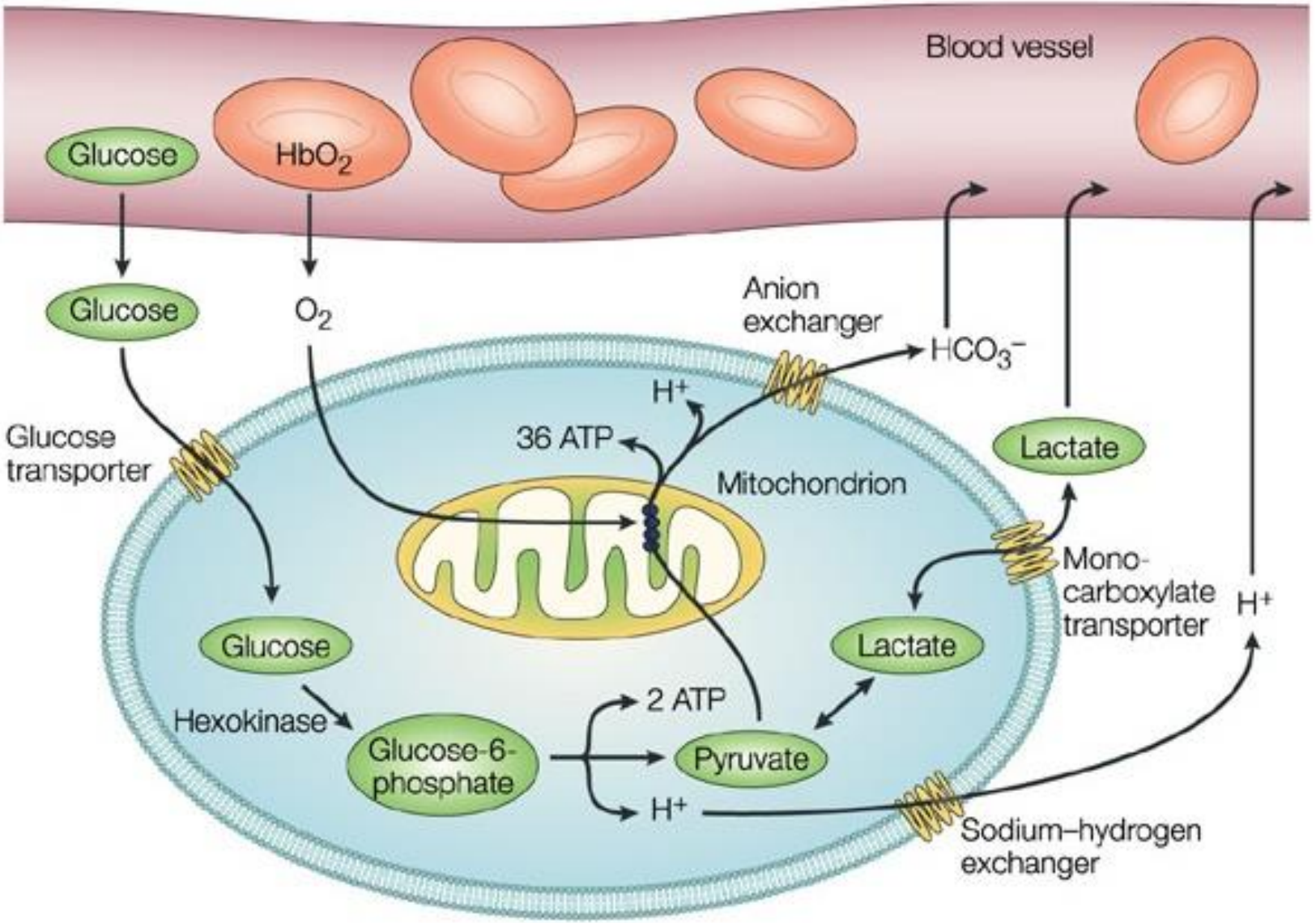
CARBOHYDRATE METABOLISM



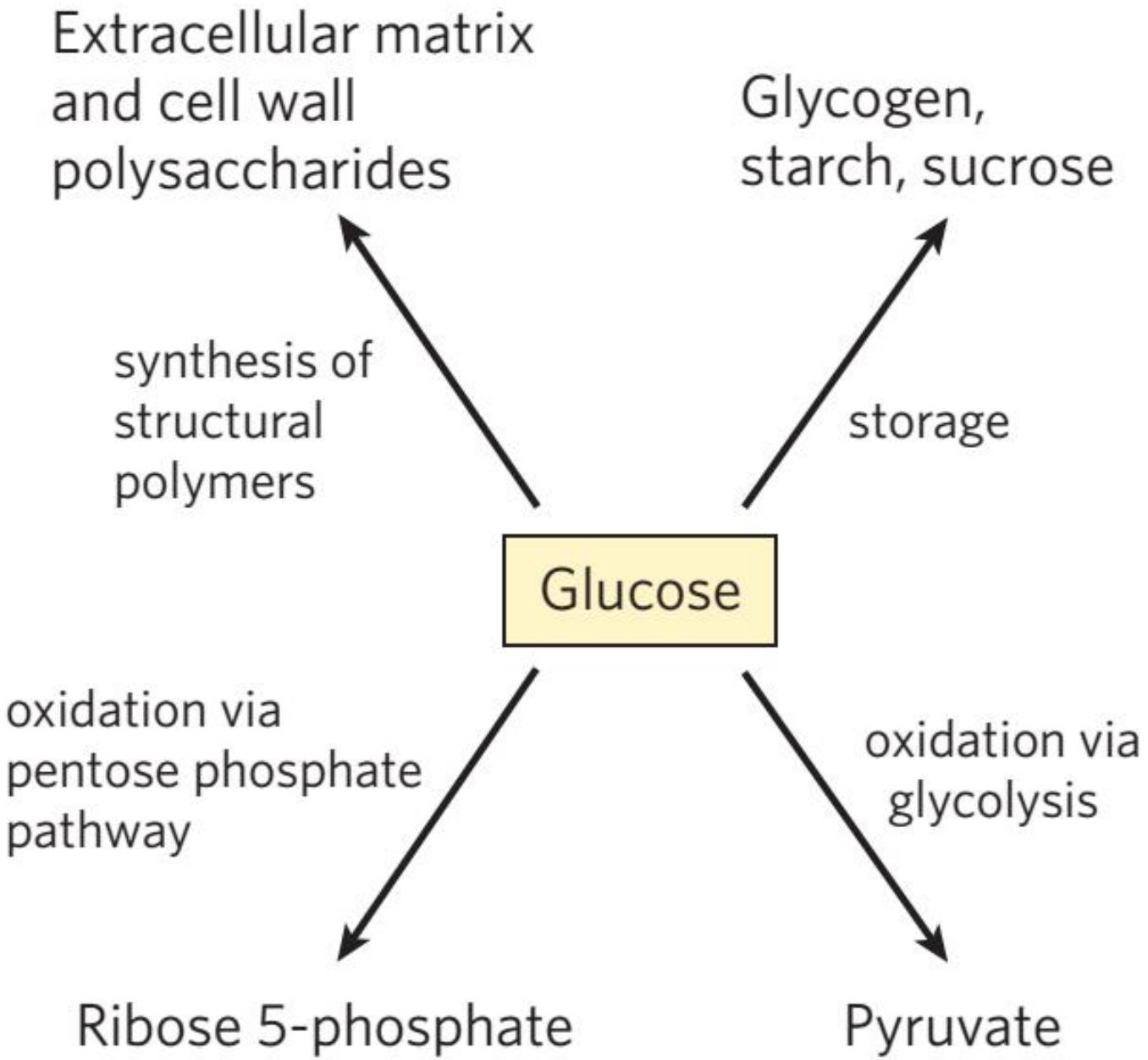
Glucose as Fuel

❖Glucose occupies a central position in the metabolism of plants, animals, and many microorganisms.

❖It is relatively rich in potential energy, and thus a good fuel; the complete oxidation of glucose to carbon dioxide and water proceeds with a standard free-energy change of 22,840 kJ/mol.

❖By storing glucose as a high molecular weight polymer such as starch or glycogen, a cell can stockpile large quantities of hexose units while maintaining a relatively low cytosolic osmolarity.

❖When energy demands increase, glucose can be released from these intracellular storage polymers and used to produce ATP either aerobically or anaerobically.


Extracellular matrix and cell wall polysaccharides
Glycogen, starch, sucrose
synthesis of structural polymers
storage
Glucose
oxidation via pentose phosphate pathway
oxidation via glycolysis
Ribose 5-phosphate
Pyruvate

❖Glucose is not only an excellent fuel, it is also a remarkably versatile precursor, capable of supplying a huge array of metabolic intermediates for biosynthetic reactions.

❖A comprehensive study of the metabolic fates of glucose would encompass hundreds or thousands of transformations.

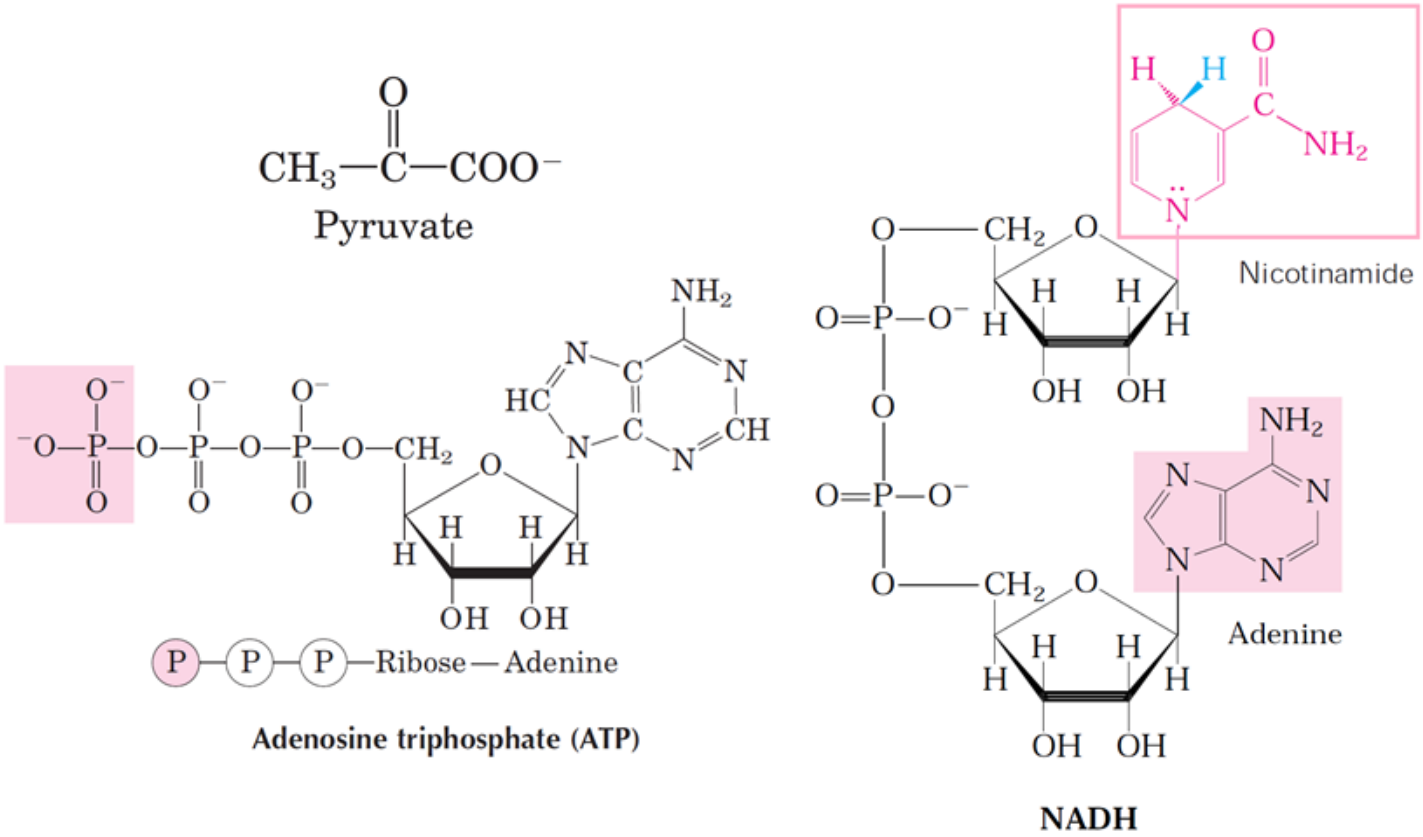
❖In animals and vascular plants, glucose has four major fates:

- it may be used in the **synthesis of complex polysaccharides** destined for the extracellular space;
- **stored in cells** (as a polysaccharide or as sucrose);
- oxidized to a three-carbon compound (pyruvate) via **glycolysis** to provide ATP and metabolic intermediates;
- or oxidized via the **pentose phosphate** (phosphogluconate) **pathway** to yield ribose 5-phosphate for nucleic acid synthesis and NADPH for reductive biosynthetic processes

Glycolysis

❖In glycolysis, a molecule of **glucose** is degraded in a series of enzyme-catalyzed reactions to yield two molecules of the three-carbon compound **pyruvate**.

❖During the sequential reactions of glycolysis, some of the free energy released from glucose is conserved in the form of ATP and NADH.



❖Glycolysis is an almost universal central pathway of glucose catabolism, the pathway with the largest flux of carbon in most cells.

❖The glycolytic breakdown of glucose is the sole source of metabolic energy in some mammalian tissues and cell types (erythrocytes, renal medulla, brain, and sperm, for example).

❖Some plant tissues that are modified to store starch and some aquatic plants derive most of their energy from glycolysis; many anaerobic microorganisms are entirely dependent on glycolysis.

❖**Fermentation** is a general term for the anaerobic degradation of glucose or other organic nutrients to obtain energy, conserved as ATP.

❖Because living organisms first arose in an atmosphere without oxygen, anaerobic breakdown of glucose is probably the most ancient biological mechanism for obtaining energy from organic fuel molecules.

❖In the course of evolution, the chemistry of this reaction sequence has been completely conserved.

❖The glycolytic enzymes of vertebrates are closely similar, in amino acid sequence and three-dimensional structure, to their homologs in yeast and spinach.

❖Glycolysis differs among species only in the details of its regulation and in the subsequent metabolic fate of the pyruvate formed.

❖The thermodynamic principles and the types of regulatory mechanisms that govern glycolysis are common to all pathways of cell metabolism.

Glycolysis Has Two Phases

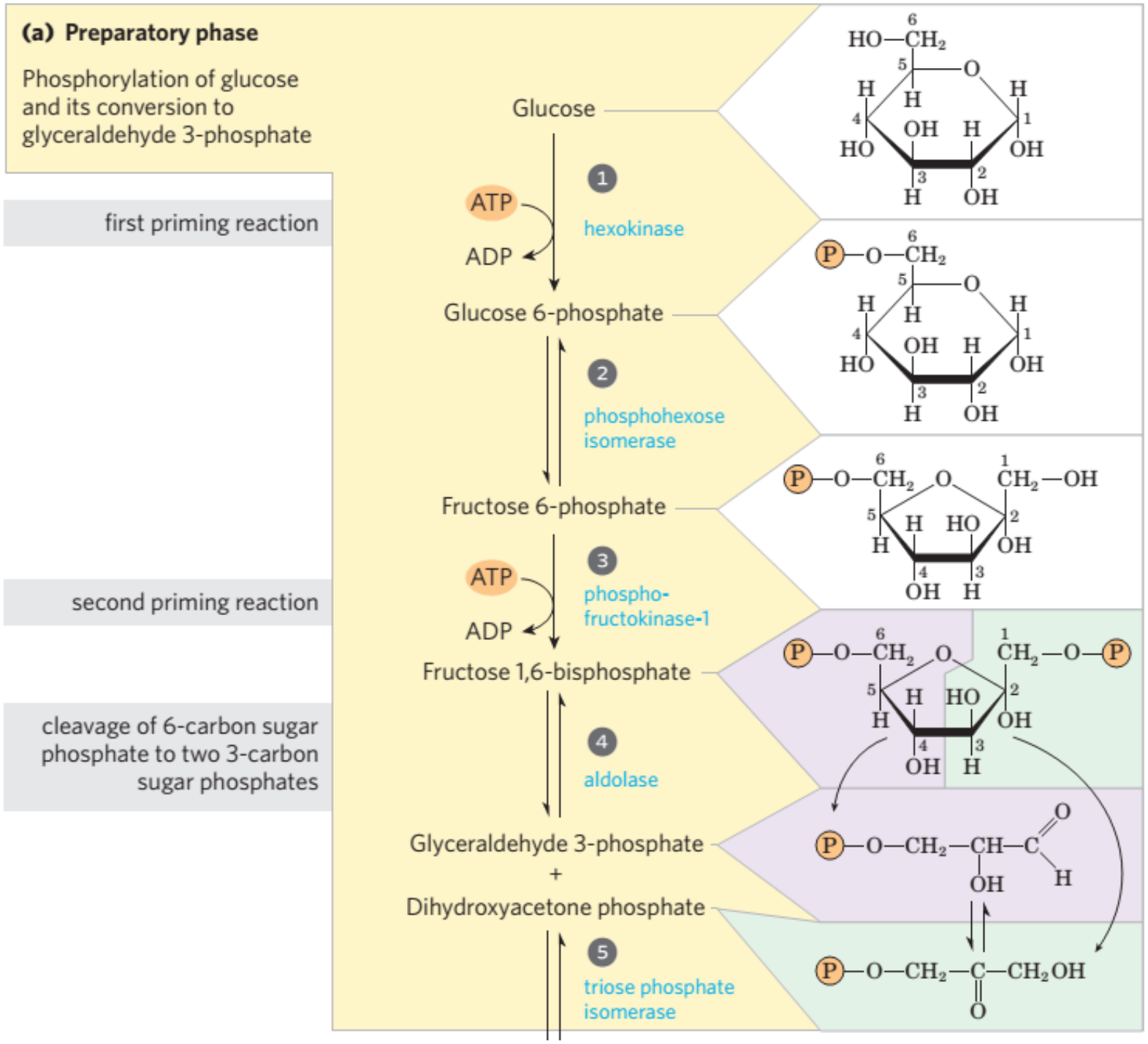
❖The breakdown of the six-carbon glucose into two molecules of the three-carbon pyruvate occurs in 10 steps, the first 5 of which constitute the **preparatory phase**.

