

VU Medical Zone

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BIOCHEMISTRY II

BIO 303 Midterm Merge ppt

Lecture 1 to 63

BIOCHEMISTRY-II

- **Welcome to the course biochemistry-II- In this course you will learn the principles of biochemistry and their application on health and disease. In the beginning we will discuss the concepts of bioenergetics.. As the terminology is self explanatory.. BIO and energetic.. Meaning that we will be dealing with the concepts of energy transfer in biological systems.**
- **Generally speaking, we know that the Living cells have to perform certain work to stay alive, to grow, and to reproduce.**
- **They should have the ability to trap energy and use it into biological work is a fundamental property of all living organisms, but they use it in different ways.**
- **Modern organisms use the chemical energy in fuels to synthesize complex, highly ordered macromolecules**
- **For example: Plants use energy in such a manner that causes them to release oxygen as a waste product, while animals (which includes humans, of course) require oxygen as a component to survive.**

BIOENERGETICS

Bioenergetics is the quantitative study of the **energy transductions**, that is.. changes of one form of energy into another—that occur in living systems, and of the nature and function of the chemical processes underlying these transductions.



fireflies convert energy into
light

Bioenergetic processes

1. **Glycolysis** is a biological process in which glucose is broken down into **pyruvate** thus generating two molecules of ATP (Adenosine triphosphate) per molecule of glucose
2. **Gluconeogenesis** is another process in which the cell synthesizes glucose from biomolecules such as proteins, amino acids and fats
3. **Citric acid cycle** generates molecules of ATP by oxidation of energy stored in food material

All animal and plant cells are powered by chemical energy stored in the chemical Bonds of organic molecules. **Animals convert the chemical energy of fuels into motion and heat and**

In a few organisms such as fireflies chemical energy is converted into light...

Lets look at some Examples of major bioenergetic processes occurring in nature

ATP is the energy currency in biological systems

Citric acid cycle, that is the process of cellular respiration generates molecules of ATP by oxidation of energy stored in food material

These processes will be discussed in detail in coming lectures.

Laws of Thermodynamics

- There are two fundamental laws of thermodynamics.

1. First law

The first law is the principle of the conservation of energy: it states that ***“for any physical or chemical change, the total amount of energy in the universe remains constant; energy may change form or it may be transported from one region to another, but it cannot be created or destroyed.”***

Entropy

- The term “entropy,” was first used in 1851 by Rudolf Clausius, which literally means “a change within,”

Entropy = disorder

- **Entropy** is a quantitative expression for the randomness or disorder in a system. When the products of a reaction are less complex and more disordered than the reactants, the reaction is said to proceed with a gain in entropy.
- Denoted by S
- ΔS has a +ve sign when entropy increase and –ve when entropy decreases
 - units of entropy are **joules/mole.Kelvin** (J/mol.K) (joules/mole **multiply by** Kelvin)

The term “entropy,” was first used in 1851 by Rudolf Clausius, which literally means “a change within” a system.. and it can be described as disorder..

By convention, ΔS has a positive sign when entropy increases and ΔH , as noted above, has a negative sign when heat is released by the system to its surroundings.

Second Law of Thermodynamics

2. Second law

The second law of thermodynamics states that ***“the universe always tends towards increasing disorder: in all natural processes, the entropy of the universe increases.”***

With this idea about entropy, we come to the 2nd law ..which states that..

“the universe always tends towards increasing disorder and in all natural processes, the entropy of the universe increases... give example of hot cup..

Now the point is that entropy is not a measure of energy ..it only describes that how energy is distributed within a system

Enthalpy (H)

- Enthalpy is the heat content of the reacting system.
- It reflects the number and kinds of chemical bonds in the reactants and products
- When a chemical reaction releases heat, it is said to be **exothermic** and **value of H is negative**
- When a chemical reaction takes up heat from surroundings, it is called as endothermic and have **positive values of H**

When a chemical reaction releases heat, it is said to be exothermic; the heat content of the products is less than that of the reactants and H has a negative value.

On the other hand, Reacting systems that takes up heat from their surroundings are endothermic and have positive values of H . Enthalpy and entropy of a system are related and help us to understand Gibb's free energy

Gibbs free energy (G)

- **Gibbs free energy, G** expresses the amount of energy capable of doing work during a reaction at constant temperature and pressure
- When a reaction proceeds with the release of free energy the free-energy change, **G , has a negative value** and the reaction is said to be **exergonic**
- In endergonic reactions, **the system gains free energy and G is positive**
- The units of **G and H** are joules/mole or calories/mole
- (1 cal is equal to 4.184 Joules)

- Under the conditions existing in biological systems (including constant temperature and pressure), changes in free energy, enthalpy, and entropy are related to each other quantitatively by the equation

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

ΔG : is the change in Gibbs free energy

ΔH : the change in enthalpy

T : the absolute temperature

ΔS : change in entropy

in which ΔG is the change in Gibbs free energy of the reacting system, ΔH is the change in enthalpy of the system, T is the absolute temperature, and ΔS is the change in entropy of the system.

Either of these conditions, which are typical of favorable processes, tend to make G negative. In fact, ΔG of a spontaneously reacting system is always negative.

BIOENERGETICS

Cells require sources of Free Energy (G)

BIOENERGETICS

Cells require sources of Free Energy (G)

- amount of energy capable of doing work during a chemical reaction (G)
- **Cells work at constant temperature and pressure (isothermal)**
- **Heat flow is NOT energy source**
- **Change in Gibbs Free energy (ΔG)**

Significance of Free-Energy Changes (ΔG)

1. Actual free-Energy changes depend on **Reactant and Product Concentrations**
2. **Allows prediction of the direction of chemical reactions, their exact equilibrium position and the amount of work done**
3. The free-energy change for a reaction is **independent of the pathway by which the reaction occurs**
4. **Free-energy changes are additive**; the net chemical reaction that results from successive reactions sharing a common intermediate has an overall free-energy change that is the sum of the ΔG values for the individual reactions

Standard free-energy G°

- The free energy of reaction at under standard conditions
- When Temperature: 298K or 25°C , when reactants are initially present at 1 M concentrations or, for gases, at partial pressures of $101.3\text{ kilopascals (kPa)}$, or 1 atm , the force driving the system toward equilibrium is defined as the standard free-energy change, ΔG°

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

But before that we should know that what is standard free energy... Under standard conditions of temperature and pressure, meaning (298 K or 25°C), when reactants and products are initially present at 1 M concentrations or, for gases, at partial pressures of $101.3\text{ kilopascals (kPa)}$, or 1 atm , the force driving the system toward equilibrium is defined as the standard free-energy change, ΔG° where Δ is the symbol to depict that the reaction occurred under standard conditions

Key conventions in biochemical reactions

- Most biochemical reactions occur near pH 7 (buffered)
- **Standard biochemical state**
 - a) (H^+) is 10^{-7} M
 - b) concentration of water is 55.5 M
- Both the pH and the concentration of water are essentially constant
- Under these conditions, ΔG is written as $\Delta G'^{\circ}$ (**with prime**)
- **Concentration of water, H^+ and Mg^{2+} are not included in equations for simplicity**

Molar concentration of water

- Density of pure water = 1000 g/L
- Molar mass of water = 18 g/mol (2H + 1 Oxygen=2+16=18)
- Molar concentration of water = d/m
= 1000 / 18.02
= 55.5 mol/L

Difference between ΔG and ΔG° ?

- the actual free-energy change, ΔG
- the standard free-energy change, ΔG°
- Each chemical reaction has a characteristic standard free-energy change, which may be positive, negative, or zero, depending on the **equilibrium constant** of the reaction

The **standard conditions**

T: 298K (25°C) **pH:** 7

Concentration: 1M

Partial pressures: 101.3
kilopascals (kPa), or 1 atm

...cont

- Thus $\Delta G'^{\circ}$ is a constant: it has a characteristic, unchanging value for a given reaction
- But the *actual* free-energy change, ΔG , is a function of reactant and product ***concentrations and of the temperature prevailing during the reaction***, none of which will necessarily match the standard conditions as defined above

under standard conditions

prime means ph conditions are under physiological conditions.. Ph=7

BIOENERGETICS

**The Standard Free-Energy is directly
related to the
Equilibrium constant**

What is Equilibrium constant (K)?

- The composition of a reacting system tends to continue changing until **equilibrium** is reached
- At the equilibrium **concentration of reactants and products**, the **rates of the forward and reverse reactions are exactly equal** and no further net change occurs in the system.
- The number that expresses the relationship between the amounts of products and reactants present at **equilibrium** in a reversible chemical reaction is known as the equilibrium constant, K_{eq}
- In the general reaction



How to calculate Equilibrium constant?

- The equilibrium constant is given by following equation where [A], [B], [C], and [D] are the molar concentrations of the reaction components at the point of equilibrium, equilibrium constant can be written as:

$$K_{eq} = \frac{[C]^c [D]^d}{[A]^a [B]^b}$$

Standard free-energy change

The standard free-energy change of a chemical reaction is an alternative mathematical way of expressing its equilibrium constant.

- ΔG is characteristic for a given reaction and can be calculated from the equilibrium constant for the reaction:

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

- where
- R is the gas constant (8.31 cal/Mol.K)
- T is standard temperature (25 C or 298K)
- K'_{eq} is the equilibrium constant

...cont

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

- If the K_{eq} is equal to 1.0

the standard free-energy change (ΔG°) of that reaction is 0.0 (the natural logarithm (**ln**) of 1.0 is zero)...**EQUILIBRIUM**

- If K_{eq} of a reaction is greater than 1.0

ΔG° is negative, **forward direction is favored**

- If K_{eq} is less than 1.0

ΔG° is positive, **reverse direction is favored**

WORKED EXAMPLE 13–1**Calculation of $\Delta G'^{\circ}$**

Calculate the standard free-energy change of the reaction catalyzed by the enzyme phosphoglucomutase



given that, starting with 20 mM glucose 1-phosphate and no glucose 6-phosphate, the final equilibrium mixture at 25°C and pH 7.0 contains 1.0 mM glucose 1-phosphate and 19 mM glucose 6-phosphate. Does the reaction in the direction of glucose 6-phosphate formation proceed with a loss or a gain of free energy?

Solution: First we calculate the equilibrium constant:

$$K'_{\text{eq}} = \frac{[\text{glucose 6-phosphate}]}{[\text{glucose 1-phosphate}]} = \frac{19 \text{ mM}}{1.0 \text{ mM}} = 19$$

We can now calculate the standard free-energy change:

$$\begin{aligned}\Delta G'^{\circ} &= -RT \ln K'_{\text{eq}} \\ &= -(8.315 \text{ J/mol} \cdot \text{K})(298 \text{ K})(\ln 19) \\ &= -7.3 \text{ kJ/mol}\end{aligned}$$

Because the standard free-energy change is negative, the conversion of glucose 1-phosphate to glucose 6-phosphate proceeds with a loss (release) of free energy. (For the reverse reaction, $\Delta G'^{\circ}$ has the same magnitude but the *opposite* sign.)

BIOENERGETICS

**The Standard Free-Energy is directly
related to the
Equilibrium constant ...cont**

ΔG and $\Delta G'^{\circ}$ are related

- The actual free-energy change, ΔG is a variable that depends on $\Delta G'^{\circ}$ and on the concentrations of reactants and products:



$$\Delta G = \Delta G'^{\circ} - RT \ln ([\text{products}]/[\text{reactants}])$$

$$\Delta G = \Delta G'^{\circ} + RT \ln \frac{[C]^c [D]^d}{[A]^a [B]^b} \quad \begin{array}{l} \text{(equation 13-4... according to Lehninger} \\ \text{6th Edition—chapter No 13)} \end{array}$$

The **free energy change** (ΔG) of a reaction determines its spontaneity. A reaction is spontaneous if ΔG is negative (if the free energy of products is less than that of reactants).

...cont

- The concentration terms in this equation express the effects commonly called mass action, and the term $[C]^c[D]^d/[A]^a[B]^b$ is called the **mass-action ratio**, Q .
- Thus the equation can be written as follows:

$$\Delta G = \Delta G'^{\circ} + RT \ln Q$$

...cont

- Lets suppose that the reaction is under standard conditions..



- But the conc. Of reactants and products is NEITHER equal to each other... Nor the conc. Is 1M
- **non-standard concentrations**
- **Actual conc. Of reactants and products but standard values of $\Delta G'^{\circ}$, R and T**
- **Value of ΔG at equilibrium (for spontaneous reaction)??**

$$0 = \Delta G = \Delta G'^{\circ} + RT \ln \frac{[C]^{\text{eq}} [D]^{\text{eq}}}{[A]^{\text{eq}} [B]^{\text{eq}}}$$

$$\Delta G'^{\circ} = -RT \ln K'^{\text{eq}}$$

...cont

- The $\Delta G'^0$ values of sequential reactions are additive.
- $$\Delta G'^0_{\text{total}} = \Delta G'^0_1 + \Delta G'^0_2$$
- This principle of bioenergetics explains how a thermodynamically unfavorable (endergonic) reaction can be driven in the forward direction by coupling it to a highly exergonic reaction through a common intermediate

Example

- For example, the synthesis of glucose 6-phosphate is the first step in the utilization of glucose by many organisms:



$$\Delta G'^{\circ} = 13.8 \text{ kJ/mol}$$

- The positive value of $\Delta G'^{\circ}$ predicts that under standard conditions the reaction will tend not to proceed spontaneously in the direction written.
- Hydrolysis of ATP to ADP and Pi, is very exergonic:



$$\Delta G'^{\circ} = -30.5 \text{ kJ/mol}$$

This principle of bioenergetics explains how a thermodynamically unfavorable (endergonic) reaction can be driven in the forward direction by coupling it to a highly exergonic reaction through a common intermediate.

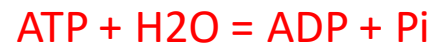
For example, the synthesis of glucose 6-phosphate is the first step in the utilization of glucose by many organisms:



$$\Delta G'^{\circ} = 13.8 \text{ kJ/mol}$$

The positive value of $\Delta G'^{\circ}$ predicts that under standard conditions the reaction will tend not to proceed spontaneously in the direction written.

Another cellular reaction, the hydrolysis of ATP to ADP and P_i , is very exergonic:



$$\Delta G'^{\circ} = -23.0 \text{ kJ/mol}$$

- These two reactions share the common intermediates Pi and H₂O and may be expressed as sequential reactions:



- The overall standard free-energy change is obtained by adding the $\Delta G'^{\circ}$ values for individual reactions:
- $\Delta G'^{\circ} = 13.8 \text{ kJ/mol} + (-30.5 \text{ kJ/mol}) = -16.7 \text{ kJ/mol}$

As $\Delta G'^{\circ}$ is a way of expressing the equilibrium constant for a reaction. For reaction (1) above,

$$K'_{eq_1} = \frac{[\text{glucose 6-phosphate}]}{[\text{glucose}][P_i]} = 3.9 \times 10^{-3} \text{ M}^{-1}$$

- The equilibrium constant for the hydrolysis of ATP is

$$K'_{eq_2} = \frac{[\text{ADP}][P_i]}{[\text{ATP}]} = 2.0 \times 10^5 \text{ M}$$

- The equilibrium constant for the two coupled reactions is

$$\begin{aligned} K'_{eq_n} &= \frac{[\text{glucose 6-phosphate}][\text{ADP}][P_i]}{[\text{glucose}][P_i][\text{ATP}]} \\ &= (K'_{eq_1})(K'_{eq_2}) = (3.9 \times 10^{-3} \text{ M}^{-1})(2.0 \times 10^5 \text{ M}) \\ &= 7.8 \times 10^2 \end{aligned}$$

- This calculation illustrates that equilibrium constants: although the $\Delta G'^{\circ}$ values for two reactions that sum to a third, overall reaction are *additive*, the K_{eq} for the overall reaction is the *product* of the individual K_{eq} values for the two reactions.
- **Equilibrium constants are *multiplicative*.**
- Energy stored in ATP is used to drive to synthesis of glucose 6-phosphate, eventhough its formation from glucose and P_i is endergonic.
- **This strategy works only if compounds such as ATP are continuously available.**

- **Homogeneous chemical reaction** **chemical reactions** in which the reactants and products are in the same phase.
- **Heterogeneous chemical reaction** have reactants in two or more phases.
- **Le Châtelier Principle** ("The Equilibrium Law") states that when a system experiences a disturbance (such as concentration, temperature, or pressure changes), it will respond to restore a new equilibrium state.

BIOENERGETICS

TYPES OF CHEMICAL REACTIONS IN CELLS

- **Chemical Logic and Common Biochemical Reactions**
- Most cells have the capacity to carry out thousands of specific, enzyme-catalyzed reactions:
- for example, transformation of a simple nutrient such as glucose into amino acids, nucleotides, or lipids
- extraction of energy from fuels by oxidation
- polymerization of monomeric subunits into macromolecules

5 types of chemical reactions in cells

The reactions going on in biological systems can be categorized into 5 types:

1. Reactions that make or break carbon-carbon bonds
2. Internal rearrangements, isomerizations, and eliminations
3. Free-radical reactions
4. Group transfer
5. Oxidation-reduction reactions

2 basic chemical principles

(1) A covalent bond can be broken in two general ways

- Homolytic cleavage
 - Heterolytic cleavage
-
- A **Covalent bond**, also called a molecular *bond*, involves the sharing of electron pairs between atoms.
 - These electron pairs are known as shared pairs or *bonding* pairs, and the stable balance of attractive and repulsive forces between atoms, when they share electrons, is known as *covalent bonding*.

And before we study the details of these chemical reactions we should revise 2 basic principles..1st principle is that..say on slide...

So what is a covalent bond.. Lets review the concept..A *covalent bond*, is a chemical *bond* that involves 2 atoms that share their electron pair. These electron pairs are known as shared pairs or *bonding* pairs, and the stable balance of attractive and repulsive forces between atoms, when they share electrons, is known as *covalent bonding*.

Atoms make covalent **bond** with other atoms in order to gain more stability, which is achieved by forming a full electron shell. By sharing their outer most (valence) electrons, atoms can fill up their outer electron shell and become more stable.

2 mechanisms for breaking a C-C or C-H bond

- **Homolytic cleavage:** each atom leaves the bond as a radical, carrying one unpaired electron
- **Heterolytic cleavage** (more common): one atom retains both bonding electrons

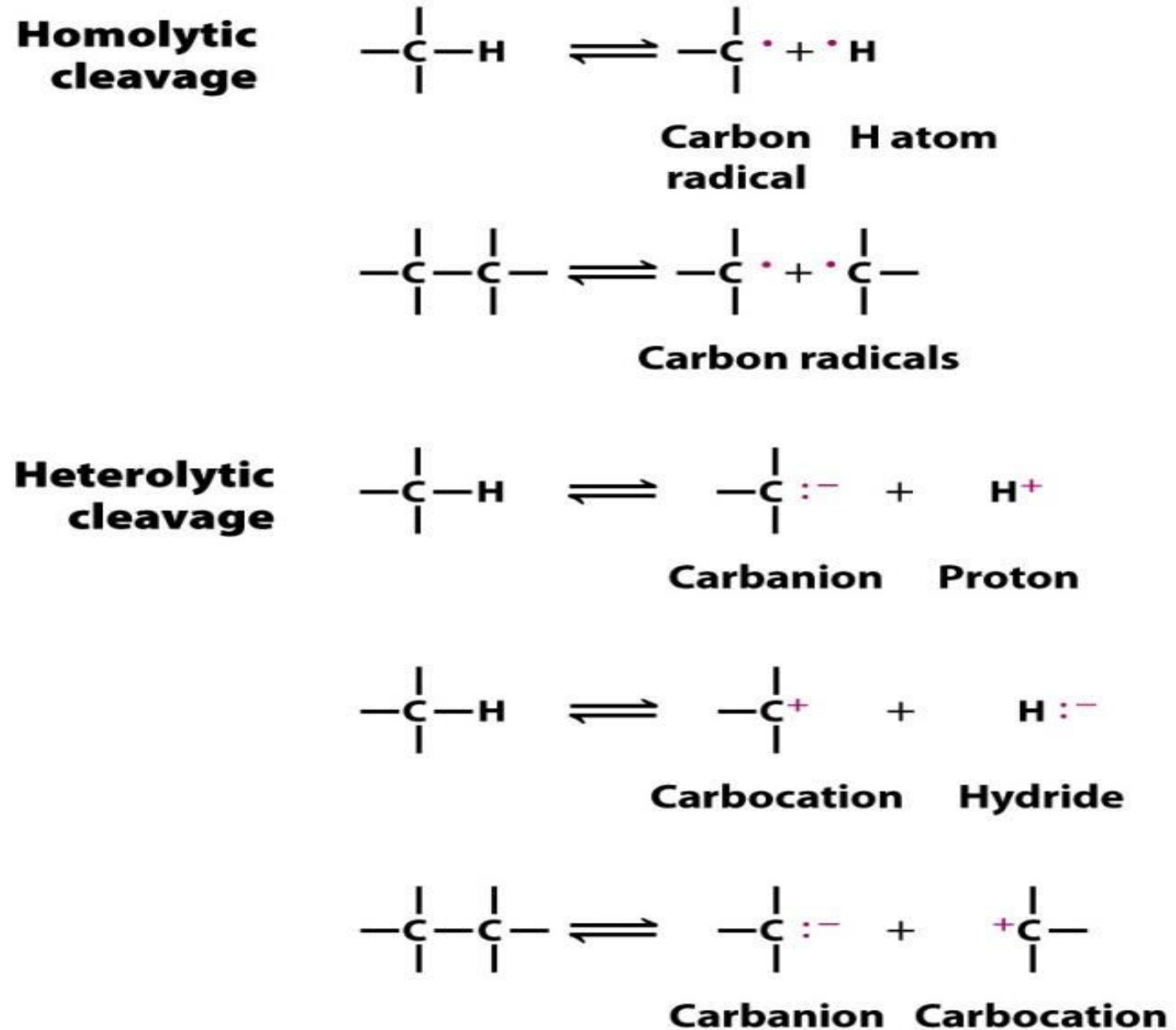


Figure 13-1
Lehninger Principles of Biochemistry, Fifth Edition
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In Homolytic cleavage: if the bond breaks, each atom leaves the bond as a radical that carries one unpaired electron

, and we know what a radical is... molecule that contains at least 1 unpaired electron

Whereas in **Heterolytic cleavage, is more common.. And 1 atom retains both the bonding electrons..**

So there can be 3 situations.. 1st is the case where the bond break in a way that.. Eg: in C-H bond in --- compound can break and carbon carries both electrons, and becomes more electronegative.. -ve charge and is known as carb-an-ion.. As it is an anion of carbon.. And hydrogen atom is deprived of electron pair, so it has positive charge and behaves as a proton..

2. In other case, if H atom will take both electrons, it will be more electronegative and become a hydride ion, that is.. an anion of hydrogen atom,, whereas carbon which will lose the electron pair will become carbo-cation

3. Consider the breakage of bond b/w 2 carbon atoms in a way that 1 carbon atom retains the electron pair and the other loses, in this case, there will be formation of 1 carb-anion and 1 carbocation in same reaction

Importantly, types of ions formed in these reactions, that is, carb-anion, carbocation and hydride ions are highly unstable.

Second principle

2. Many biochemical reactions involve interactions between nucleophiles and electrophiles

- **Nucleophiles:** functional groups rich in and capable of donating electrons
- (Nucleophiles are Lewis bases)
- **Electrophiles:** electron-deficient functional groups that seek electrons

The 2nd principle indicates that many biochemical reactions involve interaction between nucleophiles and electrophiles

- **nucleophiles: meaning** nucleus loving”, or “positive-charge loving ..are the functional groups rich in electrons and can donate them to form a covalent bond
- which is the exact definition of a Lewis base. In other words, **nucleophiles are Lewis bases.**

- **Now coming to Electrophiles:Electrophile means..** electron-loving”, or “negative-charge loving” these are the functional groups that are electron-deficient and seek electrons.. **to form a covalent bond**

Nucleophiles combine with Electrophiles and share electrons with them

There are some Common nucleophiles and electrophiles found in biochemical/biological reactions

Common nucleophiles and electrophiles in biochemical reactions

FIGURE 13-2 Common nucleophiles and electrophiles in biochemical reactions. Chemical reaction mechanisms, which trace the formation and breakage of covalent bonds, are communicated with dots and curved arrows, a convention known informally as “electron pushing.” A covalent bond consists of a shared pair of electrons. Nonbonded electrons important to the reaction mechanism are designated by dots ($:$). Curved arrows ($\curvearrowright$) represent the movement of electron pairs. For movement of a single electron (as in a free radical reaction), a single-headed (fishhook-type) arrow is used ($\frown$). Most reaction steps involve an unshared electron pair.

Electron pushing: The movement of a pair of electrons (a lone pair of electrons or a bond) from an electron rich site to an electron poor site.

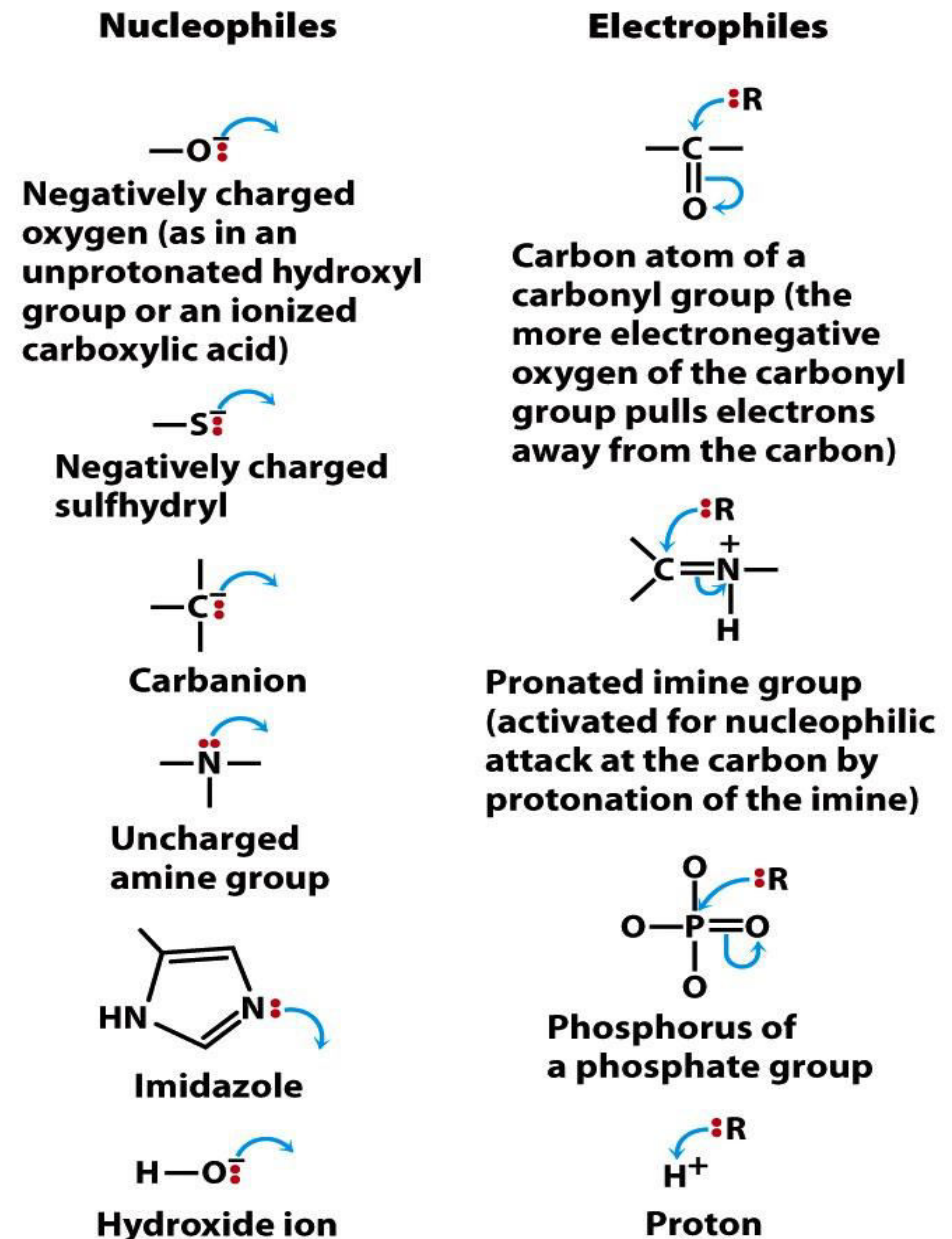


Figure 13-2
Lehninger Principles of Biochemistry, Fifth Edition
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Analysing the phosphate anion PO_4^{3-} –

it is crucial to recognise its symmetry, which is tetrahedral T_d . In this arrangement it is perfectly safe.

Very observant! What happens is that phosphorus is hybridized in the phosphate ion. Hybridization allows the central phosphate atom to form more bonds than you might expect from its *ground* state electron configuration.

Within the framework of hybridization, one may look at the bonds in the phosphate anion as having a great deal of ionic character.

The electronegativity of phosphorus is 2.19; the electronegativity of oxygen is 3.44. The difference is 1.25 – or rather sizable. As a result, electron density is largely withdrawn from the central phosphorus. Hence the negative charges you see on the three oxygen molecules in your picture.

Other depictions, which *reject* a large degree of d-orbital utilization in hybridization, might give a negative formal charge to *all* the oxygens and a positive formal charge to the central phosphorus by eschewing the $\text{P}=\text{O}$ double bond.

one might expect it to form 3 single covalent bonds and be happy. But it doesn't, it forms five bonds, presumably using each of its five electrons. My first question is - why would it do this? It seems to me that this would result in the Phosphorus atom having ten electrons in its outer shell (four from the double bond with the oxygen and two each from the single bonds with the other three oxygens).

Apart from the oxygen with a double-bond, we have three oxygens forming single bonds. Does this not leave them one electron short? Finally, why are the oxygens shown as carrying a negative charge?

phosphorus has stable oxidation states in compounds, ranging from $-III$ to $+V$.

First of all, let me state the obvious: ***Phosphorus is awesome.*** After we got that out of the way we can focus on why.

Apart from this it is possible to formulate many different cations and anions, that are derived from the above molecular structures. Just to name a few, there are P^{3+} , P^{5+} , P^{7+} , P^{9+}

But now for the most important part, phosphorus has stable oxidation states in compounds, ranging from $-III$ to $+V$. Here are a few examples: $P-IIIH_3$, $P^{2-}IH_4$, $[P-IH]_n$, $P^{4\pm 0}$, H_3P+IO_2 , $H_4P^{2+}IO_4$, $H_3P+IIIIO_3$, $H_2P^{2+}IVO_6$, H_3P+VO_4

While dealing with these compounds it is usually completely unnecessary to describe bonding with hybrid orbitals.

In case of [phosphane](#) PH_3

it would be wrong. Assuming sp^3P one would expect $\angle(H-P-H) \approx 109^\circ$, while it is found to be $\angle(H-P-H) = 93.5^\circ$, which is almost the same angle as the orbitals are having towards each other.

In general your assumption is correct, that it is possible to form only three covalent bonds to reach a stable configuration. And that will most likely be the case when phosphorus forms compounds with more electropositive elements.

Now dealing with oxygen, means dealing with a much more electronegative element, i.e. $En(O) \approx 3.4$, $En(P) \approx 2.2$. That also means that bonds are much more polarised towards the oxygen.

Apart from the oxygen with a double-bond, we have three oxygens forming single bonds. Does this not leave them one electron short?

Finally, why are the oxygens shown as carrying a negative charge?

You could draw double bonds for all the oxygens but there aren't that many valence electrons to be distributed. The structure shown above is a compromise between number of **valence electrons and electronegativity considerations** - we wouldn't want a surfeit of electrons around the central phosphorus since that's not as thermodynamically favorable as having the **more electronegative oxygen support these electrons**.

In addition, the sum of the formal charges on an atom must add up to the overall charge on the atom. Phosphate anion is triply negative charged; note how there are three oxygens bearing a single negative charge.

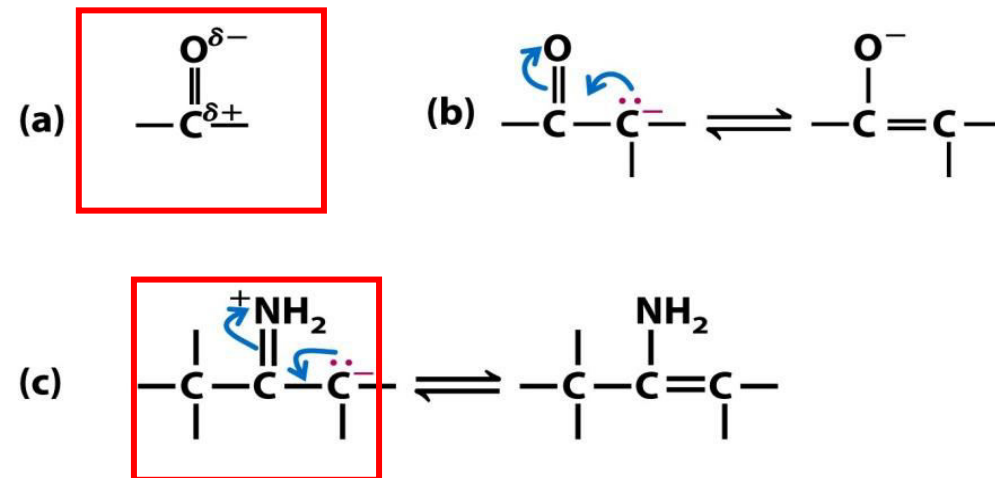
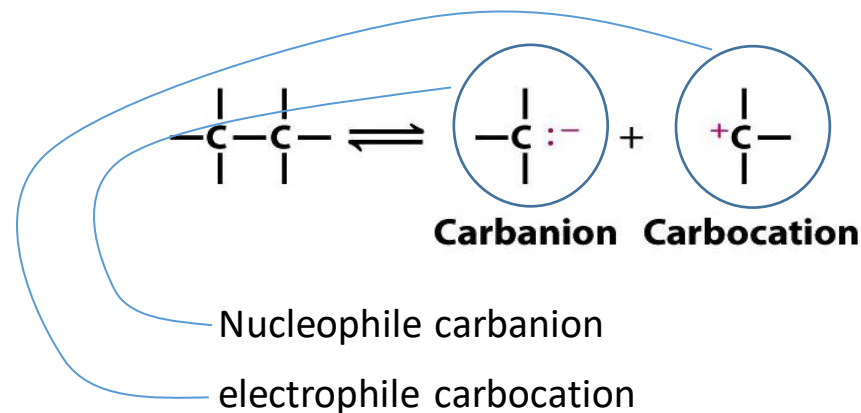
Significance of Carbonyl group

- **Carbonyl groups** are particularly important in the chemical transformations of metabolic pathways...

(1) The carbon of a **carbonyl group** has a **partial positive** charge due to the electron-withdrawing property of the **carbonyl oxygen** → **electrophilic carbon**

(2) Carbonyl group facilitate the formation of a carbanion's on an adjoining carbon by delocalizing the carbanion's negative charge

(3) An **imine group** can serve a similar function



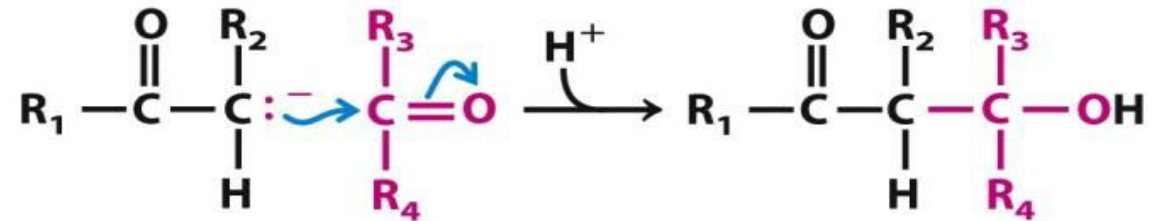
1. Types of reactions that make or break C-C bonds

- Three major class of reaction in which **carbon-carbon** bonds are formed or broken

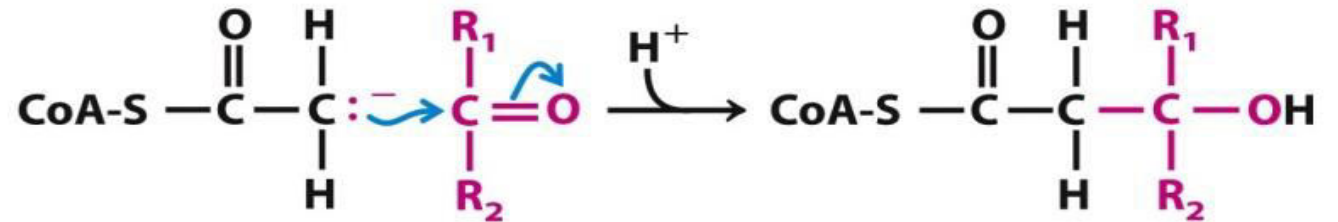
(1) Aldol condensation

(2) Claisen condensation

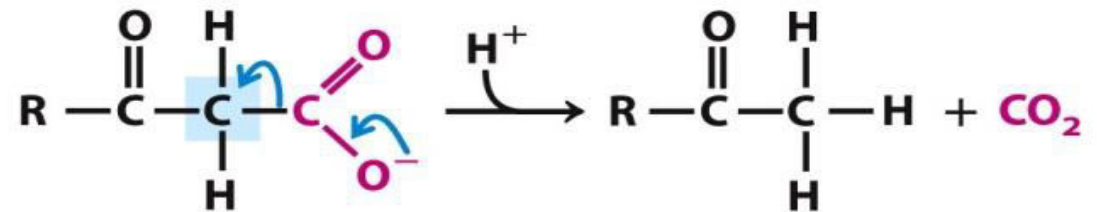
(3) Decarboxylation



Aldol condensation



Claisen ester condensation



Decarboxylation of a β -keto acid

Now coming to the types of reactions occurring in nature.. Lets take a look at the Reactions that make or break carbon-carbon bonds..

As we just studied that Heterolytic cleavage of a C-C bond yields either a carbanion and a carbocation or both at the same time.

On the other hand the formation of bond between 2 C atoms involves the joining of nucleophilic carbanion and electrophilic carbocation.

Carbanions and carbocations are generally so unstable, that *usually* They cannot be isolated from a reaction as they will react immediately with some other species to get in a stabilized condition.

to fill their empty p orbital. Because they are electron deficient, attaching electron donating groups (such as alkyl groups) to the *carbocation* will help stabilize the *carbocation*.

1. Aldol condensation:

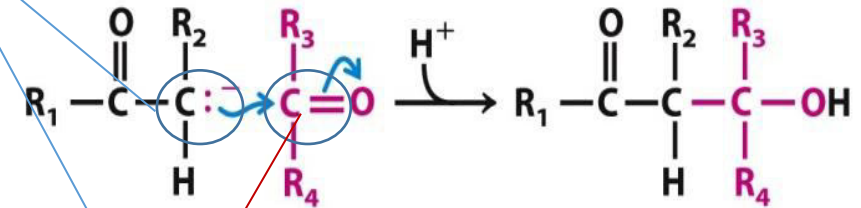
When the enolate of an aldehyde or a ketone reacts at the α -carbon with the carbonyl of another molecule to obtain β -hydroxy aldehyde or β -hydroxy ketone

2. Claisen condensation:

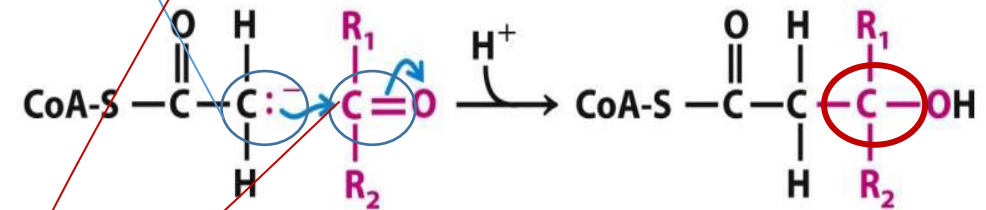
A carbon–carbon bond forming **reaction** that occurs between two esters or one ester and another carbonyl compound in the presence of a strong base, resulting in a β -keto ester.

- In **Aldol condensation and Claisen condensation** a carbanion serves as nucleophile and the carbon of a carbonyl group serves as electrophile, but, in **Claisen condensation**, the carbanion is stabilized by the carbonyl of an adjacent thioester

nucleophiles



Aldol condensation



Claisen ester condensation

electrophiles

For both the aldol condensation and the Claisen condensation, a carbanion serves as nucleophile (it donates electrons) and the carbon of a carbonyl group serves as electrophile.

In the decarboxylation reaction, a carbanion is formed on the carbon shaded blue as the CO₂ leaves. The reaction would not occur at an appreciable rate without the stabilizing effect of the carbonyl adjacent to the carbanion carbon. Wherever a carbanion is shown, a stabilizing resonance with the adjacent carbonyl, as shown in Figure 13–3b, is assumed. An imine (Fig. 13–3c) or other electron-withdrawing group (including certain enzymatic cofactors such as pyridoxal) can replace the carbonyl group in the stabilization of carbanions.

...cont

3. Decarboxylation: involves the formation of a carbanion stabilized by a carbonyl group. eg, during formation of ketone bodies during fatty acid metabolism

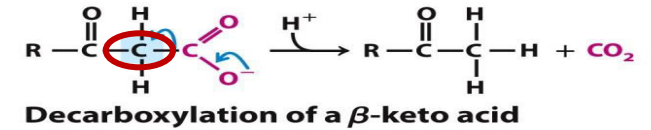


Figure 13-4
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Now coming to the types of reactions occurring in nature.. Lets take a look at the Reactions that make or break carbon-carbon bonds..

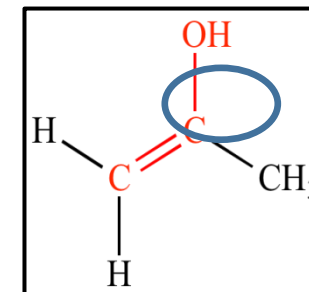
As we just studied that Heterolytic cleavage of a C-C bond yields either a carbanion and a carbocation or both at the same time.

On the other hand the formation of bond between 2 C atoms involves the joining of nucleophilic carbanion and electrophilic carbocation.

Carbanions and carbocations are generally so unstable, that *usually* They cannot be isolated from a reaction as they will react immediately with some other species to get in a stabilized condition.

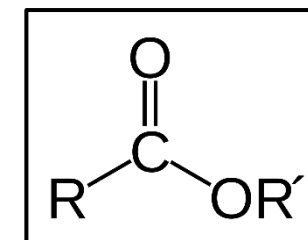
to fill their empty p orbital. Because they are electron deficient, attaching electron donating groups (such as alkyl groups) to the *carbocation* will help stabilize the *carbocation*.

- **ENOL:** also known as, alkenol, is a type of reactive structure or intermediate in organic chemistry that is represented as an alkene with a **hydroxyl group attached to one end of the alkene double bond**.



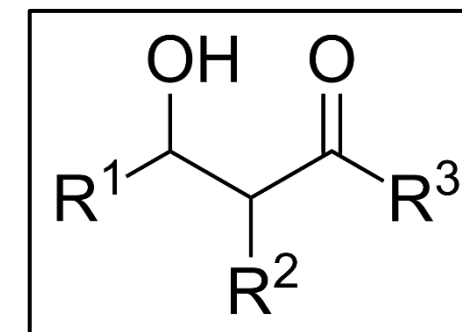
ENOL

- **ESTER:** is a chemical compound derived from an acid (organic or inorganic) in which at least one -OH (hydroxyl) group is replaced by an -O-alkyl (alkoxy) group. Usually, **esters** are derived by the reaction between a carboxylic acid and an alcohol.



Ester

- **ALDOL:** An **aldol** (from "Aldehyde alcohol") is a hydroxy ketone or aldehyde, and is the product of **aldol** addition (as opposed to **aldol** condensation).



Aldol

A **mixed anhydride** is a carboxylic acid **anhydride** that has the following general structural formula. $\text{R}^1 \neq \text{R}^2$ = hydrogen atoms, alkyl groups, aryl groups. eg: see also symmetrical **anhydride**.

BIOENERGETICS

TYPES OF CHEMICAL REACTIONS IN CELLS

...continued

5 types of chemical reactions in cells

The reactions going on in biological systems can be categorized into 5 types:

- 1. Reactions that make or break carbon-carbon bonds**
- 2. Internal rearrangements, isomerizations, and eliminations**
- 3. Free-radical reactions**
- 4. Group transfer**
- 5. Oxidation-reduction reactions**

2. Internal rearrangements, isomerizations, and eliminations

- **Intramolecular rearrangement:** redistribution of electrons results in alterations of many different types without a change in the overall oxidation state of the molecule
 - different group in the molecule may undergo oxidation-reduction, with no net change in oxidation state of the molecule
 - groups at a double bond may undergo a cis-trans rearrangement
 - the positions of double bonds may be transposed

Coming to the 2nd type of reactions taking place in biological systems, these include rearrangements within the molecule itself..this occurs by the: redistribution of electrons among the atoms and results in different types of alterations and distribution of charge .. But these rearrangements do not lead to any a change in the overall oxidation state of the molecule

- (1) different group in the molecule may undergo oxidation-reduction, with no net change in oxidation state of the molecule
- (2) groups at a double bond may undergo a cis-trans rearrangement MEANING...
- (3) the positions of double bonds may be transposed.. And how does that happen..for example...

(a) Isomerization

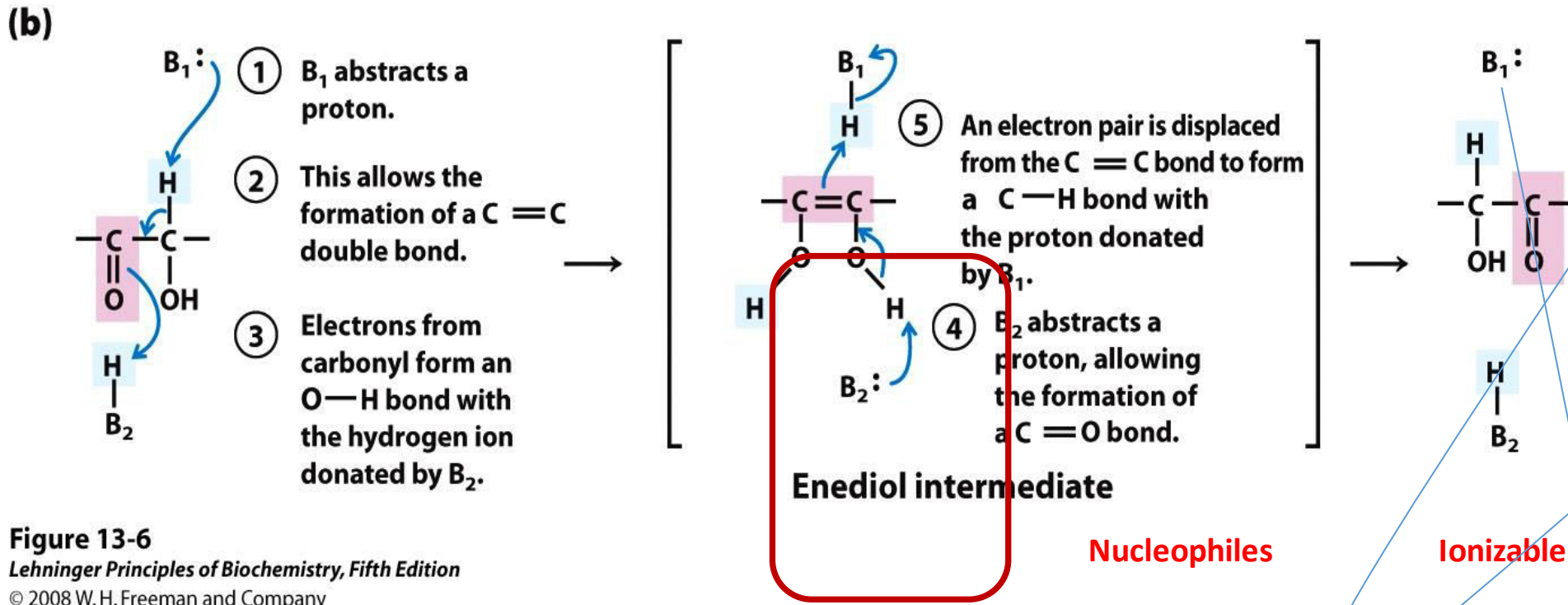
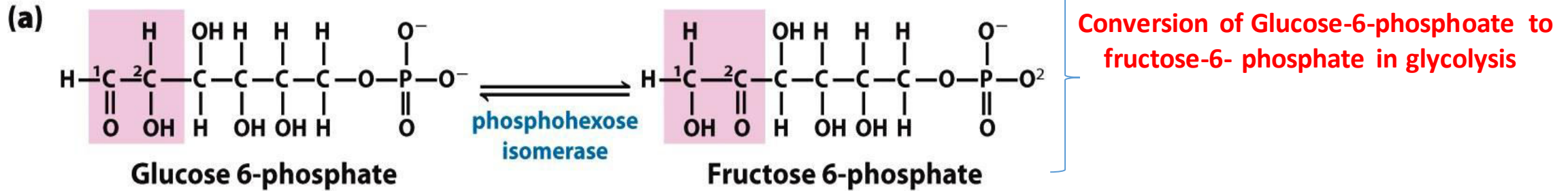


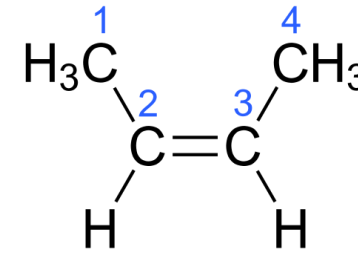
Figure 13-6
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Enediol intermediate: The **enediol** is also an **intermediate** for the epimerization of an aldose or ketose. The reactions are usually base catalyzed, but can also take place under acid or neutral conditions. A typical rearrangement reaction is that between the aldose glyceraldehyde and the ketose dihydroxyacetone in a chemical equilibrium.

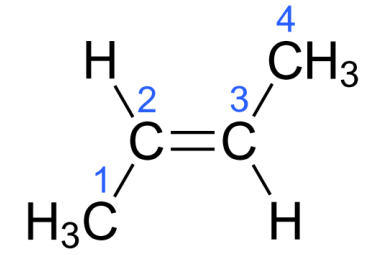
Reaction is also an example of elimination as .. Is eliminated ..

(b) Cis-trans rearrangement

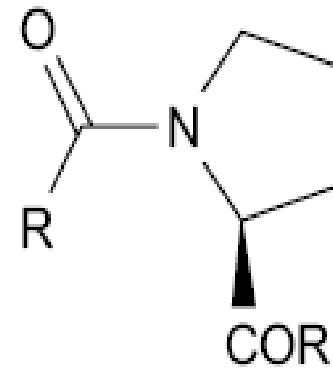
- In general, Peptide bonds formed between amino acid residues with alpha carbons in trans-configuration... (more than 99.95% have **trans configuration**.)
-
- For peptide bonds involving the **imino nitrogen of proline**, peptide bonds are able to exist both in **cis and trans configuration..** (6% are in the **cis configuration**) through peptidyl-prolyl (proline) isomerase enzyme



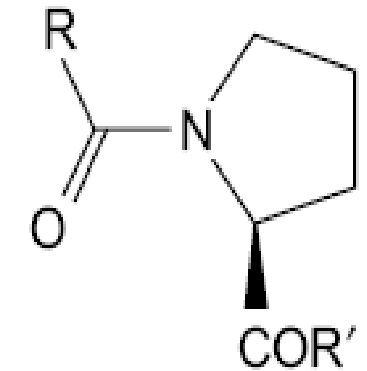
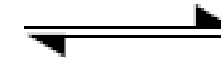
cis-butene



trans-butene



cis



trans

The *cis* and *trans* isomers of proline.
The process of the formation of *trans* and *cis* conformation is via isomerism, and the reaction is a reversible process.

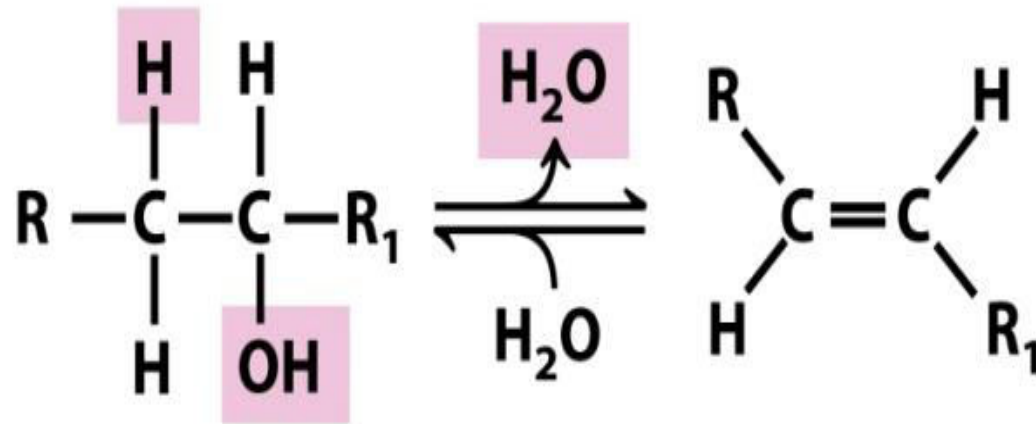
Proline

- **Cis–trans** isomerism, also known as geometric isomerism or configurational isomerism. Cis is illustrated by... The presence of functional groups on the same side of the carbon chain while **trans** conveys that functional groups are on opposing sides of the carbon chain.
- So while considering this kind of isomerism in living system, we can quote the example of proline amino acid. In general, Peptide bonds formed between amino acid residues are in trans-configuration... more than 99.95% have **However, in case of proline, Peptide bonds** formed are able to exist both as **cis and trans isomers..** through peptidyl-prolyl (proline) isomerase enzyme

Prolyl isomerase ..this enzyme is (EC 5.2.1.8) found in both prokaryotes and eukaryotes and interconverts the cis and trans isomers of peptide bonds with the amino acid proline.

(c) Elimination

- The loss of water from an alcohol, resulting in the introduction of a C=C bond b/w 2 adjacent carbon atoms
- An elimination reaction with no changes in oxidation state



Unnumbered 13 p498
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Loss of water from an alcohol → introduction of a C=C bond

3) Free-radical reactions

- Homolytic cleavage of covalent bond → produce free radical
- 1) **Isomerizations** that make use of adenosylcobalamin (vitamin B12) for methylmalonyl-CoA mutase (MCM) reactions
 - 2) Radical-initiated **decarboxylation** reaction
 - 3) Some **reduction** reaction, such as that catalyzed by **ribonucleotide reductase (converts ribonucleotides to deoxyribonucleotides)**
 - 4) Some **rearrangement reactions**, such as that catalyzed by **DNA photolyase**

Coming to free radical reactions.. From where we get these free radicals.. Remember we talked about Homolytic cleavage of covalent bond in which electrons pair is equally distributed and radicals are generated.. So cells have got some free radicals and they use them in certain reactions like/..

- 1) There are certain biological reactions in which Vit.B12 is used as a cofactor by enzymes. Among them, methylmalonyl-CoA mutase (MCM) has been extensively studied. This enzyme catalyzes the reversible isomerization of L-methylmalonyl-CoA to succinyl-CoA using adenosylcobalamin (AdoCbl) as a cofactor participating in the generation of radicals that allow isomerization of the substrate.
- 2) Certain radical-initiated decarboxylation reaction
- 3) Some reduction reaction, such as that catalyzed by ribonucleotide reductase
- 4) Some rearrangement reactions, such as that catalyzed **by DNA photolyase.. That is a DNA** repair enzyme. It binds to UV-damaged **DNA** containing pyrimidine dimers and, breaks the cyclobutane ring joining the two pyrimidines of the dimer and helps to avoid possible mutations.

Vitamin B₁₂ is an organometallic compound with important metabolic derivatives that act as cofactors of certain enzymes, which have been grouped into three subfamilies depending on their cofactors. Among them, methylmalonyl-CoA mutase (MCM) has been extensively studied. This enzyme catalyzes the reversible isomerization of L-methylmalonyl-CoA to succinyl-CoA using adenosylcobalamin (AdoCbl) as a cofactor participating in the generation of radicals that allow isomerization of the substrate. The crystal structure of MCM determined in *Propionibacterium freudenreichii* var. *shermanii* has helped to elucidate the role of this cofactor AdoCbl in the reaction to specify the mechanism by which radicals are generated from the coenzyme and to clarify the interactions between the enzyme, coenzyme, and substrate.

3) Free-radical reactions

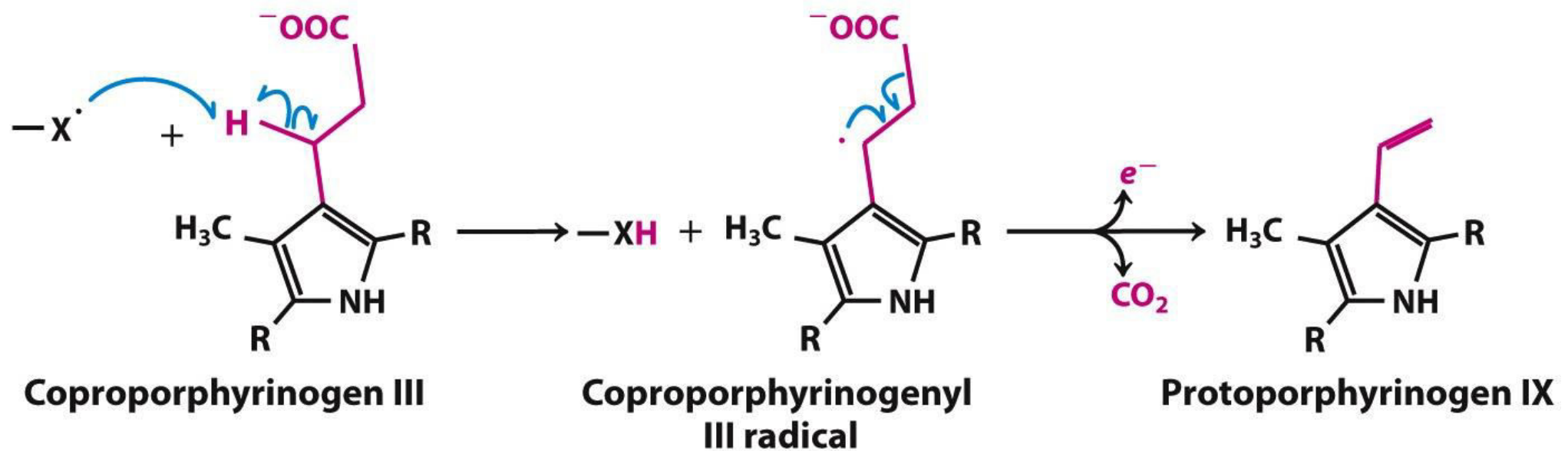


Figure 13-7
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Oxygen-independent **coproporphyrinogen III oxidase** (HemN) catalyzes decarboxylation

Oxygen-independent coproporphysinogen III oxidase catalyzes decarboxylation

In bacteria this process occurs in an oxygen-*independent fashion by the enzyme..coproporphyrinogen-III oxidase catalyzes the oxygen-independent conversion of coproporphyrinogen-III to protoporphyrinogen-IX.*and the step includes decarboxylation through free radical mechanism.. As CO₂ is released along with an electron...The acceptor for the released electron is not known.

BIOENERGETICS

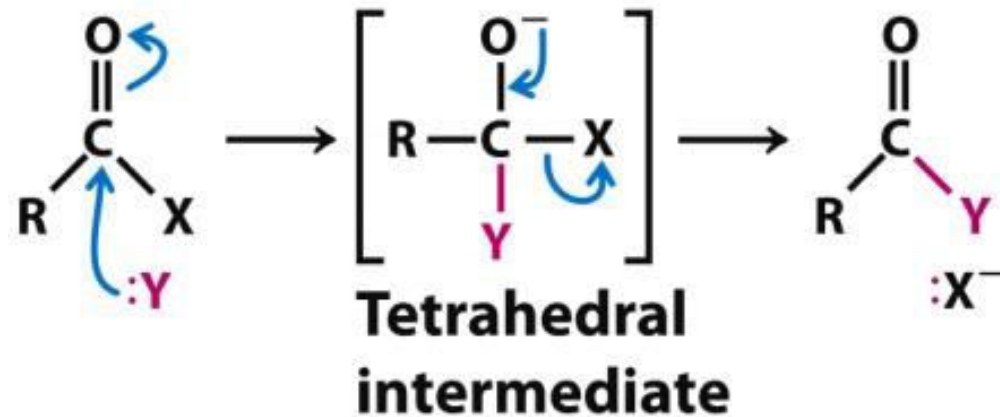
TYPES OF CHEMICAL REACTIONS IN CELLS

...continued

4) Group Transfer Reactions

- The transfer of **acyl**, **glycosyl**, and **phosphoryl** is common in living cells

1) **Acyl transfer**: acyl group transfer generally involves the addition of a nucleophile to the carbonyl carbon of an **acyl group** to form a tetrahedral intermediate, eg. Chymotrypsin reaction



- In living cells, The transfer of **acyl, glycosyl, and phosphoryl groups from one nucleophile to another** is common
- **These include The transfer of certain acyl, glycosyl, and phosphoryl groups from one nucleophile carbonyl carbon of acyl group**
- **to form a tetrahedral intermediate..and in nature, this takes place in Chymotrypsin reaction..** chymotrypsin is a proteolytic digestive enzyme of many organisms. **breaks down proteins in the small intestine. It is secreted by the pancreas and converted into an active form by trypsin.**
- **Glycosyl group transfer is enzyme catalyse reaction..occurring in ... at C1 of sugar ring**
- In organic chemistry, the **acryloyl group is form of enone** with structure $\text{H}_2\text{C}=\text{CH}-\text{C}-$; it is the acyl group derived from acrylic acid. The preferred IUPAC name for the group is prop-2-enoyl, and it is also known as acrylyl or simply acryl

...cont

- A phosphoryl group ($-\text{PO}_3^{2-}$) from ATP is transferred to an alcohol ($\rightarrow$ forming a phosphate ester)
- When nucleophile attacks the electrophilic phosphorus atom in ATP, a relatively stable pentacovalent structure forms as a reaction intermediate.
- **Phosphorous can form five covalent bonds**
- Because oxygen is more electronegative than phosphorus, the sharing of electrons is unequal: the central phosphorus bears a partial positive charge and can therefore act as an electrophile
- **Kinases: enzymes that catalyze phosphoryl group transfer) using ATP as donor)**
- Thioalcohols (thiols): the oxygen atom of an alcohol is replaced with a sulfur atom

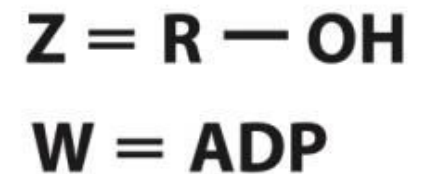


Figure 13-8d
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- Because oxygen is more electronegative than phosphorus, the sharing of electrons is unequal: the central phosphorus bears a partial positive charge and can therefore act as an electrophile
- In many metabolic reactions, A phosphoryl group ($-\text{PO}_3^{2-}$) can be transferred to an alcohol ($\rightarrow$ forming a phosphate ester), or to a carboxylic acid ($\rightarrow$ mixed anhydride)
- When a nucleophile attacks this electrophilic P in ATP, relatively stable pentacoordinate structure forms as a reaction intermediate.
- Reaction completes when ADP is released and so.. The transfer of
- **Kinases: enzymes that catalyze phosphoryl group transfer ..e.g hexokinase transfers Phosphoryl group from ATP to glucose in glycolysis.**
- Thioalcohols (thiols): the oxygen atom of an alcohol is replaced with a sulfur atom

5) Oxidation-reduction reactions

Oxidation

- Addition of oxygen
- **loss** of electrons
- **Loss** of hydrogen

Reduction

- Removal of oxygen
- **Gain** of electrons
- **Gain** of hydrogen

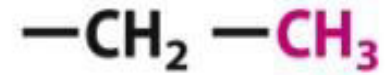
5) Oxidation-reduction reactions

- Carbon **atoms can exist in five oxidation states**
- The transition of these status are of crucial importance in metabolism
- Biological reaction (commonly), a compound loses two electrons and two hydrogen ions: **dehydrogenations (catalyzed by dehydrogenases)**
- In some biological oxidation, a carbon atom becomes covalently bonded to an oxygen atom (a enzyme catalyze these reaction calls **oxidases**)

The oxidation levels of carbon in biomolecules. Each compound is formed by oxidation of the red carbon in the compound

shown immediately above. Carbon dioxide is the most highly oxidized

form of carbon found in living systems.



Alkane



Alcohol



Aldehyde (ketone)



Carboxylic acid



Carbon dioxide

... cont

- Biological reaction (commonly), a compound loses two electrons and two hydrogen ions: dehydrogenations (catalyzed by dehydrogenases)
- In some biological oxidation (but not all), a carbon atom becomes covalently bonded to an oxygen atom (a enzyme catalyze these reaction called **oxidases**)

Oxygenases: if oxygen is derived directly from molecular oxygen (O₂).

Biological reaction (commonly), a compound loses two electrons and two hydrogen ions: dehydrogenations (catalyzed by dehydrogenases)

- In some biological oxidation (but not all), a carbon atom becomes covalently bonded to an oxygen atom (, that is an oxidation reaction catatlysed by the nzyme **oxidases**)

Oxygenases: if oxygen is derived directly from molecular oxygen (O₂).

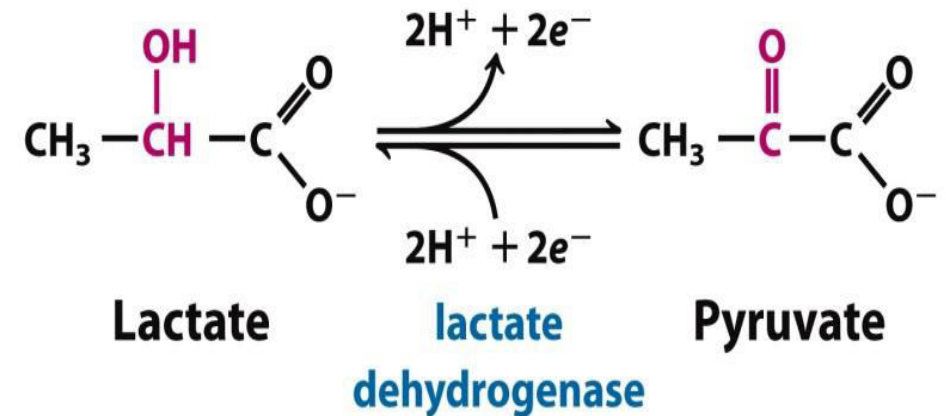
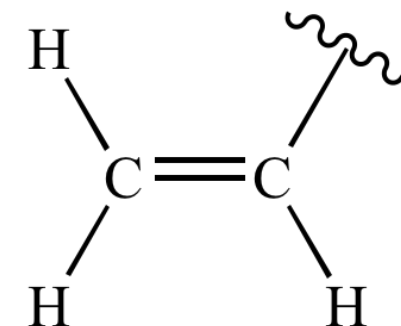


Figure 13-10
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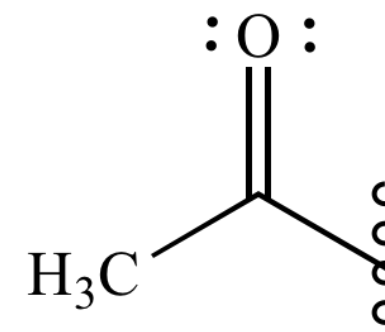
Vinyl group

In chemistry, vinyl or ethenyl is the functional group with the formula $-\text{CH}=\text{CH}_2$. It is the ethylene (IUPAC ethene) molecule ($\text{H}_2\text{C}=\text{CH}_2$) less one hydrogen atom.



Acyl group

It contains a double bonded oxygen atom and an **alkyl group**. In organic chemistry, the **acyl group** (IUPAC name: alkanoyl) is usually derived from a carboxylic acid. **Acyl group is electron withdrawing due to the electronegativity of the oxygen atom.**



Vinyl group

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Acyl group

It contains a double bonded oxygen atom and an **alkyl group**. In organic chemistry, the **acyl group** (IUPAC name: alkanoyl) is usually derived from a carboxylic acid. **Acyl group is electron withdrawing due to the electronegativity of the oxygen atom.**

The name is also used for any compound containing that group, namely $\text{R}-\text{CH}=\text{CH}_2$ where R is any other group of atoms.

An **oxoacid** is an [acid](#) that contains [oxygen](#). Specifically, it is a compound that contains hydrogen, oxygen, and at least one other [element](#), with at least one [hydrogen](#) atom bond to oxygen that can dissociate to produce the H^+ cation and the [anion](#) of the acid

In chemistry, **alkyl** refers to a **group**, a substituent, that is attached to other molecular fragments. For example, **alkyl** lithium reagents have the empirical formula $\text{Li}(\text{alkyl})$, where **alkyl** = methyl, ethyl, etc. A dialkyl ether is an ether with two **alkyl groups**, e.g., diethyl ether ($\text{O}(\text{C}_2\text{H}_5)_2$).

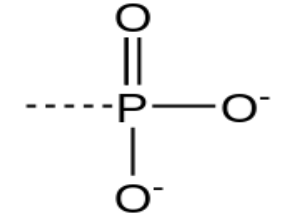
Isopropyl group

Methyl group

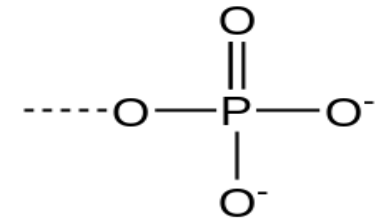
In [organic chemistry](#), an **alkyl substituent** is an [alkane](#) missing one [hydrogen](#)

Phosphoryl group

A *phosphoryl group* is the chemical entity PO_3^{2-} . The term is usually used for the compounds in which the *phosphoryl group* is attached to other atoms, e.g. *phosphoryl* chloride, or in the description of catalytic mechanism.



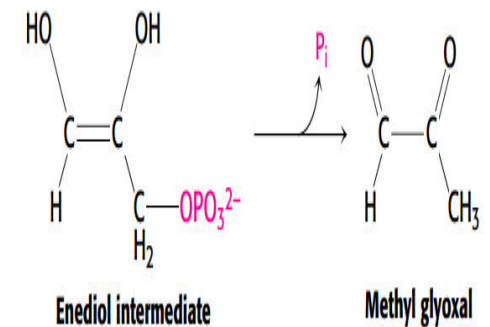
phosphoryl group



phosphate group

Enediol

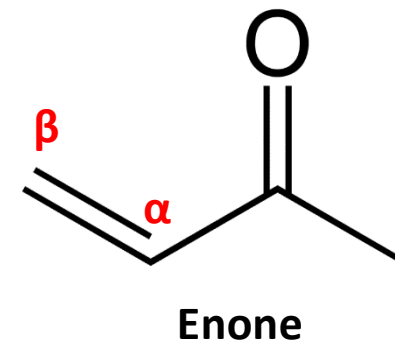
An **alkene enol** with a **hydroxyl group attached to both carbon atoms of the carbon double bond**. A reducing sugar can form an enediol.
organic compound characterized by



A **hemiacetal** or a **hemiketal** is a compound that results from the addition of an [alcohol](#) to an [aldehyde](#) or a [ketone](#), respectively. The Greek word *hèmi*, meaning half, refers to the fact that a single alcohol has been added to the [carbonyl group](#), in contrast to [acetals](#) or [ketals](#), which are formed when a second alkoxy group has been added to the structure.^[1]

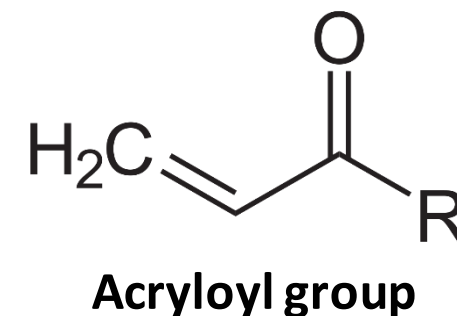
Enone:

An enone, also called an α , β -unsaturated carbonyl, is a type of organic compound consisting of an alkene conjugated to a ketone. The simplest enone is methyl vinyl ketone or $\text{CH}_2=\text{CHCOCH}_3$.



Acryloyl group

In organic chemistry, the **acryloyl group** is form of enone with structure $\text{H}_2\text{C}=\text{CH}-\text{C}(=\text{O})-$. It is the **acyl group derived from acrylic acid**. The preferred IUPAC name for the group is prop-2-enoyl, and it is also known as **acrylyl or simply acryl**.



BIOENERGETICS

Phosphoryl Group Transfers and ATP

- **Energy currency** of living cells
- ATP is used for **Phosphoryl transfer reactions**
- Heterotrophic organisms obtain energy from ATP by breakage of covalent bond, and can undergo one of the two following reactions



What we have studied so far has told us that ATP is the **Energy currency** in biological systems

And how does it do this? .. By undergoing for Phosphoryl Transfer Reactions

So let take a look at the special role of ATP that links catabolism and Anabolism and produces energy for functions of life.

Heterotrophic organisms obtain energy from ATP and this involves the breakage of covalent bond, and can undergo one of the two following reactions

A) $\text{ATP} \rightarrow \text{ADP} + \text{P}_i$ OR

B) $\text{ATP} \rightarrow \text{AMP} + 2 \text{P}_i$

Living cells use that energy to make ATP from ADP and P_i

Living cells obtain free energy in a chemical form by the catabolism of nutrient molecules

They use that energy to make ATP from ADP and P_i .

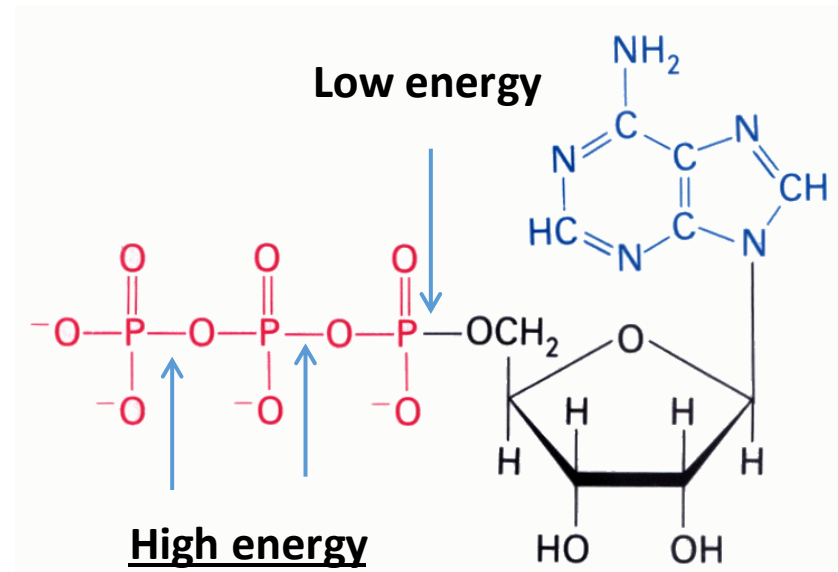
... cont

ATP donates some of its chemical energy to

1. Endergonic processes such as the synthesis of **metabolic intermediates** and macromolecules from smaller precursors
2. The **transport of substances across membranes** against concentration gradients
3. Mechanical motion (**muscle contraction**), etc.
4. Energy donation involves group transfer and hydrolysis of ATP/energy rich phosphate compounds

The Free-Energy Change for ATP Hydrolysis Is Large and Negative

1. The hydrolytic cleavage of the terminal **phosphoanhydride bond** in ATP separates one of the three negatively charged phosphates and thus relieves some of the electrostatic repulsion in ATP
2. The terminal two **phosphoanhydride bonds**, are known as high energy **bonds** because their cleavage releases tremendous amount of free energy
3. Released P_i and ADP are highly soluble



Adenosine Triphosphate

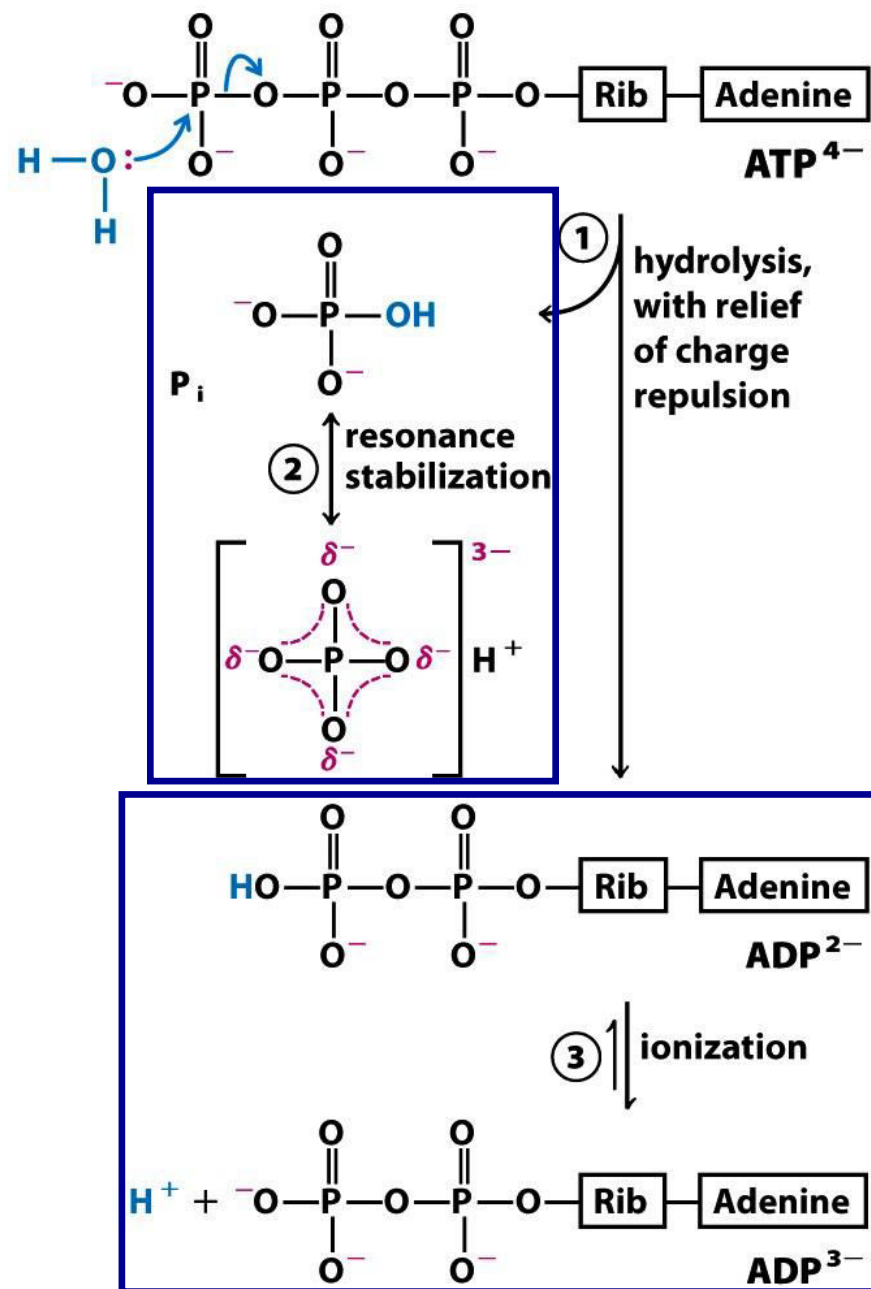
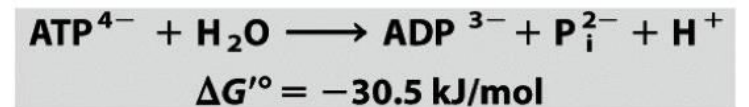
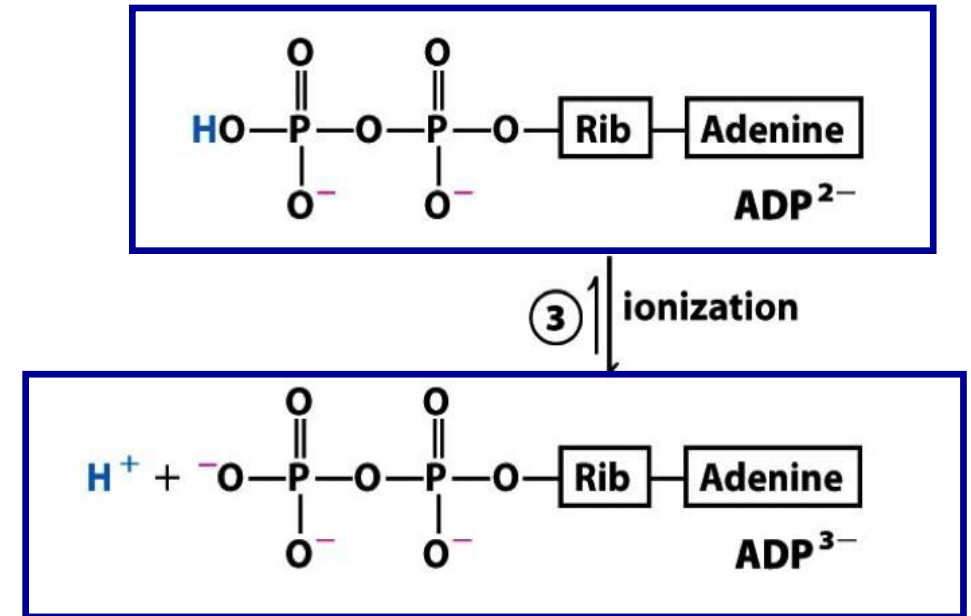


FIGURE 13-11 Chemical basis for the large free-energy change associated with ATP hydrolysis. ① The charge separation that results from hydrolysis relieves electrostatic repulsion among the four negative charges on ATP. ② The product inorganic phosphate (P_i) is stabilized by formation of a resonance hybrid, in which each of the four phosphorus-oxygen bonds has the same degree of double-bond character and the hydrogen ion is not permanently associated with any one of the oxygens. (Some degree of resonance stabilization also occurs in phosphates involved in ester or anhydride linkages, but fewer resonance forms are possible than for P_i .) A third factor (not shown) that favors ATP hydrolysis is the greater degree of solvation (hydration) of the products P_i and ADP relative to ATP, which further stabilizes the products relative to the reactants.

... cont

- ADP^{2-} is the other product of hydrolysis and it immediately ionizes, releasing H^+ into a medium of very low $[\text{H}^+]$ ($\sim 10^{-7} \text{ M}$)
- the hydrolysis of ATP is highly **exergonic** ($\Delta G'^0 = -30.5 \text{ kJ/mol}$), the ATP is stable at pH 7
- Rapid hydrolysis of ATP occurs only when catalyzed by an enzyme



...cont

- Actual free energy of hydrolysis (ΔG) of ATP in living cells is very different because

(1) the cellular concentration of ATP, ADP, and P_i are not identical

(2) ATP, ADP, and P_i concentration are much lower than the standard condition of 1M

(3) Mg^{2+} in the cytosol binds to ATP and ADP

TABLE 13-5	Adenine Nucleotide, Inorganic Phosphate, and Phosphocreatine Concentrations in Some Cells				
	Concentration (mM)*				
	ATP	ADP [†]	AMP	P_i	PCr
Rat hepatocyte	3.38	1.32	0.29	4.8	0
Rat myocyte	8.05	0.93	0.04	8.05	28
Rat neuron	2.59	0.73	0.06	2.72	4.7
Human erythrocyte	2.25	0.25	0.02	1.65	0
<i>E. coli</i> cell	7.90	1.04	0.82	7.9	0

*For erythrocytes the concentrations are those of the cytosol (human erythrocytes lack a nucleus and mitochondria). In the other types of cells the data are for the entire cell contents, although the cytosol and the mitochondria have very different concentrations of ADP. PCr is phosphocreatine, discussed on p. 510.

