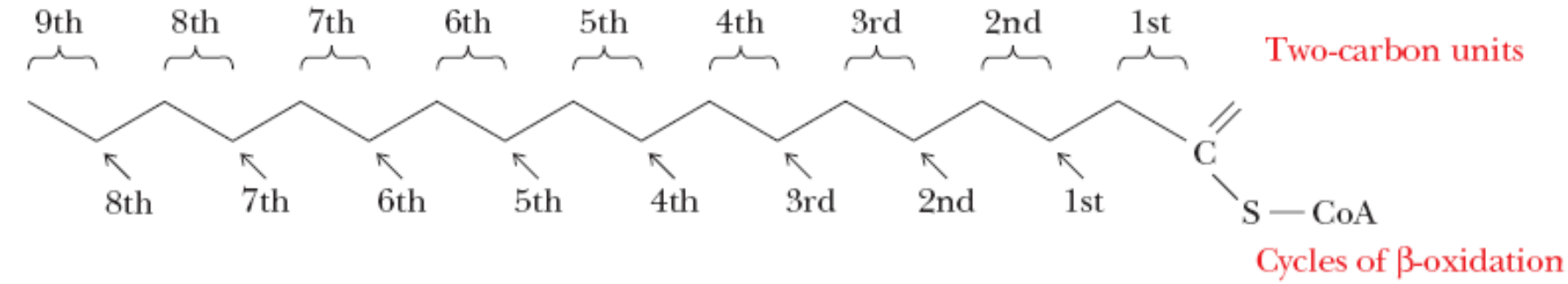
- Since 2 ATP were used for initial activation of stearic acid, the net yield is **146 ATP**

Efficiency of β-oxidation

- It has been estimated that stearic acid oxidation in calorimeter produces 1120 KJ of energy
- β-oxidation of stearic acid in the body generates 146 ATPs.
- Since 51.6 KJ of energy is needed for one ATP formation about 7280 KJ of energy is used for ATP formation.
- Thus, only 65% of energy is conserved and remainder is lost as heat. Therefore, efficiency of β-oxidation system is 65%


Stage 1
Stage 2
β Oxidation
8 Acetyl-CoA
Citric acid cycle
64e⁻
16CO2
NADH, FADH2
Stage 3
e⁻
Respiratory (electron transfer) chain
2H⁺ + ½O2
H2O
ADP + Pi
ATP


C14
C12
C10
C8
C6
C4
Acetyl -CoA
Acetyl -CoA
Acetyl -CoA
Acetyl -CoA
Acetyl -CoA
Acetyl -CoA
Acetyl -CoA
(b)



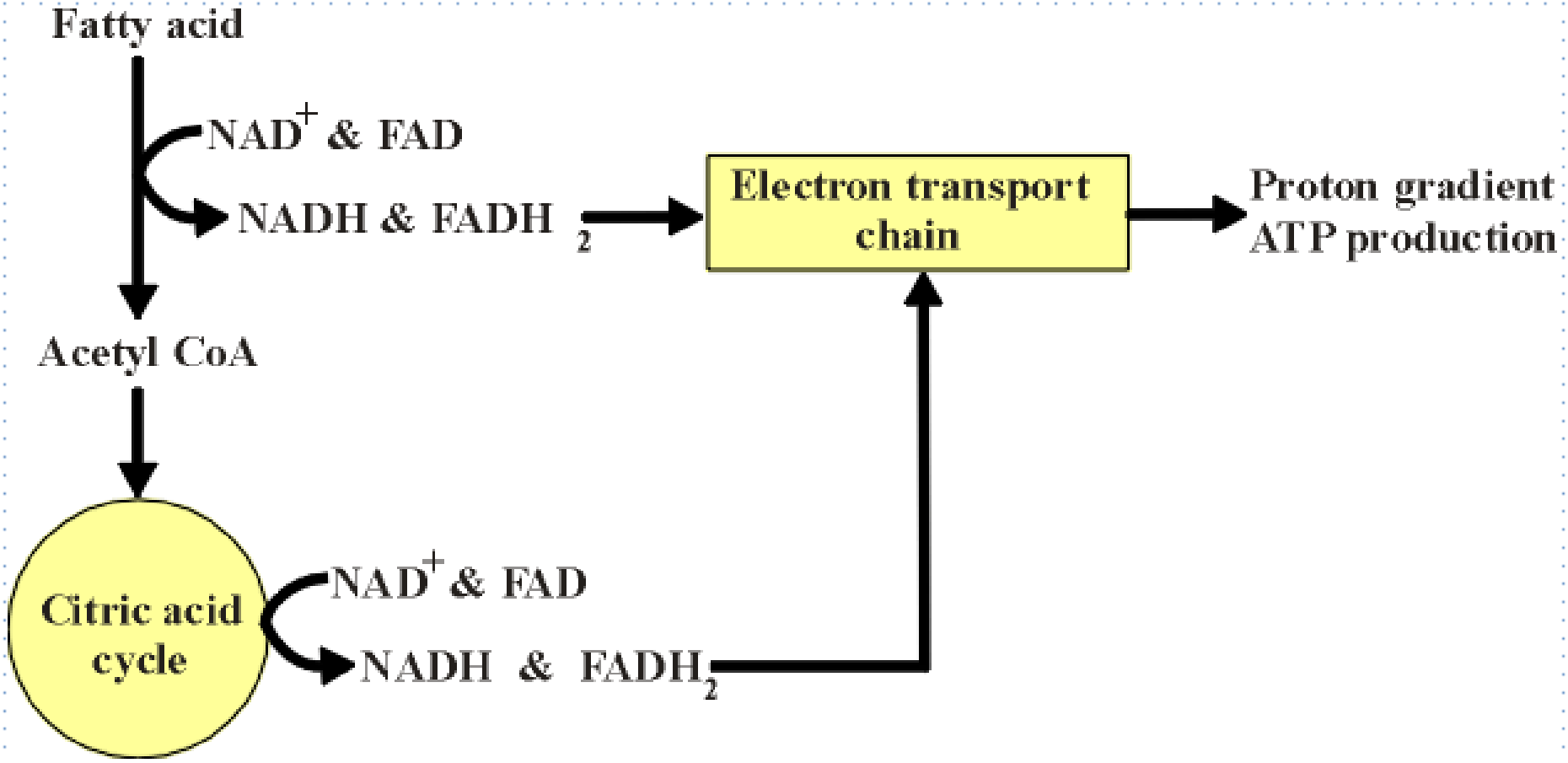
•Stearic acid (18 carbons) gives rise to nine 2-carbon units after eight cycles of oxidation.

•The ninth 2-carbon unit remains esterified to CoA after eight cycles of –oxidation have removed eight successive two-carbon units, starting at the carboxyl end on the right.

•Thus, it takes only eight rounds of -oxidation to completely process an 18-carbon fatty acid to acetyl-CoA.