❖The energy gain comes in the **payoff phase** of glycolysis, also consist of 5 steps.

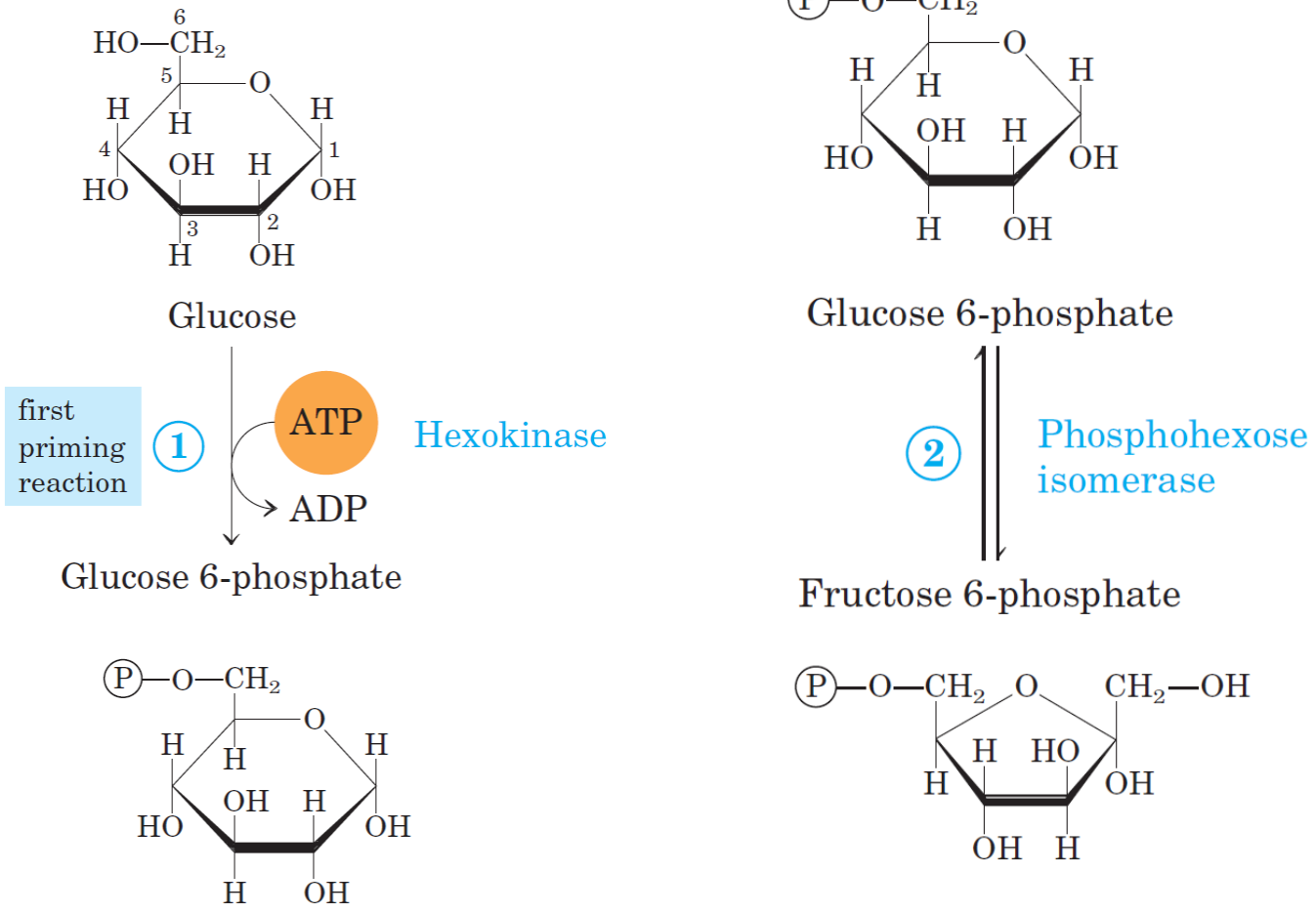
$$\text{Glucose} + 2\text{NAD}^+ + 2\text{ADP} + 2\text{P}_\text{i} \longrightarrow 2 \text{ pyruvate} + 2\text{NADH} + 2\text{H}^+ + 2\text{ATP} + 2\text{H}_2\text{O}$$

❖In the sequential reactions of glycolysis, three types of chemical transformations are particularly 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.

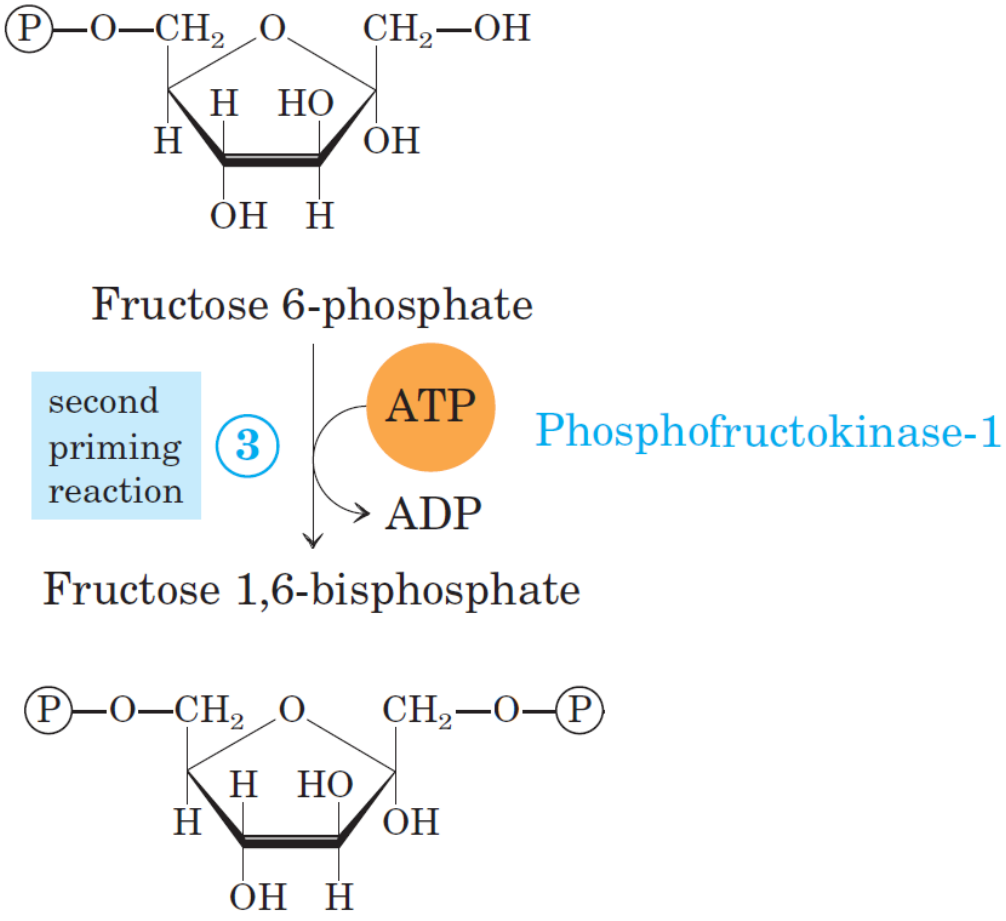

(a) Preparatory phase
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate
first priming reaction
second priming reaction
cleavage of 6-carbon sugar phosphate to two 3-carbon sugar phosphates
Glucose
ATP
ADP
1
hexokinase
Glucose 6-phosphate
2
phosphohexose isomerase
Fructose 6-phosphate
ATP
ADP
3
phospho-fructokinase-1
Fructose 1,6-bisphosphate
4
aldolase
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
triose phosphate isomerase

❖Glucose is first phosphorylated at the hydroxyl group on C-6 (step 1).

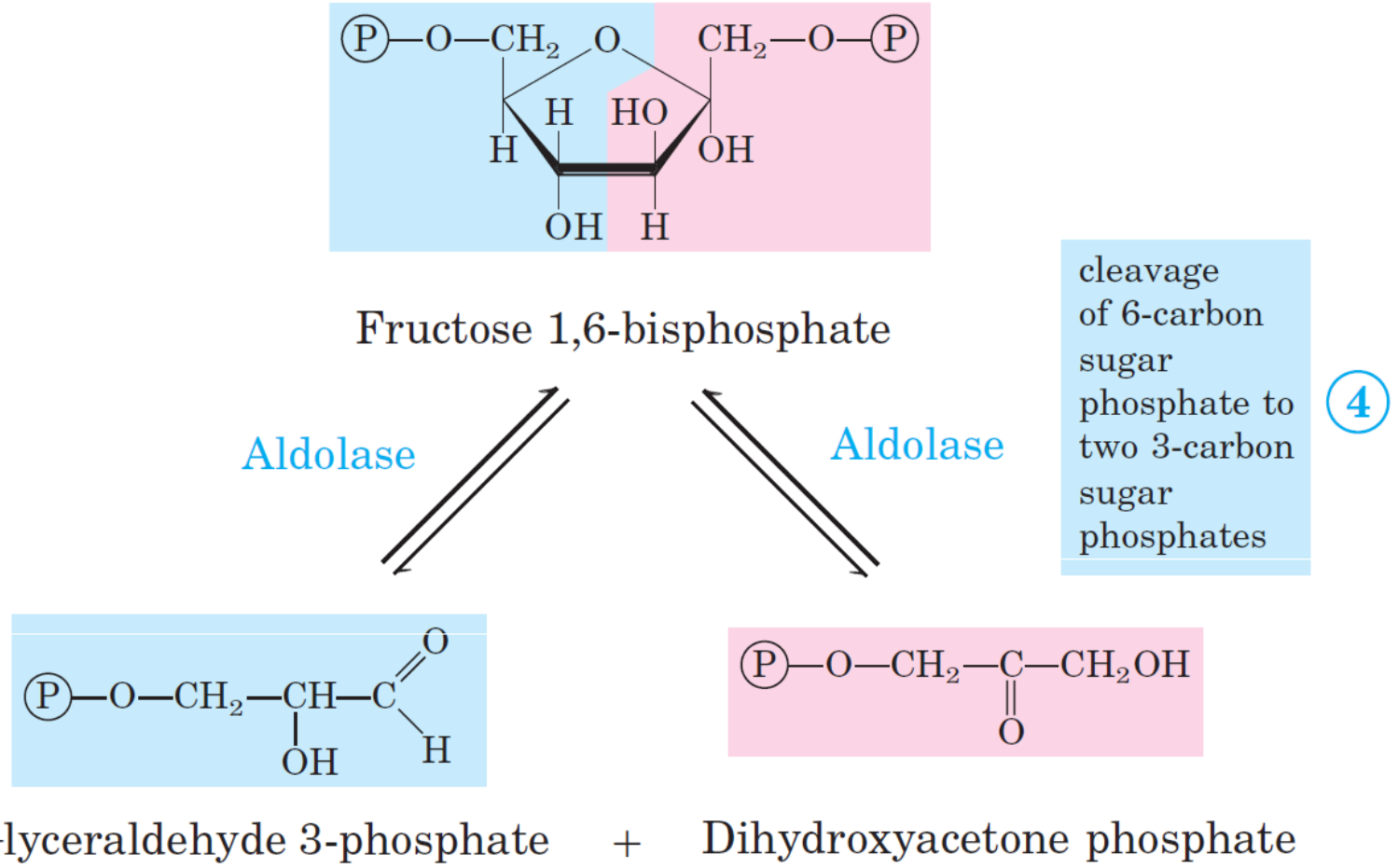


❖The Glucose 6-phosphate thus formed is converted to Fructose 6-phosphate (step 2).

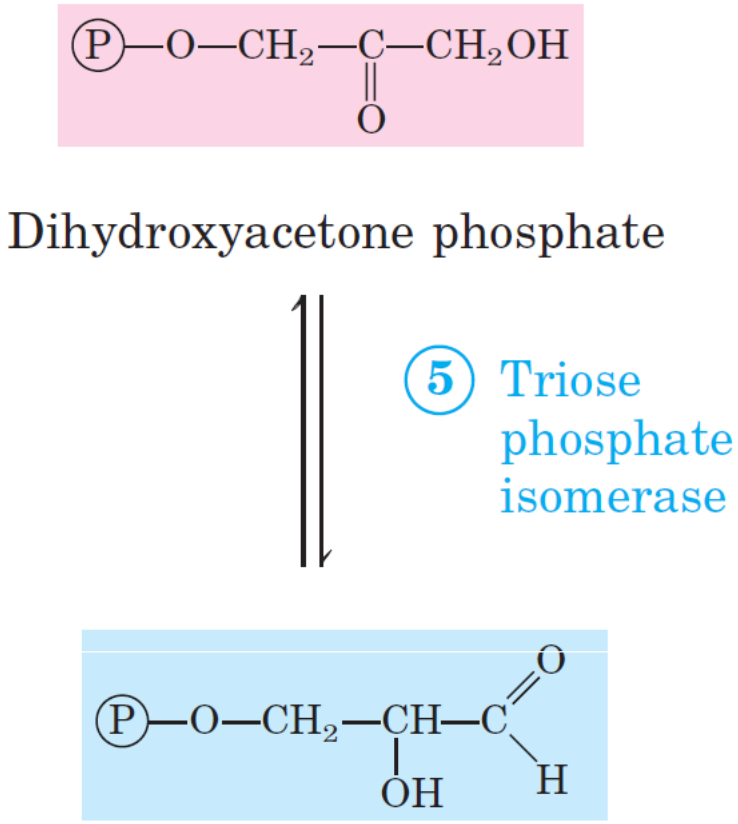
❖Fructose 6-phosphate is again phosphorylated, this time at C-1, to yield fructose 1,6-bisphosphate.



❖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.

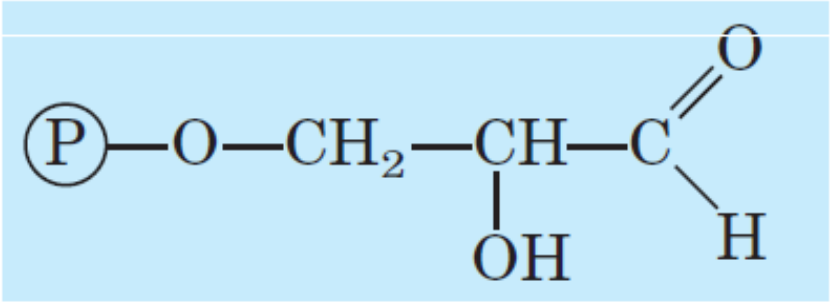


❖The dihydroxyacetone phosphate is isomerized to a second molecule of glyceraldehyde 3-phosphate (step 5), ending the first phase of glycolysis.



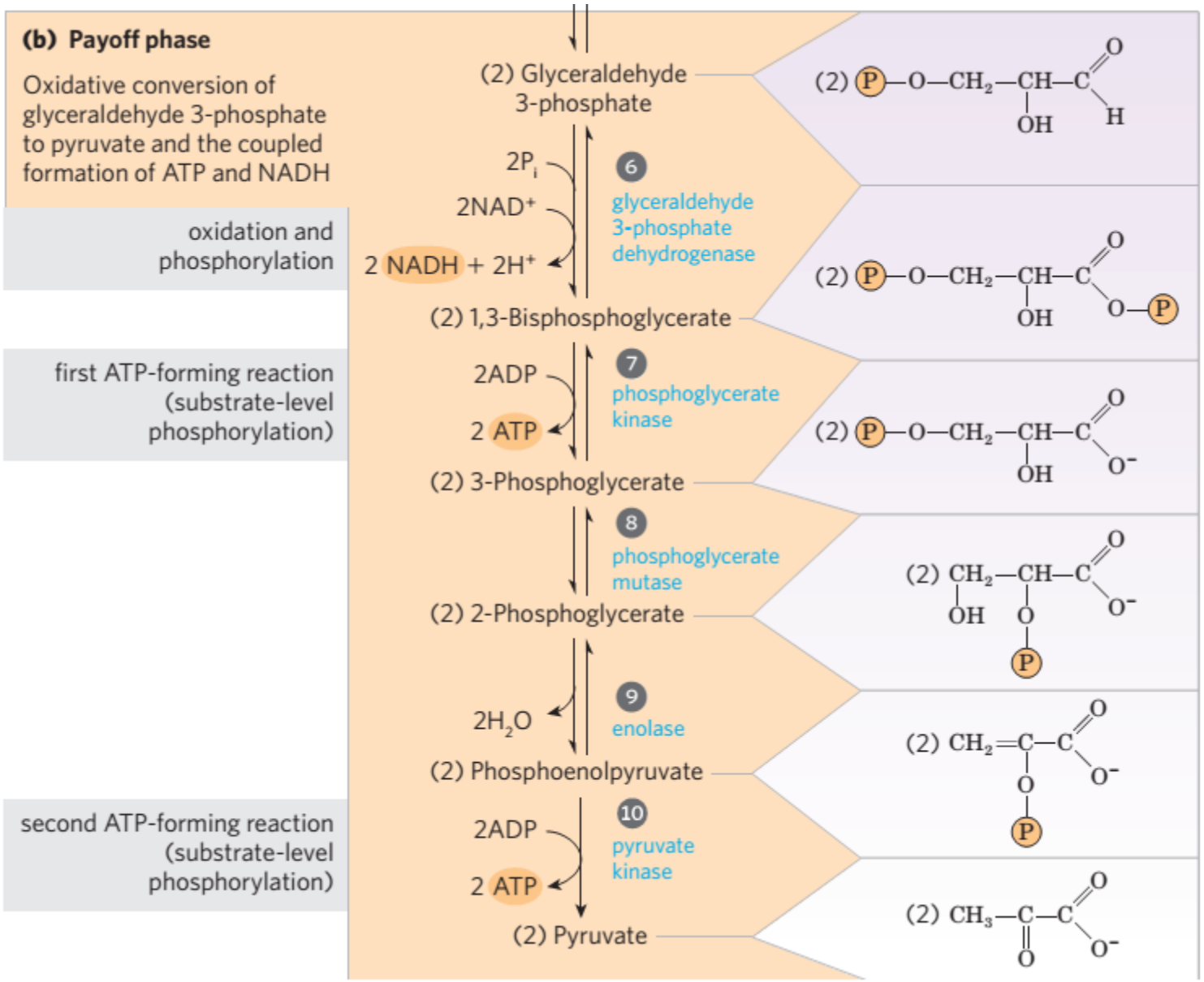
❖Note that two molecules of ATP are invested before the cleavage of glucose into two three-carbon pieces; there will be a good return on this investment.