[†]This value reflects total concentration; the true value for free ADP may be much lower (p. 503).

Table 13-5
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- For ATP as phosphoryl group donor, the true substrate is MgATP^{2-} .

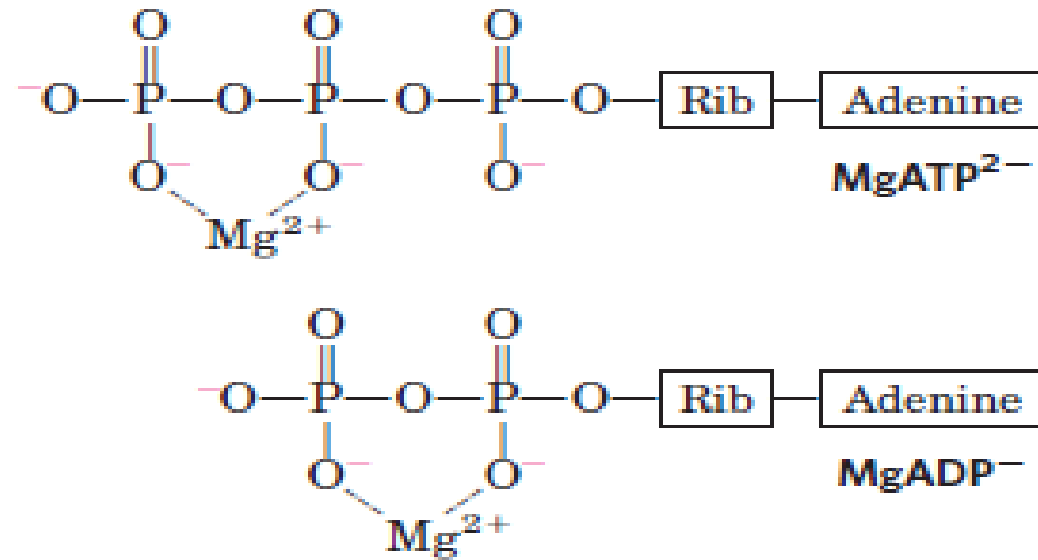


FIGURE 13-12 Mg^{2+} and ATP. Formation of Mg^{2+} complexes partially shields the negative charges and influences the conformation of the phosphate groups in nucleotides such as ATP and ADP.

Phosphorylation potential: The actual free energy of hydrolysis of ATP under intracellular conditions is often called its **phosphorylation potential**, ΔG_p .

WORKED EXAMPLE 13-2 Calculation of ΔG_p

Calculate the actual free energy of hydrolysis of ATP, ΔG_p , in human erythrocytes. The standard free energy of hydrolysis of ATP is -30.5 kJ/mol, and the concentrations of ATP, ADP, and P_i in erythrocytes are as shown in Table 13-5. Assume that the pH is 7.0 and the temperature is 37°C (body temperature). What does this reveal about the amount of energy required to *synthesize* ATP under the same cellular conditions?

Solution: The concentrations of ATP, ADP, and P_i in human erythrocytes are 2.25, 0.25, and 1.65 mM, respectively. The actual free energy of hydrolysis of ATP under these conditions is given by the relationship (see Eqn 13-4)

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



Substituting the appropriate values we get

$$\begin{aligned}\Delta G_p &= -30.5 \text{ kJ/mol} + \left[(8.315 \text{ J/mol} \cdot \text{K})(310 \text{ K}) \ln \frac{(0.25 \times 10^{-3})(1.65 \times 10^{-3})}{(2.25 \times 10^{-3})} \right] \\ &= -30.5 \text{ kJ/mol} + (2.58 \text{ kJ/mol}) \ln 1.8 \times 10^{-4} \\ &= -30.5 \text{ kJ/mol} + (2.58 \text{ kJ/mol})(-8.6) \\ &= -30.5 \text{ kJ/mol} - 22 \text{ kJ/mol} \\ &= -52 \text{ kJ/mol}\end{aligned}$$

(Note that the final answer has been rounded to the correct number of significant figures (52.5 rounded to 52), following rules for rounding a number that ends in a 5 to the nearest even number.) Thus ΔG_p , the actual free-energy change for ATP hydrolysis in the intact erythrocyte (-52 kJ/mol), is much larger than the standard free-energy change (-30.5 kJ/mol). By the same token, the free energy required to *synthesize* ATP from ADP and P_i under the conditions prevailing in the erythrocyte would be 52 kJ/mol.

Phosphorylated compounds

- Phosphoenolpyruvate
- 1,3-bisphosphoglycerate
- Phosphocreatine
- ADP
- ATP
- AMP
- PPi
- Glucose 1-phosphate
- Fructose 6-phosphate
- Glucose 6-phosphate

Large Free Energies of Hydrolysis Phosphorylated Compounds and Thioesters

- **Other compounds that have large free energy of Hydrolysis**

1. Phosphorylated compounds
2. Thioesters (Acetyl-CoA)

Orbital overlap between the O and C atoms allows resonance stabilization in oxygen esters; orbital overlap between S and C atoms is poorer and provides little resonance stabilization.

END

BIOENERGETICS

Phosphoryl Group Transfers and ATP

...continued

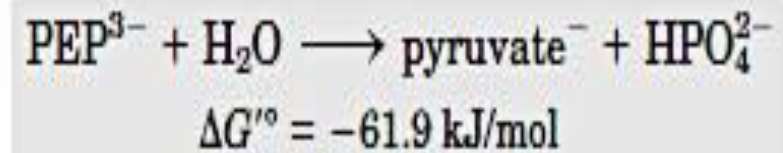
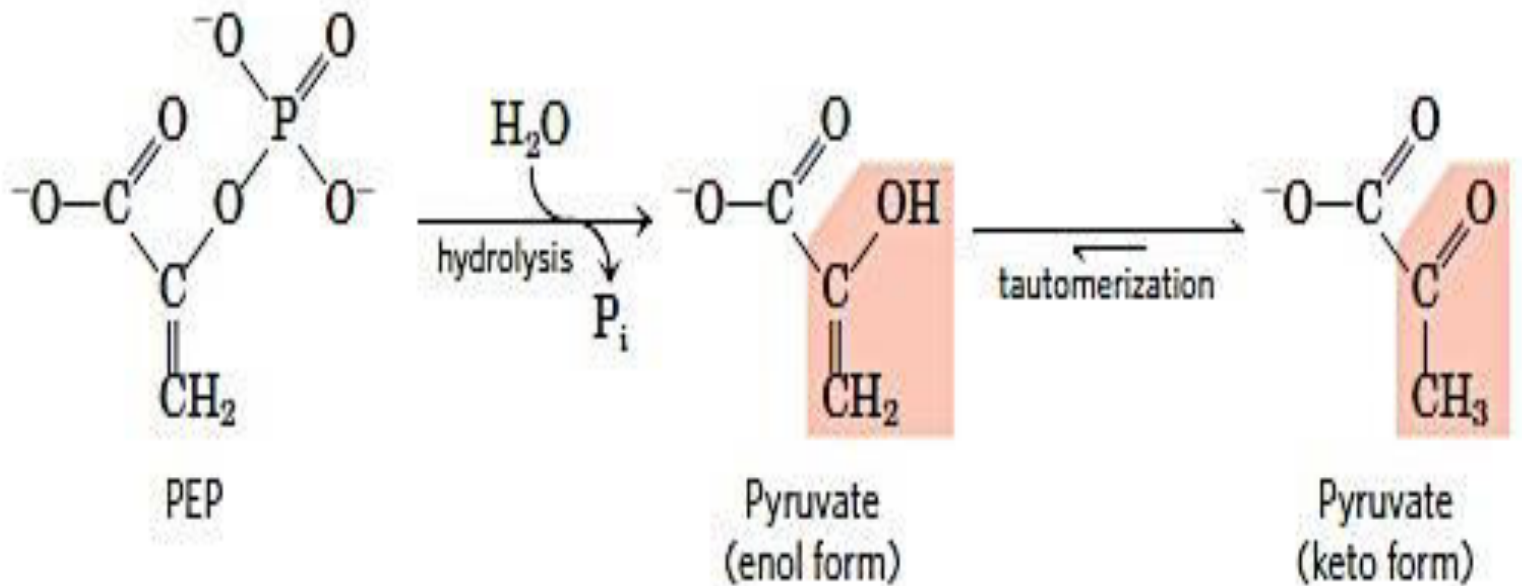
Other Phosphorylated Compounds Have Large Free Energies of Hydrolysis

- Other compounds that have large free energy change
 1. Phosphorylated compounds
 2. Thioesters (Acetyl-CoA)

Orbital overlap between the O and C atoms allows resonance stabilization in oxygen esters; orbital overlap between S and C atoms is poorer and provides little resonance stabilization. The *products* of both types of hydrolysis reaction have about the same free-energy content (G), but the **thioester** has a higher free-energy content than the oxygen ester.

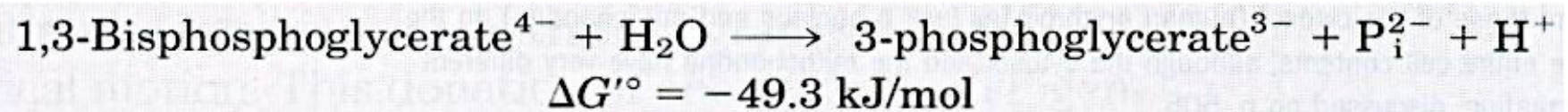
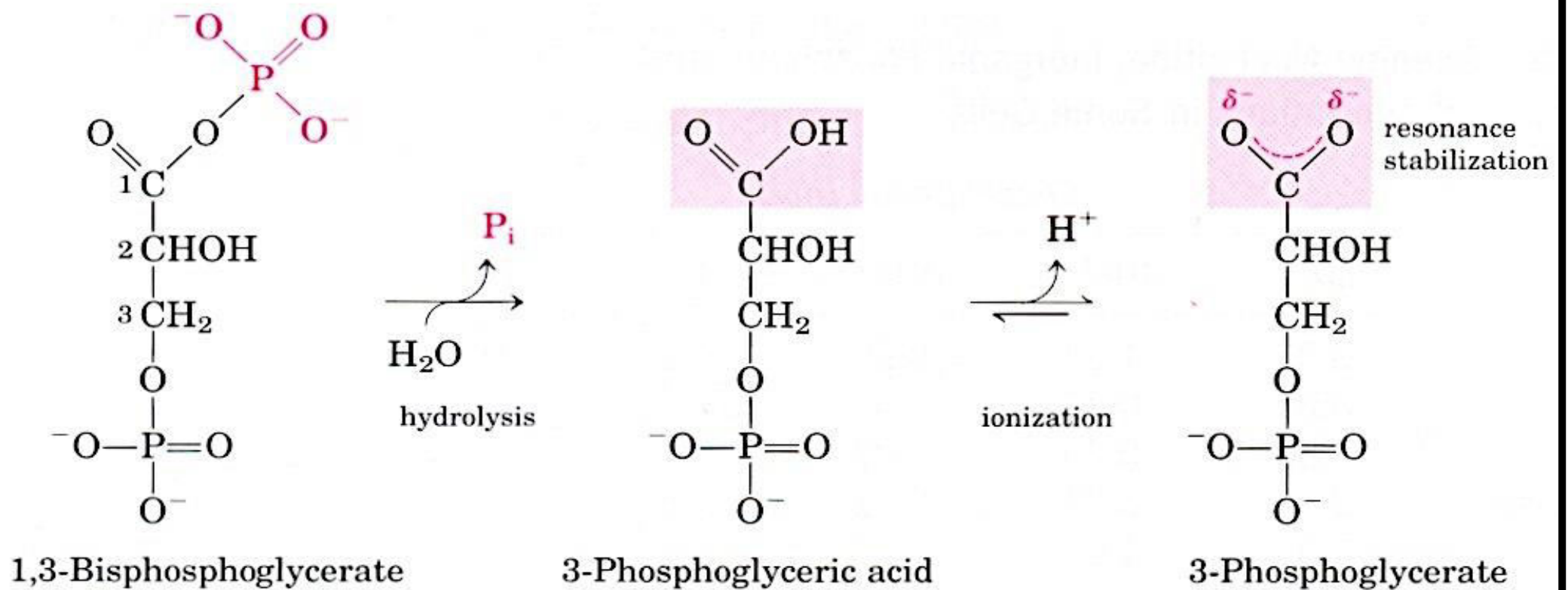
Phosphoenolpyruvate (PEP)

FIGURE 13-13 Hydrolysis of phosphoenolpyruvate (PEP). Catalyzed by pyruvate kinase, this reaction is followed by spontaneous tautomerization of the product, pyruvate. Tautomerization is not possible in PEP, and thus the products of hydrolysis are stabilized relative to the reactants. Resonance stabilization of P_i also occurs, as shown in Figure 13-11.



1,3-bisphosphoglycerate

- 1,3-bisphosphoglycerate contains an **anhydride bond** between the carboxyl group at C-1 and phosphoric acid.
- Hydrolysis of this **acyl phosphate** is accompanied by a large, negative, standard free energy change ($\Delta G'^0 = -49,3 \text{ kJ/mol}$)
- This large, negative $\Delta G'^0$ can, again, be explained in terms of the structure of reactants and products
- Addition of water to anhydride bond of 1,3-**bisphosphoglycerate**, **one of the direct products, 3-phosphoglyceric acid**, immediately leads to the lose a proton to give the **carboxylate ion**, 3-phosphoglycerate, which has two equally probable resonance forms
- Removal of a direct product, **3-phospho-glyceric acid**, and formation of resonance-stabilized ion favor the forward reaction.



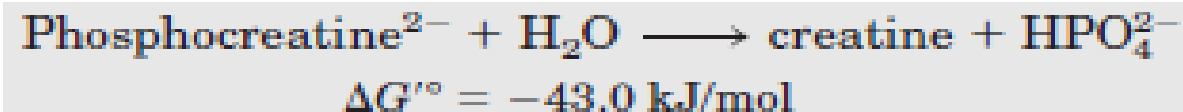
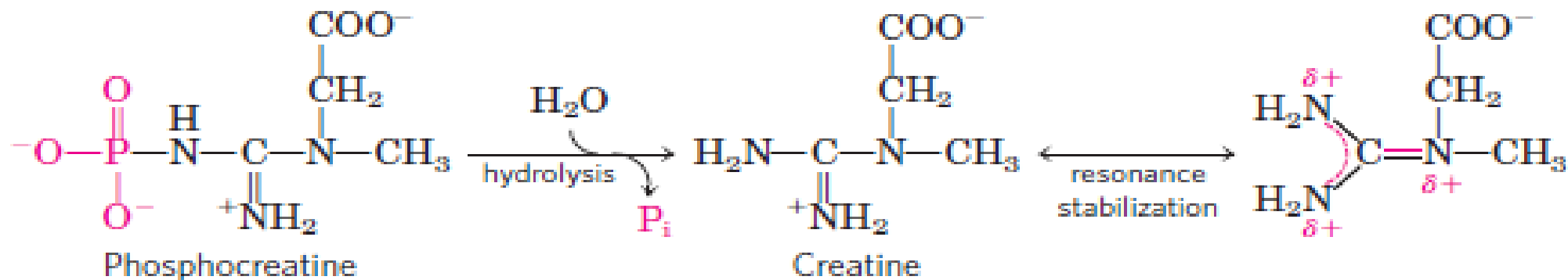
Hydrolysis of 1,3-bisphosphoglycerate.

The direct product of hydrolysis is 3-phosphoglyceric acid, with an undissociated carboxylic acid. Its dissociation allows **resonance structures** that stabilize the product relative to the reactants.

Resonance stabilization of P_i further contributes to the negative free-energy change.

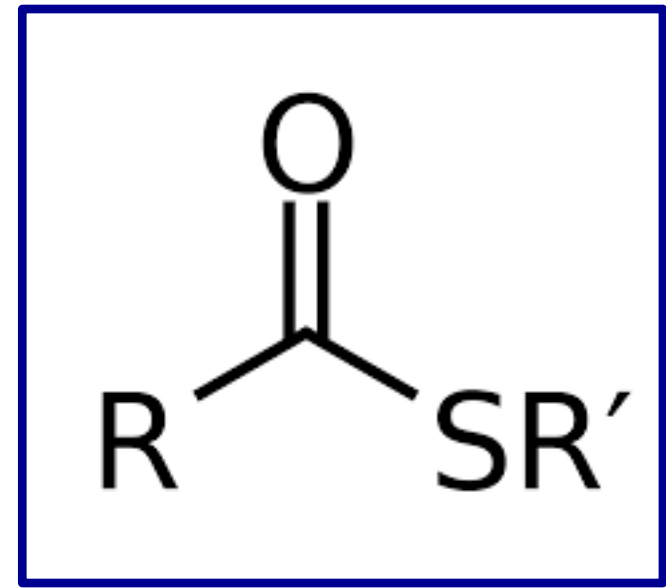
Phosphocreatine (Pcr)

- In the **phosphocreatine**, the P-N bond can be hydrolyzed to generate free **creatine** and P_i .
- The release of P_i and the resonance stabilization of creatine favor the forward reaction.
- P_i is also resonance stabilized.
- The standard free energy change of phosphocreatine is large and negative ($\Delta G'^0 = -43 \text{ kJ/mol}$).



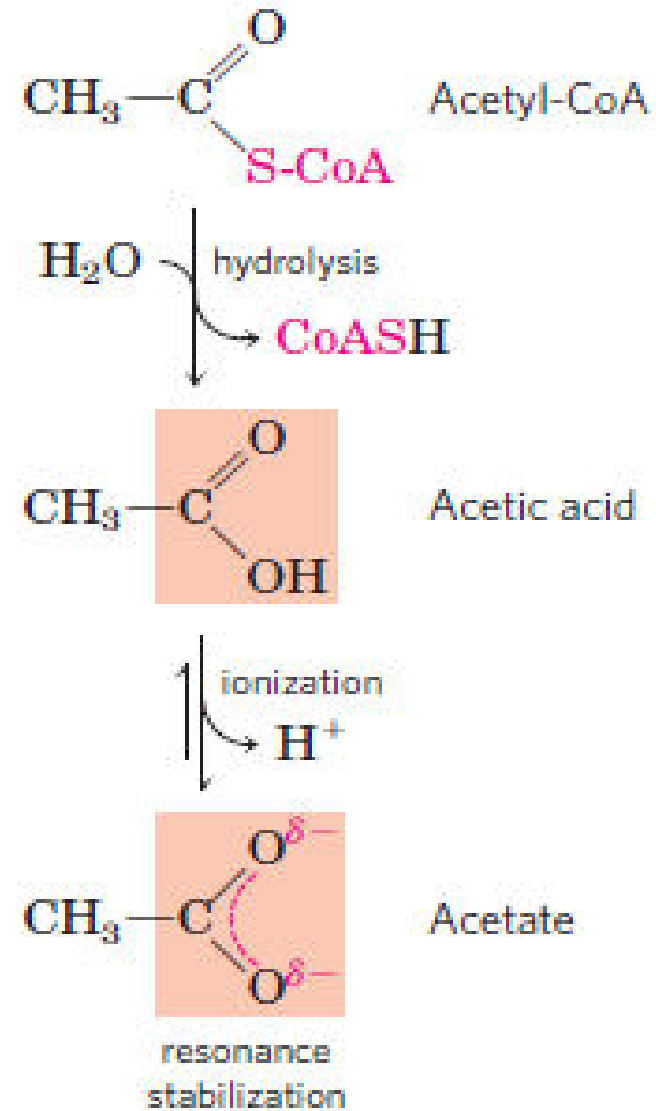
Thioesters

- Thioesters **contain a sulfur** atom in the position occupied by an oxygen
- Thioesters have large, negative standard free energy change of hydrolysis.
- **Hydrolysis of acetyl-coenzyme A**.. important in metabolism and has a large, negative, standard free energy of hydrolysis.
- **The acyl group in these compounds is activated for trans-acylation**, condensation or oxidation-reduction reactions.
- Hydrolysis of the **ester bond generates a carboxylic acid**
- $\Delta G'^0 = -31,4 \text{ kJ/mol}$ for acetyl-CoA hydrolysis
- **Acetyl coenzyme A is one of many thioesters**



Hydrolysis of acetyl-coenzyme A.

Acetyl-CoA is a thioester with a large, negative, standard free energy of hydrolysis.



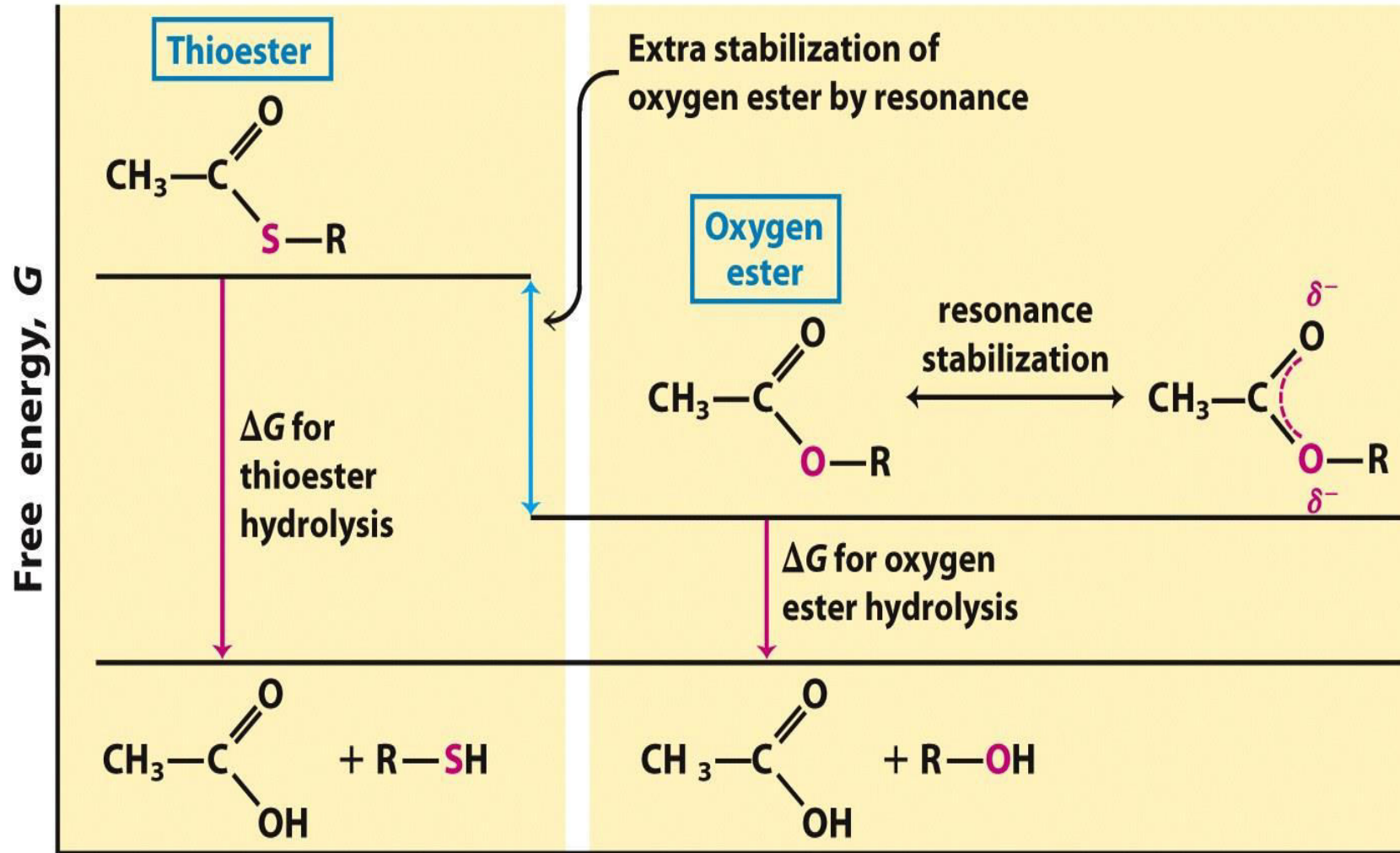


Figure 13-17
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Free energy released by hydrolysis of THIOESTERS is more than OXYGEN esters

Summary for hydrolysis reactions

For hydrolysis reactions with large, negative standard free energy changes, the products are more stable than the reactants for one or more of the following reasons:

1. The bond strain in reactants due to electrostatic repulsion is relieved by charge separation, as for ATP
2. The products are stabilized by **ionization**, as for ATP, acyl phosphates, thioesters
3. The products are stabilized by isomerization (tautomerization) as for phosphoenolpyruvate
4. The products are stabilized by **resonance** as for creatine released from phosphocreatine, **carboxylate ion released from acyl phosphates** and thioesters and phosphate released from **anhydride or ester linkages**

- The phosphate compounds found in living organisms can be arbitrarily divided into two groups based on their standard free energy changes of hydrolysis.
 1. 'High-energy' compounds have a $\Delta G'^0$ of hydrolysis more negative than -25 kJ/mol
 2. 'low-energy' compounds have a less negative $\Delta G'^0$
- ATP with a $\Delta G'^0$ of hydrolysis of -30 kJ/mol is a high-energy compound
- glucose 6-phosphate is a low-energy compound ($\Delta G'^0 = -13,8$ kJ/mol)

TABLE 13–6**Standard Free Energies of Hydrolysis of Some Phosphorylated Compounds and Acetyl-CoA (a Thioester)**

	$\Delta G'^{\circ}$	
	(kJ/mol)	(kcal/mol)
Phosphoenolpyruvate	−61.9	−14.8
1,3-bisphosphoglycerate ($\rightarrow$ 3-phosphoglycerate + P_i)	−49.3	−11.8
Phosphocreatine	−43.0	−10.3
ADP ($\rightarrow$ AMP + P_i)	−32.8	−7.8
ATP ($\rightarrow$ ADP + P_i)	−30.5	−7.3
ATP ($\rightarrow$ AMP + PP_i)	−45.6	−10.9
AMP ($\rightarrow$ adenosine + P_i)	−14.2	−3.4
PP_i ($\rightarrow$ 2P_i)	−19.2	−4.0
Glucose 3-phosphate	−20.9	−5.0
Fructose 6-phosphate	−15.9	−3.8
Glucose 6-phosphate	−13.8	−3.3
Glycerol 3-phosphate	−9.2	−2.2
Acetyl-CoA	−31.4	−7.5

Source: Data mostly from Jencks, W.P. (1976) in *Handbook of Biochemistry and Molecular Biology*, 3rd edn (Fasman, G.D., ed.), *Physical and Chemical Data*, Vol. 1, pp. 296–304, CRC Press, Boca Raton, FL. The value for the free energy of hydrolysis of PP_i is from Frey, P.A. & Arabshahi, A. (1995) Standard free-energy change for the hydrolysis of the α - β -phosphoanhydride bridge in ATP. *Biochemistry* **34**, 11,307–11,310.

Table 13-6

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BIOENERGETICS

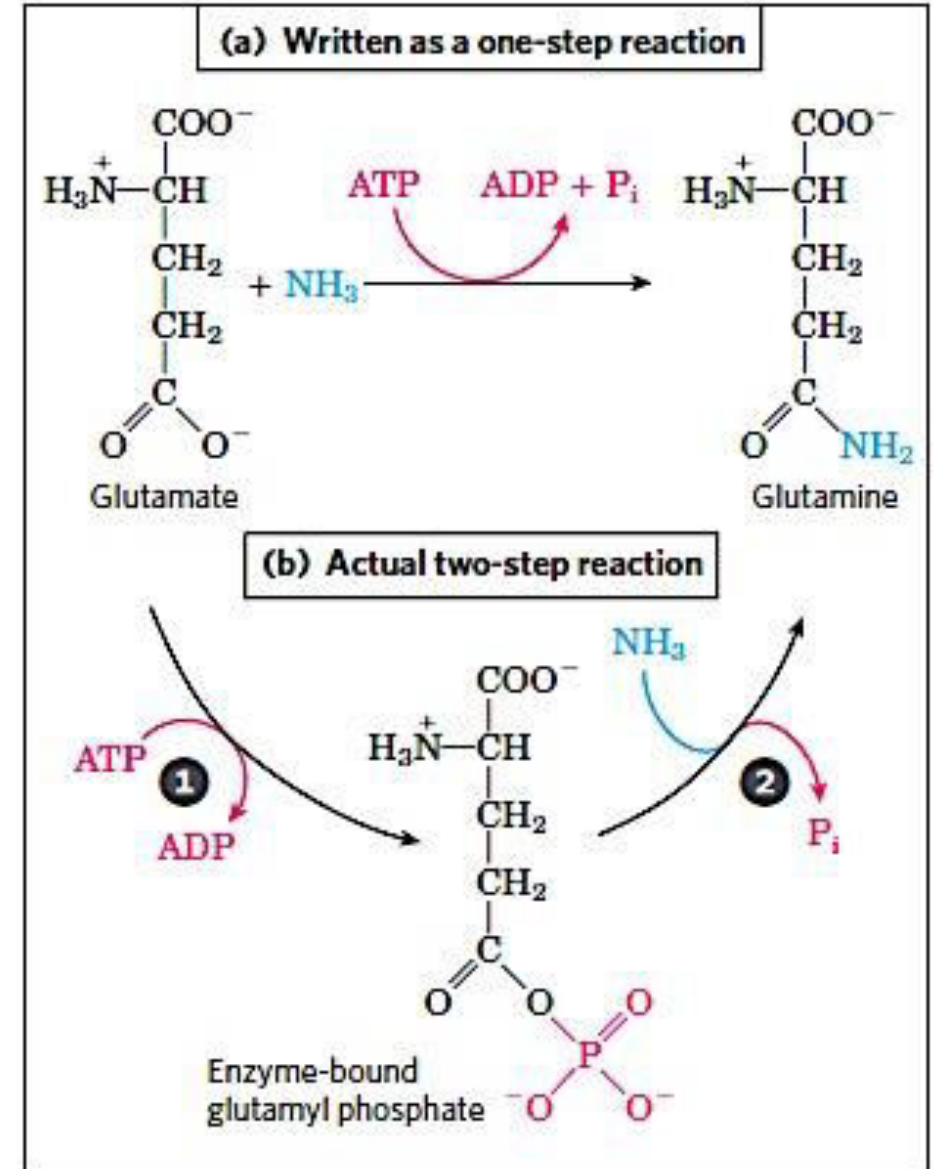
ATP Provides Energy by Group Transfers, Not by Simple Hydrolysis

ATP serves as the universal energy currency in all living cells
ATP can carry energy from high-energy phosphate compounds produced by catabolism to compounds such as glucose, converting them into more reactive species.

ATP Provides Energy by Group Transfers, Not by Simple Hydrolysis

ATP hydrolysis in two steps.

- The contribution of ATP to a reaction is often shown as a single step, but is almost always a two-step process.
 - Shown here is the reaction catalyzed by ATP-dependent **glutamine synthetase**.
-
- A phosphoryl group is transferred from ATP to glutamate
 - the phosphoryl group is displaced by NH_3 and released as P_i .



Hydrolysis accompanied biological processes

1. Noncovalent binding of ATP (or GTP), followed by its hydrolysis to ADP (or GDP) and P_i , (to cycle some proteins between two conformations), in muscle contraction
2. In the movement of enzymes along DNA
3. In the movement of ribosomes along messenger RNA
4. The energy-dependent reactions catalyzed by helicases and some topoisomerases
GTP-binding proteins that act in signaling pathways to drive conformational changes

- The phosphate compounds found in living organisms can be divided into two groups based on their standard free energy changes of hydrolysis.
 1. 'High-energy' compounds have a $\Delta G'^0$ of hydrolysis more negative than -25 kJ/mol
 2. 'low-energy' compounds have a less negative $\Delta G'^0$
- ATP with a $\Delta G'^0$ of hydrolysis of -30 kJ/mol is a high-energy compound
- glucose 6-phosphate is a low-energy compound ($\Delta G'^0 = -13,8$ kJ/mol)
- The term “high-energy phosphate bond,” used to describe the P-O bond broken in hydrolysis reactions, is incorrect and misleading as it wrongly suggests that the bond itself contains the energy.
- the breaking of all chemical bonds requires an *input* of energy

- The additivity of free energy changes of sequential reactions
- any phosphorylated compound can be synthesized by coupling the synthesis to the breakdown of another phosphorylated compound with a more negative standard free energy change of hydrolysis.

• **Example:** $\Delta G'^0$

• $\text{PEP} + \text{H}_2\text{O} \rightarrow \text{Pyruvate} + \text{P}_i$ -61,9

• $\text{ADP} + \text{P}_i \rightarrow \text{ATP} + \text{H}_2\text{O}$ +30,5

• $\text{PEP} + \text{ADP} \rightarrow \text{Pyruvate} + \text{ATP}$ -31,4

- **Cleavage of P_i from PEP releases more energy than is needed to drive the condensation of P_i with ADP, the direct donation of a phosphoryl group from PEP to ADP is thermodynamically feasible**

As is evident from the additivity of free energy changes of sequential reactions, any phosphorylated compound can be synthesized by coupling the synthesis to the breakdown of another phosphorylated compound with a more negative standard free energy change of hydrolysis.

...cont

- Notice that while the energy of overall reaction is represented as the algebraic sum of first two reactions, the overall reaction (third) does not involve P_i ; **PEP donates a phosphoryl group directly to ADP.**
- On the basis of their standard free energy changes of hydrolysis, phosphorylated compounds as having a high or low **phosphoryl group transfer potential.**
- The transfer of a phosphoryl group to a compound effectively puts free energy into that compound that can be used in subsequent metabolic transformations.

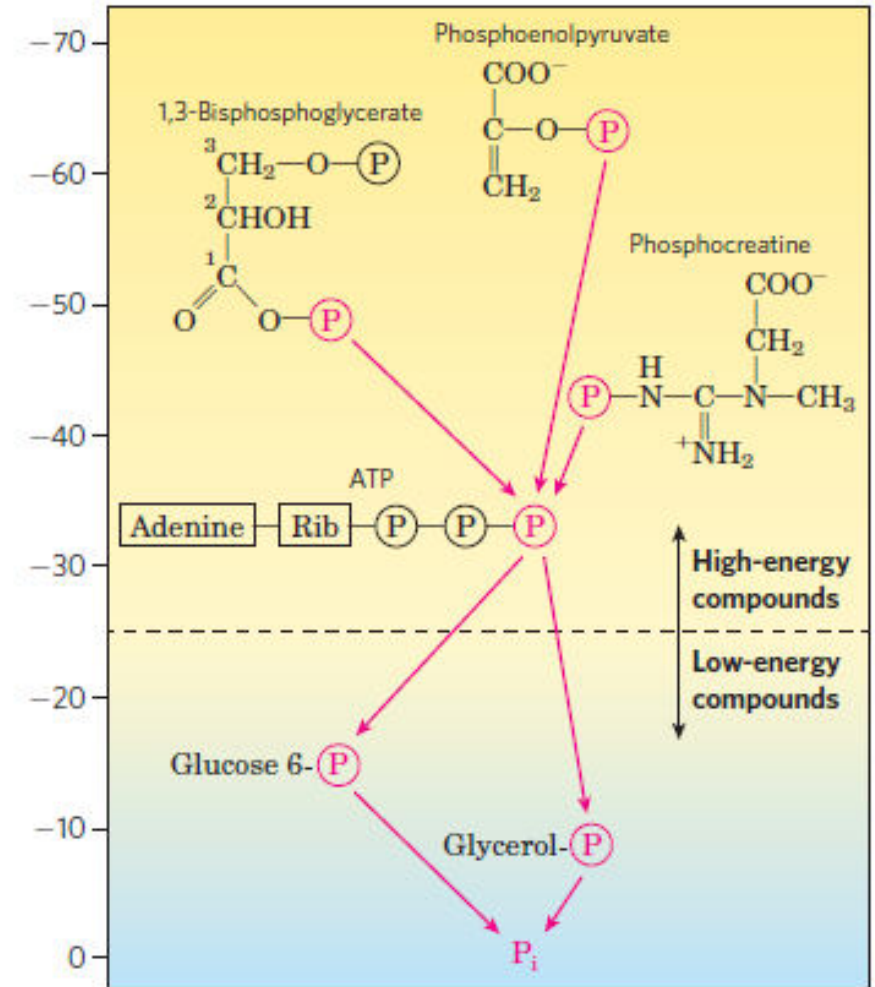
Much of catabolism is directed toward the synthesis of high-energy phosphate compounds, but their formation is not an end in itself; they are the means of activating a wide variety of compounds for further chemical transformation.

BIOENERGETICS

**ATP Provides Energy by Group Transfers,
Not by Simple Hydrolysis.. cont**

Ranking of biological phosphate compounds by standard free energies of hydrolysis

- flow of phosphoryl groups, **represented by P**, from **high-energy phosphoryl group donors via ATP to acceptor molecules** (such as glucose and glycerol) to form their low-energy phosphate derivatives.
- This flow of phosphoryl groups is catalyzed by **kinases**, and proceeds with an overall loss of free energy under intracellular conditions.



(The location of each compound's donor phosphoryl group along the scale approximately indicates the $\Delta G'^{\circ}$ of hydrolysis.)

...cont

- **in aqueous solution ATP is thermodynamically unstable and is therefore a good phosphoryl group donor, it is kinetically stable.**
- Because of high activation energies required for uncatalyzed reaction ATP does not spontaneously donate phosphoryl groups to water or to the other potential acceptors in the cell.
- **ATP hydrolysis occurs only when specific enzymes which lower the energy of activation are present**
- **thermodynamically unstable:** when energy level of products is lower than that of reactants
- kinetically stable is when reaction has high activation energy due to some strong bonds.

One more chemical feature of ATP is crucial to its role in metabolism: **although in aqueous solution ATP is thermo-dynamically unstable and is therefore a good phosphoryl group donor, it is kinetically stable.**

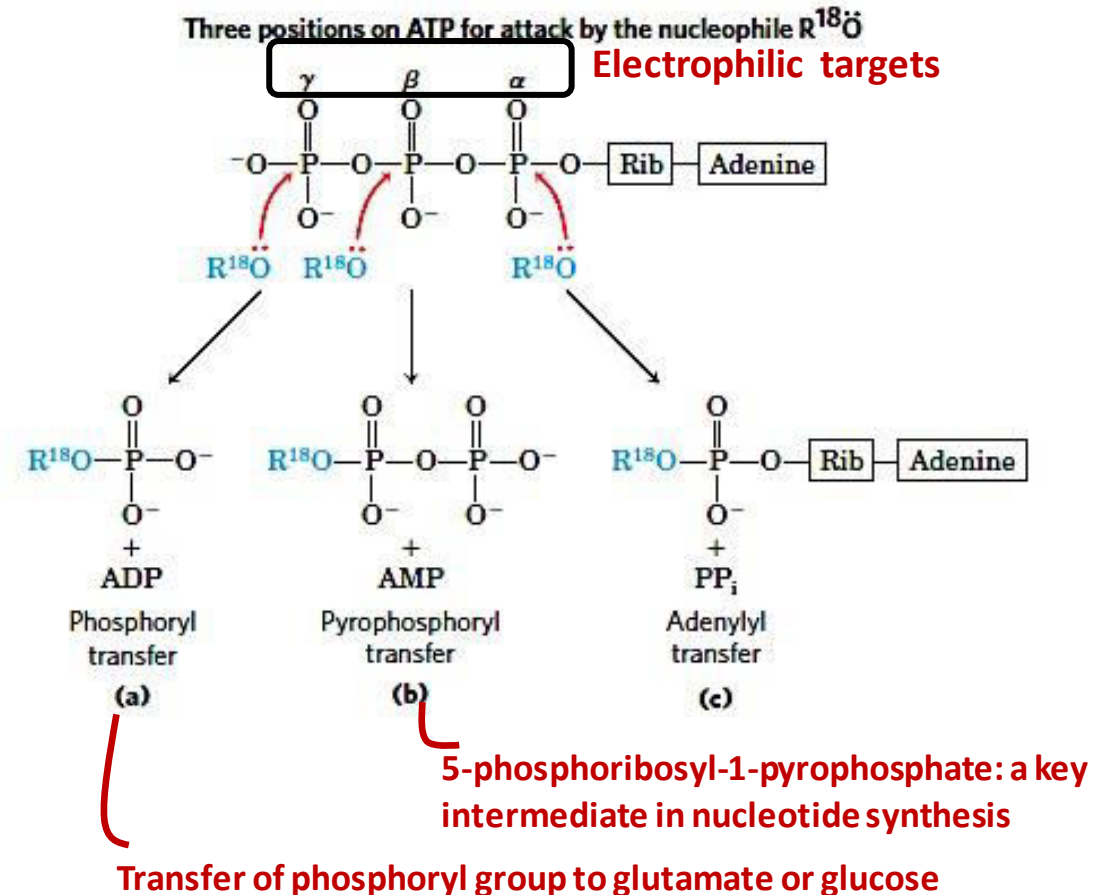
Because of high activation energies required for uncatalyzed reaction ATP does not spontaneously donate phosphoryl groups to water or to the other potential acceptors in the cell.

ATP hydrolysis occurs only when specific enzymes which lower the energy of activation are present

ATP Donates Phosphoryl, Pyrophosphoryl, and Adenylyl Groups

Nucleophilic displacement reactions of ATP.

- Any of the three P atoms (α , β , or γ) may serve as the electrophilic target for nucleophilic attack.
- By the labeled nucleophile $R-^{18}O$: The nucleophile may be an alcohol (ROH), a carboxyl group ($RCOO^-$), or a phosphoanhydride (a nucleoside mono- or diphosphate, for example).
- When the oxygen of the nucleophile attacks the position, the bridge oxygen of the product is labeled, indicating that the group transferred from ATP is a phosphoryl ($2PO_3^{-2}$), not a phosphate (OPO_3^{-2}).



Each of the three phosphates of ATP is susceptible to nucleophilic attack and each position of attack yields a different type of product

Any of the three P atoms (α , β , or γ) may serve as the electrophilic target for nucleophilic attack. In this case, by the labeled nucleophile $R\text{-}^{18}\text{O}$: The nucleophile may be an alcohol (ROH), a carboxyl group (RCOO^-), or a phosphoanhydride (a nucleoside mono- or diphosphate, for example).

When the oxygen of the nucleophile attacks the γ position, the bridge oxygen of the product is labeled, indicating that the group transferred from ATP is a phosphoryl (2PO_3^{-2}), not a phosphate (OPO_3^{-2})

Attack on the β position displaces AMP and leads to the transfer of a pyrophosphoryl (not pyrophosphate) group to the nucleophile.

Attack on the α position displaces PPi and transfers the adenylyl group to the nucleophile.

...cont

- Attack on the α position displaces AMP and leads to the transfer of a pyrophosphoryl (not pyrophosphate) group to the nucleophile.
- Attack on the β position displaces PPi and transfers the adenylyl group to the nucleophile.
- **5-phosphoribosyl-1-pyrophosphate** is a key intermediate in nucleotide synthesis
 1. Hydrolysis of α - β phosphoanhydride bond: ~ 46 kJ/mol
 2. Hydrolysis of β - γ phosphoanhydride bond: ~ 31 kJ/mol
 3. $\text{PPi} \rightarrow 2 \text{Pi}$: -19.2 kJ/mol: total = -64.8 kJ/mol (**Adenylation: produces PPi hydrolysed to 2Pi by inorganic pyrophosphatase enzyme**)

Activation of fatty acids

- a) Energy yielding oxidation
- b) Synthesis of more complex lipids (thiol esters)

1. First, adenylate (AMP) is transferred from ATP to the carboxyl group of the fatty acid, forming a mixed anhydride (fatty acyl adenylate) and liberating P_{Pi}.
2. The thiol group of coenzyme A then displaces the adenylyl group and forms a thioester with the fatty acid.
3. sum of these two reactions is energetically equivalent to the exergonic hydrolysis of ATP to AMP and P_{Pi}
4. The formation of fatty acyl–CoA is endergonic but made energetically favorable (exergonic) by hydrolysis of the P_{Pi} by inorganic pyrophosphates.
5. Thus, in the activation of a fatty acid, both phosphoanhydride bonds of ATP are broken.

The first step in the activation of a fatty acid— either for energy-yielding oxidation or for use in the synthesis of more complex lipids—is the formation of its **thiol ester**.

The direct condensation of a fatty acid with coenzyme A is endergonic, but the formation of fatty acyl–CoA is exergonic by stepwise removal of two phosphoryl groups from ATP.

Luciferin

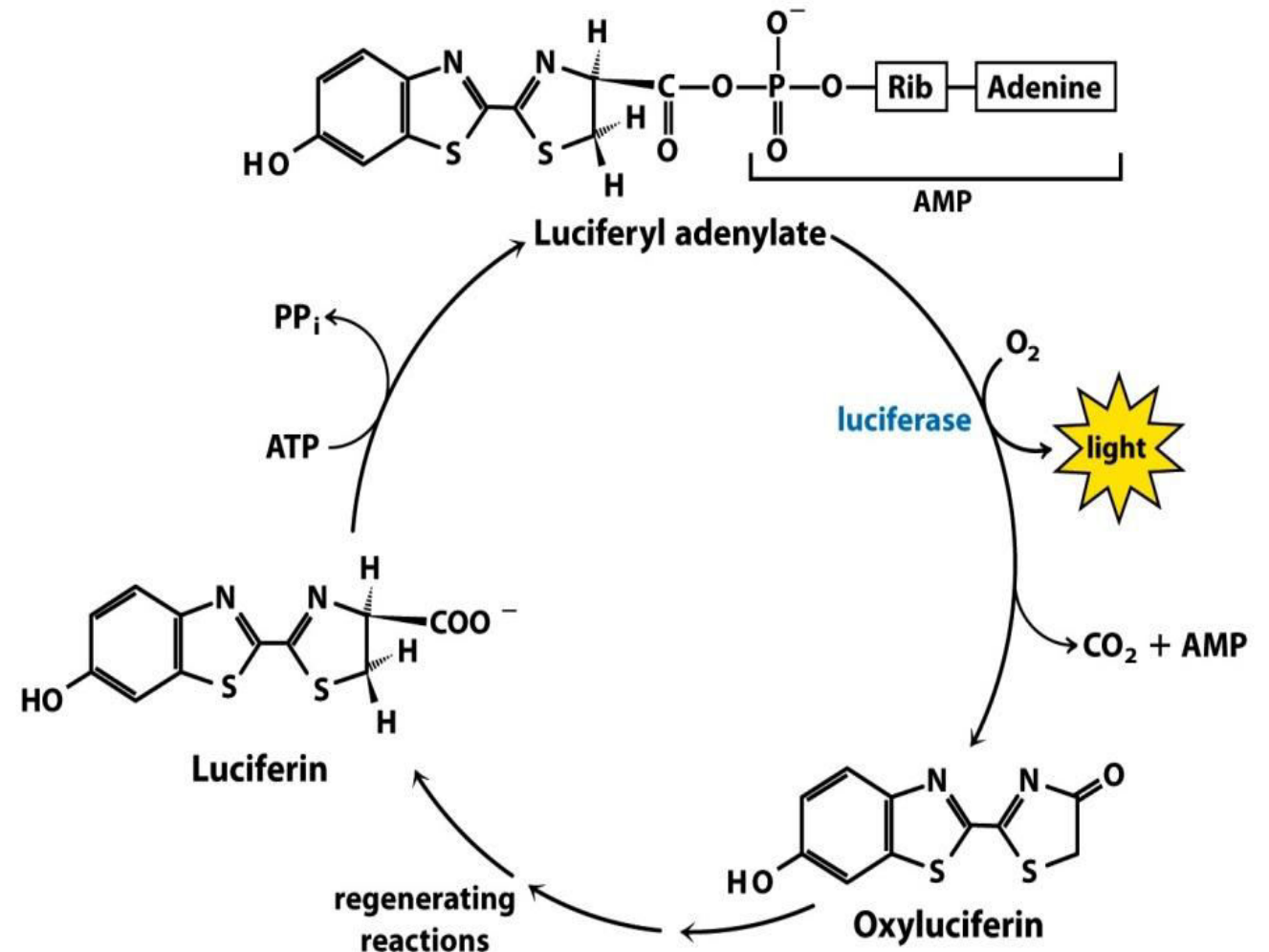
- William McElroy and his colleagues at the Johns Hopkins University isolated the principal biochemical components: **luciferin, a complex carboxylic acid**, and luciferase, an enzyme.
- The generation of a light flash requires activation of luciferin by an enzymatic reaction involving **pyrophosphate cleavage of ATP** to form **luciferyl adenylate**.
- In **the presence of molecular oxygen and luciferase**, the luciferin undergoes a multistep **oxidative decarboxylation** to **oxyluciferin**.
- This process is accompanied by emission of light.
- **Luciferin is regenerated from oxyluciferin** in a subsequent series of reactions.



Box 13-1 Figure 1a
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Firefly

- In the laboratory, pure firefly luciferin and luciferase are used to measure minute quantities of ATP by the intensity of the light flash produced. As little as a few picomoles (10^{-12} pico mol) of ATP can be measured in this way.
- **Next-gen pyrosequencing** of DNA relies on flashes of light from the luciferin-luciferase reaction to detect the presence of ATP after addition of nucleotides to a growing strand of DNA



Box 13-1 figure 1b
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Assembly of Informational Macromolecules Requires Energy

- **Pi and Ppi groups from ATP**
- The precursors for DNA and RNA synthesis are nucleoside triphosphates
- Polymerization of DNA and RNA is accompanied by cleavage of the **phosphoanhydride linkage** between α and β phosphates, with the release of **PPi**
- The moieties transferred to the growing polymer in these reactions are **adenylate (AMP), guanylate (GMP), cytidylate (CMP), or uridylate (UMP)** for RNA synthesis, and their deoxy analogs (with TMP in place of UMP) for DNA synthesis.

Adenylyl groups from ATP

- required for the **activation of amino acids for protein synthesis**, several steps on the ribosome are also accompanied by **GTP hydrolysis**.

Next heading under this topic.. The precursors for DNA and RNA synthesis are nucleoside triphosphates, and polymerization is accompanied by cleavage of the phosphoanhydride linkage between α and β phosphates, with the release of PPi (Fig. 13–20).

The moieties transferred to the growing polymer in these reactions are adenylate (AMP), guanylate (GMP), cytidylate (CMP), or uridylate (UMP) for RNA synthesis, and their deoxy analogs (with TMP in place of UMP) for DNA synthesis.

BIOENERGETICS

ATP energizes active transport and muscle contraction

- Transport processes are major consumers of energy
- In brain and kidney, two third of total is consumed to pump sodium and potassium ions across plasma membrane (Na⁺ + K⁺ ATPase)
- Na⁺ dependent phosphorylation of Na⁺ K⁺ ATPase changes protein conformation, K⁺ dependent dephosphorylation brings back original conformation.
- Phosphoryl group transfer: to enzyme, not to substrate

ATP energizes active transport and muscle contraction

- Direct hydrolysis of ATP ($\text{ATP} \rightarrow \text{ADP} + \text{P}_i$) is the source of energy in the conformational changes that produce muscle contraction
- ATP bind with head of myosin (not covalent) $\rightarrow$ dissociate from actin $\rightarrow$ ATP hydrolysis induces conformational change (still ADP and P_i bound tightly) $\rightarrow$ P_i release $\rightarrow$ conformational change of the head of myosin $\rightarrow$ actin move

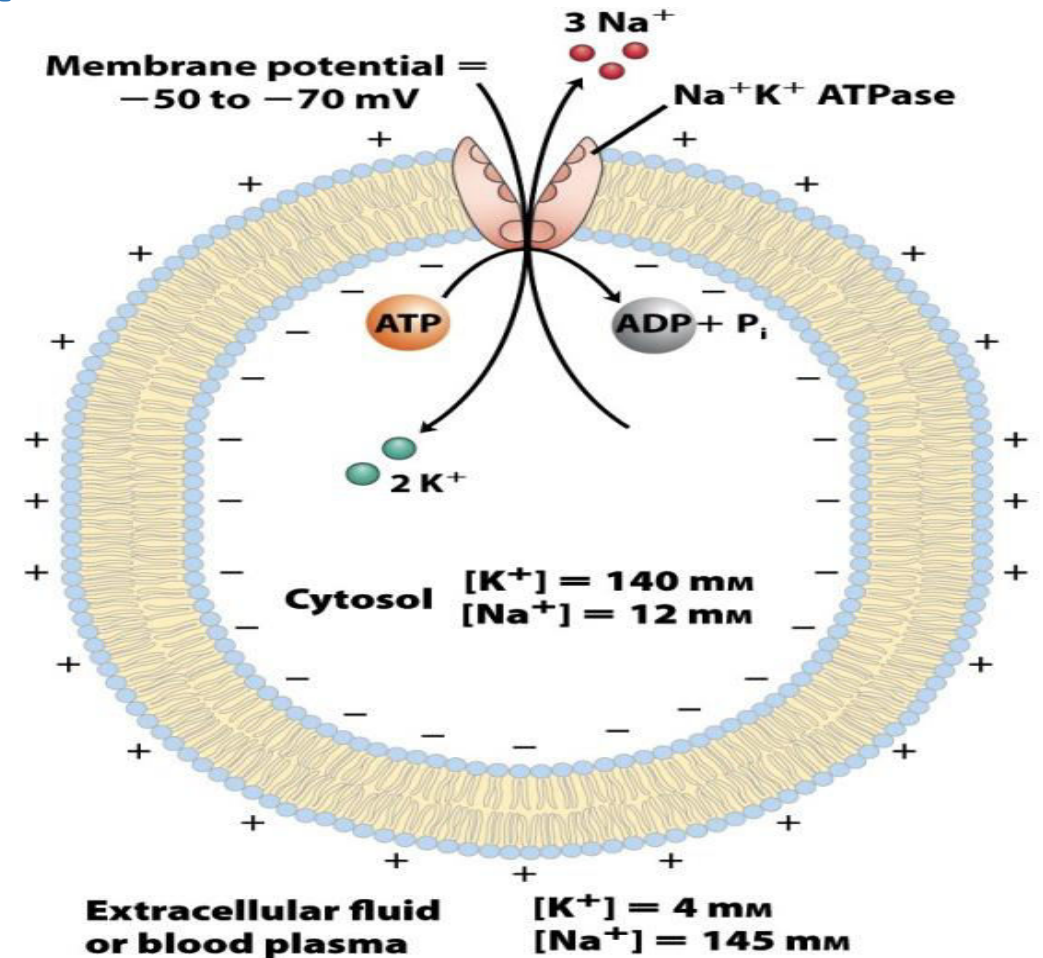


Figure 11-38
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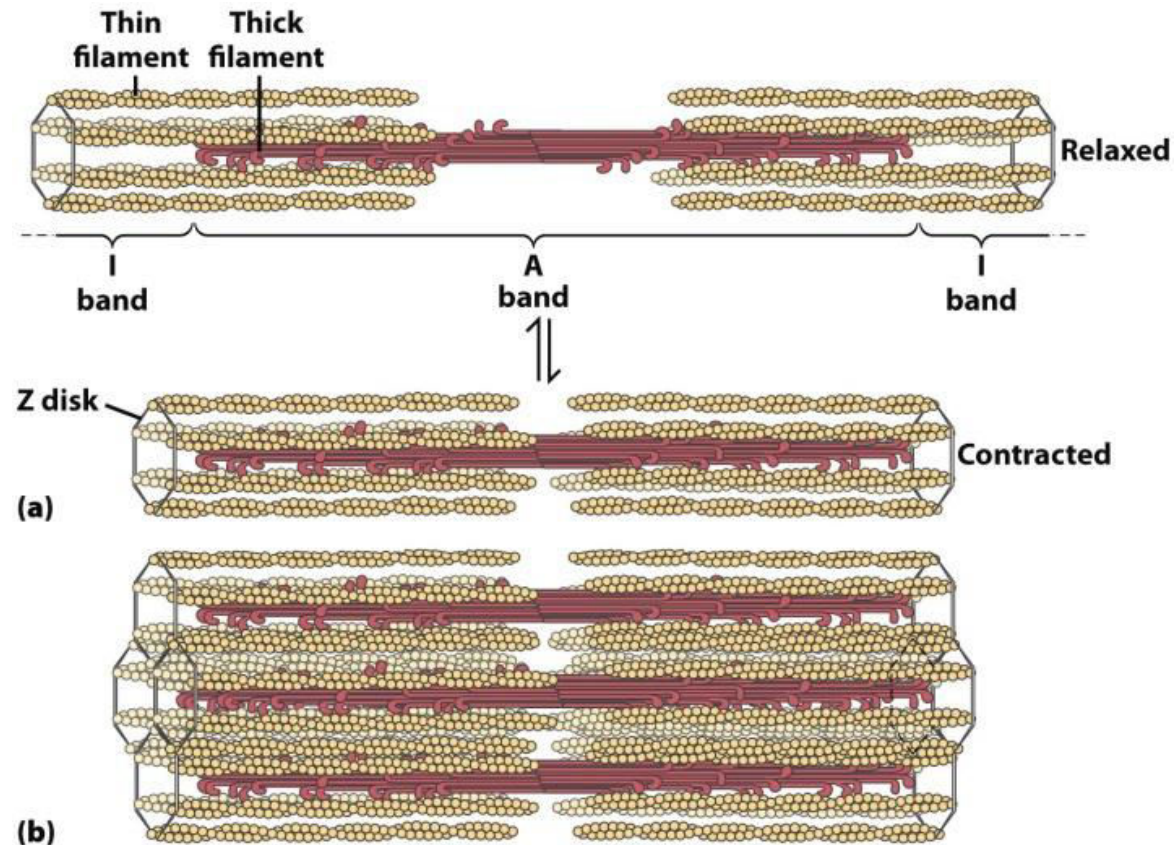


Figure 5-30
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Muscle contraction

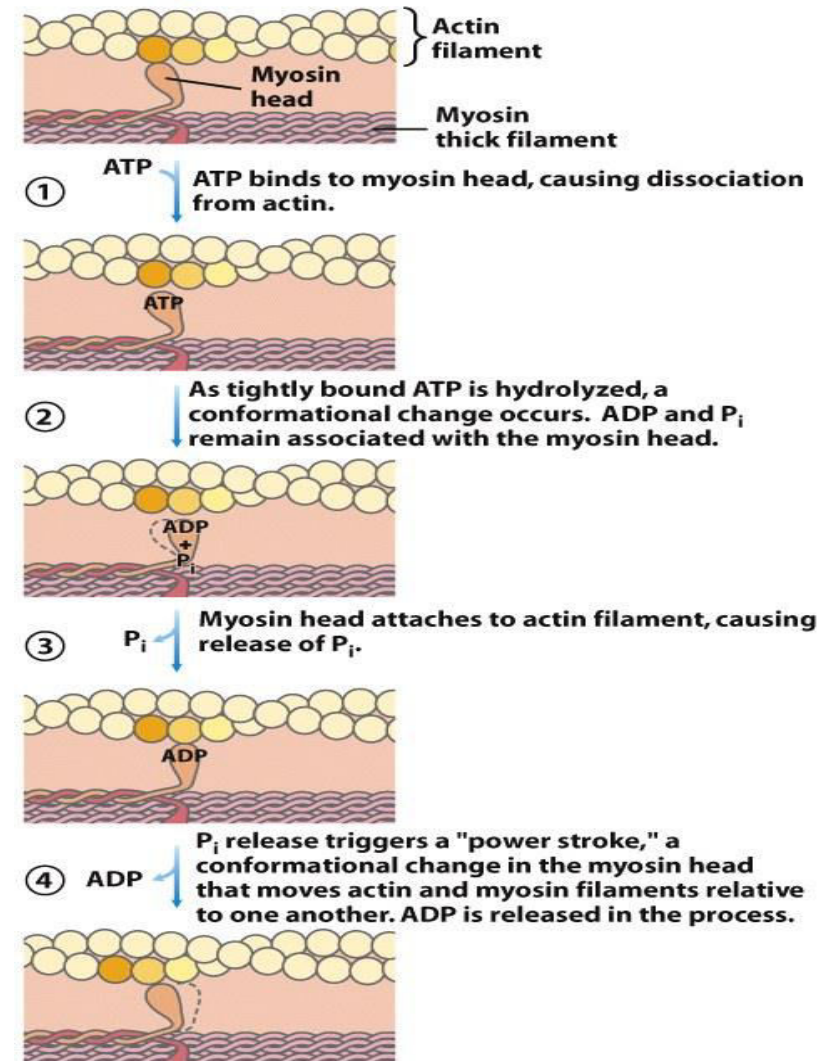
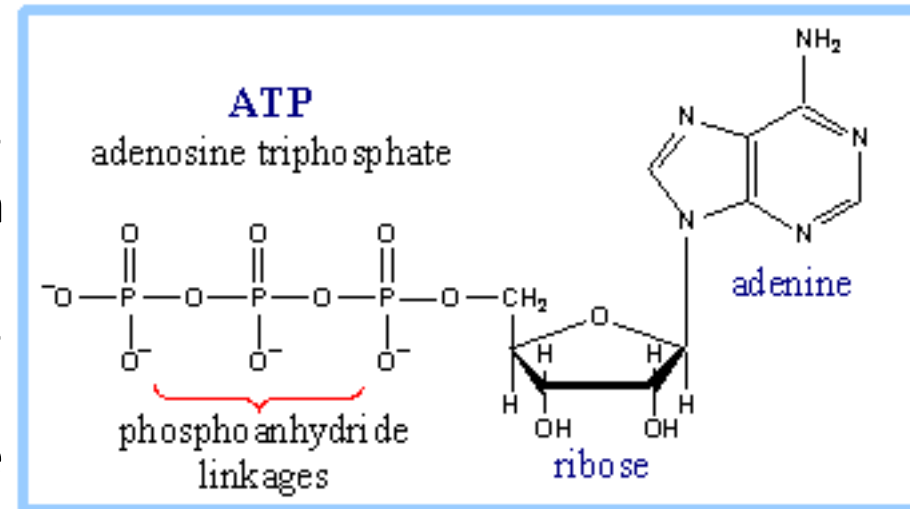


Figure 5-31
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Transphosphorylations between nucleotides occur in all cell types

- ATP is considered as the cell's energy currency and donor of phosphoryl groups, all other nucleoside triphosphates (GTP, UTP, CTP) and all the deoxynucleoside triphosphates (dATP, dGTP, dTTP and dCTP) are energetically equivalent to ATP
- The free energy changes associated with hydrolysis of their **phosphoanhydride linkages** are very nearly identical with those for ATP.
- In preparation for their various biological roles, other nucleotides are generated as the nucleoside triphosphate (NTP) forms by **phosphoryl group transfer** to the corresponding **nucleoside diphosphates (NDPs)** and **monophosphates (NMPs)**
- ATP is the primary high-energy phosphate compound produced by catabolism in the processes of glycolysis, oxidative phosphorylation. Several enzymes carry phosphoryl groups from ATP to the other nucleotides



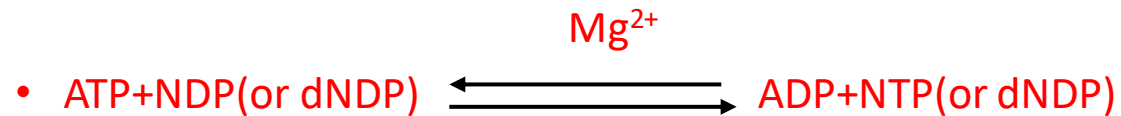
Although we have focused on ATP as the cell's energy currency and donor of phosphoryl groups, all other nucleoside triphosphates (GTP, UTP, CTP) and all the deoxynucleoside triphosphates (dATP, dGTP, dTTP and dCTP) are energetically equivalent to ATP. The free energy changes associated with hydrolysis of their phosphoanhydride linkages are very nearly identical with those for ATP.

In preparation for their various biological roles, these other nucleotides are generated as the nucleoside triphosphate (NTP) forms by phosphoryl group transfer to the corresponding nucleoside diphosphates (NDPs) and monophosphates (NMPs)

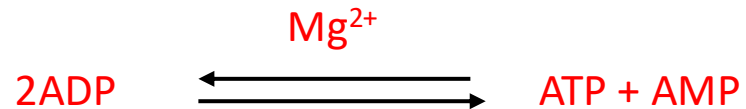
ATP is the primary high-energy phosphate compound produced by catabolism in the processes of glycolysis, oxidative phosphorylation. Several enzymes carry phosphoryl groups from ATP to the other nucleotides

...cont

- Nucleoside diphosphate kinases, found in all cells, catalyzes the reaction



- Although this reaction is fully reversible the relatively high ATP/ADP ratio in cells normally drives the reaction to the right, with the net formation of NTPs and dNTPs
- Phosphoryl group transfers from ATP results in an accumulation of ADP. eg, when muscle is contracting vigorously ADP accumulates and interferes with ATP-dependent contraction.
- During intense demand for ATP, the cell lowers the ADP concentration, and at the same time acquires ATP, by the action of **adenylate kinase**:



Ping-pong mechanism of nucleoside diphosphate kinase

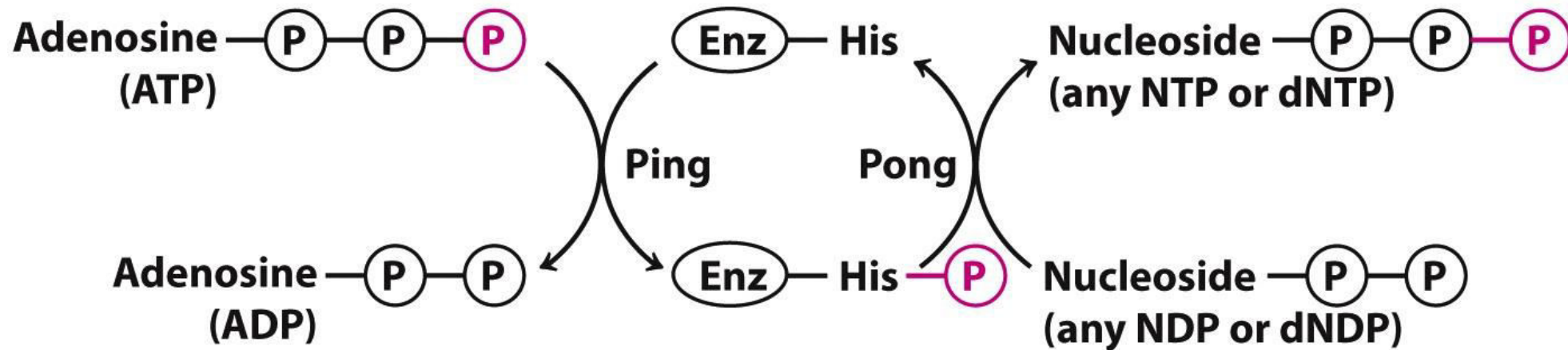


Figure 13-21

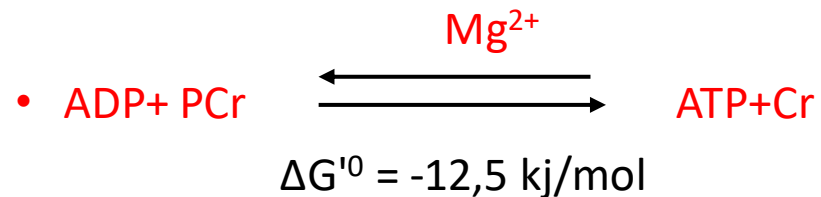
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Ping-pong mechanism of nucleoside diphosphate kinase: The enzyme binds its first substrate (ATP in our example), and a phosphoryl group is transferred to the side chain of a **His** residue. ADP departs, and another nucleoside (or deoxynucleoside) diphosphate replaces it, and this is **converted to the corresponding triphosphate by transfer of the phosphoryl group** from the phosphohistidine residue.

...cont

- This reaction is fully reversible, so after the intense demand for ATP ends, the enzyme can recycle AMP by converting it to ADP which can then be phosphorylated to ATP in mitochondria
- A similar enzyme **guanylate kinase, converts GMP to GDP** at the expense of ATP.
- **By these pathways energy conserved in the catabolic production of ATP is used to supply the cell with all required NTPs and dNTPs**
- **Phosphocreatine (PCr)** serves as a ready source of phosphoryl groups for the quick synthesis of ATP from ADP. The phosphocreatine concentration in skeletal muscle is considerably higher than those in the other tissues. The enzyme **creatine kinase** catalyzes the reversible reaction.

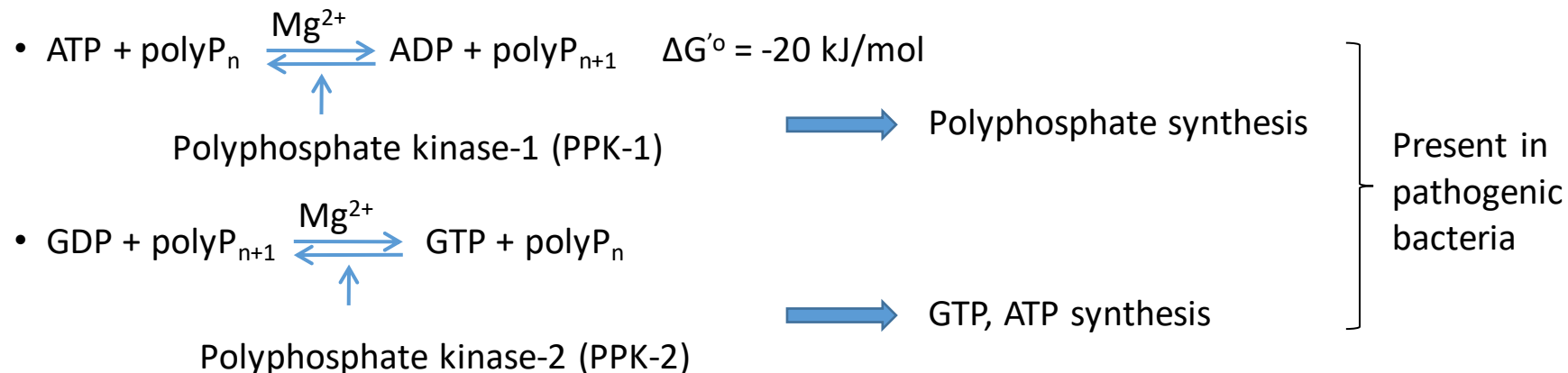


When a sudden demand for energy depletes ATP, the PCr reservoir is used to replenish ATP at a rate faster than ATP can be synthesized by catabolic pathways

When the demand for energy slackens ATP produced by catabolism is used to replenish the PCr reservoir by reversal of the creatine kinase reaction

Inorganic polyphosphate is a donates phosphoryl group

- Inorganic polyphosphate ((polyP)_n): linear polymer of Pi ... Present in simple organisms of other phyla like yeast
- In yeast, polyP accumulated in vacuoles (= 200 mM)
- Serve as a **phosphagen (Reservoir of phosphoryl group).. Like PCr**
- The shortest polyP is P₂ (n=2): can serve as the energy source for active transport of H⁺ in plant cells (across vacuolar membrane)
- **In bacteria, the enzyme polyphosphate kinase-1 (PPK-1) catalyzes the reversible reaction**
- **polyphosphate kinase-2 (PPK-2)**, catalyzes the reversible synthesis of GTP (or ATP) from polyphosphate and GDP (or ADP):



BIOENERGETICS

Biological oxidation-reduction reactions

- Central feature of metabolism

1) Phosphoryl group transfer

2) Electron transfer in oxidation-reduction

- The flow of electrons in oxidation-reduction reactions is responsible for all work done by living organisms

Oxidation

- Addition of oxygen
- **loss of electrons**
- **Loss** of hydrogen

Reduction

Removal of oxygen

Gain of electrons

Gain of hydrogen

Biological oxidation-reduction reactions

- In nonphotosynthetic organism, source of electron: food
- In photosynthetic organism, source of electron: chemical compound excited by the absorption of light
- Electrons move from various metabolic intermediates to specialized electron carriers in enzyme-catalyzed reaction → energy release

1. The flow of electrons can do biological work

- **Electromotive force (emf):** (measured in volts), is the voltage developed by any source of electrical energy such as a battery... In which two chemical species differ in their affinity for electrons, so the electrons flow spontaneously through the circuit, due to the difference in electron affinity
- **Living cells have an analogous biological “circuit”,** with relatively reduced compound such as glucose (the source of electrons)
- Glucose → enzymatically oxidized → release electron → spontaneously flow through electron-carrier intermediates to another chemical species, such as O_2 this electron flow is **exergonic reaction because O_2 has a higher affinity for electrons than do electron-carrier intermediates**
- The resulting emf provides energy to a variety of **molecular energy transducers (enzymes and other proteins)** that do biological work

The flow of electrons can do biological work

- In the mitochondrion, membrane-bound enzymes couple electron flow → a production of **transmembrane pH difference** and a **transmembrane electrical potential** → which **helps to accomplish osmotic and electrical work**
- The **proton gradient** thus formed has potential energy, sometimes called the **proton-motive force** by analogy with electromotive force.
- Another enzyme, ATP synthase in the inner mitochondrial membrane, uses the proton-motive force to do chemical work: synthesis of ATP from ADP and Pi as protons flow spontaneously across the membrane.



- Bacterial flagellar motion: use proton-motive force
- Thus, the proton potential has potential energy: electron-motive force

Oxidation-Reductions Can Be Described as Half-Reactions

Half-Reactions:

- A **half reaction** is either the oxidation or reduction reaction component of a **redox reaction**.
- A half reaction is obtained by considering the change in oxidation states of individual substances involved in the redox reaction

A half reaction is obtained by considering the change in oxidation states of individual substances involved in the redox reaction

...cont

Although oxidation and reduction occur together, we consider the two halves of an oxidation-reduction reaction separately.

- For example, the oxidation of ferrous ion by cupric ion is written as follows,



- Same can be described as 2 half reactions in the following form:



Conjugate redox pair

- The electron-donating molecule in an oxidation-reduction reaction is called the reducing agent or **reductant**
- the electron-accepting molecule is the oxidizing agent or **oxidant**.
- A given agent, such as an iron cation existing in the ferrous (Fe^{+2}) or ferric (Fe^{+3}) state functions as a **conjugate reductant-oxidant pair** (redox pair), just as an acid and corresponding base function as a **conjugate acid-base pair**.
- Acid-base reactions can be written as a general equation:
 - **proton donor $\leftrightarrow$ H^+ + proton acceptor**
- In redox reactions we can write a similar general equation:
 - **electron donor $\leftrightarrow$ e^- + electron acceptor**
- In the reversible half-reaction (1) above, Fe^{+2} is the electron donor and Fe^{+3} is the electron acceptor; together, Fe^{+2} and Fe^{+3} constitute a **conjugate redox pair**.

The electron-donating molecule in an oxidation-reduction reaction is called the reducing agent or reductant; the electron-accepting molecule is the oxidizing agent or oxidant.

A given agent, such as an iron cation existing in the ferrous (Fe^{+2}) or ferric (Fe^{+3}) state functions as a conjugate reductant-oxidant pair (redox pair), just as an acid and corresponding base function as a **conjugate acid-base pair**.

In acid-base reactions we can write a general equation:



In redox reactions we can write a similar general equation:



In the reversible half-reaction (1) above, Fe^{+2} is the electron donor and Fe^{+3} is the electron acceptor; together, Fe^{+2} and Fe^{+3} constitute a **conjugate redox pair**.

...cont

- The oxidation of reducing sugar by cupric acid
- Aldehydes and ketones with anomeric carbon can act **reducing sugars.. Having capability to reduce copper ions in medium..** The reducing sugar reduces the [copper\(II\)](#) ions in these test solutions to copper(I), which forms a brick red [copper\(I\) oxide](#) precipitate
- Basis of qualitative carbohydrate test (Benedict's test)

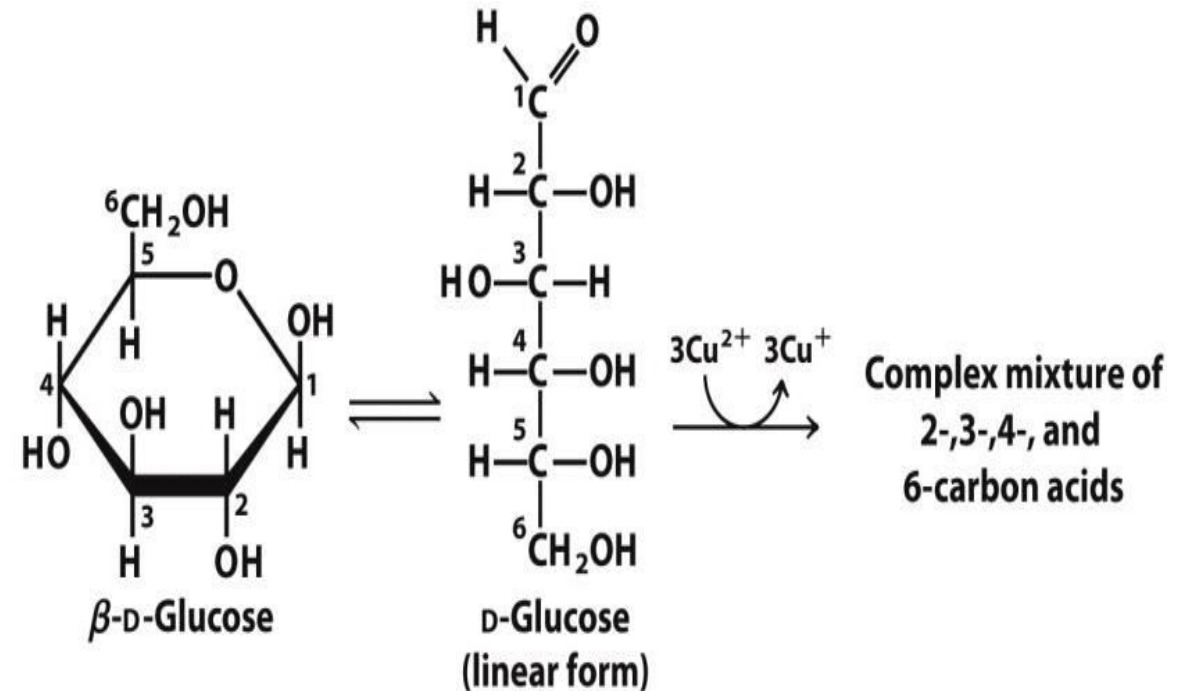


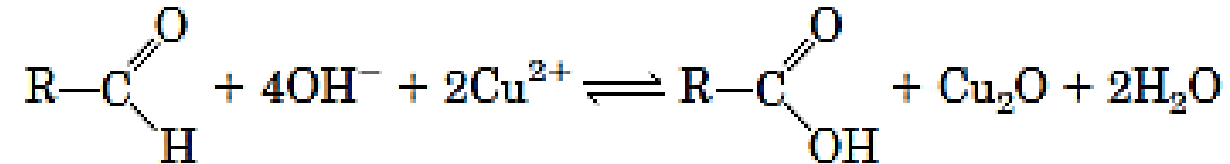
Figure 7-10
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The *reducing end* of a disaccharide is the monosaccharide with a free anomeric carbon that is not involved in a glycosidic bond and is thus capable of converting to the open-chain form.

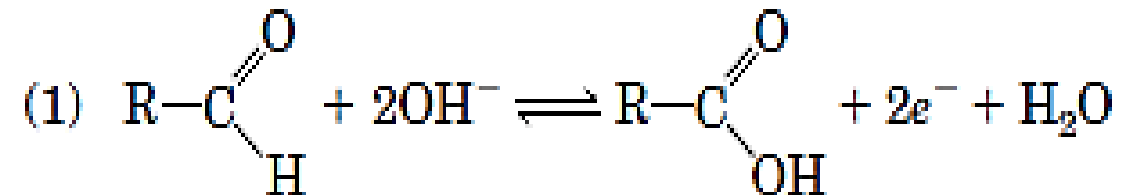
A nonreducing disaccharide has both anomeric carbons tied up in the glycosidic bond...

...cont

- consider the oxidation of a reducing sugar by cupric ion:



- This overall reaction can be expressed as two half reactions:



- Because two electrons are removed from the aldehyde carbon, the second half-reaction (the one-electron reduction of cupric to cuprous ion) must be doubled to balance the overall equation.

The monosaccharides can be divided into two groups: the aldoses, which have an **aldehyde** group, and the ketoses, which have a **ketone** group. Ketoses must first tautomerize to aldoses before they can act as **reducing sugars**.. **Having capability to reduce copper ions in medium**.. The reducing sugar reduces the [copper\(II\)](#) ions in these test solutions to copper(I), which then forms a brick red [copper\(I\) oxide](#) precipitate has a free [aldehyde](#) group or a free [ketone](#) group

The *reducing end* of a disaccharide is the monosaccharide with a free [anomeric carbon](#) that is not involved in a glycosidic bond and is thus capable of converting to the open-chain form.

A nonreducing disaccharide has both anomeric carbons tied up in the glycosidic bond...

. The common dietary monosaccharides galactose, **glucose** and fructose are all **reducing sugars**.

Biological Oxidations Often Involve Dehydrogenation

- In biological Systems oxidation (loss of electrons) is often synonymous with **dehydrogenation** and many enzymes that catalyze oxidation reactions are **dehydrogenases**.
- The carbon in living cells exists in a range of oxidation status
- **Electronegativity: $H < C < S < N < O$**

for attached C atoms, *i.e.* C-C bonds electrons shared, \count 0

for attached X atoms, *i.e.* C-X bonds (X more electronegative), \count -1
(per bond)

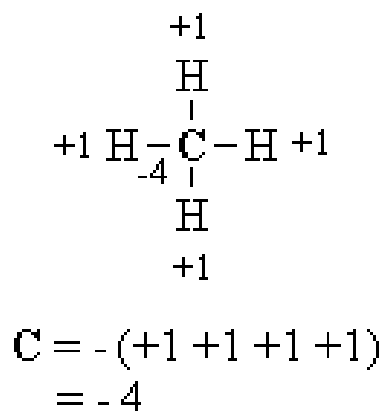
for attached H atoms, *i.e.* C-H bonds (H is less electronegative than C), \count +1

...cont

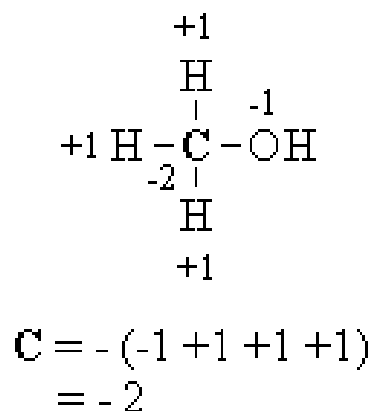
OXIDATION STATE

It is calculated by counting all the electrons bonded to carbon and add them in following way:

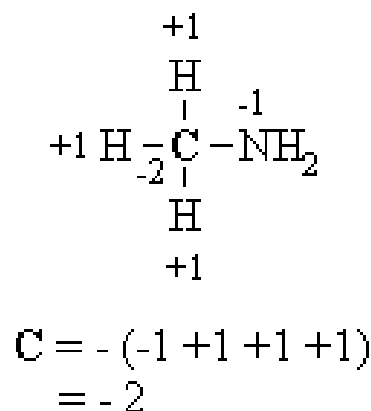
- for attached C atoms, *i.e.* C-C bonds electrons shared, count 0
- for attached X atoms, *i.e.* C-X bonds (X more electronegative), count -1 (per bond)
- for attached H atoms, *i.e.* C-H bonds (H is less electronegative than C), count +1
- Add the total for atoms attached to the C in question, then switch the sign.



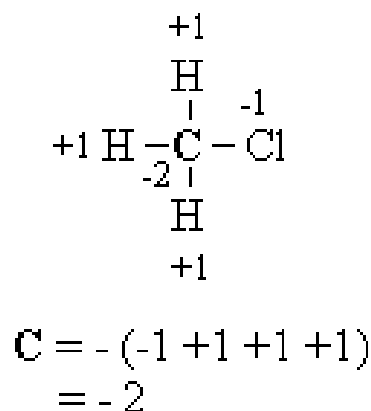
Methane



Methanol



AminoMethane



Methayl chloride

Oxidation Levels of carbon compounds

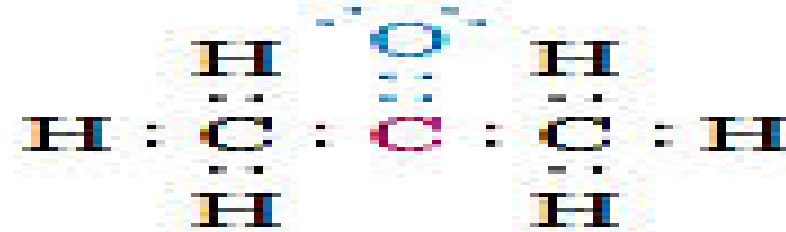
For the figure given on right:

Different levels of oxidation of carbon compounds in the biosphere.