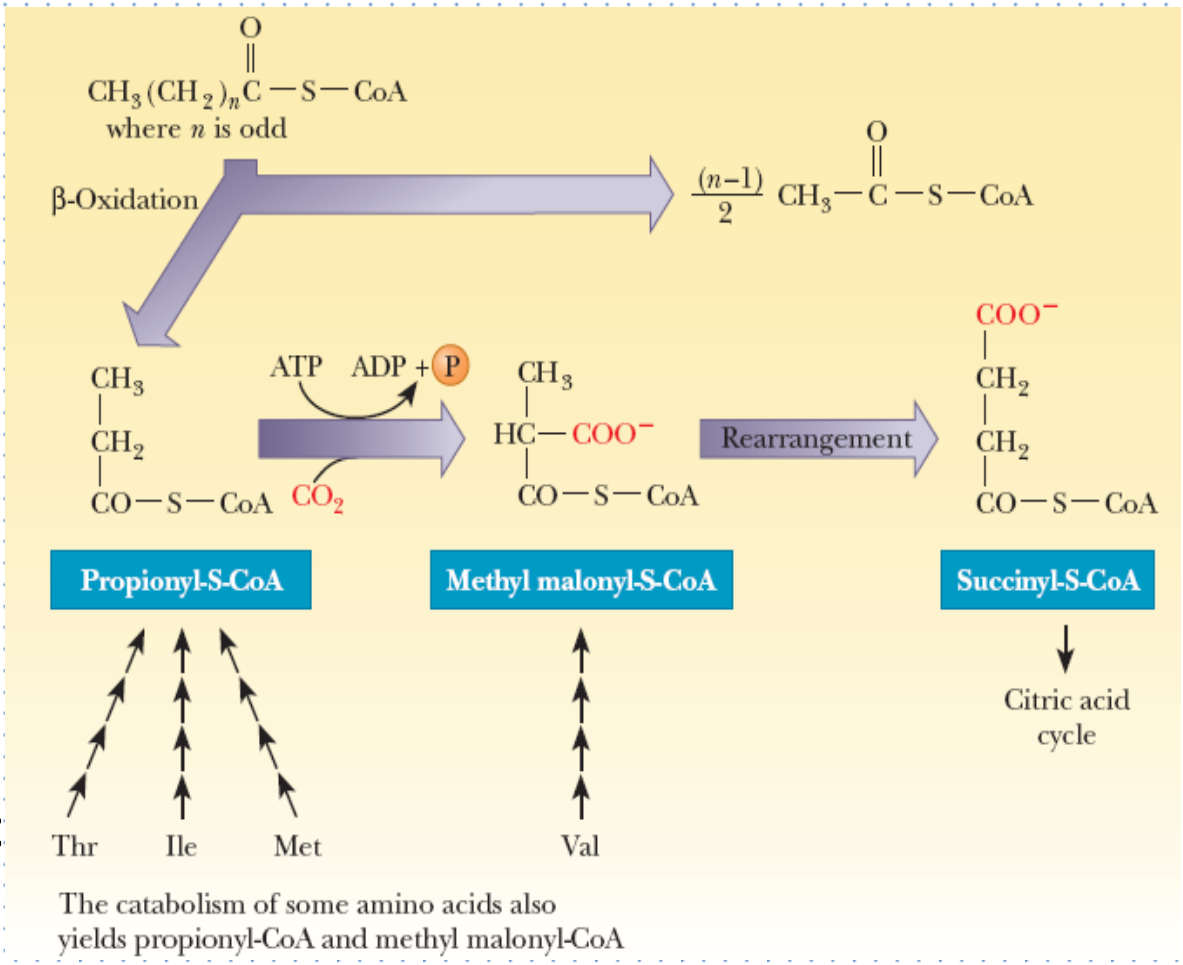
Essential Information

The breakdown of fatty acids takes place in the mitochondrial matrix and proceeds by successive removal of two-carbon units as acetyl-CoA. Each cleavage of a two-carbon moiety requires a four-step reaction sequence called β-oxidation. The complete oxidation of fatty acids by the citric acid cycle and the electron-transport chain releases large amounts of energy.


Fatty acid
NAD+ & FAD
NADH & FADH2
Electron transport chain
Proton gradient
ATP production
Acetyl CoA
Citric acid cycle
NAD+ & FAD
NADH & FADH2

Catabolism of Unsaturated Fatty Acids and Odd-Carbon Fatty Acids

- Odd-numbered fatty acids also undergo b-oxidation.
- The last cycle of beta-oxidation produces one molecule of propionyl-CoA.
- Propionyl-CoA is converted to succinyl-CoA, which then enters the citric acid cycle.



The catabolism of some amino acids also yields propionyl-CoA and methyl malonyl-CoA

Difference between the oxidation of unsaturated and saturated fatty acids?

- The conversion of unsaturated fatty acids differently requires a *cis–trans* isomerization reaction

- The oxidation of unsaturated fatty acids does not generate as many ATPs as it would for a saturated fatty acid with the same number of carbons.

- This is because the presence of a double bond means that the acyl-CoA dehydrogenase step will be skipped. Thus, fewer $FADH_2$ will be produced.

Regulation of β-oxidation

- Carnitineacyl transferase activity regulates fatty acid oxidation.
- It is inhibited by malonyl-CoA.
- In fed condition, more malonyl-CoA is produced. As a result, carnitineacyl transferase is inhibited and fatty acid oxidation diminishes.
- In contrast, during fasting or starvation, malonyl-CoA concentration decreases and hence inhibition of carnitineacyl transferase is relieved.
- As a result β-oxidation is activated. Thus, β-oxidation is regulated at entry level.

Ketone Bodies

- Ketone bodies (i.e., β -*hydroxybutyrate, acetone, acetoacetate)* are produced when an excess of acetyl-CoA arises from b-oxidation.

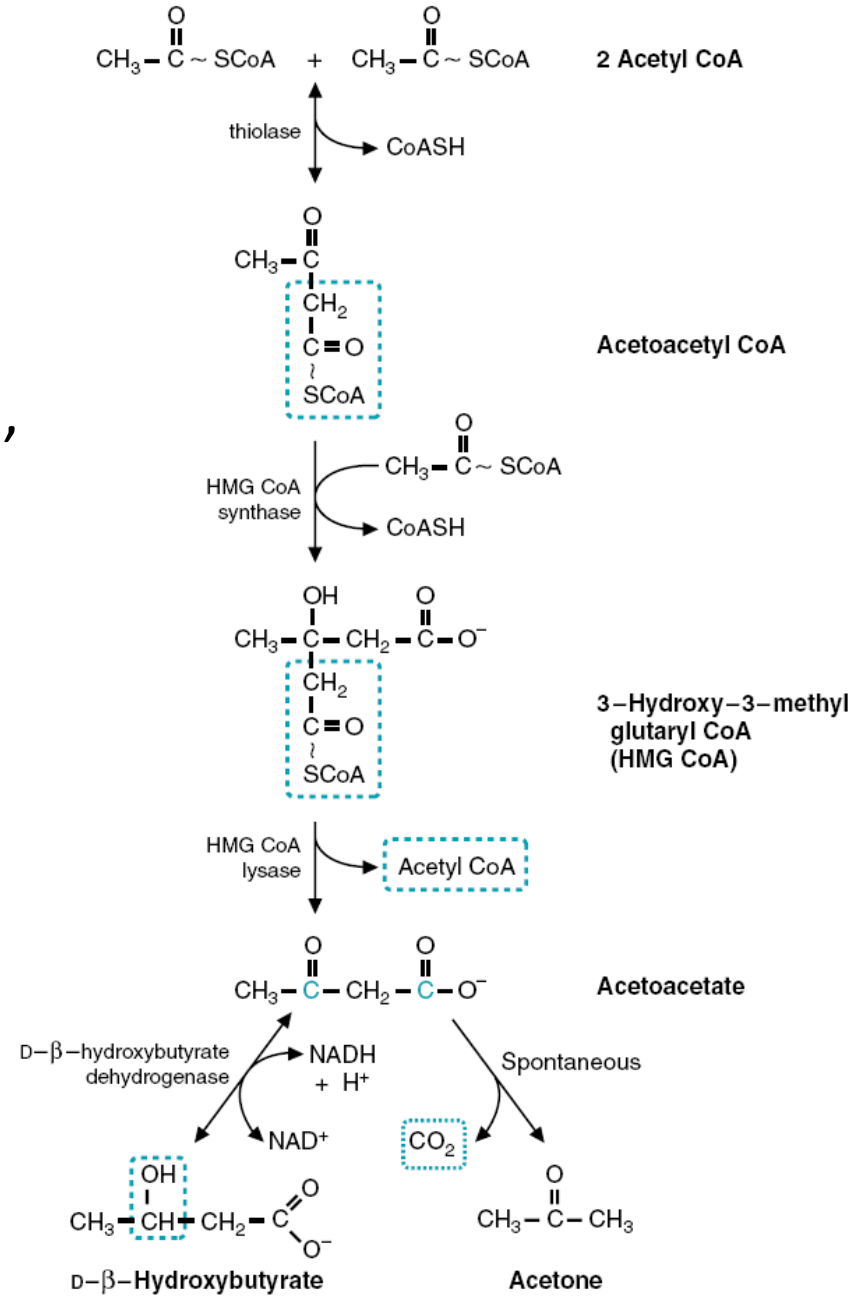
- This condition occurs when not enough oxaloacetate is available to react with the large amounts of acetyl-CoA that could enter the citric acid cycle.

- The reactions that result in ketone bodies start with the condensation of two molecules of acetyl-CoA to produce acetoacetyl-CoA.

- *Acetoacetate is produced* from acetoacetyl-CoA through condensation with another acetyl-CoA to form β-hydroxy- β -methylglutaryl-CoA (HMG-CoA)

- HMG-CoA is a compound used in cholesterol synthesis.

Synthesis of the ketone bodies acetoacetate, -hydroxybutyrate, and acetone.



- The odor of acetone can frequently be detected on the breath of people with diabetes whose disease is not controlled by suitable treatment.

- The excess of acetoacetate, and consequently of acetone, is a pathological condition known as *ketosis.*

- *Because acetoacetate and β-hydroxybutyrate are acidic,* their presence at high concentration overwhelms the buffering capacity of the blood.