❖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.

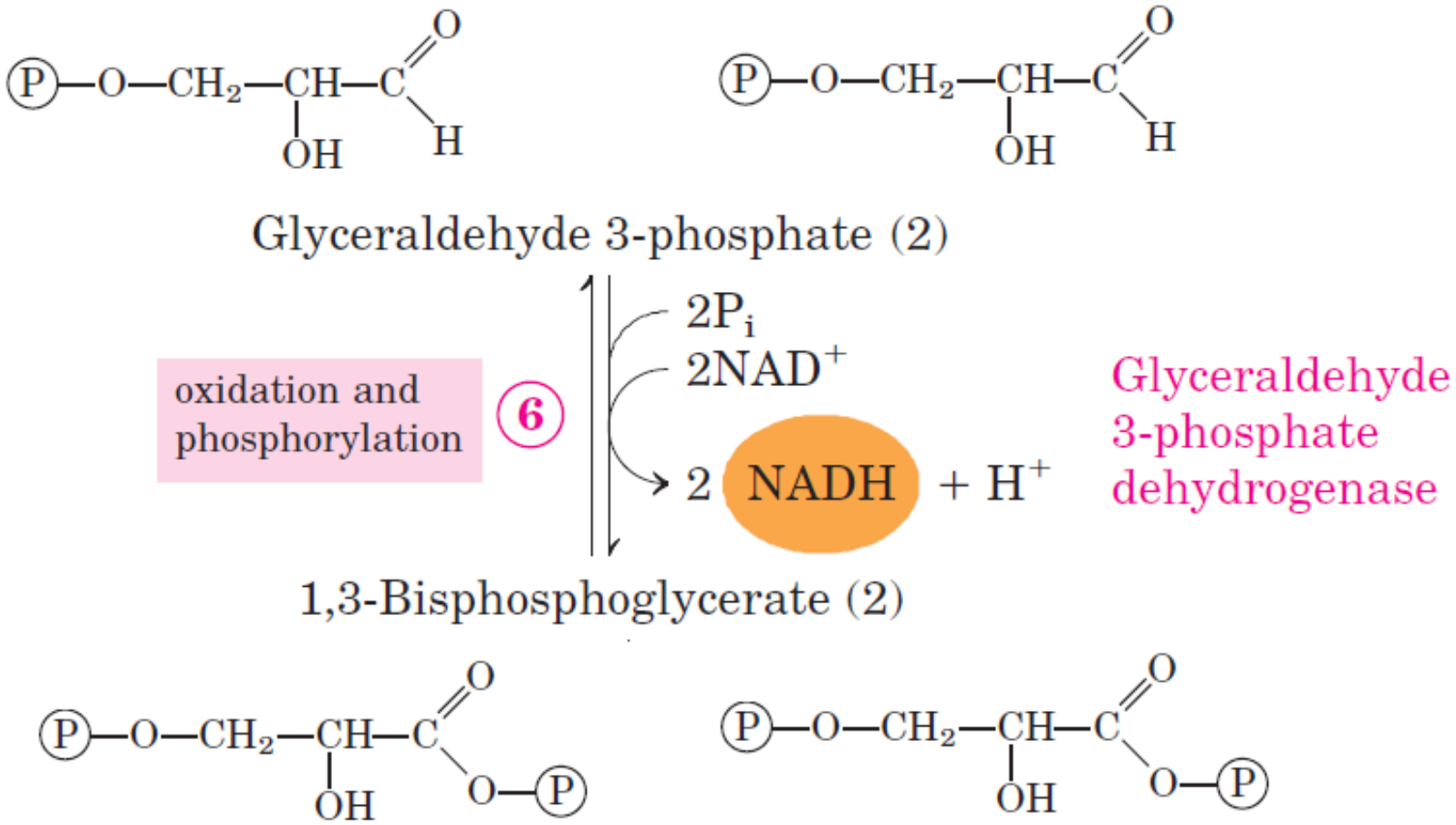


Glyceraldehyde 3-phosphate

❖The energy gain comes in the payoff phase of glycolysis.

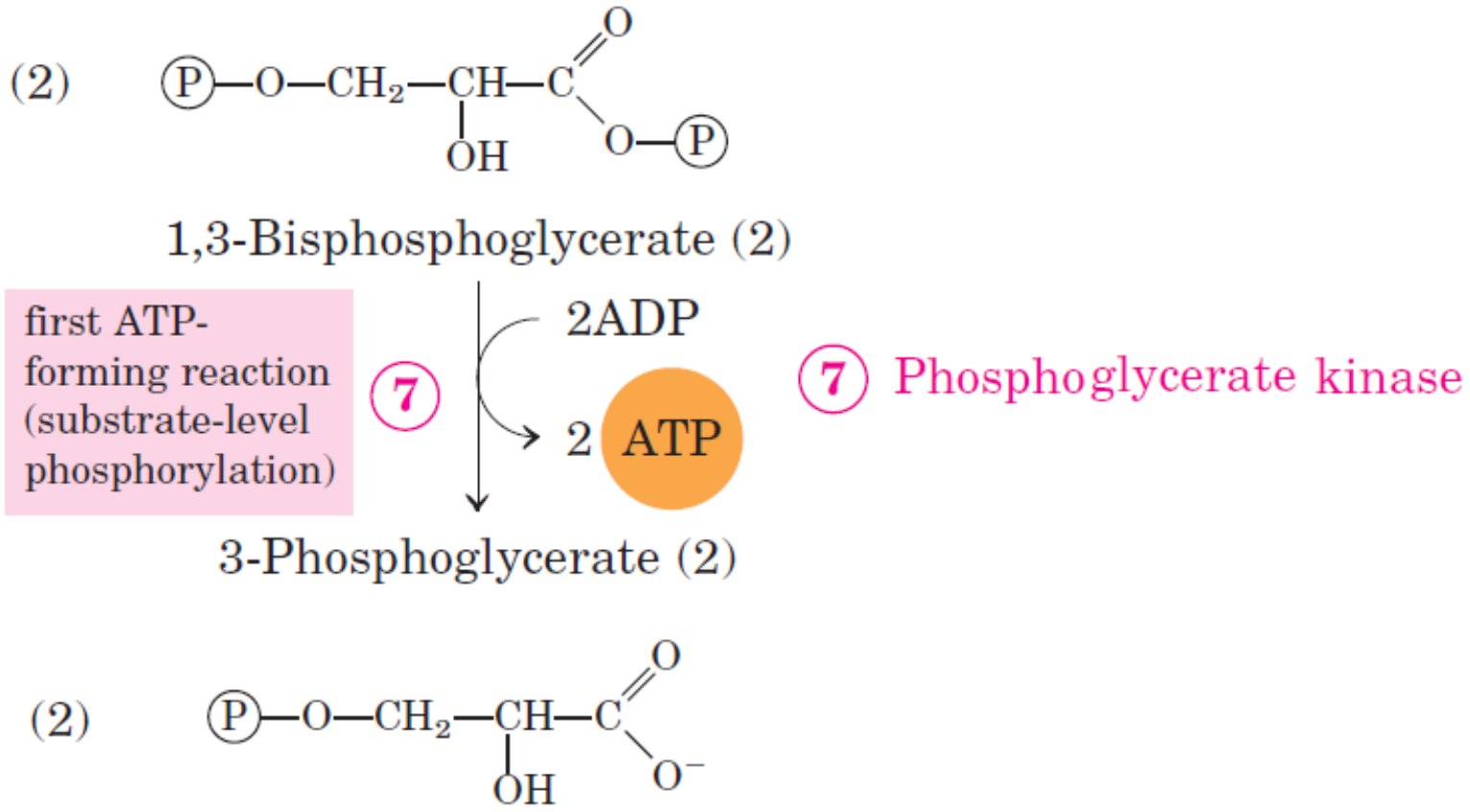


❖Each molecule of glyceraldehyde 3-phosphate is oxidized and phosphorylated by inorganic phosphate (not by ATP) to form 1,3-bisphosphoglycerate (step 6).

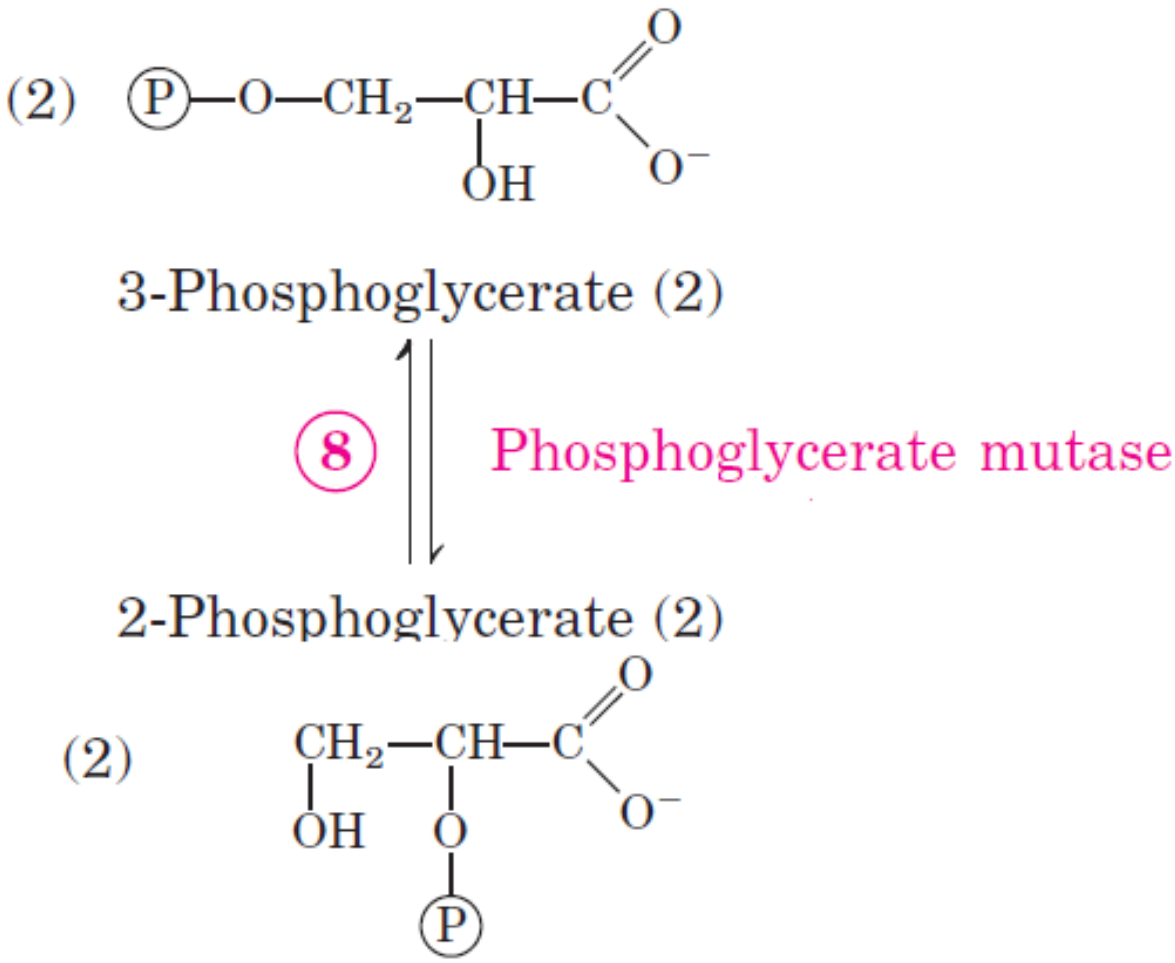


❖1,3-bisphosphoglycerate transfer one phosphoryl group to ADP, which turn into ATP (step 7).

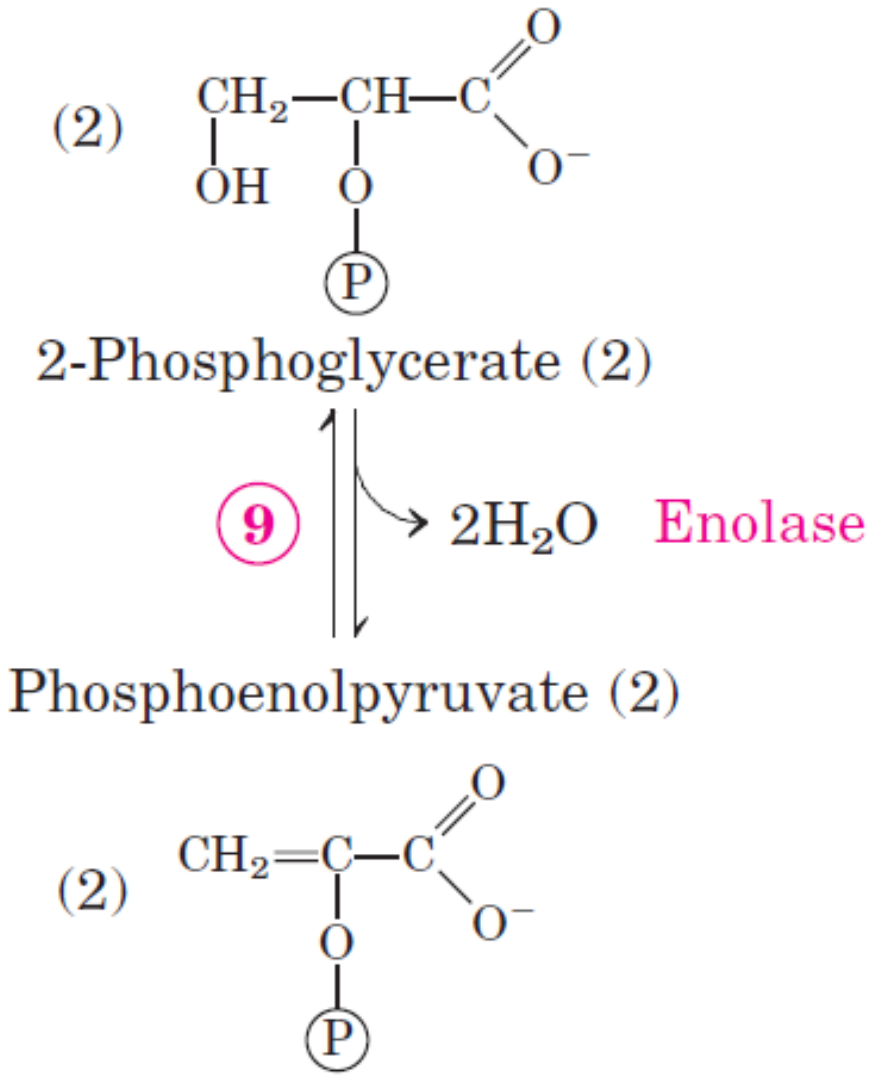
❖This step is the first ATP-forming reaction (substrate-level phosphorylation).



❖Resulting 3-phosphoglycerate turn into 2-phosphoglycerate (step 8).

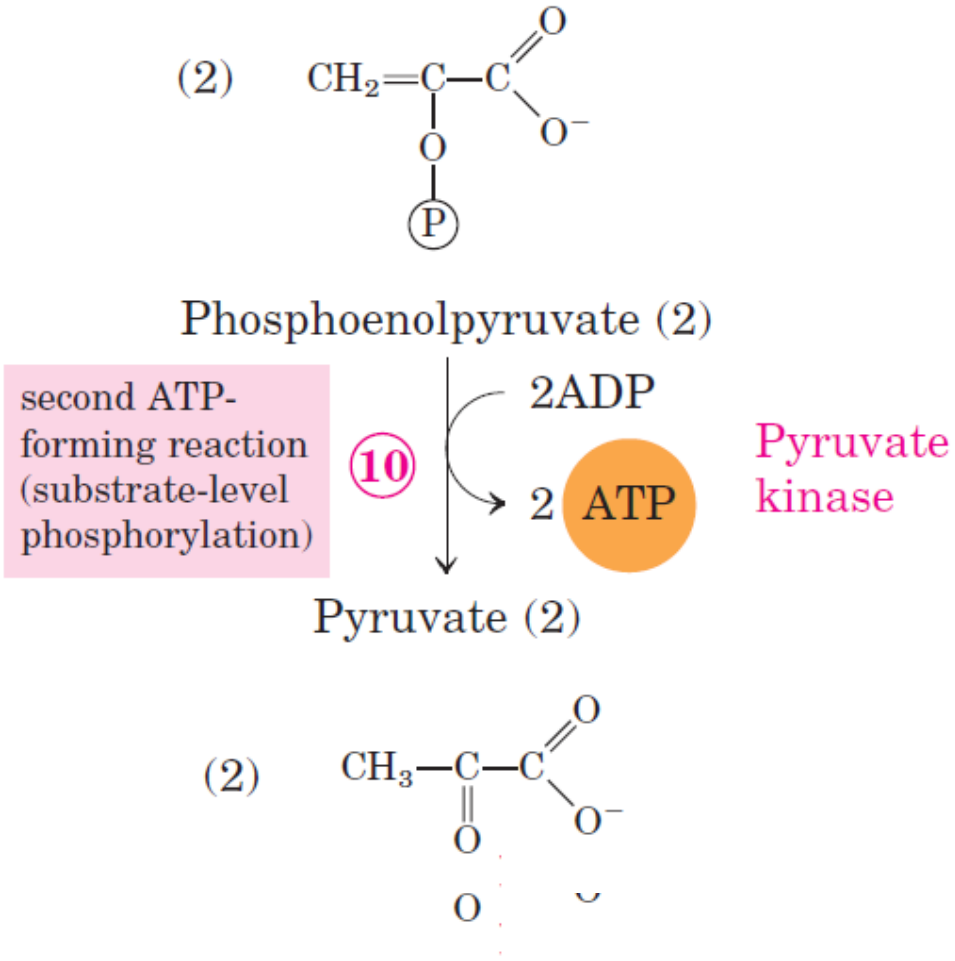


❖2-phosphoglycerate turn into phosphoenolpyruvate (step 9).