- In order to estimate the level of oxidation of these compounds, focus on the red carbon atom and its bonding electrons.
- **When this carbon is bonded to the less electronegative H atom, both bonding electrons (red) are assigned to the carbon.**
- When carbon is bonded to another carbon, bonding electrons are shared equally, one of the two electrons is assigned to the red carbon. When the **red carbon** is bonded to the **more electronegative O atom**, the bonding electrons are assigned to the oxygen.
- **The number to the right of each compound is the number of electrons “owned” by the red carbon**, a rough expression of the degree of oxidation of that compound. As the red carbon undergoes oxidation (loses electrons), the number gets smaller.

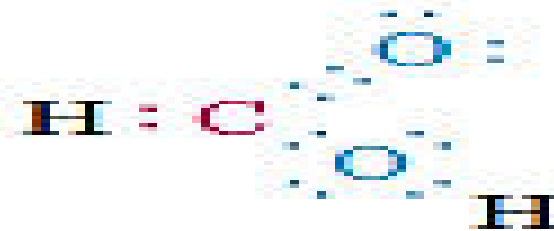
Methane	$\begin{array}{c} \text{H} \\ \vdots \\ \text{H} : \text{C} : \text{H} \\ \vdots \\ \text{H} \end{array}$	8
Ethane (alkane)	$\begin{array}{c} \text{H} \quad \text{H} \\ \vdots \quad \vdots \\ \text{H} : \text{C} : \text{C} : \text{H} \\ \vdots \quad \vdots \\ \text{H} \quad \text{H} \end{array}$	7
Ethene (alkene)	$\begin{array}{c} \text{H} \quad \quad \text{H} \\ \vdots \quad \quad \vdots \\ \text{H} : \text{C} :: \text{C} : \text{H} \\ \vdots \quad \quad \vdots \\ \text{H} \quad \quad \text{H} \end{array}$	6
Ethanol (alcohol)	$\begin{array}{c} \text{H} \quad \text{H} \\ \vdots \quad \vdots \\ \text{H} : \text{C} : \text{C} : \text{O} : \text{H} \\ \vdots \quad \vdots \\ \text{H} \quad \text{H} \end{array}$	5
Acetylene (alkyne)	$\text{H} : \text{C} :: \text{C} : \text{H}$	5
Formaldehyde	$\begin{array}{c} \text{H} \\ \vdots \\ \text{H} : \text{C} :: \text{O} : \\ \vdots \\ \text{H} \end{array}$	4
Acetaldehyde (aldehyde)	$\begin{array}{c} \text{H} \quad \quad \text{H} \\ \vdots \quad \quad \vdots \\ \text{H} : \text{C} : \text{C} : \text{O} : \\ \vdots \quad \quad \vdots \\ \text{H} \quad \quad \text{O} : \end{array}$	3

Acetone
(ketone)



2

Formic acid
(carboxylic acid)



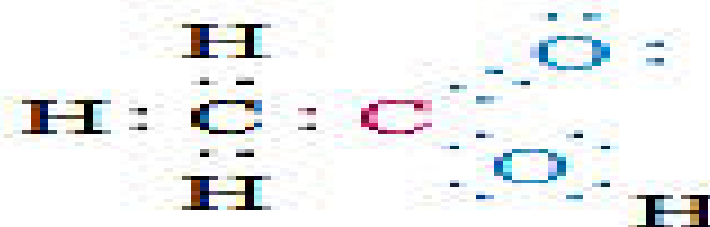
2

Carbon
monoxide



2

Acetic acid
(carboxylic acid)



1

Carbon
dioxide



0

Oxidation states of C reducing sugar (an aldehyde or ketone)
by carbon in various compounds; from fully reduced (methane
to fully oxidized (carbon dioxide)

Electrons are transferred from one molecule to another in 4 ways

1. Directly as electrons: $\text{Fe}^{2+}/\text{Fe}^{3+}$ redox pair can transfer an electron to the Cu/Cu^{2+} redox pair



2. As hydrogen atoms: hydrogen atom consists of a proton (H^{+}) and a single electron (e^{-})



AH_2/A : conjugate redox pair $\rightarrow$ can reduce another compound B



3. As a hydride ion ($:\text{H}^{-}$) .. has two electrons

This reaction occurs in NAD-linked dehydrogenase

4. Through direct combination with oxygen, where Oxygen covalently incorporated into product



END

BIOENERGETICS

Reduction potential measures affinity for electrons

The tendency for a reduction reaction is measured by its **reduction potential**

- **Reduction potential:**

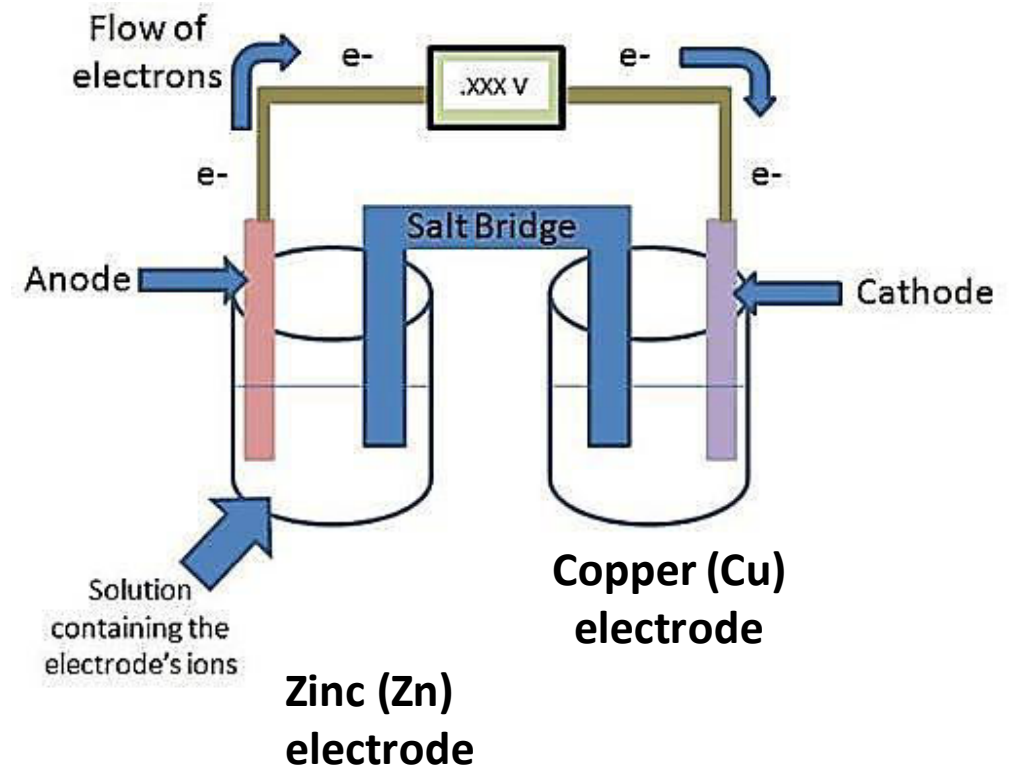
Reduction potential (redox potential, oxidation/reduction potential), is a measure of the tendency of a chemical species to acquire electrons and thereby get **reduced**. Units: Volts (V), or millivolts (mV).. denoted by **E** .

- **Standard reduction potential:**

The tendency for a chemical species to be reduced, **at standard conditions**. The **more positive the potential is the more likely it will be reduced...** denoted by **E°** .

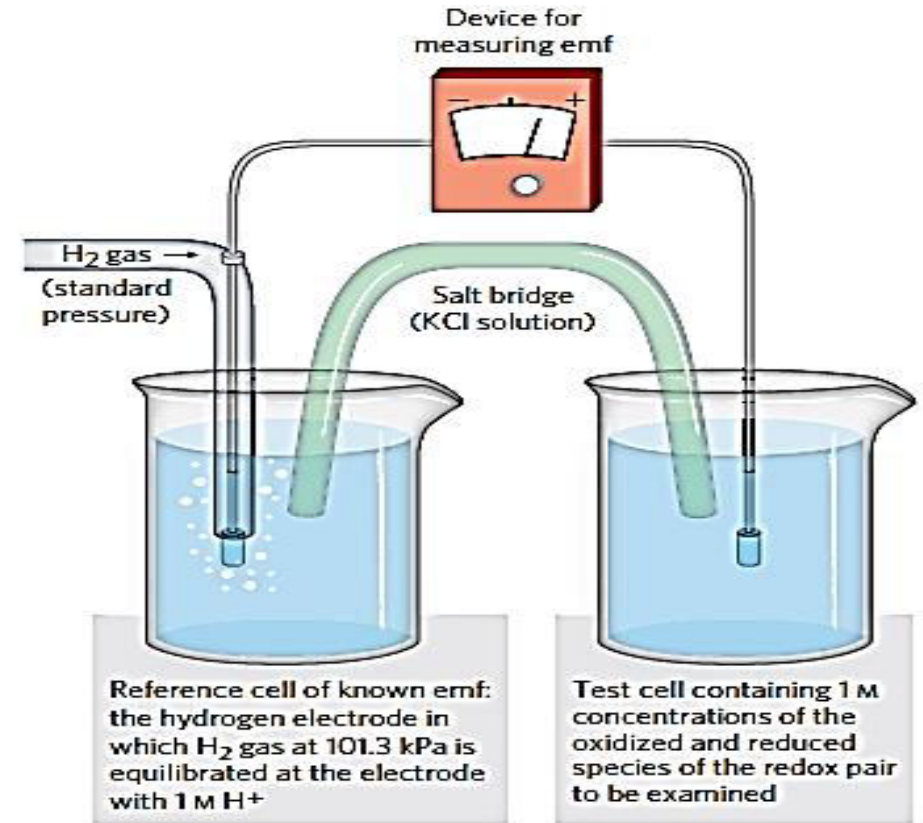
A Half-cell

- Reduction potential of a half-cell depends on the activity of reduced and oxidized species which is approximated by their concentrations
- On the anode, oxidation takes place.
 $\text{Zn} \longrightarrow \text{Zn}^{2+} + 2 \text{e}^{-}$
Zn is the reducing agent, and Zn^{2+} the oxidizing agent.
- On the cathode, reduction takes place.
 $\text{Cu}^{2+} + 2 \text{e}^{-} \longrightarrow \text{Cu}$
 Cu^{2+} is the oxidizing agent and Cu the reducing agent.



A standard Half-cell

- Standard Hydrogen electrode..(SHE) is the primary reference electrode with platinum electrode dipped in solution with H ions under standard conditions
- The electromotive force (emf) of this electrode is designated 0.00 V. (At pH 7, 25 °C)
- Using observed emf (of test solution) and the known emf of the reference cell, the emf of the test cell with the redox pair can be calculated.
- **The cell that gains electrons has, by convention, the more positive reduction potential.**
- **The electrode at which this half-reaction occurs (called a half-cell) is arbitrarily assigned an E^0 of 0.00 V.**



Standard reduction potentials can be used to calculate free-energy change

- Electrons tend to flow to the half-cell with the more positive E , and the strength of that tendency is proportional to ΔE , the difference in reduction potential
- We can then predict the direction in which electrons will tend to flow when the two half-cells are connected through an external circuit
- Electrons tend to flow to the half-cell with the more positive $E \rightarrow$ the strength of flow depends on ΔE (difference in reduction potential)
- The energy made available by this spontaneous electron flow (the free-energy change, ΔG , for the oxidation-reduction reaction) is proportional to ΔE :

$$\Delta G = -nF\Delta E \quad \text{or} \quad \Delta G^\circ = -nF\Delta E^\circ$$

TABLE 13–7 **Standard Reduction Potentials of Some Biologically Important Half-Reactions**

Half-reaction	E'° (V)
$\frac{1}{2}\text{O}_2 + 2\text{H}^+ + 2e^- \longrightarrow \text{H}_2\text{O}$	0.816
$\text{Fe}^{3+} + e^- \longrightarrow \text{Fe}^{2+}$	0.771
$\text{NO}_3^- + 2\text{H}^+ + 2e^- \longrightarrow \text{NO}_2^- + \text{H}_2\text{O}$	0.421
Cytochrome <i>f</i> (Fe^{3+}) + $e^- \longrightarrow$ cytochrome <i>f</i> (Fe^{2+})	0.365
$\text{Fe}(\text{CN})_6^{3-}$ (ferricyanide) + $e^- \longrightarrow \text{Fe}(\text{CN})_6^{4-}$	0.36
Cytochrome <i>a</i> ₃ (Fe^{3+}) + $e^- \longrightarrow$ cytochrome <i>a</i> ₃ (Fe^{2+})	0.35
$\text{O}_2 + 2\text{H}^+ + 2e^- \longrightarrow \text{H}_2\text{O}_2$	0.295
Cytochrome <i>a</i> (Fe^{3+}) + $e^- \longrightarrow$ cytochrome <i>a</i> (Fe^{2+})	0.29
Cytochrome <i>c</i> (Fe^{3+}) + $e^- \longrightarrow$ cytochrome <i>c</i> (Fe^{2+})	0.254
Cytochrome <i>c</i> ₁ (Fe^{3+}) + $e^- \longrightarrow$ cytochrome <i>c</i> ₁ (Fe^{2+})	0.22
Cytochrome <i>b</i> (Fe^{3+}) + $e^- \longrightarrow$ cytochrome <i>b</i> (Fe^{2+})	0.077
Ubiquinone + $2\text{H}^+ + 2e^- \longrightarrow$ ubiquinol + H_2	0.045
Fumarate ²⁻ + $2\text{H}^+ + 2e^- \longrightarrow$ succinate ²⁻	0.031

Half-reaction	E'° (V)
$2\text{H}^+ + 2e^- \longrightarrow \text{H}_2$ (at standard conditions, pH 0)	0.000
Crotonyl-CoA + $2\text{H}^+ + 2e^- \longrightarrow$ butyryl-CoA	−0.015
Oxaloacetate ²⁻ + $2\text{H}^+ + 2e^- \longrightarrow$ malate ²⁻	−0.166
Pyruvate ⁻ + $2\text{H}^+ + 2e^- \longrightarrow$ lactate ⁻	−0.185
Acetaldehyde + $2\text{H}^+ + 2e^- \longrightarrow$ ethanol	−0.197
$\text{FAD} + 2\text{H}^+ + 2e^- \longrightarrow \text{FADH}_2$	−0.219*
Glutathione + $2\text{H}^+ + 2e^- \longrightarrow$ 2 reduced glutathione	−0.23
$\text{S} + 2\text{H}^+ + 2e^- \longrightarrow \text{H}_2\text{S}$	−0.243
Lipoic acid + $2\text{H}^+ + 2e^- \longrightarrow$ dihydrolipoic acid	−0.29
$\text{NAD}^+ + \text{H}^+ + 2e^- \longrightarrow \text{NADH}$	−0.320
$\text{NADP}^+ + \text{H}^+ + 2e^- \longrightarrow \text{NADPH}$	−0.324
Acetoacetate + $2\text{H}^+ + 2e^- \longrightarrow$ β -hydroxybutyrate	−0.346
α -Ketoglutarate + $\text{CO}_2 + 2\text{H}^+ + 2e^- \longrightarrow$ isocitrate	−0.38
$2\text{H}^+ + 2e^- \longrightarrow \text{H}_2$ (at pH 7)	−0.414
Ferredoxin (Fe^{3+}) + $e^- \longrightarrow$ ferredoxin (Fe^{2+})	−0.432

Source: Data mostly from Loach, R.A. (1976) In *Handbook of Biochemistry and Molecular Biology*, 3rd edn (Fasman, G.D., ed.), *Physical and Chemical Data*, Vol. 1, pp. 122–130, CRC Press, Boca Raton, FL.

* This is the value for free FAD; FAD bound to a specific flavoprotein (e.g., succinate dehydrogenase) has a different E'° that depends on its protein environment.

Table 13-7

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...cont

- where n is the number of electrons transferred in the reaction
- **F is Faraday (unit of electricity)**, used in the study of electrochemical reactions and equal to the amount of electric charge that liberates one gram equivalent of any ion from an electrolytic solution
- With this equation we can calculate the actual free-energy change for any oxidation-reduction reaction from the values of E'° (reduction potentials) and the concentrations of reacting species
- When acetaldehyde is reduced by the biological electron carrier NADH, $\Delta G = -35.3 \text{ kJ/mol}$

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Table 13-7

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- **Conjugate redox pair:** An electron donor and its corresponding electron acceptor form; for example, Cu^+ (donor) and Cu^{2+} (acceptor), or NADH (donor) and NAD^+ (acceptor) form **Conjugate redox pair**.
- **Half cell:** A **half cell** is one of the two electrodes in a galvanic **cell** or simple battery. For example, in the Zn-Cu battery, the two **half cells** make an oxidizing-reducing couple.

Reduction potential (also known as **redox potential**, oxidation/**reduction potential**, or E_h) **measures** the tendency of a chemical species to acquire **electrons** and thereby be reduced.

END

BIOENERGETICS

Universal Electron Carriers

Cellular oxidation of glucose to carbon dioxide requires specialized electron carriers

- The principles of oxidation-reduction energetics → can apply to the many metabolic reactions that involve electron transfers

Glucose oxidation:



- Cells does not convert to CO_2 in a single, high energy releasing reaction but rather in a series of controlled reactions, some of which are oxidation for the ATP synthesis from ADP through NAD^+ and FAD

<https://www.khanacademy.org/test-prep/mcat/biomolecules/overview-metabolism/v/electron-carrier-molecules>

So what kind of processes produce electrons and why.. glucose is the basic fuel of life.. And is converted to CO_2 during catabolic reactions and we studied that the oxidation state of C in CO_2 is zero.. Due to the fact that....more electro-negative oxygen is attached in CO_2 whereas in glucose hydrogen is attached

..so in CO_2 .. WHO is more oxidized...(having less electron density)and this is the duty of e carriers to harness energy from e produced at each step ..in oxidation process

Finally these collected e have to be forwarded to the e transport chain..(mitochondria) And at this point enzymes are present in our body that accept the es..and from there to the final e acceptor in our body.. That's oxygen

So why is this so complicated.. Consider the equation that writes that glucose is converted to CO_2 and .. With energy..it depicts a general combustion process.. Where our glucose is the fuel..+ O_2 ...

b/c in this case .. Energy is produced in the form of heat energy.. That's unusable.. As our body uses energy only in form of ATP..luckily energy prod is in a stepwise manner.. ~~And this is kinetically unfavourable~~... high temp is able to overcome activation energy.. To allow combustion to occur?

Our bodies hv temp much lower than combustion requires...our body uses enzymes to overcome high act energy

Useful as produces large no of usable metabolites.. We hv a controlled oxidation of glucose..e carriers are alwz called as coenzymes enzymes in g. glycolysis are dehydrogenases while taking away H they remove e.. And do this by taking a proton with 2 e.. Called hydride..

NAD and FAD... nad has positive charge in most oxidized form that fad does not..

Each reacts with 2 protons and 2 e⁻.. Meaning with 1 hydride ion ..and 1 H... once the carrier carries them its now reduced..nad can carry only 1 e so it becomes NADH but FADH₂ ...and these reduced forms of e carriers can transport e to etc to allow prod of ATP.

That's the idea here

At each step of break down of glucose.. Oxidation of f--- occurs and electrons are released..and metabolites are more and more oxidized..

Energy generation is not only by glucose and ATP, but there are some other Behind the scene contributors too.. These are electron carrier molecules..

Glucose

A Few Types of Coenzymes and Proteins Serve as Universal Electron Carriers

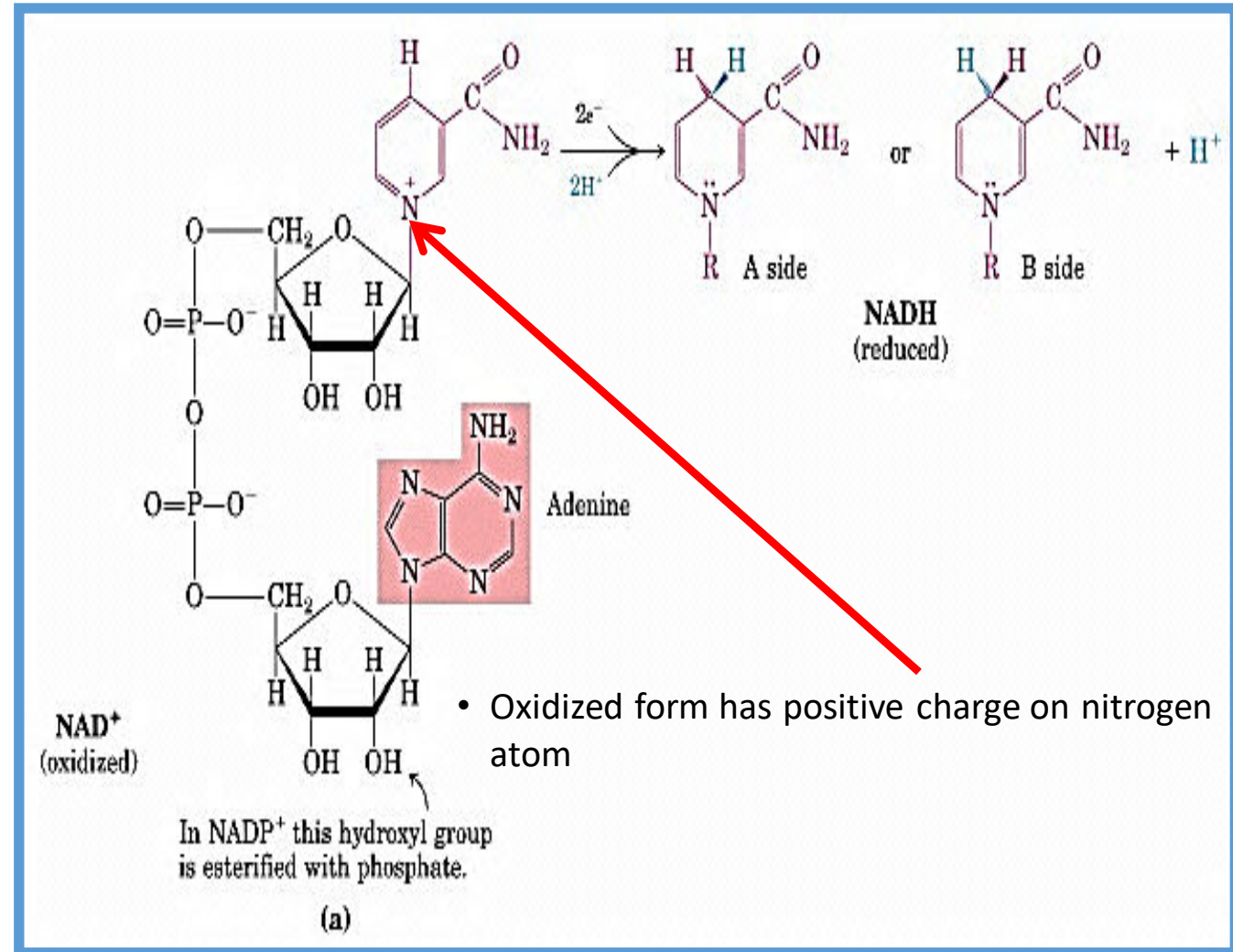
1. The nucleotides NAD and NADP move readily from one enzyme to another
 2. The **flavin nucleotides FMN and FAD** are usually very tightly bound to the enzymes, called **flavoproteins**, for which they serve as **prosthetic groups**
 3. **Lipid-soluble quinones: ubiquinone and plastoquinone** act as **electron carriers and proton donors in the non-aqueous environment** of membranes
 4. **Iron-sulfur proteins and cytochromes**, which have tightly bound prosthetic groups that undergo reversible oxidation and reduction, serve as electron carriers in many oxidation-reduction reactions
- **NAD, NADP, FMN, and FAD** are water-soluble coenzymes and undergo reversible oxidation and reduction during many of the electron-transfer reactions of metabolism.

Energy generation is not only by glucose and ATP, but there are some other Behind the scene contributors too.. These are electron carrier molecules.. Glucose . Some of these proteins are water-soluble, but others are peripheral or integral membrane proteins

1. The reduction of these carriers in catabolic processes results in the conservation of free energy released by substrate oxidation.
2. **NAD, NADP, FMN, and FAD** are water-soluble coenzymes and undergo reversible oxidation and reduction in many of the electron-transfer reactions of metabolism.
3. The nucleotides NAD and NAD move readily from one enzyme to another
4. The **flavin nucleotides FMN and FAD** are usually very tightly bound to the enzymes, called **flavoproteins**, for which they serve as **prosthetic groups**.
5. **Lipid-soluble quinones: ubiquinone and plastoquinone** act as **electron carriers and proton donors in the non-aqueous environment** of membranes.
6. **Iron-sulfur proteins and cytochromes**, which have tightly bound prosthetic groups that undergo reversible oxidation and reduction, also serve as electron carriers in many oxidation-reduction reactions.

NAD and NADP are soluble electron carriers that act with dehydrogenase enzymes

- Activated electron carriers, involved in oxidation-reduction and coupling reactions
- NAD⁺ (nicotinamide adenine dinucleotide)
- NADP⁺ (nicotinamide adenine dinucleotide phosphate)
- **For NAD: → NAD⁺: oxidized form**
- **For NADP: → NADP⁺: oxidized form**
- Joined by phosphoanhydride bond
- Pyridine nucleotides
- Nicotinamide moiety comes from **NIACIN Vitamin**



NAD⁺ (nicotinamide adenine dinucleotide)

NADP⁺ (nicotinamide adenine dinucleotide phosphate)

And it's the flow of e⁻ in etc that is used to produce ATP ..

There are other important activated carriers involved in biosynthesis of molecules. These activated carriers are involved in oxidation-reduction reactions and involved in coupling reactions.

The most important of these carriers are NAD⁺ (nicotinamide adenine dinucleotide) and closely related molecule NADP⁺ (nicotinamide adenine dinucleotide phosphate).

A conjugate acid, within the Brønsted–Lowry acid–base theory, is a species formed by the reception of a proton (H⁺) by a base—in other words, it is a base with a hydrogen ion added to it. On the other hand, a conjugate base is merely what is left over after an acid has donated a proton during a chemical reaction

Mechanism. The **reaction** mechanism generally begins with the oxidative addition of an **organic** halide to the catalyst. ... The final step is reductive elimination of the two **coupling** fragments to regenerate the catalyst and give the **organic** product.

... cont

- Both coenzymes undergo reversible reduction of the nicotinamide ring
- As a substrate molecule undergoes oxidation (dehydrogenation), giving up two hydrogen atoms, the oxidized form of the nucleotide (NAD^+ or NADP^+) accepts a hydride ion (:H_2 , the equivalent of a proton and two electrons) and is reduced (to NADH or NADPH)
- The second proton removed from the substrate is released to the aqueous solvent.

The half-reactions for these nucleotide cofactors are:

- $\text{NAD}^+ + 2\text{e}^- + 2\text{H}^+ + \text{NADH} \longrightarrow \text{NADH} + \text{H}^+$
- $\text{NADP}^+ + 2\text{e}^- + 2\text{H}^+ + \text{NADH} \longrightarrow \text{NADPH} + \text{H}^+$

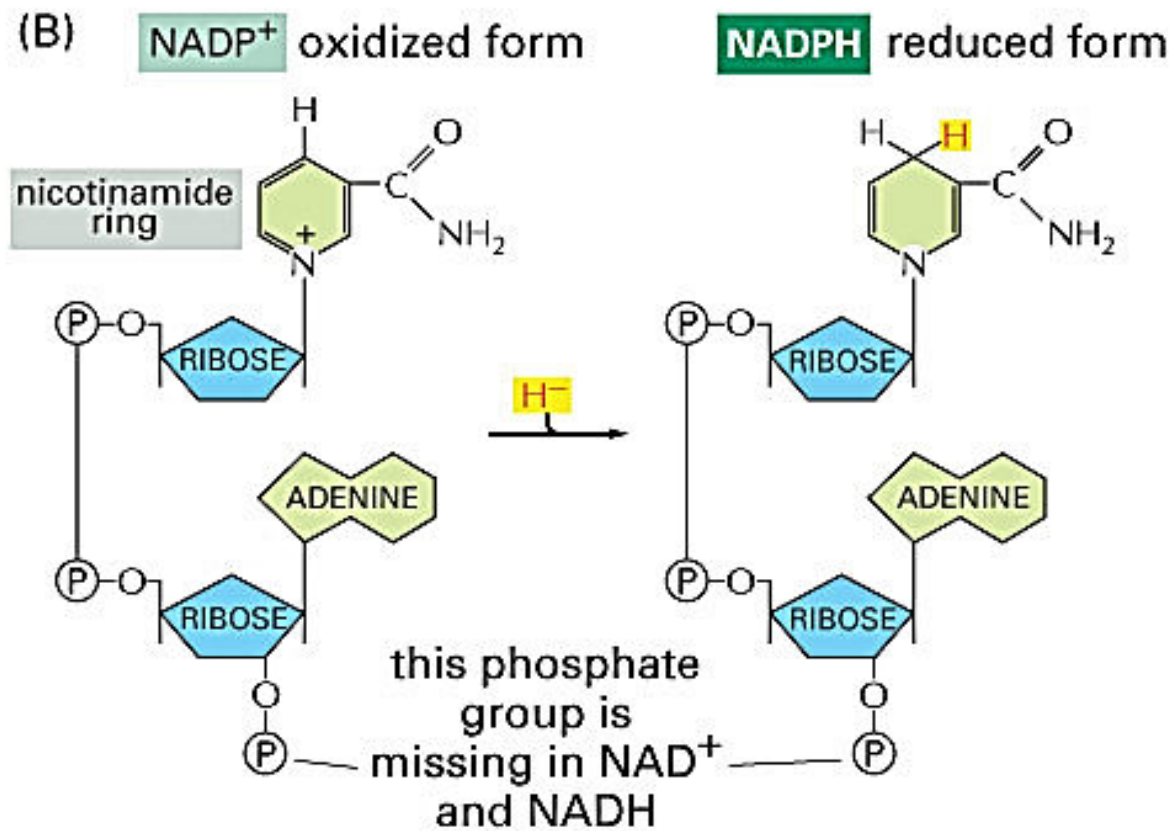
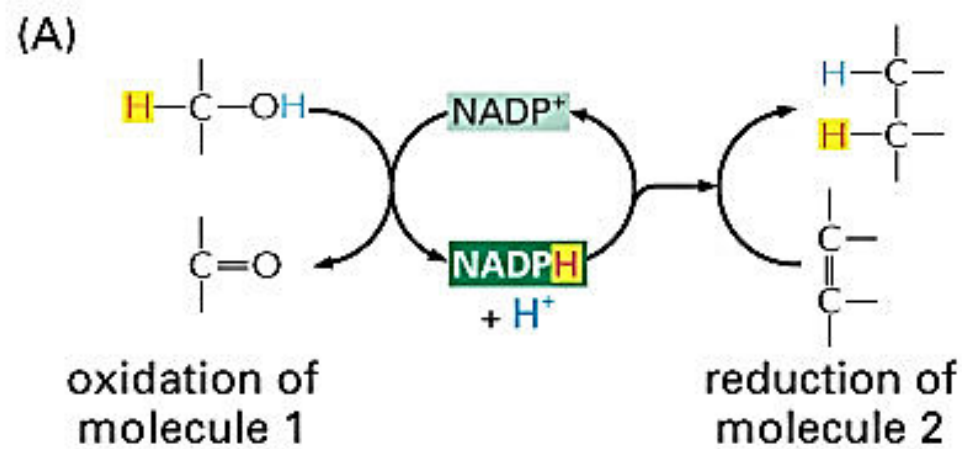


Figure 3-35 Essential Cell Biology, 2/e. (© 2004 Garland Science)

...cont

- Reduction of NAD or NADP converts the benzenoid ring of the nicotinamide moiety (with a fixed positive charge on the ring nitrogen) to the **quinonoid** form (with no charge on the nitrogen).
- The reduced nucleotides absorb light at **340 nm**; the oxidized forms do not (used for detection)
- Plus sign with NAD and NADP does *not* indicate the net charge (both are negatively charged) rather, it indicates that the nicotinamide ring is in its oxidized form, with a positive charge on the **Nitrogen atom**
- **“H” in NADH and NADPH, the denotes the added hydride ion**

Reduction of NAD⁺ or NADP⁺ converts the benzenoid ring of the nicotinamide moiety (with a fixed positive charge on the ring nitrogen) to the quinonoid form (with no charge on the nitrogen).

The reduced nucleotides absorb light at 340 nm; the oxidized forms do not (used for detection)

Note that the plus sign in the abbreviations NAD⁺ and NADP⁺ does *not* indicate the net charge on these molecules

in fact, both are negatively charged); rather, it indicates that the nicotinamide ring is in its oxidized form, with a positive charge on the nitrogen atom. In the abbreviations NADH and NADPH, the “H” denotes the added hydride ion. To refer to these nucleotides without specifying their oxidation state, we use NAD and NADP.

...cont

- Total concentration of $\text{NAD}^+ + \text{NADH}$ in most tissues: 10^{-5} M
- Total concentration of $\text{NADP}^+ + \text{NADPH}$ in most tissues: 10^{-6} M
- Concentration ratio: $\text{NAD}^+ > \text{NADH} \rightarrow$ favoring hydride transfer from a substrate to NAD^+ to form $\text{NADH} \rightarrow$ function in oxidation, occur in mitochondrial matrix
- Concentration ratio: $\text{NADP}^+ < \text{NADPH} \rightarrow$ favoring hydride transfer from NADPH to a substrate $\rightarrow$ function in reduction $\rightarrow$ occur in cytosol

...cont

- More than 200 enzymes are known to catalyze reactions in which NAD⁺ (or NADP⁺) accepts a hydride ion from a reduced substrate or NADPH (or NADH) donates a hydride ion to an oxidized substrate
 - **General reaction**
 - $\text{AH}_2 + \text{NAD}^+ \rightleftharpoons \text{A} + \text{NADH} + \text{H}^+$ (NADH is Oxidized substrate) (AH₂ is reduced)
 - $\text{A} + \text{NADPH} + \text{H}^+ \rightleftharpoons \text{AH}_2 + \text{NADP}^+$ (NADP⁺ is reduced substrate, A is oxidized)
- oxidoreductase
-
- $\text{CH}_3\text{CH}_2\text{OH} + \text{NAD}^+ \longrightarrow \text{CH}_3\text{CHO} + \text{NADH} + \text{H}^+$

Ethanol acetaldehyde

}

Alcohol dehydrogenase
enzyme

...cont

NAD and NADP:

- NAD and its phosphorylated analog NADP undergo reduction to NADH and NADPH, accepting a hydride ion from an oxidizable substrate.
- The hydride ion is added to either the front (the A side) or the back (the B side) of the planar nicotinamide ring

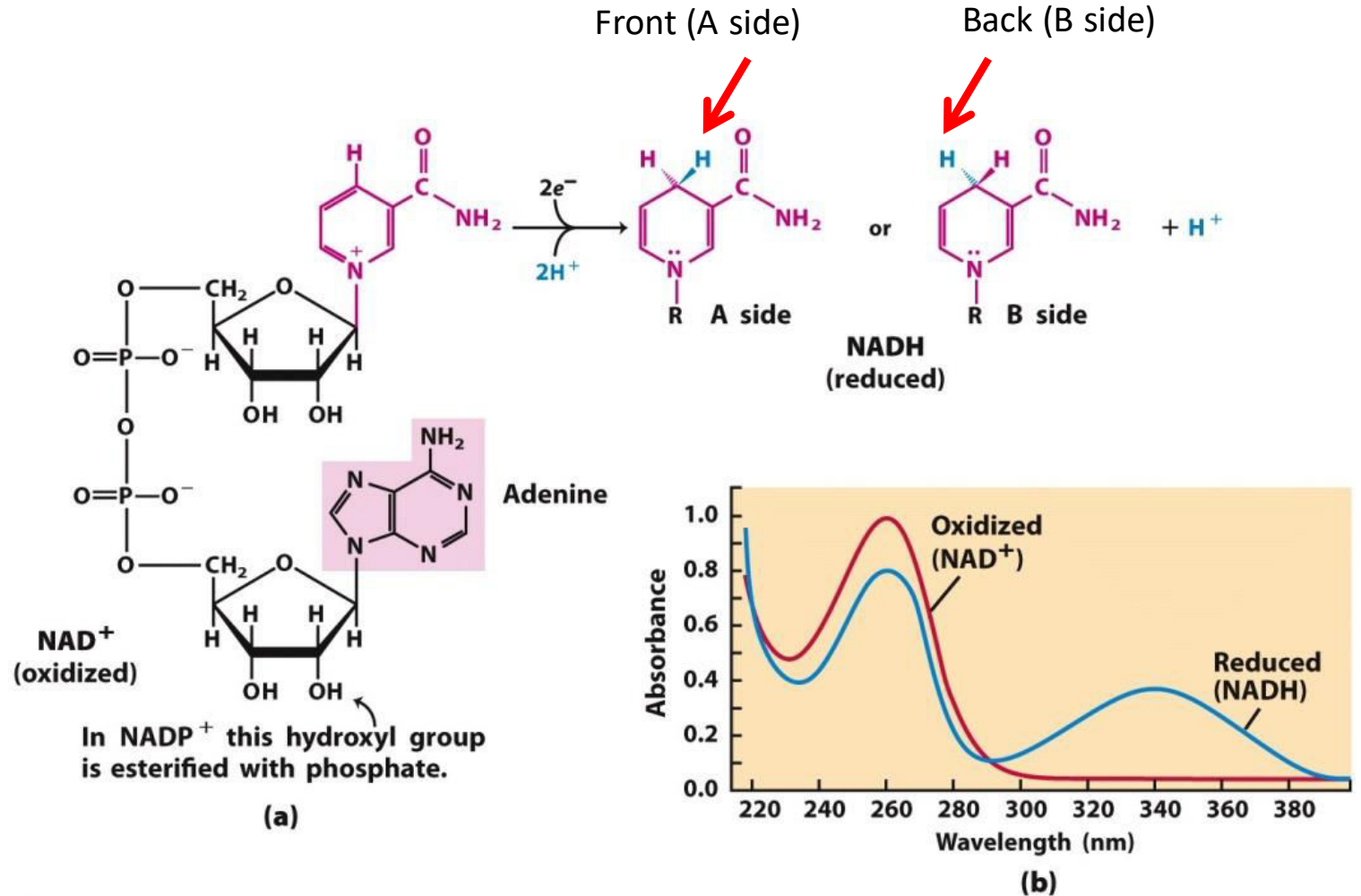


Figure 13-24
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The UV absorption spectra of NAD and NADH

TABLE 13–8 Stereospecificity of Dehydrogenases That Employ NAD^+ or NADP^+ as Coenzymes

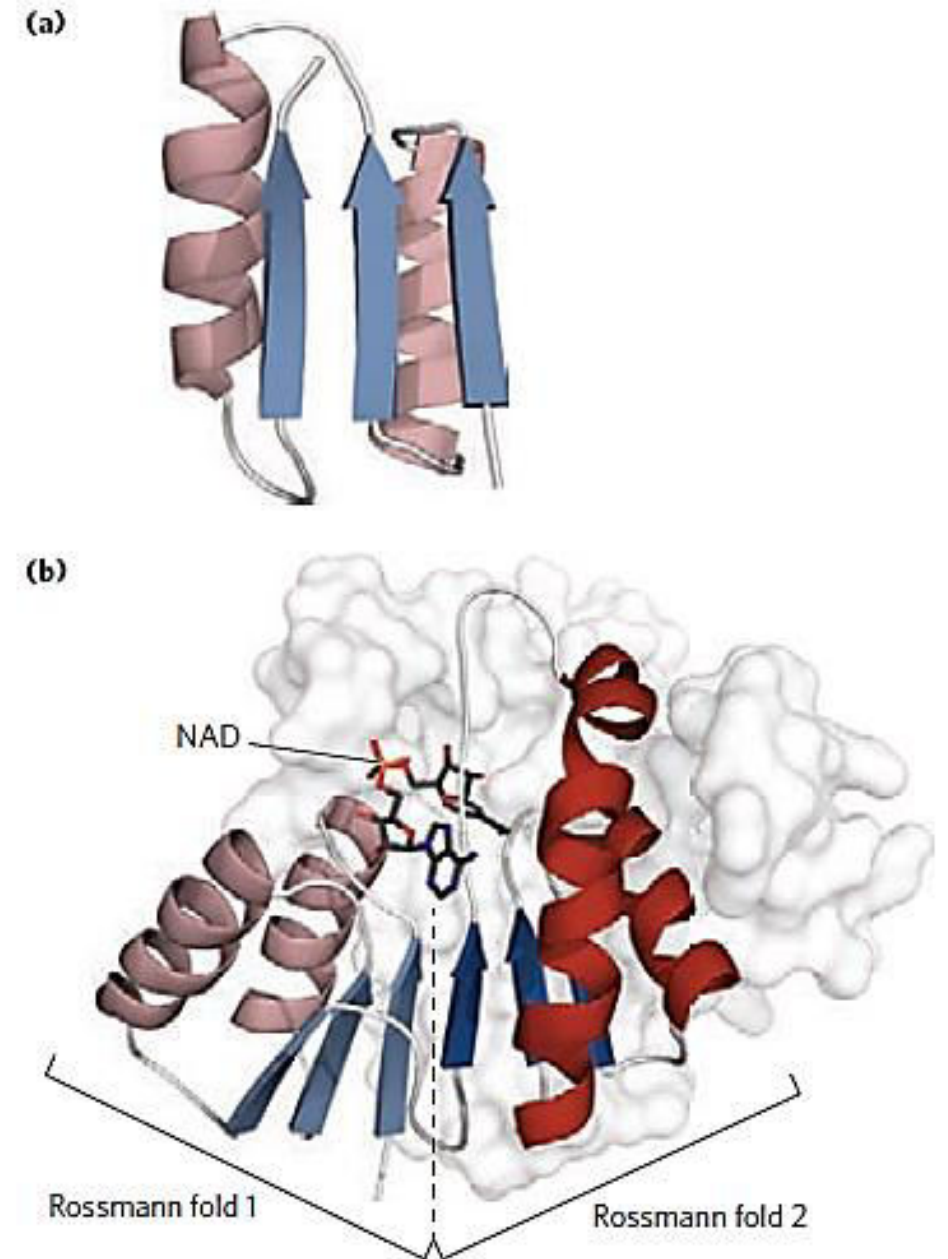
Enzyme	Coenzyme	Stereochemical specificity for nicotinamide ring (A or B)	Text page
Isocitrate dehydrogenase	NAD^+	A	643
α -Ketoglutarate dehydrogenase	NAD^+	B	644
Glucose 6-phosphate dehydrogenase	NADP^+	B	577
Malate dehydrogenase	NAD^+	A	647
Glutamate dehydrogenase	NAD^+ or NADP^+	B	702
Glyceraldehyde 3-phosphate dehydrogenase	NAD^+	B	553
Lactate dehydrogenase	NAD^+	A	563
Alcohol dehydrogenase	NAD^+	A	565

TABLE 11-7 Some Diseases Resulting from Ion Channel Defects

Ion channel	Affected gene	Disease
Na^+ (voltage-gated, skeletal muscle)	<i>SCN4A</i>	Hyperkalemic periodic paralysis (or paramyotonia congenita)
Na^+ (voltage-gated, neuronal)	<i>SCN1A</i>	Generalized epilepsy with febrile seizures
Na^+ (voltage-gated, cardiac muscle)	<i>SCN5A</i>	Long QT syndrome 3
Ca^{2+} (neuronal)	<i>CACNA1A</i>	Familial hemiplegic migraine
Ca^{2+} (voltage-gated, retina)	<i>CACNA1F</i>	Congenital stationary night blindness
Ca^{2+} (polycystin-1)	<i>PKD1</i>	Polycystic kidney disease
K^+ (neuronal)	<i>KCNQ4</i>	Dominant deafness
K^+ (voltage-gated, neuronal)	<i>KCNQ2</i>	Benign familial neonatal convulsions
Nonspecific cation (cGMP-gated, retinal)	<i>CNCG1</i>	Retinitis pigmentosa
Acetylcholine receptor (skeletal muscle)	<i>CHRNA1</i>	Congenital myasthenic syndrome
Cl^-	<i>CFTR</i>	Cystic fibrosis

Rossmann fold

- Most dehydrogenases that use NAD or NADH contain a conserved protein binding domain called the Rossmann fold
- **Rossmann fold**: consists of a six-stranded parallel beta sheet and four associated alpha helices
- Nucleotide binding domain of lactate dehydrogenase with NAD is shown
- NAD is bound to paired beta-alpha motifs of Rossmann fold

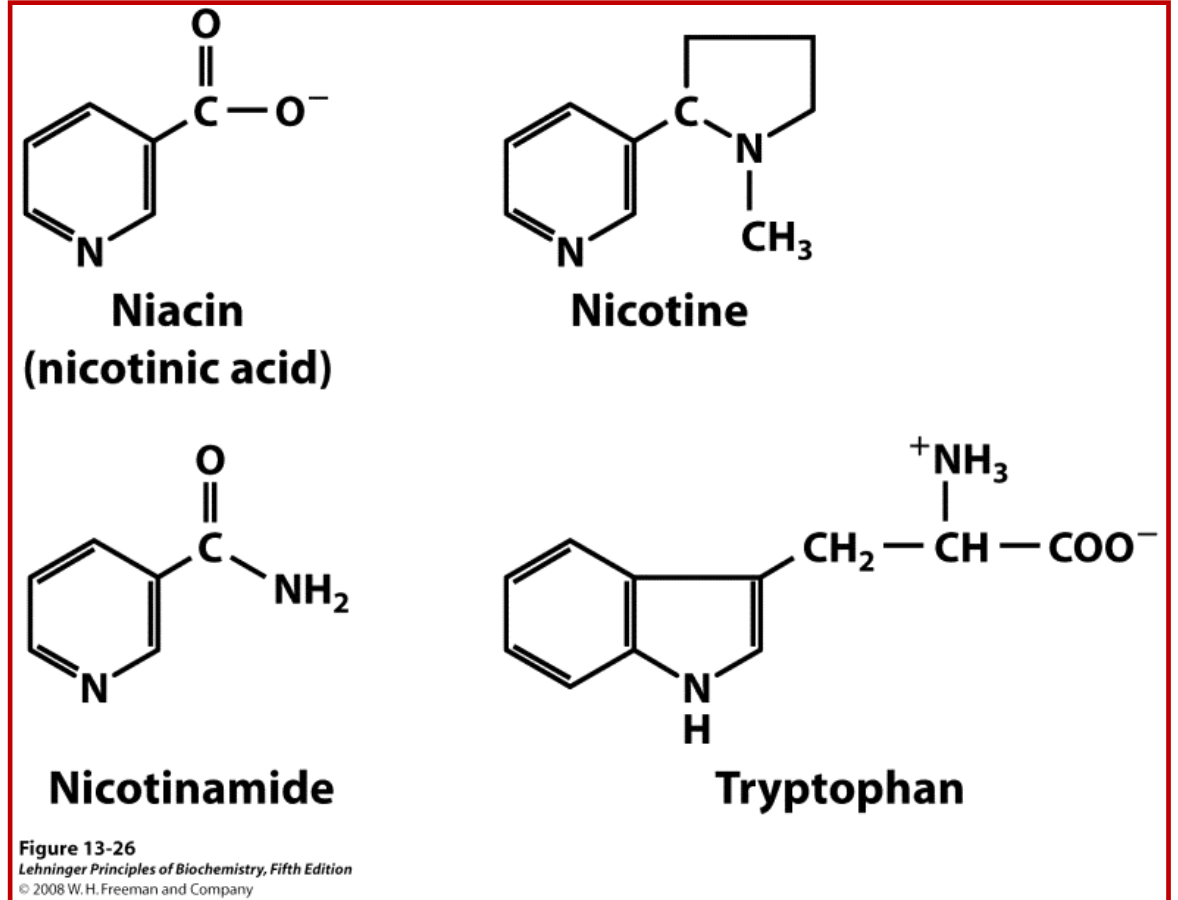


- The association between dehydrogenases and cofactor is loose → the coenzyme readily diffuse from one enzyme to another → act as **a water-soluble carrier of electron form one metabolite to another**
- In overall reaction, there is not net production or consumption of NAD^+ or NADH → no concentration change of NAD^+ or NADH .. and NAD^+ or NADH get recycled repeatedly



Dietary deficiency of Niacin causes Pellagra

- Most coenzyme: derived from vitamins
- NAD and NADP: derived from vitamin niacin (=nicotinic acid)
- **Human cannot synthesize sufficient quantities of niacin**
- **Niacin** derived from tryptophan
- In the laboratory, nicotinic acid was first produced by oxidation of the natural product.. nicotine—thus the name



Most coenzyme: derived from vitamins

NAD and NADP: derived from vitamin niacin (=nicotinic acid)

Human cannot synthesize sufficient quantities of niacin

Maize: content low tryptophan

Niacin deficiency: affects all the NAD(P)-dependent dehydrogenase

→ causes the serious human disease pellagra (=rough skin):

characterized by the Three Ds (dermatitis, diarrhea and dementia)

→ death

Too much alcohol drinking → reduce niacin absorption from the intestine

.. cont

- Niacin deficiency: affects all the NAD(P)-dependent dehydrogenase → causes the serious human disease pellagra (=rough skin): characterized by the **Three Ds (dermatitis, diarrhea and dementia)**
→ death
- similar disease **black tongue in dogs**
- **Niacin is curative agent (scientists)**
- Too much alcohol drinking → reduce niacin absorption from the intestine
- Both nicotinic acid and nicotinamide cure pellagra, but nicotine (from cigarettes or elsewhere) has no curative activity



Niacin (nicotinic acid) and its derivative nicotinamide

- Niacin (nicotinic acid) and its derivative nicotinamide.
- The biosynthetic precursor of these compounds is tryptophan.
- In the laboratory, nicotinic acid was first produced by oxidation of the natural product.. nicotine—thus the name.
- Both nicotinic acid and nicotinamide cure pellagra, but nicotine (from cigarettes or elsewhere) has no curative activity

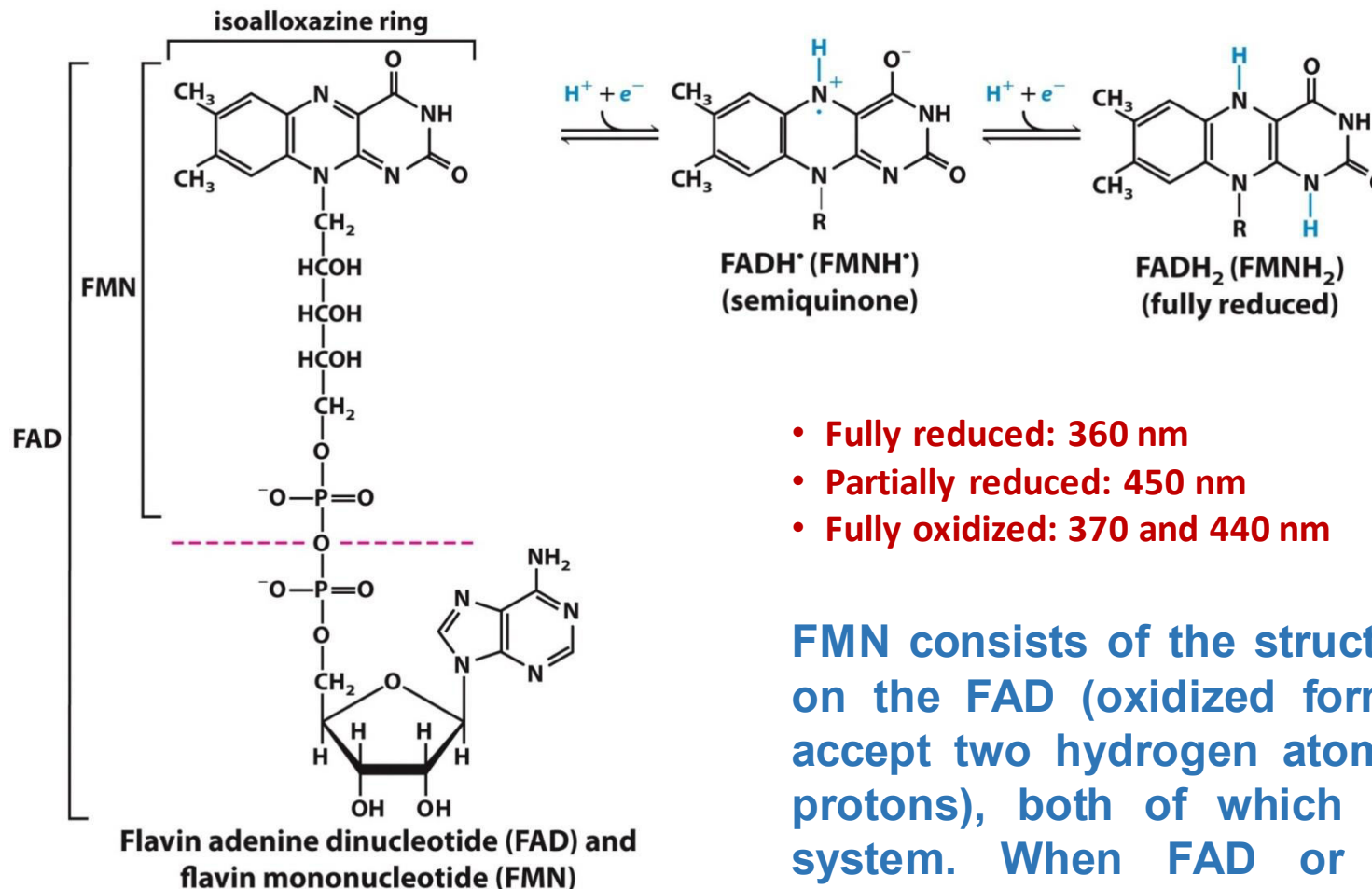
Flavoproteins

- Flavoproteins: enzymes that catalyze oxidation-reduction reactions using coenzyme
 1. Flavin mononucleotide (FMN)
 2. Flavin adenine dinucleotide (FAD)
 3. Flavin nucleotides → derived from the vitamin riboflavin
 4. Flavoproteins act in a quite different role, as light receptors
- The fused ring structure of the flavin nucleotide (isoalloxazine ring)
- **Isoalloxazine ring** undergoes reversible reduction, accepting either one or two electrons in the form of **one or two hydrogen atoms** (each atom an electron plus a proton) from a reduced substrate
- When a fully oxidized flavin nucleotide accepts only one electron (one hydrogen atom), **the semiquinone form of the isoalloxazine ring** is produced
- The fully reduced forms are abbreviated FADH₂ and FMNH₂

Oxidized and reduced forms of FAD and FMN

Some enzymes use flavin nucleotides for oxidation-reduction

- Flavin nucleotides are tightly bound in flavoproteins



- Fully reduced: 360 nm
- Partially reduced: 450 nm
- Fully oxidized: 370 and 440 nm

FMN consists of the structure above the dashed line on the FAD (oxidized form). The flavin nucleotides accept two hydrogen atoms (two electrons and two protons), both of which appear in the flavin ring system. When FAD or FMN accepts only one hydrogen atom, the semiquinone, a stable free radical, forms.

Enzymes that use FAD or FMN as coenzymes

TABLE 13-9 Some Enzymes (Flavoproteins) That Employ Flavin Nucleotide Coenzymes

Enzyme	Flavin nucleotide	Text page(s)
Acyl-CoA dehydrogenase	FAD	673
Dihydrolipoyl dehydrogenase	FAD	637
Succinate dehydrogenase	FAD	646
Glycerol 3-phosphate dehydrogenase	FAD	759
Thioredoxin reductase	FAD	917
NADH dehydrogenase (Complex I)	FMN	738–739
Glycolate oxidase	FMN	813

Table 13-9
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Cryptochromes

- Family of flavoproteins,
- Found in both bacteria and eukaryotes
- mediate the effects of blue light on plant development and the effects of light on mammalian circadian rhythms (oscillations in physiology and biochemistry, with a 24-hour period)
- **Homologs of Photolyases**
- **Photolyases: Use energy of light to repair defects in DNA**

Cryptochromes: Family of flavoproteins, widely distributed in the eukaryotic phyla, that mediate the effects of blue light on plant development and the effects of light on mammalian circadian rhythms (oscillations in physiology and biochemistry, with a 24-hour period).

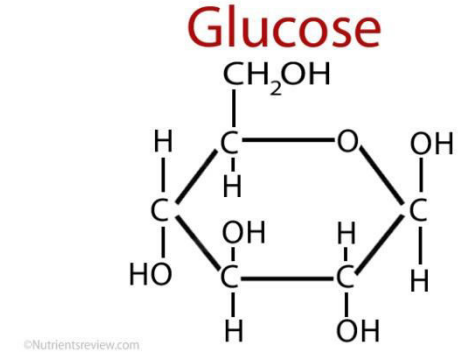
The cryptochromes are homologs of another family of flavoproteins, the photolyases. Found in both bacteria and eukaryotes, **photolyases** use the energy of absorbed light to repair chemical defects in DNA

End

GLUCOSE METABOLISM

In animals and vascular plants, glucose has three major fates:

1. It may be stored (as a polysaccharide , i-e., glycogen or starch)
2. Oxidized to a three-carbon compound (pyruvate) via glycolysis to provide ATP and metabolic intermediates.
3. Oxidized via the pentose phosphate (phosphogluconate) pathway to yield ribose 5-phosphate for nucleic acid synthesis and NADPH for reductive biosynthetic processes.



Glucose is a simple sugar with the molecular formula $C_6H_{12}O_6$, which means that it is a molecule that is made of six carbon atoms, twelve hydrogen atoms, and six oxygen atoms. Glucose circulates in the blood of animals as blood sugar.

- Organisms that do not have access to glucose from other sources must make it
- **Photosynthetic organisms** make glucose by first reducing atmospheric CO₂ to trioses, then converting the trioses to glucose.
- **Non-photosynthetic cells** make glucose from simpler three and four-carbon precursors by the process of **gluconeogenesis**, effectively reversing glycolysis in a pathway that uses many of the glycolytic enzymes.

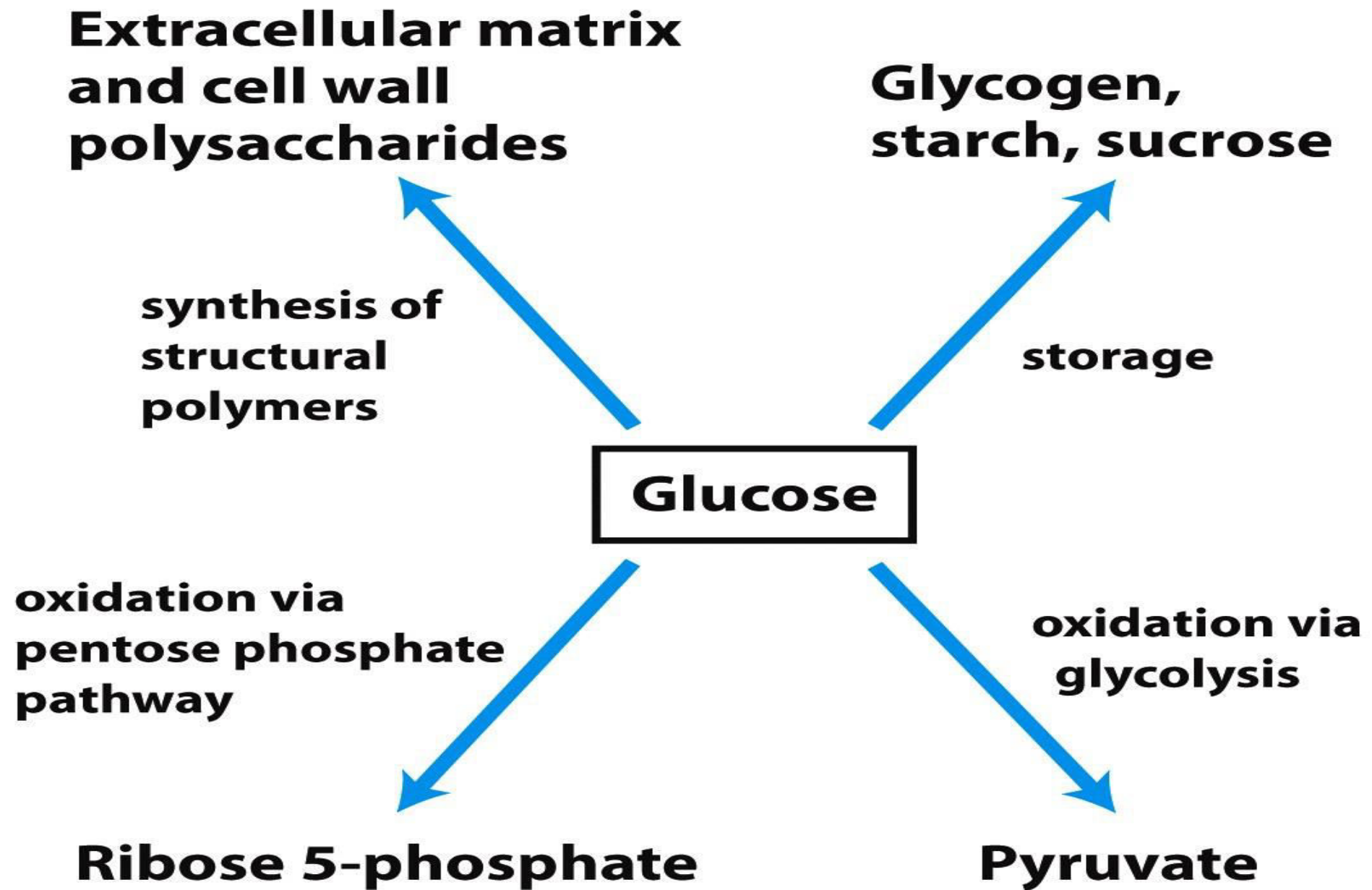


Figure 14-1
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GLYCOLYSIS

- In **glycolysis** (from the Greek *glykys*, “sweet” or “sugar,” and *lysis*, “splitting”), a molecule of glucose $C_6H_{12}O_6$ is degraded in a series of enzyme-catalyzed reactions to yield two molecules of the three-carbon compound **pyruvate** $CH_3COCOO^- + H^+$.
- During glycolysis, the free energy released is used to form the high-energy compounds **ATP** (adenosine triphosphate) and **NADH** (reduced nicotinamide adenine dinucleotide).
- The pathway of glycolysis was explained by Otto Warburg and Hans von Euler-Chelpin **in yeast** and in **muscle** by Gustav Embden and Otto Meyerhof in 1930s.

- The most common type of glycolysis is the *Embden–Meyerhof–Parnas* (**EMP pathway**), which was discovered by Embden and Meyerhof and J.K. Parnas.
- Glycolysis occurs in most organisms in the **cytosol** of the cell.
- **It is an oxygen independent metabolic pathway**, meaning that it does not use molecular oxygen (i.e. atmospheric oxygen) for any of its reactions.
- **The products of glycolysis, that is, pyruvate and NADH + H⁺)** are sometimes disposed off using atmospheric oxygen.
- **Aerobic Glycolysis:** When molecular oxygen is used in the disposal of the products of glycolysis the process is usually referred to as **Aerobic Glycolysis**.
- **Anaerobic Glycolysis:** When no oxygen is used, the process is said to be **Anaerobic**.

2 phases of GLYCOLYSIS

1. The Preparatory Phase – in which ATP is consumed, hence also known as the **investment phase**
 2. The Pay Off Phase – in which ATP is produced
- Occurs in 10 steps, **the first 5** of which constitute the ***preparatory phase.***

The Preparatory Phase

- **step 1:** The first step in glycolysis is phosphorylation of glucose at the hydroxyl group on C-6 by a family of enzymes called **hexokinases** to form **glucose-6-phosphate** (G6P).
- **step 2:** The D-glucose 6-phosphate thus formed is converted to D-fructose 6-phosphate,
- **step 3:** which is again phosphorylated, this time at C-1, to yield D-fructose 1,6-bisphosphate
- **For both phosphorylations, ATP is the phosphoryl group donor.**
- **step 4:** Fructose 1,6-bisphosphate is split to yield two three-carbon molecules, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (step 4); this is the “lysis” step that gives the pathway its name.
- **step 5:** The dihydroxyacetone phosphate is isomerized to a second molecule of glyceraldehyde 3-phosphate (step 5), ending the first phase of glycolysis.

The Preparatory Phase

To summarize:

In the preparatory phase of glycolysis the energy of ATP is invested, raising the free-energy content of the intermediates, and the carbon chains of all the metabolized hexoses are converted to a common product, glyceraldehyde 3-phosphate.

Note that during preparatory phase, 2 molecules of ATP are invested before the cleavage of glucose into two three-carbon pieces.

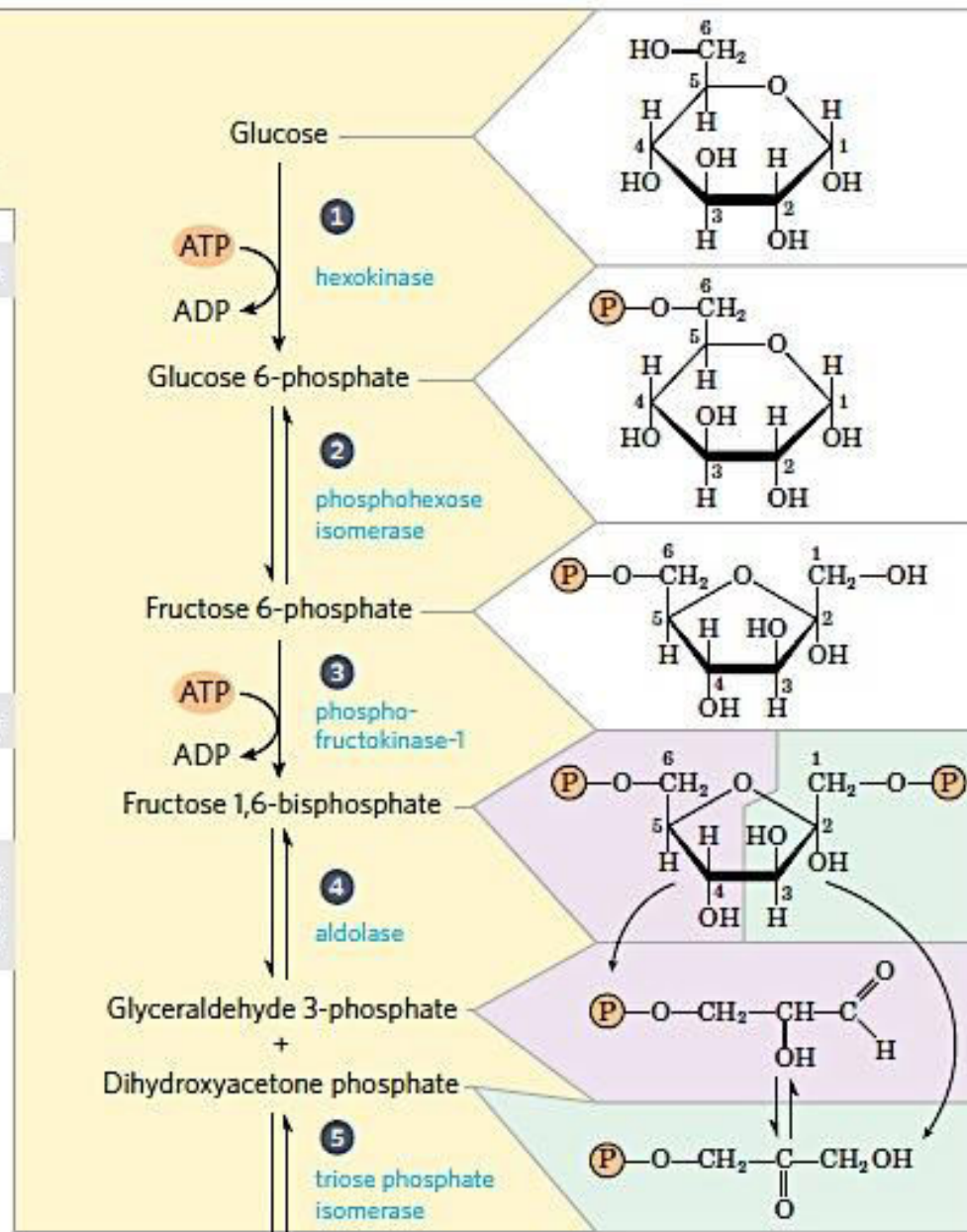
(a) Preparatory phase

Phosphorylation of glucose
and its conversion to
glyceraldehyde 3-phosphate

first priming reaction

second priming reaction

cleavage of 6-carbon sugar
phosphate to two 3-carbon
sugar phosphates



Preparatory phase of Glycolysis

For each molecule of glucose that passes through the preparatory phase (a), two molecules of glyceraldehyde 3-phosphate are formed; both pass through the payoff phase

GLUCOSE METABOLISM

GLYCOLYSIS

The Pay-off Phase

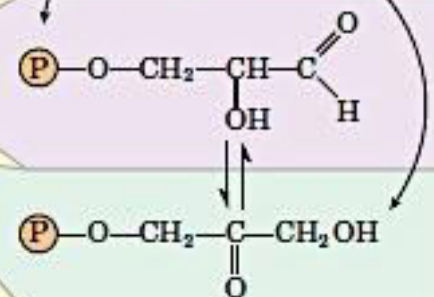
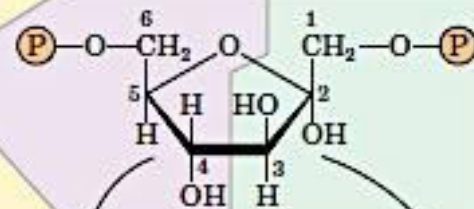
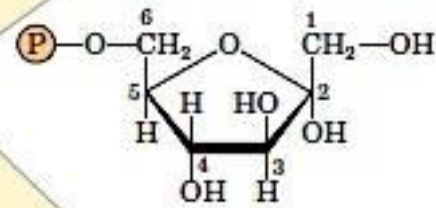
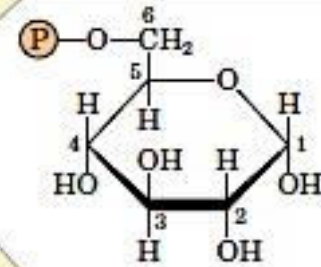
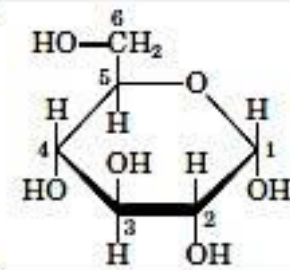
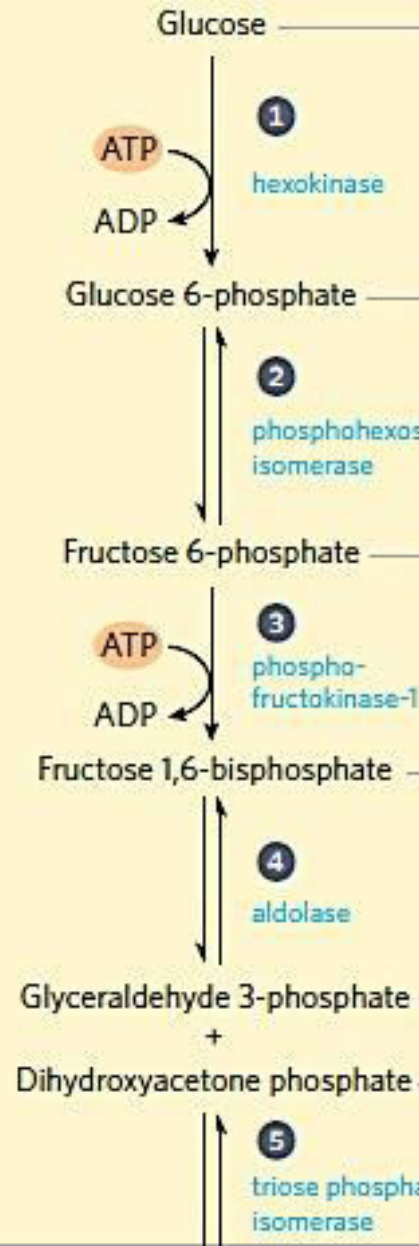
(a) Preparatory phase

Phosphorylation of glucose
and its conversion to
glyceraldehyde 3-phosphate

first priming reaction

second priming reaction

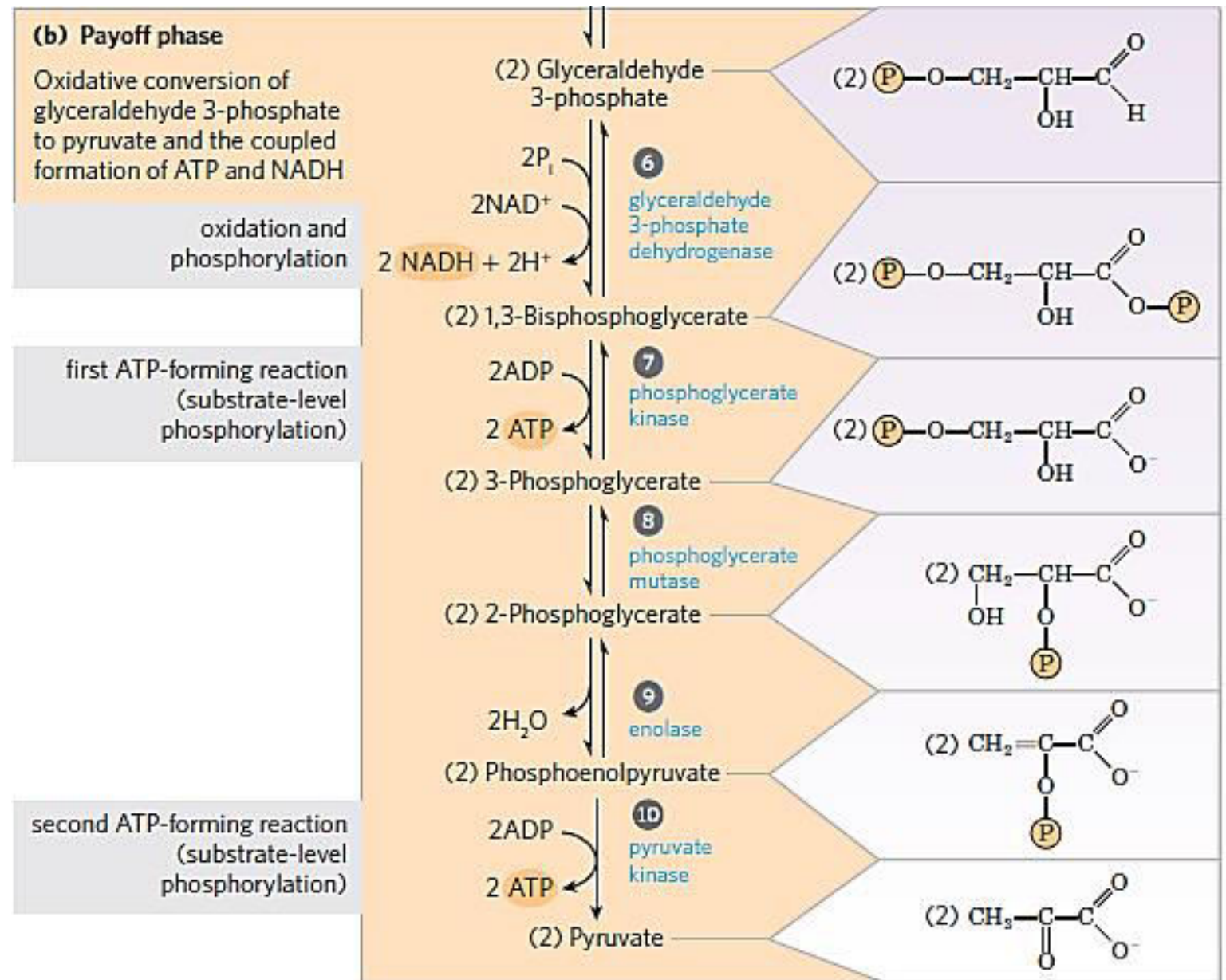
cleavage of 6-carbon sugar
phosphate to two 3-carbon
sugar phosphates



The Pay-off Phase

- The energy gain comes in the *payoff phase* of glycolysis
- **step 6:** Each molecule of glyceraldehyde 3-phosphate is oxidized and phosphorylated by inorganic phosphate (*not* by ATP) to form 1,3-bisphosphoglycerate
- Energy is also conserved in the formation of **two molecules of the electron carrier NADH per molecule of glucose**
- **steps 7 through 10:** Much of this energy is conserved by the coupled **phosphorylation of four molecules of ADP to ATP.**
- The net yield is two molecules of ATP per molecule of glucose, because two molecules of ATP were invested in the preparatory phase.

- Pyruvate is the end product of the **second phase of glycolysis**.
- For each glucose molecule, **two ATP are consumed in the preparatory phase and four ATP are produced in the payoff phase**,
- This gives a net yield of two ATP per molecule of glucose converted to pyruvate.
- each phosphoryl group, represented here as P, has two negative charges



Pay-Off phase of Glycolysis

Energy yield during Glycolysis

- Two ATP are consumed in the preparatory phase and four ATP are produced in the payoff phase, giving a net yield of **two ATP** per molecule of glucose converted to pyruvate. Note that each phosphoryl group, represented as P, has two negative charges (2PO_3^{-2})
- Much of this energy is conserved by the coupled phosphorylation of four molecules of ADP to ATP.
- Energy is also conserved in the payoff phase in the formation of **two molecules of the electron carrier NADH per molecule of glucose.**

- In the sequential reactions of glycolysis, three types of chemical transformations are noteworthy:

- (1) degradation of the carbon skeleton of glucose to yield pyruvate

- (2) phosphorylation of ADP to ATP by compounds with high phosphoryl group transfer potential, formed during glycolysis

- (3) transfer of a hydride ion to NAD⁺ forming NADH.

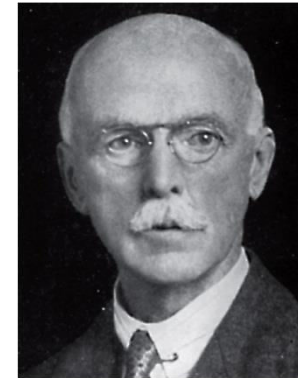
High Phosphoryl Transfer Potential Compounds. These compounds have a **higher phosphoryl transfer potential** than that of ATP and can be used to phosphorylate ADP to form ATP.

GLUCOSE METABOLISM

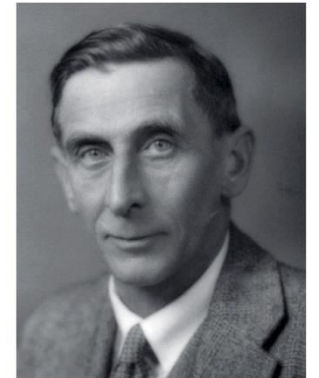
**Preparatory phase of Glycolysis Requires
ATP**

Experiment by A. Harden and W. Young

- Preparatory phase of glycolysis, two molecules of ATP are invested and the hexose chain is cleaved into **two triose phosphates**
- In 1906, **Arthur Harden and William Young** tested their hypothesis that **inhibitors of proteolytic enzymes would stabilize the glucose fermenting enzymes in yeast extract.**
- They added blood serum (**known to contain inhibitors of proteolytic enzymes**) to yeast extracts and observed the predicted stimulation of glucose metabolism.
- However, in a control experiment with the boiling serum, they discovered that boiled serum was just as effective at stimulating glycolysis



Arthur Harden
1865–1940



William Young
1878–1942

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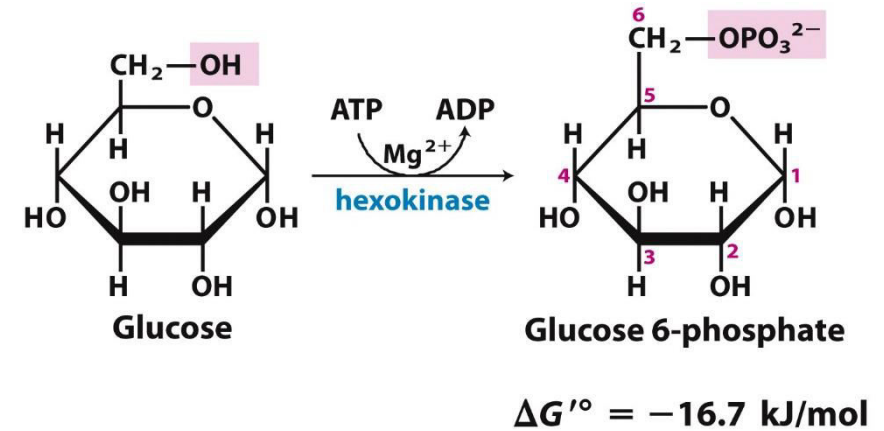
Cont..

- **Harden and Young** soon discovered that glucose added to their yeast extract was converted to a hexose bisphosphate (the “Harden- Young ester,” eventually identified as **fructose 1,6-bisphosphate**).
- Careful examination revealed that inorganic phosphate was responsible for the stimulation.
- This was the beginning of investigations on the role of organic esters and anhydrides of phosphate in biochemistry, which has led to our current understanding of the central role of **phosphoryl group transfer in biology**.

Preparatory phase of Glycolysis Requires ATP

1. *Phosphorylation of Glucose*

- In the first step of glycolysis, glucose is activated for subsequent reactions by its phosphorylation at C-6 to yield **glucose 6-phosphate**, with ATP as the phosphoryl donor:
- This reaction is irreversible under intracellular conditions, and catalyzed by **hexokinase**.
- The acceptor in the case of **hexokinase** is a **hexose**, normally **D-glucose**, hexokinase also catalyzes the phosphorylation of other common hexoses, such as D-fructose and D-mannose, in some tissues.



Unnumbered 14 p532a
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- Hexokinase requires Mg^{+2} for its activity, because the true substrate of the enzyme is not ATP^{-4} but the MgATP^{-2} complex
- Hexokinase and other nine enzymes of are soluble cytosolic proteins

Note:

- **Isozymes**

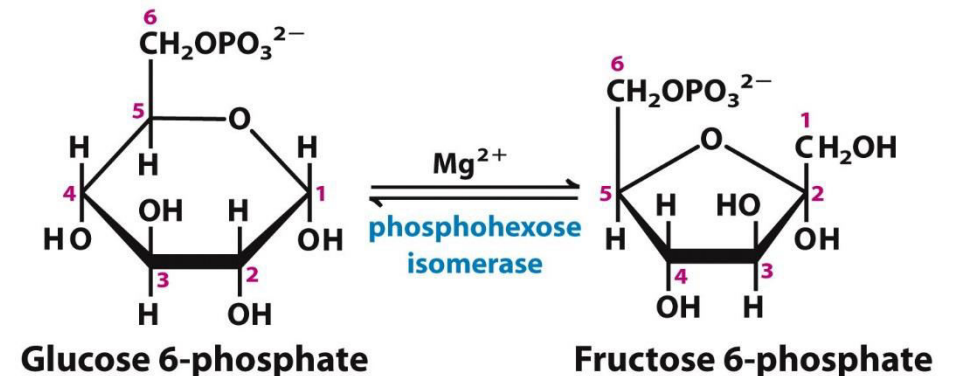
Two or more enzymes that catalyze the same reaction but are encoded by different genes are called **isozymes**

- **Kinase:**

- Kinases are a subclass of transferases...catalyse transfer of phosphate group from phosphate donating molecule to specific substrates

2. Conversion of Glucose 6-Phosphate to Fructose 6-Phosphate

- The enzyme **phosphohexose isomerase** (**phosphoglucose isomerase**) catalyzes the reversible isomerization of glucose 6-phosphate, an **aldose**, to **fructose 6-phosphate**, a **ketose**.
- The mechanism for this reaction involves an enediol intermediate
- Enediol**: An **enediol** is an alkene enol with a hydroxyl group attached to both carbon atoms of the carbon double bond.



$$\Delta G'^{\circ} = 1.7 \text{ kJ/mol}$$

..cont

- 3 steps for ***Conversion of Glucose 6-Phosphate to Fructose 6-Phosphate*** (phosphohexose isomerase reaction)

1. opening of ring

2. Isomerization of glucose to fructose through formation of enediol intermediate

3. Closing of fructose ring

Fig 14-5: The phosphohexose isomerase reaction.