- The body deals with the consequent lowering of blood pH (ketoacidosis) by excreting H^+ into the urine, accompanied by excretion of Na^+, K^+, and water.

- Severe dehydration can result (excessive thirst is a classic symptom of diabetes); diabetic coma is another possible danger.

- The principal site of synthesis of ketone bodies is liver mitochondria, but they are not used there because the liver lacks the enzymes necessary to recover acetyl-CoA from ketone bodies.

- It is easy to transport ketone bodies in the bloodstream because, unlike fatty acids, they are water-soluble and do not need to be bound to proteins, such as serum albumin.

- Organs other than the liver can use ketone bodies, particularly acetoacetate.

- Even though glucose is the usual fuel in most tissues and organs, acetoacetate can be used as a fuel. In heart muscle and the renal cortex, acetoacetate is the preferred source of energy.

Fats vs Sugars

If we continue with our pamitate example: ATP/C oxidized = 129/16 = 8 ATP/C.

For a glucose molecule enetering glycolysis, PDH, Krebs Cycle:

- 2ATP from glycolysis (Substrat-level phosphotylation)
- 2 NADH from glycolysis = 6 ATP (ETS and OP)
- 2 NADH from PDH = 6 ATP (ETS and OP)
- 2 GTP = 2ATP from Krebs Cycle (Substrat-level phosphotylation)
- 6 NADH from Krebs Cycle = 18 ATP (ETS and OP)
- 2 $FADH_2$ from Krebs Cycle = 4 ATP (ETS and OP)

Thus from 1 molecule of glucose the net yield = 2 + 6 + 6 + 18 + 4 + 2 = 38 ATP (38/6 = 6.3 ATP/C)

However, in fatty acids this yield is 8 ATP/C. Thus, this shows that fats are more reduced than sugars.

Fats vs Sugars

Since there are more oxygen atoms in sugars than fats, the latter has İs more reduced and has more potential energy than the former.

For example, let's compare palmitic acid with glucose:

Palmitic acid (16:0) CH3-CH2-CH2-CH2-CH2-CH2-CH2-CH2-CH2-CH2-CH2-CH2-CH2-CH2-CH2-COOH

16 C; 16 x 12 =192
32 H; 32 x 1 = 32
2 O; 16 x 2 = 32
Total 256 g/mol

Glucose= $C_6H_{12}O_6$
6 C; 6 x 12 = 72
12 H; 12 x 1=12
6 O; 6 x 16 =96
Total 180 g/mol

Thus,
1 g palmitic acid is equal to 0.5 mol ATP (129 ATP/256 g)

1 g sugar, however, is about 0.2 mol ATP (38 ATP/180 g).

In other words, the same gram amount of fatty acid has 2.5 fold more energy than sugar.

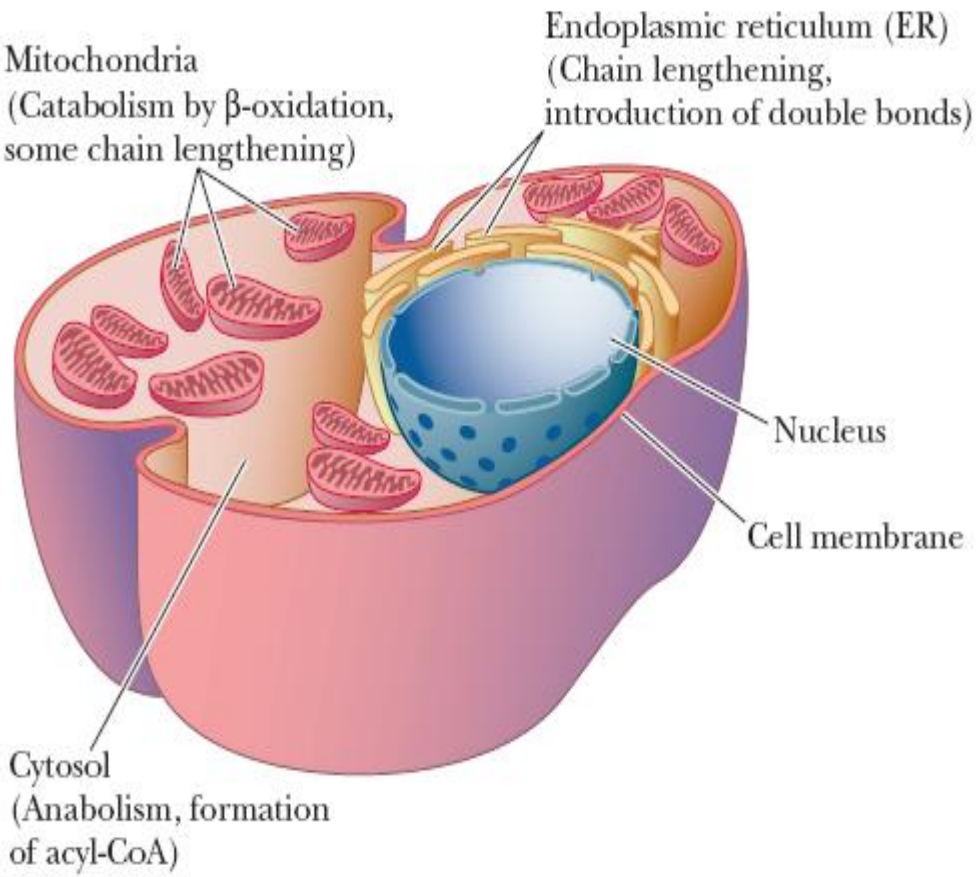
Brown Fat

- Brown adipose tissue (BAT) dissipates energy as heat to maintain optimal thermogenesis and to contribute to energy expenditure in rodents and possibly humans.
- BAT also possesses a great capacity for glucose uptake and metabolism, and an ability to regulate insulin sensitivity.
- These properties make BAT an appealing target for the treatment of obesity, dia- betes, and other metabolic disorders.
- The main function of BAT is to dissipate energy in the form of heat, a property driven by the presence of the mitochon- drial protein UCP1 (uncoupling protein 1) that uncouples mitochondrial respiration.
- The thermogenic capacity of BAT may be important for heat production in newborns, essential for rodents and hibernating mammals, and possibly helps burn excess dietary energy consumption.

Fatty-Acid Biosynthesis

- The anabolism of fatty acids is not simply a reversal of the reactions of b-oxidation.

- The degradative reactions of b-oxidation take place in the mitochondrial matrix, while the anabolic reactions take place in the cytosol.



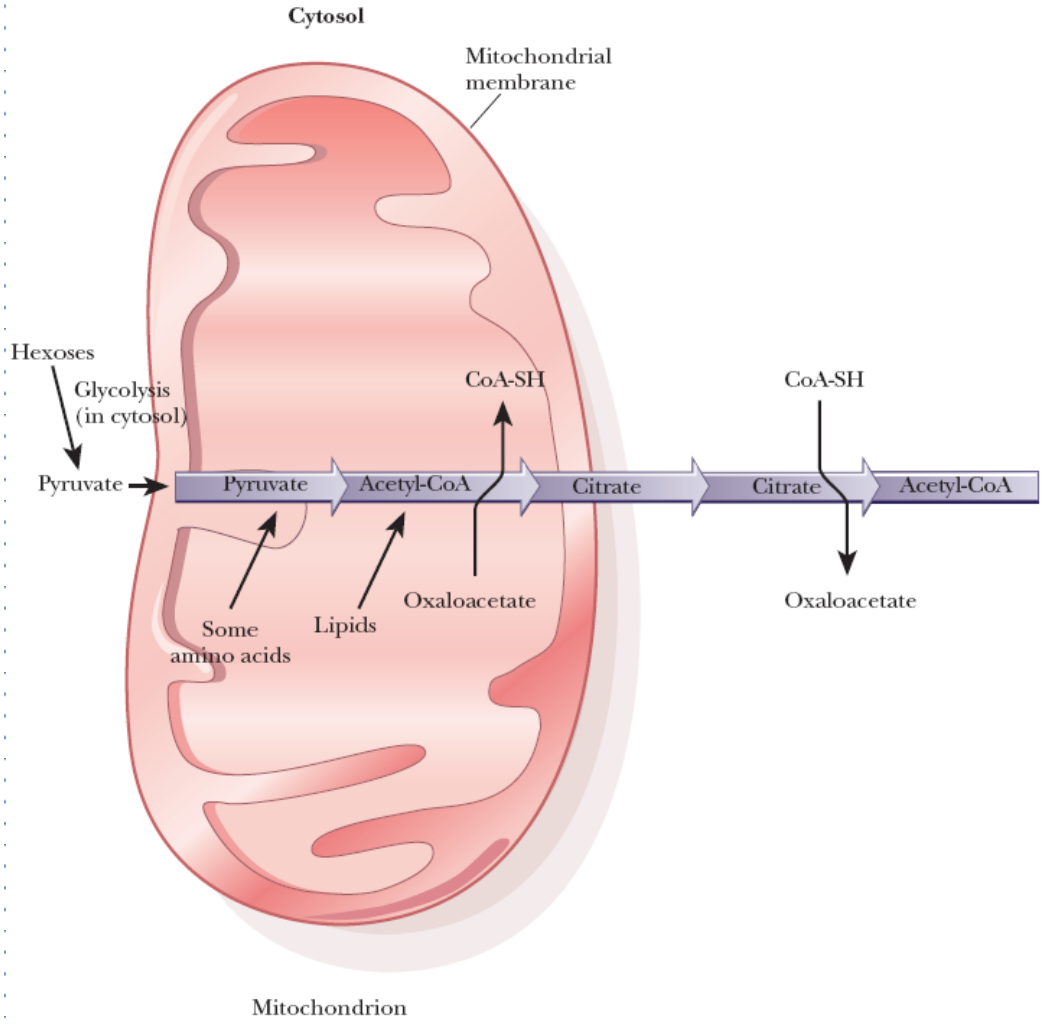
Fatty acid synthesis is different from fatty acid oxidation