❖The last step in glycolysis is the transfer of the phosphoryl group from phosphoenolpyruvate to ADP, catalyzed by pyruvate kinase (step 10).

❖This reaction is also a substrate-level phosphorylation.

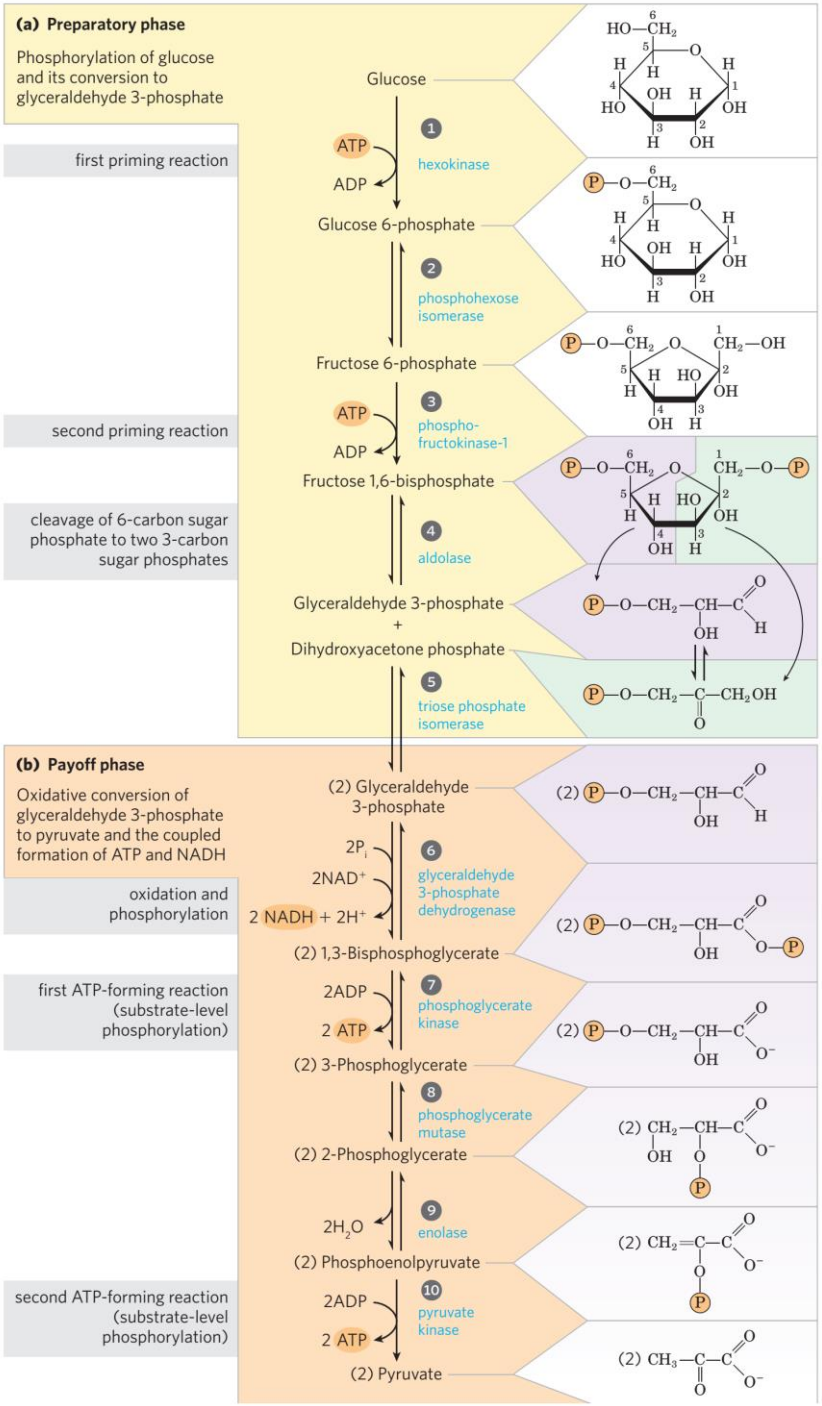


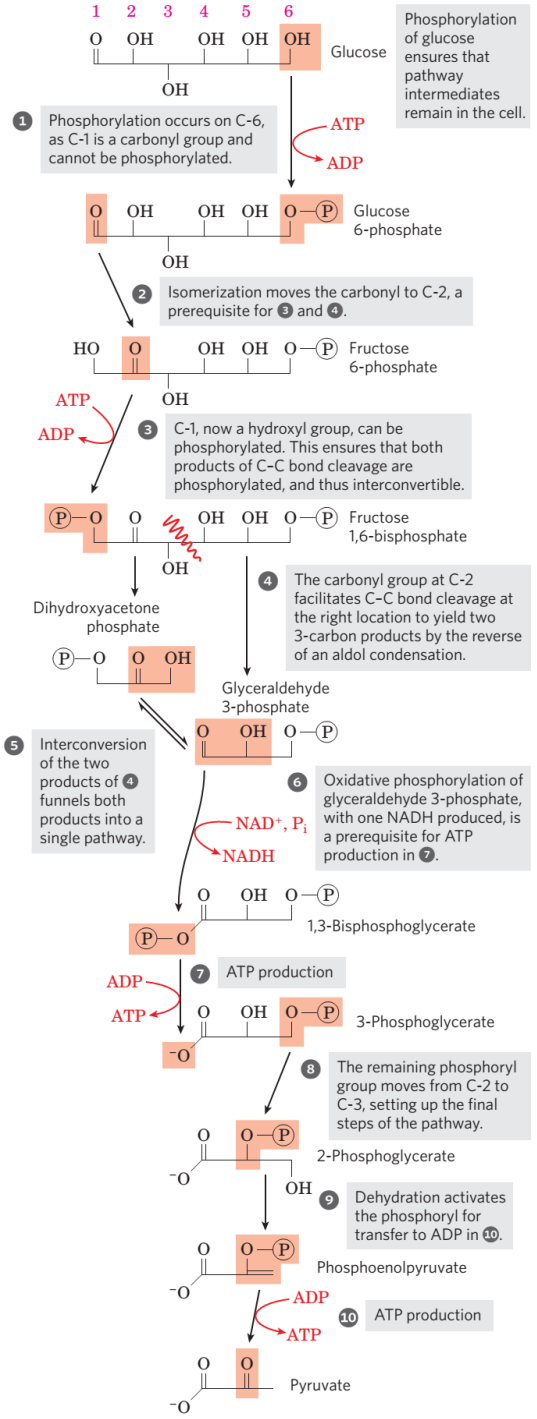
❖In the sequential reactions of glycolysis, three types of chemical transformations are particularly noteworthy:

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

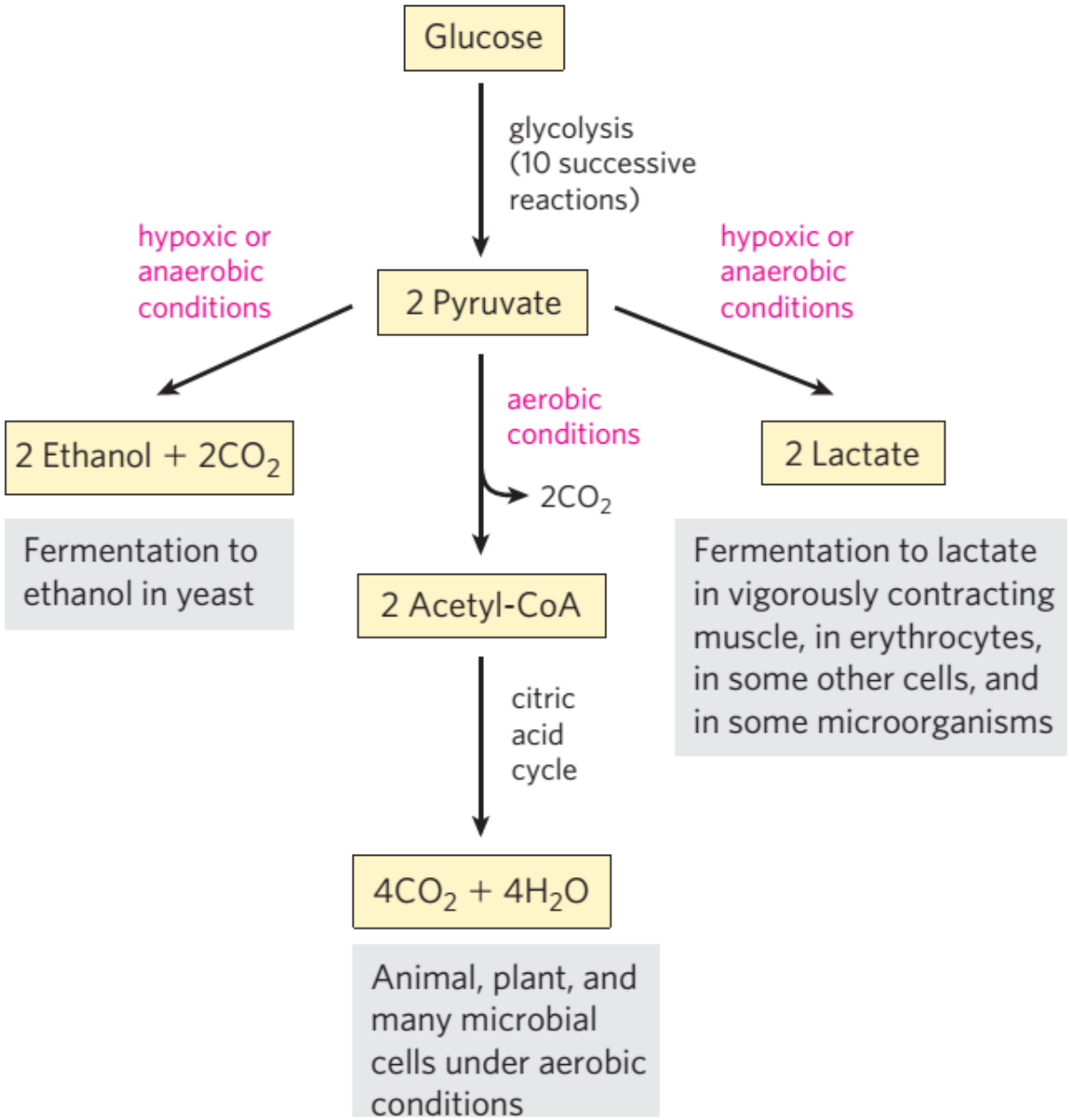
❖(2) phosphorylation of ADP to ATP by compounds with high phosphoryl group transfer potential, formed during glycolysis; and

❖(3) transfer of a hydride ion to NAD^+, forming NADH.


(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
Glucose
ATP
ADP
1
hexokinase
Glucose 6-phosphate
2
phosphohexose isomerase
Fructose 6-phosphate
ATP
ADP
3
phospho-fructokinase-1
Fructose 1,6-bisphosphate
4
aldolase
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
triose phosphate isomerase
(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)
(2) Glyceraldehyde 3-phosphate
2Pi
2NAD+
2 NADH + 2H+
6
glyceraldehyde 3-phosphate dehydrogenase
(2) 1,3-Bisphosphoglycerate
2ADP
2 ATP
7
phosphoglycerate kinase
(2) 3-Phosphoglycerate
8
phosphoglycerate mutase
(2) 2-Phosphoglycerate
2H2O
9
enolase
(2) Phosphoenolpyruvate
2ADP
2 ATP
10
pyruvate kinase
(2) Pyruvate


Glucose
Phosphorylation of glucose ensures that pathway intermediates remain in the cell.
1 Phosphorylation occurs on C-6, as C-1 is a carbonyl group and cannot be phosphorylated.
ATP
ADP
Glucose 6-phosphate
2 Isomerization moves the carbonyl to C-2, a prerequisite for 3 and 4.
Fructose 6-phosphate
ATP
ADP
3 C-1, now a hydroxyl group, can be phosphorylated. This ensures that both products of C–C bond cleavage are phosphorylated, and thus interconvertible.
Fructose 1,6-bisphosphate
4 The carbonyl group at C-2 facilitates C–C bond cleavage at the right location to yield two 3-carbon products by the reverse of an aldol condensation.
Dihydroxyacetone phosphate
Glyceraldehyde 3-phosphate
5 Interconversion of the two products of 4 funnels both products into a single pathway.
6 Oxidative phosphorylation of glyceraldehyde 3-phosphate, with one NADH produced, is a prerequisite for ATP production in 7.
NAD+, Pi
NADH
1,3-Bisphosphoglycerate
ADP
ATP
7 ATP production
3-Phosphoglycerate
8 The remaining phosphoryl group moves from C-2 to C-3, setting up the final steps of the pathway.
2-Phosphoglycerate
9 Dehydration activates the phosphoryl for transfer to ADP in 10.
Phosphoenolpyruvate
ADP
ATP
10 ATP production
Pyruvate

Glucose Catabolism



❖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.

❖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.

❖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, and

❖Lactate is also the product of glycolysis under anaerobic conditions in some microorganisms.

❖ The third major route of pyruvate catabolism leads to ethanol. In some plant tissues and in certain invertebrates, protists, and microorganisms such as brewer's or baker's yeast, pyruvate is converted under hypoxic or anaerobic conditions to ethanol and CO_2, a process called ethanol (alcohol) fermentation.

❖The oxidation of pyruvate is an important catabolic process, but pyruvate has anabolic fates as well. It can, for example, provide the carbon skeleton for the synthesis of the amino acid alanine or for the synthesis of fatty acids.

❖During glycolysis some of the energy of the glucose molecule is conserved in ATP, while much remains in the product, pyruvate.

❖The overall equation for glycolysis is

$$\text{Glucose} + 2\text{NAD}^+ + 2\text{ADP} + 2\text{P}_\text{i} \longrightarrow 2\ \text{pyruvate} + 2\text{NADH} + 2\text{H}^+ + 2\text{ATP} + 2\text{H}_2\text{O}$$

❖For each molecule of glucose degraded to pyruvate, two molecules of ATP are generated from ADP and P_i, and two molecules of NADH are produced by the reduction of NAD^+.

❖We can now resolve the equation of glycolysis into two processes;

$$\text{Glucose} + 2\text{NAD}^+ \longrightarrow 2\ \text{pyruvate} + 2\text{NADH} + 2\text{H}^+ \quad \Delta G_1'^\circ = -146\ \text{kJ/mol}$$

$$2\text{ADP} + 2\text{P}_\text{i} \longrightarrow 2\text{ATP} + 2\text{H}_2\text{O} \quad \Delta G_2'^\circ = 2(30.5\ \text{kJ/mol}) = 61.0\ \text{kJ/mol}$$

$$\begin{aligned}\Delta G_\text{s}'^\circ = \Delta G_1'^\circ + \Delta G_2'^\circ &= -146\ \text{kJ/mol} + 61.0\ \text{kJ/mol} \\ &= -85\ \text{kJ/mol}\end{aligned}$$

❖Under standard conditions, and under the (nonstandard) conditions that prevail in a cell, glycolysis is an essentially irreversible process, driven to completion by a large net decrease in free energy.

❖Glycolysis releases only a small fraction of the total available energy of the glucose molecule.

❖The two molecules of pyruvate formed by glycolysis still contain most of the chemical potential energy of glucose.

❖That energy can be extracted by oxidative reactions in the citric acid cycle and oxidative phosphorylation.

❖Each of the nine glycolytic intermediates between glucose and pyruvate is phosphorylated.

❖The phosphoryl groups seem to have three functions.

❖1. Because the plasma membrane generally lacks transporters for phosphorylated sugars, the phosphorylated glycolytic intermediates cannot leave the cell.

❖2. Phosphoryl groups are essential components in the enzymatic conservation of metabolic energy.

❖Energy released in the breakage of phosphoanhydride bonds (such as those in ATP) is partially conserved in the formation of phosphate esters such as glucose 6-phosphate. High-energy phosphate compounds formed in glycolysis (1,3-bisphosphoglycerate and phosphoenolpyruvate) donate phosphoryl groups to ADP to form ATP.