The ring opening and closing reactions (steps 1 and 4) are catalyzed by an active-site **His residue**, by mechanisms omitted here for simplicity.

The proton (light red) initially at C-2 is made more easily abstractable by electron withdrawal by the adjacent carbonyl and nearby hydroxyl groups. After its transfer from C-2 to the active-site **Glu residue (a weak acid)**, the proton is freely exchanged with the surrounding solution; that is, the proton abstracted from C-2 in step 2 is not necessarily the same one that is added to C-1 in step 3.

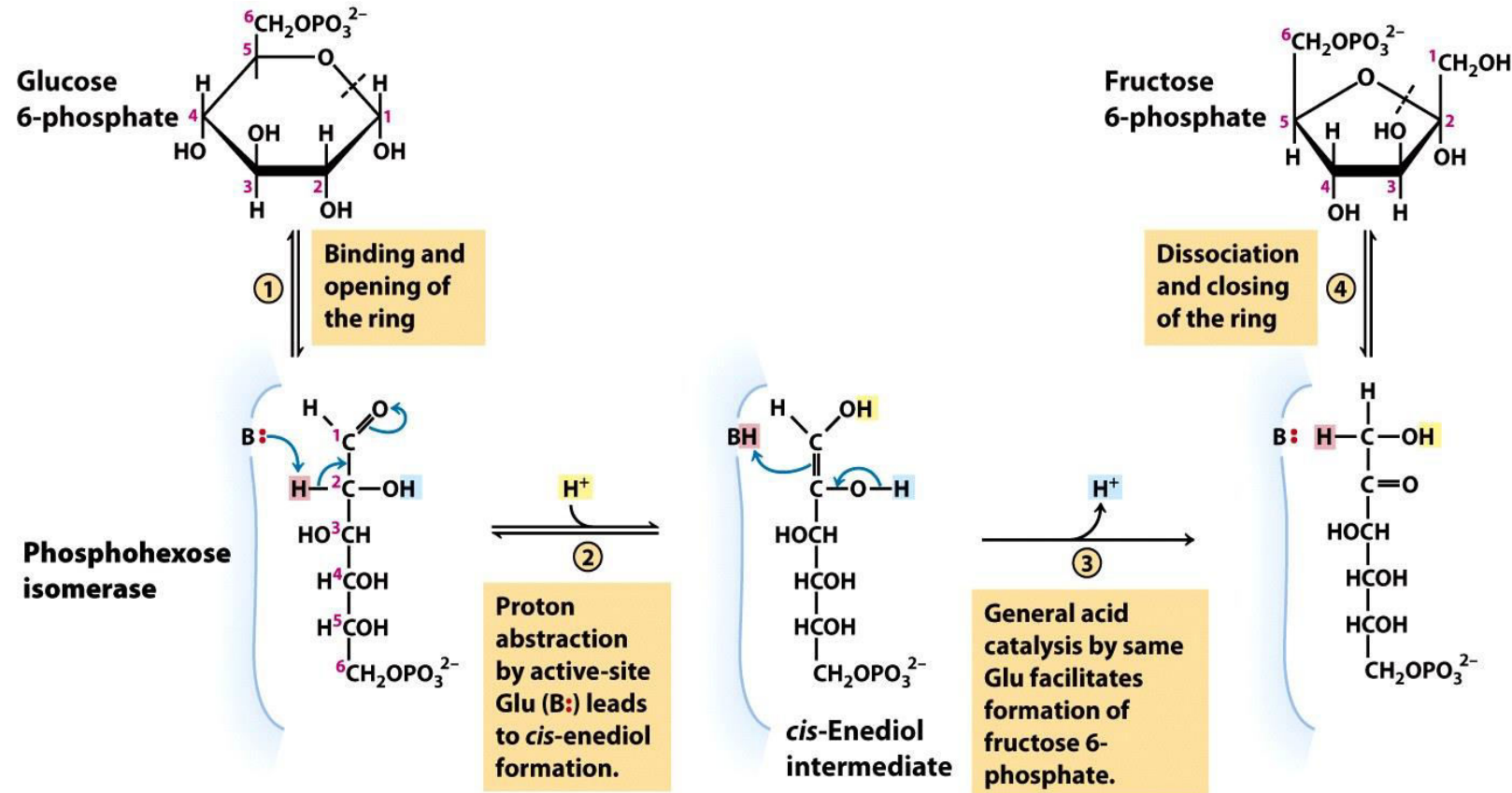
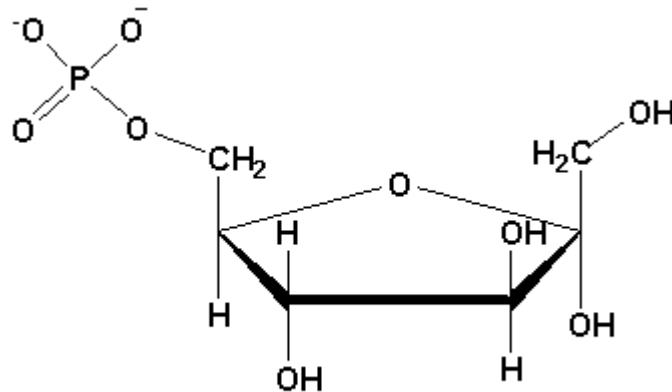


Figure 14-4
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GLUCOSE METABOLISM

**Preparatory phase of Glycolysis Requires
ATP - 2**



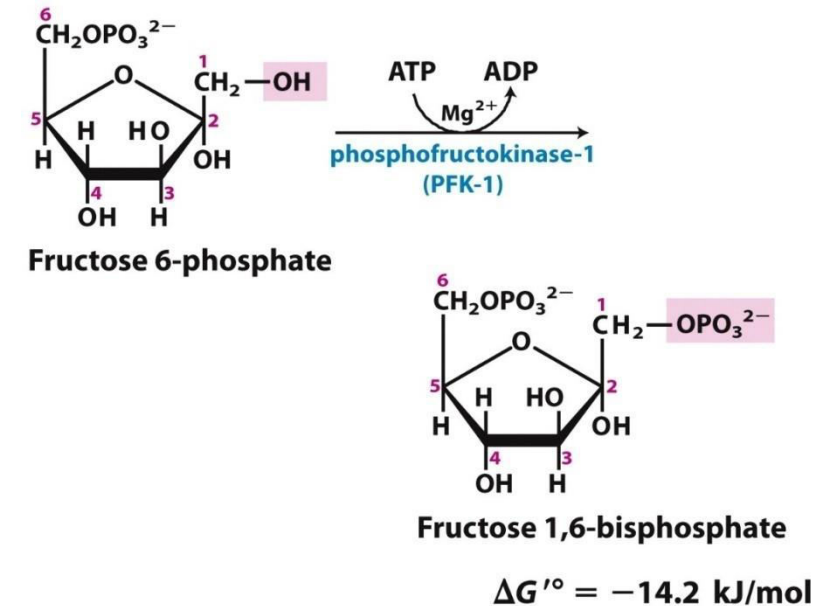
Fructose-6-phosphate (F6P)

3. Phosphorylation of Fructose 6-Phosphate to Fructose 1,6-Bisphosphate

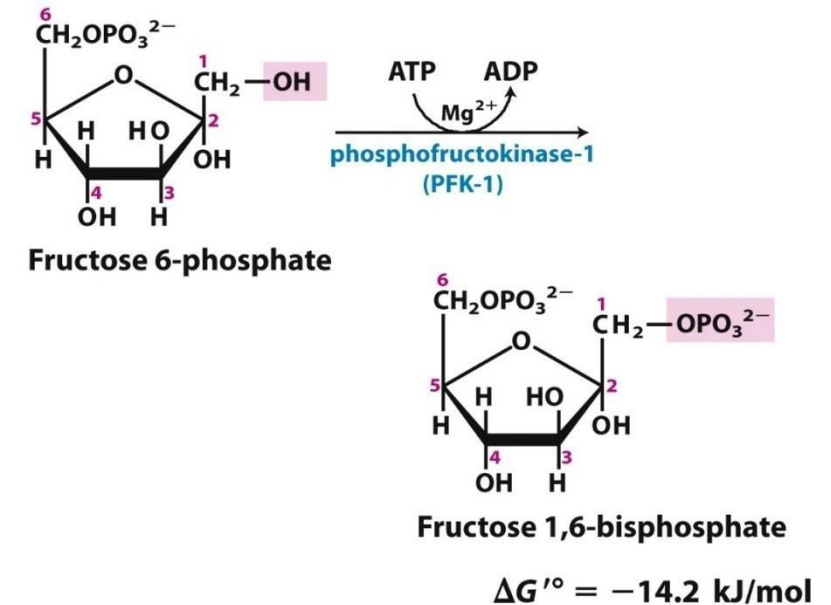
Phosphofructokinase-1 (PFK-1) catalyzes the transfer of a phosphoryl group from ATP to fructose 6-phosphate to yield **fructose 1,6-bisphosphate**.

KEY CONVENTION:

Compounds that contain two phosphate or phosphoryl groups attached at different positions in the molecule are named **bisphosphates (or bisphospho compounds)**; for example, fructose 1,6-bisphosphate and 1,3-bisphosphoglycerate. **Compounds with two phosphates linked together as a pyrophosphoryl group are named diphosphates**; for example, adenosine diphosphate (ADP). **Similar rules apply for the naming of trisphosphates** (such as inositol 1,4,5-trisphosphate;) and **triphosphates** (such as adenosine triphosphate, ATP)



- The **PFK-1 reaction** is essentially irreversible under cellular conditions, and it is the first “committed” step in the glycolytic pathway
- Glucose 6-phosphate and fructose 6-phosphate have other possible fates, but **fructose 1,6-bisphosphate is targeted for glycolysis**
- Some bacteria and protists and perhaps all plants have a phosphofructokinase that uses pyrophosphate (PPi), not ATP, as the phosphoryl group donor in the synthesis of fructose 1,6-bisphosphate:



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- In metabolic pathways, enzymes catalyzing essentially irreversible reactions are potential sites of control
- **Phosphofructokinase-1** is subject to complex **allosteric regulation**
- The PFK1 has got binding sites for both, ATP-Mg²⁺ and F6P binding sites
- **The activity of PFK-1 enzyme and glycolysis is inhibited whenever the cell has high levels of ATP**
- In this way, the cell can increase or decrease the rate of glycolysis in response to the cell's energy requirements
- In Eukaryotes, **fructose 2,6-bisphosphate** (not to be confused with the **PFK-1 reaction product, fructose 1,6-bisphosphate**) is a potent allosteric activator of PFK-1
- Ribulose 5-phosphate, an intermediate in the pentose phosphate pathway also activates phosphofructokinase indirectly.

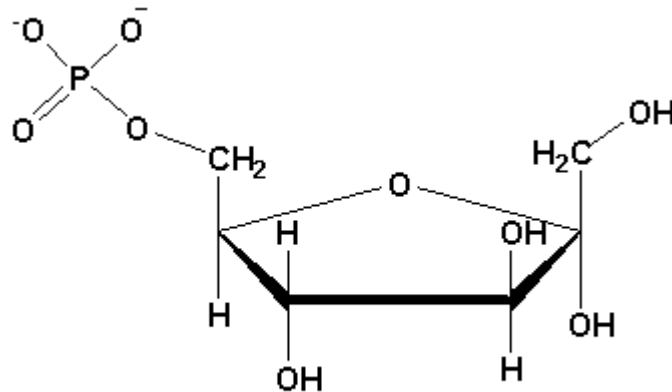
- **Imine:** An **imine** is a functional group or chemical compound containing a carbon–nitrogen double bond. The nitrogen atom can be attached to a hydrogen or an organic group.
- **Schiff base intermediate:**

They can be considered a sub-class of imines, being either secondary ketimines or secondary aldimines depending on their structure. ($R-CH=NR'$ where $R' \neq H$)

Amine: an organic compound derived from ammonia by replacement of one or more hydrogen atoms by organic groups.

GLUCOSE METABOLISM

**Preparatory phase of Glycolysis Requires
ATP - 2**

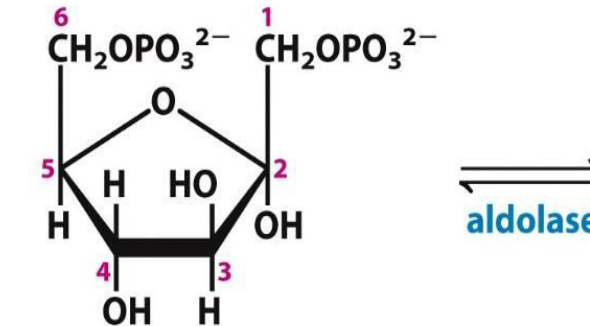


Fructose-6-phosphate (F6P)

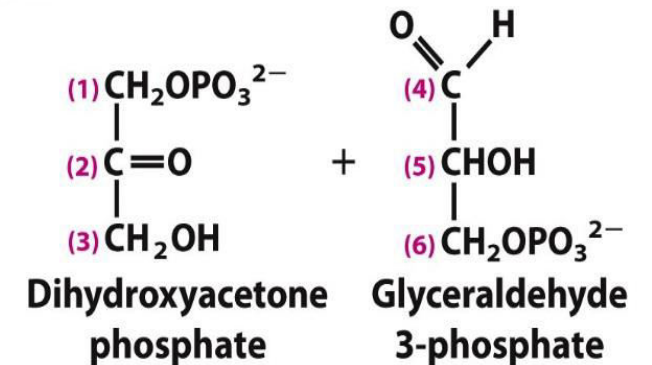
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- In Eukaryotes, **fructose 2,6-bisphosphate** (not to be confused with the **PFK-1 reaction product, fructose 1,6-bisphosphate**) is a potent allosteric activator of PFK-1
- Ribulose 5-phosphate, an intermediate in the pentose phosphate pathway also activates phosphofructokinase indirectly.

4. Cleavage of Fructose 1,6-Bisphosphate

- The enzyme **fructose 1,6-bisphosphate aldolase**, often called simply **aldolase**, catalyzes a reversible aldol condensation (see Fig. 13–4..Lehninger Edition 6).
- Fructose 1,6-bisphosphate is cleaved to yield two different triose phosphates, **glyceraldehyde 3-phosphate**, an aldose, and **dihydroxyacetone phosphate**, a ketose.



Fructose 1,6-bisphosphate



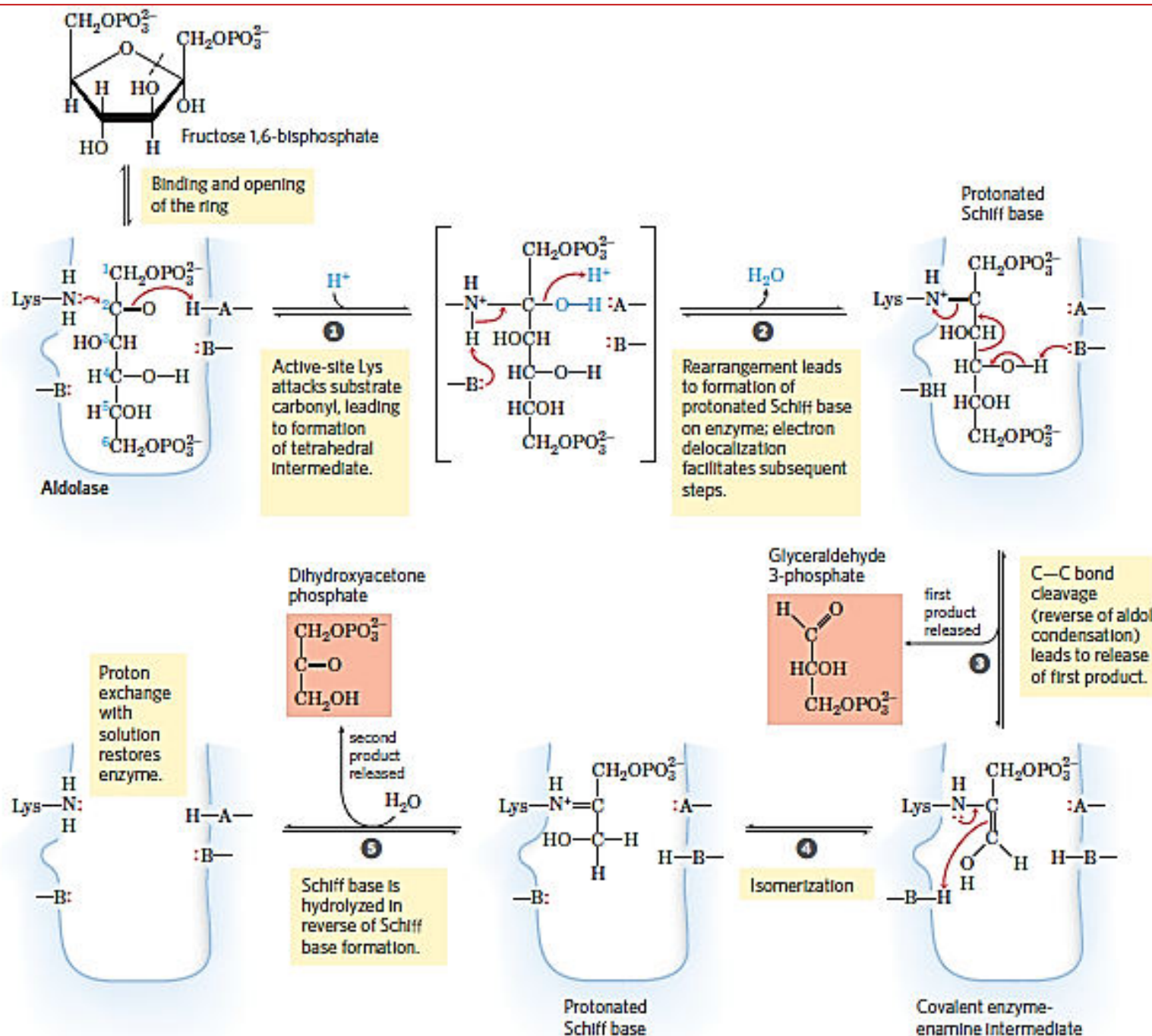
$$\Delta G'^{\circ} = 23.8 \text{ kJ/mol}$$

Two classes of aldolases

- There are two classes of aldolases.
- **Class I**, found in animals and plants, form the Schiff base intermediate
- **Class II**, in fungi and bacteria, do not form the Schiff base intermediate

- **Imine:** An **imine** is a functional group or chemical compound containing a carbon–nitrogen double bond. The nitrogen atom can be attached to a hydrogen or an organic group.
- **Schiff base intermediate:**

They can be considered a sub-class of imines, being either secondary ketimines or secondary aldimines depending on their structure. ($R-CH=NR'$ where $R' \neq H$)



• MECHANISM

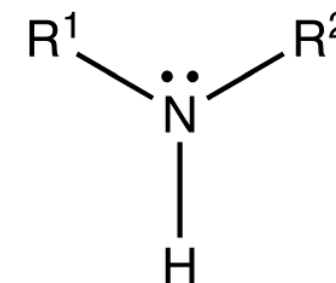
- FBPA, class I aldolase reaction. The reaction shown here is the reverse of **an aldol condensation**.
- Cleavage between C-3 and C-4 depends on the presence of the **carbonyl group at C-2**, which is converted to an imine on the enzyme.
- A and B represent amino acid residues that serve as general acid (A) or base (B).

- **Imine:** An **imine** is a functional group or chemical compound containing a carbon–nitrogen double bond. The nitrogen atom can be attached to a hydrogen or an organic group.
- **Schiff base intermediate:**

They can be considered a sub-class of imines, being either secondary ketimines or secondary aldimines depending on their structure. ($R-CH=NR'$ where $R' \neq H$)

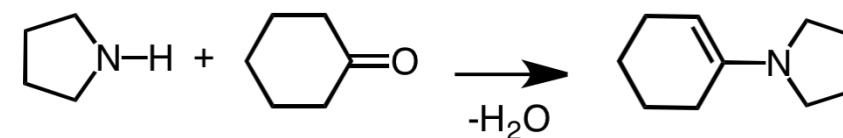
- **Secondary amine:**

Secondary amines have two organic substituents (alkyl, aryl or both) bound to the nitrogen together with one hydrogen.



- **Enamine:**

An enamine is an unsaturated compound derived by the condensation of an aldehyde or ketone with a secondary amine.

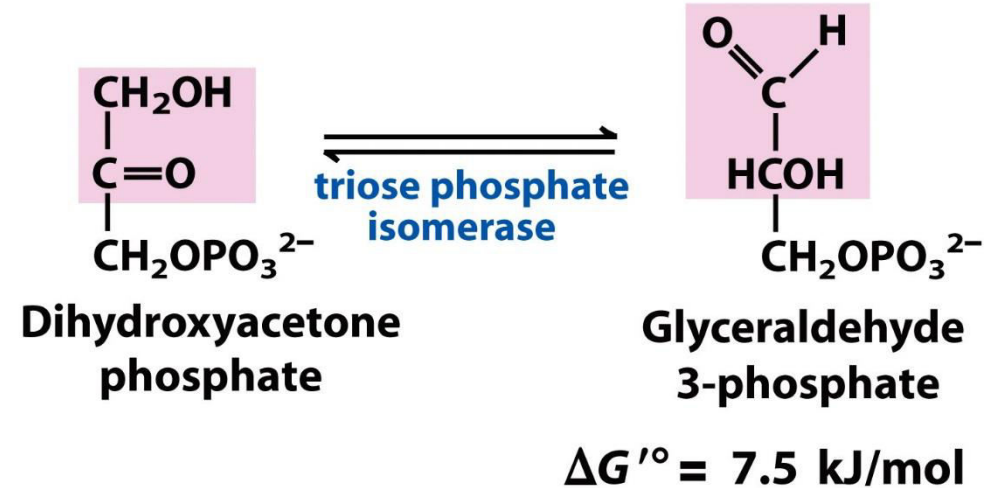


GLUCOSE METABOLISM

Lesson 21

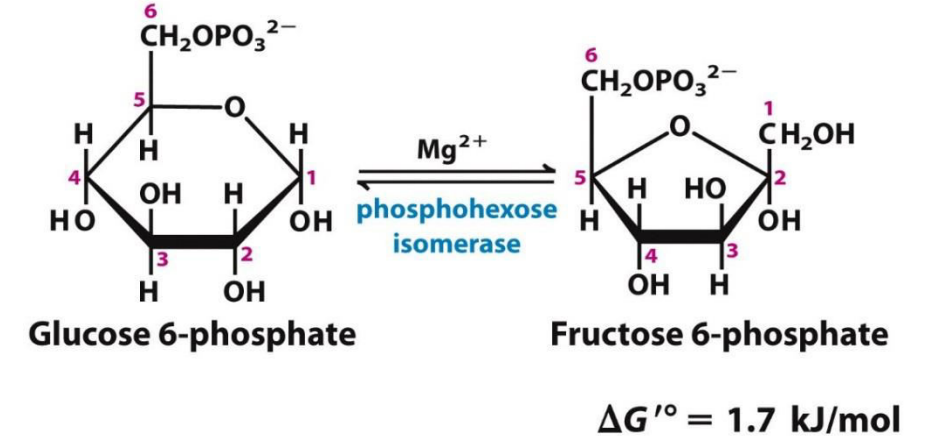
5. Interconversion of the Triose Phosphates

- Only one of the two triose phosphates formed by aldolase, glyceraldehyde 3-phosphate, can be directly degraded in the subsequent steps of glycolysis.
- The other product, **dihydroxyacetone phosphate**, is rapidly and reversibly converted to glyceraldehyde 3-phosphate by the **fifth** enzyme of the glycolytic sequence, **triose phosphate isomerase**:

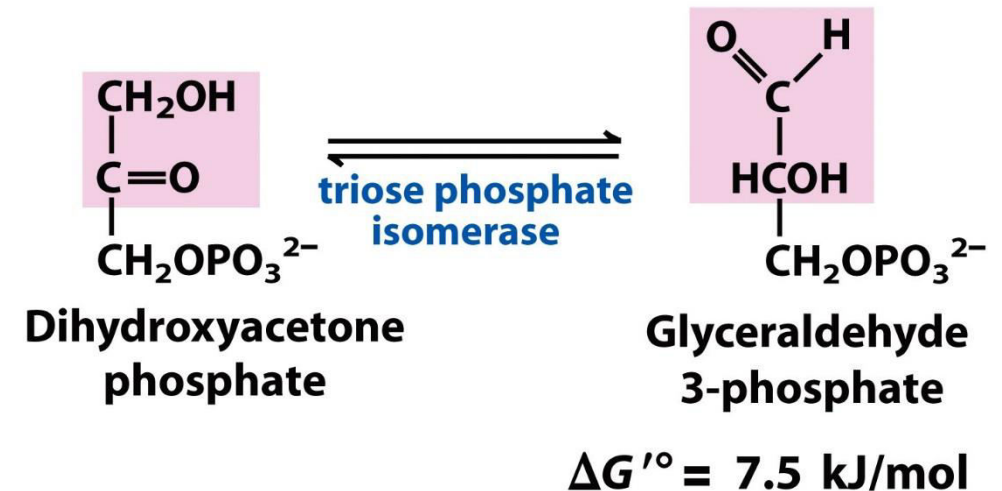


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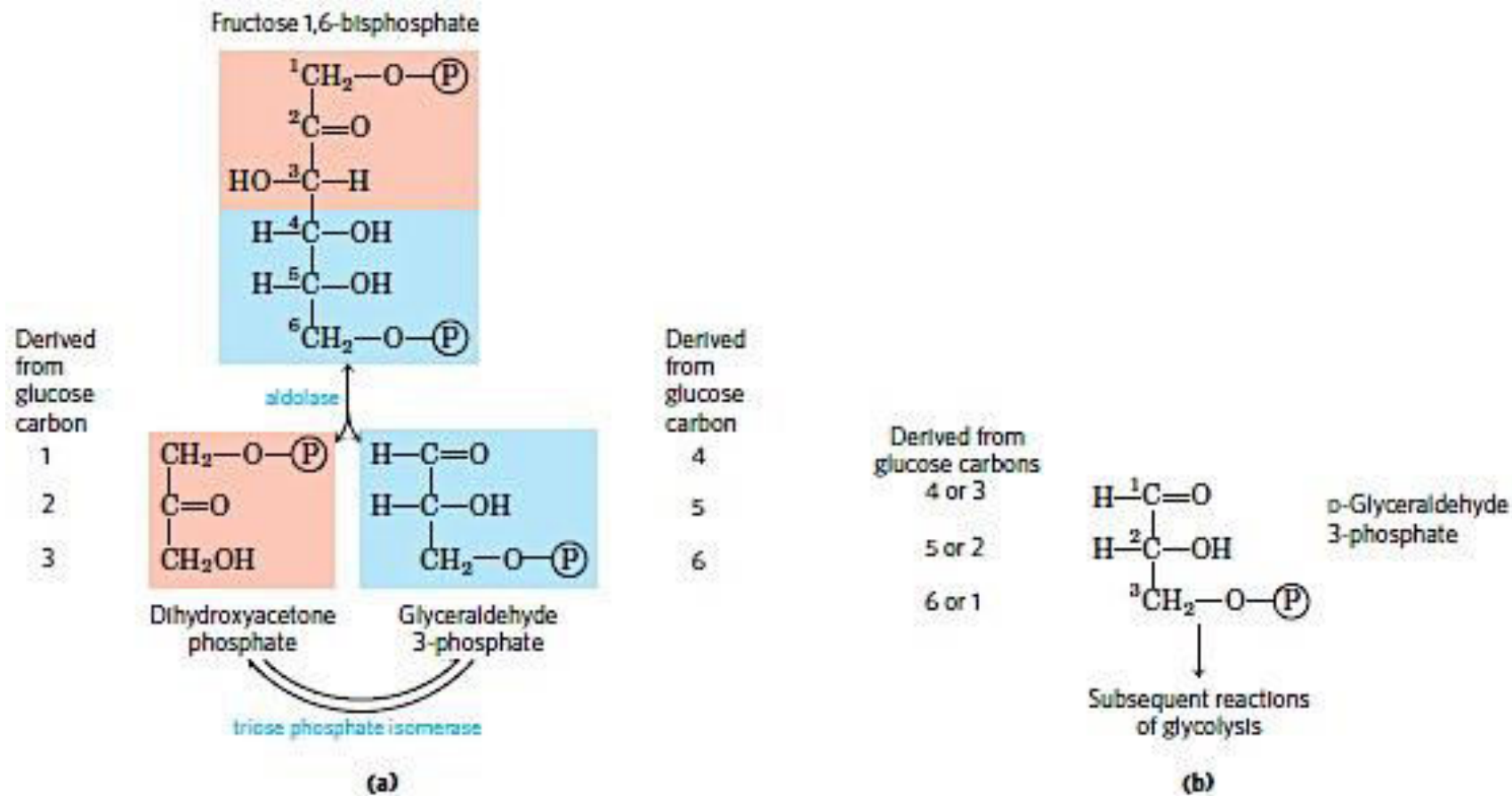
- The reaction mechanism is similar to the reaction promoted by phosphohexose isomerase in step 2 of glycolysis (Fig. 14–5, Lehninger; edition 6).
- After the triose phosphate isomerase reaction, the carbon atoms derived from C-1, C-2, and C-3 of the starting glucose are chemically indistinguishable
- from C-6, C-5, and C-4, respectively, the two “halves” of glucose have both yielded glyceraldehyde 3-phosphate.
- This reaction completes the preparatory phase of glycolysis.
- The hexose molecule has been phosphorylated at C-1 and C-6 and then cleaved to form two molecules of glyceraldehyde 3-phosphate.



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Fate of the glucose carbons in the formation of glyceraldehyde 3-phosphate.

- The origin of the carbons in the two 3-carbon products of the aldolase and triose phosphate isomerase reactions. The end product of the two reactions is glyceraldehyde 3-phosphate (two molecules).
- Each carbon of glyceraldehyde 3-phosphate is derived from either of two specific carbons of glucose. Note that the numbering of the carbon atoms of glyceraldehyde 3-phosphate differs from that of the glucose from which it is derived. In glyceraldehyde 3-phosphate, the most complex functional group (the carbonyl) is specified as C-1. This numbering change is important for interpreting experiments with glucose in which a single carbon is labeled with a radioisotope.

Radioisotopes:

Atomic number (Z): no. of protons

Mass number (A): the total **number** of protons and neutrons in an atomic nucleus (together known as nucleons).

- Isotopes are the atoms in an element that have the same atomic number but a different atomic mass; that is, the same number of protons and thus identical chemical properties, but different numbers of neutrons and consequently different physical properties.
- Isotopes can be stable or unstable or radioisotopes.
- In the latter, their nuclei have a special property: they emit energy in the form of ionizing radiation while searching for a more stable configuration.

GLUCOSE METABOLISM

The Payoff Phase of Glycolysis Yields ATP and NADH

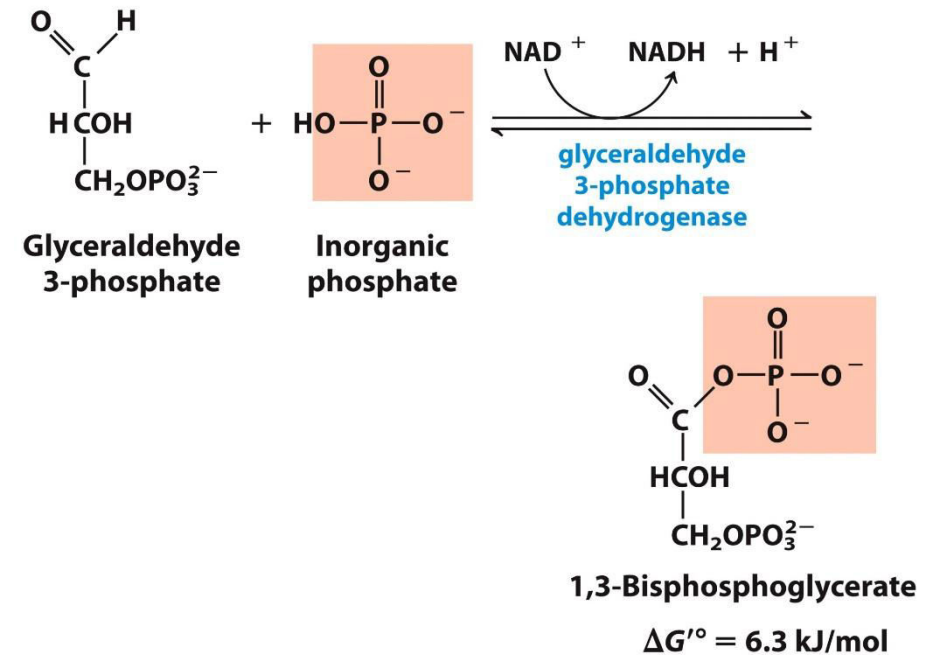
**Reference Book: Lehninger Principles of Biochemistry.
Edition 6**

The Payoff Phase of Glycolysis Yields ATP and NADH

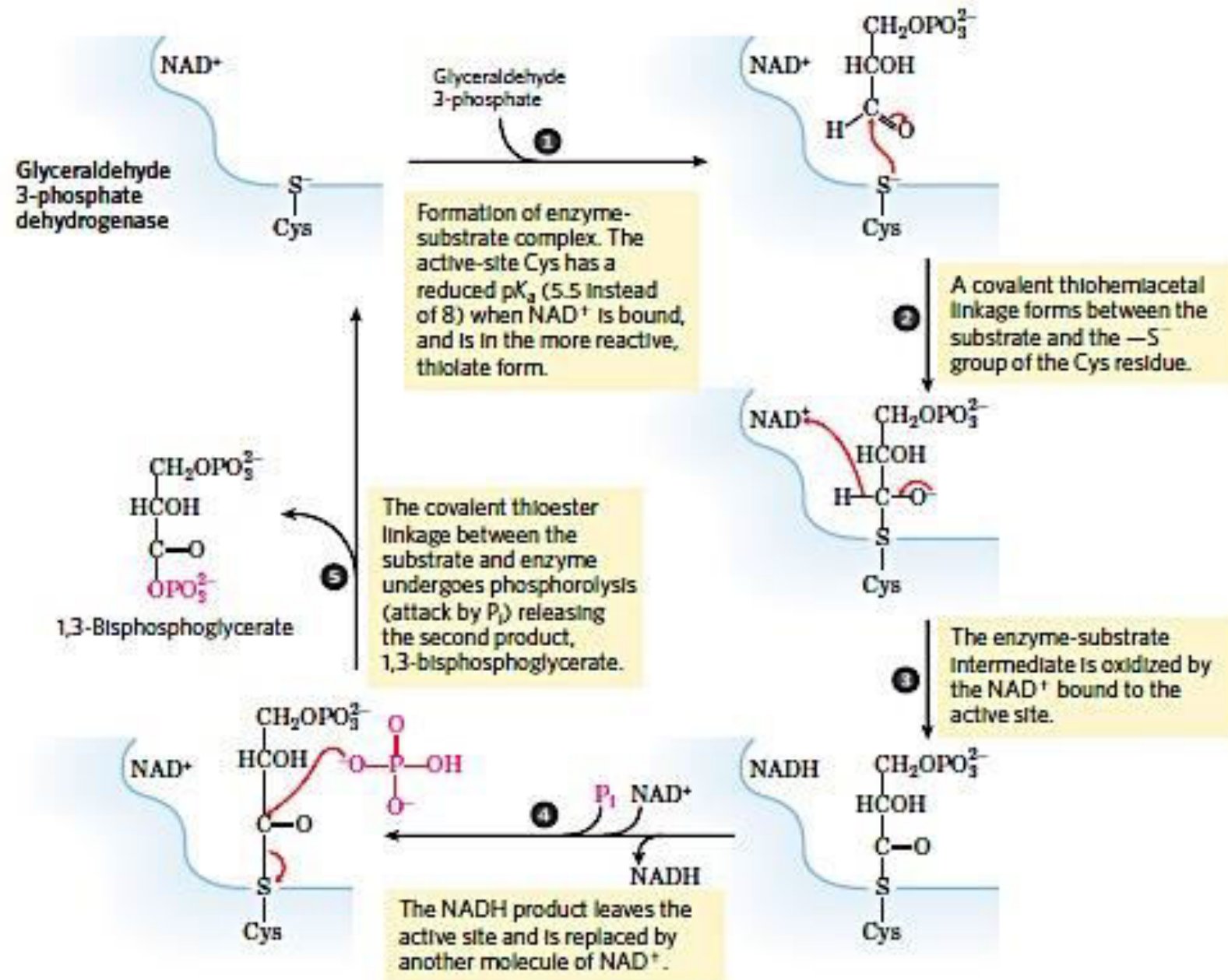
- The payoff phase of glycolysis includes the **energy-conserving phosphorylation steps**
- The chemical energy of the glucose molecule is conserved in the form of **ATP and NADH**
- The conversion of two molecules of glyceraldehyde 3-phosphate to two molecules of pyruvate is accompanied **by the formation of four molecules of ATP from ADP.**
- the net yield of ATP per molecule of glucose degraded is only two, because two ATP were invested in the preparatory phase of glycolysis to phosphorylate the two ends of the hexose molecule.

6. Oxidation of Glyceraldehyde 3-Phosphate to 1,3-Bisphosphoglycerate

- The first step in the payoff phase is the oxidation of glyceraldehyde 3-phosphate to **1,3-bisphosphoglycerate**, catalyzed by **glyceraldehyde 3-phosphate dehydrogenase**
- This is the **first of the two energy-conserving reactions** of glycolysis that eventually lead to the formation of ATP.
- The aldehyde group of glyceraldehyde 3-phosphate (G3P) is oxidized, not to a free carboxyl group but to a carboxylic acid anhydride with phosphoric acid.
- This type of **anhydride**, called an **acyl phosphate**, has a very high standard free energy of hydrolysis (-49.3 kJ/mol)



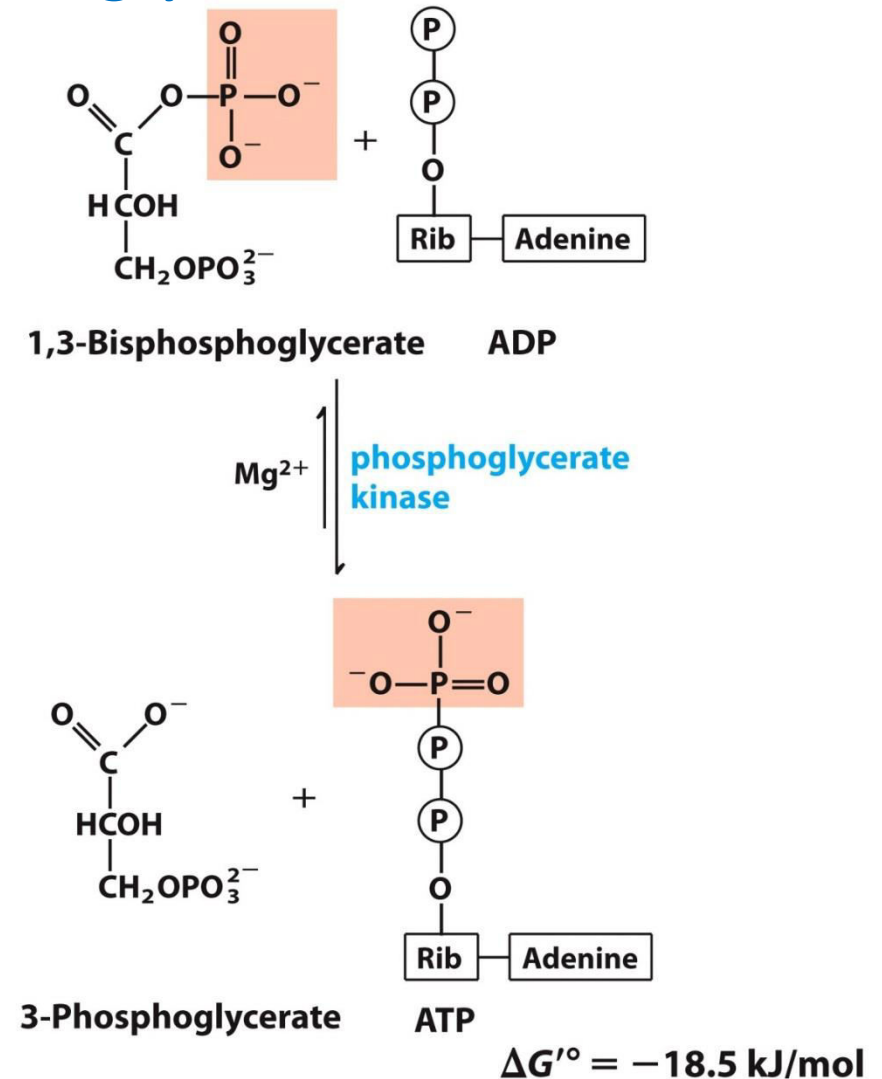
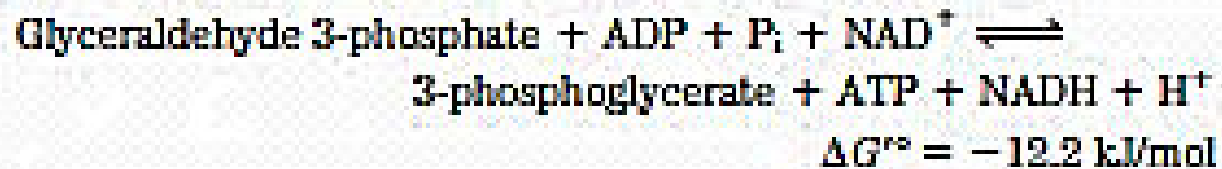
- Much of the free energy of oxidation of the aldehyde group of glyceraldehyde 3-phosphate is conserved by formation of the acyl phosphate group at **C-1 of 1,3-bisphosphoglycerate**.
- NAD⁺ is the e⁻ acceptor (bound to Rossmann fold of dehydrogenase)
- G3P is covalently bound to the **dehydrogenase**
- The aldehyde group of glyceraldehyde 3-phosphate reacts with the **—SH group** of an essential **Cys residue** in the active site, in a reaction analogous to the formation of a **hemiacetal** producing a *thiohemiacetal*.
- Reaction of the essential Cys residue with a heavy metal such as Hg²⁺ irreversibly inhibits the enzyme.
- The amount of NAD⁺ in a cell is far smaller than the amount of glucose metabolized in a few minutes.
- Glycolysis would soon come to a halt if the NADH formed in this step of glycolysis were not continuously reoxidized and recycled.



MECHANISM FIGURE 14-8 The glyceraldehyde 3-phosphate dehydrogenase reaction.

7. Phosphoryl Transfer from 1,3-Bisphosphoglycerate to ADP

- The enzyme **phosphoglycerate kinase** transfers the high-energy phosphoryl group from the carboxyl group of 1,3-bisphosphoglycerate to ADP, forming ATP and **3-phosphoglycerate**.
- phosphoglycerate kinase is named for the reverse reaction, in which it transfers a phosphoryl group to an enzyme, it catalyzes the reaction in both directions.
- In glycolysis, the reaction it catalyzes proceeds in the direction of ATP synthesis.
- Steps 6 and 7 of glycolysis together constitute an energy-coupling process in which 1,3-bisphosphoglycerate is the common intermediate; it is formed in the first reaction (which would be endergonic in isolation), and its acyl phosphate group is transferred to ADP in the second reaction (which is strongly exergonic). Overall reaction is exergonic.. The sum of these two reactions is

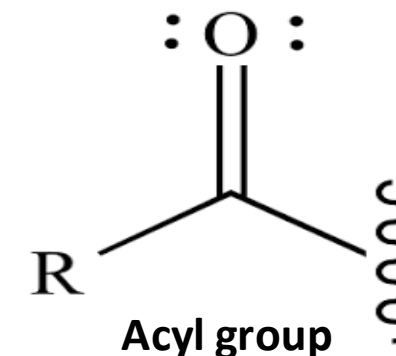


..cont

- The outcome of these reactions, both reversible under cellular conditions, is that the energy released on **oxidation of an aldehyde to a carboxylate** group is conserved by the coupled formation of ATP from ADP and Pi.
- The formation of ATP by phosphoryl group transfer from a substrate such as 1,3-bisphosphoglycerate is referred to as a **substrate-level phosphorylation**, to distinguish this mechanism from **respiration-linked phosphorylation**.
- **Substrate-level phosphorylation**
- **Substrate-level phosphorylation** is a metabolic reaction that results in the formation of ATP or GTP by the direct transfer of a phosphoryl (PO_3) group to ADP or GDP from another **phosphorylated** compound.
- Substrate-level phosphorylations involve soluble enzymes and chemical intermediates (1,3-bisphosphoglycerate in this case).
- Respiration-linked phosphorylations, involve membrane-bound enzymes and transmembrane gradients of protons.

Acyl group

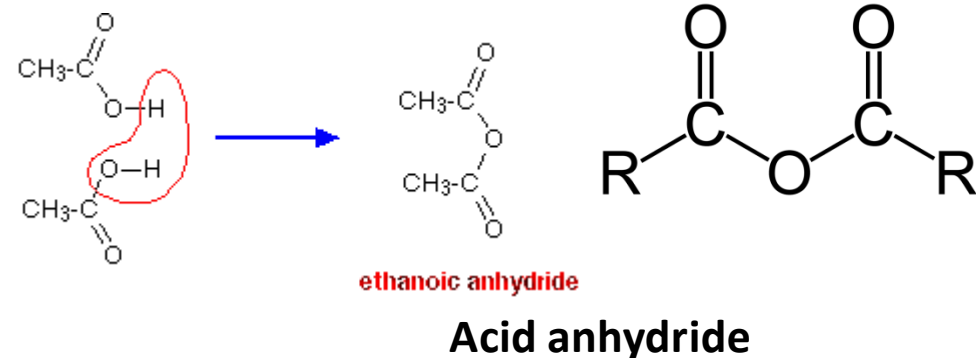
It contains a double bonded oxygen atom and an **alkyl group**.
($\text{R}-\text{C}=\text{O}$ **group** is called **Acyl Group**). In organic chemistry, the **acyl group** (IUPAC name: alkanoyl) is usually derived from a carboxylic acid.



Acid anhydride

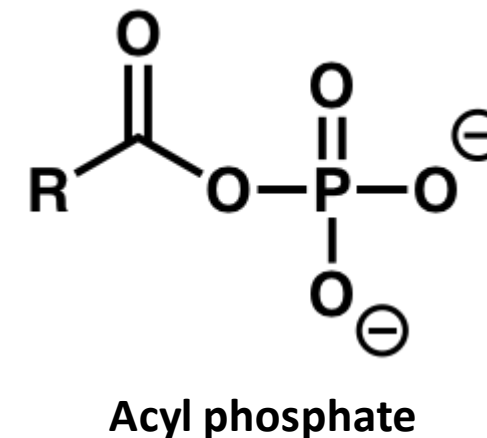
An acid anhydride is a compound that has two acyl groups bonded to the same oxygen atom.

Thus, $(\text{CH}_3\text{CO})_2\text{O}$ is called **acetic anhydride**. Mixed (or unsymmetrical) **acid anhydrides**, such as **acetic formic anhydride**.



Acyl phosphate

A phosphate group is connected to the acyl group by a single bond and that the bond occurs between one of the oxygen atoms of the phosphate and the carbonyl carbon of the acyl group.



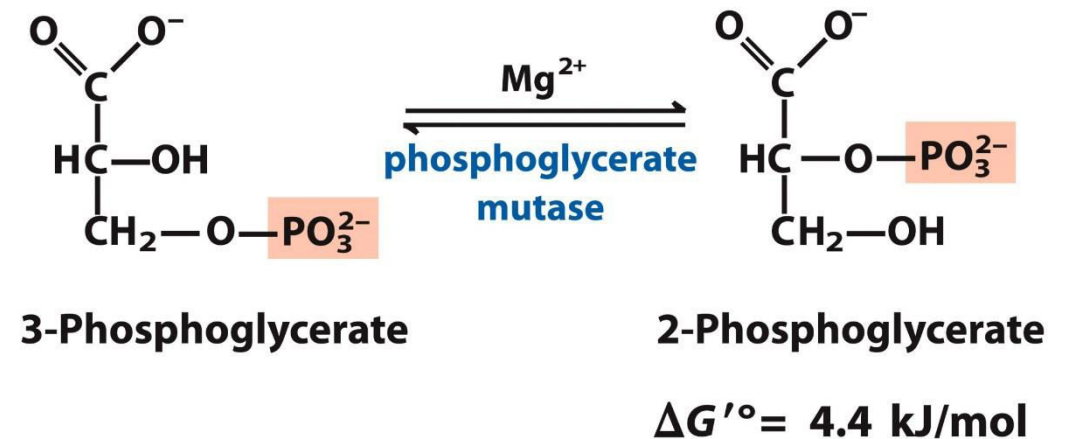
GLUCOSE METABOLISM

The Payoff Phase of Glycolysis Yields ATP and NADH-2

Step 8-10

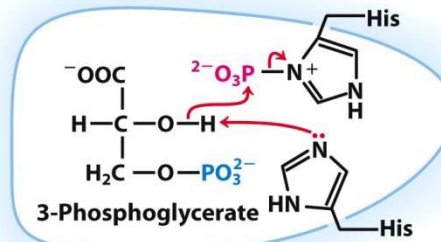
8. Conversion of 3-Phosphoglycerate to 2-Phosphoglycerate

- The enzyme **phosphoglycerate mutase** catalyzes a reversible shift of the phosphoryl group between C-2 and C-3 of glycerate
- Mg²⁺ is essential for this reaction. The reaction occurs in two steps **A phosphoryl group** initially attached to a His residue of the mutase is transferred to the hydroxyl group at C-2 of 3-phosphoglycerate, forming 2,3-bisphosphoglycerate (2,3-BPG).
- The phosphoryl group at C-3 of 2,3-BPG is then transferred to the same His residue, producing 2-phosphoglycerate and regenerating the phosphorylated enzyme.
- Phosphoglycerate mutase is initially phosphorylated by phosphoryl transfer from 2,3-BPG, which is required in small quantities to initiate the catalytic cycle and is continuously regenerated by that cycle

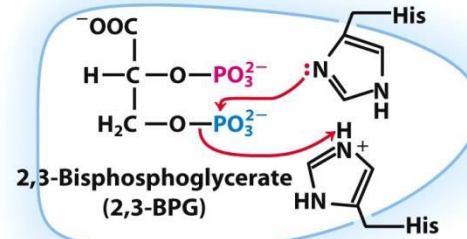


The phosphoglycerate mutase reaction.

Phosphoglycerate mutase



1 Phosphoryl transfer occurs between an active-site His and C-2 (OH) of the substrate. A second active-site His acts as general base catalyst.



2 Phosphoryl transfer from C-3 of the substrate to the first active-site His. The second active-site His acts as general acid catalyst.

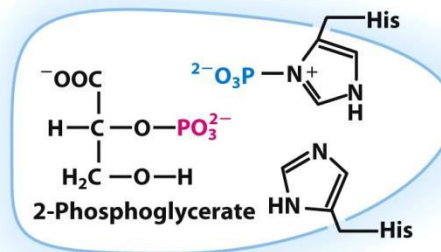
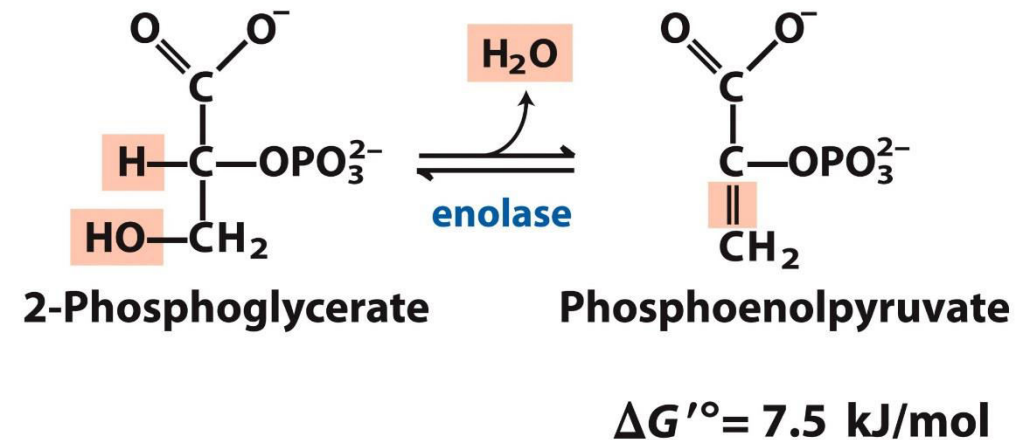


Figure 14-9

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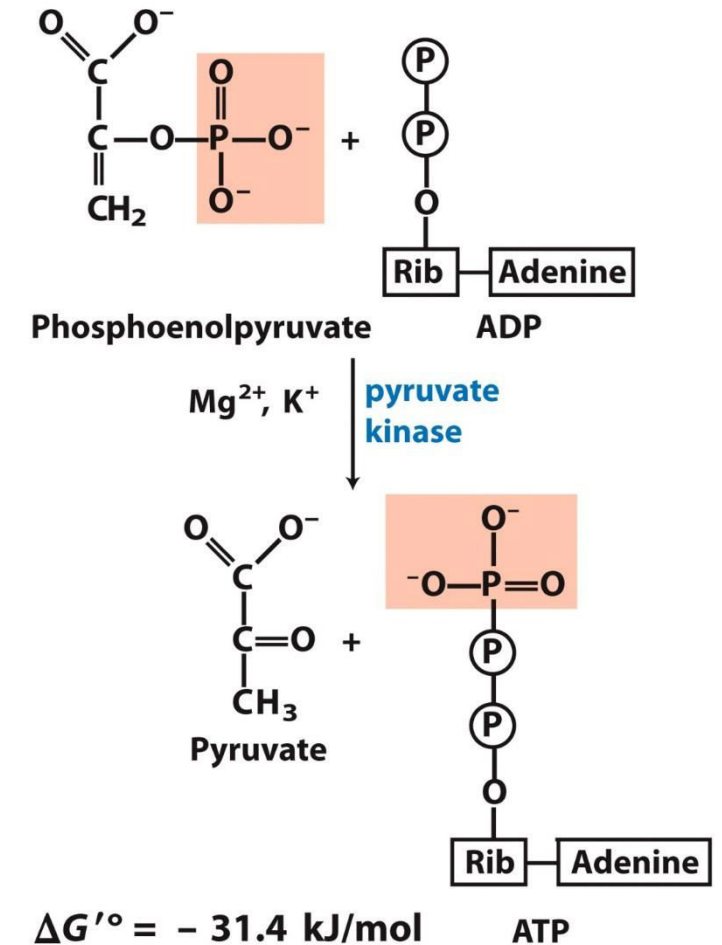
9. Dehydration of 2-Phosphoglycerate to Phosphoenolpyruvate

- In the second glycolytic reaction that generates a compound with high phosphoryl group transfer potential
- (the first was step 6), **enolase** promotes reversible removal of a molecule of water from 2-phosphoglycerate
- The mechanism of the enolase reaction involves an enolic intermediate stabilized by Mg^{+2}
- The reaction converts a compound with a relatively low phosphoryl group transfer potential ($\Delta G'^{\circ}$ for hydrolysis of 2-phosphoglycerate is -17.6 kJ/mol) to one with high phosphoryl group transfer potential ($\Delta G'^{\circ}$ for PEP hydrolysis is -61.9 kJ/mol)



10. Transfer of the Phosphoryl Group from Phosphoenolpyruvate to ADP

- The last step in glycolysis is the transfer of the phosphoryl group from phosphoenolpyruvate to ADP, catalyzed by **pyruvate kinase**, which requires K^+ (potassium ions) and either Mg^{+2} or Mn^{+2}
- In this substrate-level phosphorylation, the product **pyruvate** first appears in its enol form, then tautomerizes rapidly and nonenzymatically to its keto form, which predominates at pH 7.
- The overall reaction change, spontaneous conversion of the enol form of pyruvate to the keto form has a large, negative standard free energy
- About half of the energy released by PEP hydrolysis (-61.9 kJ/mol) is conserved in the formation of the phosphoanhydride bond of ATP (-30.5 kJ/mol), and the rest (-31.4 kJ/mol) constitutes a large driving force pushing the reaction toward ATP synthesis.



GLUCOSE METABOLISM

GLYCOLYSIS

ATP Formation Coupled to Glycolysis

- ***ATP Formation Coupled to Glycolysis*** During glycolysis some of the energy of the glucose molecule is conserved in the form of ATP, while much remains in the product, pyruvate.
- The overall equation for glycolysis is



- For each molecule of glucose degraded to pyruvate, two molecules of ATP are generated from ADP and Pi.
- We can now resolve the equation of glycolysis into two processes:
(1) the conversion of glucose into pyruvate is exergonic:



(2) and the formation of ATP from ADP and Pi is endergonic:



- If we now write the sum of Equations (II) and (III), we can also determine the overall standard free-energy change of glycolysis (Eqn I), including ATP formation, as the algebraic sum, $\Delta G^{\circ'}_s$, of $\Delta G^{\circ'}_1$ and $\Delta G^{\circ'}_2$

$$\Delta G^{\circ'}_s = \Delta G^{\circ'}_1 + \Delta G^{\circ'}_2 = -146 \text{ kJ/mol} + 61 \text{ kJ/mol} = -85 \text{ kJ/mol}$$

Under standard and intracellular conditions, glycolysis is an essentially irreversible process, driven to completion by this large net decrease in free energy.

Energy Remaining in the Pyruvate Produced by Glycolysis

- The two molecules of pyruvate formed by glycolysis still contain most of the chemical potential energy of glucose
- **This energy that can be extracted by oxidative reactions in the citric acid cycle and oxidative phosphorylation**
- 4 electrons..in the form of 2 hydride ions are transferred from 2 G3P to 2NAD⁺
- 2 molecules of NADH are reoxidized to NAD⁺ by transfer of electrons to electron transport chain (ETC) within mitochondria
- ETC passes these electrons to oxygen, that provides energy for ATP synthesis during respiration

“Pasteur effect”

- The ATP yield from glycolysis under anaerobic conditions (2 ATP per molecule of glucose) is much smaller than that from the complete oxidation of glucose to CO₂ under aerobic conditions (30 or 32 ATP per glucose)
- About 15 times more glucose would be consumed in anaerobic conditions to produce same amount of energy (ATP)
- This was discovered by Louis Pasteur while studying process of fermentation in yeast, and is known as the “Pasteur effect”

“Warburg effect”

- The German biochemist Otto Warburg first observed in 1928 that tumors of nearly all types carry out glycolysis at a much higher rate than normal tissue, *even when oxygen is available*.
- This is known as “Warburg effect” and is the basis for several methods of detecting and treating cancer
- Isolated 7 enzymes of glycolysis from tumor tissues
- Warburg manometer: experimental tool to measure amount oxygen consumed ..so oxidative activity
- Nobel prize of Physiology in 1931

Why glycolysis in tumor tissue?

- Most tumor cells grow under hypoxic conditions (i.e., with limited oxygen supply) because, at least initially, they lack the capillary network to supply sufficient oxygen.
- Cancer cells located more than 100 to 200 μm from the nearest capillaries must depend on glycolysis alone (without further oxidation of pyruvate) for much of their ATP production. The energy yield (2 ATP per glucose) is far lower than can be obtained by the complete oxidation of pyruvate to CO_2 in mitochondria (about 30 ATP per glucose)
- To make the same amount of ATP, tumor cells must take up much more glucose than do normal cells, converting it to pyruvate and then to lactate as they recycle NADH.
- In general, the more aggressive the tumor, the greater is its rate of glycolysis. This increase in glycolysis is achieved at least in part by increased synthesis of the glycolytic enzymes and of the plasma membrane transporters GLUT1 and GLUT3 that carry glucose into cells.
- The **hypoxia-inducible transcription factor (HIF-1)** is a protein stimulates the production of at least eight glycolytic enzymes and the glucose transporters when oxygen supply is limited
- Another protein induced by HIF-1 is the peptide hormone VEGF (vascular endothelial growth factor), which stimulates the growth of blood vessels (angiogenesis) toward the tumor
- **This heavier reliance of tumors than of normal tissue on glycolysis suggests a possibility for anticancer therapy: inhibitors of glycolysis might target and kill tumors by depleting their supply of ATP. Three inhibitors of hexokinase have shown promise as chemotherapeutic agents: 2-deoxyglucose, lonidamine, and 3-bromopyruvate.**

Glucose Uptake Is Deficient in Type 1 Diabetes Mellitus

- The metabolism of glucose in mammals is limited by the rate of glucose uptake into cells and its phosphorylation by **hexokinase**.
- Glucose uptake from the blood is mediated by the **GLUT family** of glucose transporters
- The transporters of hepatocytes (GLUT1, GLUT2) and of brain neurons (GLUT3) are always present in plasma membranes.
- In contrast, the main glucose transporter in the cells of skeletal muscle, cardiac muscle, and adipose tissue (GLUT4) is sequestered in small intracellular vesicles and moves into the plasma membrane only in response to an insulin signal
- Thus in skeletal muscle, heart, and adipose tissue, glucose uptake and metabolism
- depend on the normal release of insulin by **pancreatic beta cells** in response to elevated blood glucose

Individuals with type 1 diabetes mellitus have too few β cells and cannot release sufficient insulin to trigger glucose uptake by the cells of skeletal muscle, heart, or adipose tissue.

Thus, after a meal containing carbohydrates, glucose accumulates to abnormally high levels in the blood, a condition known as hyperglycemia. Unable to take up glucose, muscle and fat tissue use the fatty acids of stored triacylglycerols as their principal fuel.

In the liver, acetyl-CoA derived from this fatty acid breakdown is converted to “ketone bodies”—acetoacetate and β -hydroxybutyrate—which are exported and carried to other tissues to be used as fuel.

These compounds are especially critical to the brain, which uses ketone bodies as alternative fuel when glucose is unavailable. (Fatty acids cannot pass through the blood-brain barrier and thus are not a fuel for brain neurons.)

In untreated type 1 diabetes, overproduction of **acetoacetate and hydroxybutyrate** leads to their accumulation in the blood, and the consequent lowering of blood pH produces **ketoacidosis**, a life-threatening condition.

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... cont

- **Phosphate groups are essential components in the enzymatic conservation of metabolic energy.**
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- **High-energy phosphate compounds formed in glycolysis (1,3-bisphosphoglycerate and phosphoenol pyruvate) donate phosphate groups to ADP to form ATP.**

... cont

- Binding of phosphate groups to the active sites of enzymes provides binding energy that contributes to lowering the activation energy and increasing the specificity of enzyme-catalyzed reactions.
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- Nearly all the glycolytic enzymes require Mg^{2+} for activity.

GLUCOSE METABOLISM

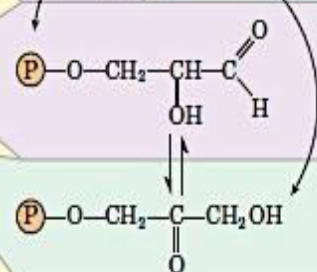
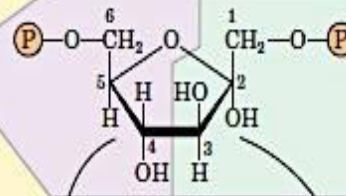
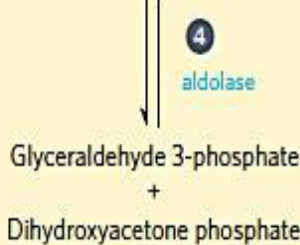
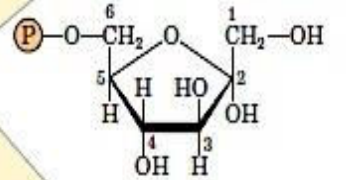
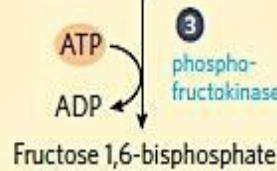
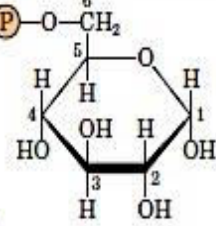
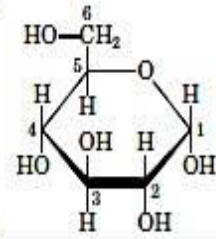
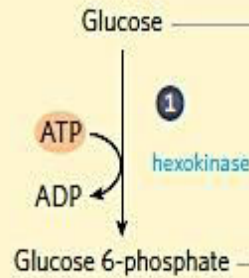
GLYCOLYSIS

Importance of Phosphorylated Intermediates

(a) Preparatory phase

Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate

first priming reaction



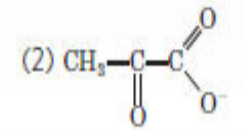
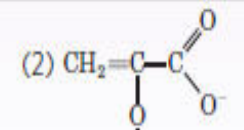
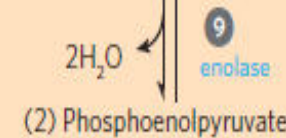
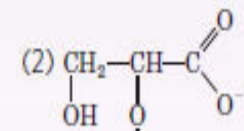
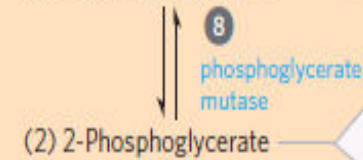
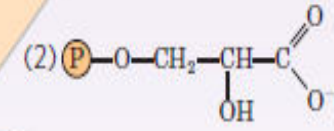
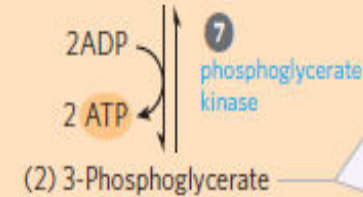
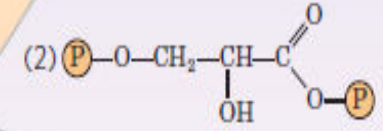
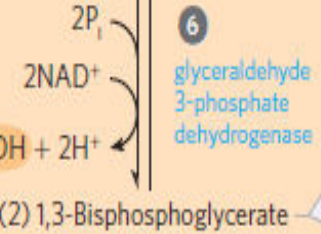
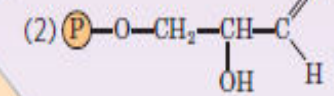
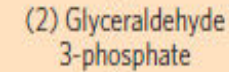
(b) Payoff phase

Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH

oxidation and phosphorylation

first ATP-forming reaction (substrate-level phosphorylation)

second ATP-forming reaction (substrate-level phosphorylation)



GLYCOLYSIS

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- **Nearly all the glycolytic enzymes require Mg^{2+} for activity.**

Glucose Uptake Is Deficient in Type 1 Diabetes Mellitus

- The metabolism of glucose in mammals is limited by the rate of glucose uptake into cells and its phosphorylation by **hexokinase**.
- Glucose uptake from the blood is mediated by the **GLUT family** of glucose transporters
- The transporters of hepatocytes (GLUT1, GLUT2) and of brain neurons (GLUT3) are always present in plasma membranes.
- In contrast, the main glucose transporter in the cells of skeletal muscle, cardiac muscle, and adipose tissue (GLUT4) is sequestered in small intracellular vesicles and moves into the plasma membrane only in response to an insulin signal
- Thus in skeletal muscle, heart, and adipose tissue glucose uptake and metabolism depend on the normal release of insulin by **pancreatic beta cells** in response to elevated blood glucose

- Patients with type 1 diabetes mellitus have too few cells and cannot release sufficient insulin to trigger glucose uptake by the cells of skeletal muscle, heart, or adipose tissue
- Thus, after a meal containing carbohydrates, glucose accumulates to abnormally high levels in the blood, a condition known as hyperglycemia
- Unable to take up glucose, muscle and fat tissue use the fatty acids of stored triacylglycerols as their principal fuel
- In the liver, acetyl-CoA derived from this fatty acid breakdown is converted to “ketone bodies”—acetoacetate and –hydroxybutyrate

- These compounds are especially critical to the brain, which uses ketone bodies as alternative fuel when glucose is unavailable. (Fatty acids cannot pass through the blood-brain barrier and thus are not a fuel for brain neurons)
- In untreated type 1 diabetes, overproduction of **acetoacetate and hydroxybutyrate** leads to their accumulation in the blood, and the consequent lowering of blood pH produces **ketoacidosis**, a life-threatening condition.
- **Insulin injection reverses this sequence of events:**
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GLUCOSE METABOLISM

Fates of Pyruvate

Fates of Pyruvate

- The pyruvate formed by glycolysis is further metabolized via one of three catabolic routes.
- In aerobic organisms or tissues, under aerobic conditions, glycolysis is only the first stage in the complete degradation of glucose

1. Oxidation of Pyruvate:

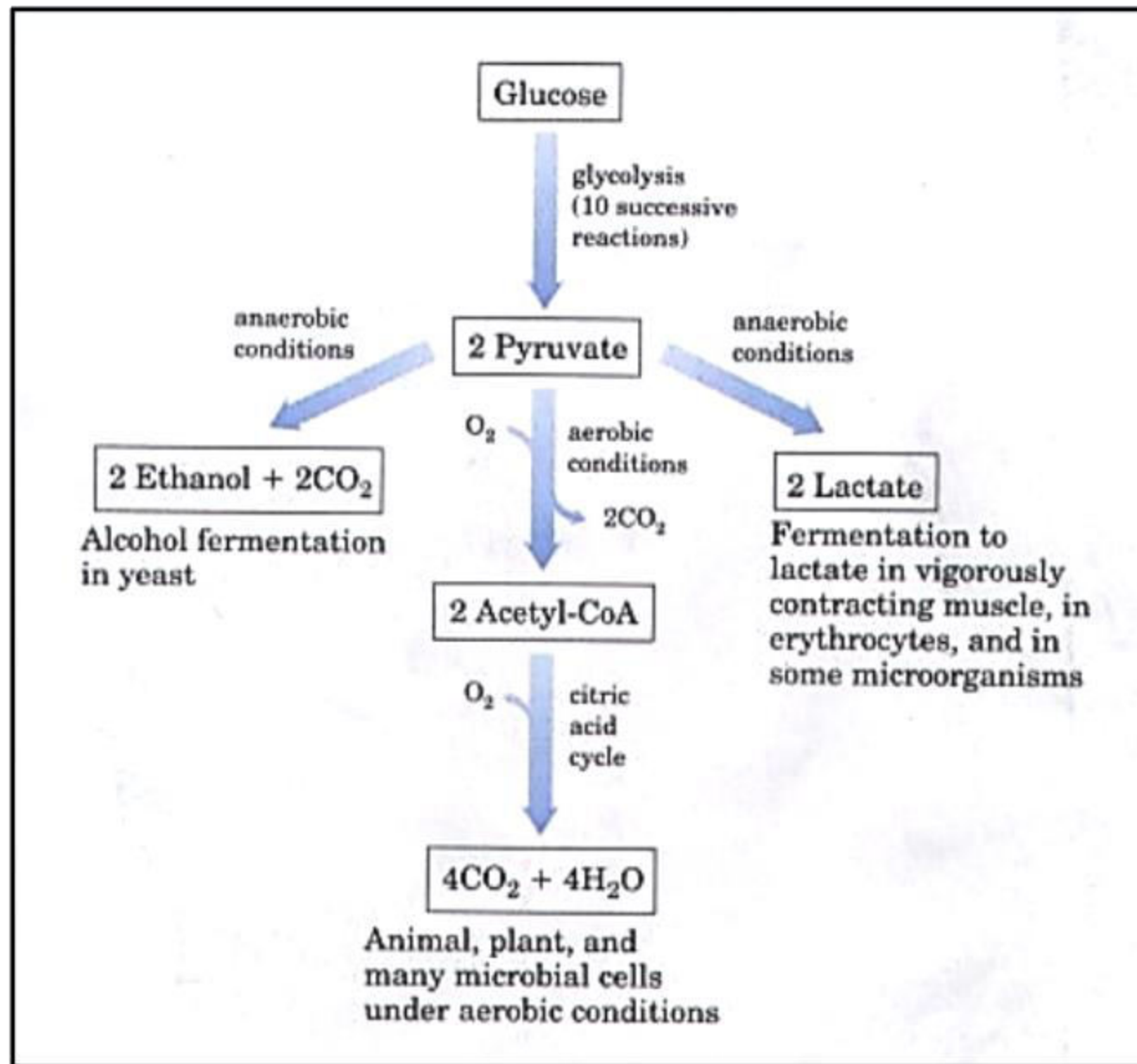
Pyruvate is oxidized, with loss of its carboxyl group as CO_2 , to yield the acetyl group of acetyl-coenzyme A; the acetyl group is then oxidized completely to CO_2 by the citric acid cycle. The electrons from these oxidations are passed to O_2 through a chain of carriers in mitochondria, to form H_2O . The energy from the electron-transfer reactions drives the synthesis of ATP in mitochondria

2. Reduction to lactate

- **The second route** for pyruvate is its **reduction to lactate** via **lactic acid fermentation**.
- When vigorously contracting skeletal muscle must function under low oxygen conditions (**hypoxia**), NADH cannot be reoxidized to NAD⁺, but NAD⁺ is required as an electron acceptor for the further oxidation of pyruvate.
- Under these conditions pyruvate is reduced to lactate, accepting electrons from NADH and thereby regenerating the NAD⁺ necessary for glycolysis to continue. Certain tissues and cell types (retina and erythrocytes, for example) convert glucose to lactate even under aerobic conditions.
- Lactate is also the product of glycolysis under anaerobic conditions in some microorganisms.

3. ethanol (alcohol) fermentation

- The third major route of pyruvate catabolism leads to formation of ethanol.
- In some plant tissues and in certain invertebrates, protists, and microorganisms such as yeast, pyruvate is converted under hypoxic or anaerobic conditions to ethanol and CO₂, a process called **ethanol (alcohol) fermentation**
- The oxidation of pyruvate is an important catabolic process, but pyruvate has anabolic fates as well. It can, provide the carbon skeleton for the synthesis of the **amino acid alanine** or for the synthesis of **fatty acids**.



Three possible catabolic fates of the pyruvate formed in the payoff phase of glycolysis

GLUCOSE METABOLISM

Fates of Pyruvate -1

Fates of Pyruvate under Aerobic Conditions

- **Under Aerobic conditions**, the pyruvate formed in the final step of glycolysis is oxidized to **acetate (acetyl- CoA)**, which **enters the citric acid cycle** and is oxidized to CO₂ and H₂O.
- The NADH formed by **dehydrogenation of glyceraldehyde 3-phosphate** is ultimately **reoxidized** to **NAD⁺** by passage of its electrons to O₂ in mitochondrial respiration.

Fates of Pyruvate under Anaerobic Conditions

- **Under hypoxic (low-oxygen) conditions**, as in very active skeletal muscle, in submerged plant tissues, in solid tumors, or in lactic acid bacteria—**NADH generated by glycolysis cannot be reoxidized by O₂**.
- Failure to regenerate NAD⁺ can leave the cell with **no electron acceptor** for the oxidation of glyceraldehyde 3-phosphate, and the energy-yielding reactions of glycolysis would stop.
- **There must be another way to regenerate NAD⁺**
- modern organisms continually regenerate NAD⁺ during anaerobic glycolysis **by transferring electrons from NADH to form a reduced end product such as lactate or ethanol (by fermentation)**.

Fermentation

- Fermentation actually comes from Latin word “**fervere**” which means “**to boil**”
- So the word “**Fermentation**” is referred to physical state of boiling/bubbling
- The bubbling appearance in fermentation is due to the production of CO₂ bubbles caused by anaerobic catabolism of sugars
- **Fermentation** is a metabolic process that converts sugars to **acids, gases, or alcohol**
- The science of fermentation is known as **ZYMOLOGY**
- French microbiologist Louis Pasteur is often remembered for his insights into fermentation and its microbial causes.
- It occurs in yeast and bacteria, and also in oxygen-starved muscle cells
- In terms of **BIOCHEMISTRY** **Fermentation relates to the generation of energy by catabolism of organic compounds**
- Industrial Fermentation is used to refer to the bulk growth of microorganisms on a growth medium, often with the goal of producing a specific chemical product.

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The bubbling appearance in fermentation is due to the production of CO₂ bubbles caused by

anaerobic catabolism of sugars

Fermentation is a metabolic process that converts sugars to **acids, gases, or alcohol only in the absence of oxygen.**

It occurs in yeast and bacteria, and also in oxygen-starved muscle cells, as in the case of lactic acid fermentation.

Fermentation is also used more broadly to refer to the bulk growth of microorganisms on a growth medium, often with the goal of producing a specific chemical product.

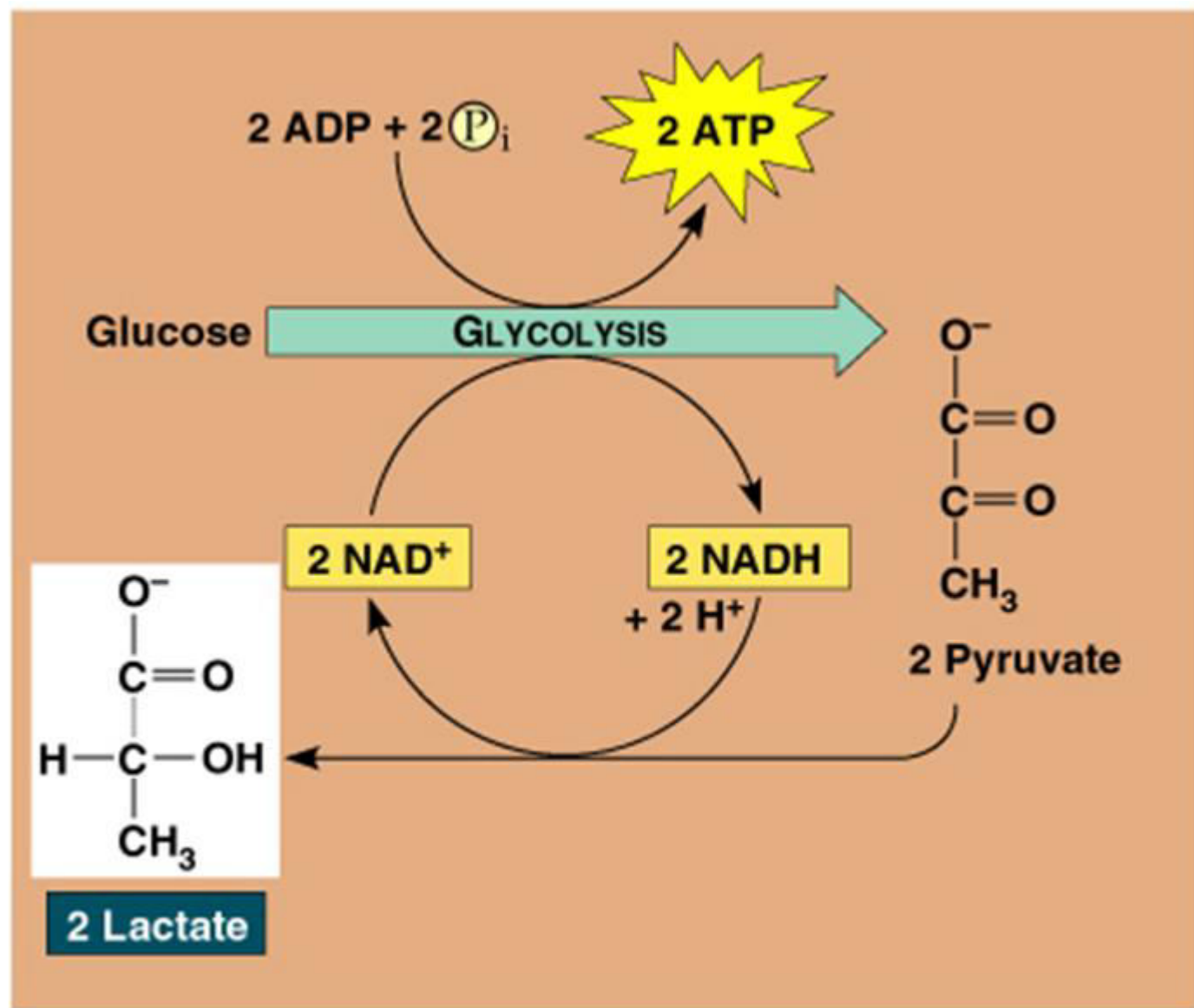
French microbiologist Louis Pasteur is often remembered for his insights into fermentation and its microbial causes.

The science of fermentation is known as zymology.

Fermentations are carried out by a wide range of organisms, many of which occupy anaerobic niches, and they yield a variety of end products, some of which are used commercially.

Lactic Acid Fermentation

- Lactic acid fermentation is done by some fungi, some bacteria like in yogurt, and sometimes by our muscles.
- Glucose $\longrightarrow$ carbon dioxide + lactic acid
- In the process of lactic acid fermentation, the 3-carbon pyruvic acid molecules are turned into lactic acid
- Allows glycolysis to continue
- Normally our muscles do cellular respiration like the rest of our bodies, using O_2 supplied by our lungs and blood. However, under greater exertion when the oxygen supplied by the lungs and blood system can't get there fast enough to keep up with the muscles' needs, our muscles can switch over and do **lactic acid fermentation**.



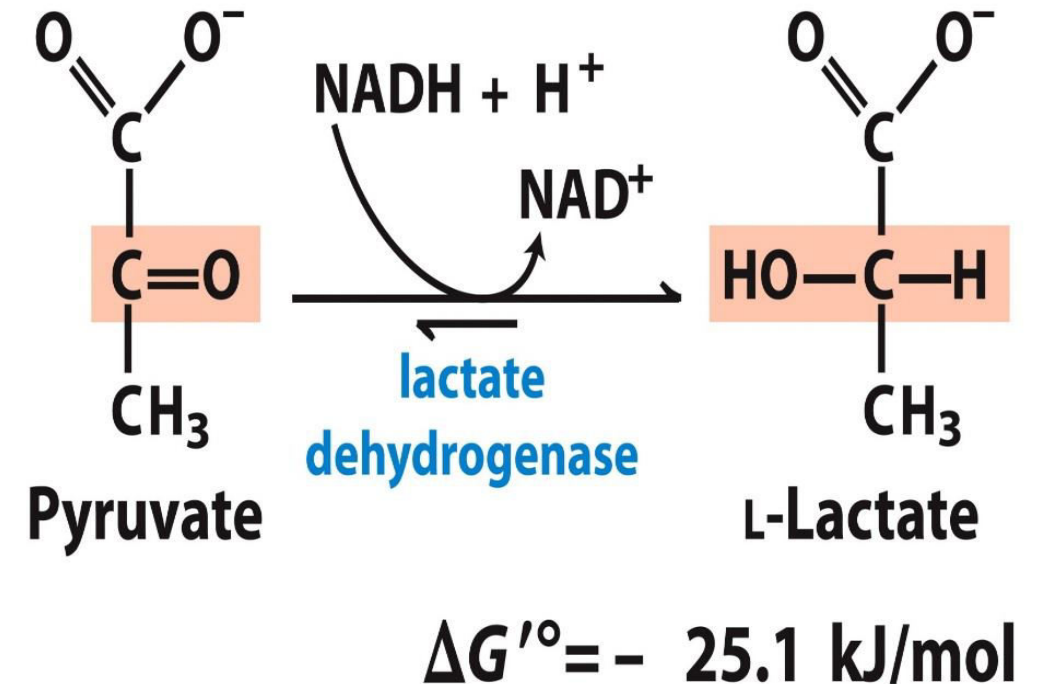
(b) Lactic acid fermentation

©1999 Addison Wesley Longman, Inc.