- Intermediates in fatty acid synthesis are linked covalently to the sulfhydryl groups of special proteins, **the acyl carrier proteins (ACPs)**. In contrast, fatty acid breakdown intermediates are bound to the -SH group of coenzyme A.

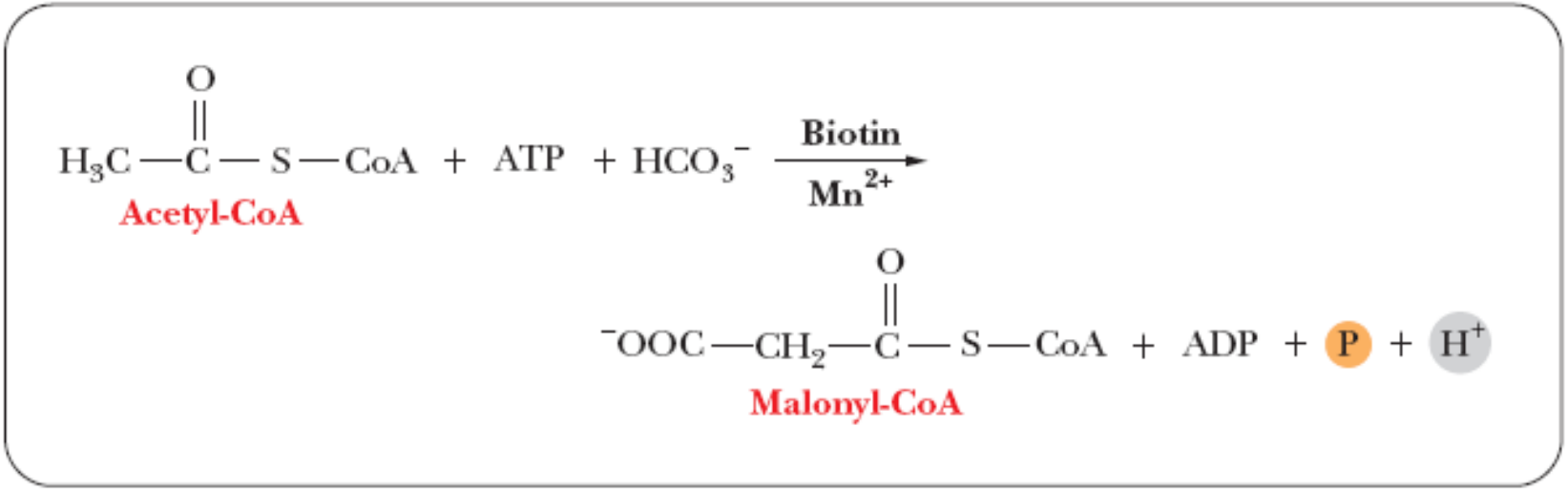
- Fatty acid synthesis occurs in the cytosol, whereas fatty acid degradation takes place in mitochondria.

- The coenzyme for the reactions of fatty acid synthesis is NADP/NADPH, whereas degradation involves the NAD/NADH couple.

- The first step in fatty-acid biosynthesis is transport of acetyl-CoA to the cytosol.
- The transport mechanism is based on the fact that citrate can cross the mitochondrial membrane.
- Acetyl-CoA condenses with oxaloacetate, which cannot cross the mitochondrial membrane, to form citrate.
- Once transported into the cytosol, citrate undergoes the reverse reaction and reproduce oxaloacetate and acetyl-CoA.
- Acetyl-CoA enters the pathway for fatty-acid biosynthesis.



- In the cytosol, acetyl-CoA is carboxylated, producing **malonyl-CoA**, a key intermediate in fatty-acid biosynthesis



The formation of malonyl-CoA, catalyzed by acetyl-CoA carboxylase.

- The biosynthesis of fatty acids involves the successive addition of two-carbon units to the growing chain.

- Two of the three carbon atoms of the malonyl group of malonyl-CoA are added to the growing fatty-acid chain with each cycle of the biosynthetic reaction.

- This reaction is made possible by a multienzyme complex called **fatty-acid synthase.**

- The usual product of fatty-acid anabolism is *palmitate,* the 16-carbon saturated fatty acid.

A Comparison of Fatty-Acid Degradation and Biosynthesis	
Degradation	**Biosynthesis**
1. Product is acetyl-CoA	Precursor is acetyl-CoA
2. Malonyl-CoA is not involved; no requirement for biotin	Malonyl-CoA is source of two-carbon units; biotin required
3. Oxidative process; requires NAD^+ and FAD and produces ATP	Reductive process; requires NADPH and ATP
4. Fatty acids form thioesters with CoA-SH	Fatty acids form thioesters with acyl carrier proteins (ACP-SH)
5. Starts at carboxyl end ($CH_3CO_2^-$)	Starts at methyl end ($CH_3CH_2^-$)
6. Occurs in the mitochondrial matrix, with no ordered aggregate of enzymes	Occurs in the cytosol, catalyzed by an ordered multienzyme complex
7. β-Hydroxyacyl intermediates have the L configuration	β-Hydroxyacyl intermediates have the D configuration

- Triacylglycerols, phosphoacylglycerols, and steroids are derived from fatty acids.

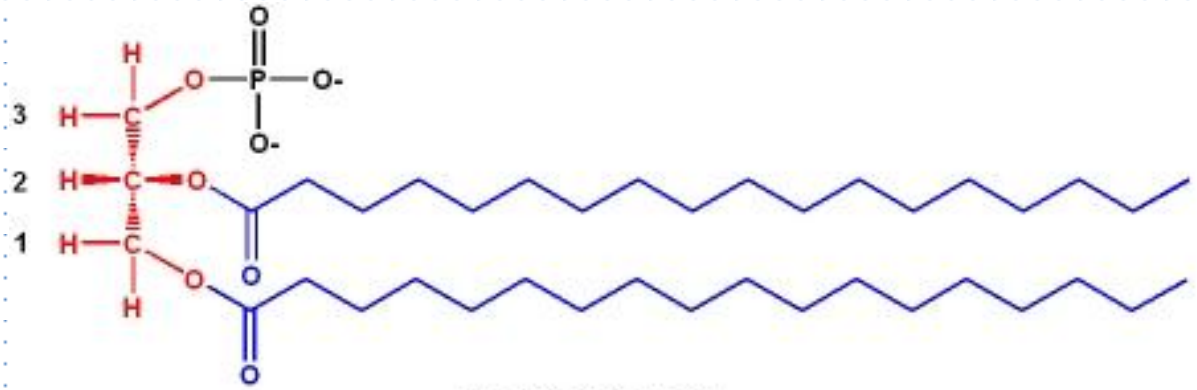
- Free fatty acids do not occur in the cell to any great extent; they are normally found incorporated in triacylglycerols and phosphoacylglycerols.

- The biosynthesis of these two types of compounds takes place principally on the ER of liver cells or adipocytes.

- The glycerol portion of lipids is derived from glycerol-3-phosphate, a compound available from glycolysis.

- **Phosphatidic acids** are the simplest diacyl-glycerophospholipids and precursors of other phospholipids.

- Phosphatidic acid (PA) consists of glycerol backbone with generally a saturated fatty acid bonded to C-1, an unsaturated fatty acid bonded to C-2, and a phosphate group bonded to C-3.