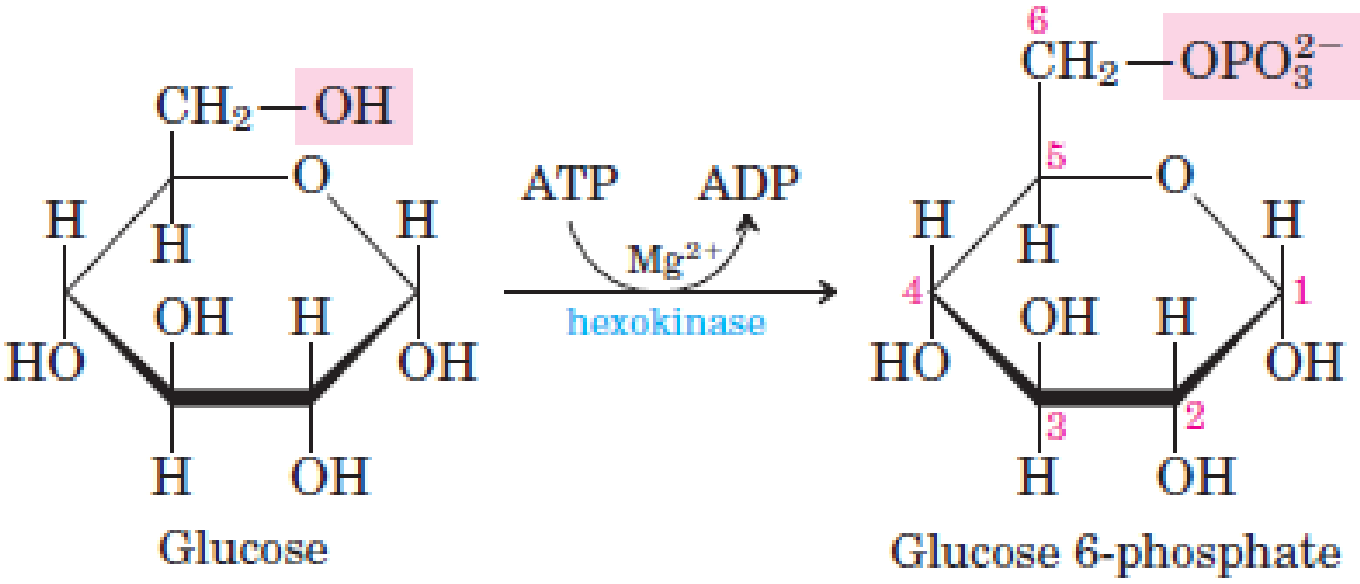
❖3. Binding energy resulting from the binding of phosphate groups to the active sites of enzymes lowers the activation energy and increases the specificity of the enzymatic reactions

❖The phosphate groups of ADP, ATP, and the glycolytic intermediates form complexes with Mg^{2+}, and the substrate binding sites of many glycolytic enzymes are specific for these Mg^{2+} complexes.

The Preparatory Phase of Glycolysis

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:



$$\Delta G'^{\circ} = -16.7 \text{ kJ/mol}$$

❖This reaction, which is irreversible under intracellular conditions, is catalyzed by **hexokinase**.

❖Kinases are enzymes that catalyze the transfer of the terminal phosphoryl group from ATP to an acceptor nucleophile. Kinases are a subclass of transferases.

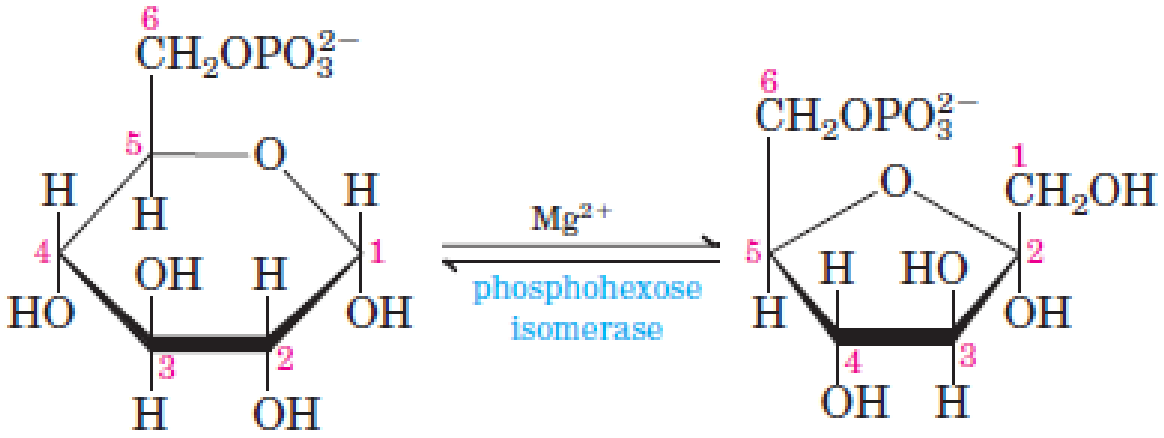
❖The acceptor in the case of hexokinase is a hexose, normally D-glucose, although hexokinase also catalyzes the phosphorylation of other common hexoses, such as D-fructose and D-mannose, in some tissues.

❖Hexokinase, like many other kinases, requires Mg^{2+} for its activity, because the true substrate of the enzyme is not ATP^{4-} but the $MgATP^{2-}$ complex.

❖Mg^{2+} shields the negative charges of the phosphoryl groups in ATP, making the terminal phosphorus atom an easier target for nucleophilic attack by an —OH of glucose.

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

❖**Phosphohexose isomerase** catalyzes the reversible isomerization of glucose 6-phosphate, an aldose, to fructose 6- phosphate, a ketose:



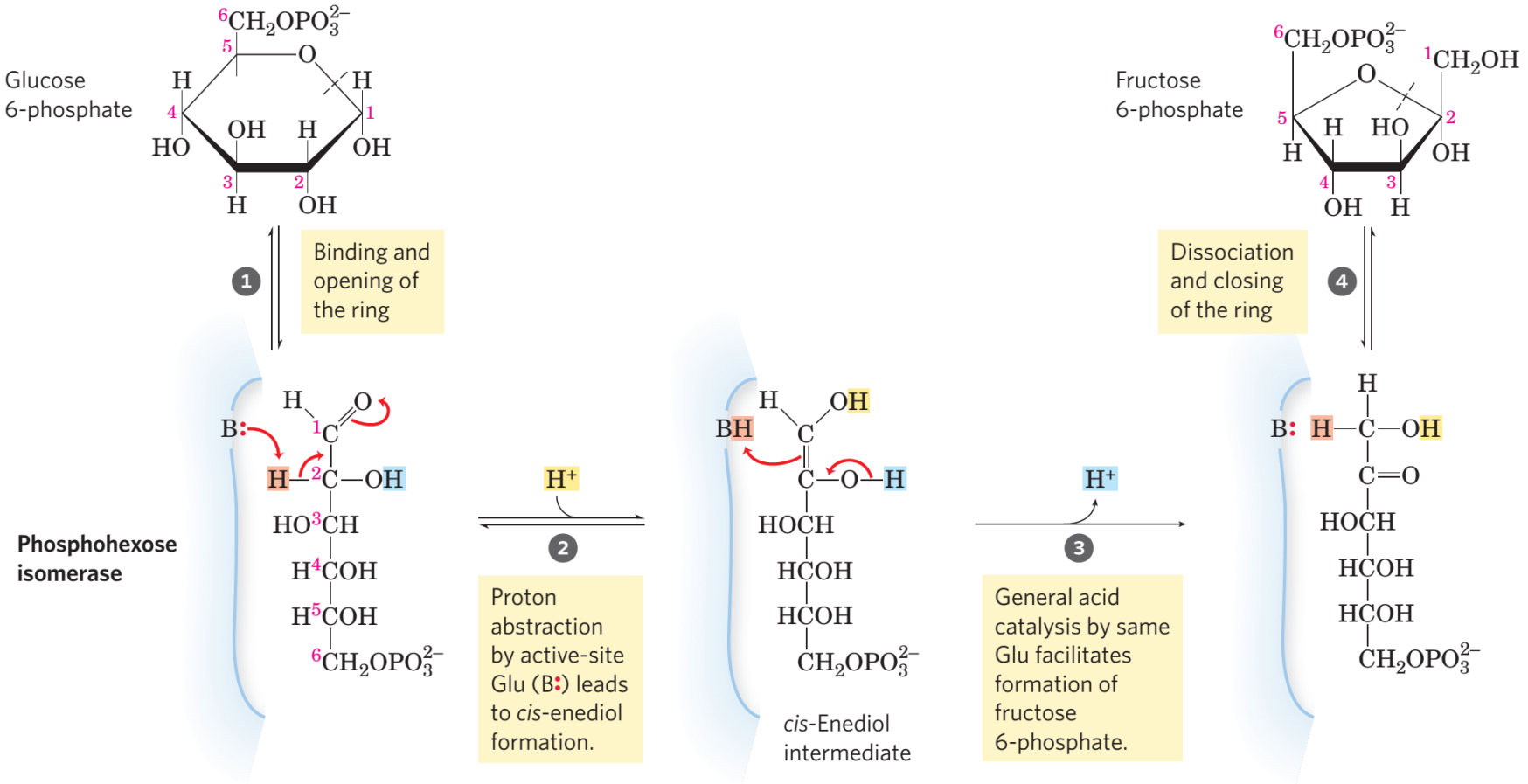
Glucose 6-phosphate

Fructose 6-phosphate

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

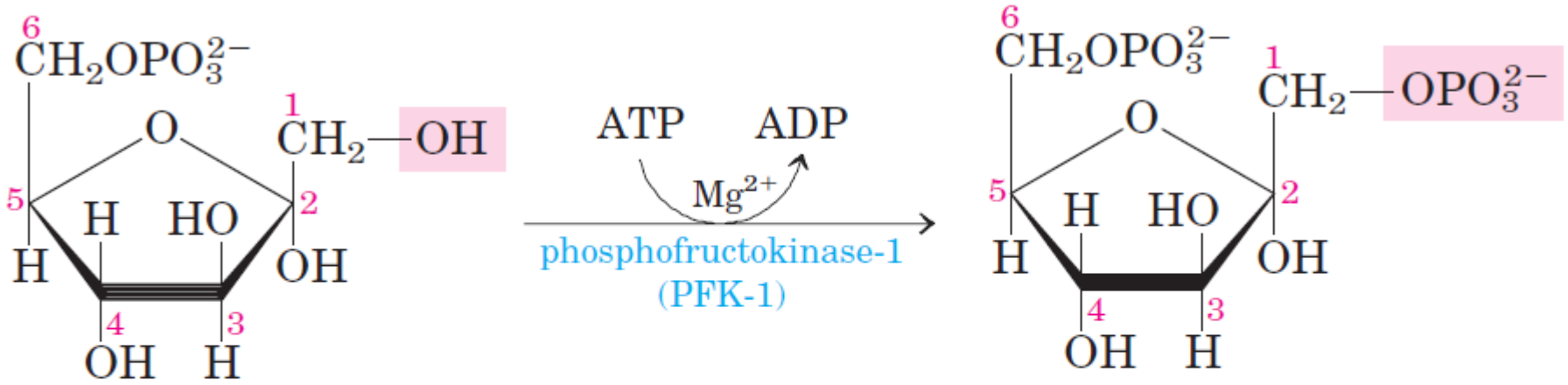
❖The mechanism for this reaction involves an enediol intermediate.

❖The reaction proceeds readily in either direction, as might be expected from the relatively small change in standard free energy.


Glucose 6-phosphate
Fructose 6-phosphate
Phosphohexose isomerase
1
Binding and opening of the ring
2
Proton abstraction by active-site Glu (B:) leads to cis-enediol formation.
cis-Enediol intermediate
3
General acid catalysis by same Glu facilitates formation of fructose 6-phosphate.
4
Dissociation and closing of the ring

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

❖In the second of the two priming reactions of glycolysis, **phosphofructokinase-1 (PFK-1)** catalyzes the transfer of a phosphoryl group from ATP to fructose 6-phosphate to yield fructose 1,6-bisphosphate.



Fructose 6-phosphate

Fructose 1,6-bisphosphate

$$\Delta G'^{\circ} = -14.2 \text{ kJ/mol}$$

❖The enzyme that forms fructose 1,6-bisphosphate is called PFK-1 to distinguish it from a second enzyme (PFK-2) that catalyzes the formation of fructose 2,6-bisphosphate from fructose 6-phosphate in a separate pathway.

❖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:

❖Phosphofructokinase-1 is subject to complex allosteric regulation; its activity is increased whenever the cell's ATP supply is depleted or when the ATP breakdown products, ADP and AMP (particularly the latter), accumulate.

❖The enzyme is inhibited whenever the cell has ample ATP and is well supplied by other fuels such as fatty acids.

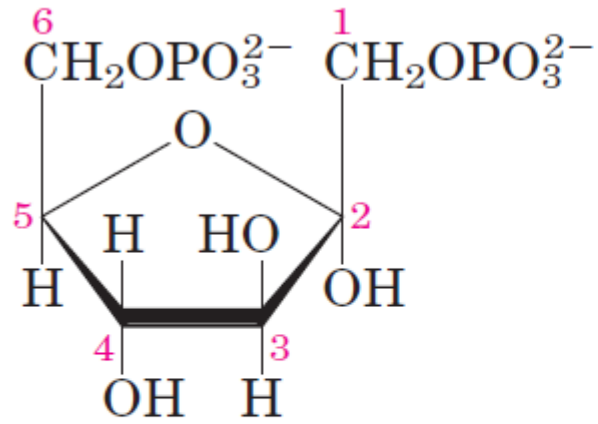
❖In some organisms, fructose 2,6-bisphosphate (not to be confused with the PFK-1 reaction product, fructose 1,6-bisphosphate) is a potent allosteric activator of PFK-1.

❖Ribulose 5-phosphate, an intermediate in the pentose phosphate pathway, also activates phosphofructokinase indirectly.

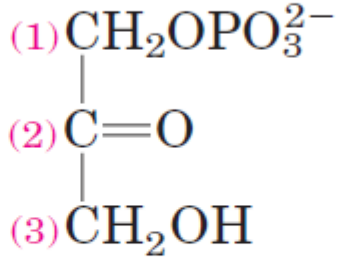
4- Cleavage of Fructose 1,6-Bisphosphate

❖The enzyme fructose 1,6-bisphosphate aldolase, often called simply **aldolase**, catalyzes a reversible aldol condensation.

❖Fructose 1,6-bisphosphate is cleaved to yield two different triose phosphates, glyceraldehyde 3-phosphate, an aldose, and dihydroxyacetone phosphate, a ketose:

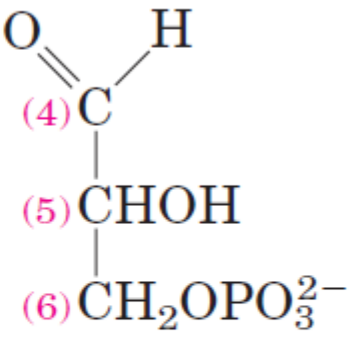


Fructose 1,6-bisphosphate



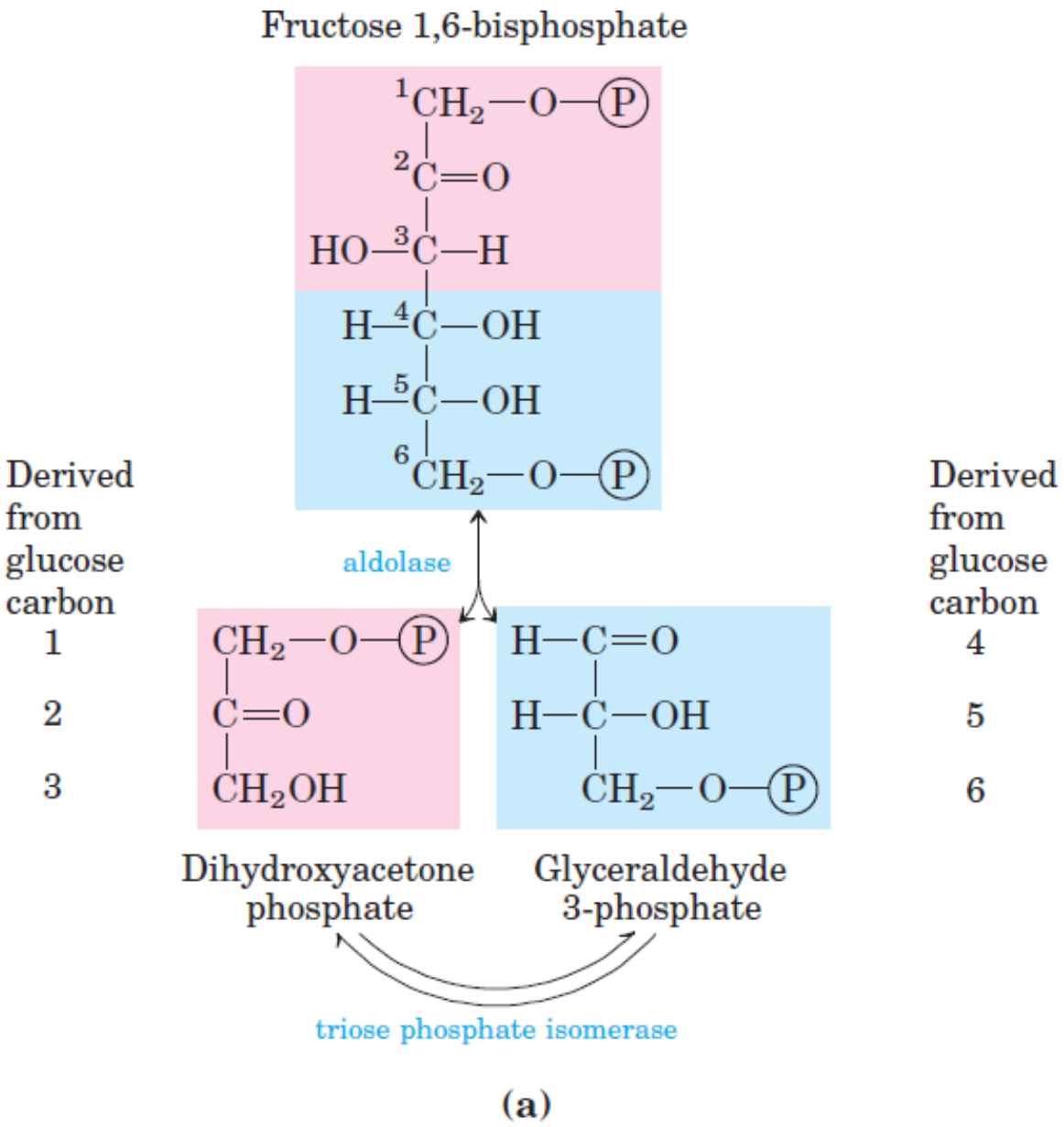
Dihydroxyacetone phosphate

+



Glyceraldehyde 3-phosphate

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


Fructose 1,6-bisphosphate
Derived from glucose carbon
1
2
3
aldolase
Derived from glucose carbon
4
5
6
Dihydroxyacetone phosphate
Glyceraldehyde 3-phosphate
triose phosphate isomerase
(a)