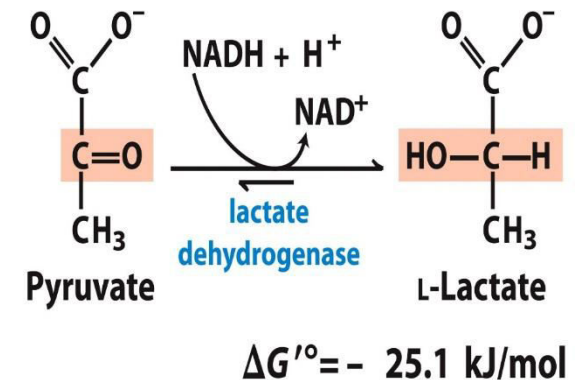
Pyruvate Is the Terminal Electron Acceptor in Lactic Acid Fermentation

- NAD⁺ is regenerated from NADH by the reduction of pyruvate to **lactate**.
- some tissues and cell types (such as erythrocytes, which have no mitochondria and cannot oxidize pyruvate to CO₂) produce **lactate from glucose even under aerobic conditions**.
- The reduction of pyruvate in this pathway is catalyzed by **lactate dehydrogenase**, which forms lactate at pH 7.

- **Under aerobic conditions NADH transfers its electrons eventually to oxygen, regenerating NAD⁺.**
- **Under anaerobic conditions pyruvate is reduced to lactate to regenerate NAD⁺ for glycolysis to continue.**



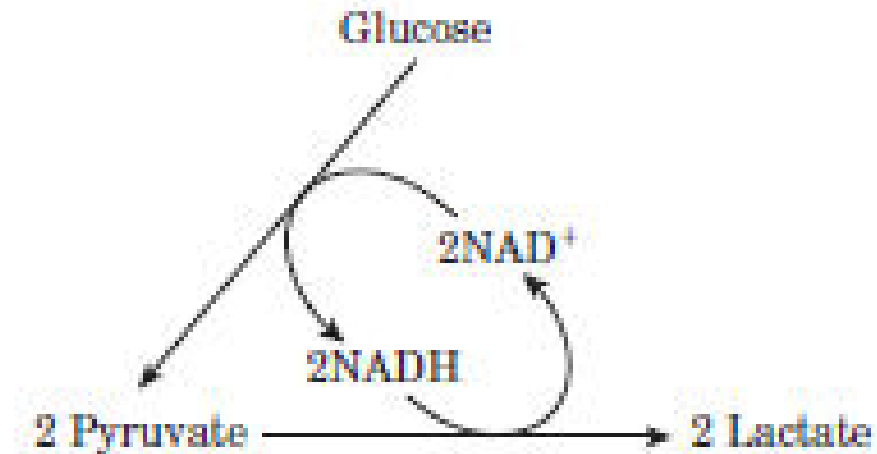
- The overall equilibrium of the reaction strongly favors lactate formation, as shown by the large negative standard free-energy change.
- In glycolysis, dehydrogenation of the two molecules of glyceraldehyde 3-phosphate derived from each molecule of **glucose converts two molecules of NAD⁺ to two of NADH**.
- Because the **reduction of two molecules of pyruvate** to two of lactate regenerates **two molecules of NAD⁺**, there is no net change in NAD⁺ or NADH.
- The lactate formed by active skeletal muscles (or by erythrocytes) can be recycled; it is carried in the blood to the liver, where it is converted to glucose during the recovery from strenuous muscular activity.
- When lactate is produced in large quantities during vigorous muscle contraction (during a sprint, for example), the acidification that results from ionization of lactic acid in muscle and blood limits the period of vigorous activity.



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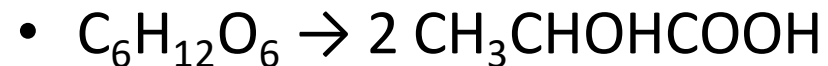
- Although conversion of glucose to lactate includes two oxidation-reduction steps, there is no net change in the oxidation state of carbon; in glucose ($\text{C}_6\text{H}_{12}\text{O}_6$) and lactic acid ($\text{C}_3\text{H}_6\text{O}_3$), the H:C ratio is the same.



Lactic Acid Fermentation

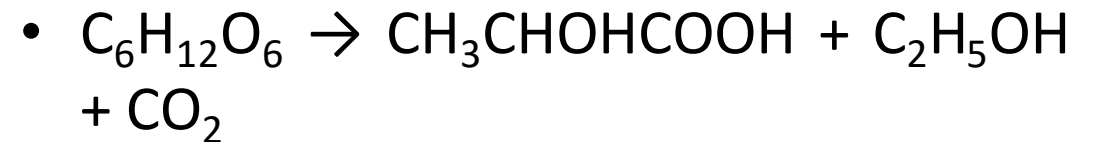
- **Homolactic Fermentation**

- **HOMOLACTIC** In homolactic fermentation, one glucose molecule is converted into two molecules of lactic acid



- **Heterolactic Fermentation**

- In Heterolactic Fermentation, one glucose molecule is converted into one molecule of lactic acid, one molecule of ethanol and one molecule of carbondioxide



Products of Lactic Acid Fermentation



Yogurt

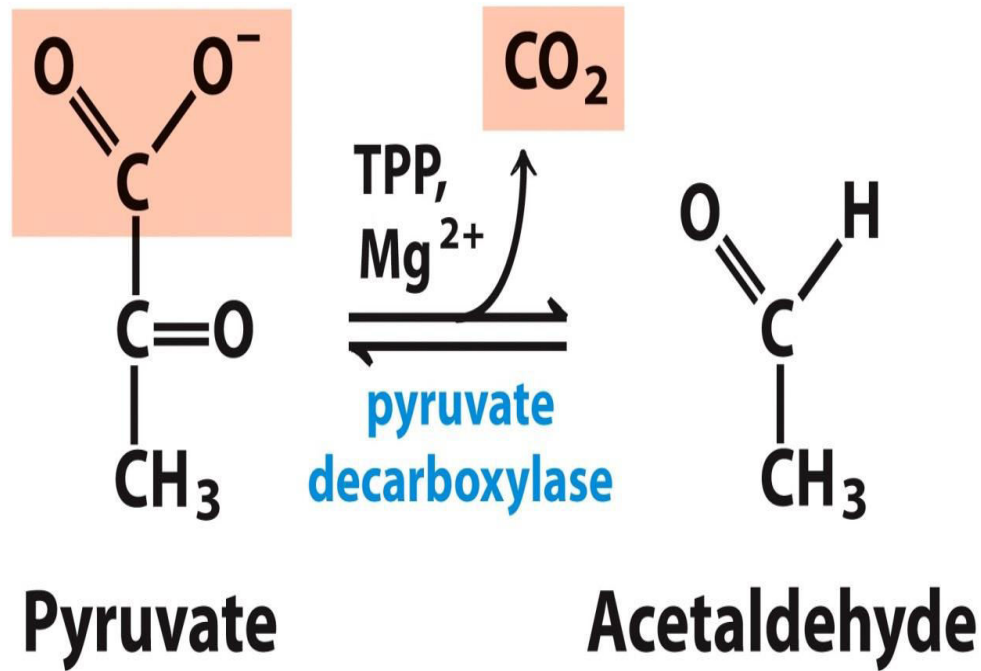
Ethanol Fermentation

- **Alcohol fermentation** is done by yeast and some kinds of bacteria.
- Products: glucose undergoes fermentation to produce **ethanol and CO₂**, instead of lactate.
- Substrate: glucose



Ethanol Fermentation

- **Alcohol fermentation** is done by yeast and some kinds of bacteria.
- Products: glucose undergoes fermentation to produce **ethanol and CO₂**, instead of lactate.
- Substrate: glucose
- **two-step process:**
 - Yeast contain the enzyme **pyruvate decarboxylase** that **decarboxylates pyruvate to form acetaldehyde**.
 - .. Acetaldehyde is **reduced** to ethanol under **anaerobic conditions**.

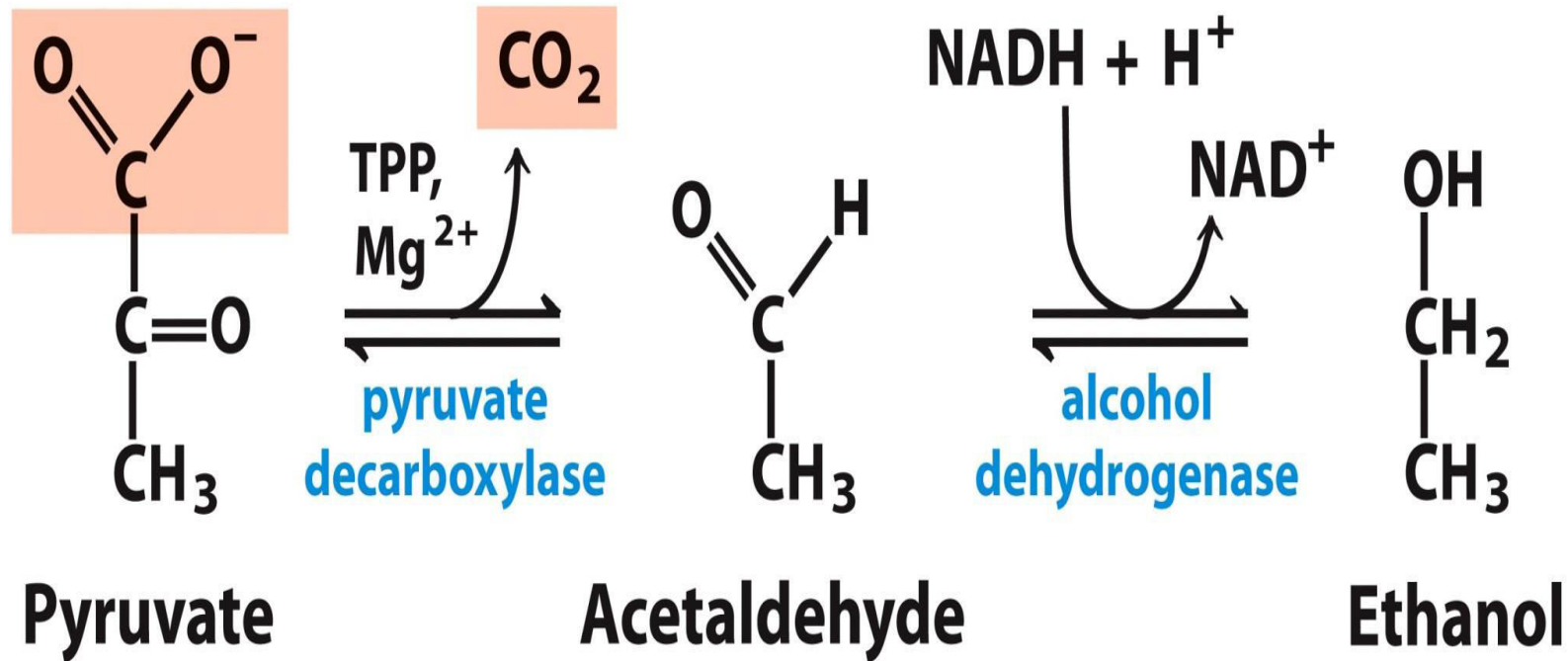


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Ethanol Is the Reduced Product in Ethanol Fermentation

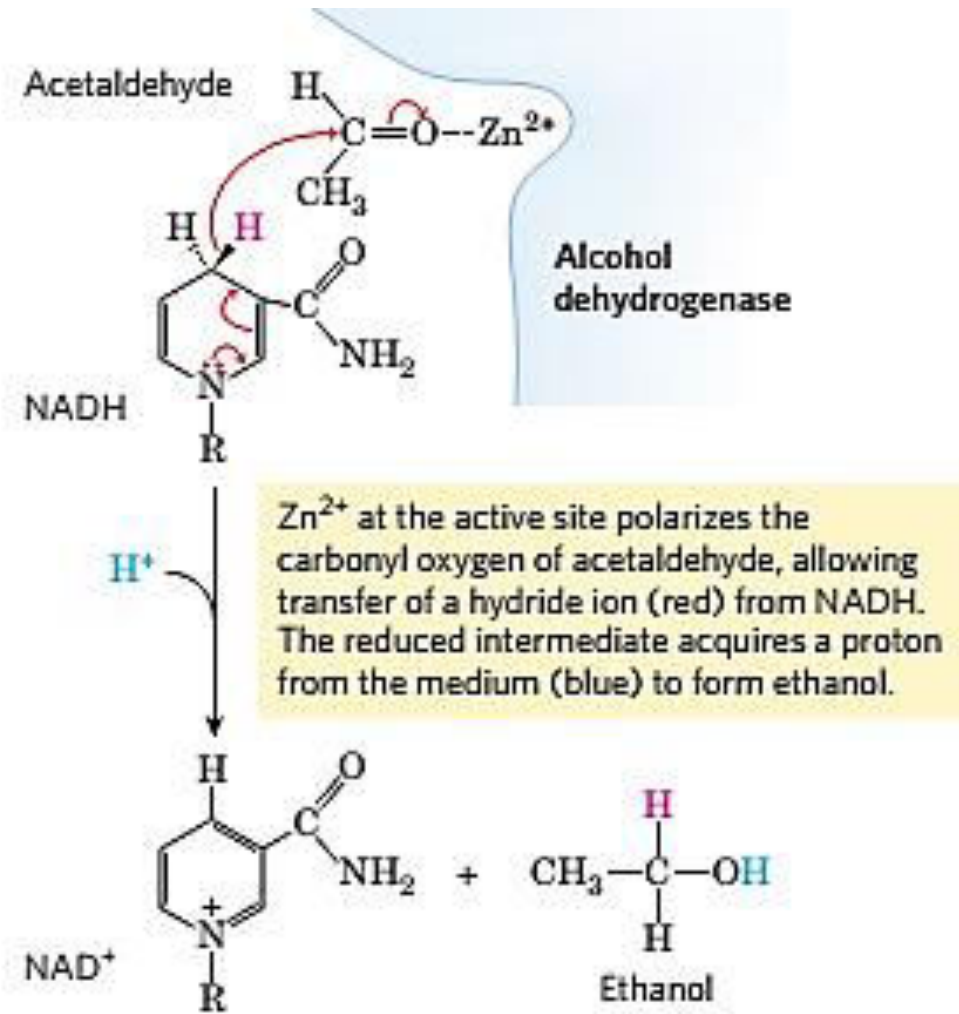


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Ethanol Is the Reduced Product in Ethanol Fermentation



The alcohol dehydrogenase reaction.

...cont

- Final equation:



- As in lactic acid fermentation, there is no net change in the ratio of hydrogen to carbon atoms
- when glucose (H:C ratio = $12/6 = 2$) is fermented to two ethanol and two CO₂ (combined H:C ratio = $12/6 = 2$).
- In all fermentations, the H:C ratio of the reactants and products remains the same.
- Alcohol dehydrogenase is present in many organisms that metabolize ethanol, including humans.
- In the liver it catalyzes the oxidation of ethanol, either ingested or produced by intestinal microorganisms, **with the reduction of NAD⁺ to NADH.**

...cont

- **Pyruvate decarboxylase** is present in brewer's and baker's yeast (*Saccharomyces cerevisiae*) and in all other organisms that ferment glucose to ethanol, including some plants.
- The CO₂ produced by pyruvate decarboxylation in brewer's yeast is responsible for the characteristic carbonation of champagne (ethanol fermentation).
- In **baking**, CO₂ released by **pyruvate decarboxylase** when yeast is mixed with a fermentable sugar causes dough to rise.
- The enzyme is absent in organisms that carry out lactic acid fermentation.



Fermentation

Use of Fermentation In Industry

Industrial Fermentation

- Oldest form of microbiology and biotechnology which was used to make wine, beer, bread with use of bacteria and yeasts without knowing scientific basis
- Fermentation has different meaning to biochemistry and industrial microbiologists.
- Biochemical meaning relates to the generation of energy by the catabolism of organic compounds

- Microbiologists have extended the term - fermentation - to describe: “A process that generates a product by the mass culture of microorganism.”
- **In Industrial Biotechnology,** fermentation means a process in which microorganisms that are cultured on a large-scale under aerobic or anaerobic conditions, convert a substrate into a product which is useful to man.
- The industrial microorganisms are grown under controlled conditions with an aim of optimizing the growth of the organism for production of a target microbial product.

Use of Fermentation In Industry

- In 1910 Chaim Weizmann (later to become the first president of Israel) discovered that the bacterium *Clostridium acetobutyricum* ferments starch to **butanol and acetone**.
- This discovery opened the field of industrial fermentations, in which some readily available material rich in carbohydrate (corn starch or molasses, for example) is supplied to a pure culture of a specific microorganism, which ferments it into a product of greater commercial value.

Some important fermentation products

- Fermentations Are Used to Produce Some Common Foods and Industrial Chemicals

Product	Organism	Use
Ethanol	<i>Saccharomyces cerevisiae</i>	Industrial solvents, beverages
Glycerol	<i>Saccharomyces cerevisiae</i>	Production of explosives
Lactic acid	<i>Lactobacillus bulgaricus</i>	Food and pharmaceutical
Acetone and butanol	<i>Clostridium acetobutylicum</i>	Solvents
α -amylase	<i>Bacillus subtilis</i>	Starch hydrolysis

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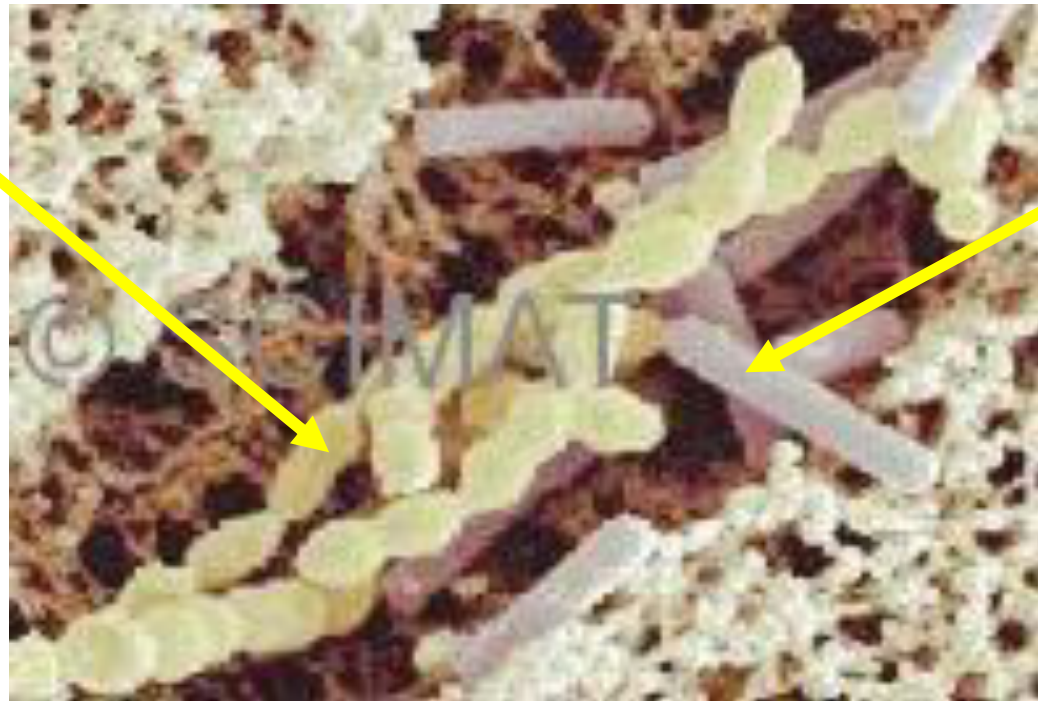
- **Yogurt**, is produced when the bacterium *Lactobacillus bulgaricus* ferments the carbohydrate in milk, producing lactic acid; the resulting **drop in pH causes the milk proteins to precipitate**, producing the thick texture and sour taste of unsweetened yogurt.
- **Cheese** is produced by using *Propionibacterium freudenreichii*, which ferments milk to produce **propionic acid and CO₂**; the propionic acid
- precipitates milk proteins, and bubbles of CO₂ cause the holes characteristic of **Swiss Cheese**.

Commercial Yogurt

Contains 2 species of bacteria specialized to grow well in milk (but can't survive inside the human body):

These bacteria work in *symbiosis*. Each bacterium stimulates the growth of the other
=> acidifies the milk more rapidly than either partner on its own.

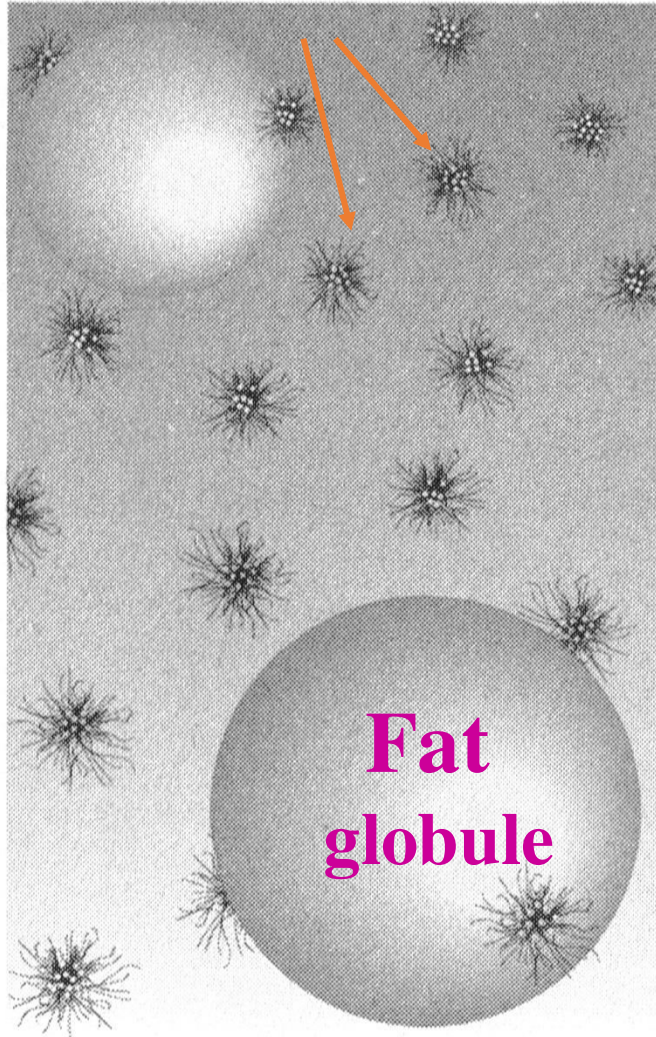
First,
Streptococcus
thermophilus is
more active, then
slows down
when acidity
reaches 0.5%



Next, Lactobacillus
bulgaricus is more
acid tolerant and
takes over until
acidity $\geq 1\%$

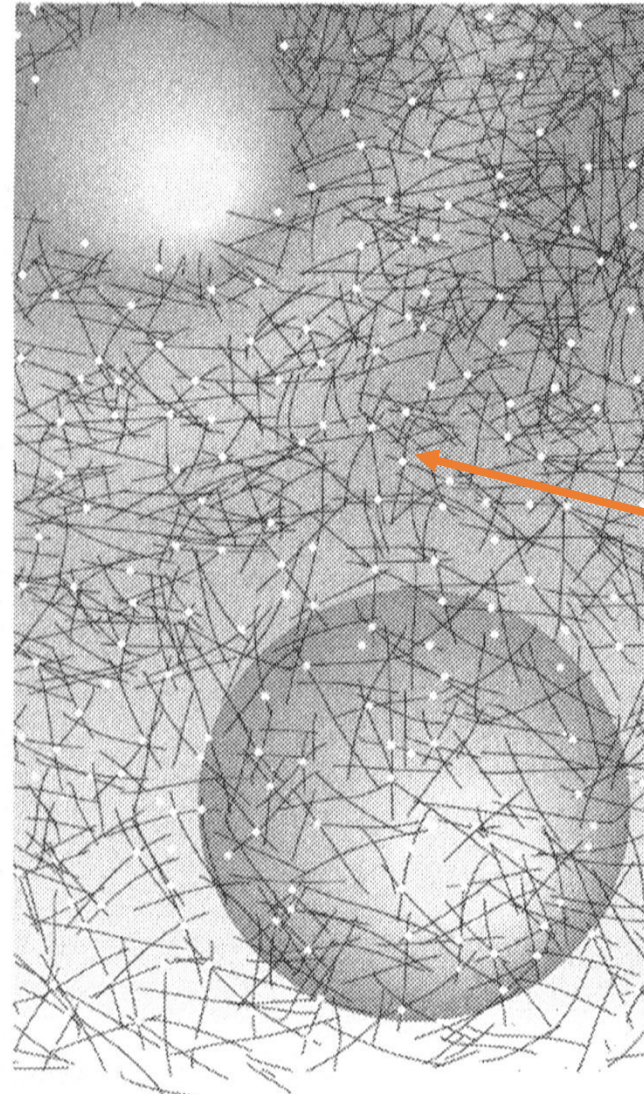
Milk

Casein protein micelles (bundles)
 10^{-7} meters in diameter



Yogurt

Bacteria produce acid



Acid causes Casein bundles to fall apart into separate casein molecules.

These rebind to each other in a network that traps water.

=> makes a gel

Use of Fermentation In agriculture

- In agriculture, plant byproducts such as corn stalks are preserved for use as animal feed by packing them into a large container (a silo) with limited access to air; microbial fermentation produces acids that lower the pH.
- **The silage** that results from this fermentation process can be kept as animal feed for long time without spoilage.

Industrial Fermentation..II

Fermentation Products and steps of industrial fermentation

Fermentation Products

There are five major groups of commercially important fermentations:

1. Those that produce microbial cells (or biomass) as the product.
2. Those that produce microbial metabolites.
3. **Those that produce microbial enzymes.**
4. Those that produce recombinant products.
5. Those that modify a compound which is added to the fermentation – the transformation process.

Fermentation Products

➤ **Biomass Production**

- Baker's Yeast
- SCP (Single Cell Protein)
- Probiotics
- Bio fertilizers
- Vaccines
- Bioweapons

1. Upstream processing

- Upstream processing includes *formulation of the fermentation medium*, *sterilisation* of air, fermentation medium and the fermenter, *inoculum preparation* and *inoculation* of the medium.

The *fermentation medium* should contain an **energy source**, a **carbon source**, a **nitrogen source** and **micronutrients** required for the growth of the microorganism along with **water** and **oxygen**, if necessary.

A medium which is used for a large scale fermentation, in order to ensure the sustainability of the operation, should have the following characteristics;

1. It should be cheap and easily available
2. It should maximise the growth of the microorganism, productivity and the rate of formation of the desired product
3. It should minimise the formation of undesired products

- Usually, waste products from other industrial processes, such as molasses, cheese whey and corn steep liquor, after modifying with the incorporation of additional nutrients, are used as the substrate for many industrial fermentation

- ***Sterilisation*** is essential for preventing the contamination with any undesired microorganisms. **Air** is sterilised by **membrane filtration** while the **medium** is usually **heat sterilised**. Any nutrient component which is **heat labile** is **filter-sterilised** and later added to the sterilised medium. The fermenter may be sterilised together with the medium or separately.
- ***Inoculum build up*** is the preparation of the seed culture in amounts sufficient to be used in the large fermenter vessel. This involves growing the microorganisms **obtained from the pure stock culture** in **several consecutive fermenters**. This process cuts down the time required for the growth of microorganisms in the fermenter, thereby increasing the rate of productivity. Then the seed culture obtained through this process is used to inoculate the fermentation medium.

2. The fermentation process

- The fermentation process involves the *propagation of the microorganism* and *production of the desired product*. The fermentation process can be categorised depending on various parameters.

It can be either **aerobic fermentation**, carried out in the presence of oxygen or **anaerobic fermentation**, carried out in the absence of oxygen. Many industrial fermentations are carried out under aerobic conditions where a few processes such as ethanol production by yeast require strictly anaerobic environments.

3. Downstream Processing

- **Downstream Processing** includes the **recovery of the products** in a pure state and the **effluent treatment**. **Product recovery** is carried out through a series of operations including *cell separation* by settling, centrifugation or filtration; *product recovery* by disruption of cells (if the product is produced intracellularly); *extraction* and *purification* of the product. Finally, the *effluents are treated* by chemical, physical or biological methods.

Industrial Fermentation..III... cont

General Aspects of Fermentation Processes-

What is fermentation technique?

Techniques for **large-scale production** of microbial products. It must both provide an **optimum environment** for the microbial synthesis of the desired product and be **economically feasible** on a large scale.

What is fermentation technique?

- Fermentation technique can be divided into following on the basis of moisture requirement

1. surface (emersion) fermentation

2. submersion fermentation technique

- The latter may be run in **batch, fed batch, continuous reactors**

1. Surface techniques:

- In the **surface techniques**, the microorganisms are cultivated on the surface of a liquid or solid substrate.
- These techniques are very complicated and used in industry for producing bread, cocoa

SSF (Maize crop)



2. Submersion processes

- In the **submersion processes**, the microorganisms grow in a liquid medium provided with the substrate
- Except in traditional beer and wine fermentation, the medium is held in fermenters and stirred to obtain a homogeneous distribution of cells and medium.
- Most processes are aerobic, and for these the medium must be vigorously aerated.
- **Used** for pickling vegetables and producing soy sauce.
- All important industrial processes (production of biomass and protein, antibiotics, enzymes) are carried out by submersion processes.



Fermenter/Bioreactors

- The heart of the fermentation process is the fermenter.
- **A bioreactor is a device in which the organisms are cultivated and motivated to form a desired product**
- Closed vessel or containment designed to give a right environment for optimal growth and metabolic activity of the organism
- Fermenter: for microbes/ Bioreactor: for eukaryotic cells
- Size variable ranging from 20-250 million litres or Large scale production (..to1000-million L capacity)

Design of a Fermenter

The success of a fermentation process is highly dependent on environmental factors

Factors to consider when designing a fermenter

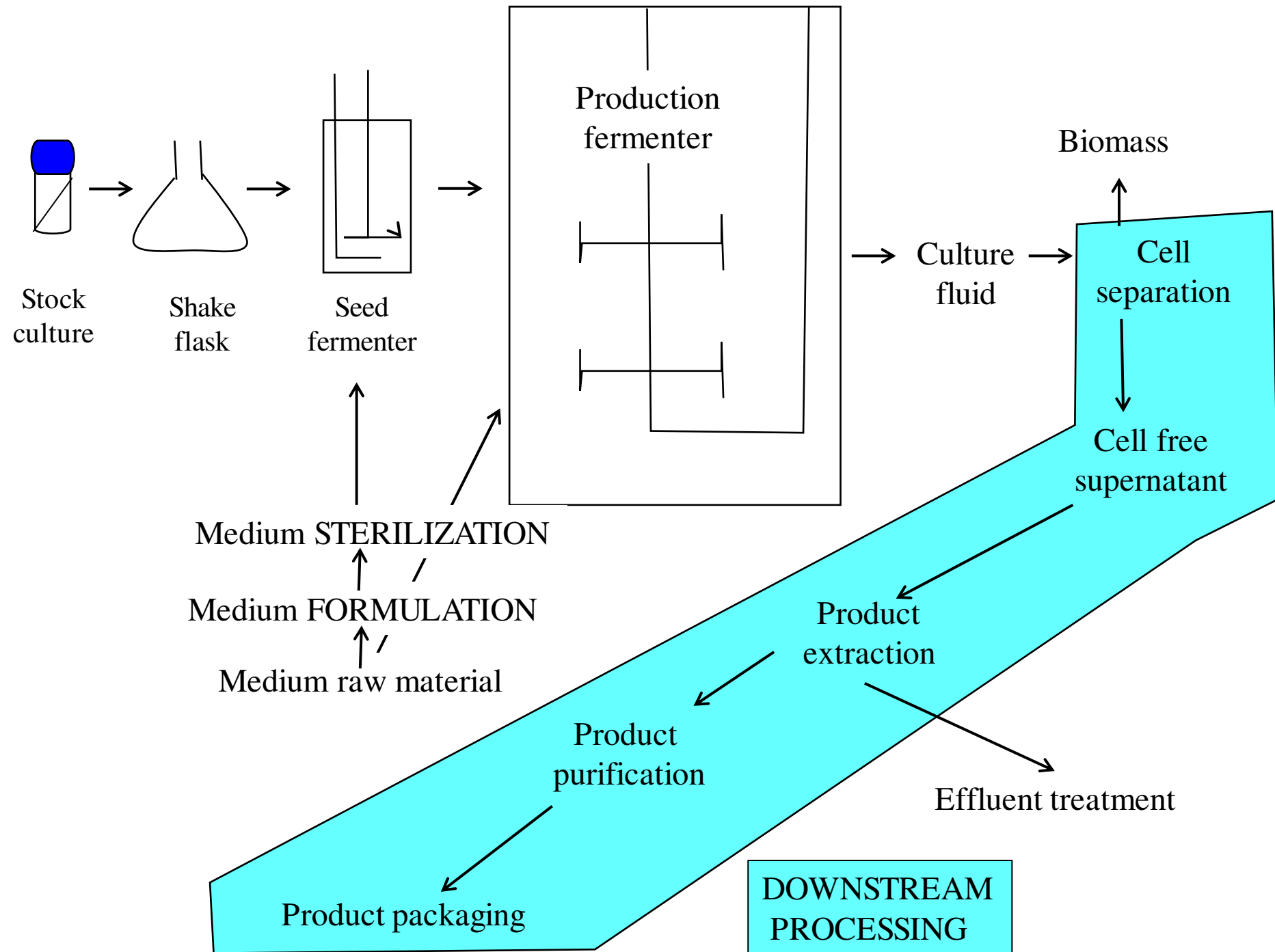
- Aseptic and long-term reliability
- Adequate for aeration and agitation
- Low power consumption
- Temperature and pH controls
- Sampling facilities
- drain or overflow
- control systems
- sensors
- cooling to achieve maximum microbial yield

(14 L fermenter shown is a copyright of New Brunswick Scientific)

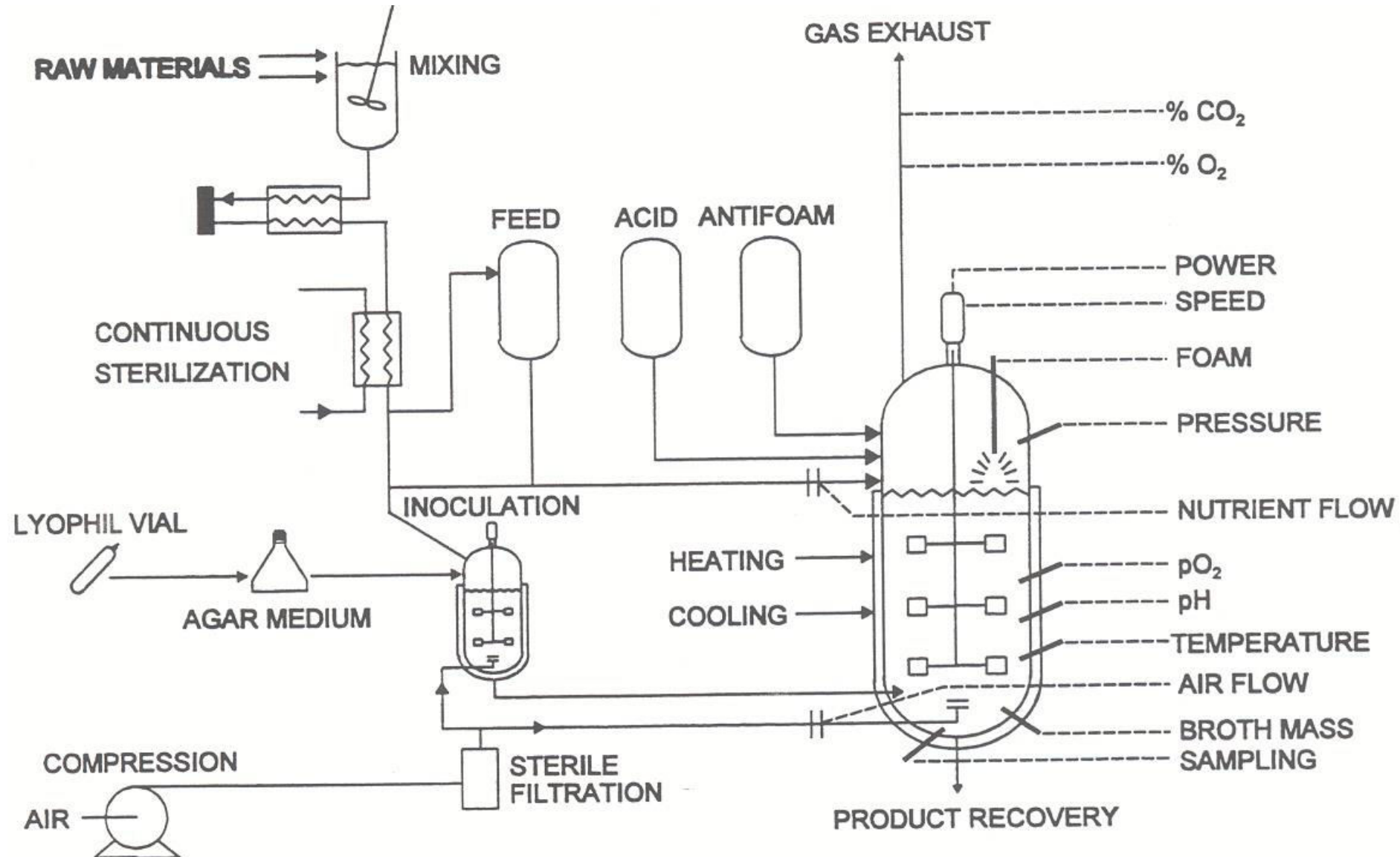


Types of Bioreactors

- Simple fermenters (batch and continuous)
- Fed batch fermenter
- Air-lift or bubble fermenter
- Cyclone column fermenter
- Tower fermenter
- Packed bed bioreactor
- photobioreactor



Flow sheet of a multipurpose fermenter and its auxiliary equipment



Industrial Fermentation..III... cont

Basic modes for operating a fermenter

Types of fermentation on the basis of Process

Basic modes for operating a fermenter

- Batch fermentation
- Closed system
- Batch fermentation refers to “A technique used to grow microorganisms or cells.
- A limited supply of Sterile nutrients (substrate) for growth is inoculated (**starter culture**) when these are used up, or some other factor becomes limiting, the **culture** declines.
- The time for which fermentation occurs is called ‘fermentation time’ or ‘**batch time**’
- The only material added and removed during the course of a batch fermentation is oxygen (in the form of air, if the process is aerobic) and pH control solutions.

... cont

- The culture is allowed to grow until no more of the product is being made
- Cells, or products that the organisms have made, are "harvested" and fermenter is cleaned out for another run.
- The **principal disadvantage** of batch processing is the high proportion of unproductive time (down-time) between batches, comprising the charge and discharge of the fermenter vessel, the cleaning, sterilization and re-start process

... cont

- We take the example of *Aspergillus niger* and *Lactobacillus* are the microbes used to commercially produce citric acid and lactic acid, respectively.
- The production takes place in a batch fermenter.
- Rules of fermentation are applied on batch fermentation
 - *Microbial Growth Kinetics*
 - *Media for Industrial Fermentations*
 - *Sterilization*
 - *The Development of Inocula for Industrial Fermentations*
 - *Design of a Fermenter*
 - *Instrumentation and Control*
 - *Aeration and Agitation*

Phases of Batch fermentation

- ❑ **lag phase** (adapt to their surroundings)
- ❑ **exponential growth** (grow in numbers)
- ❑ **stationary phase** (stop growing)
- ❑ **death phase**

Microbial Growth Kinetics

- Lag Phase
 - This is the first phase in the fermentation process
 - The cells have just been injected into a new environment and they need time to adjust accordingly
 - Cell growth is minimal in this phase.

Microbial Growth Kinetics

- Exponential Phase
 - The second phase in the fermentation process
 - The cells have adjusted to their environment and rapid growth takes place
 - Cell growth rate is highest in this phase

Microbial Growth Kinetics

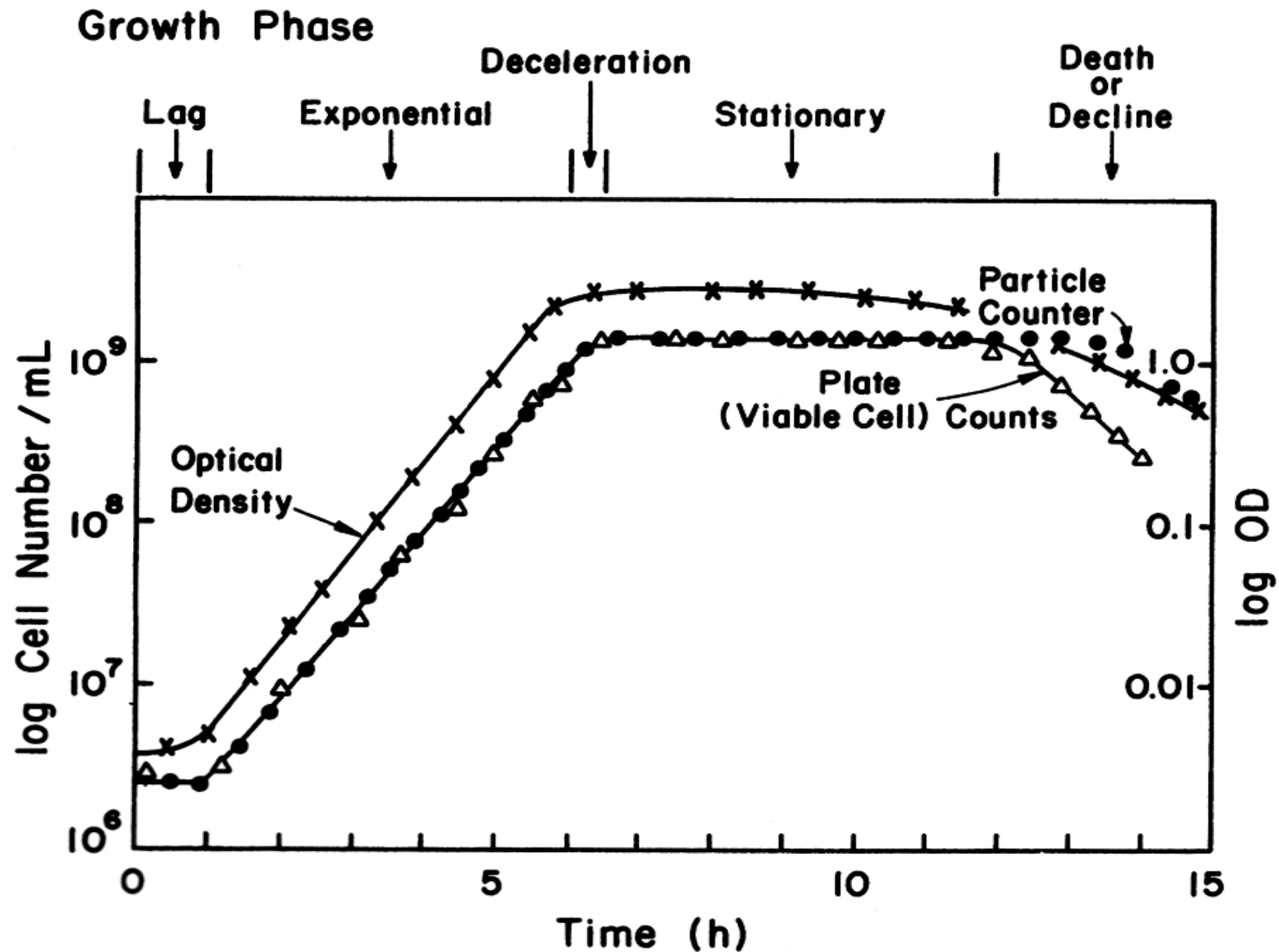
- Exponential Phase (Continued)
 - At some point the cell growth rate will level off and become constant
 - The most likely cause of this leveling off is substrate limited inhibition
 - Substrate limited inhibition means that the microbes do not have enough nutrients in the medium to continue multiplying.

Microbial Growth Kinetics

- Stationary phase
 - This is the third phase in the fermentation process
 - The cell growth rate has leveled off and become constant
 - The number of cells multiplying equals the number of cells dying

Microbial Growth Kinetics

- Death phase
 - The fourth phase in the fermentation process
 - The number of cells dying is greater than the number of cells multiplying
 - The cause of the death phase is usually that the cells have consumed most of the nutrients in the medium and there is not enough left for sustainability



Lecture No. 32

2. Continuous Fermentation

- A Continuous fermentation is a process in which fresh medium (substrate) is **continuously** added in the bioreactor , and biomass or products containing left over nutrients and microorganisms are **continuously** removed at the same rate to keep the **culture** volume constant.
- Under these conditions the cells remain in the logarithmic phase of growth

3. Fed-batch Fermentation

- The fed-batch technique was originally devised by yeast producers in the early 1900s to regulate the growth in batch culture of *Saccharomyces* Yeast producers observed that in the presence of high concentrations of malt, a by-product - ethanol - was produced, while in low concentrations of malt, the yeast growth was restricted. The problem was then solved by a controlled feeding regime, so that yeast growth remained substrate limited.
- The concept was then extended to the production of other products, such as some enzymes, antibiotics, growth hormones, microbial cells, vitamins, amino acids and other organic acids.

... cont

- ***Fed-batch culture*** is, in the broad sense, defined as an operational technique in biotechnological processes where one or more nutrients (substrates) are fed (supplied) to the bioreactor during cultivation and in which the **product(s)** remain in the bioreactor until the end of the run.
- An alternative description of the method is that of a culture in which "a base medium supports initial cell culture and a feed medium is added to prevent nutrient depletion".
- It is also a type of **semi-batch culture**. In some cases, all the nutrients are fed into the bioreactor.

... cont

- ***Advantage of the fed-batch culture*** is that one can control concentration of fed-substrate in the culture liquid at arbitrarily desired levels (in many cases, at low levels).
- Generally speaking, fed-batch culture is superior to **conventional batch culture** when controlling concentrations of a nutrient (or nutrients) affect the yield or productivity of the desired metabolite.

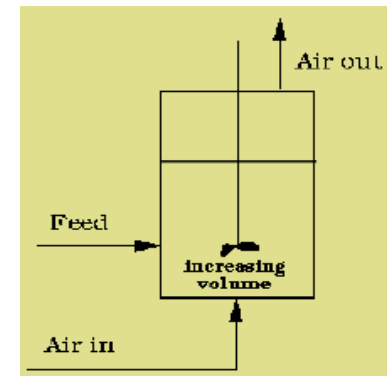
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Basically, cells are grown under a batch regime for some time, usually until close to the end of the exponential growth phase.

At this point, the reactor is fed with a solution of substrates, without the removal of culture fluid.

This feed should be balanced enough to keep the growth of the microorganisms at a desired specific growth rate and reducing simultaneously the production of by-products (that can be growth or product production inhibitory and make the system not as effective).

By products may lead to cell death



GLUCONEOGENESIS

The process of synthesis of glucose from non-carbohydrate sources is known as Gluconeogenesis (abbreviated GNG) (“new formation of sugar”), OR

Gluconeogenesis is the pathway, which converts pyruvate and related non-carbohydrate carbon compounds to glucose.

Why the body needs Gluconeogenesis?

- For the human brain and nervous system, as well as the erythrocytes, testes, renal medulla, and embryonic tissues, glucose from the blood is the sole or major fuel source.
- The brain alone requires about 120 g of glucose each day
- more than half of all the glucose stored as glycogen in muscle and liver
- between meals and longer fasts, after vigorous exercise, glycogen is depleted
- For these times, organisms need a method for synthesizing glucose from **noncarbohydrate precursors**.

Site of Gluconeogenesis?

Organisms doing Gluconeogenesis: all animals, plants, fungi, and microorganisms

In many microorganisms, gluconeogenesis starts from simple organic compounds of two or three carbons, such as acetate, lactate, and propionate, in their growth medium.

Precursors of glucose in Gluconeogenesis: The important precursors of glucose in animals are three-carbon compounds such as lactate, pyruvate, and glycerol, as well as certain amino acids

.. cont

Organs doing Gluconeogenesis:

- mainly in the liver
- To a lesser extent in renal cortex
- in the epithelial cells that line the inside of the small intestine
- In cytosol and mitochondria depending upon the substrate

Where does this glucose go?

The glucose produced passes into the blood to supply other tissues.

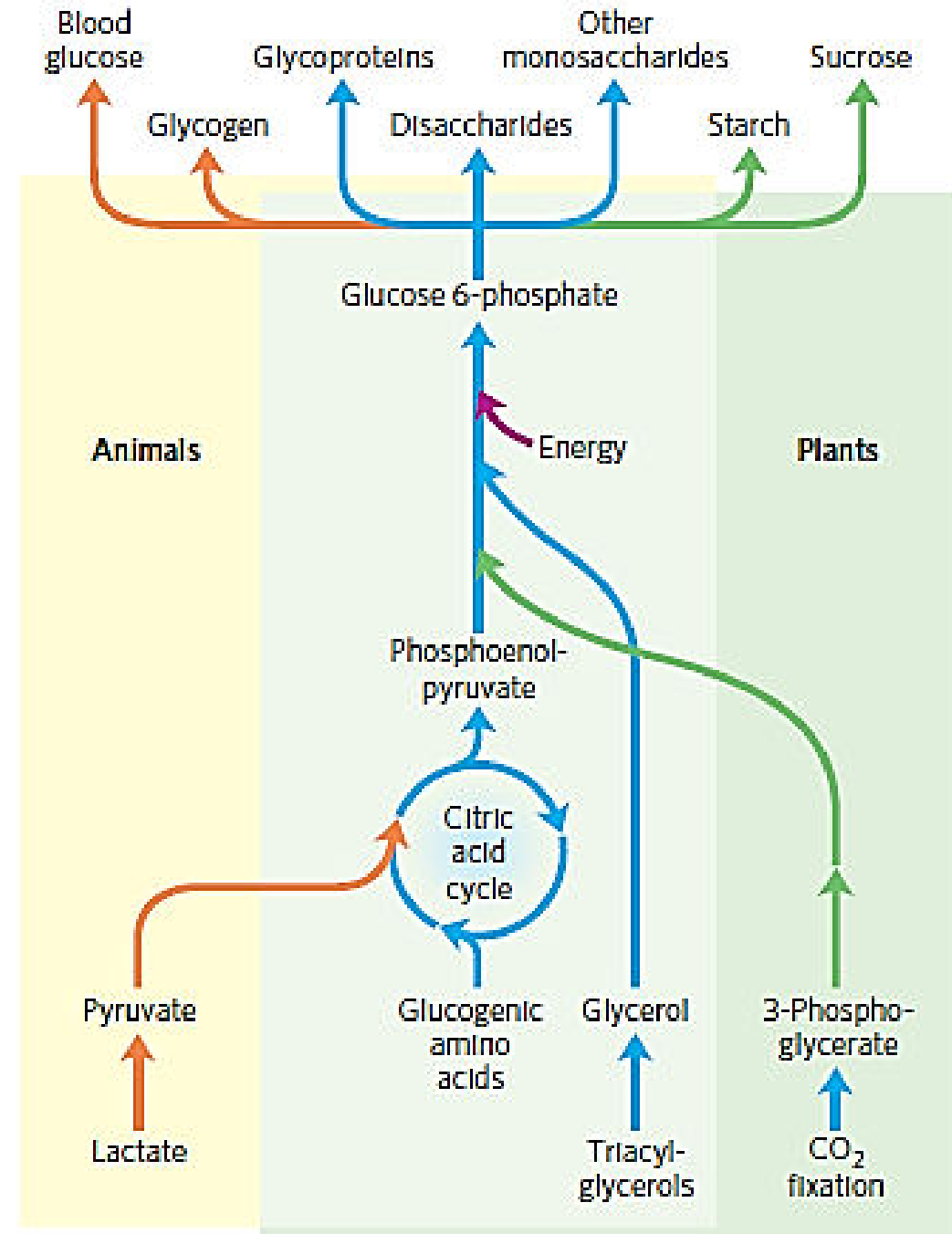
Nature: Anabolic: 4 ATP are required for synthesis of 1 glucose from 1 pyruvate or lactate

Pathways involved in Gluconeogenesis

- Reverse glycolysis (PEP to G6P)
- Tri carbocyclic acid cycle (TCA) or Krebs's cycle
- Cori cycle
- Glucose-alanine cycle

Precursors of glucose

- Gluconeogenesis occurs in all animals, plants, fungi, and microorganisms.
- The reactions are essentially the same in all tissues and all species.



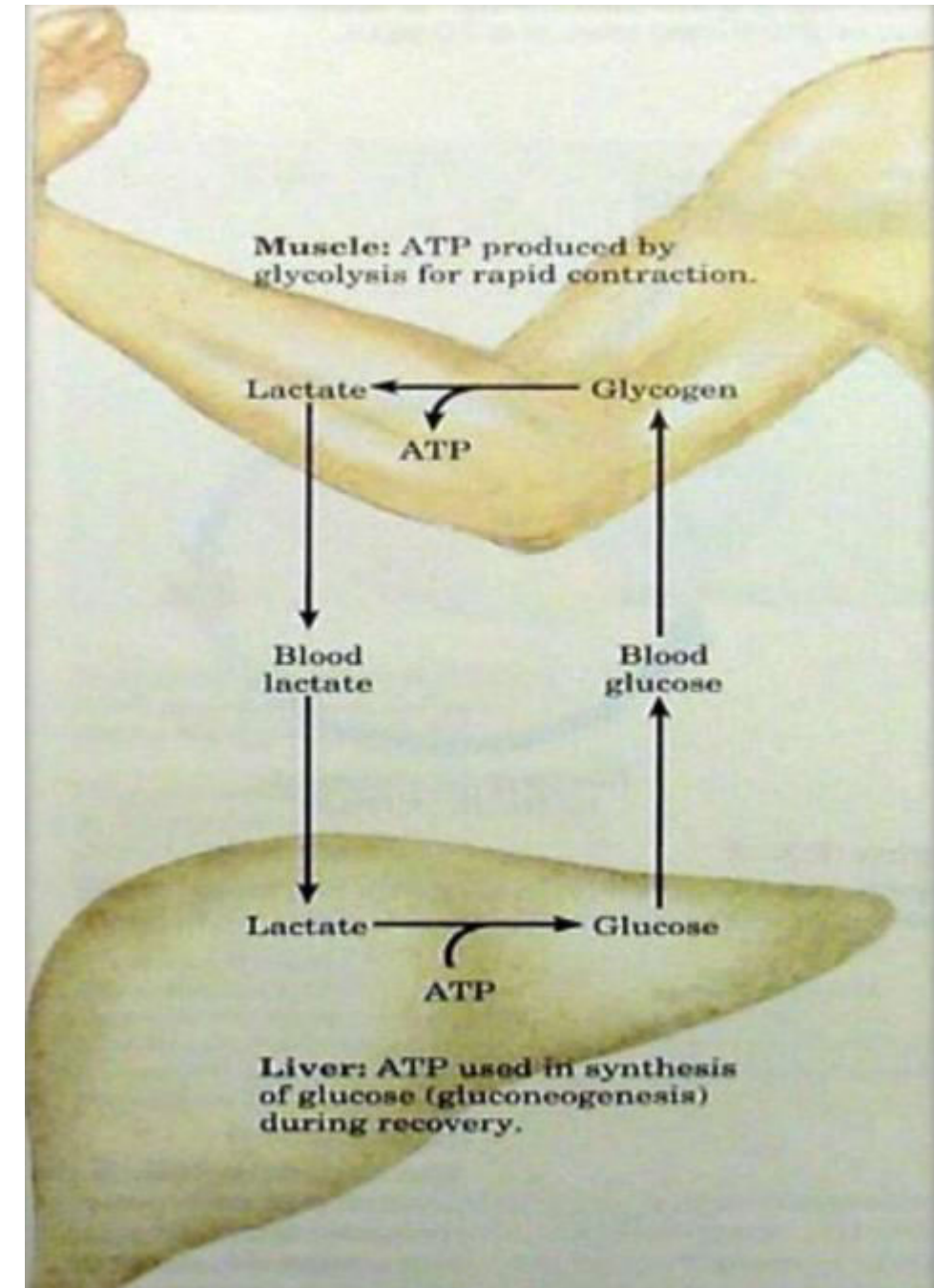
GLUCONEOGENESIS..cont

Biomedical importance of Gluconeogenesis

- To maintain the blood glucose concentration during prolonged fasting or starvation when sufficient carbohydrate is not available from the diet or glycogen reserves.
- It maintains the level of intermediates of the TCA cycle even when fatty acids are the main source of acetyl coA in the tissues.
- It clears the lactate produced by muscle and erythrocytes and glycerol produced by adipose tissue.

Cori cycle (the Lactic acid cycle)

- The pathway through which lactate produced by anaerobic glycolysis in skeletal muscle returns to the liver and is converted to glucose
- This moves back to muscle and is converted to glycogen is called the Cori cycle
- **Site: Liver**
- **Substrate: Lactate**
- “Occurs due to absence of glucose-6-phosphate in liver”



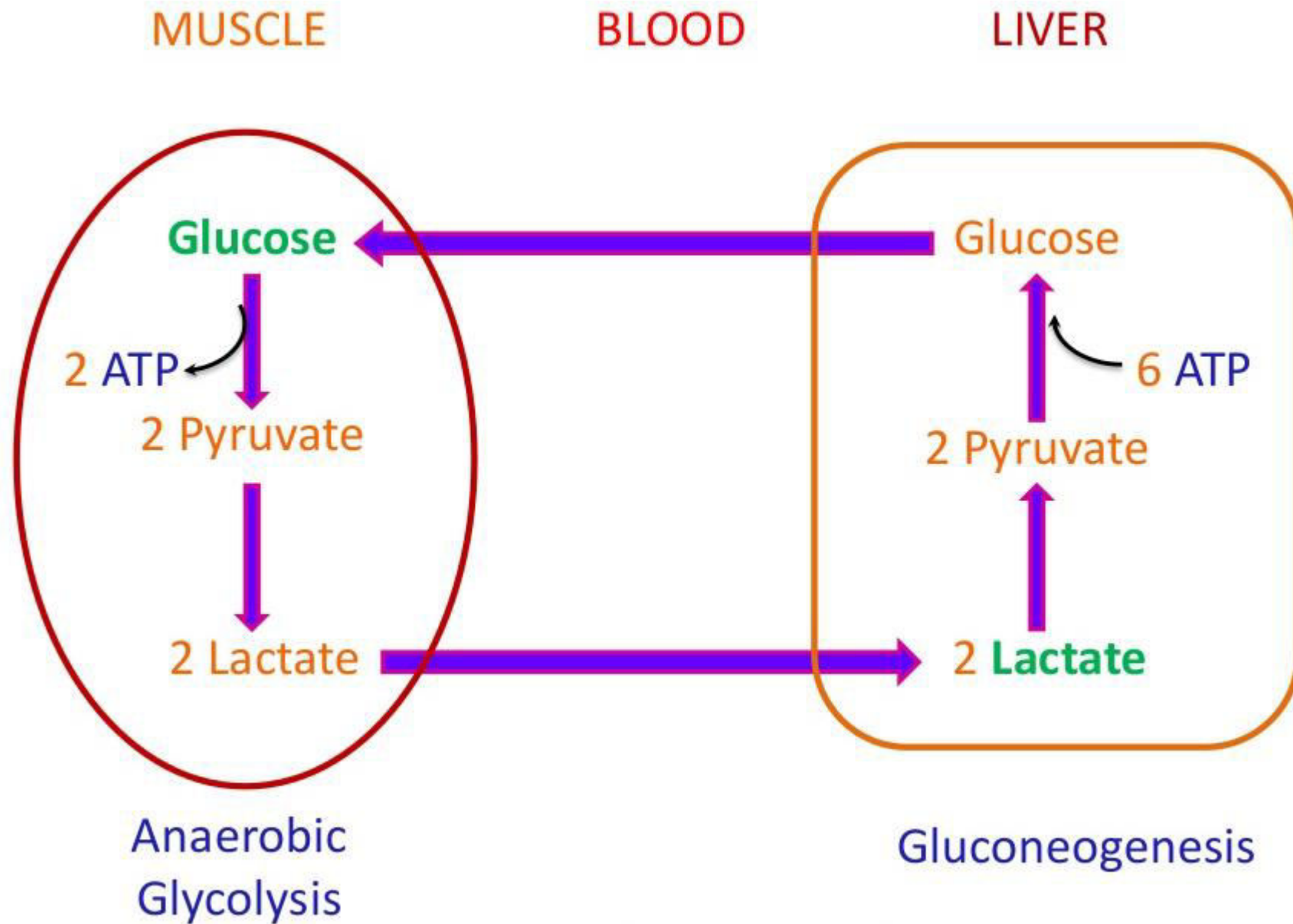


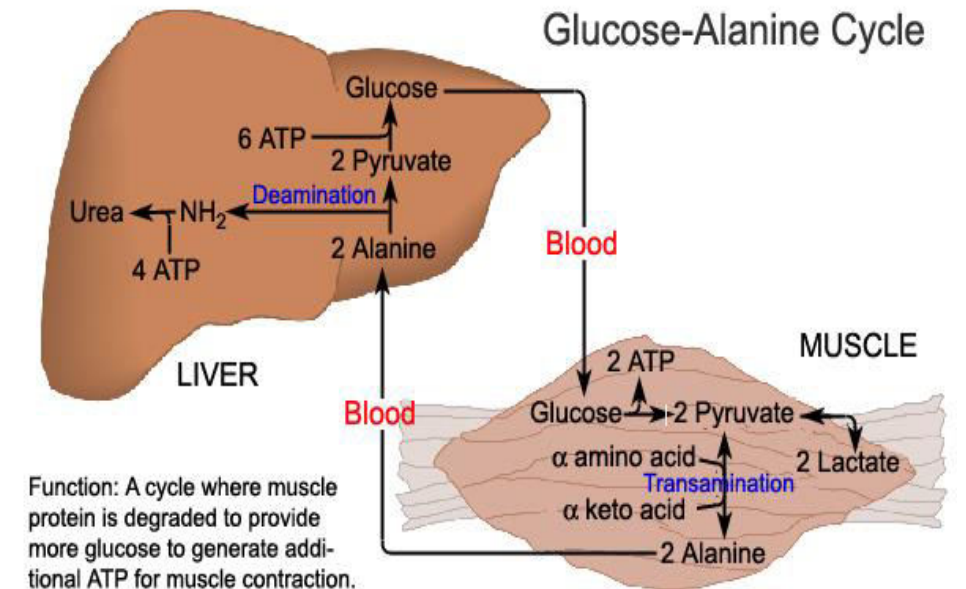
Fig: The Cori Cycle

Significance of Cori cycle

1. Prevents lactic acidosis in the muscle under anaerobic conditions
2. The cycle is also important in producing ATP, an energy source, during muscle activity
3. The Cori cycle is a much more important source of substrate for gluconeogenesis than food.
4. The drug **METFORMIN** can cause lactic acidosis in patients because metformin inhibits the hepatic gluconeogenesis of the Cori cycle

Glucose-Alanine cycle

- When in extrahepatic tissues amino acids are used for energy, pyruvate, derived from GLYCOLYSIS, is used as amino group acceptor, forming **alanine**
- Alanine diffuses into the bloodstream and reaches the liver
- In the liver, the amino group of alanine is transferred to **α -ketoglutarate** to form pyruvate
- The amino group enters the urea cycle, and in part acts as a nitrogen donor in many biosynthetic pathways.
- Pyruvate enters the GLUCONEOGENESIS and is used for glucose synthesis
- The newly formed glucose diffuses into the bloodstream and reaches the peripheral tissues where, due to GLYCOLYSIS, is converted into pyruvate that can accept amino groups from the free amino acids, thus closing the cycle.



Glucose → Pyruvate → Alanine → Pyruvate → Glucose

consists of a series of steps through which extrahepatic tissues, for example the skeletal muscle, export **pyruvate and amino groups as alanine** to the liver, and receive glucose from the liver via the bloodstream.

The main steps of the glucose-alanine cycle are summarized below.

When in extrahepatic tissues amino acids are used for energy, pyruvate, derived from the [glycolytic pathway](#), is used as amino group acceptor, forming **alanine**, a nonessential amino acid.

Alanine diffuses into the bloodstream and reaches the liver.

In the liver, the amino group of alanine is transferred to α -ketoglutarate to form pyruvate and glutamate, respectively.

The amino group of glutamate mostly enters the urea cycle, and in part acts as a nitrogen donor in many biosynthetic pathways.

Pyruvate enters the [gluconeogenesis pathway](#) and is used for glucose synthesis.

The newly formed glucose diffuses into the bloodstream and reaches the peripheral tissues where, due to [glycolysis](#), is converted into pyruvate that can accept amino groups from the free amino acids, thus closing the cycle.

Therefore, the glucose-alanine cycle provides a link between [carbohydrate](#) and **amino acid metabolism**, as schematically described below.

Glucose → Pyruvate → Alanine → Pyruvate → Glucose

Significance of Glucose-Alanine cycle

1. The Alanine cycle is less productive than the Cori cycle, which uses lactate, since a byproduct of energy production from alanine is production of Urea
2. Removal of the urea is energy-dependent, requiring four "high-energy" phosphate bonds
3. This pathway requires the presence of Alanine aminotransferase, restricted to muscle, liver and intestine
4. Therefore, this pathway is used instead of the Cori cycle only when an aminotransferase is present, or a need to transfer ammonia to the liver
5. The alanine cycle also Recycles carbon skeletons between muscle and liver
6. Transports ammonium to the liver and is converted into urea.

Gluconeogenesis and glycolysis

Gluconeogenesis and glycolysis are not identical pathways running in opposite directions, although they do share several steps

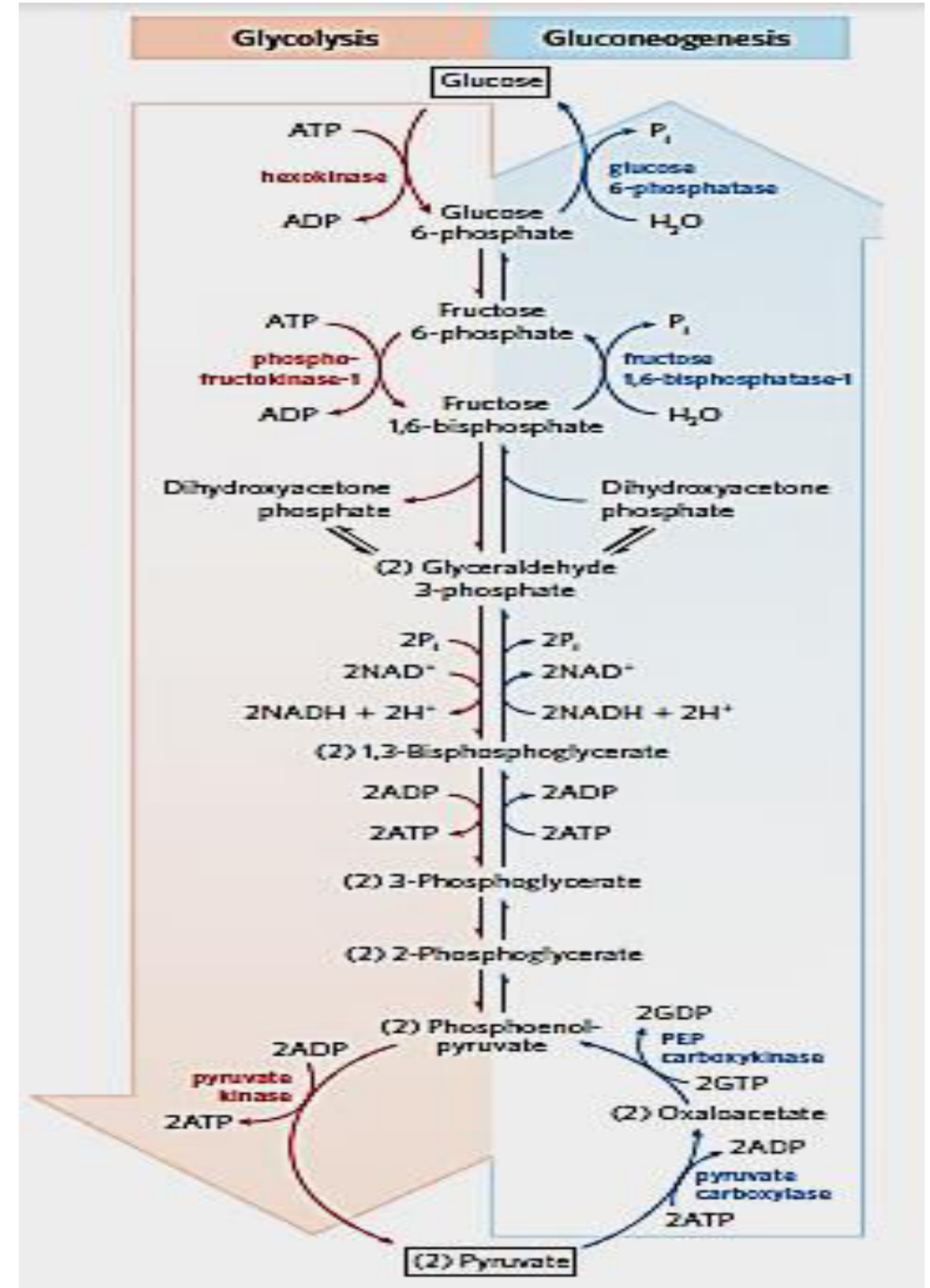
7 of the 10 enzymatic reactions of gluconeogenesis are the reverse of glycolytic reactions

- three reactions of glycolysis are essentially irreversible in vivo and cannot be used in gluconeogenesis:
 1. the conversion of **glucose to glucose 6-phosphate** by **hexokinase**,
 2. the **phosphorylation** of fructose 6-phosphate to fructose 1,6-bisphosphate by **phosphofructokinase-1**
 3. the conversion of **phosphoenolpyruvate to pyruvate** by **pyruvate kinase**

Glycolysis VS Gluconeogenesis

FIGURE 14–17 (Lehninger Ed. 6)

The reactions of glycolysis are on the left side, in red; the opposing pathway of gluconeogenesis is on the right, in blue.

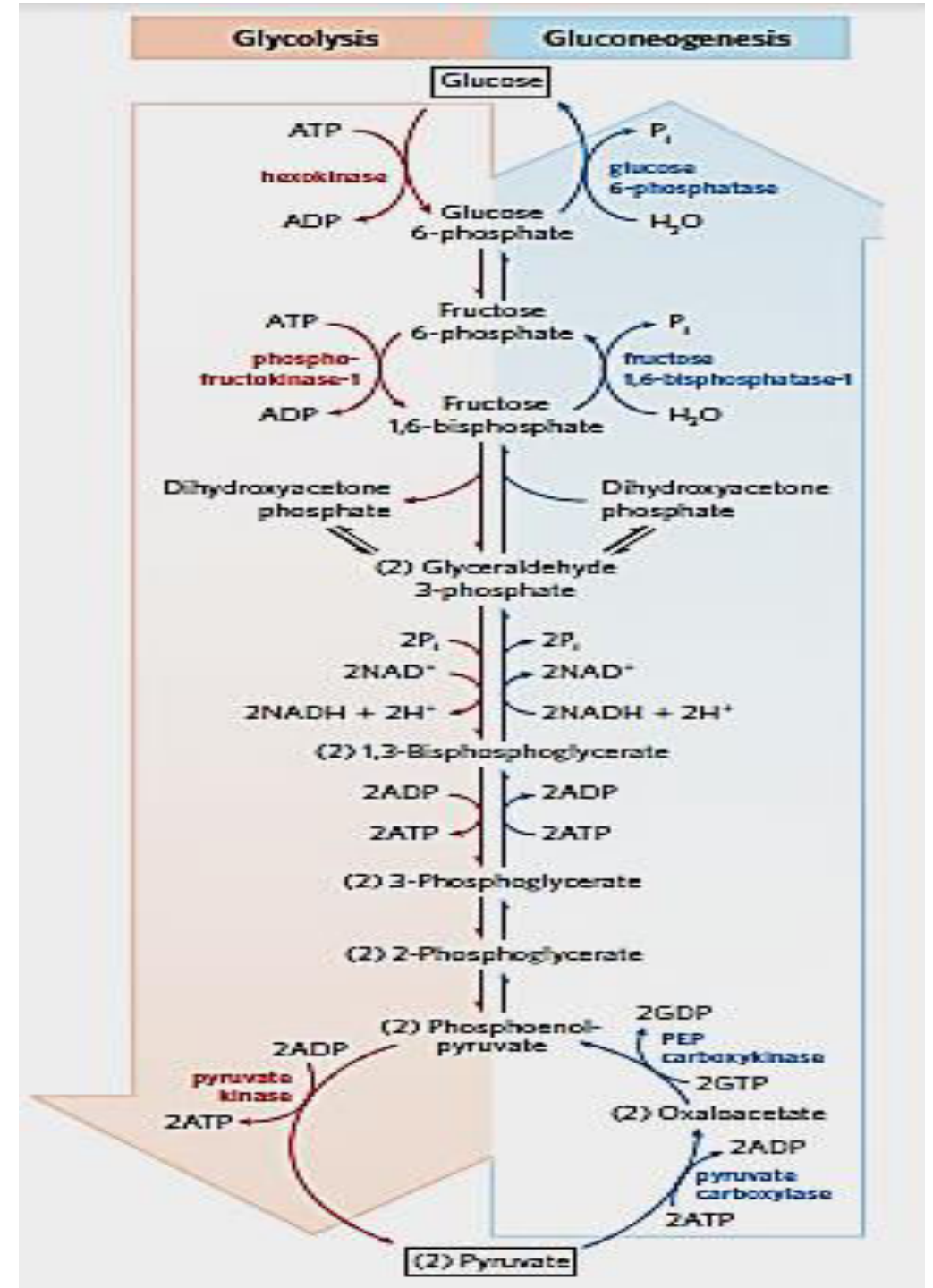


GLUCONEOGENESIS..cont..3

Bypass reactions in Gluconeogenesis

Glycolysis VS Gluconeogenesis

The reactions of glycolysis are on the left side, in red; the opposing pathway of gluconeogenesis is on the right, in blue.



Glycolysis VS Gluconeogenesis

- Both are energy generating pathways-**NOT identical**
- At one time only one of 2 can take place within a cell
- Glycolysis takes place essentially in the cytoplasm
- Gluconeogenesis occurs mutually in the cytosol and mitochondria
- Requirement of gluconeogenesis-**Glycogen**
- **Gluconeogenesis** from pyruvate **share 7 reversible** steps of glycolysis
- **Seven out of the 10 enzymes** used in the glycolytic pathway **are** used..

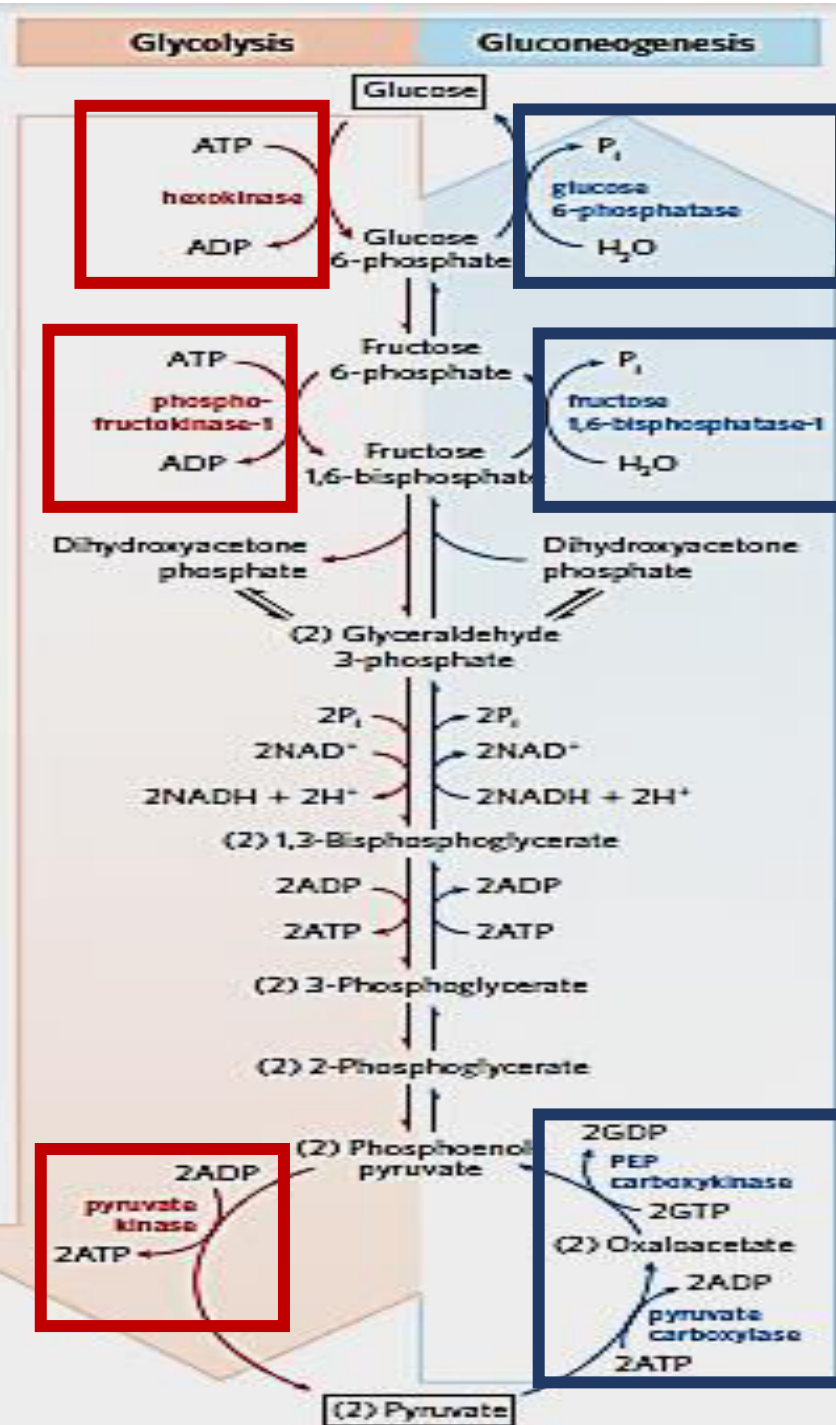
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- The 3 irreversible steps of glycolysis that proceed with a large negative free energy change are bypassed during **gluconeogenesis** by using different enzymes.
- These three bypass reactions (during glycolysis) are catalyzed by:
 1. Pyruvate kinase
 2. Phosphofructokinase-1 (PFK-1)
 3. Hexokinase/glucokinase

TABLE 14–2 Free-Energy Changes of Glycolytic Reactions in Erythrocytes

Glycolytic reaction step	$\Delta G'^{\circ}$ (kJ/mol)	ΔG (kJ/mol)
1 Glucose + ATP $\longrightarrow$ glucose 6-phosphate + ADP	−16.7	−33.4
2 Glucose 6-phosphate $\rightleftharpoons$ fructose 6-phosphate	1.7	0 to 25
3 Fructose 6-phosphate + ATP $\longrightarrow$ fructose 1,6-bisphosphate + ADP	−14.2	−22.2
4 Fructose 1,6-bisphosphate $\rightleftharpoons$ dihydroxyacetone phosphate + glyceraldehyde 3-phosphate	23.8	−6 to 0
5 Dihydroxyacetone phosphate $\rightleftharpoons$ glyceraldehyde 3-phosphate	7.5	0 to 4
6 Glyceraldehyde 3-phosphate + P_i + NAD^+ $\rightleftharpoons$ 1,3-bisphosphoglycerate + NADH + H^+	6.3	−2 to 2
7 1,3-Bisphosphoglycerate + ADP $\rightleftharpoons$ 3-phosphoglycerate + ATP	−18.8	0 to 2
8 3-Phosphoglycerate $\rightleftharpoons$ 2-phosphoglycerate	4.4	0 to 0.8
9 2-Phosphoglycerate $\rightleftharpoons$ phosphoenolpyruvate + H_2O	7.5	0 to 3.3
10 Phosphoenolpyruvate + ADP $\longrightarrow$ pyruvate + ATP	−31.4	−16.7

Note: $\Delta G'^{\circ}$ is the standard free-energy change, as defined in Chapter 13 (pp. 507–508). ΔG is the free-energy change calculated from the actual concentrations of glycolytic intermediates present under physiological conditions in erythrocytes, at pH 7. The glycolytic reactions bypassed in gluconeogenesis are shown in red. Biochemical equations are not necessarily balanced for H or charge (p. 517).



1. Conversion of Pyruvate to Phosphoenolpyruvate

- The first of the bypass reactions in gluconeogenesis is the conversion of pyruvate to phosphoenolpyruvate (PEP).
- This reaction cannot occur by simple reversal of the pyruvate kinase reaction
- Instead, the phosphorylation of pyruvate is achieved by a sequence of reactions that in eukaryotes requires 2 enzymes in both the **cytosol and mitochondria**.