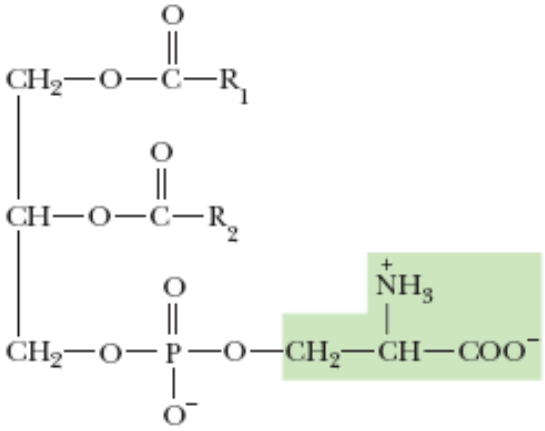


Phosphatidic Acid

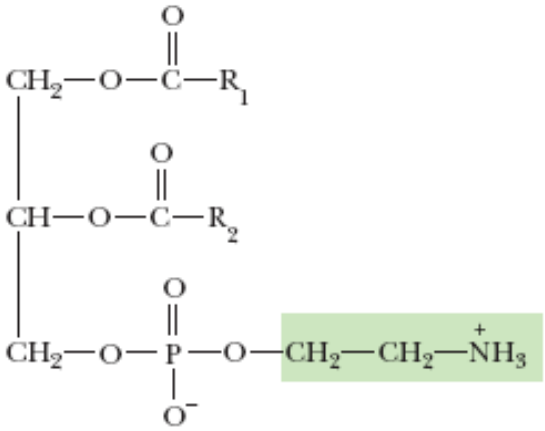
- Phosphoacylglycerols (phosphoglycerides) are based on phosphatidates, with the phosphate group esterifi ed to another alcohol.

- The conversion of PA into diacylglycerol (DAG) is the commitment step for the production of *phosphatidylcholine*, *phosphatidylethanolamine*, and *phosphatidylserine*.

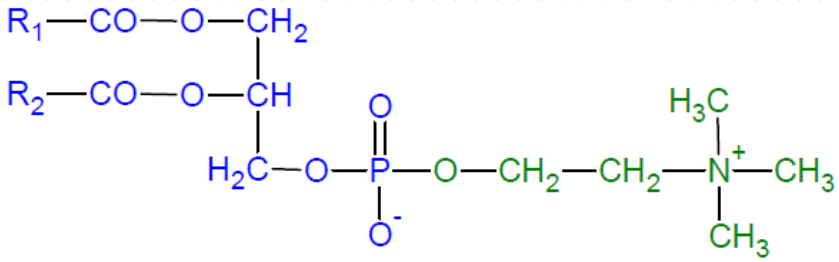
- In addition, DAG is also converted into *phosphatidylglycerol*, *phosphatidylinositol* and *phosphoinositides*.



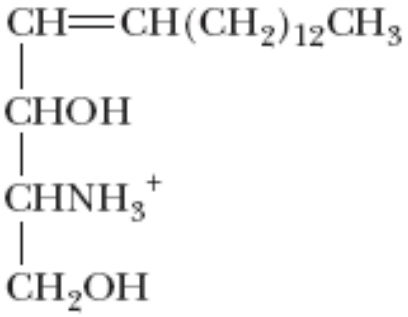
Phosphatidylserine



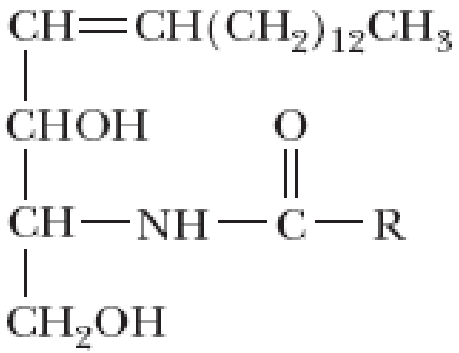
Phosphatidylethanolamine



Phosphatidylcholine



Sphingosine



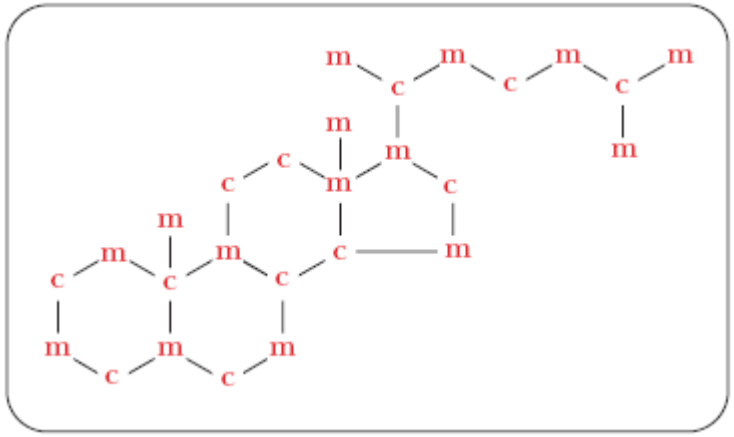
A ceramide

Cholesterol Biosynthesis

- The ultimate precursor of all the carbon atoms in cholesterol and in the other steroids that are derived from cholesterol is the acetyl group of acetyl-CoA.
- There are many steps in the biosynthesis of steroids.
- The condensation of three acetyl groups produces mevalonate, which contains six carbons.
- Decarboxylation of mevalonate produces the five-carbon ***isoprene unit*** frequently encountered in the structure of lipids.
- The involvement of isoprene units is a key point in the biosynthesis of steroids and of many other compounds that have the generic name *terpenes*.
- Vitamins A, E, and K come from reactions involving terpenes that humans cannot carry out.

- Isoprene units are involved in the biosynthesis of ubiquinone (coenzyme Q).
- Isoprene units are often added to proteins to act as anchors when the protein is attached to a membrane.
- Six isoprene units condense to form *squalene*, which contains 30 carbon atoms.
- Finally, squalene is converted to **cholesterol**, which contains 27 carbon atoms.

$$\underset{C_2}{\text{Acetate}} \rightarrow \underset{C_6}{\text{Mevalonate}} \rightarrow \underset{C_5}{[\text{Isoprene}]} \rightarrow \underset{C_{30}}{\text{Squalene}} \rightarrow \underset{C_{27}}{\text{Cholesterol}}$$



- 12 of the carbon atoms of cholesterol arise from the carboxyl carbon of the acetyl group; these are the carbon atoms labeled “c”.

- The other 15 carbon atoms arise from the methyl carbon of the acetyl group; these are the carbon atoms labeled “m.”

- The major fatty acids oxidized are the long-chain fatty acids, palmitate, oleate, and stearate, because they are highest in dietary lipids and are also synthesized in the human.