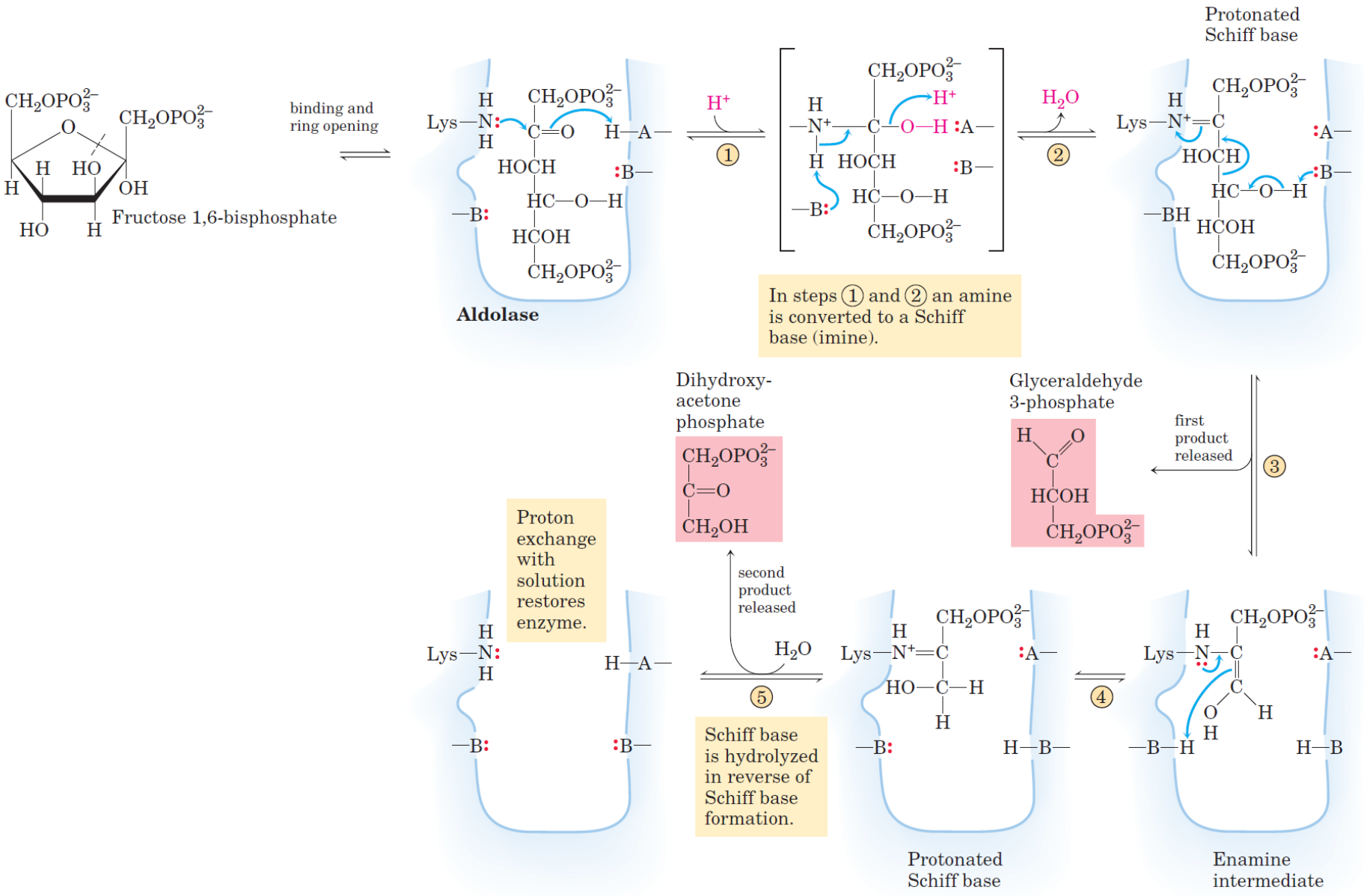
❖There are two classes of aldolases.

❖Class I aldolases, found in animals and plants, use the mechanism shown in the first part of the following figure.

❖Class II enzymes, in fungi and bacteria, do not form the Schiff base intermediate.

❖Instead, a zinc ion at the active site is coordinated with the carbonyl oxygen at C-2; the Zn^{2+} polarizes the carbonyl group and stabilizes the enolate intermediate created in the C—C bond cleavage step.

❖Although the aldolase reaction has a strongly positive standard free-energy change in the direction of fructose 1,6-bisphosphate cleavage, at the lower concentrations of reactants present in cells the actual free energy change is small and the aldolase reaction is readily reversible.

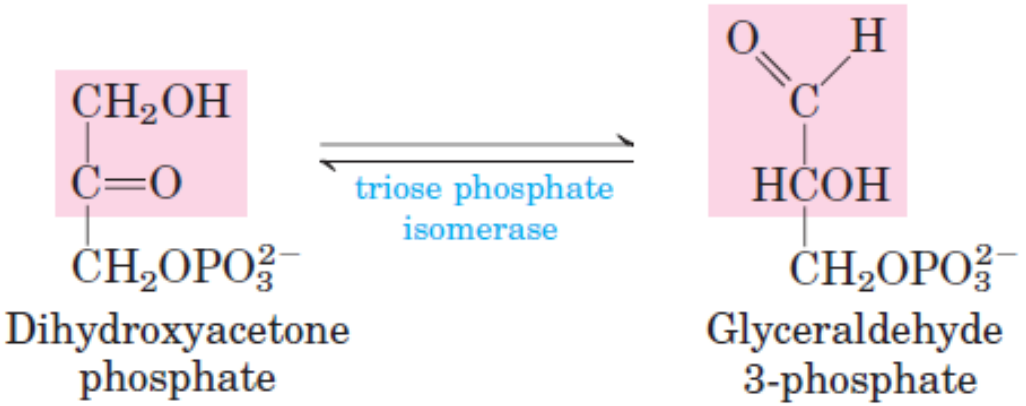

Fructose 1,6-bisphosphate
binding and ring opening
Aldolase
H+
1
2
H2O
Protonated Schiff base
In steps 1 and 2 an amine is converted to a Schiff base (imine).
Dihydroxy-acetone phosphate
Glyceraldehyde 3-phosphate
first product released
3
second product released
5
Proton exchange with solution restores enzyme.
Schiff base is hydrolyzed in reverse of Schiff base formation.
4
Protonated Schiff base
Enamine intermediate

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**:

❖The reaction mechanism is similar to the reaction promoted by phosphohexose isomerase in step 2 of glycolysis.



Dihydroxyacetone phosphate

Glyceraldehyde 3-phosphate

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

The Payoff Phase of Glycolysis

❖The payoff phase of glycolysis includes the energy-conserving phosphorylation steps in which some of the chemical energy of the glucose molecule is conserved in the form of ATP and NADH.

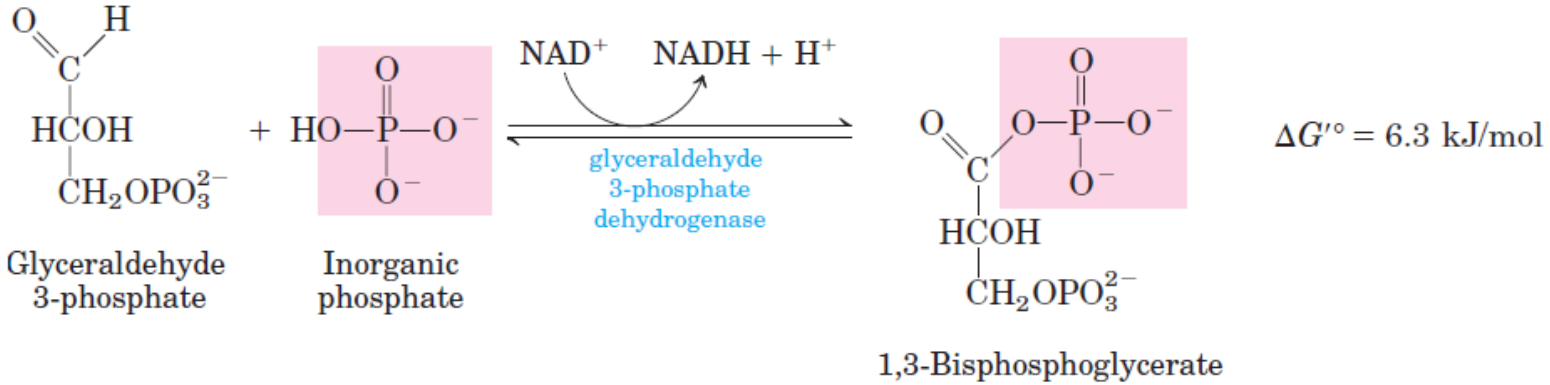
❖Remember that one molecule of glucose yields two molecules of glyceraldehyde 3-phosphate, and both halves of the glucose molecule follow the same pathway in the second phase of glycolysis.

❖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.

❖However, 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

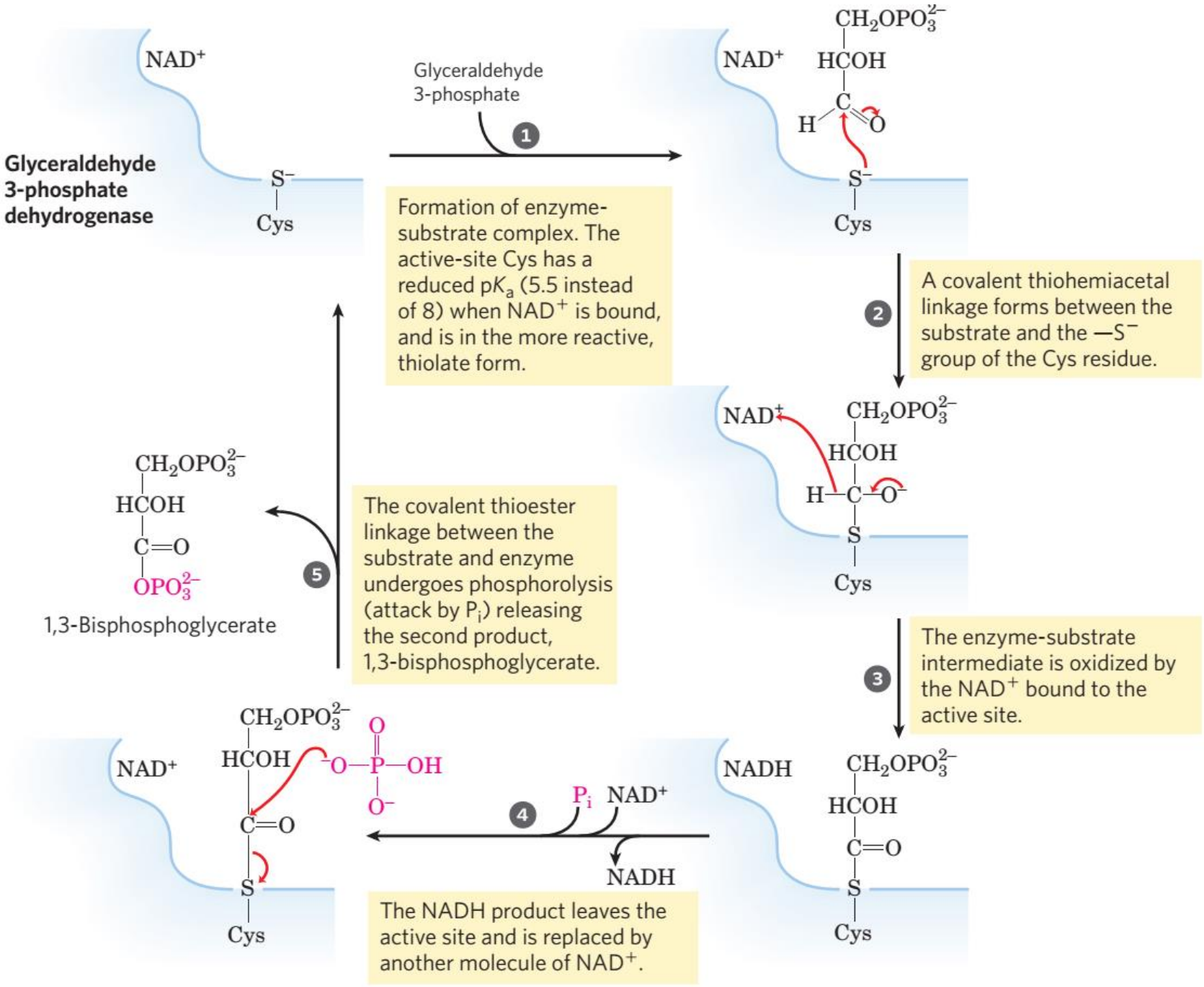
❖The first step in the payoff phase is the oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate, catalyzed by **glyceraldehyde 3-phosphate dehydrogenase**:



❖This is the first of the two energy-conserving reactions of glycolysis that eventually lead to the formation of ATP.

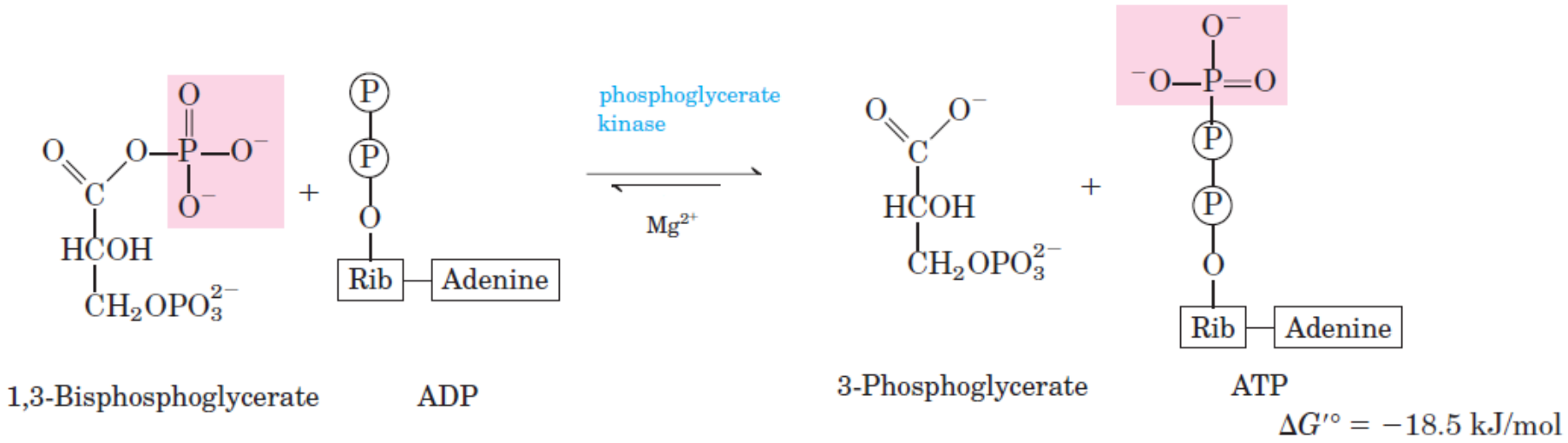
❖The aldehyde group of glyceraldehyde 3-phosphate is oxidized, not to a free carboxyl group but to a carboxylic acid anhydride with phosphoric acid.

❖This type of anhydride, called an acyl phosphate, has a very high standard free energy of hydrolysis


Glyceraldehyde 3-phosphate dehydrogenase
NAD+
S−
Cys
Glyceraldehyde 3-phosphate
1
Formation of enzyme-substrate complex. The active-site Cys has a reduced pKa (5.5 instead of 8) when NAD+ is bound, and is in the more reactive, thiolate form.
CH2OPO3 2−
HCOH
C
H
O
2
A covalent thiohemiacetal linkage forms between the substrate and the —S− group of the Cys residue.
3
The enzyme-substrate intermediate is oxidized by the NAD+ bound to the active site.
NADH
4
Pi
NAD+
NADH
The NADH product leaves the active site and is replaced by another molecule of NAD+.
5
The covalent thioester linkage between the substrate and enzyme undergoes phosphorolysis (attack by Pi) releasing the second product, 1,3-bisphosphoglycerate.
C=O
OPO3 2−
1,3-Bisphosphoglycerate

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:



1,3-Bisphosphoglycerate ADP 3-Phosphoglycerate ATP

$\Delta G'^{\circ} = -18.5$ kJ/mol

❖Notice that phosphoglycerate kinase is named for the reverse reaction, in which it transfers a phosphoryl group from ATP to 3-phosphoglycerate.

❖This enzyme acts in the direction suggested by its name during gluconeogenesis.