..cont

- Pyruvate is first transported from the cytosol into mitochondria or is generated from alanine within mitochondria by **transamination**, in which the **alpha -amino group is transferred from alanine (leaving pyruvate) to an alpha-keto carboxylic acid**
- In mitochondria, pyruvate is converted to oxaloacetate in a biotin (**coenzyme**)-requiring reaction catalyzed by **pyruvate carboxylase**, a **mitochondrial enzyme**



Bicarbonate Pyruvate

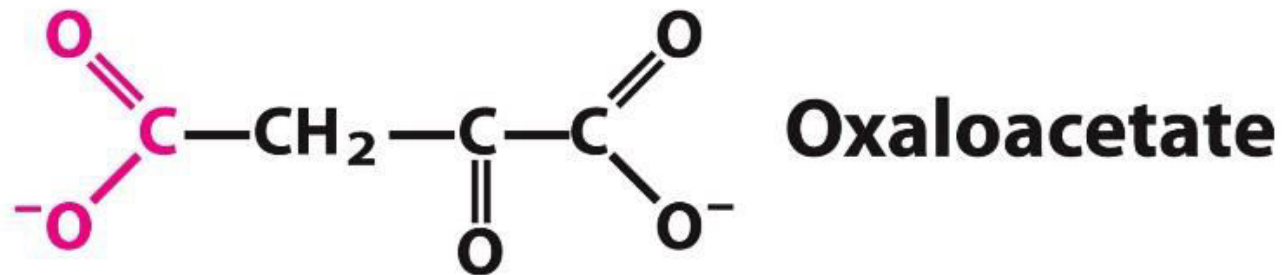
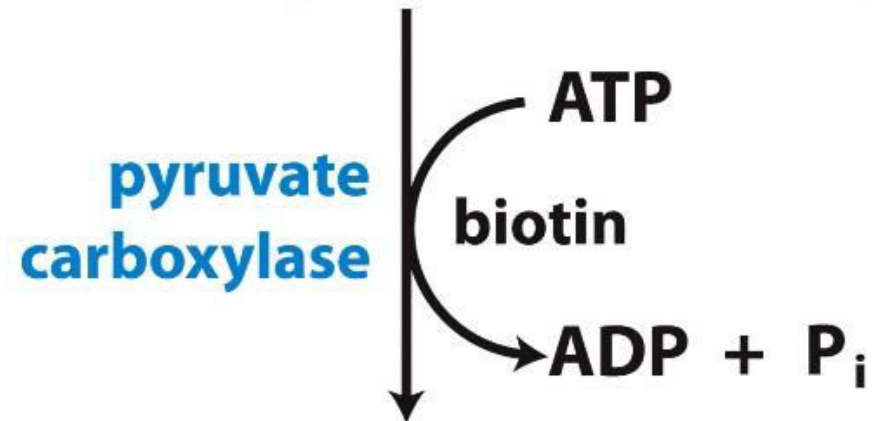
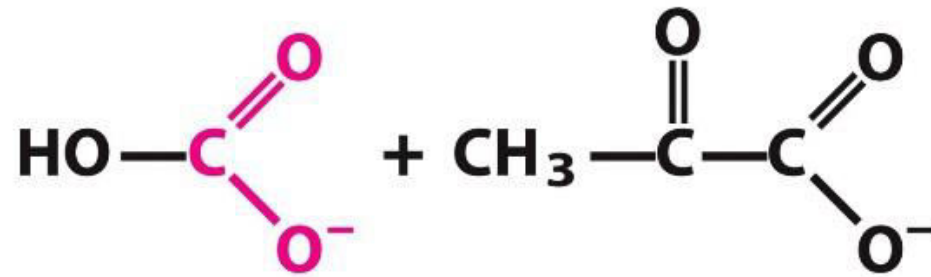


Figure 14-18a
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..cont

- **Pyruvate carboxylase** is the first regulatory enzyme in the gluconeogenic pathway
- **Pyruvate carboxylase requires acetyl-CoA as a positive effector. (Acetyl-CoA is produced by fatty acid oxidation)**
- the mitochondrial membrane has no transporter for oxaloacetate, before export to the cytosol the oxaloacetate formed from pyruvate must be reduced to malate by mitochondrial **malate dehydrogenase**, at the expense of NADH

Alternative paths from pyruvate to phosphoenolpyruvate

- Oxalo acetic acid (OAA) produced in mitochondria is impermeable through mitochondrial membrane
- Oxaloacetate + NADH + H⁺ $\longleftrightarrow$ L-malate + NAD⁺
- Therefore, Oxalo acetic acid is converted **into malate**, exported from the mitochondrion into the cytoplasm, **converted back to oxaloacetate** in order to allow gluconeogenesis
- In both mitochondria and cytoplasm, the transporter of malate is **malate dehydrogenase**

- (a) **In the cytosol**, oxaloacetate is converted to phosphoenolpyruvate by **PEP carboxykinase**.
- (b) The CO₂ incorporated in the pyruvate carboxylase reaction is lost here as CO₂. The decarboxylation leads to a rearrangement of electrons that facilitates attack of the carbonyl oxygen of the pyruvate moiety on the phosphate of GTP

(a)

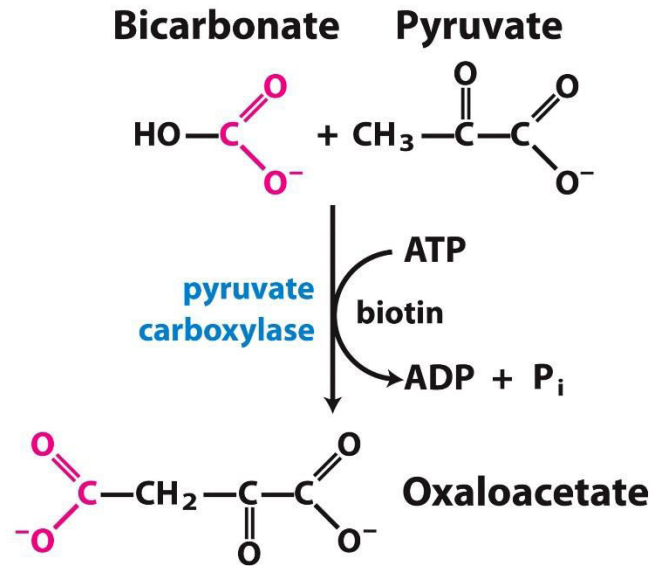


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(b)

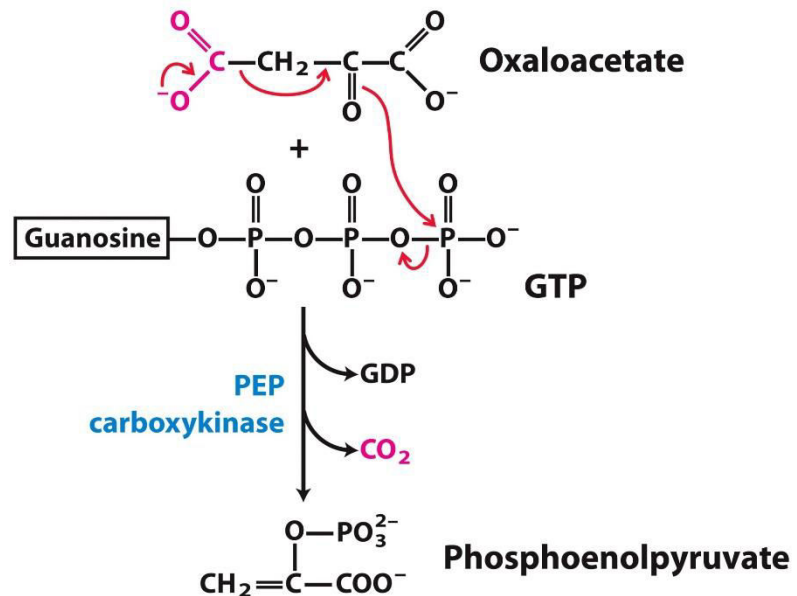
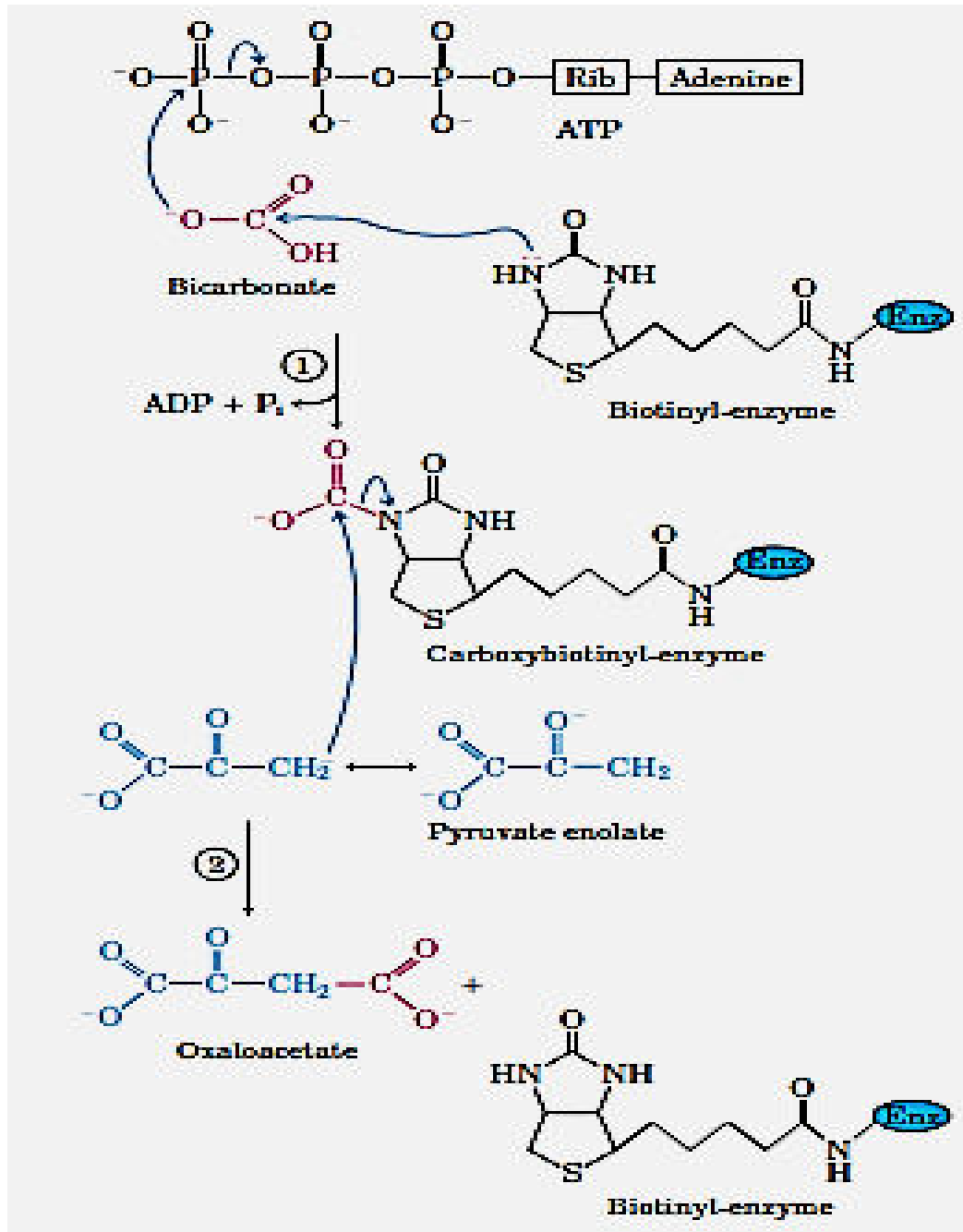


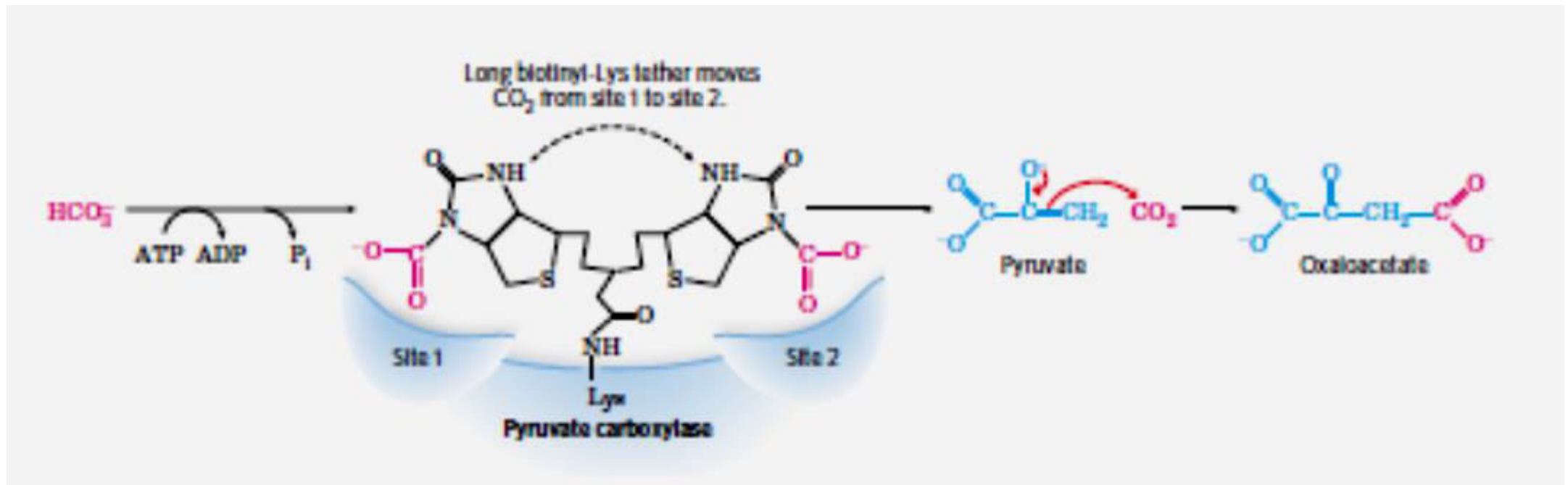
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- Synthesis of phosphoenolpyruvate from pyruvate.
- (a) In mitochondria, pyruvate is converted to oxaloacetate in a **biotin-requiring reaction** catalyzed by pyruvate carboxylase.
- (b) In the cytosol, oxaloacetate is converted to phosphoenolpyruvate by PEP carboxykinase.
- The CO₂ incorporated in the pyruvate carboxylase reaction is lost here as CO₂.
- The **decarboxylation** leads to a rearrangement of electrons that facilitates attack of the **carbonyl oxygen** of the pyruvate moiety on **the phosphate of GTP**.



Role of biotin in the pyruvate carboxylase reaction.

- The cofactor biotin is covalently attached to the enzyme through an amide linkage to the amino group of a Lys residue, forming a biotinyl-enzyme.
- The reaction occurs in two phases, which occur at two different sites in the enzyme.
- At catalytic site 1, bicarbonate ion is converted to CO_2 at the expense of ATP. Then CO_2 reacts with biotin, forming carboxybiotinyl-enzyme.
- The long arm composed of biotin and the Lys side chain to which it is attached then carry the CO_2 of carboxybiotinyl enzyme to catalytic site 2 on the enzyme surface, where CO_2 is released and reacts with the pyruvate, forming oxaloacetate and regenerating the biotinyl-enzyme.



Role of biotin in the pyruvate carboxylase reaction.

- The cofactor biotin is covalently attached to the enzyme through an amide linkage to the ϵ -amino group of a Lys residue, forming a biotinyl-enzyme.
- The reaction occurs in two phases, which occur at two different sites in the enzyme.
- At catalytic site 1, bicarbonate ion is converted to CO_2 at the expense of ATP. Then CO_2 reacts with biotin, forming carboxybiotinyl-enzyme.
- The long arm composed of biotin and the Lys side chain to which it is attached then carry the CO_2 of carboxybiotinyl-enzyme to catalytic site 2 on the enzyme surface, where CO_2 is released and reacts with the pyruvate, forming oxaloacetate and regenerating the biotinyl-enzyme.

(a)

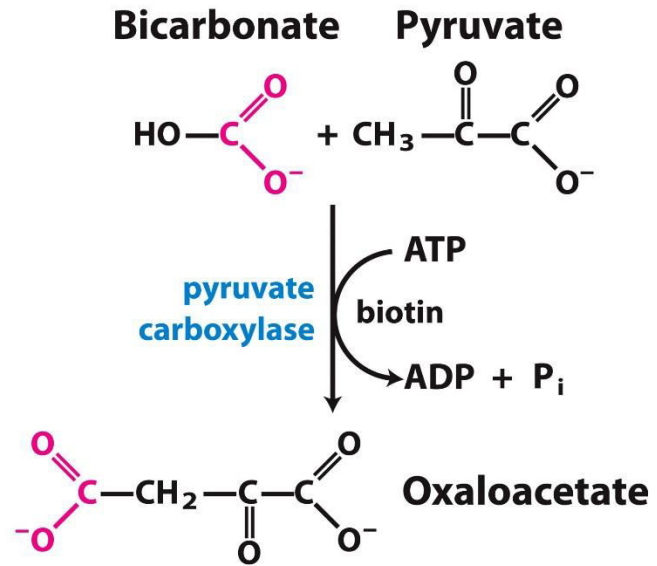


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(b)

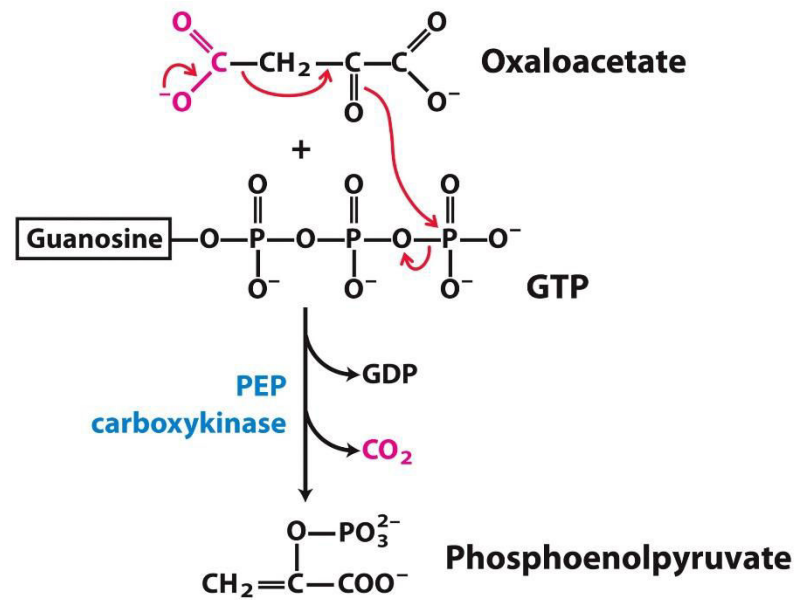
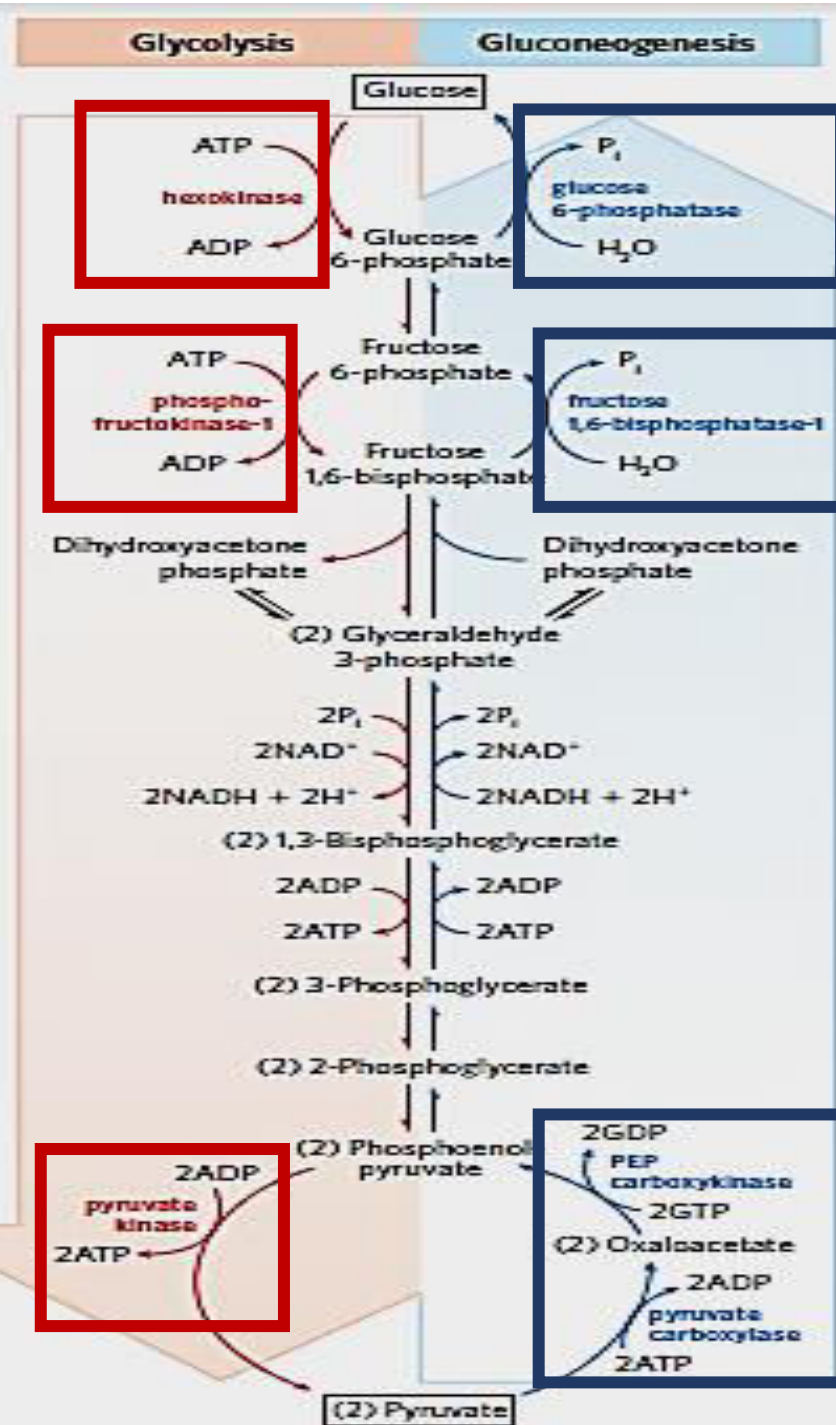


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..cont

- There is a logic to the route of these reactions through the mitochondrion.
- The $[NADH]/[NAD^+]$ ratio in the cytosol is 8×10^{-4} , about 10 times lower than in mitochondria.
- Because cytosolic NADH is consumed in gluconeogenesis (in the conversion of 1,3-bisphosphoglycerate to glyceraldehyde 3-phosphate)
- Glucose biosynthesis cannot proceed unless NADH is available.
- The transport of malate from the mitochondrion to the cytosol and its reconversion there to oxaloacetate effectively moves NAD to the cytosol, and converts it into NADH.
- This path from pyruvate to PEP therefore provides an important balance between NADH produced and consumed in the cytosol during gluconeogenesis.

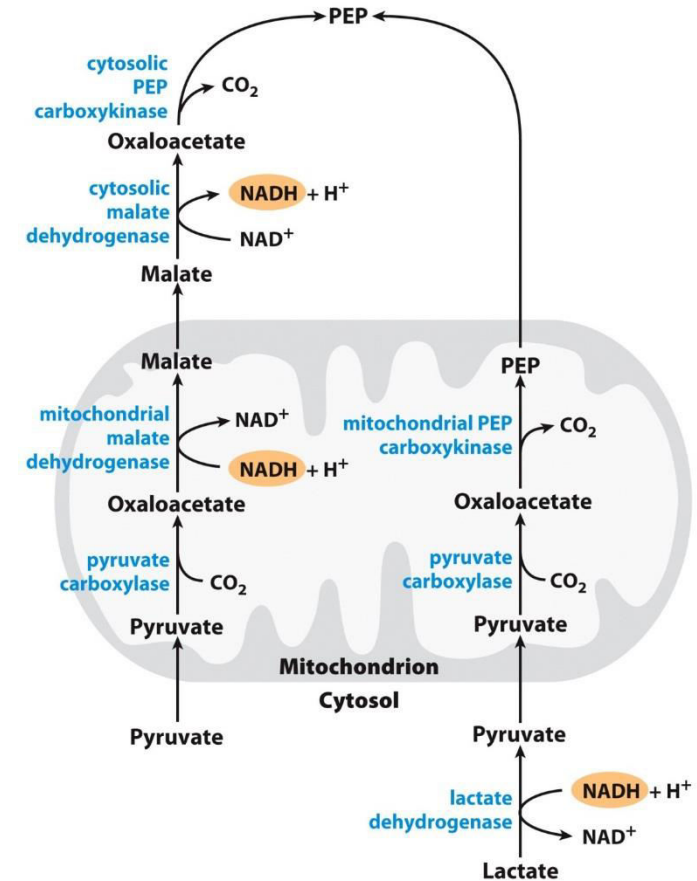


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- **Alternative paths from pyruvate to phosphoenolpyruvate**
- The relative importance of the two pathways depends on the availability of lactate or pyruvate and the cytosolic requirements for NADH for gluconeogenesis.
- The path on the right predominates when lactate is the precursor, because cytosolic NADH is generated in the lactate dehydrogenase reaction and does not have to be shuttled out of the mitochondrion
- The requirements of ATP for pyruvate carboxylase and GTP for PEP carboxykinase are omitted for simplicity.

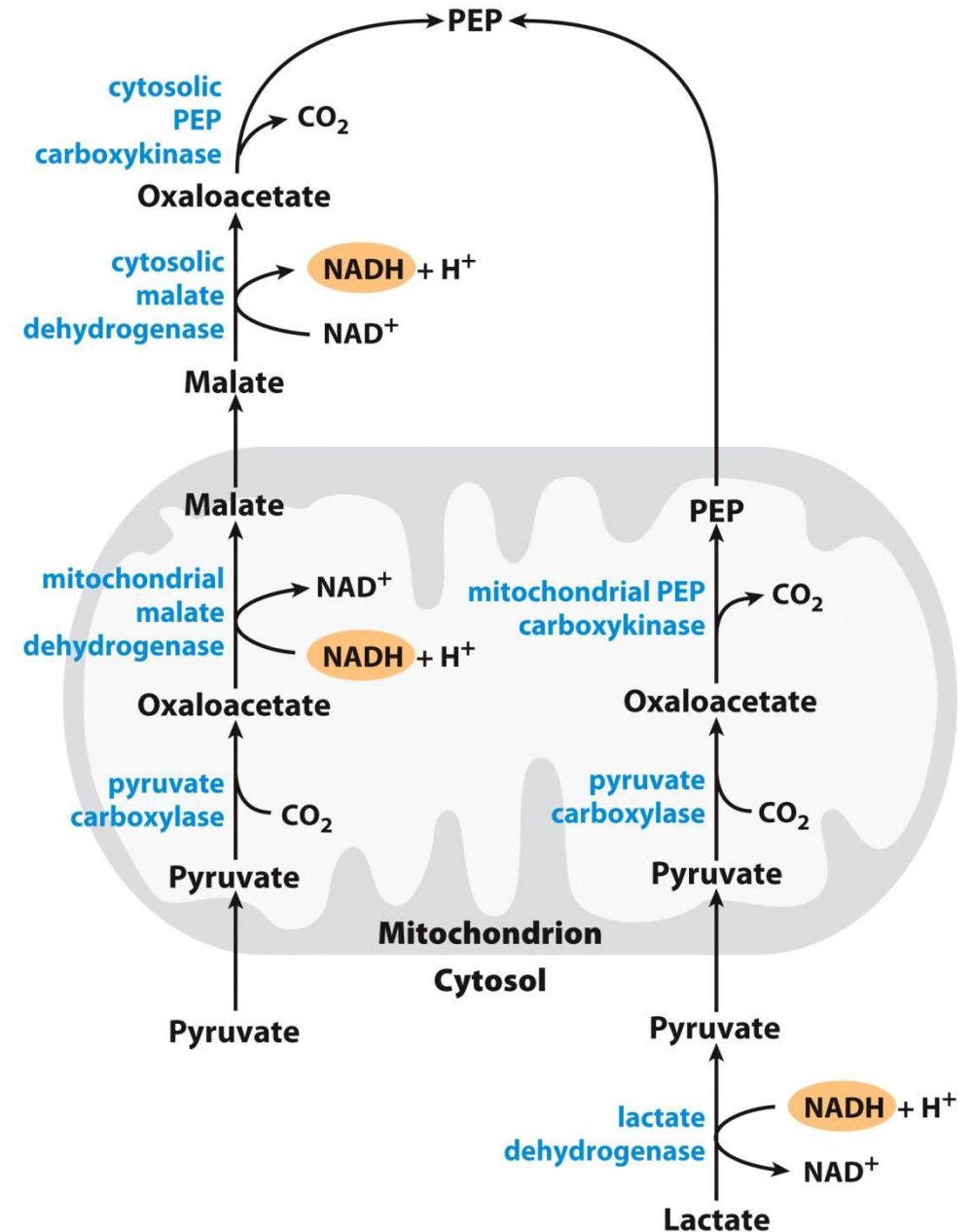


Figure 14-20

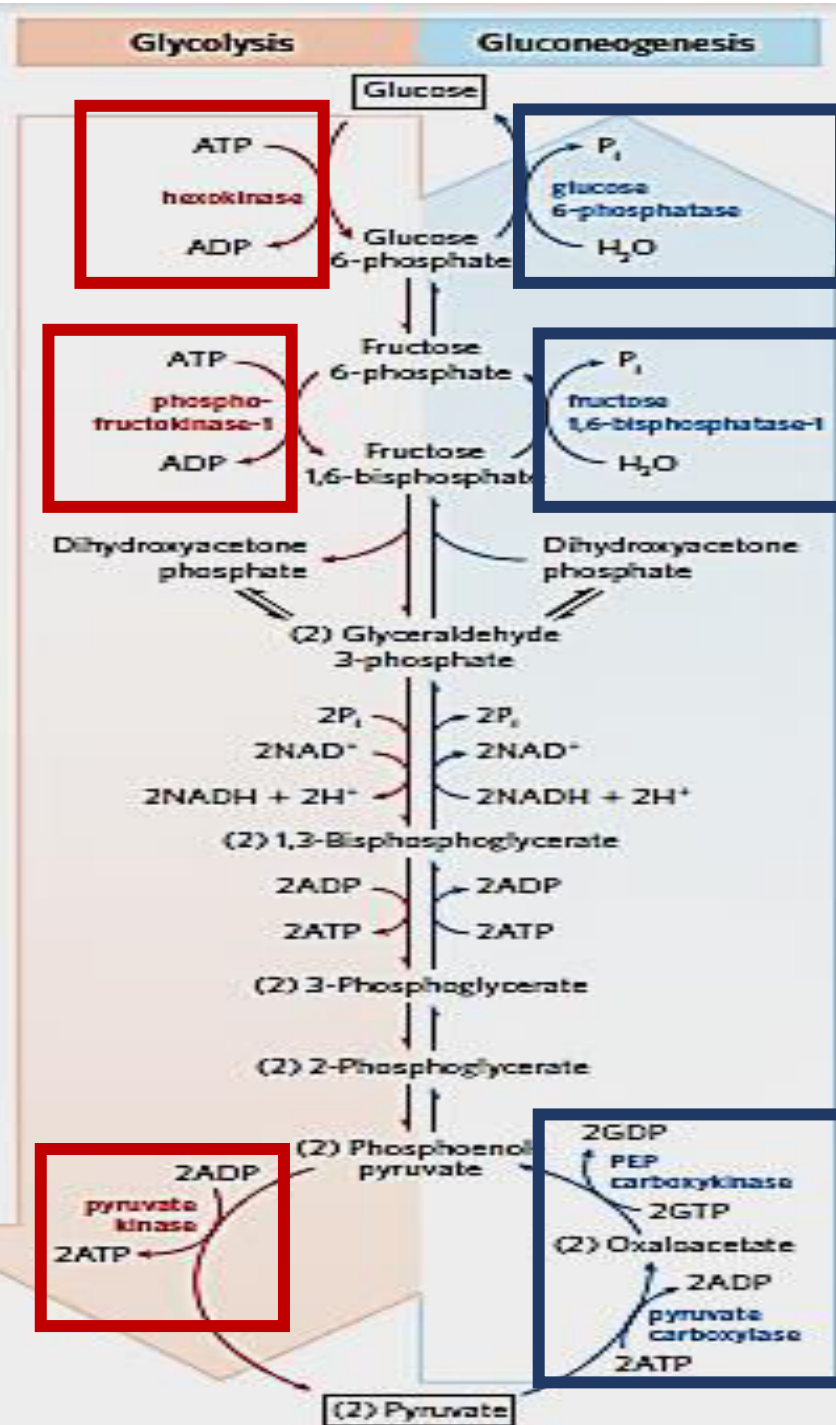
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GLUCONEOGENESIS..cont..3

Bypass reactions in Gluconeogenesis-2

Conversion of Fructose 1,6-Bisphosphate to Fructose 6-Phosphate Is the Second Bypass

- The second glycolytic reaction that cannot participate in gluconeogenesis is the phosphorylation of fructose 6-phosphate by PFK-1
- Because this reaction is **highly exergonic** and therefore irreversible, the generation of fructose 6-phosphate from fructose 1,6-bisphosphate is catalyzed by a different enzyme, **fructose 1,6-bisphosphatase (FBPase-1)**



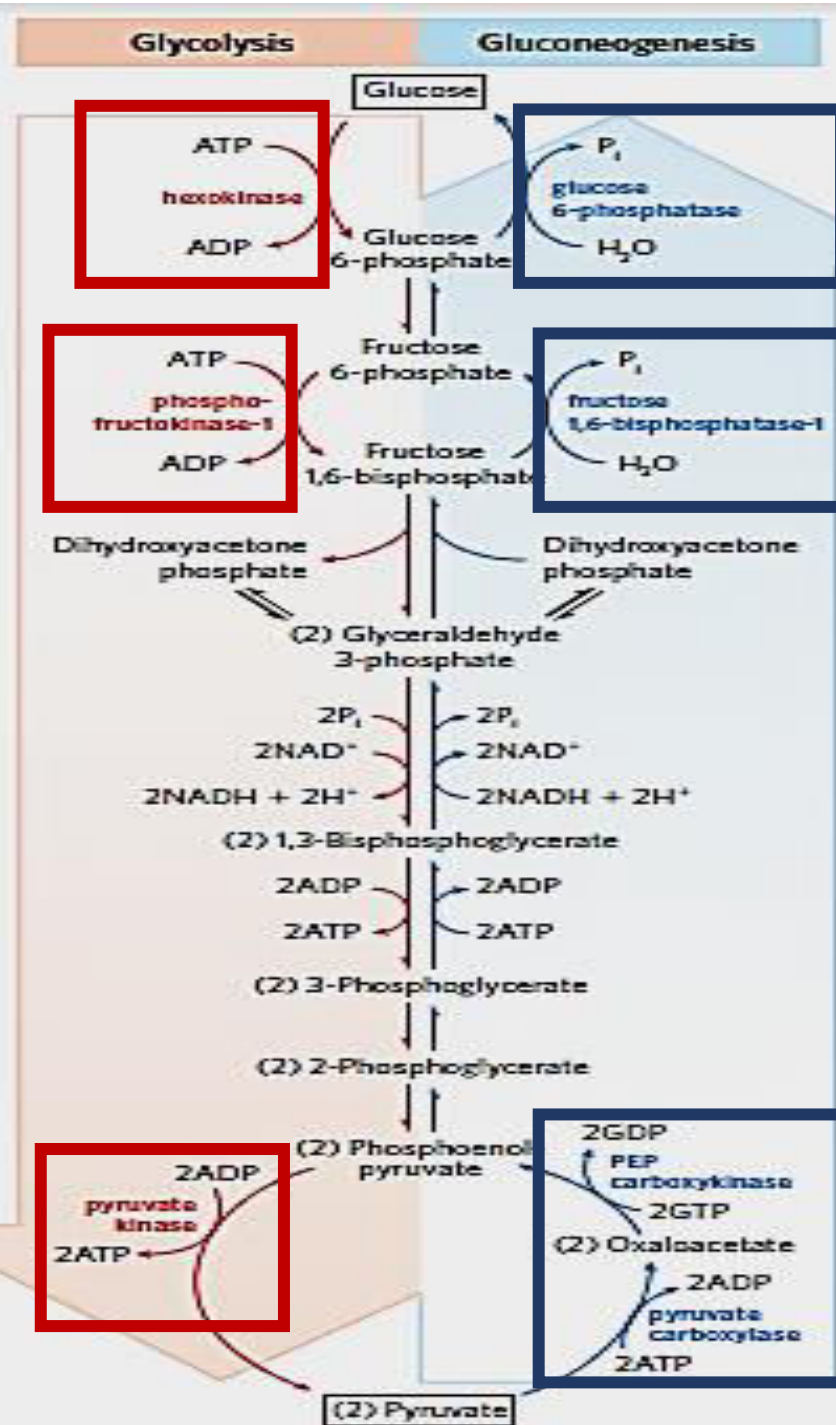
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- **fructose 1,6-bisphosphatase (FBPase-1)** is Mg^{+2} -dependent
- **FBPase-1** promotes the essentially irreversible *hydrolysis* of the C-1 phosphate (*not* phosphoryl group transfer to ADP)

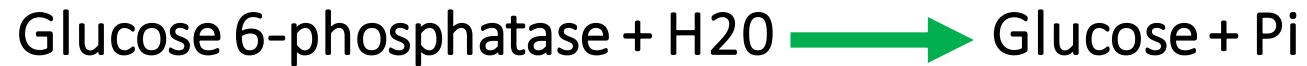


Conversion of Glucose 6-Phosphate to Glucose Is the Third Bypass

- The third bypass is the final reaction of gluconeogenesis, the dephosphorylation of glucose 6-phosphate to yield glucose
- Reversal of the hexokinase reaction would require phosphoryl group transfer from glucose 6-phosphate to ADP, forming ATP... An energetically unfavorable reaction
- The reaction catalyzed by **glucose 6-phosphatase** does not require synthesis of ATP; it is a simple hydrolysis of a phosphate ester



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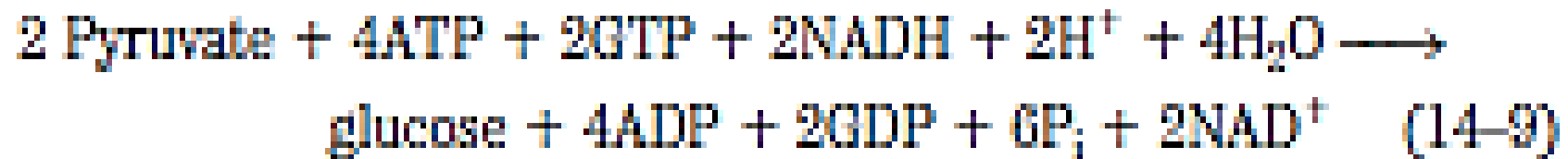


- This Mg²⁺-activated enzyme (**glucose 6-phosphatase**) is found on the luminal side of the endoplasmic reticulum of hepatocytes, renal cells, and epithelial cells of the small intestine but not in other tissues, therefore other organs are unable to supply glucose to the blood.
- If other tissues had glucose 6-phosphatase, this enzyme's activity would hydrolyze **the glucose 6-phosphate needed within those tissues for glycolysis**.
- Glucose produced by gluconeogenesis in the liver or kidney or ingested in the diet is delivered to these other tissues, including brain and muscle, through the bloodstream.

GLUCONEOGENESIS..cont..3

Gluconeogenesis Is Energetically Expensive, but Essential

- The sum of the biosynthetic reactions leading from pyruvate to free blood glucose is as follows



..cont

- For each molecule of glucose formed from pyruvate, **six high-energy phosphate** groups are required, **four from ATP and two from GTP**.
- In addition, **two molecules of NADH** are required for the reduction of two molecules of 1,3-bisphosphoglycerate.
- Clearly, Glycolysis is not simply the reverse of the equation for conversion of glucose to pyruvate by glycolysis, which would require **only two molecules of ATP**



..cont

- The synthesis of glucose from pyruvate is a relatively expensive process
- this high energy cost is necessary to ensure the irreversibility of gluconeogenesis
- Under intracellular conditions, the overall free-energy change of glycolysis is at least -63 kJ/mol.
- Under the same conditions the overall ΔG of gluconeogenesis is -16 kJ/mol.
- Thus both glycolysis and gluconeogenesis are essentially irreversible processes in cells.
- A second advantage to investing energy to convert pyruvate to glucose is **that if pyruvate were instead excreted, its considerable potential for ATP production by complete, aerobic oxidation would be lost (more than 10 ATP are produced per pyruvate)**

TABLE 14-3 Sequential Reactions in Gluconeogenesis Starting from Pyruvate

Pyruvate + HCO ₃ ⁻ + ATP → oxaloacetate + ADP + P _i	×2
Oxaloacetate + GTP ⇌ phosphoenolpyruvate + CO ₂ + GDP	×2
Phosphoenolpyruvate + H ₂ O ⇌ 2-phosphoglycerate	×2
2-Phosphoglycerate ⇌ 3-phosphoglycerate	×2
3-Phosphoglycerate + ATP ⇌ 1,3-bisphosphoglycerate + ADP	×2
1,3-Bisphosphoglycerate + NADH + H ⁺ ⇌ glyceraldehyde 3-phosphate + NAD ⁺ + P _i	×2
Glyceraldehyde 3-phosphate ⇌ dihydroxyacetone phosphate	
Glyceraldehyde 3-phosphate + dihydroxyacetone phosphate ⇌ fructose 1,6-bisphosphate	
Fructose 1,6-bisphosphate → fructose 6-phosphate + P _i	
Fructose 6-phosphate ⇌ glucose 6-phosphate	
Glucose 6-phosphate + H ₂ O → glucose + P _i	
<i>Sum:</i> 2 Pyruvate + 4ATP + 2GTP + 2NADH + 2H ⁺ + 4H ₂ O → glucose + 4ADP + 2GDP + 6P _i + 2NAD ⁺	

The bypass reactions are in red; all other reactions are reversible steps of glycolysis. The figures at the right indicate that the reaction is to be counted twice, because two three-carbon precursors are required to make a molecule of glucose. The reactions required to replace the cytosolic NADH consumed in the glyceraldehyde 3-phosphate dehydrogenase reaction (**the conversion of lactate to pyruvate in the cytosol** or the **transport of reducing equivalents from mitochondria to the cytosol in the form of malate**) are not considered in this summary. Biochemical equations are not necessarily balanced for H and charge.

GLUCONEOGENESIS..cont..

Gluconeogenesis and Glycolysis are Reciprocally regulated

Reciprocal Regulation of Gluconeogenesis and Glycolysis in the Liver

- Gluconeogenesis and glycolysis are coordinated so that within a cell one pathway is relatively inactive while the other is highly active.
- The rate of glycolysis is determined by the concentration of glucose
- The rate of gluconeogenesis by the concentrations of **lactate and other precursors of glucose**
- **Pyruvate kinase** is inhibited by phosphorylation during starvation.
- The level of fructose 2,6-bisphosphate is high in the fed state and low in starvation.

- The interconversion of fructose 6-phosphate and fructose 1,6-bisphosphate is stringently controlled
- **AMP** stimulates phosphofructokinase, whereas **ATP and citrate** inhibit it.
- Fructose 1,6-bisphosphatase, is inhibited by AMP and activated by citrate.
- **A high level of AMP indicates that the energy charge is low and signals the need for ATP generation.**
- Conversely, high levels of ATP and citrate indicate that the energy charge is high and that biosynthetic intermediates are abundant.
- Under these conditions, glycolysis is nearly switched off and gluconeogenesis is promoted.

- Phosphofructokinase and fructose 1,6-bisphosphatase are also reciprocally controlled by *fructose 2,6-bisphosphate in the liver*
- **The level of F-2,6-BP is low during starvation** and high in the fed state, because of the antagonistic effects of glucagon and insulin on the production and degradation of this signal molecule.
- *Fructose 2,6-bisphosphate strongly stimulates phosphofructokinase and inhibits fructose 1,6-bisphosphatase.*
- Hence, glycolysis is accelerated and gluconeogenesis is diminished in the fed state.
- **During starvation**, gluconeogenesis predominates because the level of F-2,6-BP is very low. Glucose formed by the liver under these conditions is essential for the viability of brain and muscle.

- The interconversion of phosphoenolpyruvate and pyruvate also is precisely regulated.
- High levels of [ATP](#) and alanine, which signal that the energy charge is high and that building blocks are abundant, inhibit the enzyme in liver.
- Conversely, pyruvate carboxylase, which catalyzes the first step in gluconeogenesis from pyruvate, is activated by **Acetyl CoA** and inhibited by **ADP**.
- Likewise, **ADP inhibits phosphoenolpyruvate carboxykinase**.
- Hence, gluconeogenesis is favored when the cell is rich in biosynthetic precursors and ATP

Regulatory effect of hormones

- The regulators in this case are hormones.
- Hormones affect gene expression primarily by changing the rate of transcription, as well as by regulating the **degradation of mRNA**
- Insulin, which rises subsequent to eating, stimulates the expression of phosphofructokinase, pyruvate kinase, and the bifunctional enzyme that makes and degrades F-2,6-BP.
- Glucagon, which rises during starvation, inhibits the expression of these enzymes and stimulates instead the production of two key gluconeogenic enzymes, phosphoenolpyruvate carboxykinase and fructose 1,6-bisphosphatase.

Pentose Phosphate Pathway (PPP) of Glucose Oxidation

- The *oxidative* pentose phosphate pathway
- **Phosphogluconate pathway**
- **Hexose monophosphate pathway**

- The major catabolic fate of **glucose 6-phosphate** is glycolytic breakdown to **pyruvate**, much of which is then oxidized via the **citric acid cycle**, leading to ATP formation .
- Glucose 6-phosphate does have other catabolic fates, which lead to specialized products needed by the cell.
- E.g. the **oxidation of glucose 6-phosphate to pentose phosphates** by the **pentose phosphate pathway**.
- Since it results in the synthesis of pentoses and NADPH, it is considered as an **anaerobic and anabolic pathway**.
- Occurs in cytosol

Outputs of PPP

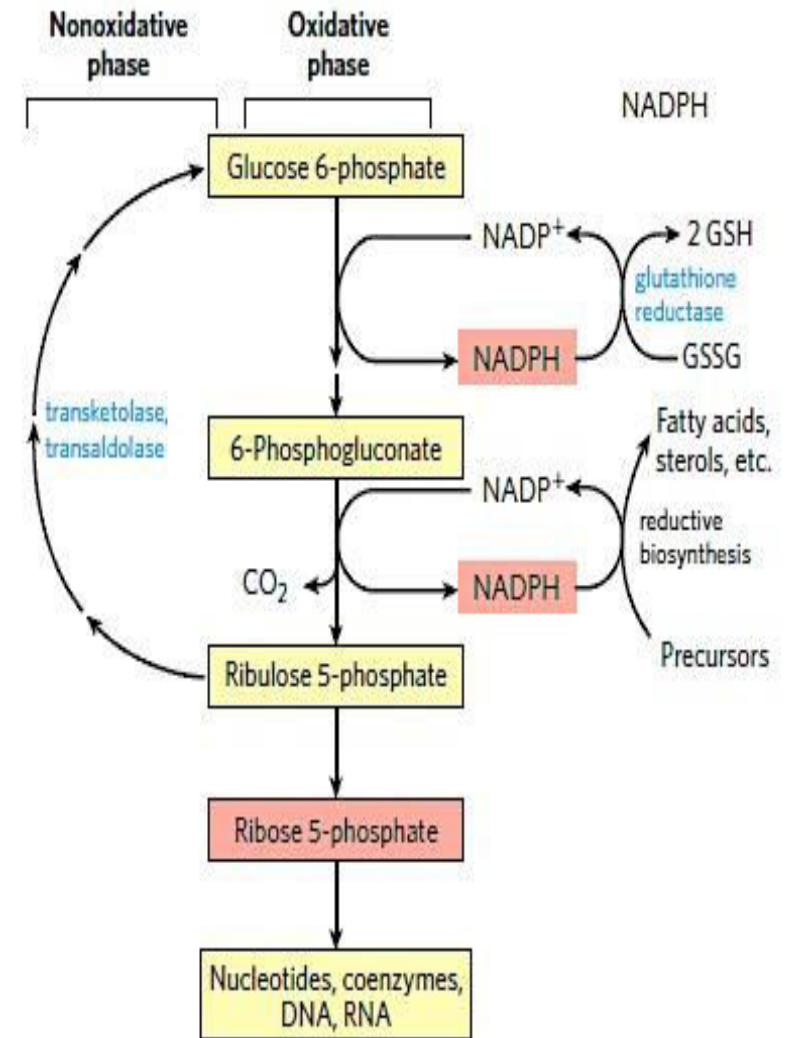
- Reducing NADP⁺ to **NADPH** and producing **pentose phosphates** . .
- brings about **oxidation and decarboxylation** of glucose 6-phosphate (G6P) at C-1, to form **pentoses**.. (bone marrow, skin, intestinal mucosa) to make RNA, DNA, ATP, NADH, FADH₂ and coenzyme A
- NADPH: to counter damaging effects of oxygen radicals (liver, adipose, erythrocytes, cornea)
- Erythrose 4 phosphate (E4P) used in the synthesis of aromatic amino acids

Two phases of PPP

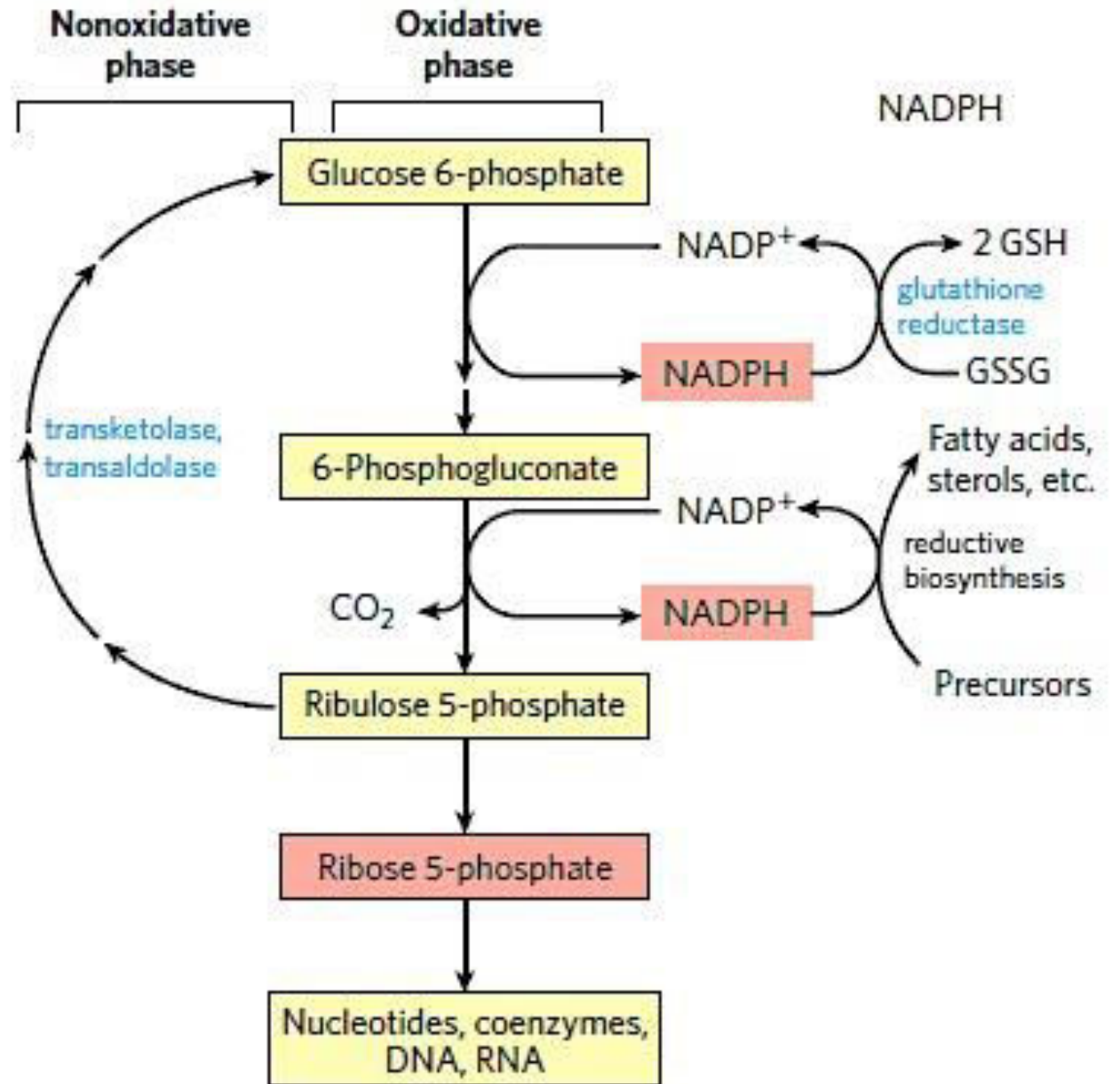
- There are **two phases** of the pentose phosphate pathway:
 1. The oxidative phase
 2. The non-oxidative phase

1. The Oxidative Phase

1. The Oxidative Phase Produces Pentose Phosphates and NADPH
2. The first reaction of the pentose phosphate pathway is the oxidation of glucose 6-phosphate by **glucose 6-phosphate dehydrogenase (G6PD)** to form 6-phosphoglucono-delta-lactone, an intramolecular ester.
3. NADP^+ is the electron acceptor, and overall equilibrium is in the direction of NADPH formation.
4. The **lactone** is hydrolyzed to the free acid 6-phosphogluconate by a specific enzyme **lactonase**, then 6-phosphogluconate undergoes oxidation and decarboxylation by **6-phosphogluconate dehydrogenase**



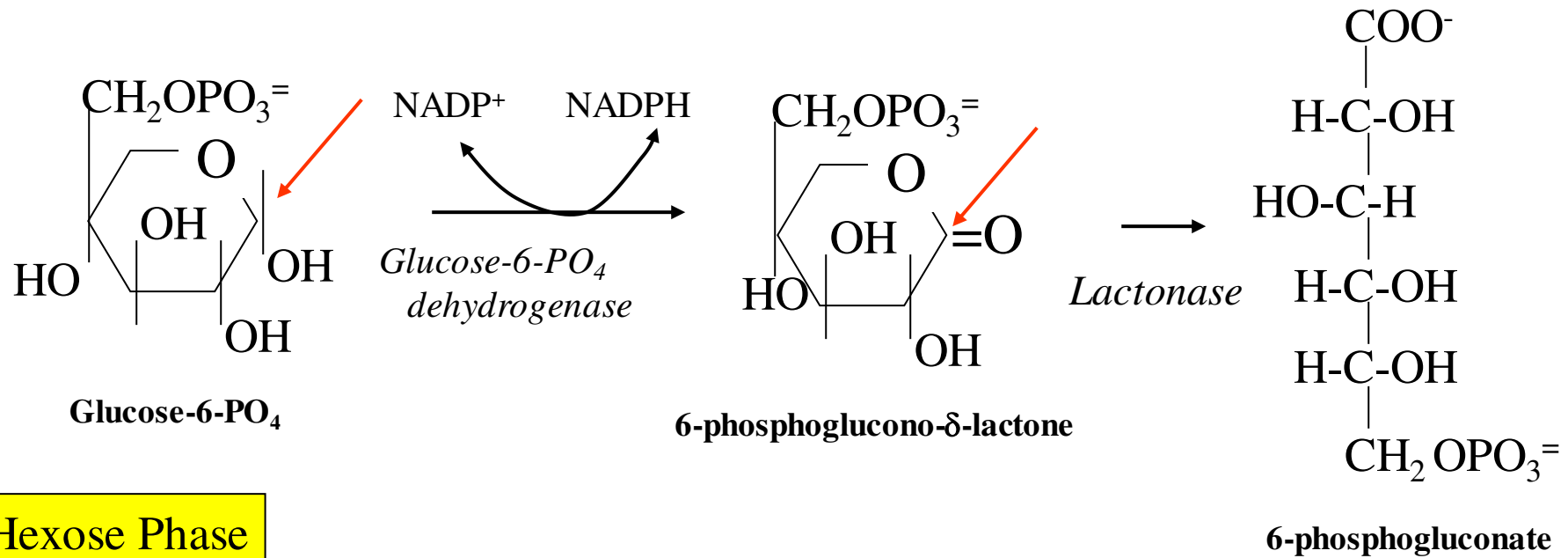
Pentose Phosphate Pathway NADPH formed in the oxidative phase is used to reduce glutathione to GSSG (Glutathione disulfide) and to support reductive biosynthesis. The other product of the oxidative phase is **ribose 5-phosphate**, which serves as a precursor for nucleotides, coenzymes, and nucleic acids. In cells that are not using ribose 5-phosphate for biosynthesis, the **non-oxidative phase** recycles six molecules of the pentose into five molecules of the hexose glucose 6-phosphate, allowing continued production of NADPH and converting glucose 6-phosphate to CO₂.



Pentose Phosphate Pathway (PPP) ...cont

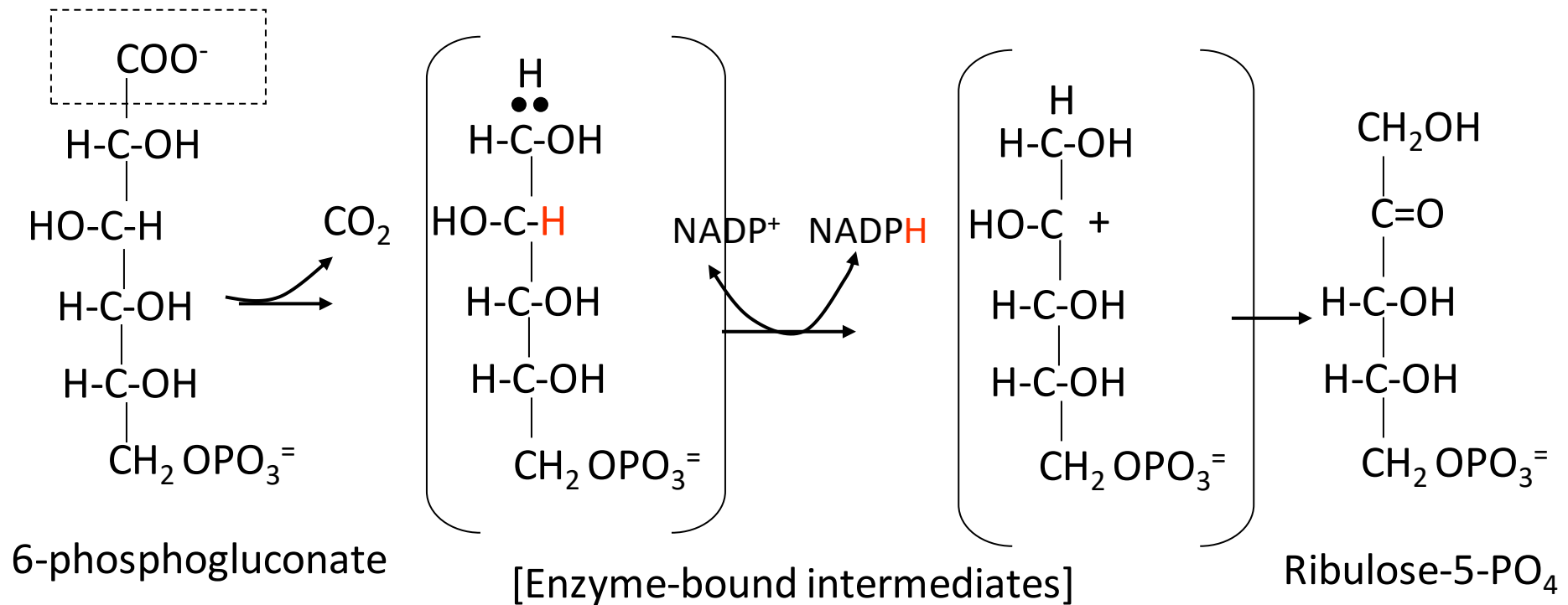
- The reaction generates a second molecule of NADPH.
- **Phosphopentose isomerase** converts ribulose 5-phosphate to its aldose isomer, ribose 5-phosphate.
- In some tissues, the pentose phosphate pathway ends at this point, and its overall equation for reactions is
- **$G6-P + 2NADP^+ + H_2O \longrightarrow \text{ribose 5-phosphate} + CO_2 + 2NADPH + 2H^+$**
- The net result is the production of NADPH, a reducing agent for biosynthetic reactions, and ribose 5-phosphate, a precursor for nucleotide synthesis.

...cont



...cont

6-phosphogluconate dehydrogenase



Summary of The Oxidative Phase

Reactants	Products	Enzyme	Description
Glucose 6-phosphate + NADP ⁺	→ 6-phosphoglucono-δ-lactone + NADPH	glucose 6-phosphate dehydrogenase	Dehydrogenation . The hydroxyl on carbon 1 of glucose 6-phosphate turns into a carbonyl, generating a lactone, and, in the process, NADPH is generated.
6-phosphoglucono-δ-lactone + H ₂ O	→ 6-phosphogluconate + H ⁺	6-phosphogluconolactonase	Hydrolysis
6-phosphogluconate + NADP ⁺	→ ribulose 5-phosphate + NADPH + CO ₂	6-phosphogluconate dehydrogenase	Oxidative decarboxylation . NADP ⁺ is the electron acceptor, generating another molecule of NADPH , a CO ₂ , and ribulose 5-phosphate .

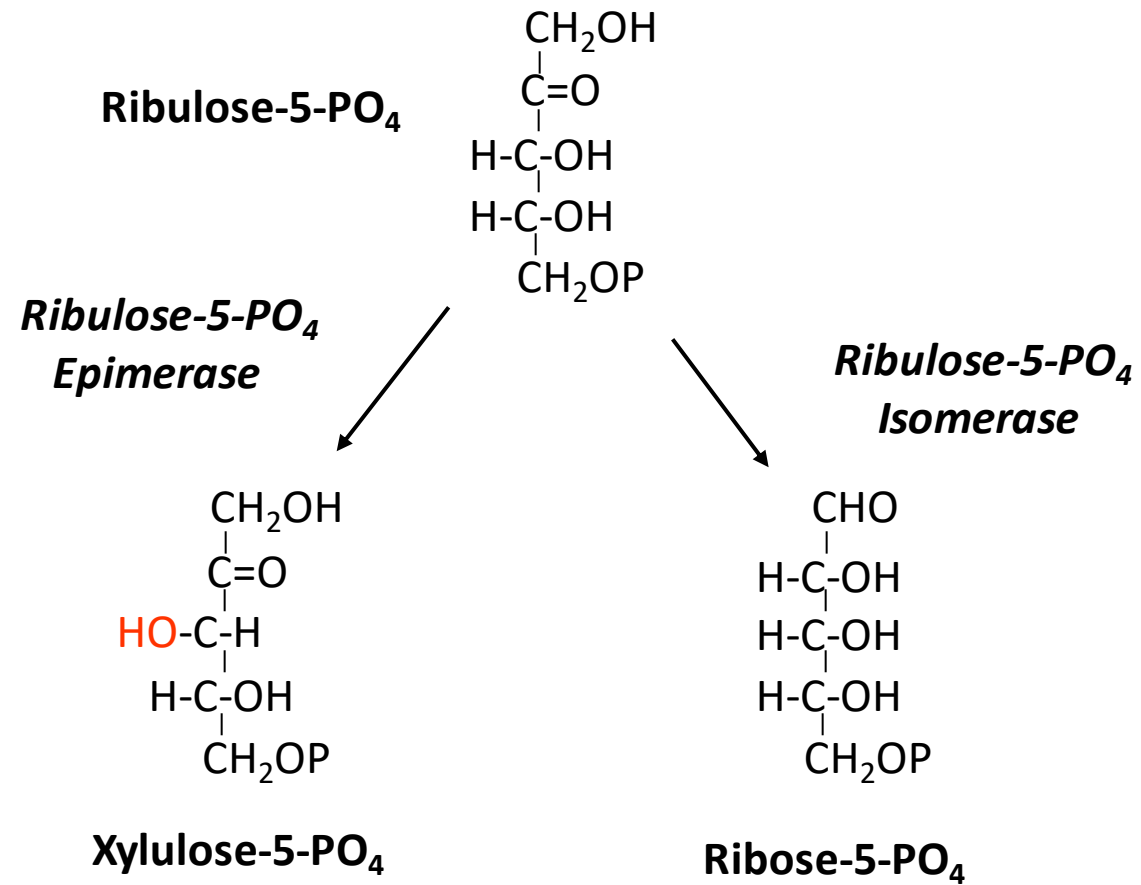
Pentose Phosphate Pathway (PPP) ...cont

2. The Non-oxidative Phase

2. The Non-oxidative Phase

- The Non-oxidative Phase Recycles Pentose Phosphates to Glucose 6-Phosphate
- In tissues that require **primarily NADPH**, the **pentose phosphates** produced in the oxidative phase of the pathway are **recycled into glucose 6-phosphate**.
- A series of rearrangements of the carbon skeletons takes place during which **6 five-carbon sugar phosphates are converted to 5 six-carbon sugar phosphates**, completing the cycle and allowing continued oxidation of glucose 6-phosphate with production of NADPH
- In nonoxidative phase, ribulose 5-phosphate is first **epimerized** to xylulose 5-phosphate

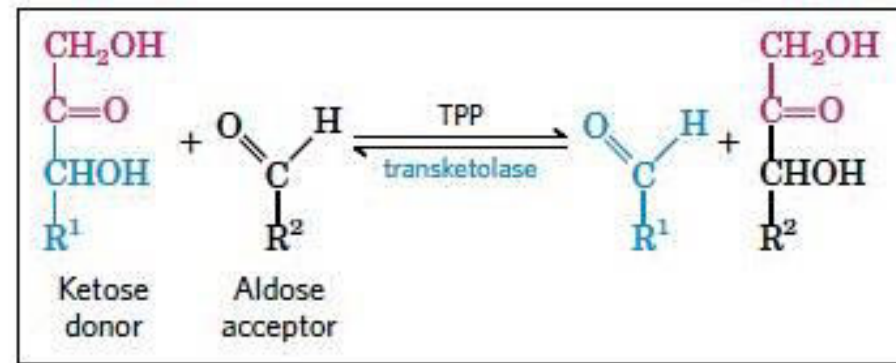
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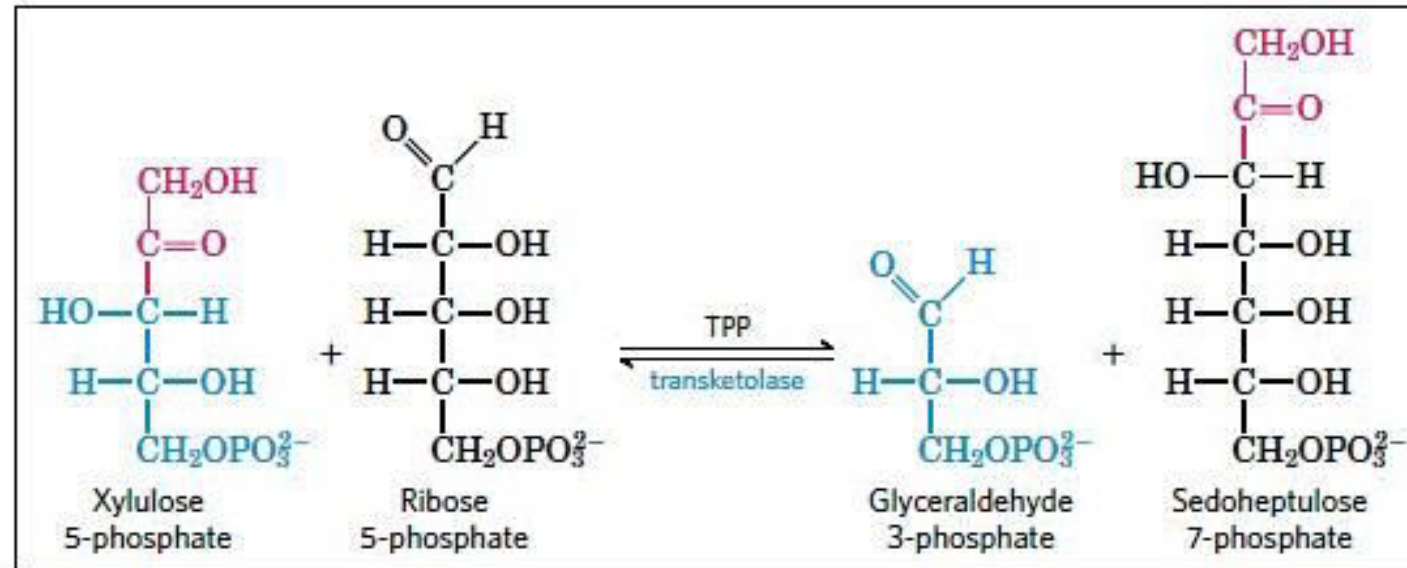
- Continued recycling leads ultimately to the conversion of glucose 6-phosphate to six CO_2 .
- **Two enzymes unique to the pentose phosphate pathway act in these interconversions of sugars:** transketolase and transaldolase.

a. Transketolase

- Transketolase catalyzes the transfer of a two carbon fragment from **a ketose donor to an aldose acceptor** In its first appearance in the pentose phosphate pathway, transketolase transfers C-1 and C-2 of xylulose 5-phosphate to ribose 5-phosphate, forming the seven-carbon product sedoheptulose 7-phosphate.
- The remaining three carbon fragment from xylulose forms glyceraldehyde 3-phosphate.



(a)



The first reaction catalyzed by transketolase. (a) The general reaction catalyzed by transketolase is the transfer of a two carbon group, carried temporarily on enzyme-bound TPP, (cofactor thymine pyrophosphate) from a ketose donor to an aldose acceptor. (b) Conversion of two pentose phosphates to a triose phosphate and a seven-carbon sugar phosphate, sedoheptulose 7-phosphate.

b. Transaldolase

- Transaldolase catalyzes a reaction similar to the aldolase reaction of glycolysis, that is, a **three-carbon fragment is removed from sedoheptulose 7-phosphate** and condensed with glyceraldehyde 3-phosphate, **forming fructose 6-phosphate and the tetrose erythrose 4-phosphate.**
- Now transketolase acts again, forming fructose 6-phosphate and glyceraldehyde 3-phosphate from erythrose 4-phosphate and xylulose 5-phosphate. Two molecules of glyceraldehyde 3-phosphate formed by two repetition of the process can be converted to a molecule of fructose 1,6-bisphosphate as in gluconeogenesis
- finally FBPase-1 and phosphohexose isomerase convert fructose 1,6-bisphosphate to glucose 6-phosphate.
- Overall, six pentose phosphates have been converted to five hexose phosphates—the cycle is now complete.
- Transketolase requires the cofactor thiamine pyrophosphate (TPP), which stabilizes a two-carbon carbanion in this reaction

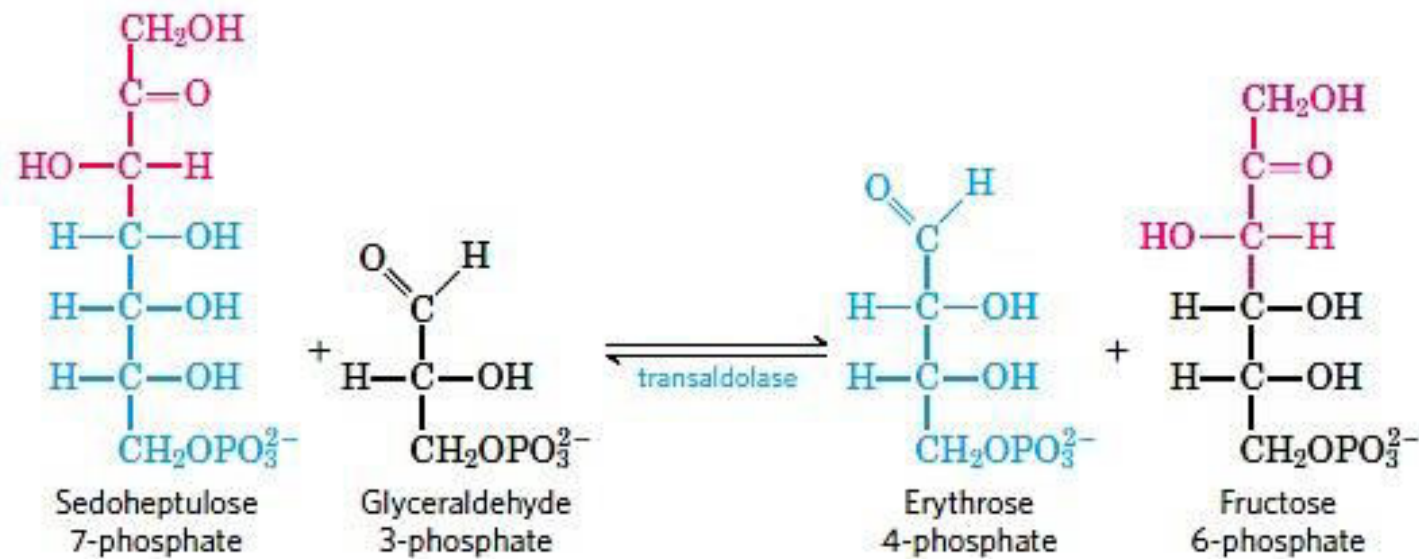


FIGURE 14-25 The reaction catalyzed by transaldolase.

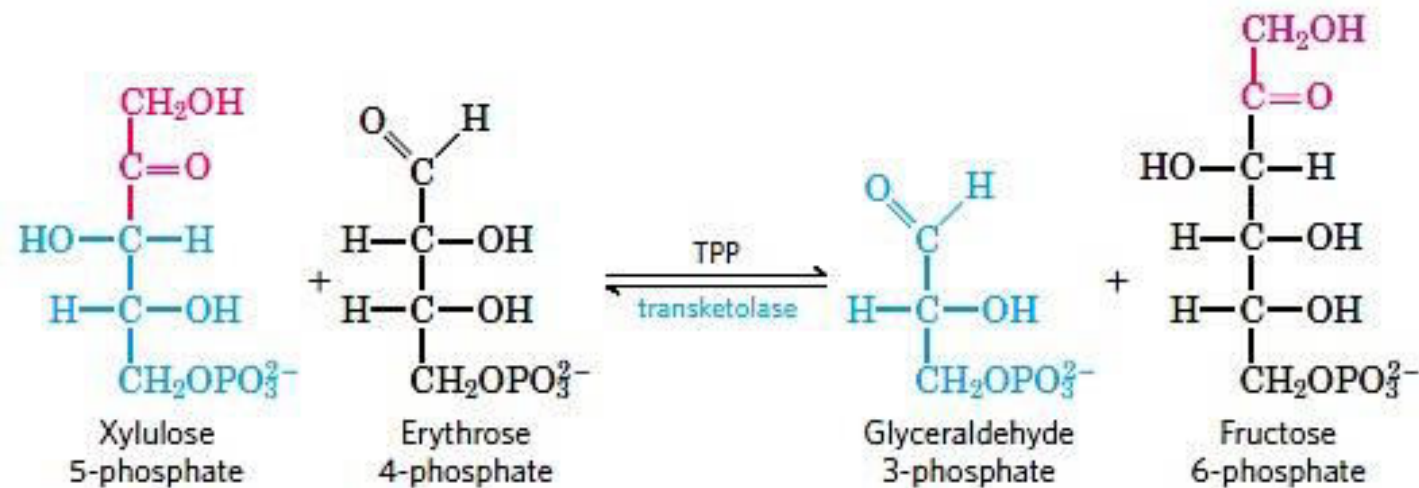
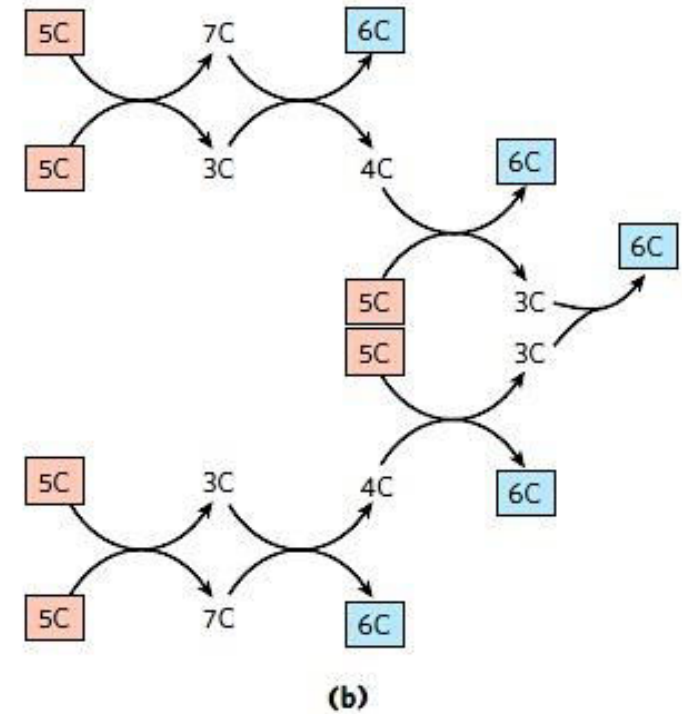
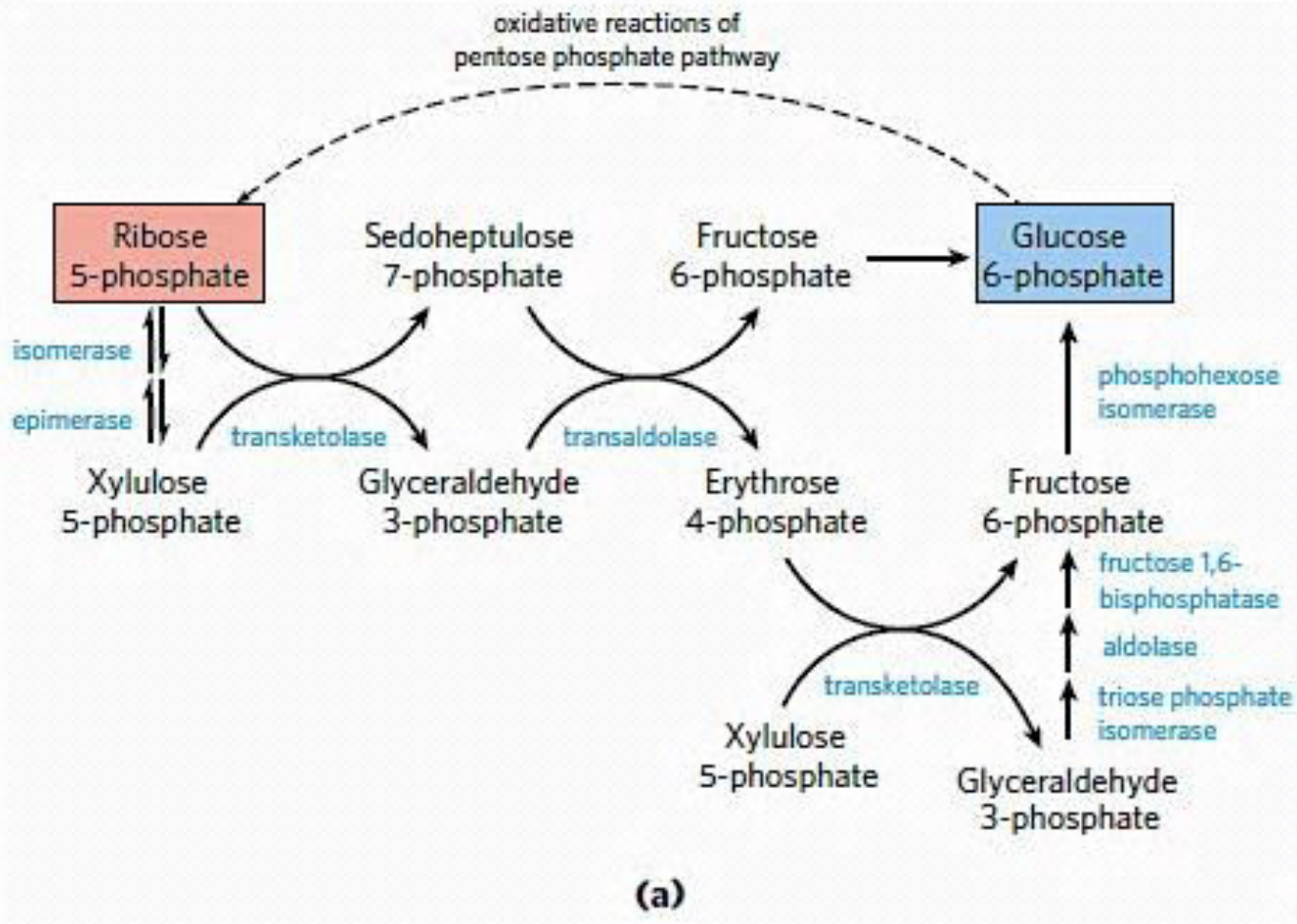


FIGURE 14-26 The second reaction catalyzed by transketolase.



Nonoxidative reactions of the pentose phosphate pathway.

(a) These reactions convert pentose phosphates to hexose phosphates, allowing the oxidative reactions. Transketolase and transaldolase are specific to this pathway; the other enzymes also serve in the glycolytic or gluconeogenic pathways.

(b) A schematic diagram showing the pathway from six pentoses (5C) to five hexoses (6C). Note that this involves two sets of the interconversions shown in (a). Every reaction shown here is reversible; unidirectional arrows are used only to make clear the direction of the reactions during continuous oxidation of glucose 6-phosphate. In the light-independent reactions of photosynthesis, the direction of these reactions is reversed.

Summary of The Non-Oxidative Phase

Reactants	Products	Enzymes
<u>ribulose 5-phosphate</u>	→ <u>ribose 5-phosphate</u>	Ribulose 5-Phosphate Isomerase
<u>ribulose 5-phosphate</u>	→ <u>xylulose 5-phosphate</u>	Ribulose 5-Phosphate 3-Epimerase
<u>xylulose 5-phosphate</u> + <u>ribose 5-phosphate</u>	→ <u>glyceraldehyde 3-phosphate</u> + <u>sedoheptulose 7-phosphate</u>	<u>transketolase</u>
<u>sedoheptulose 7-phosphate</u> + <u>glyceraldehyde 3-phosphate</u>	→ <u>erythrose 4-phosphate</u> + <u>fructose 6-phosphate</u>	<u>transaldolase</u>
<u>xylulose 5-phosphate</u> + <u>erythrose 4-phosphate</u>	→ <u>glyceraldehyde 3-phosphate</u> + <u>fructose 6-phosphate</u>	<u>transketolase</u>

Pentose Phosphate Pathway (PPP) ...cont..3

3. Glucose 6-Phosphate enters
Glycolysis OR the Pentose Phosphate Pathway

Summary of The Oxidative Phase

Reactants	Products	Enzyme	Description
Glucose 6-phosphate + NADP ⁺	→ 6-phosphoglucono-δ-lactone + NADPH	glucose 6-phosphate dehydrogenase	Dehydrogenation . The hydroxyl on carbon 1 of glucose 6-phosphate turns into a carbonyl, generating a lactone, and, in the process, NADPH is generated.
6-phosphoglucono-δ-lactone + H ₂ O	→ 6-phosphogluconate + H ⁺	6-phosphogluconolactonase	Hydrolysis
6-phosphogluconate + NADP ⁺	→ ribulose 5-phosphate + NADPH + CO ₂	6-phosphogluconate dehydrogenase	Oxidative decarboxylation . NADP ⁺ is the electron acceptor, generating another molecule of NADPH , a CO ₂ , and ribulose 5-phosphate .

Summary of The Non-Oxidative Phase

Reactants	Products	Enzymes
<u>ribulose 5-phosphate</u>	→ <u>ribose 5-phosphate</u>	Ribulose 5-Phosphate Isomerase
<u>ribulose 5-phosphate</u>	→ <u>xylulose 5-phosphate</u>	Ribulose 5-Phosphate 3-Epimerase
<u>xylulose 5-phosphate</u> + <u>ribose 5-phosphate</u>	→ <u>glyceraldehyde 3-phosphate</u> + <u>sedoheptulose 7-phosphate</u>	<u>transketolase</u>
<u>sedoheptulose 7-phosphate</u> + <u>glyceraldehyde 3-phosphate</u>	→ <u>erythrose 4-phosphate</u> + <u>fructose 6-phosphate</u>	<u>transaldolase</u>
<u>xylulose 5-phosphate</u> + <u>erythrose 4-phosphate</u>	→ <u>glyceraldehyde 3-phosphate</u> + <u>fructose 6-phosphate</u>	<u>transketolase</u>

Glucose 6-Phosphate Is Partitioned between Glycolysis and the Pentose Phosphate Pathway

- Whether glucose 6-phosphate enters glycolysis or the pentose phosphate pathway depends on the current needs of the cell and on the concentration of NADP^+ in the cytosol. Without this electron acceptor, the first reaction of the pentose phosphate pathway (catalyzed by G6PD) cannot proceed.
- When a cell is rapidly converting NADPH to NADP^+ in biosynthetic reductions, the level of NADP^+ rises, allosterically stimulating G6PD and increasing the flux of glucose 6-phosphate through the pentose phosphate pathway
- When the demand for NADPH slows, the level of NADP^+ drops, the pentose phosphate pathway slows, and glucose 6-phosphate is instead used to fuel glycolysis.
- The ratio of NADPH to NADP^+ is 100:1 in liver cells (cytosol is a highly reducing environment)

Wernicke-Korsakoff Syndrome

- **Wernicke–Korsakoff** syndrome results from thiamine (Vitamin B12), deficiency. a component of TPP, by a Defect in Transketolase
- The syndrome is more common among **chronic** alcoholics
- can be caused by a mutation in the gene for transketolase that results in an enzyme with a lowered affinity for TPP—an affinity one-tenth that of the normal enzyme.
- This defect makes individuals much more sensitive to a thiamine deficiency: even a moderate thiamine deficiency (tolerable in individuals with an unmutated transketolase) can drop the level of TPP below that needed to saturate the enzyme.
- The result is a slowing down of the whole pentose phosphate pathway.
- Symptoms: **memory loss, mental confusion, ataxia and partial paralysis.**

Wernicke–Korsakoff syndrome results from thiamine deficiency by a Defect in Transketolase

by a severe deficiency of thiamine (Vitamin B12), a component of TPP

The syndrome is more common among **chronic** alcoholics people with alcoholism because chronic, heavy alcohol consumption interferes with the intestinal absorption of thiamine. The syndrome can be caused by a mutation in the gene for transketolase that results in an enzyme with a lowered affinity for TPP—an affinity one-tenth that of the normal enzyme.