MBG304 Biochemistry

Lecture 9: Amino acid metabolism

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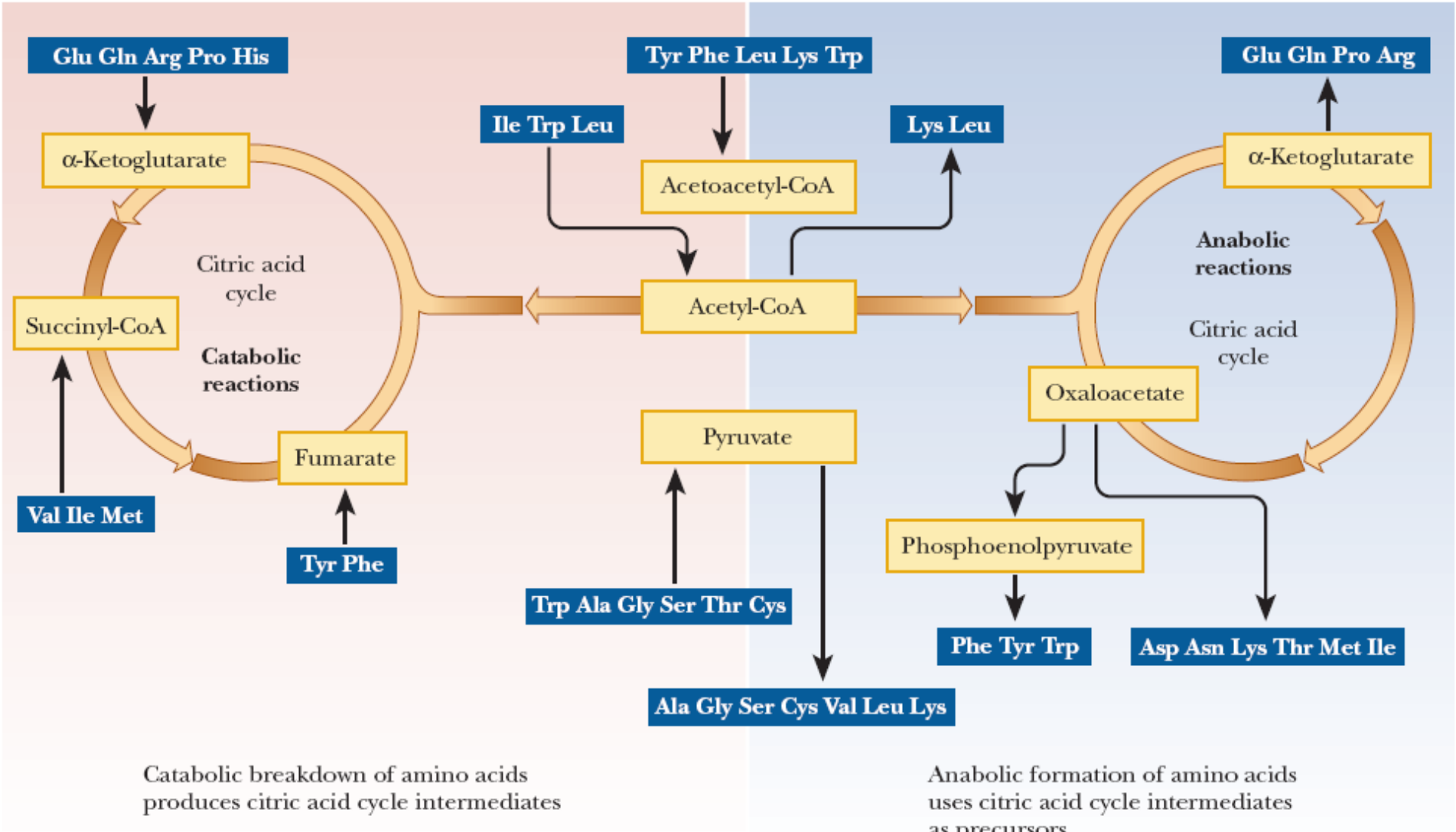
The biosynthesis of amino acids

- We can also make some generalizations about amino acid metabolism in terms of the relationship of the carbon skeleton to the *citric acid cycle* and the related reactions of *pyruvate* and *acetyl-CoA*.

- The citric acid cycle is *amphibolic*; it has a part in both catabolism and anabolism.

- The anabolic aspect of the citric acid cycle is of interest in amino acid biosynthesis.

- The catabolic aspect is apparent in the breakdown of amino acids, leading to their eventual excretion, which takes place in reactions related to the citric acid cycle.


Glu Gln Arg Pro His
α-Ketoglutarate
Citric acid cycle
Catabolic reactions
Succinyl-CoA
Fumarate
Val Ile Met
Tyr Phe
Ile Trp Leu
Tyr Phe Leu Lys Trp
Acetoacetyl-CoA
Lys Leu
Acetyl-CoA
Pyruvate
Trp Ala Gly Ser Thr Cys
Ala Gly Ser Cys Val Leu Lys
Glu Gln Pro Arg
α-Ketoglutarate
Anabolic reactions
Citric acid cycle
Oxaloacetate
Phosphoenolpyruvate
Phe Tyr Trp
Asp Asn Lys Thr Met Ile
Catabolic breakdown of amino acids produces citric acid cycle intermediates
Anabolic formation of amino acids uses citric acid cycle intermediates as precursors

- Glutamate is formed from NH_4^+ and α-ketoglutarate in a reductive amination that requires NADPH.

- This reaction is reversible and is catalyzed by *glutamate dehydrogenase (GDH).*

- Glutamate is a major donor of amino groups in reactions, and α-ketoglutarate is a major acceptor of amino groups.

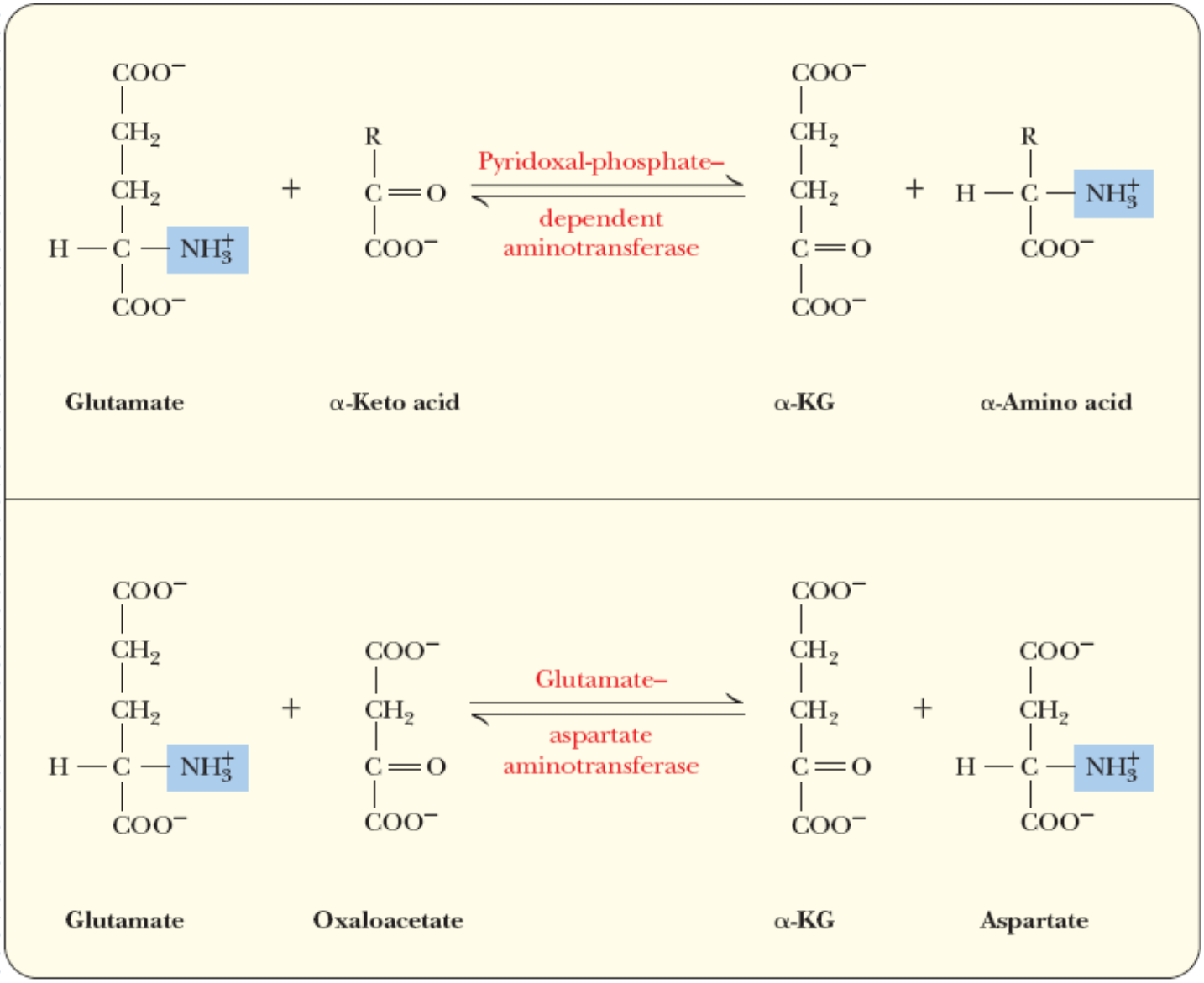
$$NH_4^+ + \alpha\text{-ketoglutarate} + NADPH + H^+ \rightarrow \text{Glutamate} + NADP^+ + H_2O$$

- The conversion of glutamate to glutamine is catalyzed by **glutamine synthetase (GS)** in a reaction that requires ATP.

$$NH_4^+ + \text{Glutamate} + ATP \rightarrow \text{Glutamine} + ADP + P_i + H_2O$$

- These reactions fix inorganic nitrogen (NH3), forming organic nitrogen compounds, such as amino acids.

- The combination of GDH and GS is responsible for most of the assimilation of ammonia into organic compounds.



Transamination reactions switch an amino group from one amino acid to an a-keto acid.

- Enzymes that catalyze transamination reactions require *pyridoxal phosphate* as a coenzyme.

- In addition to transamination reactions, *one-carbon transfer* reactions occur frequently in amino acid biosynthesis.

- **Tetrahydrofolate**, a derivative of folic acid, is a frequent carrier of one-carbon units in metabolic pathways.