❖Steps 6 and 7 of glycolysis together constitute an energy-coupling process in which 1,3-bisphosphoglycerate is the common intermediate;

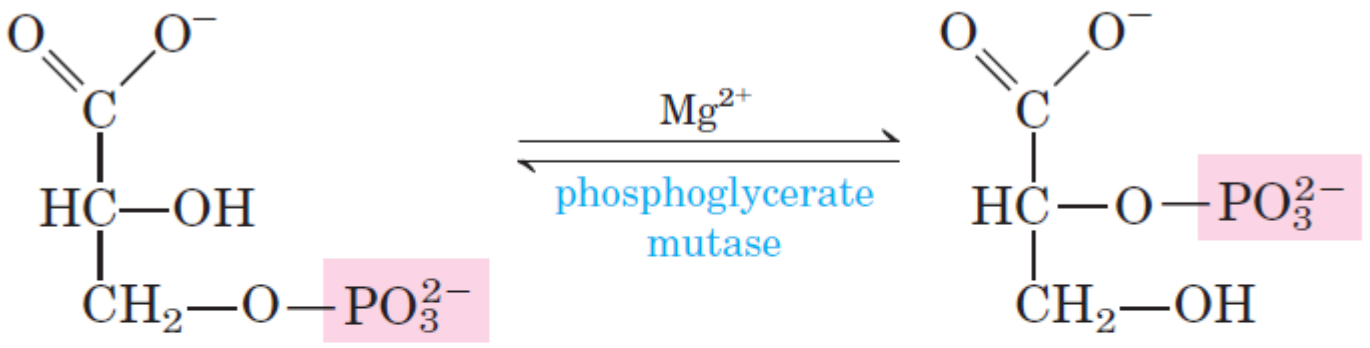
$$\text{Glyceraldehyde 3-phosphate} + \text{ADP} + \text{P}_\text{i} + \text{NAD}^+ \rightleftharpoons \text{3-phosphoglycerate} + \text{ATP} + \text{NADH} + \text{H}^+$$
$$\Delta G'^\circ = -12.5 \text{ kJ/mol}$$

❖The outcome of these coupled 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 (oxidative) phosphorylation**.

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; Mg^{2+} is essential for this reaction:

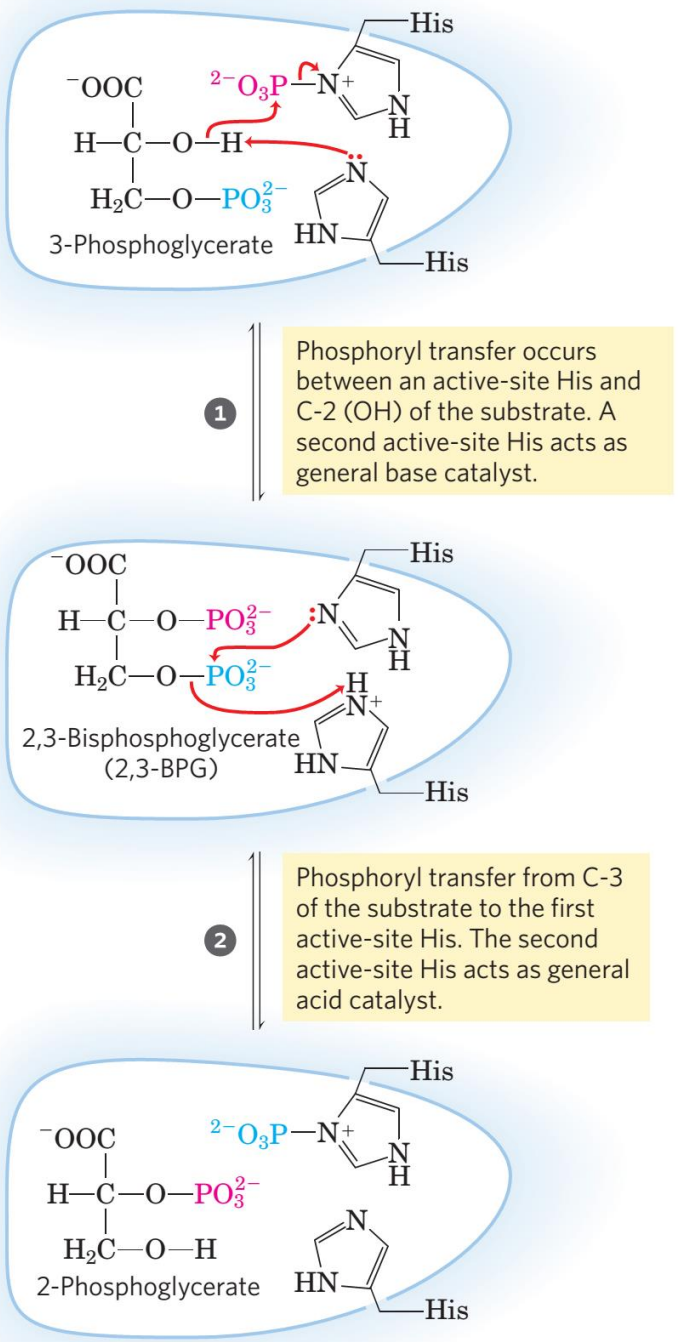


3-Phosphoglycerate

2-Phosphoglycerate

$\Delta G'^{\circ} = 4.4$ kJ/mol

Phosphoglycerate mutase

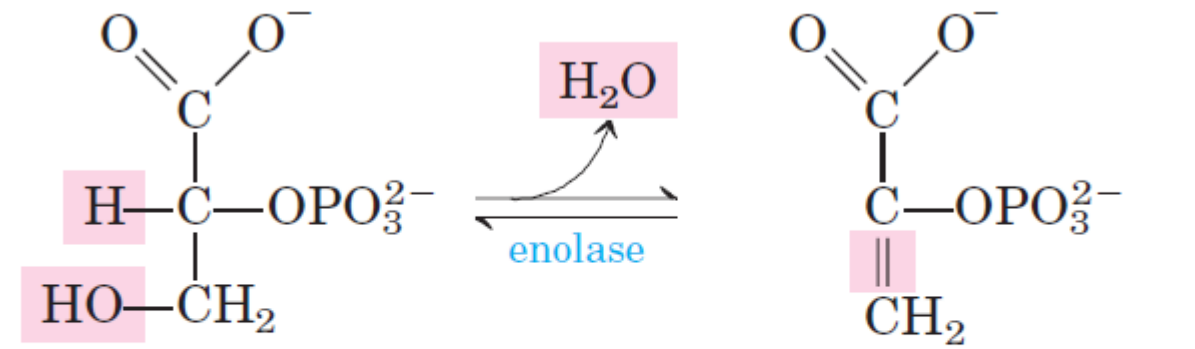


❖The reaction occurs in two steps. A phosphoryl group initially attached to a His residue of the mutase is transferred to the hydroxyl group at C-2 of 3-phosphoglycerate, forming 2,3-bisphosphoglycerate(2,3-BPG).

❖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.

9- Dehydration of 2-Phosphoglycerate to Phosphoenolpyruvate

❖In the second glycolytic reaction that generates a compound with high phosphoryl group transfer potential, **enolase** promotes reversible removal of a molecule of water from 2-phosphoglycerate to yield phosphoenolpyruvate (PEP):

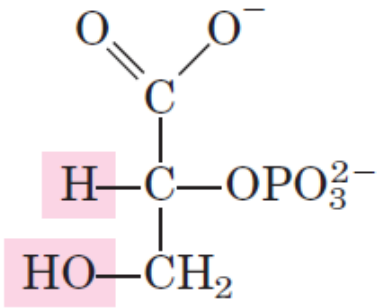


2-Phosphoglycerate Phosphoenolpyruvate

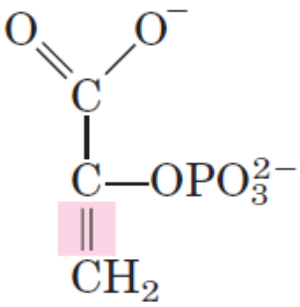
$\Delta G'^{\circ} = 7.5$ kJ/mol

❖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 (ΔG'° for hydrolysis of 2-phosphoglycerate is -17.6 kJ/mol) to one with high phosphoryl group transfer potential (ΔG'° for PEP hydrolysis is -61.9 kJ/mol).



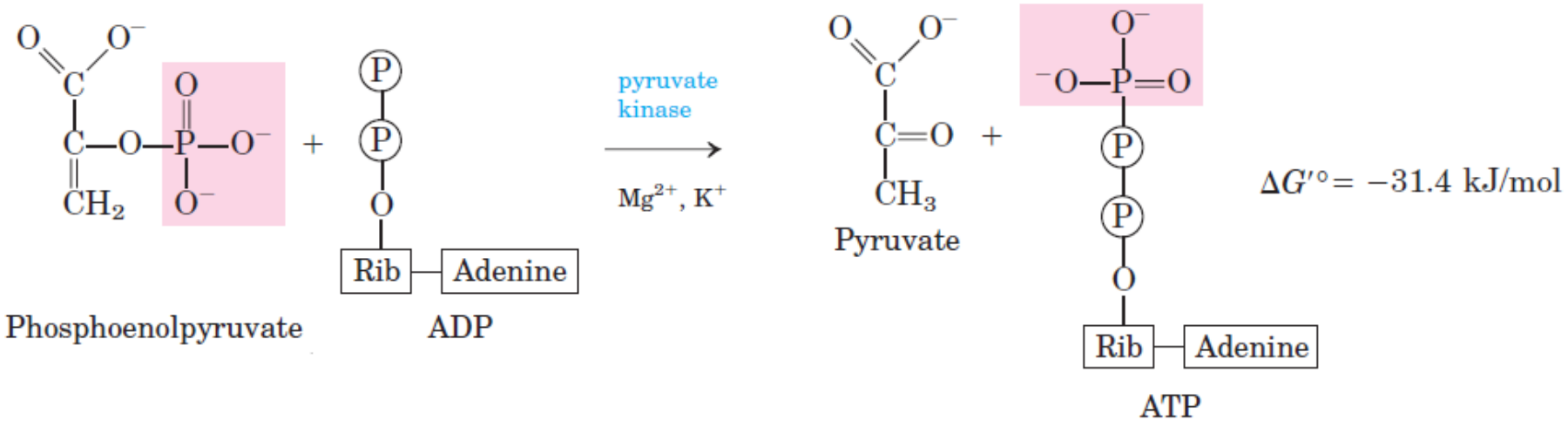
2-Phosphoglycerate



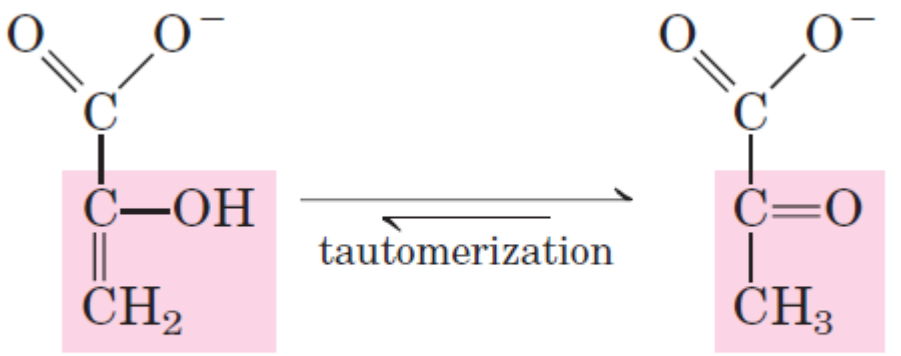
Phosphoenolpyruvate

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^+ 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 has a large, negative standard free energy change, due in large part to the spontaneous conversion of the enol form of pyruvate to the keto form.

❖About half of the energy released by PEP hydrolysis (ΔG'° -61.9 kJ/mol) is conserved in the formation of the phosphoanhydride bond of ATP (ΔG'° -30.5 kJ/mol), and the rest (-31.4 kJ/mol) constitutes a large driving force pushing the reaction toward ATP synthesis

❖We can now construct a balance sheet for glycolysis :

$$\text{Glucose} + 2\text{ATP} + 2\text{NAD}^+ + 4\text{ADP} + 2\text{P}_i \longrightarrow 2\ \text{pyruvate} + 2\text{ADP} + 2\text{NADH} + 2\text{H}^+ + 4\text{ATP} + 2\text{H}_2\text{O}$$

❖Canceling out common terms on both sides of the equation gives the overall equation for glycolysis under aerobic conditions:

$$\text{Glucose} + 2\text{NAD}^+ + 2\text{ADP} + 2\text{P}_i \longrightarrow 2\ \text{pyruvate} + 2\text{NADH} + 2\text{H}^+ + 2\text{ATP} + 2\text{H}_2\text{O}$$

❖The two molecules of NADH formed by glycolysis in the cytosol are, under aerobic conditions, reoxidized to NAD^+ by transfer of their electrons to the electrontransfer chain, which in eukaryotic cells is located in the mitochondria.

❖The electron-transfer chain passes these electrons to their ultimate destination, O_2:

$$2\text{NADH} + 2\text{H}^+ + \text{O}_2 \longrightarrow 2\text{NAD}^+ + 2\text{H}_2\text{O}$$

❖Electron transfer from NADH to O_2 in mitochondria provides the energy for synthesis of ATP by oxidative phosphorylation.

❖In the overall glycolytic process, one molecule of glucose is converted to two molecules of pyruvate (the pathway of carbon).

❖Two molecules of ADP and two of Pi are converted to two molecules of ATP (the pathway of phosphoryl groups).

❖Four electrons, as two hydride ions, are transferred from two molecules of glyceraldehyde 3-phosphate to two of NAD^+ (the pathway of electrons).

❖During his studies on the fermentation of glucose by yeast, Louis Pasteur discovered that both the rate and the total amount of glucose consumption were many times greater under anaerobic than aerobic conditions.

❖Later studies of muscle showed the same large difference in the rates of anaerobic and aerobic glycolysis.

❖The biochemical basis of this "**Pasteur effect**" is now clear.

❖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 as much glucose must therefore be consumed anaerobically as aerobically to yield the same amount of ATP.

❖The flux of glucose through the glycolytic pathway is regulated to maintain nearly constant ATP levels (as well as adequate supplies of glycolytic intermediates that serve biosynthetic roles).

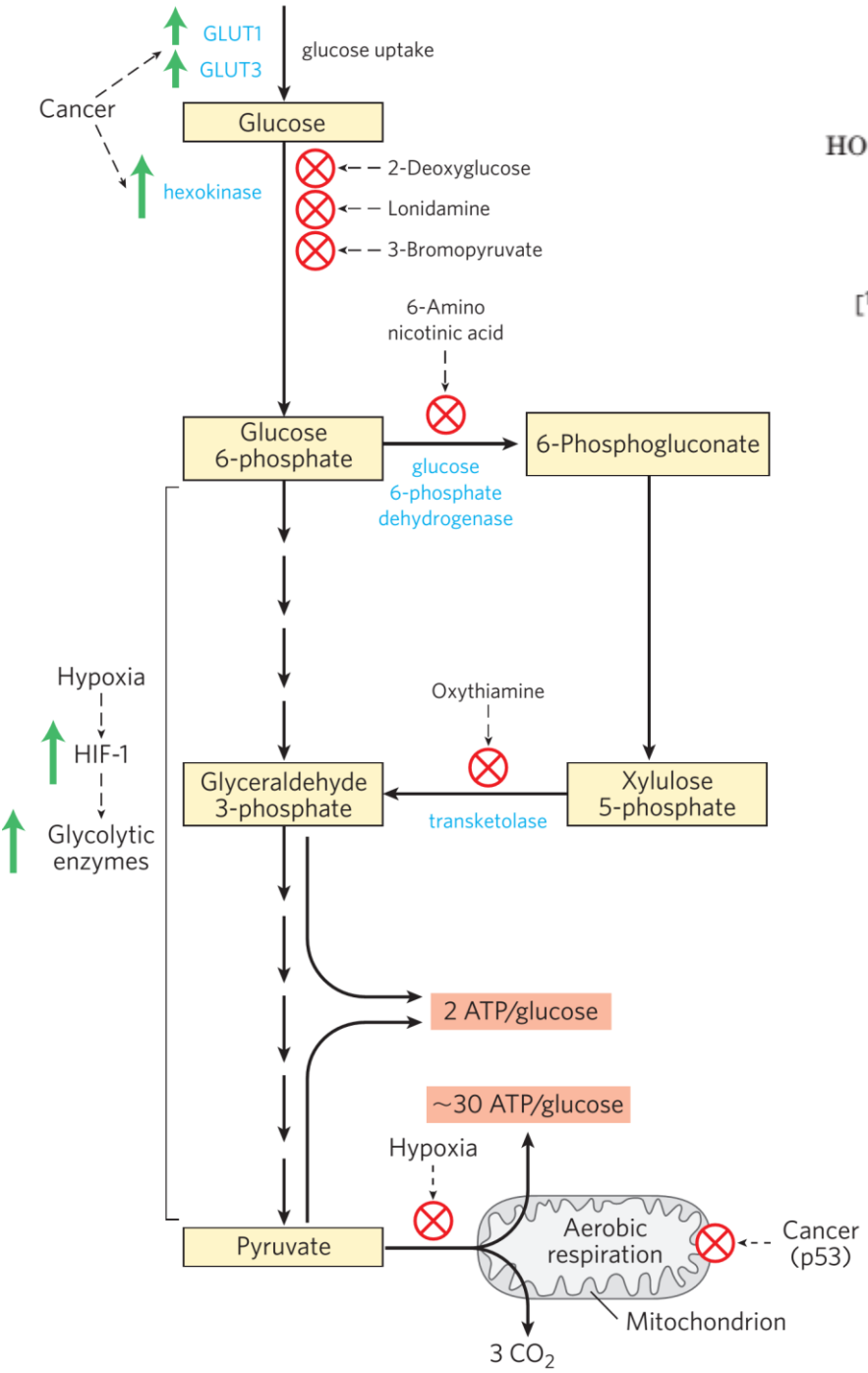
❖The required adjustment in the rate of glycolysis is achieved by a complex interplay among ATP consumption, NADH regeneration, and allosteric regulation of several glycolytic enzymes —including hexokinase, PFK-1, and pyruvate kinase.