This defect makes individuals much more sensitive to a thiamine deficiency: even a moderate thiamine deficiency (tolerable in individuals with an unmutated transketolase) can drop the level of TPP below that needed to saturate the enzyme.

The result is a slowing down of the whole pentose phosphate pathway.

In people with Wernicke-Korsakoff syndrome this results in a worsening of symptoms, which can include severe **memory loss, mental confusion, and partial paralysis.**

GLUCOSE METABOLISM

Feeder pathways of GLYCOLYSIS

Feeder pathways of glycolysis

- **Polysaccharides** : Glycogen and starch (Complex carbs)
- **Disaccharides**: Maltose, lactose, trehalose, sucrose
- **Monosaccharides**: Fructose, mannose, galactose

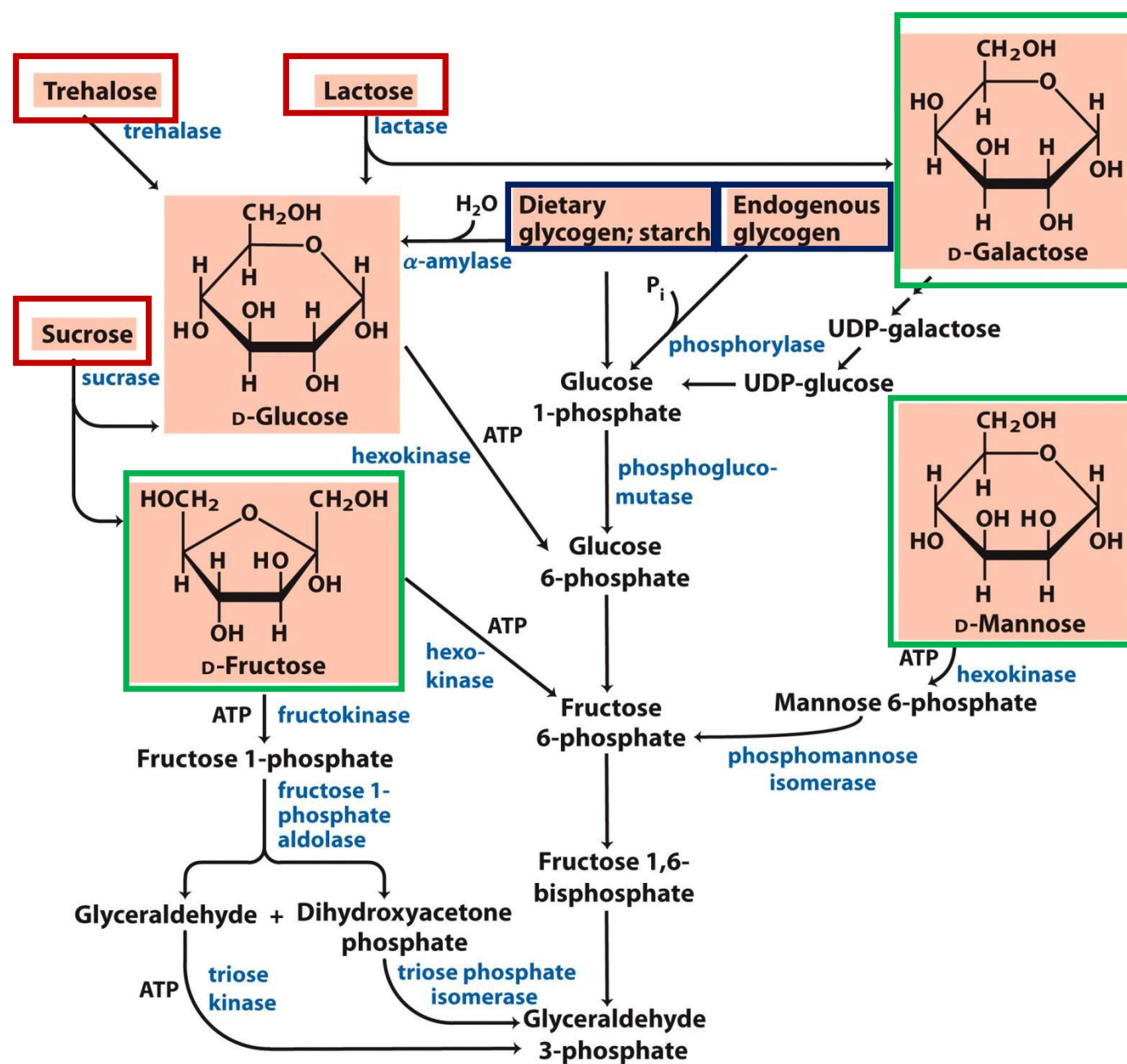
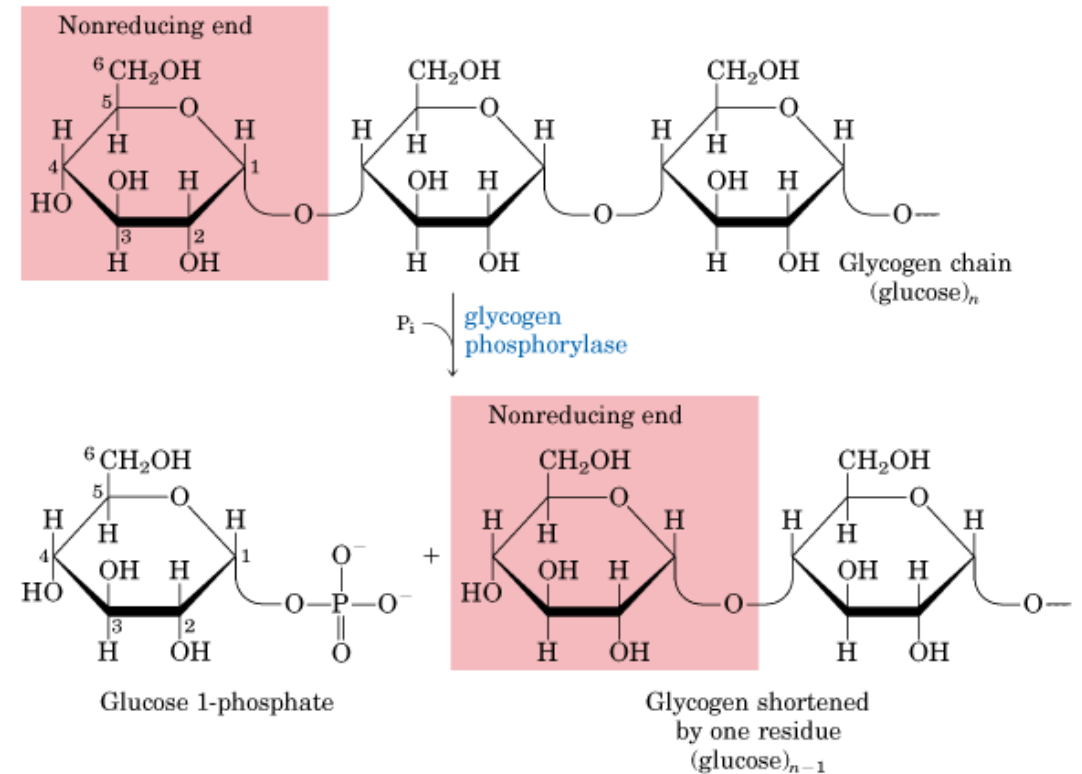


Figure 14-11
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1. Catabolism of Polysachharides

- Alpha amylase in saliva and intestine breaks starch to oligosaccharides, maltose, dextrins, and finally glucose
- In ruminants, in the rumen, symbiotic microorganisms that produce cellulase break down cellulose into glucose molecules.
- These microorganisms use the resulting glucose in an anaerobic fermentation that produces propionate, used in gluconeogenesis to produce lactose of milk
- Glycogen phosphorylase (starch phosphorylase in plants) catalyzes an attack by P_i on the ($\alpha 1 \rightarrow 4$) glycosidic linkage that joins the last two glucose residues at a nonreducing end, generating glucose 1-phosphate and a polymer shortened by one residue



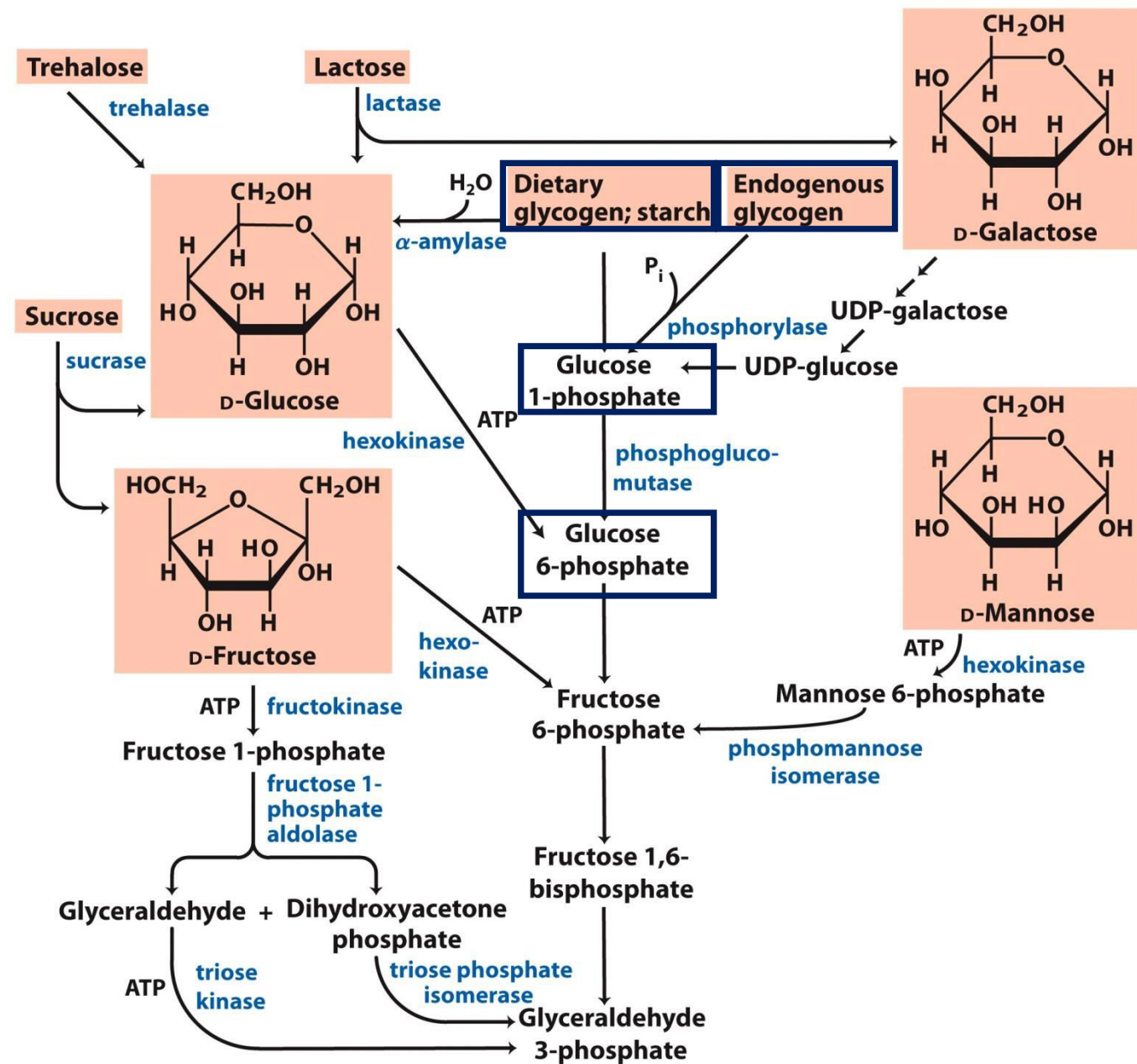
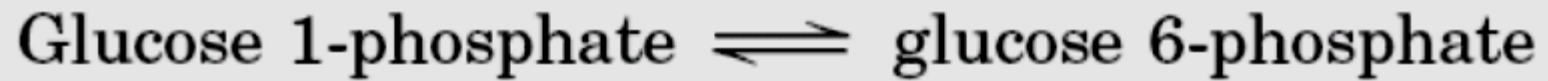


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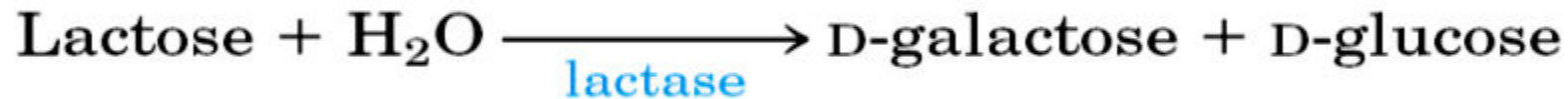
- Glucose 1-phosphate produced by glycogen phosphorylase is converted to glucose 6-phosphate by **phosphoglucomutase**, which catalyzes the reversible reaction



1. Glycolysis
2. Pentose phosphate pathway

2. Metabolism of dietary polysaccharides and disaccharides

Dietary polysaccharides and disaccharides are hydrolyzed to monosaccharides by various enzymes



GLUCOSE METABOLISM

Feeder pathways of GLYCOLYSIS-2

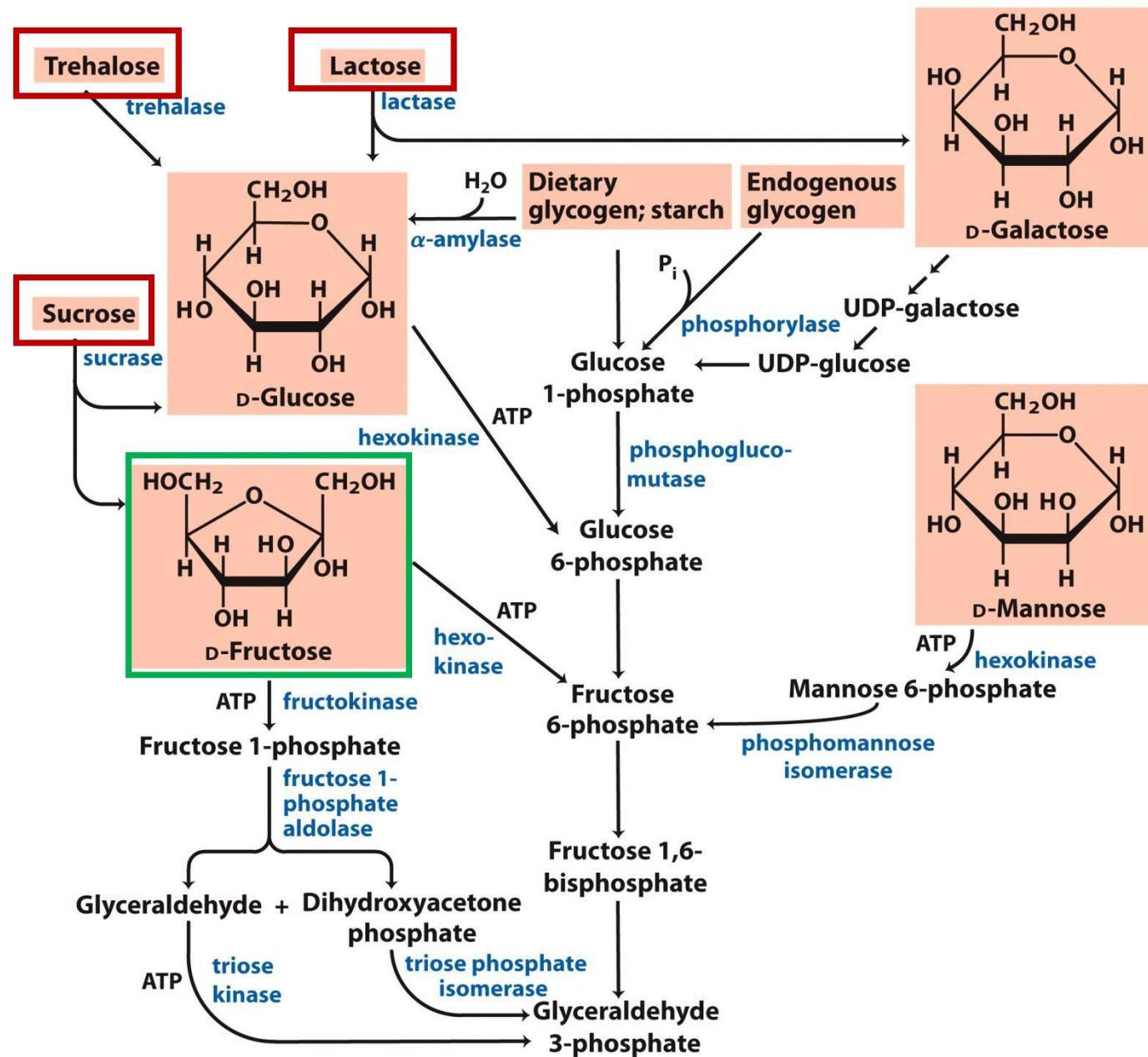
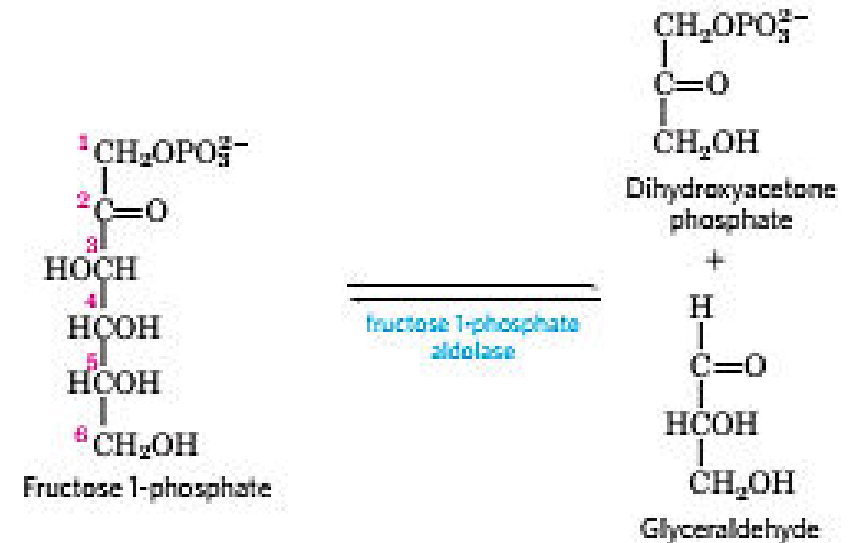


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2. Metabolism of monosaccharides

- In most organisms, hexoses other than glucose can undergo glycolysis after conversion to a phosphorylated derivative.
- **FRUCTOSE:**
 - D-Fructose, present in free form in many fruits and formed by hydrolysis of sucrose in the small intestine of vertebrates, is phosphorylated by hexokinase
 - Major pathway of fructose entry into glycolysis in the muscles and kidney.
 - The liver enzyme **fructokinase** catalyzes the phosphorylation of fructose at C-1 rather than C-6
 - The fructose 1-phosphate is then cleaved to glyceraldehyde and dihydroxyacetone phosphate by **fructose 1-phosphate aldolase**



- **MANNOSE:**

- D-Mannose, released in the digestion of various polysaccharides and glycoproteins of foods, can be phosphorylated at C-6 by hexokinase
- Mannose 6-phosphate is isomerized by **phosphomannose isomerase** to yield fructose 6-phosphate, an intermediate of glycolysis.

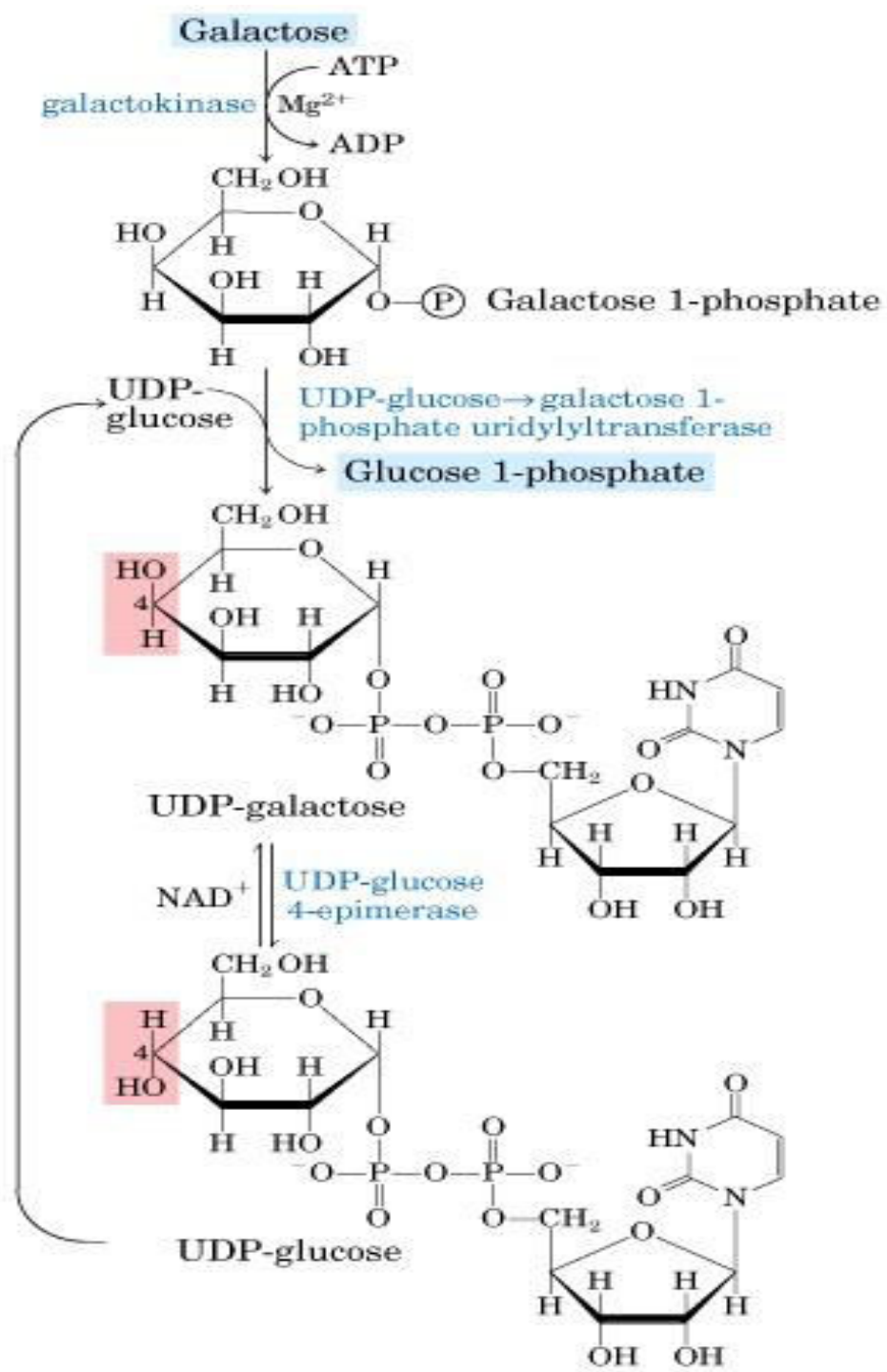


- **GALACTOSE:**

- D-Galactose, a product of the hydrolysis of lactose (milk sugar), passes in the blood from the intestine to the liver, where it is first phosphorylated at C-1, at the expense of ATP, by the enzyme **galactokinase**



Conversion of galactose to glucose 1-phosphate



GLUCOSE METABOLISM

Feeder pathways of GLYCOLYSIS-2

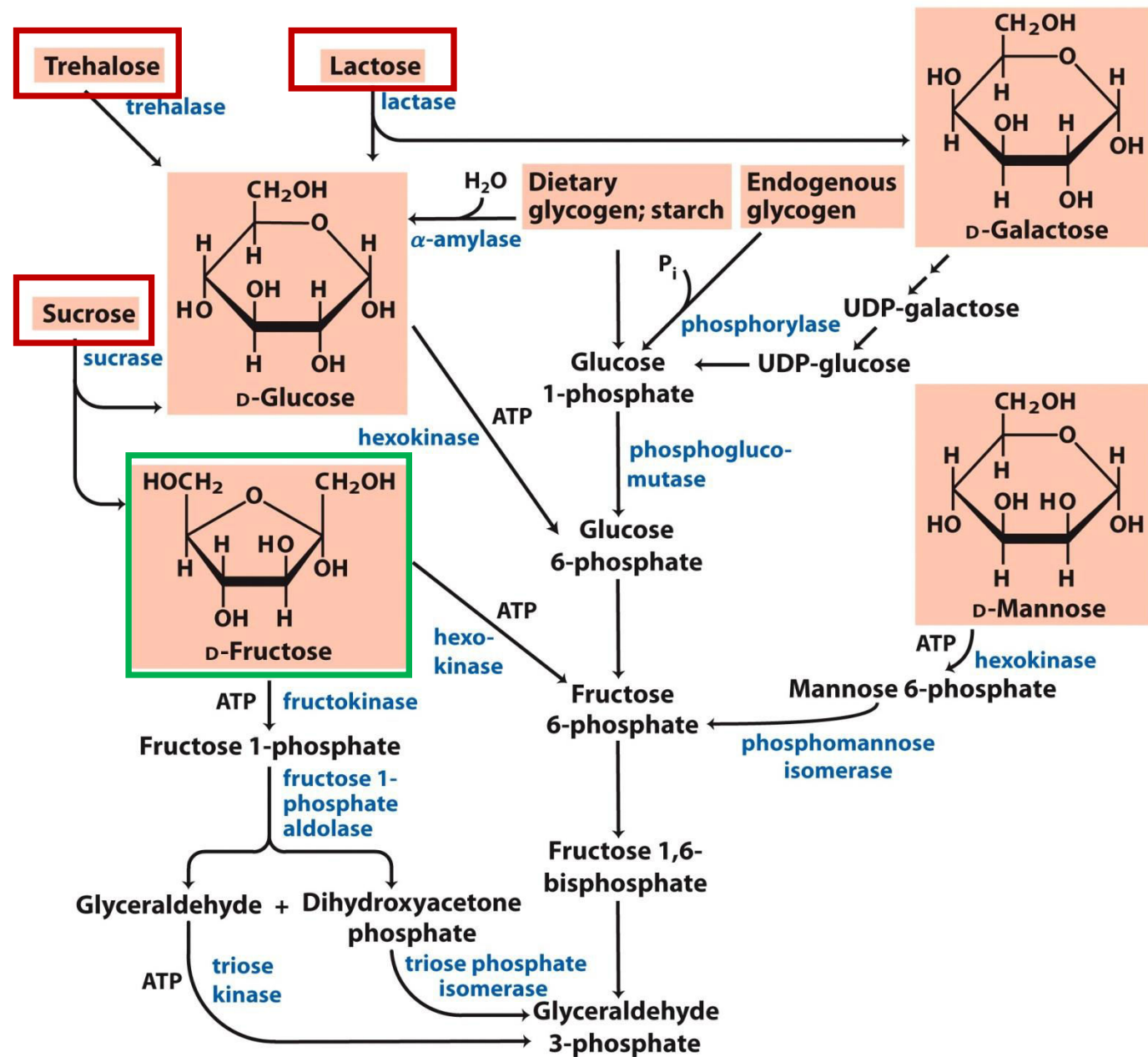


Figure 14-11

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Galactosemia

- A defect in any of the three enzymes in this pathway causes **galactosemia**

1. Galactokinase deficiency galactosemia

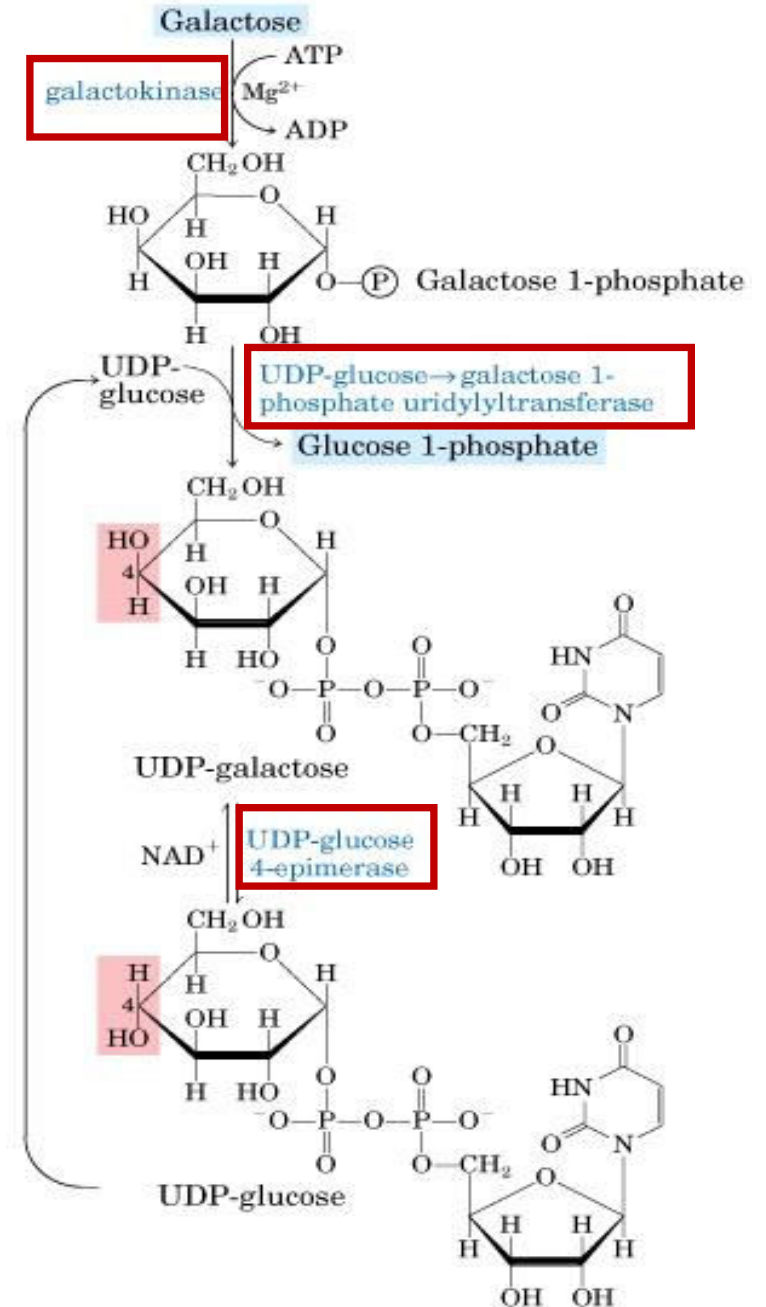
- high conc. are found in blood and urine.
- Infant develop cataracts, caused by deposition metabolite **galactitol in the lens**

2. Transferase deficiency galactosemia

- more serious
- characterized by poor growth in children, mental deficiency and liver damage that may be fatal

3. Epimerase deficiency galactosemia

- similar symptoms, less severe when dietary galactose is carefully controlled



Lactose intolerance

- due to the lack of lactase synthesis
- common among adults
- Without intestinal lactase, lactose cannot be completely digested and absorbed in the small intestine, and it passes into the large intestine, where bacteria convert it to toxic products that cause abdominal cramps and diarrhea.
- The problem is further complicated because undigested lactose and its metabolites increase the osmolarity of the intestinal contents, favoring retention of water in the intestine.
- In most parts of the world where lactose intolerance is prevalent, milk is not used as a food by adults, although milk products predigested with lactase are commercially available in some countries.
- The digestive disturbances can sometimes be minimized by a controlled diet
- **Mutase:**

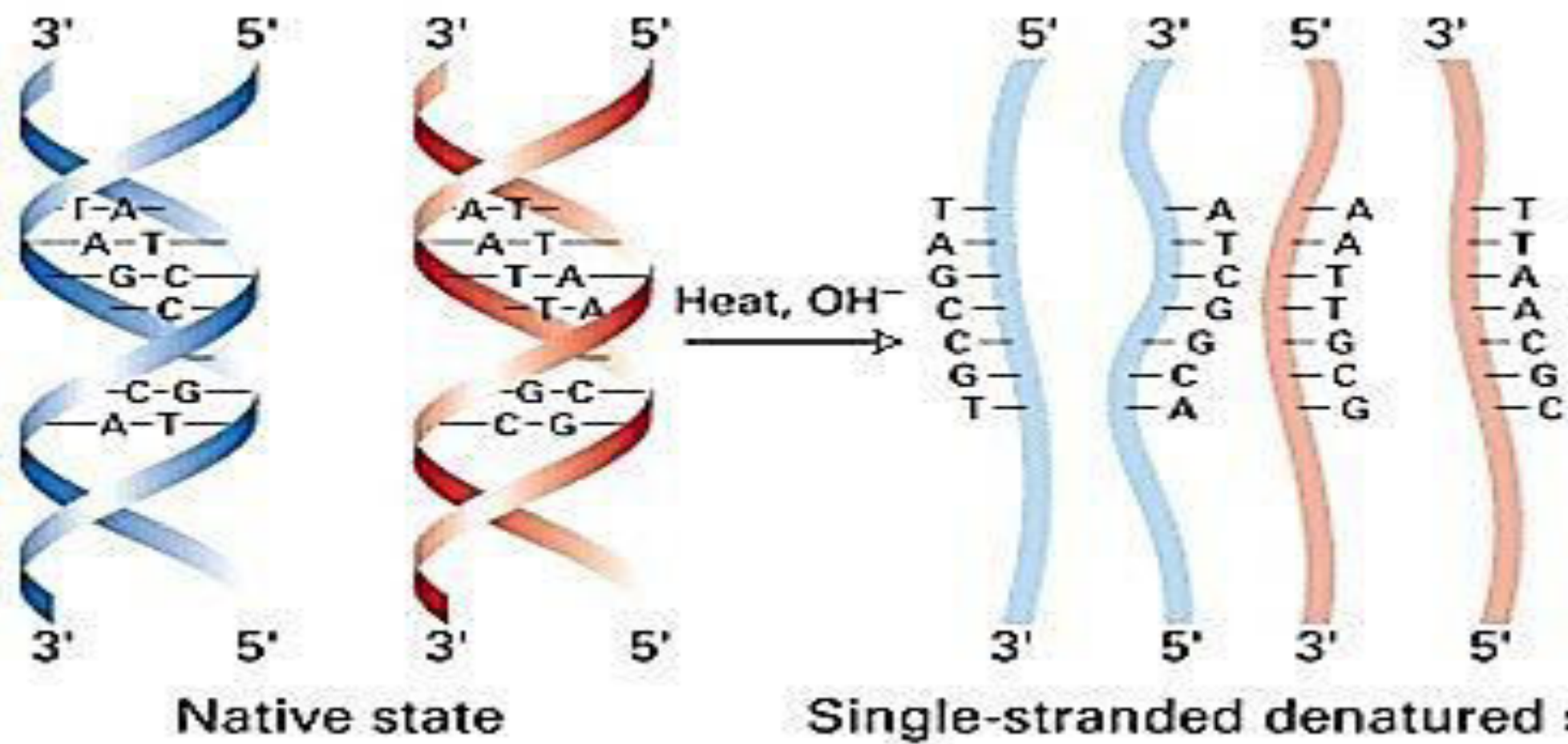
A **mutase** is an enzyme of the isomerase class that catalyzes the transfer of a functional group from one position to another within the same molecule

DNA Denaturation

- Separation of the two strands of the double helix when hydrogen bonds between the paired bases are disrupted.

- Disruption can occur
in the laboratory if
 - the pH or the salt concentration of the DNA solution is altered
 - if the solution is heated above 80°C

- Disruption of the hydrogen bonds between paired bases and of base stacking causes **unwinding of the double helix** to form two single strands complete separation of DNA stands **along the entire length**.
- No covalent bonds in the DNA are broken



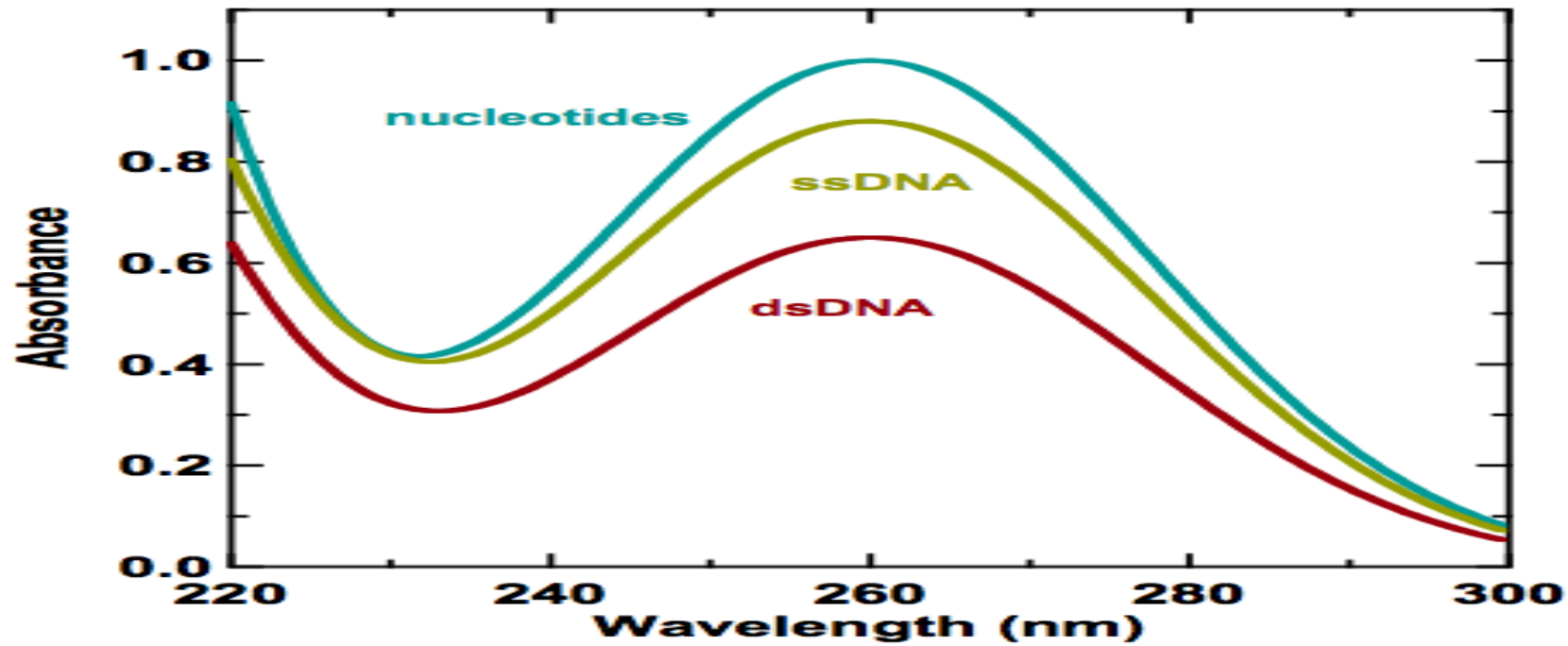
- T_m : When DNA is heated, the temperature at which one half of the helical structure is lost is defined as the melting temperature.

- A specific sequence of DNA has a characteristic denaturation temperature, or melting point (T_m)

- Careful determination of the melting temperature of a DNA specimen, under fixed conditions of pH and ionic strength, can yield an estimate of a DNA base composition.

END

- **DNA Denaturation (Contd.)**
- Concomitant with this denaturation of the DNA molecule is an increase in the optical absorbance of the purine and pyrimidine bases—a phenomenon referred to as hyperchromicity of denaturation.



The absorbance of double-stranded DNA (dsDNA) at 260 nm is less than that of either single-stranded DNA (ssDNA) or the free bases. This is called “hyperchromism.”

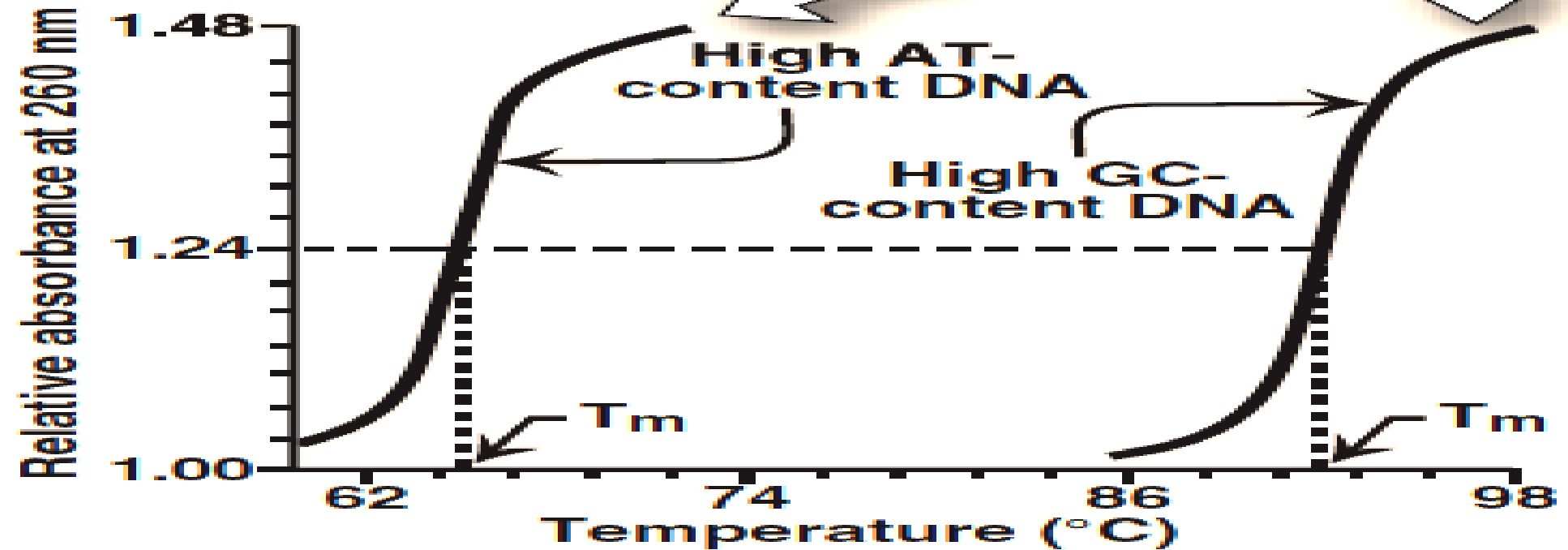
- So single-stranded DNA has a higher relative absorbance at this wavelength than does double-stranded DNA.
- Denaturation can be monitored by measuring its absorbance at 260 nm

Factors affecting T_m

- The T_m is influenced by
- the base composition of the DNA
- the salt concentration of the solution

- The higher the content of GC base pairs, the higher the melting point of the DNA.
- This is because GC base pairs, with three hydrogen bonds, require more heat energy to dissociate than AT base pairs.

At temperatures above the T_m , DNA is present as a single strand.



- An *increase* in salt concentration *increases* and a
- decrease in salt concentration decreases the T_m

END

DNA Renaturation

- Under appropriate conditions (*temp. & salt concentration*), separated strands of DNA will renature or reassociate and form the double helix by the process called **renaturation** (or **reannealing**).

- This reannealing process is also referred to as hybridization.

- When the **temperature or pH** is returned to the range in which most organisms live, the unwound segments of the two strands spontaneously rewind, or **anneal**, to yield the **intact duplex**

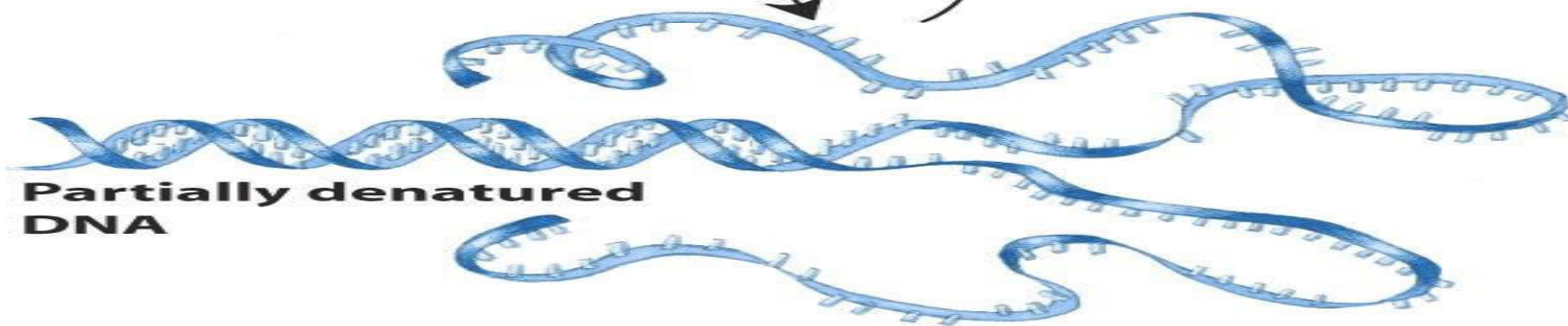
**Double-helical
DNA**



Denaturation

Annealing

**Partially denatured
DNA**



**Separation
of strands**

**Association of
strands by base
pairing**

**Separated strands
of DNA in random coils**



- Renaturation of a DNA molecule is a rapid **one-step process**.
- The rate of reassociation depends upon the concentration of the complementary strands.

- At a given temperature and salt concentration, a particular nucleic acid strand will associate tightly only with a complementary strand
- This property is utilized in analyzing nucleotide sequencing of a given nucleic acid.

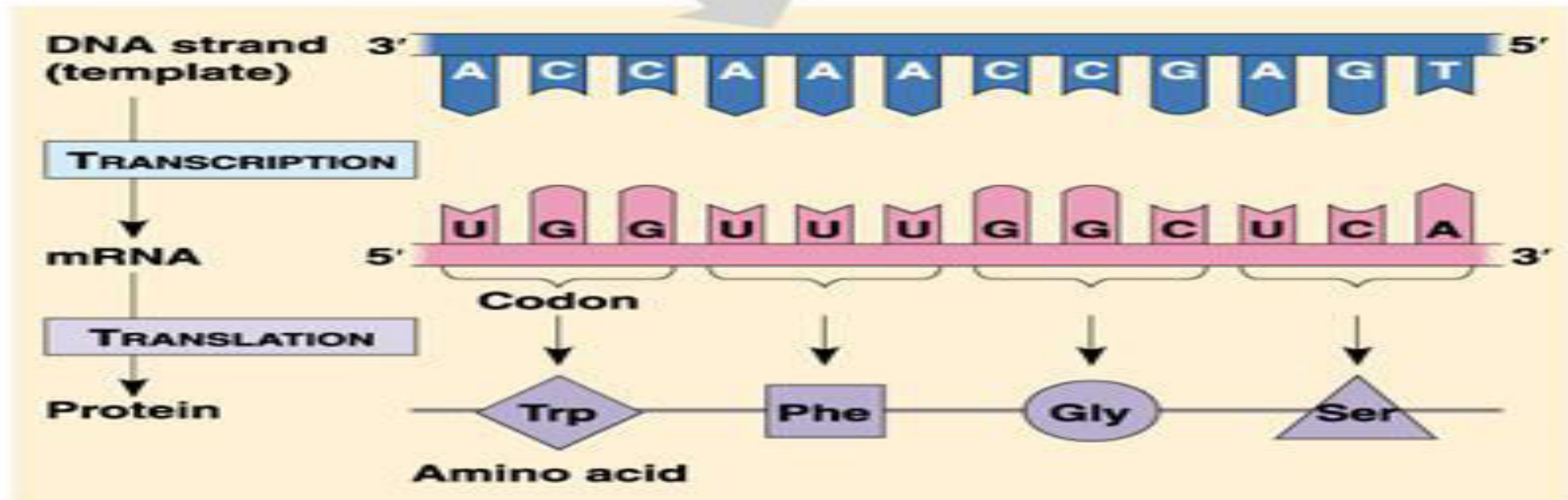
- Under appropriate conditions DNA will form a hybrid with a complementary DNA or with a complementary RNA

- Hybridization is combined with gel electrophoresis techniques that separate nucleic acids by size,
- coupled with radioactive or fluorescent probe labeling to provide a detection of a nucleotide sequence.

END

RNA

- The genetic master plan is contained in the nucleotide sequence of DNA.
- It is through the ribonucleic acid (RNA)—the "working copies" of the (DNA) — that the master plan is expressed

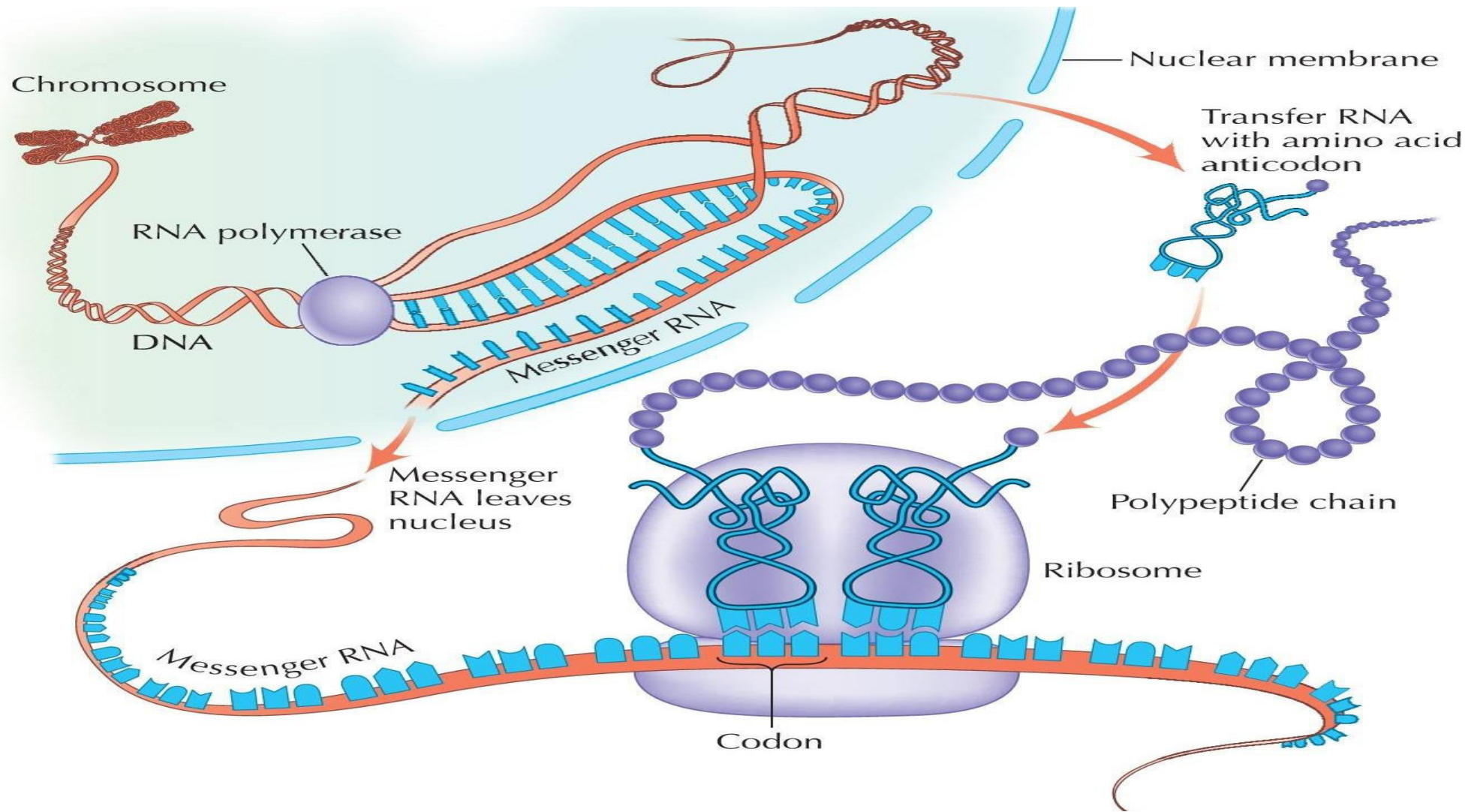


- RNA is a polymer of ribonucleotides of Adenine, Uracil, Guanine and Cytosine, joined together by 3'-5' phosphodiester bonds.
- RNA does not contain thymine except in rare cases.

- The pentose sugar of RNA is D-ribose.
- Location: RNA is found in the nucleolus, ribosomes, mitochondria, and cytoplasm.
- The genetic material for some animal and plant viruses is RNA rather than DNA.

- There is a wide variety of RNAs,
- messenger RNAs (mRNAs)-
transfer genetic information from
DNA to the protein-synthesizing
machinery.
- ribosomal RNAs (rRNAs)-
contribute to the formation and
function of ribosomes

- transfer RNAs (tRNAs)- adapter molecules that carry specific amino acids for protein synthesis



- small nuclear RNA (snRNA)- play pivotal roles in RNA processing, particularly mRNA processing
- ribozymes — some RNA molecules have intrinsic catalytic activity these RNA enzymes, are called ribozymes

END

Differences between DNA and RNA

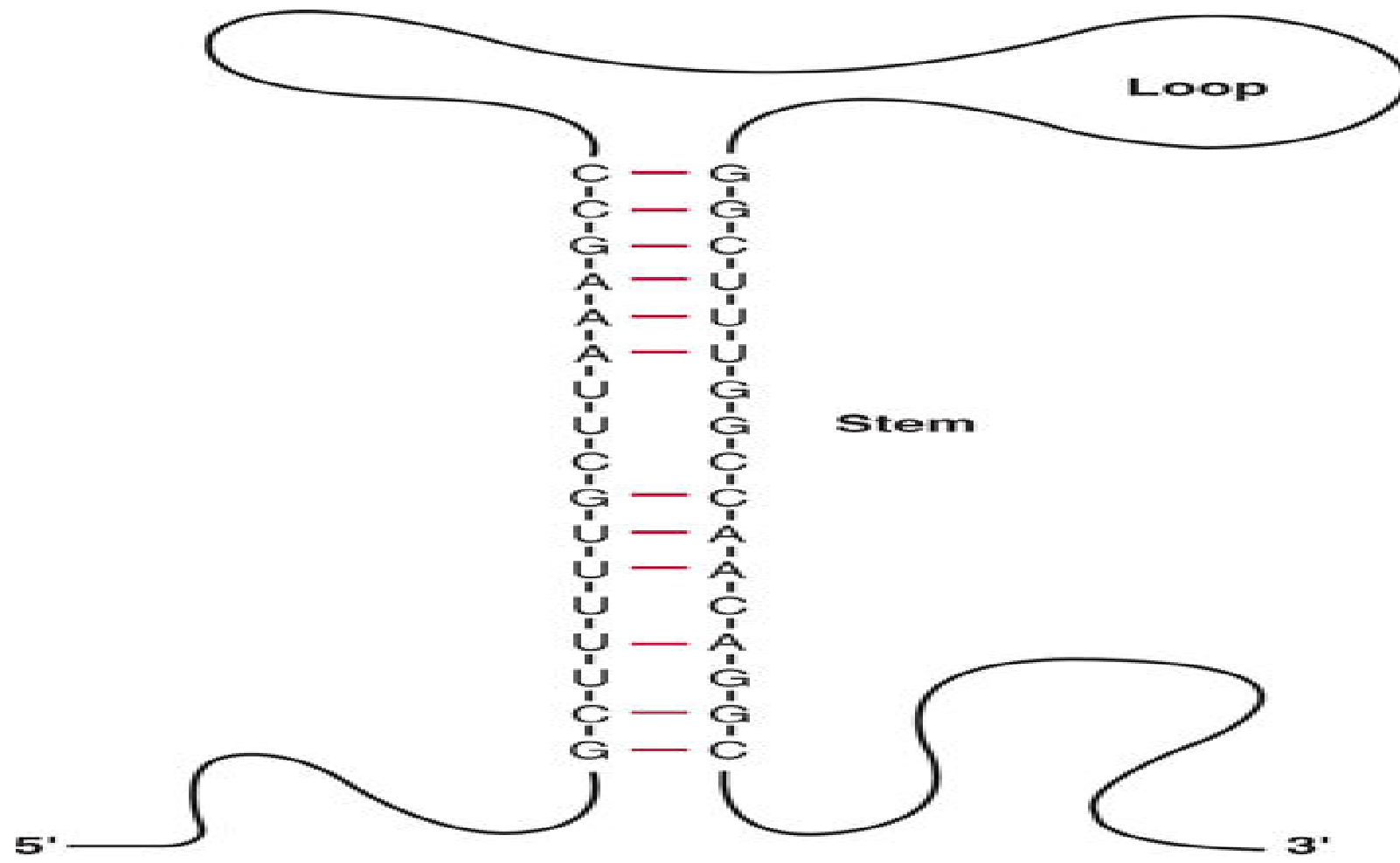
- Although sharing many features with DNA, RNA possesses several specific differences.

- **Size:** They are considerably smaller than DNA
- **Sugar:** In RNA, the sugar moiety to which the phosphates and purine and pyrimidine bases are attached is **ribose** rather than the **2'-deoxyribose** of DNA

- **Pyrimidine:** The pyrimidine components of RNA differ from those of DNA.
- Instead of **thymine**, RNA contains the ribonucleotide of **uracil**.
- **Thymine** is present in the **rare** case of tRNA

- Single Strand:
- RNA typically exists as a single strand whereas DNA exists as a double-stranded helical molecule.

- However, given the proper complementary base sequence with opposite polarity, the single strand of RNA is capable of self-folding like a hairpin and thus acquiring double-stranded characteristics: G pairing with C, and A with U.



- Chargaff's Rules Do Not Apply:
- Since the RNA molecule is a single strand complementary to only one of the two strands of a gene,
- $G \neq C$
- $A \neq U$
- Purines $\neq$ Pyrimidines

- **Hydrolysis:** RNA can be hydrolyzed by alkali to 2',3' cyclic diesters of the mononucleotides, compounds that cannot be formed from alkali-treated DNA because of the absence of a 2'-hydroxyl group.

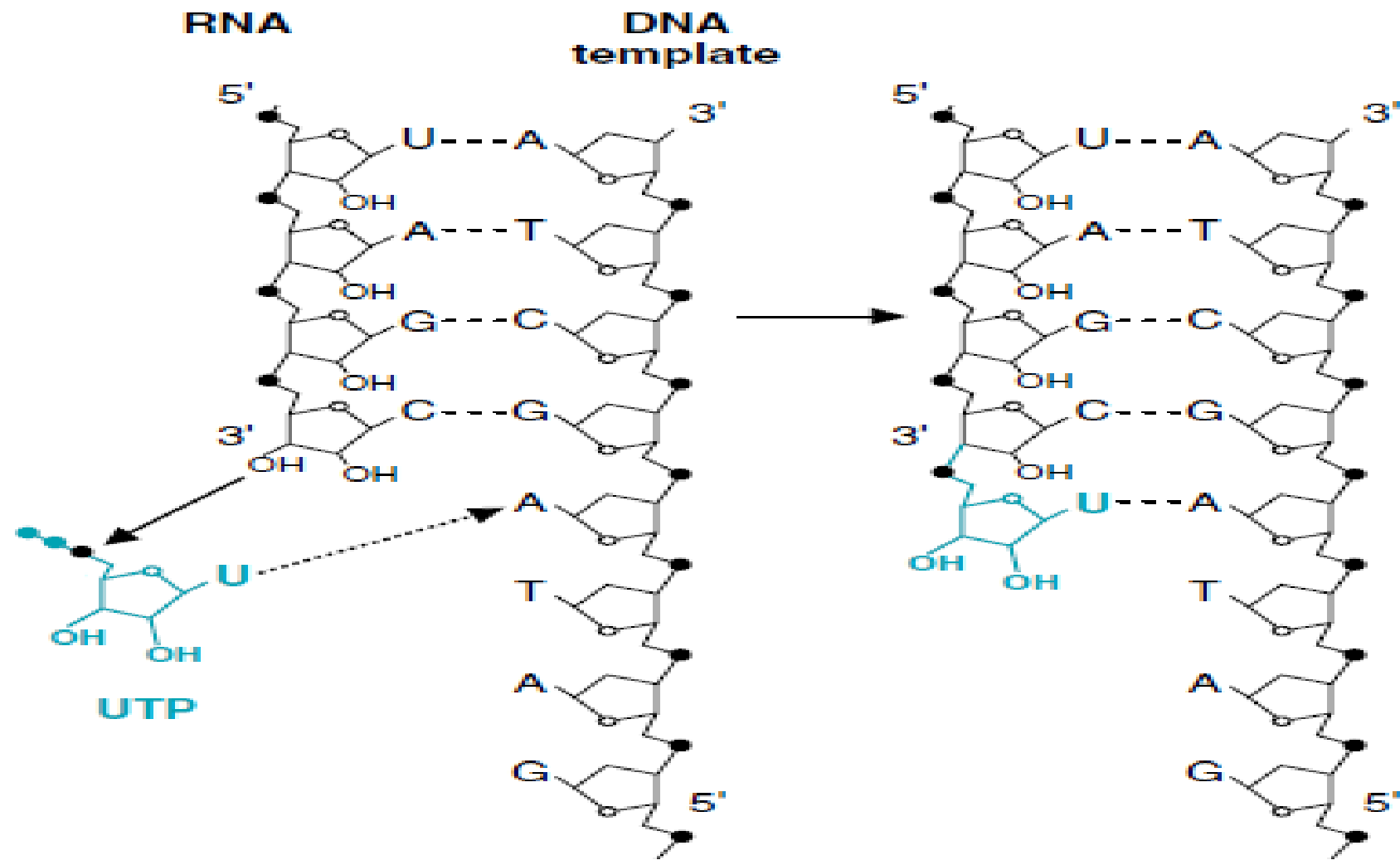
- **Location:** In addition to nucleus, RNA is found in cytoplasm.
- **Reverse Transcription:** DNA forms RNA by transcription whereas the process by which RNA form DNA is called **reverse transcription**.

- **RNA Structural Hierarchy**
- RNA has no simple, structural hierarchy that serves as a reference point, as does the double helix for DNA.
- The three-dimensional structures of many RNAs, like those of proteins, are complex and unique.

Primary Structure of RNA

- It is defined as the **number and sequence** of ribonucleotides in the RNA chain.

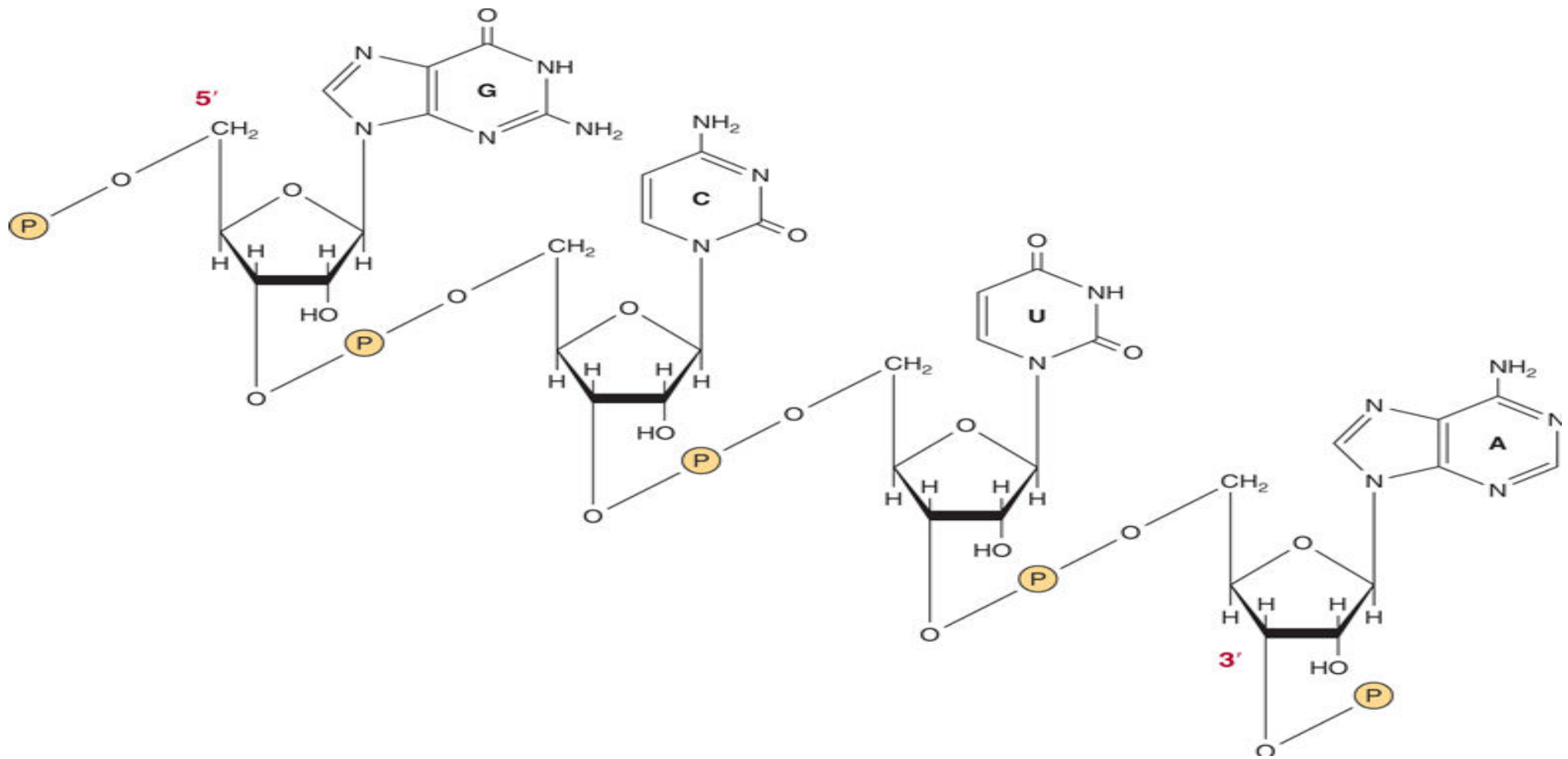
- The sequence is complementary to the template strand of the gene from which it was transcribed.



template strand, the sequence of DNA that is copied.

Non Template (coding strand).

- The ribonucleotides are held together by 3'-5' phosphodiester bonds.
- 3'-OH group of one nucleotide is bound to 5'-PO₄ of the other nucleotide and form a linear strand.

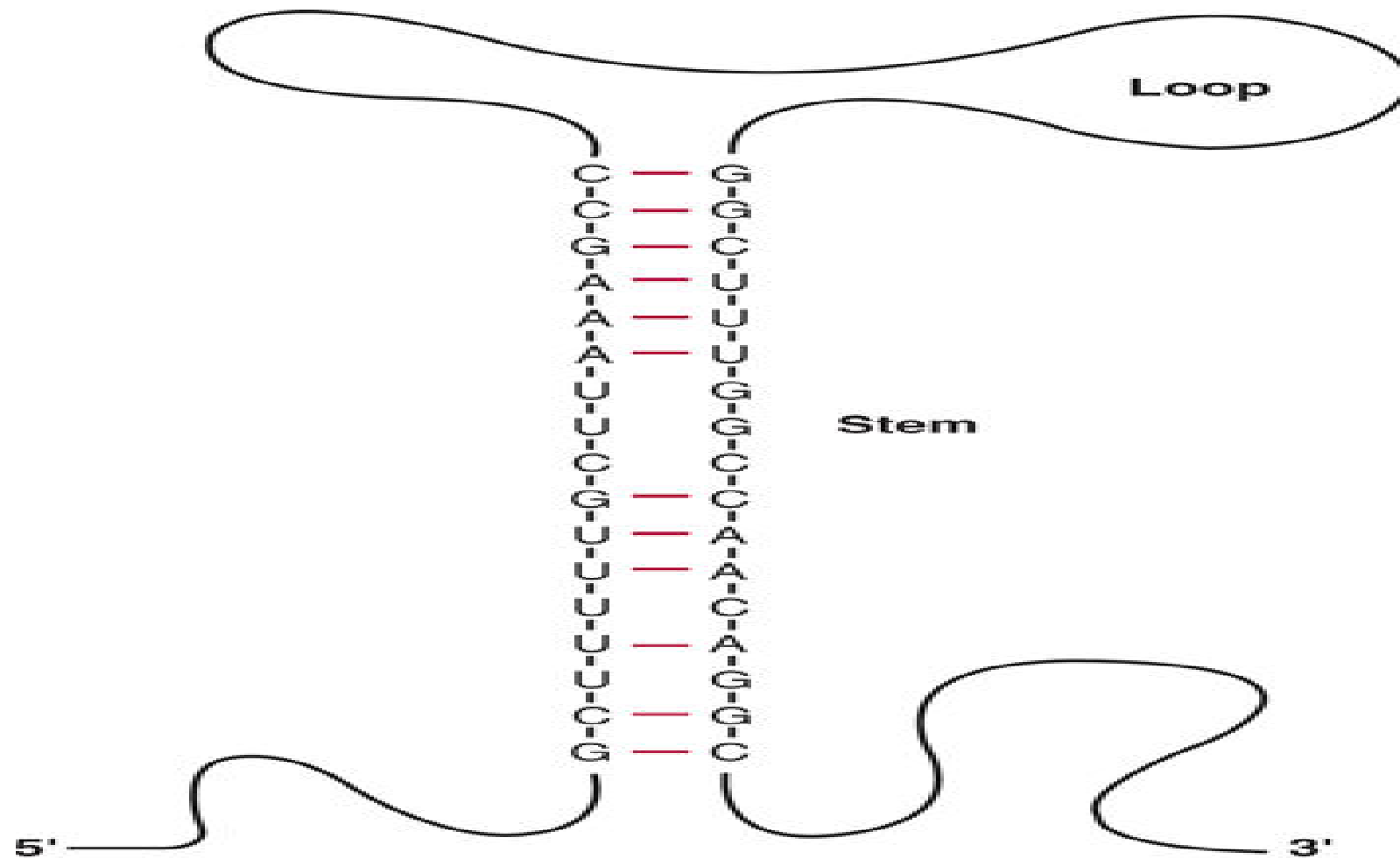


- The ribosyl moieties are attached to the nucleobases by *N*-glycosidic bonds
- Similar to DNA RNA polymer also has polarity

- **Secondary Structure of RNA**

- Secondary structure involves **coil formation** of the polyribonucleotide chain.

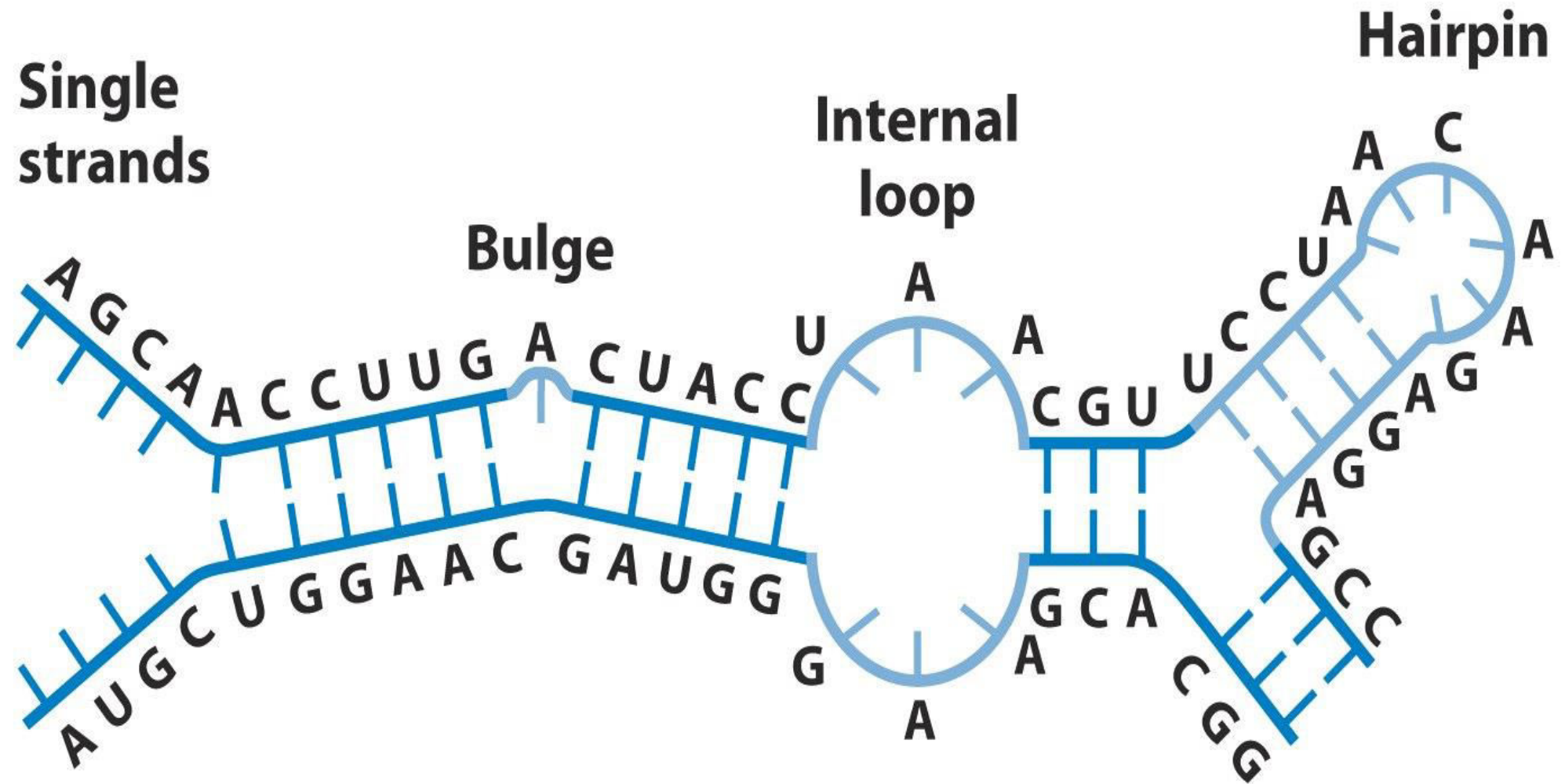
- The coiled structures are stabilized by
- Hydrophobic interactions between purine and pyrimidine bases.
- Intra-chain hydrogen bonds between G-C and A-U.



Note: Adenine pairs with Uracil in RNA

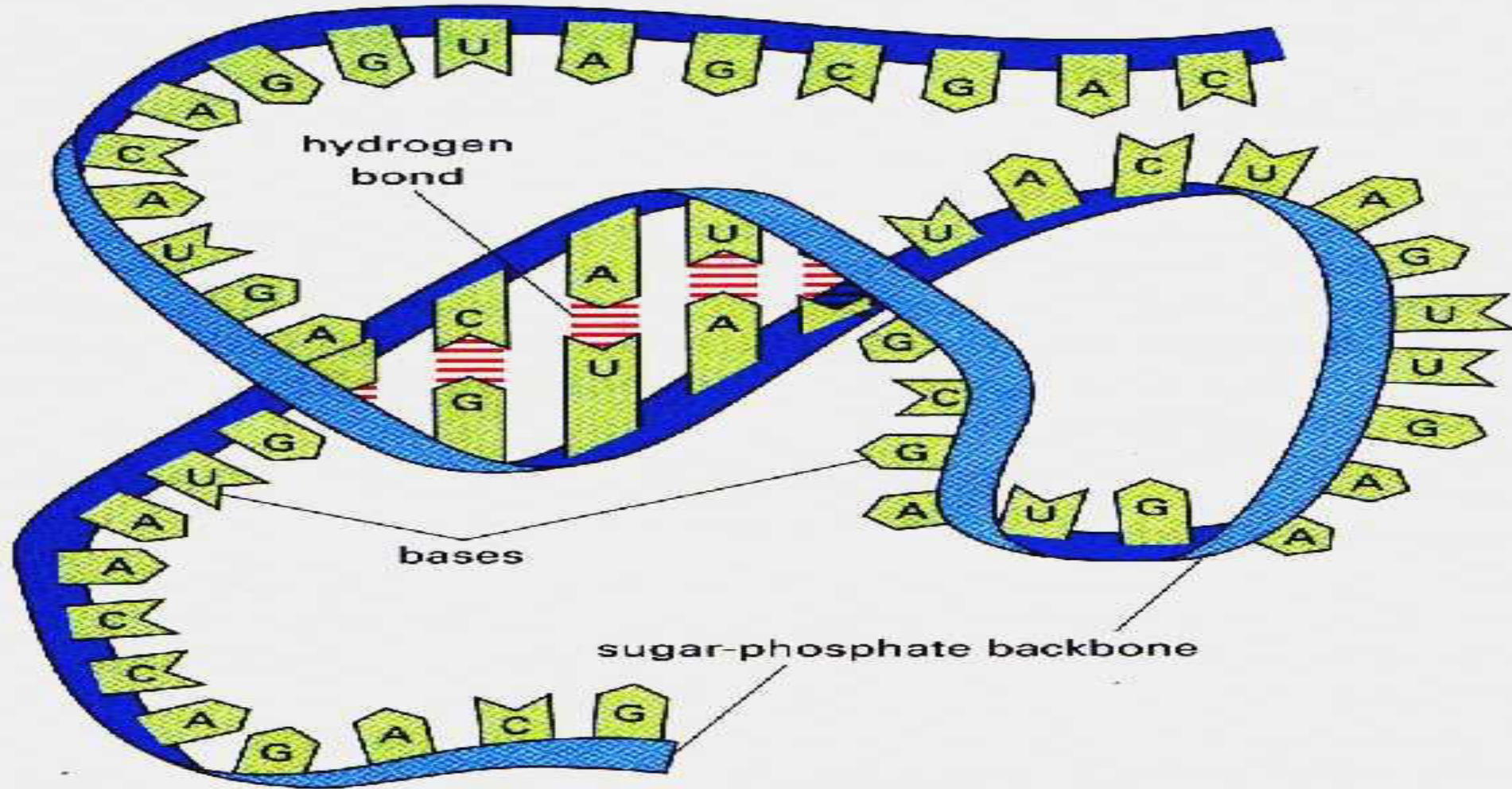
- Weak interactions, especially base-stacking interactions.
- Where complementary sequences are present, the predominant double-stranded structure is formed.

- Common RNA secondary structures
- **Internal loops:** a short series of unpaired bases in a longer paired helix and
- **bulges:** regions in which one strand of a helix has "extra" inserted bases with no counterparts in the opposite strand



Tertiary Structure of RNA

- It is the folding of the molecule into three dimensional structure.
- cross-linking at various sites stabilized by hydrophobic and hydrogen bonds produces a compactly coiled globular structure.

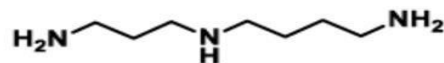


- The stacking of helices, together with specific **helix–helix** contacts or **helix–loop interactions**, lead to compact tertiary structure of the RNA assemblies, generally in the presence of **divalent ions** or **polyamines**

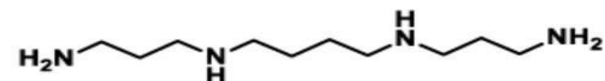
Chemical structures of the polyamines and polyamine analogues discussed throughout this review.