- Three important carriers of one-carbon units:
  1. biotin, a carrier of CO_2
  2. tetrahydrofolate (FH_4), a carrier of methylene and formyl groups
  3. *S-adenosylmethionine, a carrier of methyl* groups

- ***S-adenosylmethionine (SAM)*** *is fromed when m*ethionine reacts with ATP. *With its highly reactive methyl groups,* SAM is an important carrier of methyl groups in many reactions.

Amino Acid Requirements in Humans	
Essential	**Nonessential**
Arginine*	Alanine
Histidine†	Asparagine
Isoleucine	Aspartate
Leucine	Cysteine
Lysine	Glutamate
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Proline
Tryptophan	Serine
Valine	Tyrosine

* Mammals synthesize arginine but cleave most of it to urea (Section 23.6).
† Essential for children, but not necessarily for adults.

- The biosynthesis of proteins requires all 20 amino acids.
- If one of the 20 amino acids is missing or in short supply, protein biosynthesis is inhibited.
- Some organisms, such as *Escherichia coli*, can synthesize all *the* amino acids they need.
- Other species, including humans, must obtain some amino acids from dietary sources.

Amino Acid Catabolism

- In the catabolism of amino acids, the first step we consider is the removal of nitrogen by transamination.
- Transamination reactions are also important in the anabolism of amino acids.
- In catabolism, the amino nitrogen of the original amino acid is transferred to α-ketoglutarate to produce glutamate, leaving behind the carbon skeletons.
- The fates of the carbon skeleton and of the nitrogen can be considered separately.

*The fate of the **carbon skeleton** in amino acid breakdown*

- Breakdown of the carbon skeletons of amino acids follows two general pathways:
  1. A **glucogenic** amino acid yields *pyruvate* or *oxaloacetate* on degradation. Oxaloacetate is the starting point for the production of glucose by gluconeogenesis.
  2. A **ketogenic** amino acid breaks down to *acetyl-CoA* or *acetoacetyl-CoA*, leading to the formation of ketone bodies

Glucogenic and Ketogenic Amino Acids		
Glucogenic	**Ketogenic**	**Glucogenic and Ketogenic**
Aspartate	Leucine	Isoleucine
Asparagine	Lysine	Phenylalanine
Alanine		Tryptophan
Glycine		Tyrosine
Serine		
Threonine		
Cysteine		
Glutamate		
Glutamine		
Arginine		
Proline		
Histidine		
Valine		
Methionine		

- The carbon skeletons of the amino acids mainly give rise to metabolic intermediates such as:
  - *pyruvate*
  - *acetyl-CoA*
  - *acetoacetyl-CoA*
  - *α-ketoglutarate*
  - *succinyl-CoA*
  - *fumarate*
  - *oxaloacetate*

- **Glucogenic** amino acids can be converted to glucose, with oxaloacetate as an intermediate

- **Ketogenic** amino acids cannot be converted to glucose.

- Some amino acids have more than one pathway for catabolism, which explains why four of the amino acids are listed as both glucogenic and ketogenic.

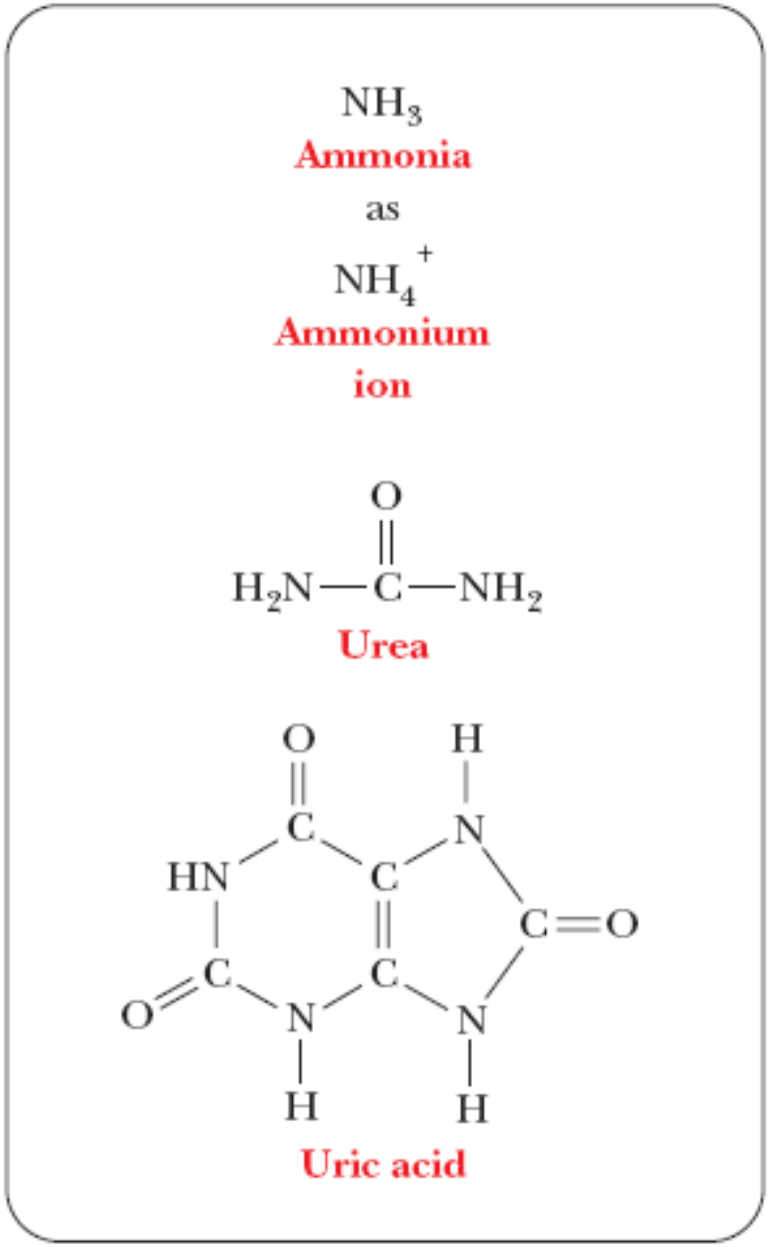
*The fate of the **nitrogen** in amino acid breakdown*

- The nitrogen portion of amino acids is involved in transamination reactions in breakdown as well as in biosynthesis.

- Excess nitrogen is excreted in one of three forms: *ammonia (as ammonium ion, NH_4^+), urea, and uric acid.*

Nitrogen-containing products of amino acid catabolism

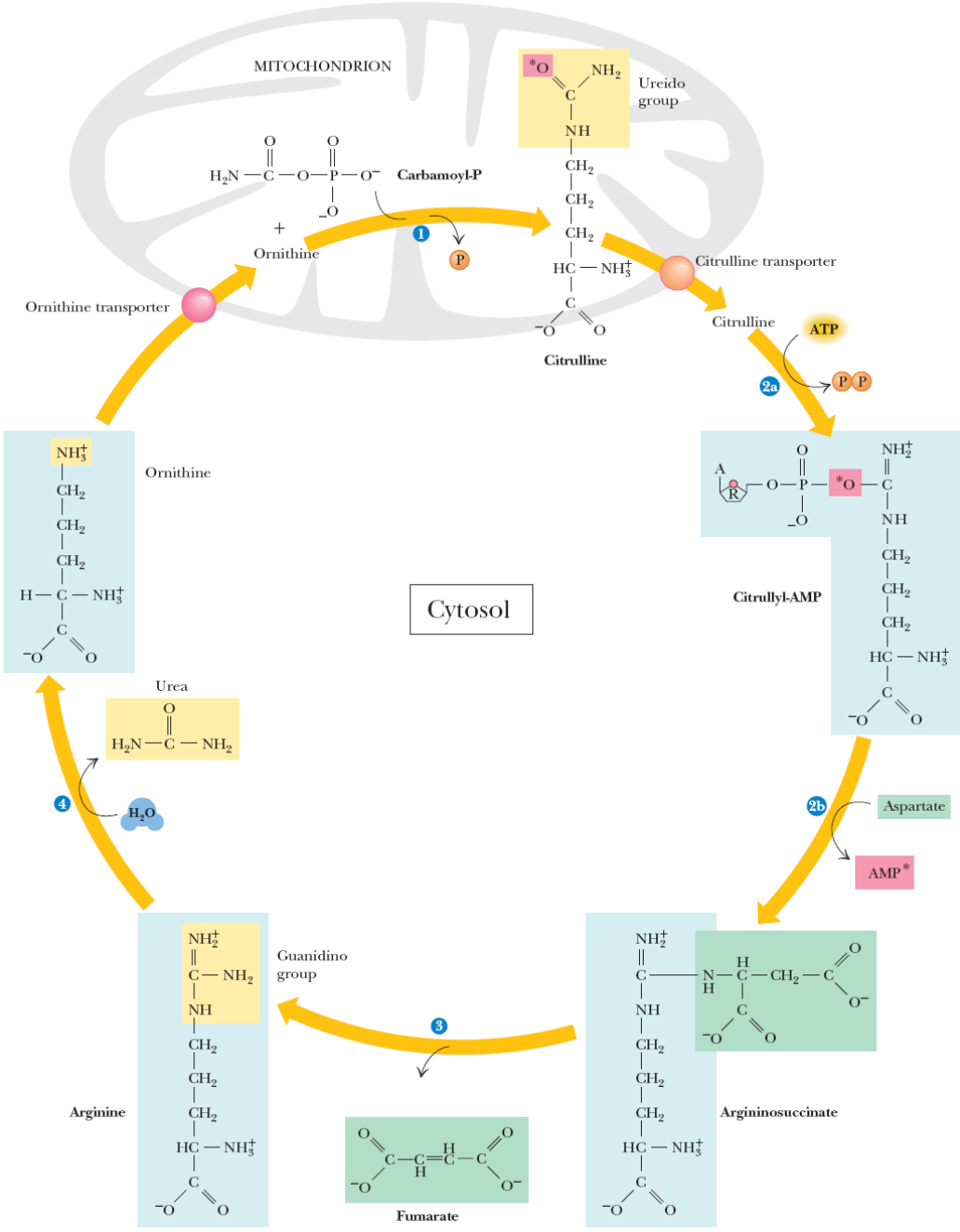
Animals, such as fish excrete nitrogen as *ammonia*, terrestrial animals as *urea*, birds excrete nitrogen in the form of *uric acid*