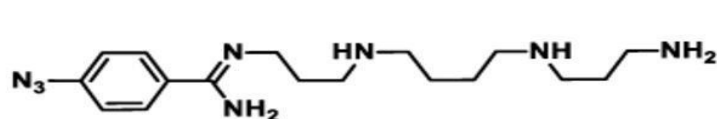
Putrescine



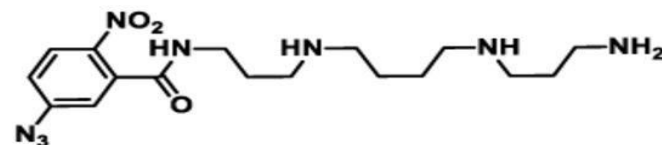
Spermidine



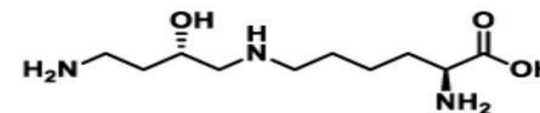
Spermine



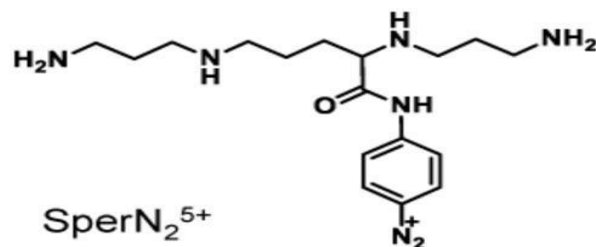
ABA-spermine



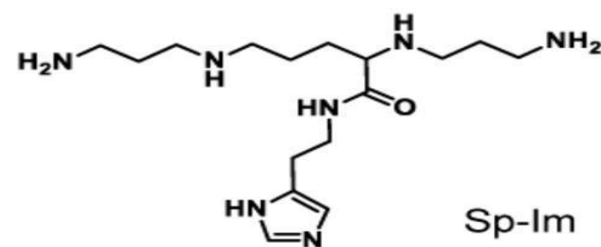
ANB-spermine



Hypusine



SperN₂⁵⁺



Sp-Im

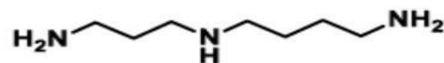
Helen L. Lightfoot, and Jonathan Hall Nucl. Acids Res. 2014;nar.gku837

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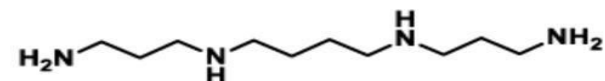
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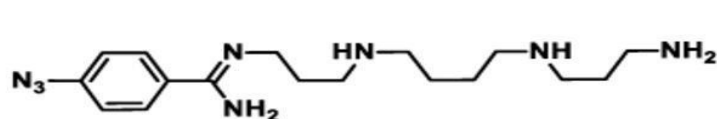
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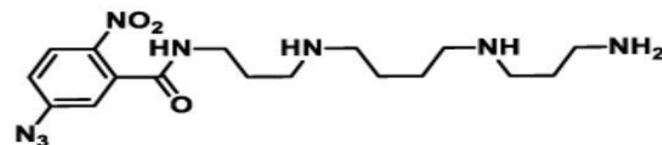
Spermidine



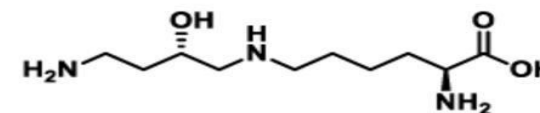
Spermine



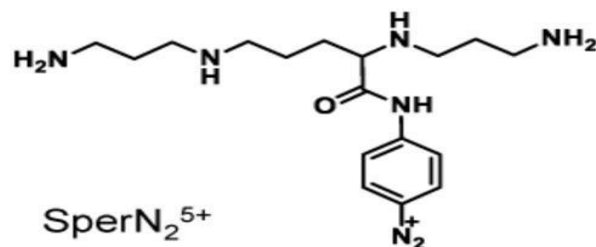
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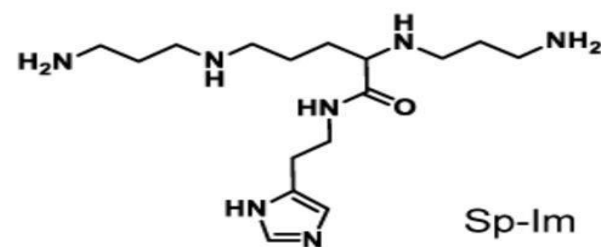
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Hypusine



SperN₂⁵⁺



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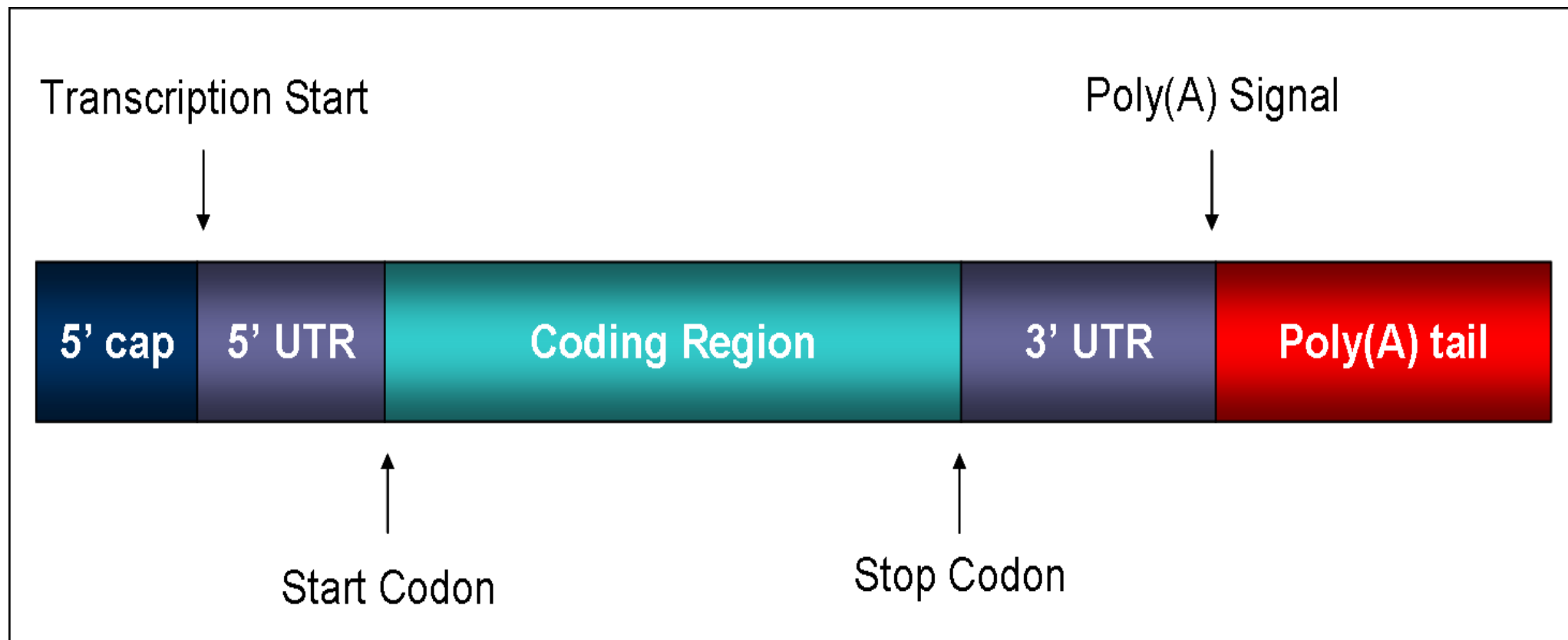
Helen L. Lightfoot, and Jonathan Hall Nucl. Acids Res. 2014;nar.gku837

Messenger RNA (mRNA)

- This class is the most heterogeneous in
- Abundance
- Size (500-6000 nucleotides)
- base sequence
- Stability

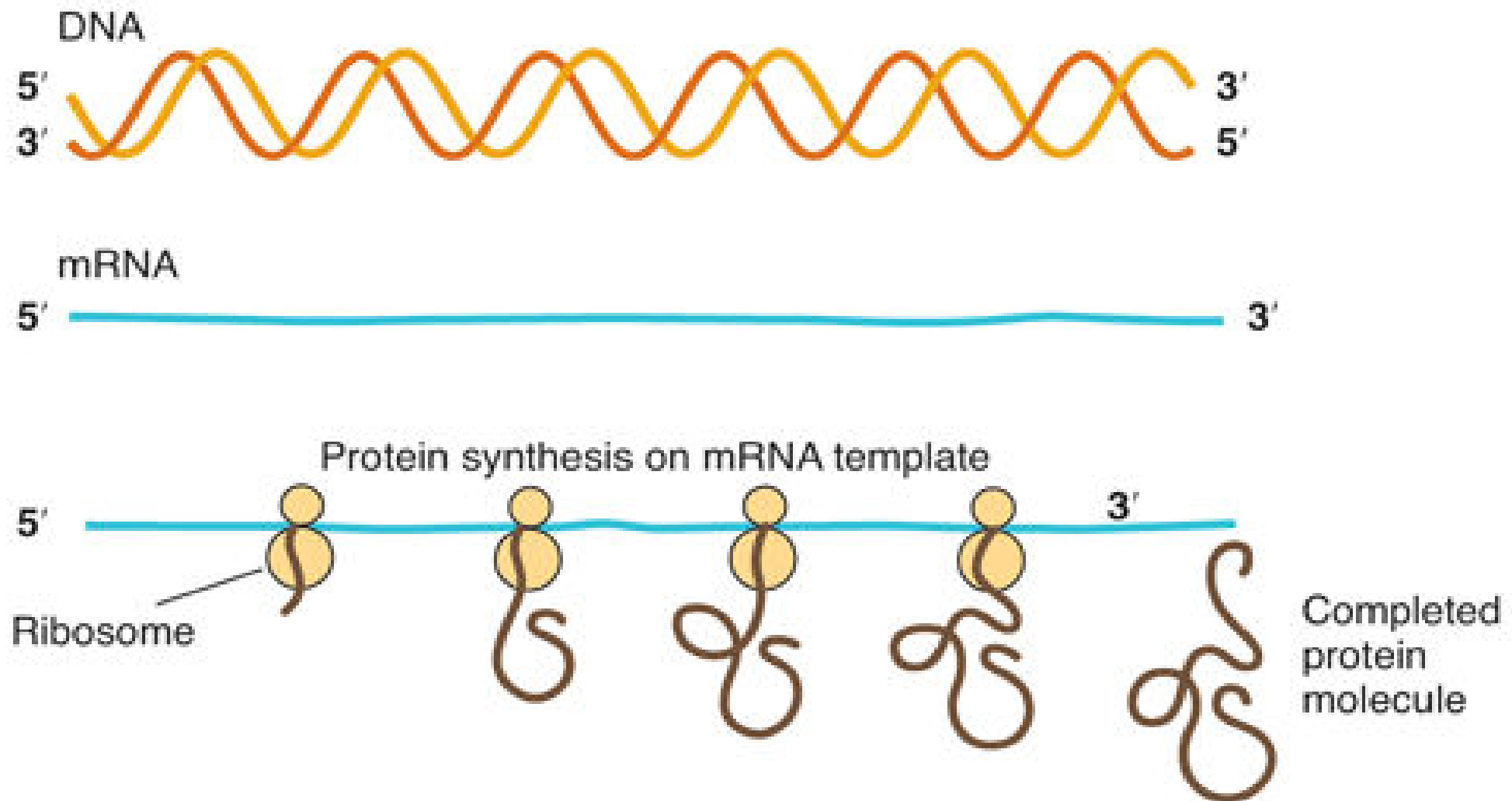
- mRNA comprise about 2–5% of total cellular RNA
- mRNA molecules are formed with the help of DNA template strand (3′-5′) during the process called transcription.

- In addition to the protein coding regions in the mature eukaryotic mRNA that can be translated,
- there are untranslated regions at its 5' and 3' ends
- Moreover, there is a 5' cap and
- a poly A tail at 3' end



Function of mRNA

- The members of this class function as messengers to convey the information in a gene to the protein synthesizing machinery.



- The mRNA carries genetic information from the nuclear DNA to the cytosol, where it is used as a **template for protein synthesis**.

Transfer RNA (tRNA)

- t RNA is the **smallest** of the three major species of RNA (4S).
- They are **single stranded globular molecules**.
- They remain largely in cytoplasm.

- They are generated by nuclear processing of a precursor molecule.
- tRNAs compose roughly 20% of total cellular RNA
- There are at least 20 species of tRNA molecules in every cell.

- Although each specific tRNA differs from the others in its sequence of nucleotides, the tRNA molecules as a class have many features in common

Primary structure

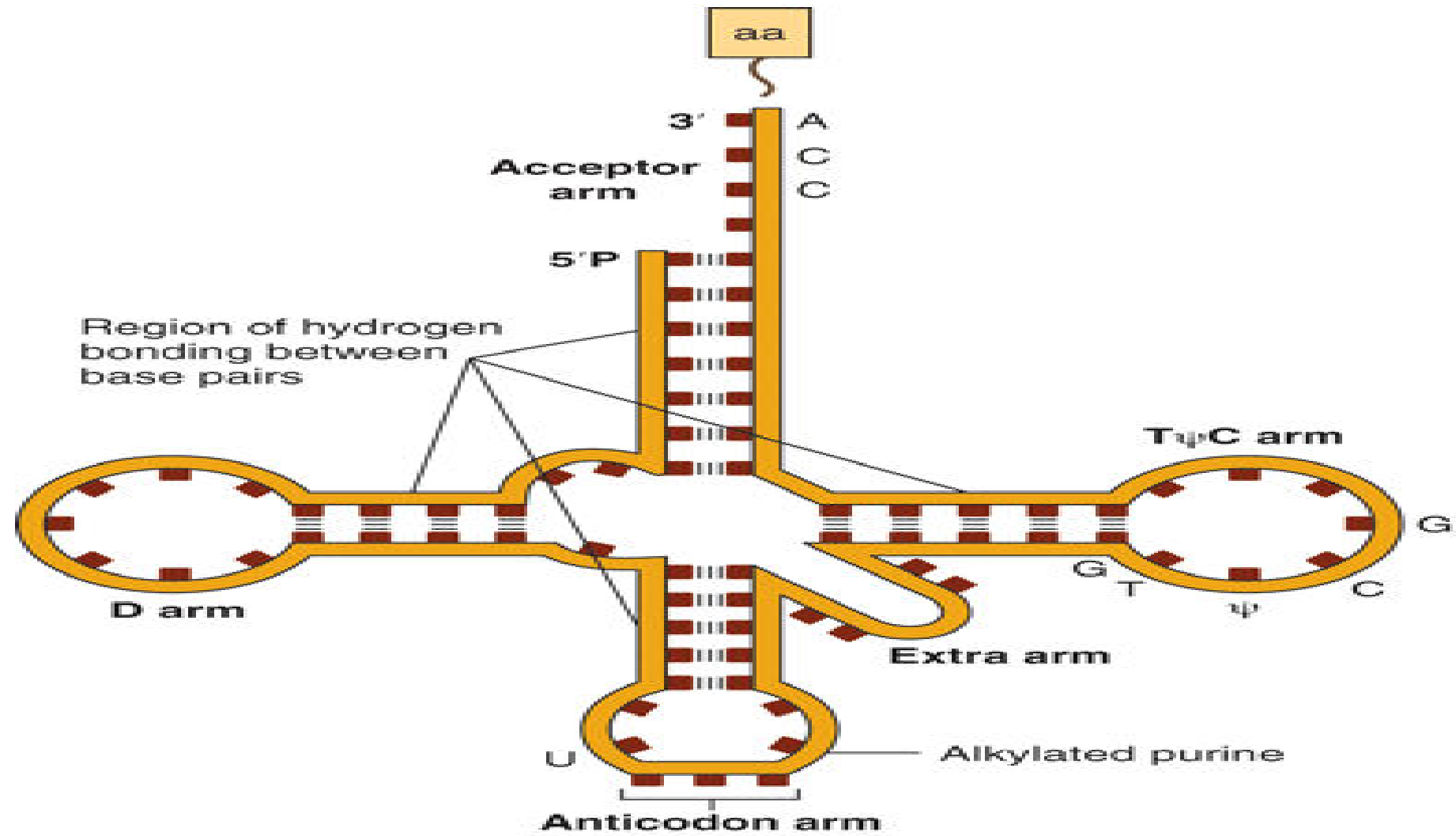
- t RNA molecules consist of 74-95 nucleotides in a particular sequence.
- The t RNA molecules contain not only the usual bases like adenine, guanine, cytosine, uracil but also contain unusual bases

- These unusual bases(also called modified bases) include
- Dihydrouracil
- Pseudouridine
- Thymine.

Secondary Structure

Pseudouridine

- Each single stranded t RNA is folded extensively.
- Extensive intra chain base pairing which leads to a characteristic CLOVER-LEAF structure.



- These folds are stabilized by hydrogen bonds between complementary bases of the same strand.

Transfer RNA (tRNA) (Countinued)

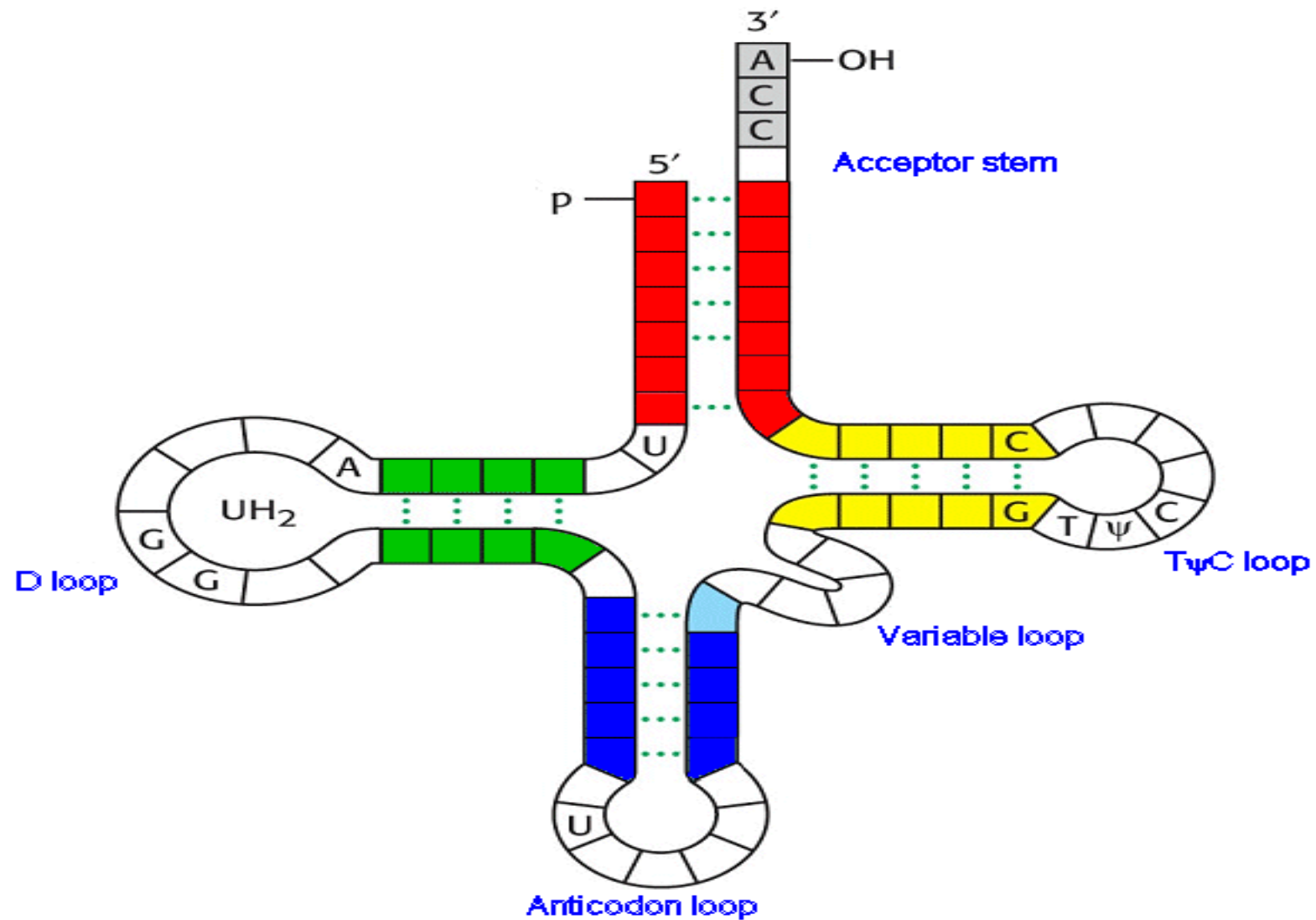
Arms or loops of tRNA

- All tRNA molecules contain 4 main arms or loops.
- **1-Acceptor arm:** This is made up of unpaired sequences of cytosine-cytosine-adenine (CCA) at the 3' end.

- The 3'OH group of adenine binds with the carboxylic group of a specific amino acid and carries it to ribosomes for protein synthesis.

- **2-Anticodon arm:** It is in the form of a loop and carries specific sequences of **three bases** which constitute the anticodon.
- The bases of anticodon are bonded with three **complementary bases of codon** on mRNA.

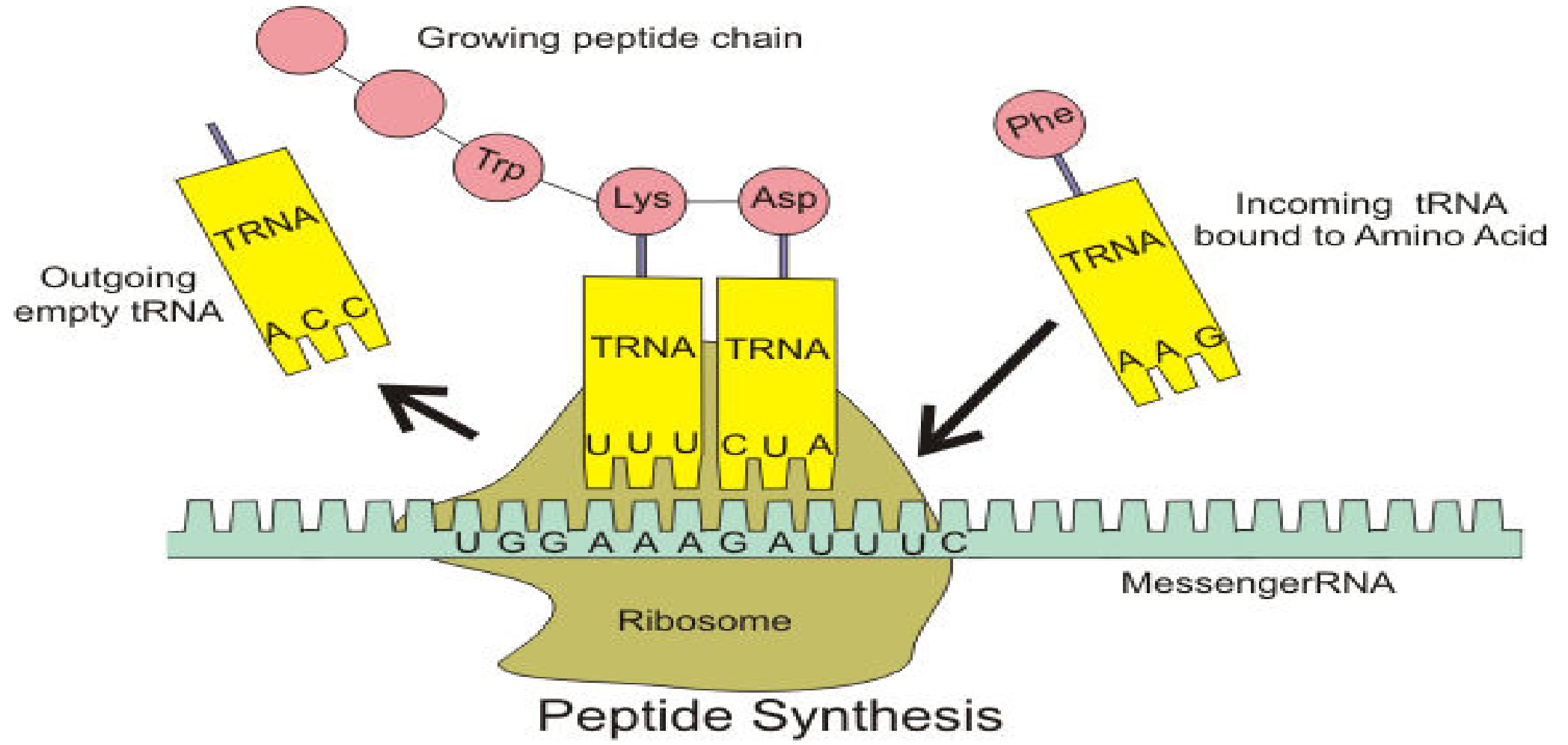
- **3-D arm:** It contains the base dihydrouridine.
- **4-TΨC arm:** It contains thymine, pseudouridine and cytosine.
- The extra arm and the TΨC arms help to define a specific tRNA.



D arm: contains dihydrouridine. **TΨC arm:** thymine, pseudouridine and cytosine.

Function of tRNA

- The t RNA molecules serve as **ADAPTERS** for the **translation** of information in the sequence of nucleotides of the mRNA into specific **amino acids**.



- There is at least one (and often several) specific type of tRNA molecule for each of the amino acids commonly found in proteins.
- Each t RNA carries its **specific amino acid** to the site of protein synthesis.

- There it recognizes the genetic code word on mRNA (codon) and this specifies the addition of its amino acids to the growing peptide chain.

Transfer RNA (tRNA) (Countinued)

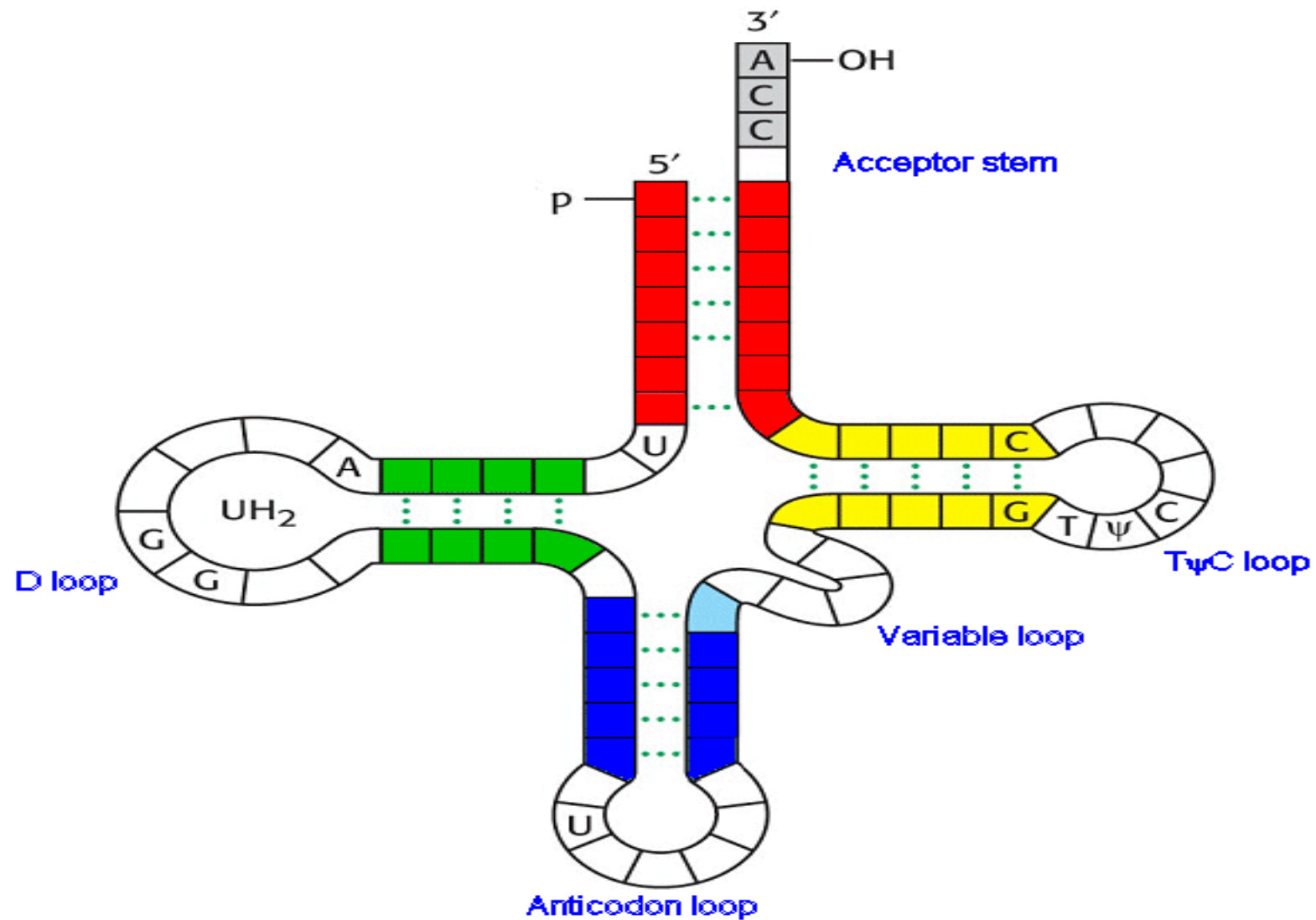
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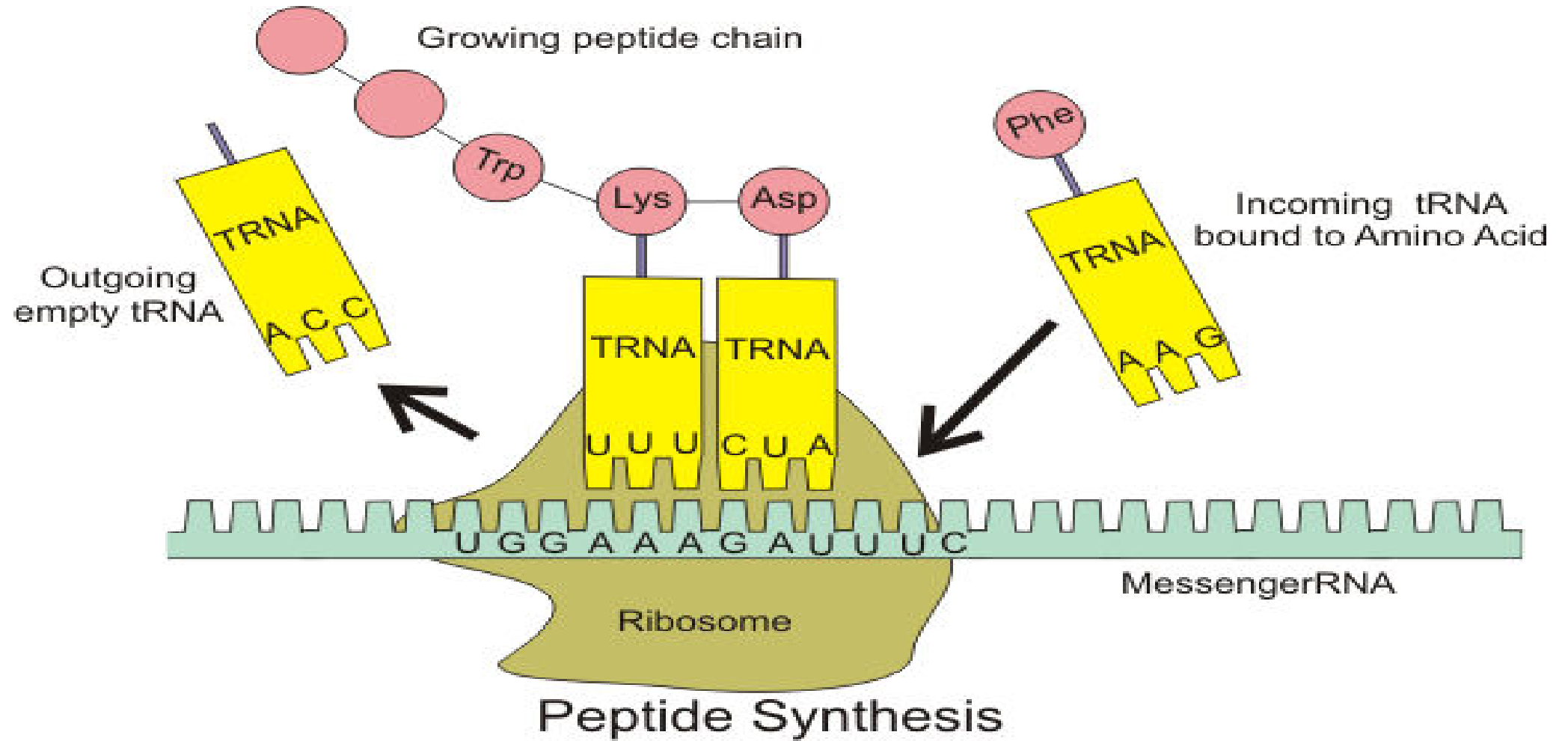
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Ribosomal RNA (rRNA)

- found in association with several proteins as a component of ribosomes--- a cytoplasmic nucleoprotein structure that acts as the machinery for the synthesis of proteins from the mRNA template.

- RNAs make up 80% of the total RNA in the cell.
- The ribosomal subunits are defined according to their sedimentation velocity in Svedberg units.

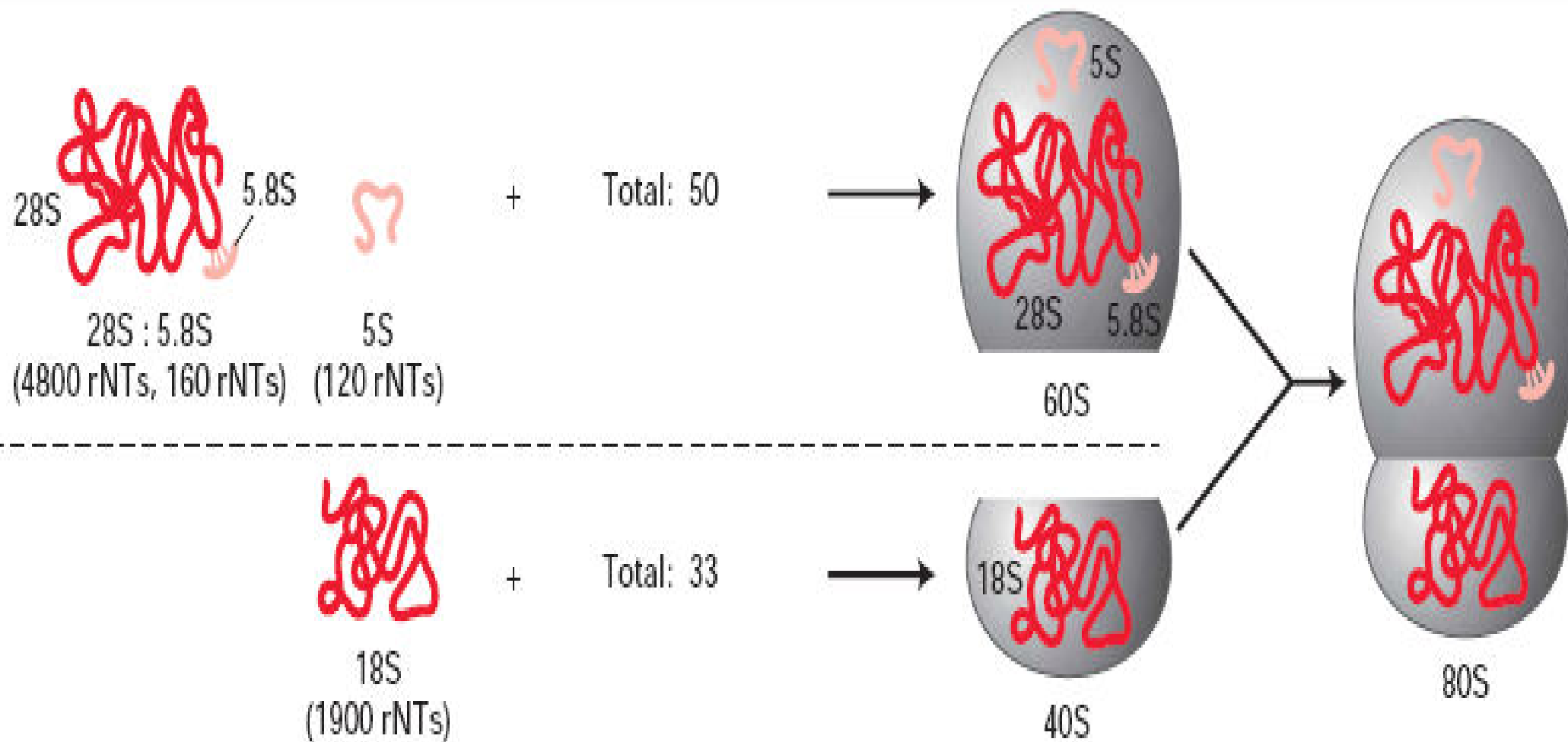
- Svedberg unit is related to the molecular weight and shape of the compound.
- The bases in r RNA are mainly adenine, guanine, cytosine and uracil and a few pseudouridine

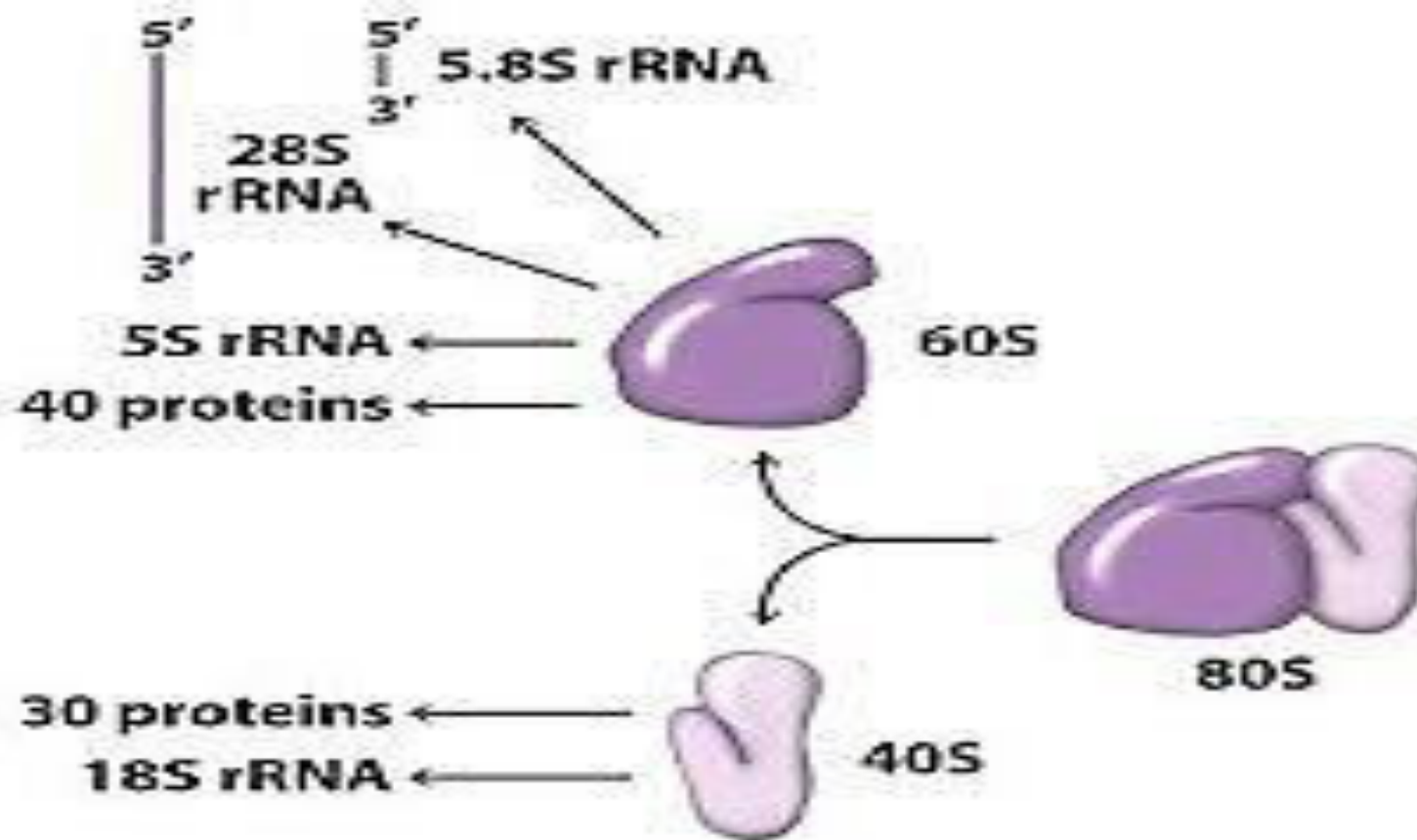
Eukaryotic Ribosome

- The mammalian ribosome contains two major nucleoprotein subunits:
- a larger one with 60S
- a smaller one with 40S.

- The **60S** subunit contains
- a 5S rRNA
- a 5.8S rRNA
- a 28S rRNA
- more than 50 specific polypeptides.

- The **40S** subunit is smaller and contains
- a single 18S rRNA
- Approx. 30 distinct polypeptide chains.





Alternate diagram

Eukaryote

- In eukaryotes, all of the ribosomal RNA molecules **except the 5S rRNA**, which is independently transcribed, are **processed from a single 45S precursor RNA molecule** in the **nucleolus**- packed with the specific ribosomal proteins.

- The rRNA are necessary for **ribosomal assembly** and play a key role in the binding of mRNA to ribosomes and its translation
- In the cytoplasm, the ribosomes remain quite stable and **capable of many translation cycles**

Other types of RNA

Small nuclear RNA (snRNA)

- Small nuclear RNA (snRNA) are large number of small stable RNA species found in eukaryotic cells.
- Most of them are complexed with proteins to form ribonucleoproteins.

- They are distributed in the nucleus, in the cytoplasm or in both.
- They are significantly involved in rRNA and mRNA processing and gene regulation.

- **Large & Small Noncoding Regulatory RNAs**
- One of the most exciting discoveries in the last decade of eukaryotic regulatory biology has been the identification and characterization of **regulatory nonprotein coding RNAs (ncRNAs)**.

- NcRNAs exist in two general size classes,
- small consisting of microRNA (miRNAs) and silencing (siRNAs) and
- Large consisting of long noncoding RNAs (lncRNAs)

- The small ncRNAs termed microRNA (miRNAs) and silencing (siRNAs) typically inhibit gene expression at the level of specific protein production by
- targeting mRNAs through one of several distinct mechanisms.

- Both siRNAs and miRNAs typically hybridize, via the formation of RNA–RNA hybridization to their targeted mRNAs

- **long noncoding RNAs (lncRNAs).**
- LncRNAs, which as their name implies, do not code for protein (ie, the mRNA encoding genes).
- ncRNAs make up a significant portion of eukaryotic transcription

- ncRNAs play many roles ranging from contributing to structural aspects of chromatin to regulation of mRNA gene transcription by RNA polymerase II.

END

IUB Classification of Enzymes

- International Union of Biochemists (IUB) developed an unambiguous system of enzyme nomenclature in which each enzyme has a
- unique name and
- code number

- As an example, the formal systematic name of the enzyme (**hexokinase**) catalyzing the reaction
- $\text{ATP} + \text{D-glucose} \rightarrow \text{ADP} + \text{D-glucose-6 phosphate}$ is
- **ATP:glucose phosphotransferase,**

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- Its Enzyme Commission (E.C.) number is 2.7.1.1.
- (2) denotes the class name (transferase)
- (7) the subclass phosphotransferase
- (1) denotes a hydroxyl group as acceptor
- (1) D-glucose as the phosphoryl group acceptor.

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- In the systematic naming system, enzymes are divided into **six major classes** each with numerous subgroups

CLASSIFICATION OF ENZYMES

Group of Enzyme	Reaction Catalysed	Examples
1. Oxidoreductases	Transfer of hydrogen and oxygen atoms or electrons from one substrate to another.	Dehydrogenases Oxidases
2. Transferases	Transfer of a specific group (a phosphate or methyl etc.) from one substrate to another.	Transaminase Kinases
3. Hydrolases	Hydrolysis of a substrate.	Estrases Digestive enzymes
4. Isomerases	Change of the molecular form of the substrate.	Phospho hexo Isomerase, Fumarase
5. Lyases	Nonhydrolytic removal of a group or addition of a group to a substrate.	Decarboxylases Aldolases
6. Ligases (Synthetases)	Joining of two molecules by the formation of new bonds.	Citric acid synthetase

CLASSIFICATION OF ENZYMES

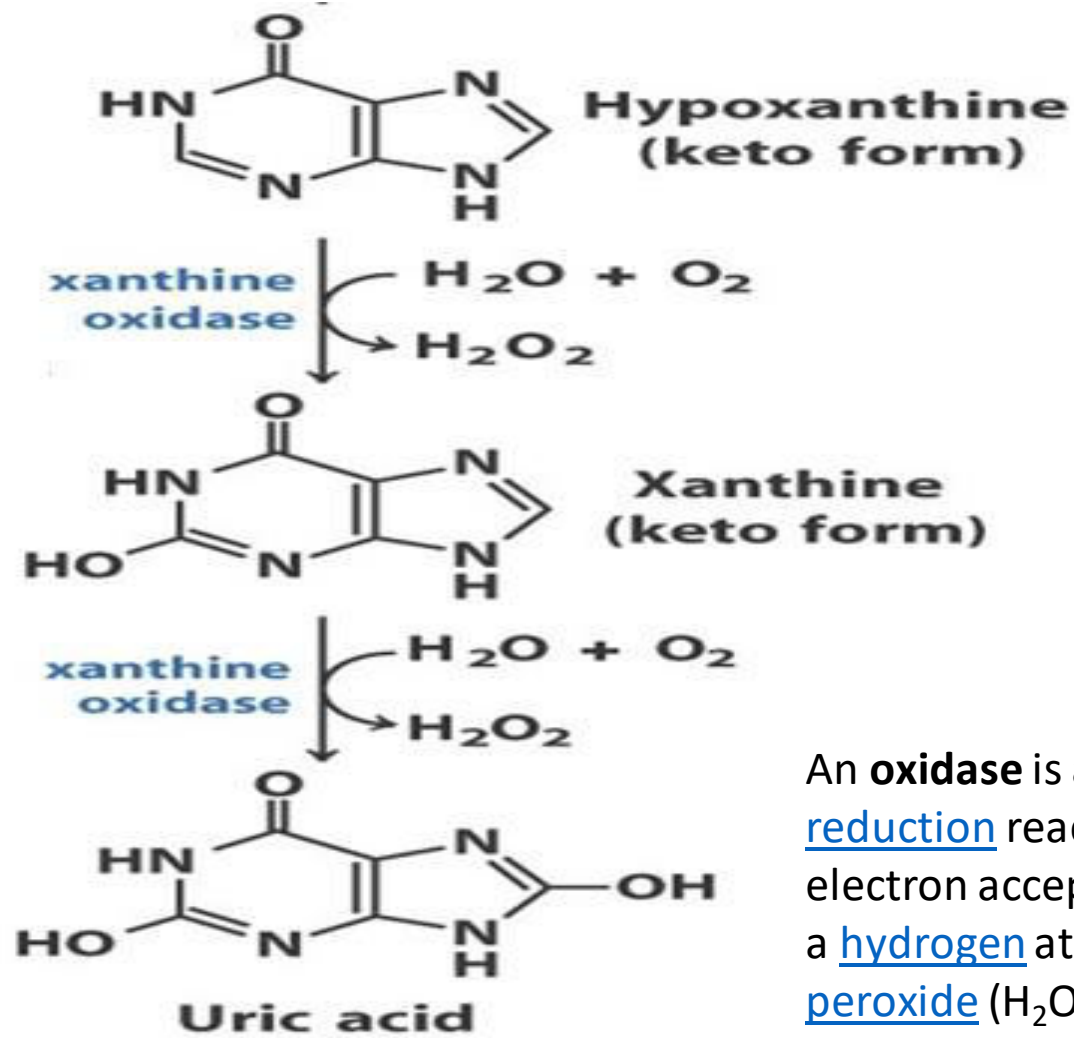
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3. Hydrolases	Hydrolysis of a substrate.	Estrases Digestive enzymes

CLASSIFICATION OF ENZYMES

Group of Enzyme	Reaction Catalysed	Examples
4. Isomerases	Change of the molecular form of the substrate.	Phospho hexo Isomerase, Fumarase
5. Lyases	Nonhydrolytic removal of a group or addition of a group to a substrate.	Decarboxylases Aldolases
6. Ligases (Synthetases)	Joining of two molecules by the formation of new bonds.	Citric acid Synthetase

1) Oxidoreductases

- catalyze oxidation reduction reactions
- further divided into four subgroups;
- Oxidase,
- Dehydrogenases,
- Hydroperoxidases
- Oxygenases.



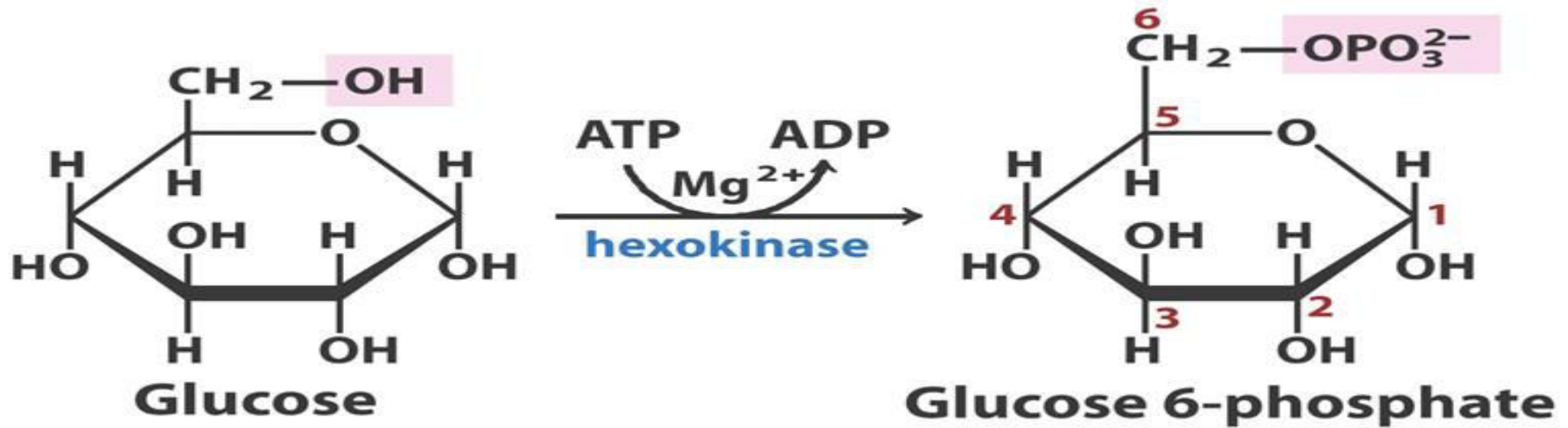
- Two reactions both catalyzed by Xanthine oxidase are given
- Hypoxanthine $\rightarrow$ xanthine
- Xanthine $\rightarrow$ Uric acid

An **oxidase** is an [enzyme](#) that [catalyzes](#) an [oxidation-reduction](#) reaction, especially one involving [dioxygen](#) (O_2) as the electron acceptor. In reactions involving donation of a [hydrogen](#) atom, oxygen is reduced to [water](#) (H_2O) or [hydrogen peroxide](#) (H_2O_2). Some oxidation reactions, such as those involving [monoamine oxidase](#) or [xanthine oxidase](#), typically do not involve free molecular oxygen.

2) Transferases

- These bring about a transfer of functional groups such as
- phosphate and
- amino group
- from one molecule to another molecule called donor and acceptor molecules respectively.

- The common examples of this group are
- Transminases
- Phosphotransfrases (Kinases)



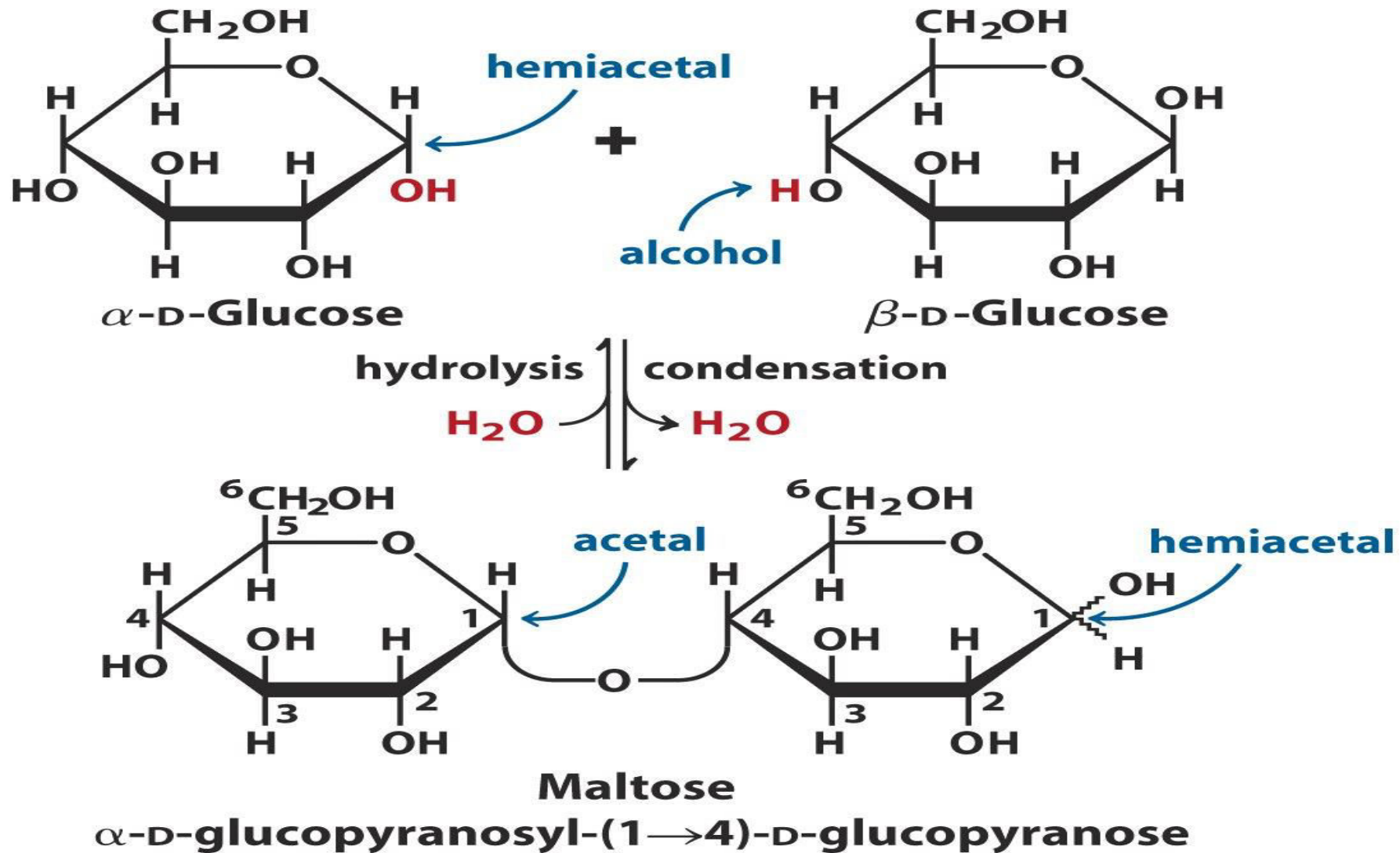
- Hexokinase is a phosphotransferase which catalyzes the transfer of phosphate groups.
- $\text{Glucose} + \text{ATP} \rightarrow \text{Glucose 6-phosphate} + \text{ADP}$.

IUB Classification of Enzymes (Contd.)

3) Hydrolases

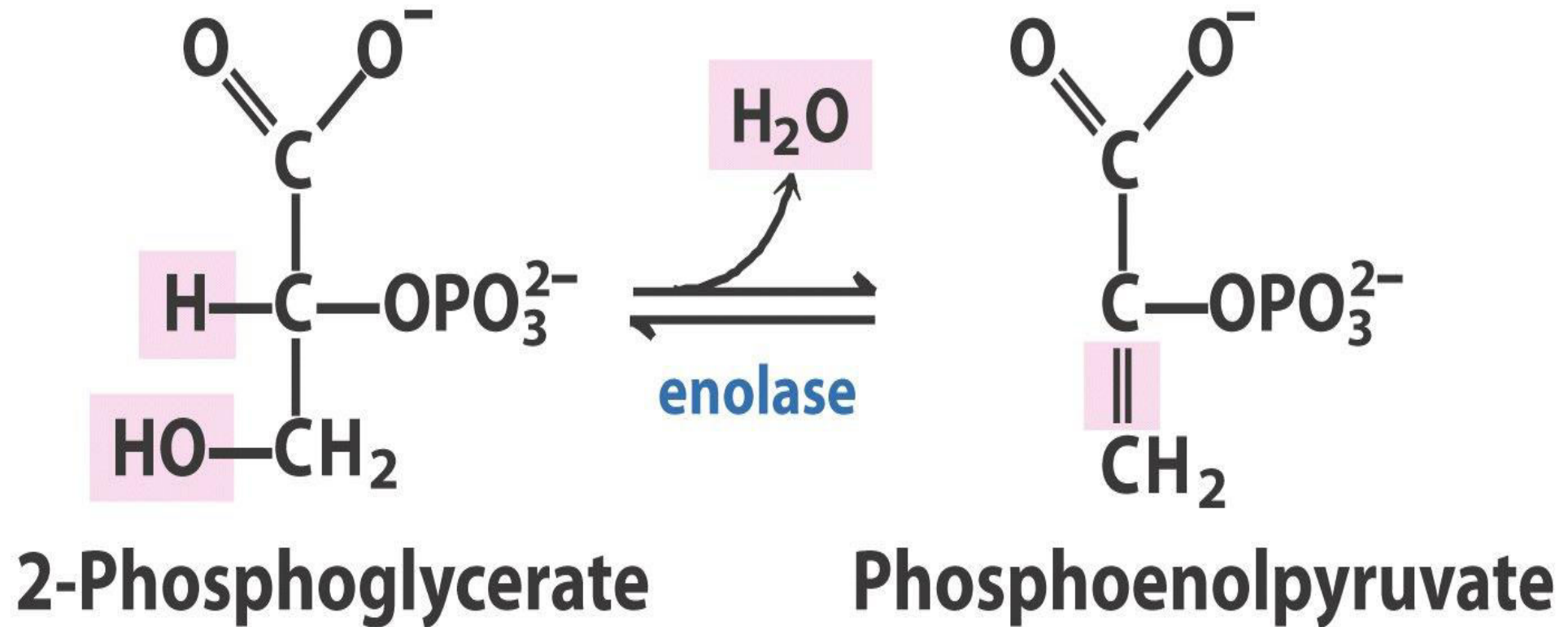
- These enzymes catalyze **hydrolysis**, i.e.
- add water molecule to the substrate which is simultaneously decomposed; the functional group of substrate is transferred to water.

- Common example of hydrolases are:
- Protein hydrolyzing Enzymes (peptidases).
- Carbohydrases
- Lipid hydrolyzing enzymes e.g. Lipases and
- Phospholipases.

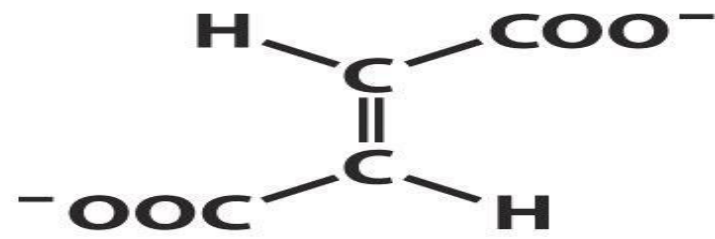


4) Lyases

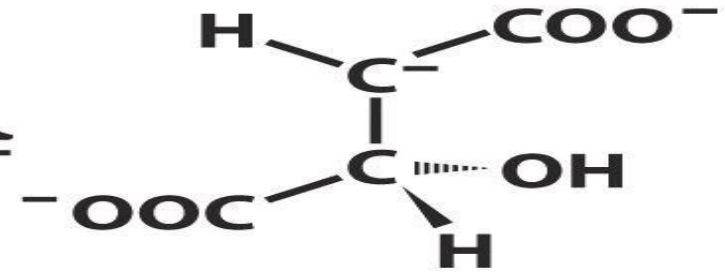
- These enzymes catalyze the addition of
- NH_3 ,
- H_2O or
- CO_2 to double bonds or
- the removal of these groups leaving behind double bonds.



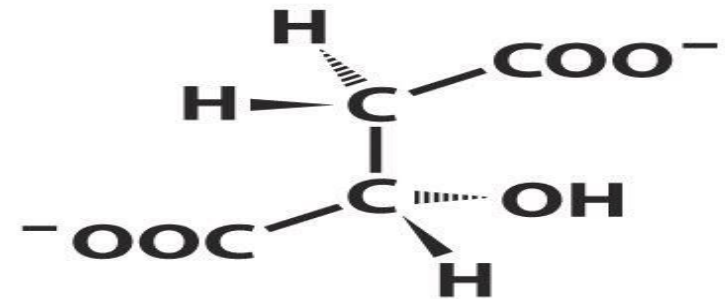
$$\Delta G'^{\circ} = 7.5 \text{ kJ/mol}$$



Fumarate



**Carbanion
transition state**



Malate

$$\Delta G'^{\circ} = -3.8 \text{ kJ/mol}$$

- Lyases are included in a separate class because they catalyze these reactions by means other than hydrolysis or oxidation.

END

Enzymes

IUB Classification of Enzymes (Contd.)

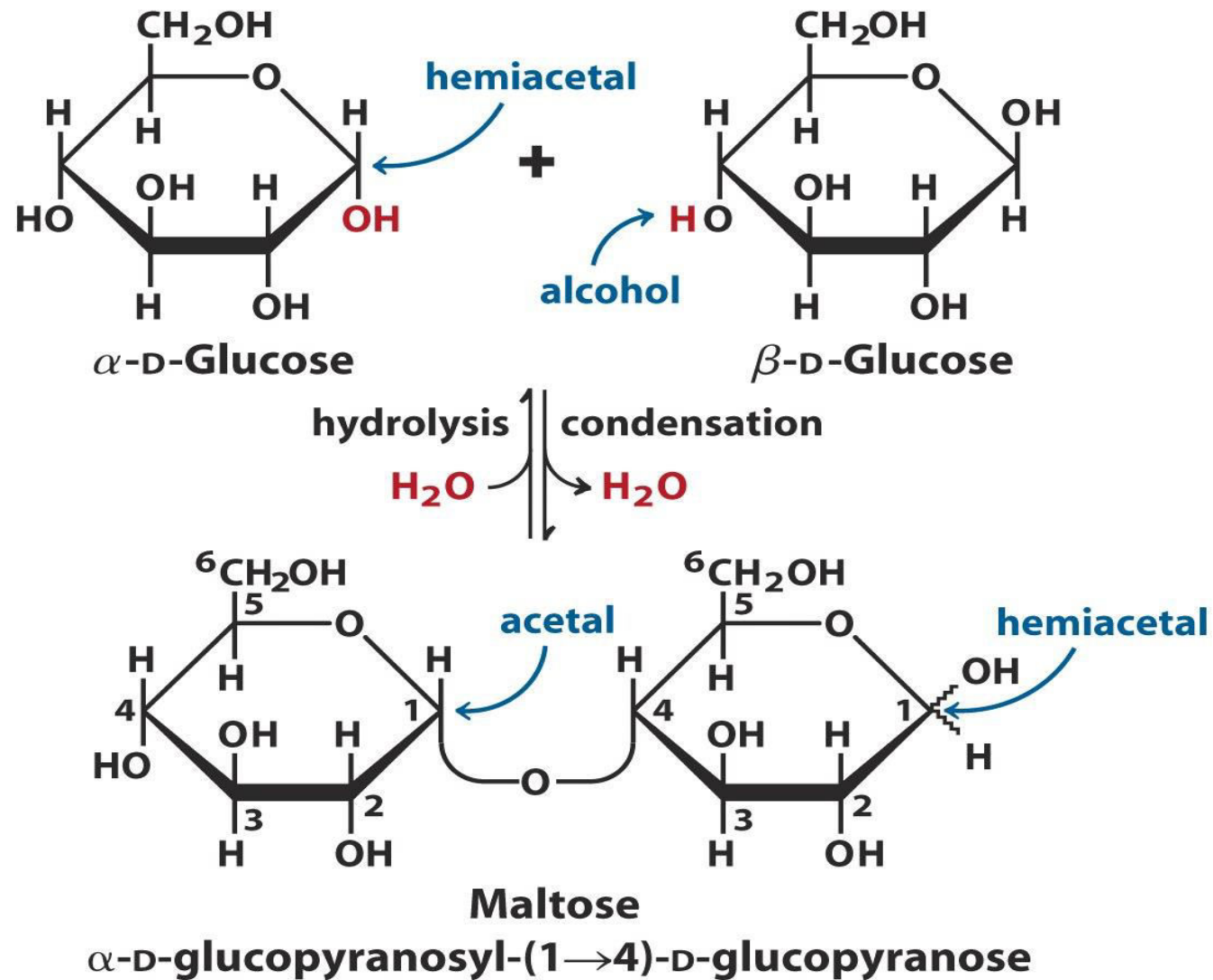
3) Hydrolases

- These enzymes catalyze **hydrolysis**, i.e.
- add water molecule to the substrate which is simultaneously decomposed; the functional group of substrate is transferred to water.

Enzymes

- Common example of hydrolases are:
- Protein hydrolyzing Enzymes (**peptidases**).
- **Carbohydases**
- Lipid hydrolyzing enzymes e.g. **Lipases** and
- **Phospholipases**.

Enzymes

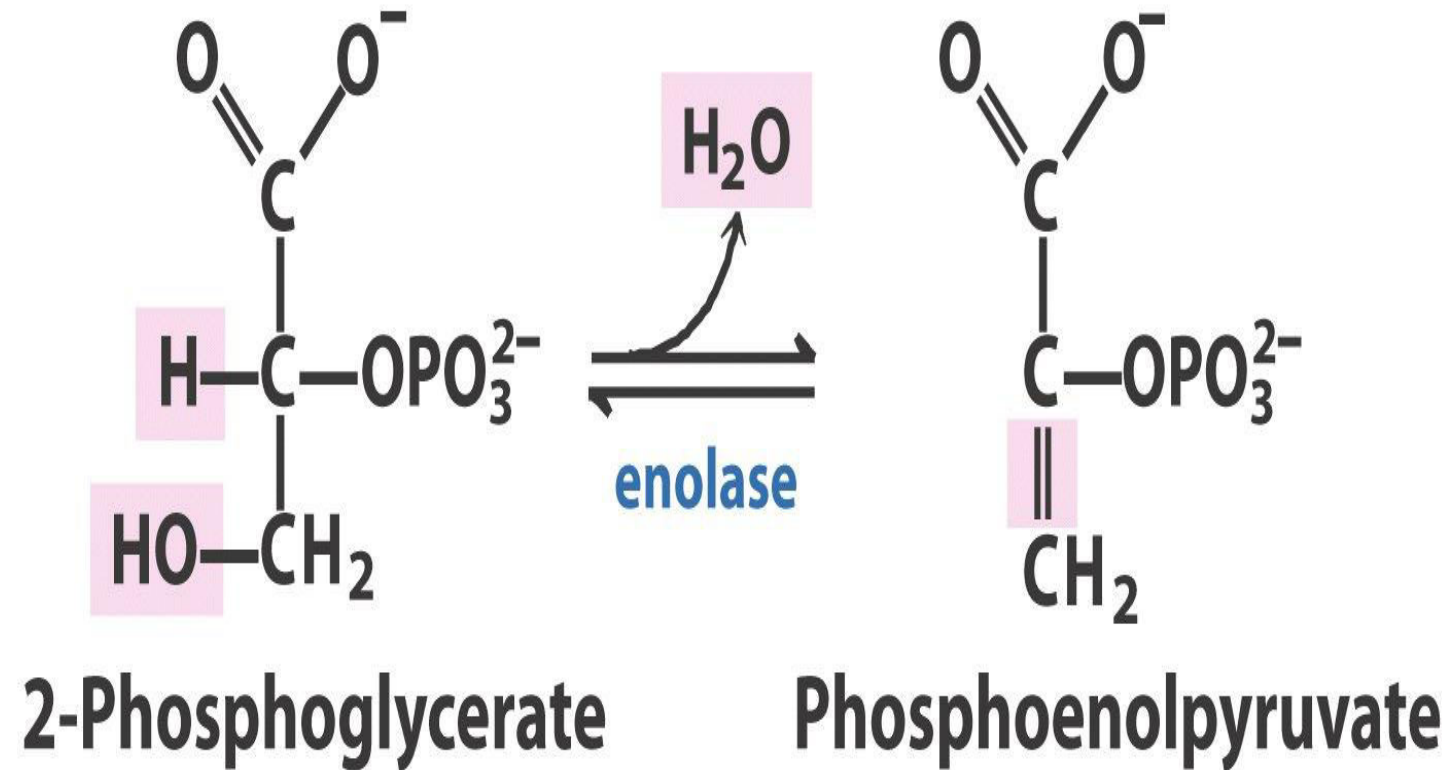


Enzymes

4) Lyases

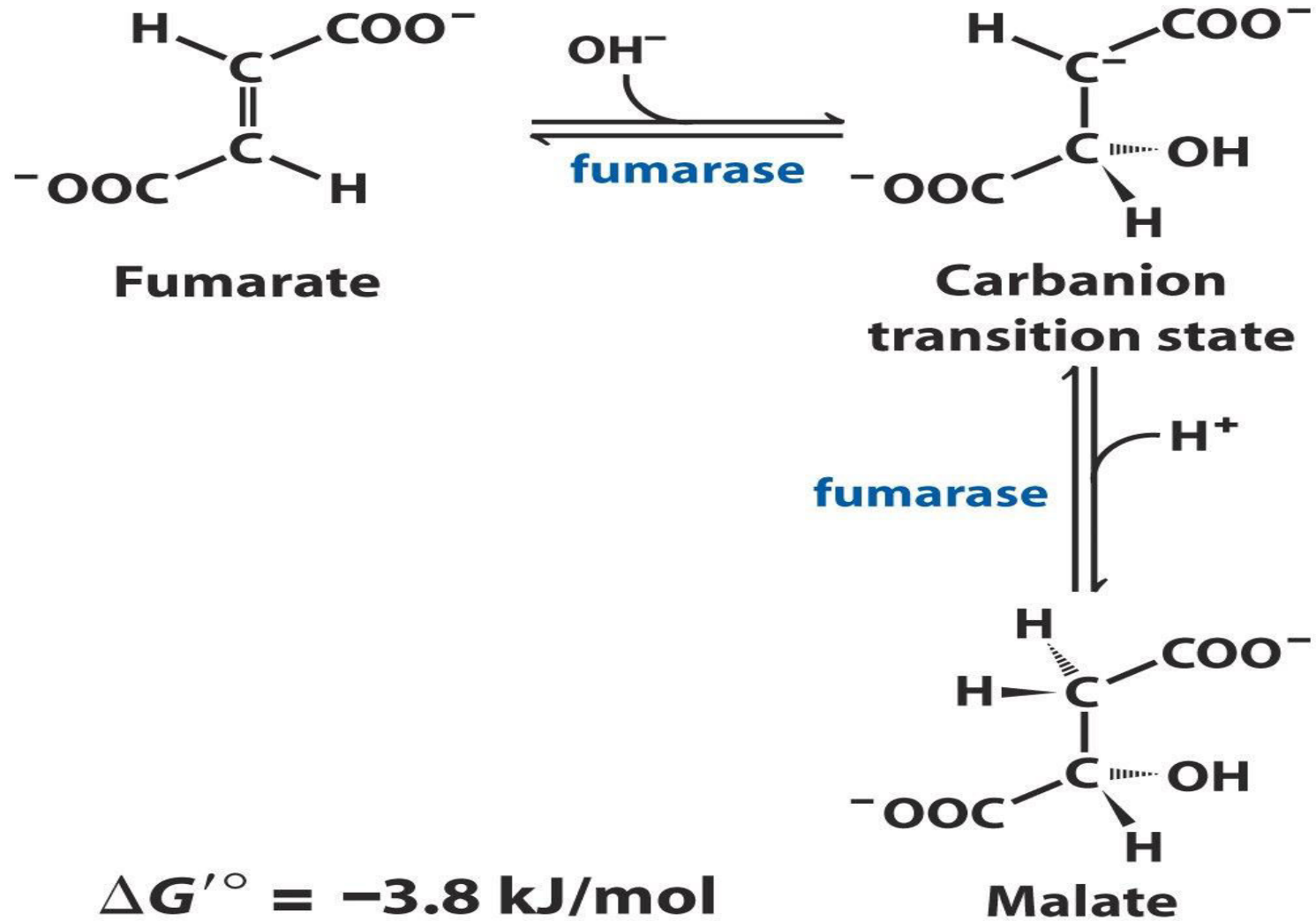
- These enzymes catalyze the **addition** of
- NH_3 ,
- H_2O or
- CO_2 to double bonds
or
- the **removal** of these groups leaving behind double bonds.

Enzymes



$$\Delta G'^{\circ} = 7.5 \text{ kJ/mol}$$

Enzymes



Enzymes

- Lyases are included in a separate class because they catalyze these reactions by means other than hydrolysis or oxidation.

END

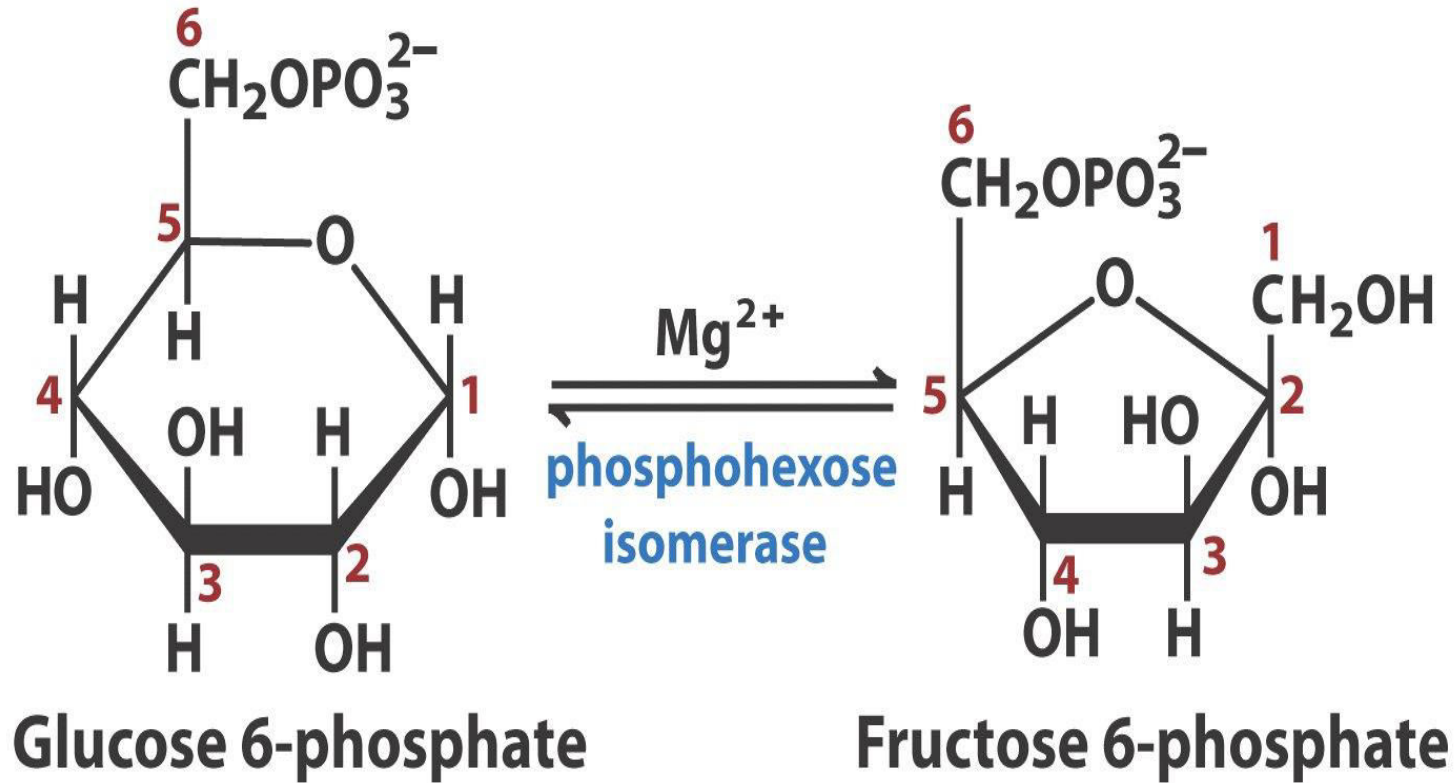
Enzymes

IUB Classification of Enzymes (Contd.)

5) Isomerases

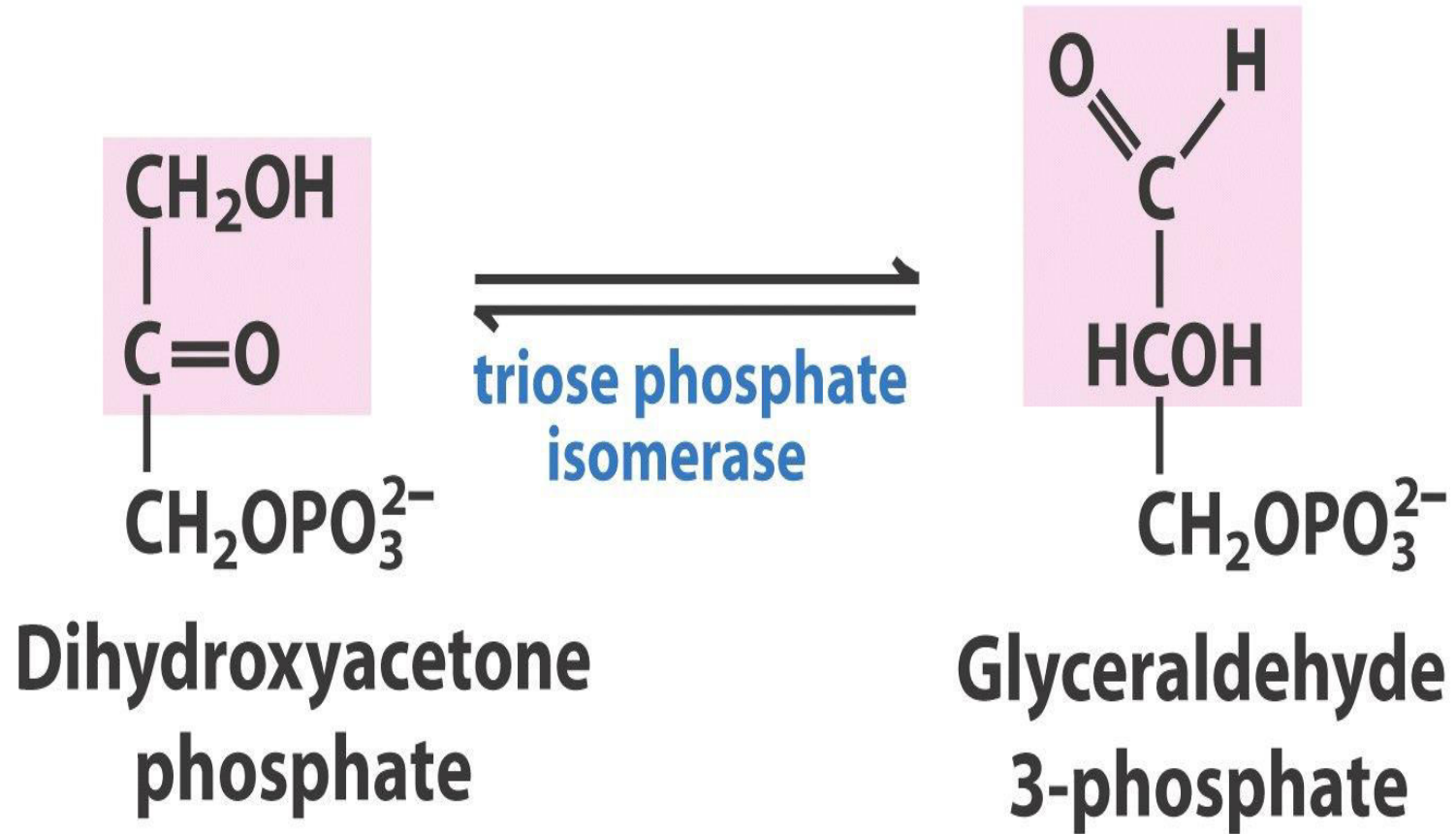
- These enzymes catalyze the **structural change** within a single molecule by the **transfer of groups within** it, resulting in the formation of an isomeric form of substrate.

Enzymes



$$\Delta G'^{\circ} = 1.7 \text{ kJ/mol}$$

Enzymes



$$\Delta G'^{\circ} = 7.5 \text{ kJ/mol}$$

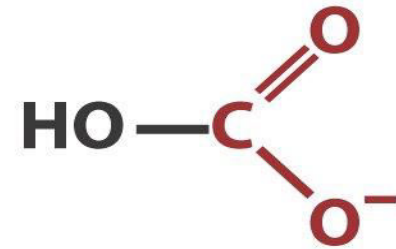
Enzymes

6) Ligases

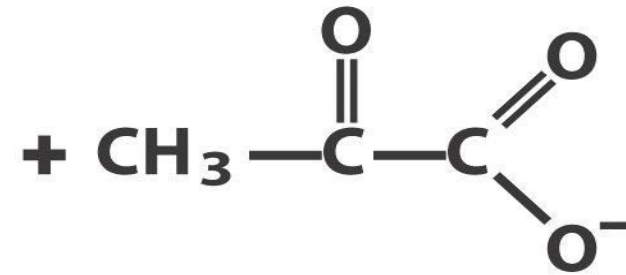
- These enzymes catalyze **condensation reactions** joining two molecules by forming
- C-O,
- C-S,
- C-N and
- C-C bonds.

Enzymes

Bicarbonate



Pyruvate

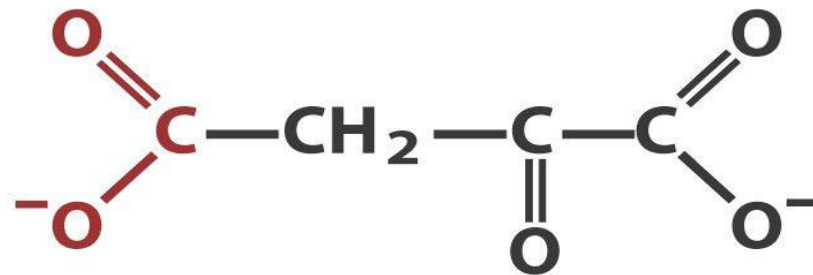


**pyruvate
carboxylase**

ATP

biotin

ADP + P_i



Oxaloacetate

Enzymes

- The energy for condensation is provided by cleavage of high energy phosphates, e.g. ATP, GTP etc.

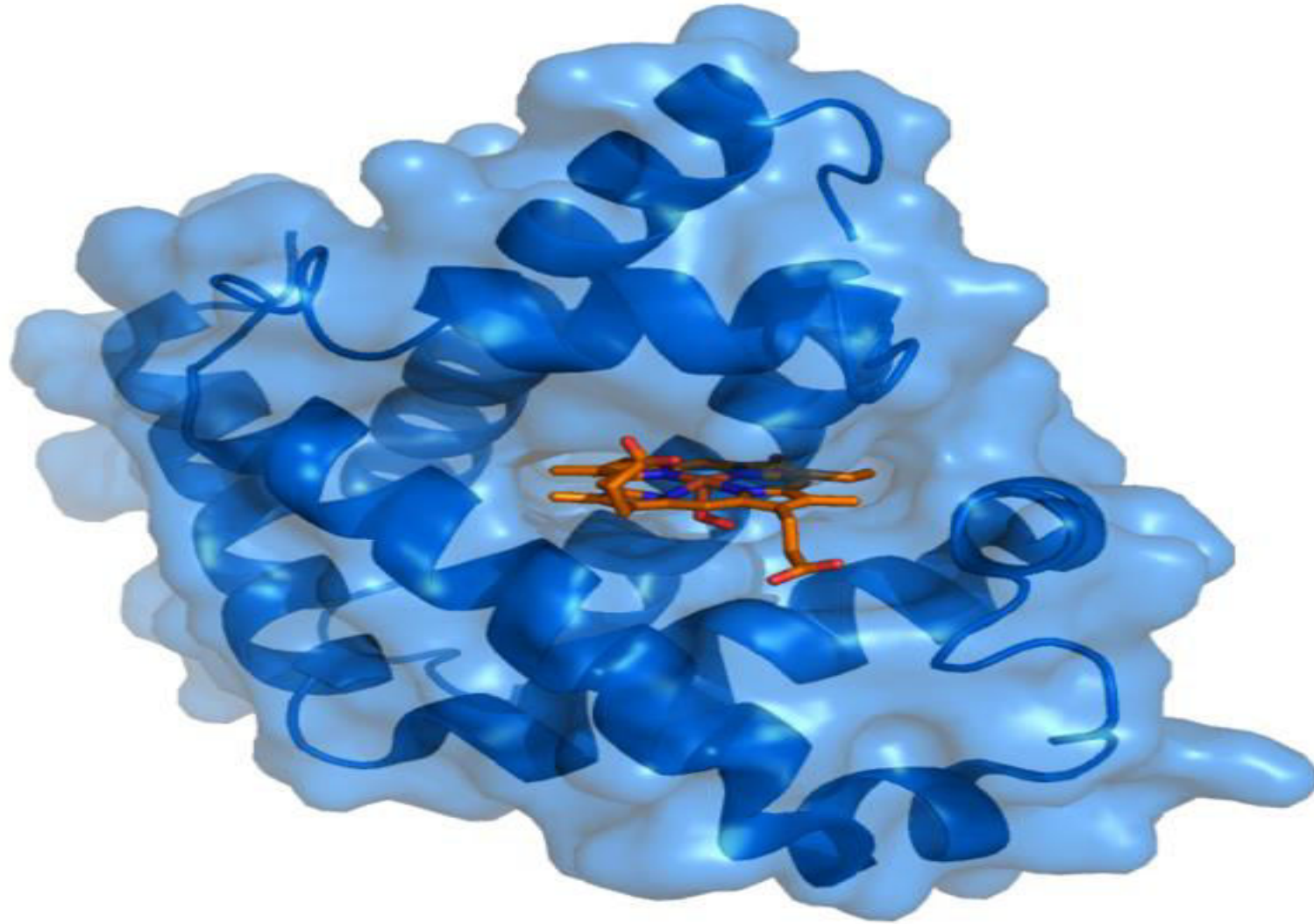
END

Enzymes

Ligand

- In biochemistry and pharmacology, a **ligand** (from the Latin *ligandum, binding*) is a substance (usually a small molecule),
- that forms a complex with a biomolecule to serve a biological purpose.

Enzymes



Enzymes

- In a narrower sense, it is a **signal triggering molecule**, binding to a site on a target protein
- A molecule bound reversibly by a protein is called a **ligand**.
- Ligands include substrates, inhibitors, activators, and neurotransmitters

Enzymes

- A ligand may be any kind of molecule, including another protein.
- A ligand binds at a site on the protein called the **binding site**,

Enzymes

- **binding site** is complementary to the ligand in
- size,
- shape,
- charge, and
- hydrophobic or
- hydrophilic character.

Enzymes

- Furthermore, the interaction is **specific**: the protein can **discriminate** among the thousands of different molecules in its environment and **selectively bind** only one or a few.

Enzymes

- The binding of a protein and ligand is often coupled to a **conformational change** in the protein that makes the binding site more complementary to the ligand, permitting **tighter binding** called **induced fit**.

Enzymes

- A given protein may have **separate binding sites** for several different ligands.
- In a multi-subunit protein, a conformational change in one subunit often affects the conformation of other subunits.

Enzymes

- Interactions between **ligands** and proteins may be regulated, through interactions with additional ligands.
- These other ligands may cause **conformational changes** in the protein that **affect** the binding of the **first ligand**.

END

Midterm Syllabus Finished

Lecture 1 to 63

Effort By

Amaan Khan