The role of the urea cycle in amino acid breakdown

- A central pathway in nitrogen metabolism is the **urea cycle**
- The nitrogens that enter the urea cycle come from several sources.
- One of the nitrogens of urea is added in the mitochondria, and its immediate precursor is glutamate.
- A condensation reaction between the ammonium ion and carbon dioxide produces **carbamoyl phosphate** in a reaction that requires two molecules of ATP.

The urea cycle



- Carbamoyl phosphate reacts with **ornithine** to form **citrulline.**

- Citrulline is then transported to the cytosol. A second nitrogen enters the urea cycle when aspartate reacts with citrulline to form **argininosuccinate** in another reaction that requires ATP.

- The amino group of the aspartate is the source of the second nitrogen in the urea that will be formed in this series of reactions.

- Argininosuccinate is split to produce **arginine and fumarate.**

- Finally, arginine is hydrolyzed to give urea and to regenerate ornithine, which is transported back to the mitochondrion.

- The synthesis of fumarate is a link between the urea cycle and the citric acid cycle. Fumarate is, of course, an intermediate of the citric acid cycle, and it can be converted to oxaloacetate.

- A transamination reaction can convert oxaloacetate to aspartate, providing another link between the two cycles.

Nitrogen Fixation

- **Nitrogen fi xation** is the process by which inorganic molecular nitrogen (N_2) from the atmosphere is incorporated first into ammonia (NH_3) and then into organic compounds (such as amino acids)

- Nitrate ion (NO_3^-) is the form in which nitrogen is found in the soil and many fertilizers contain nitrates.

- The process of **nitrification** (nitrate reduction to ammonia) provides another way for organisms to obtain nitrogen.

- Nitrate ion and nitrite ion (NO_2-) are also involved in **denitrification reactions**, which return nitrogen to the atmosphere

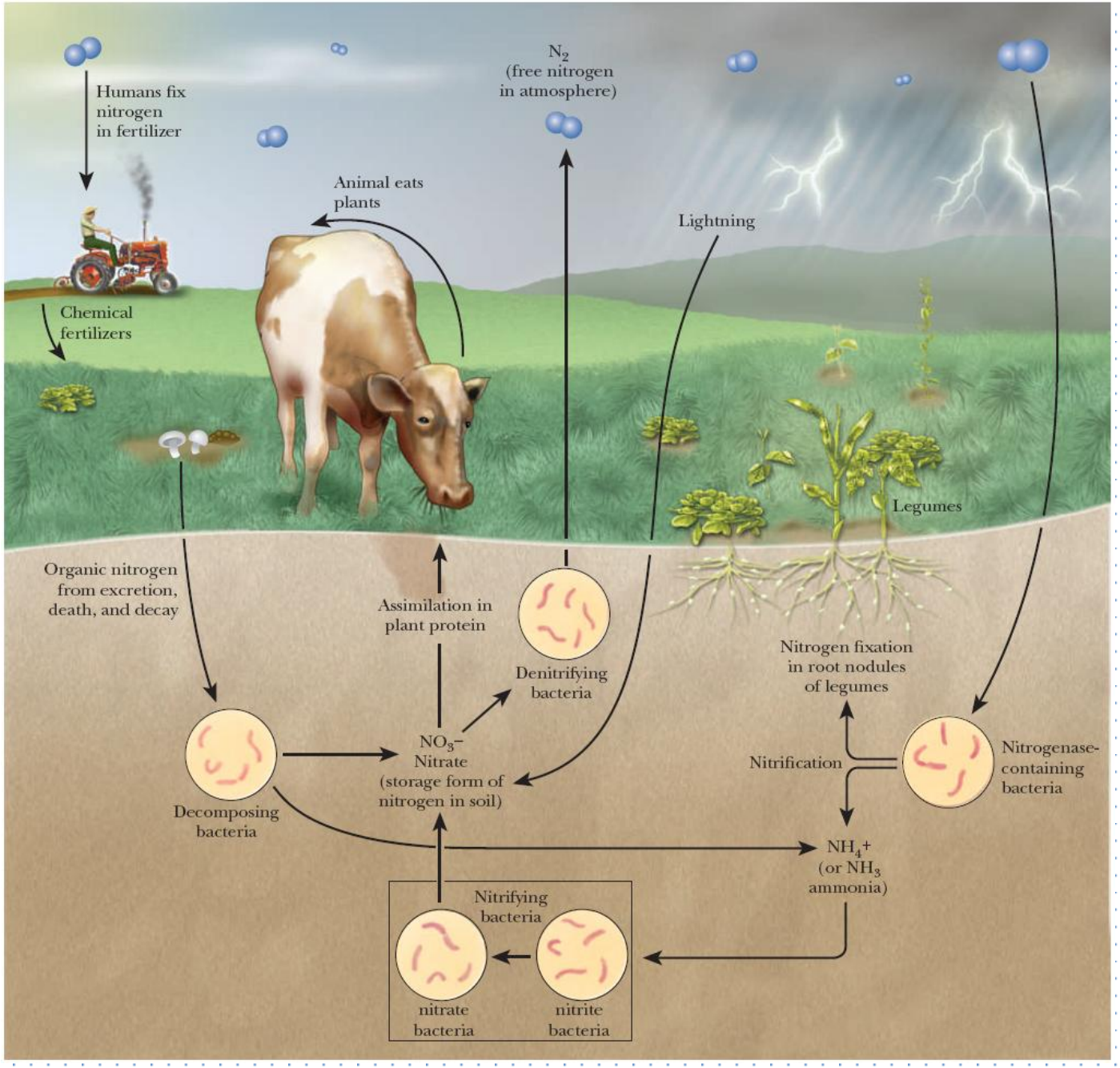
- Ammonia formed by either pathway, nitrogen fixation or nitrification, enters the biosphere.

- Ammonia is converted to organic nitrogen by plants, and organic nitrogen is passed to animals through food chains.

- Finally, animal waste products, such as urea, are excreted and degraded to ammonia by microorganisms.

- Bacteria are responsible for the reduction of N_2 to ammonia (NH_3).
- Typical nitrogen-fixing bacteria are symbiotic organisms that form nodules on the roots of leguminous plants, such as beans and alfalfa.
- Many free-living microbes and some cyanobacteria also fix nitrogen.
- Plants and animals cannot carry out nitrogen fixation.
- This conversion of molecular nitrogen to ammonia is the only source of nitrogen in the biosphere except for that provided by nitrates.

The flow of nitrogen in the biosphere



How is nitrogen from the atmosphere incorporated into biologically useful compounds?

- The **nitrogenase** enzyme complex found in nitrogen-fi xing bacteria catalyzes the production of ammonia from molecular nitrogen.

Nitrogen-fixing bacteria form nodules on alfalfa roots.

$$N_2 + 8e^- + 16ATP + 10H^+ \rightarrow 2NH_4^+ + 16ADP + 16P_i + H_2$$