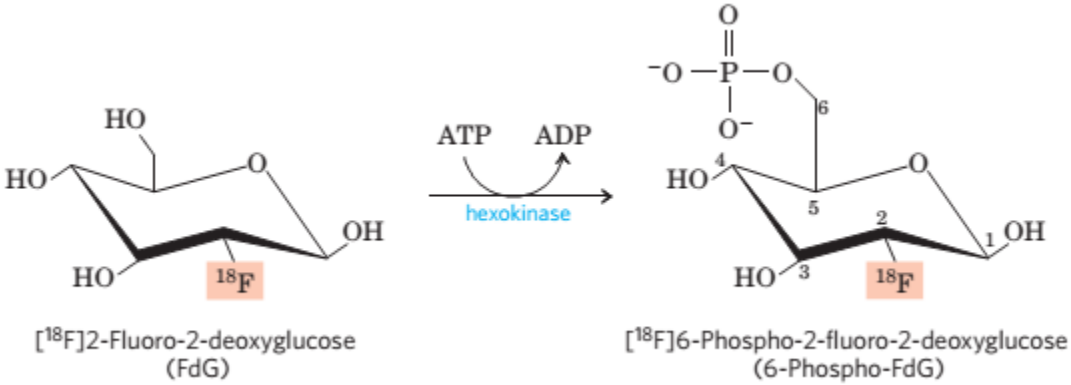
❖On a slightly longer time scale, glycolysis is regulated by the hormones glucagon, epinephrine, and insulin, and by changes in the expression of the genes for several glycolytic enzymes.

❖An especially interesting case of abnormal regulation of glycolysis is seen in cancer.

❖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.

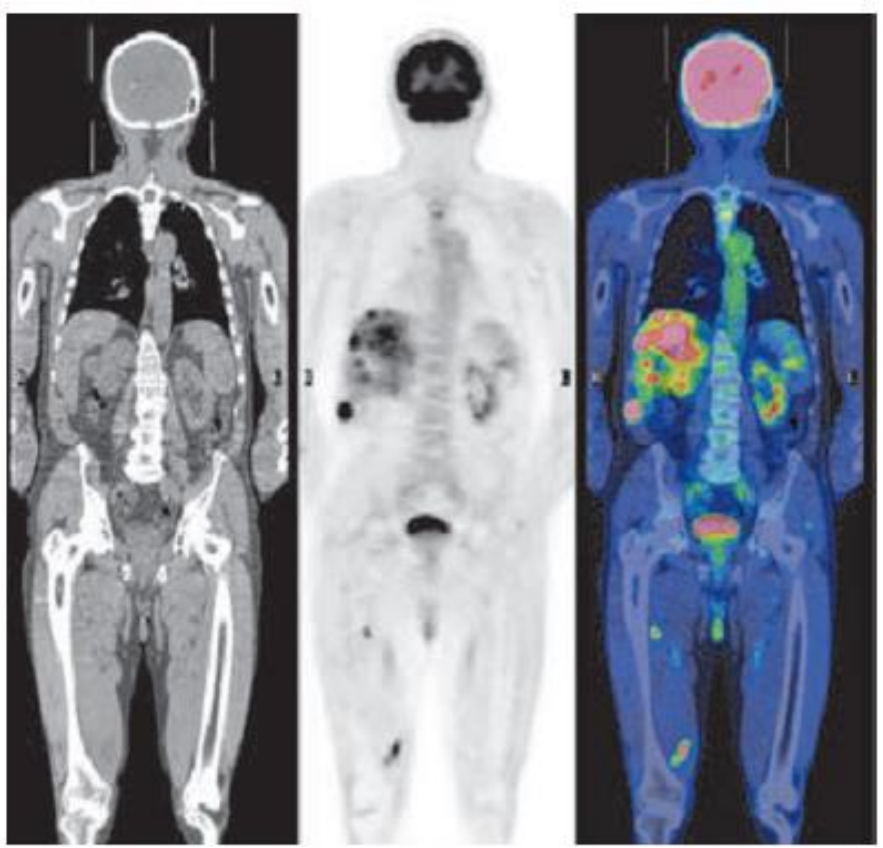
❖This “Warburg effect” is the basis for several methods of detecting and treating cancer.





[18F]2-Fluoro-2-deoxyglucose
(FdG)

[18F]6-Phospho-2-fluoro-2-deoxyglucose
(6-Phospho-FdG)



❖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 β cells in response to elevated blood glucose.

❖Individuals with type 1 diabetes mellitus (also called insulin-dependent diabetes) 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.

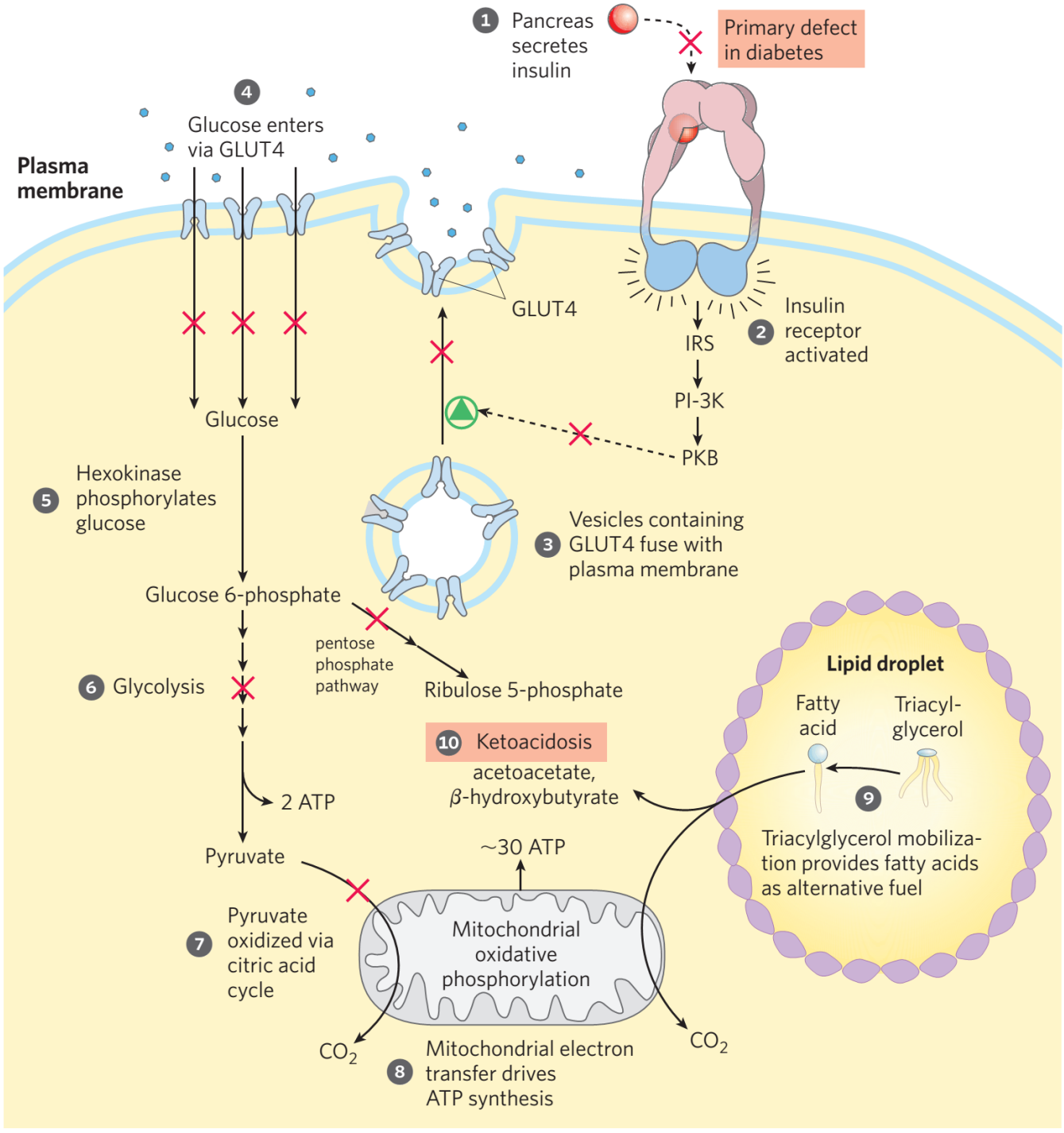
❖In the liver, acetyl-CoA derived from this fatty acid breakdown is converted to “ketone bodies”—acetoacetate and β-hydroxybutyrate—which are exported and carried to other tissues to be used as fuel.

❖These compounds are especially critical to the brain, which uses ketone bodies as alternative fuel when glucose is unavailable.

❖In untreated type 1 diabetes, overproduction of acetoacetate and β-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: GLUT4 moves into the plasma membranes of hepatocytes and adipocytes, glucose is taken up into the cells and phosphorylated, and the blood glucose level falls, greatly reducing the production of ketone bodies.

❖Diabetes mellitus has profound effects on the metabolism of both carbohydrates and fats.

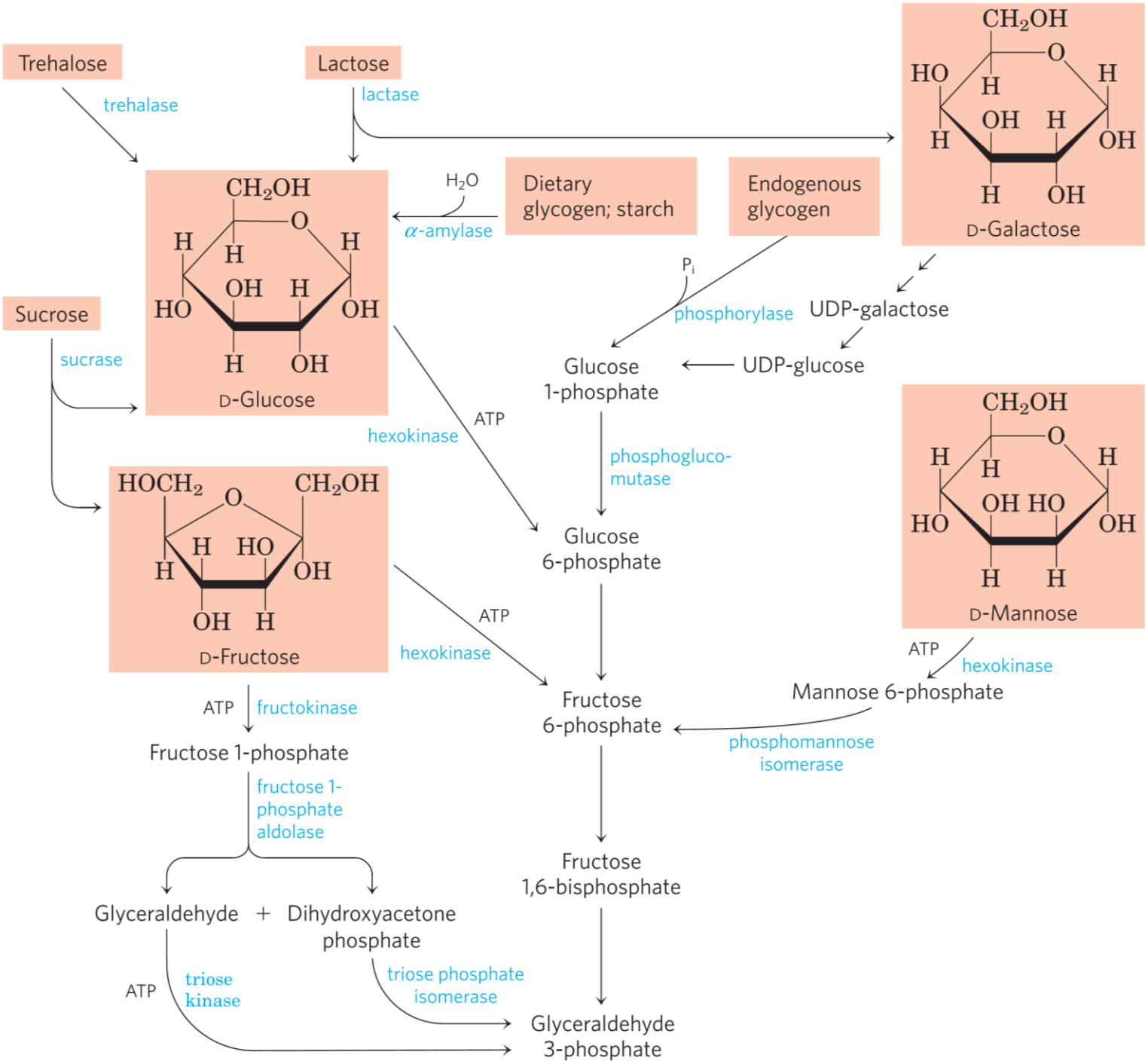

1 Pancreas secretes insulin
Primary defect in diabetes
4 Glucose enters via GLUT4
Plasma membrane
GLUT4
2 Insulin receptor activated
IRS
PI-3K
PKB
Glucose
5 Hexokinase phosphorylates glucose
3 Vesicles containing GLUT4 fuse with plasma membrane
Glucose 6-phosphate
pentose phosphate pathway
Ribulose 5-phosphate
6 Glycolysis
Lipid droplet
Fatty acid
Triacyl-glycerol
10 Ketoacidosis
acetoacetate, β-hydroxybutyrate
9
Triacylglycerol mobilization provides fatty acids as alternative fuel
2 ATP
~30 ATP
Pyruvate
7 Pyruvate oxidized via citric acid cycle
Mitochondrial oxidative phosphorylation
CO2
CO2
8 Mitochondrial electron transfer drives ATP synthesis

Feeder Pathways for Glycolysis

❖Many carbohydrates besides glucose meet their catabolic fate in glycolysis, after being transformed into one of the glycolytic intermediates.

❖The most significant are

- the storage polysaccharides glycogen and starch, either within cells (endogenous) or obtained in the diet;
- The disaccharides maltose, lactose, trehalose, and sucrose;
- and the monosaccharides fructose, mannose, and galactose.

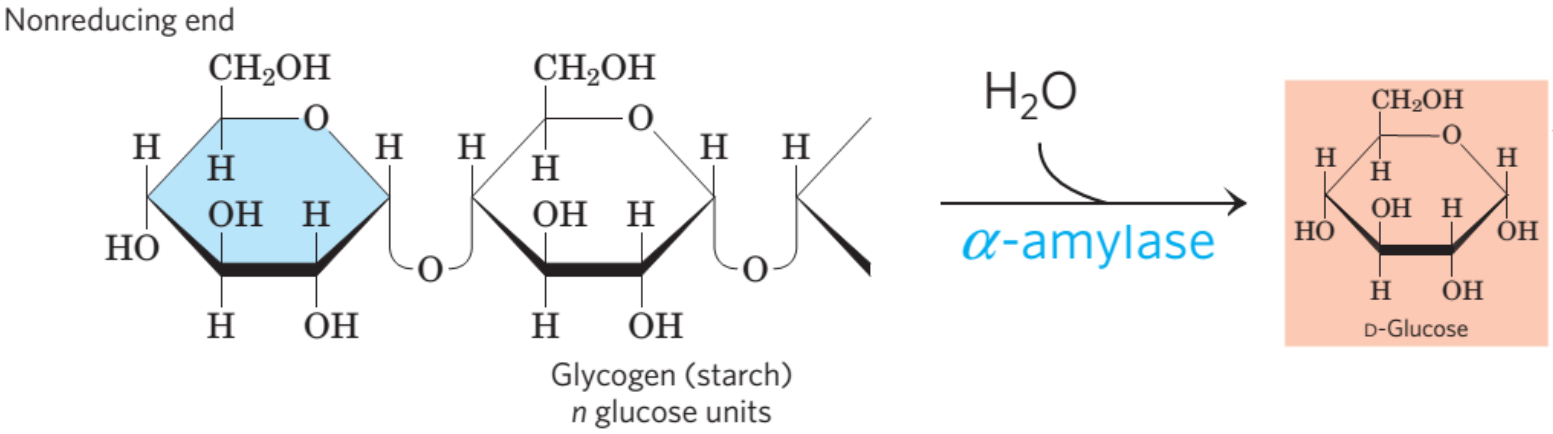

Trehalose
trehalase
Lactose
lactase
D-Galactose
H2O
α-amylase
Dietary glycogen; starch
Endogenous glycogen
Pi
phosphorylase
UDP-galactose
Sucrose
sucrase
D-Glucose
Glucose 1-phosphate
UDP-glucose
hexokinase
ATP
phosphogluco-mutase
D-Mannose
Glucose 6-phosphate
D-Fructose
ATP
hexokinase
ATP
hexokinase
Mannose 6-phosphate
ATP
fructokinase
Fructose 6-phosphate
phosphomannose isomerase
Fructose 1-phosphate
fructose 1-phosphate aldolase
Fructose 1,6-bisphosphate
Glyceraldehyde + Dihydroxyacetone phosphate
ATP
triose kinase
triose phosphate isomerase
Glyceraldehyde 3-phosphate

Dietary Polysaccharides and Disaccharides Undergo Hydrolysis to Monosaccharides

❖For most humans, starch is the major source of carbohydrates in the diet.

❖Digestion begins in the mouth, where salivary α-amylase hydrolyzes the internal (α1→4) glycosidic linkages of starch, producing short polysaccharide fragments or oligosaccharides.

❖(Note that in this hydrolysis reaction, water, not Pi, is the attacking species.)



❖Pancreatic α-amylase yields mainly maltose and maltotriose (the di- and trisaccharides of glucose) and oligosaccharides called limit dextrins, fragments of amylopectin containing (α1→6) branch points.

❖Maltose and dextrins are degraded to glucose by enzymes of the intestinal brush border (the fingerlike microvilli of intestinal epithelial cells, which greatly increase the area of the intestinal surface).

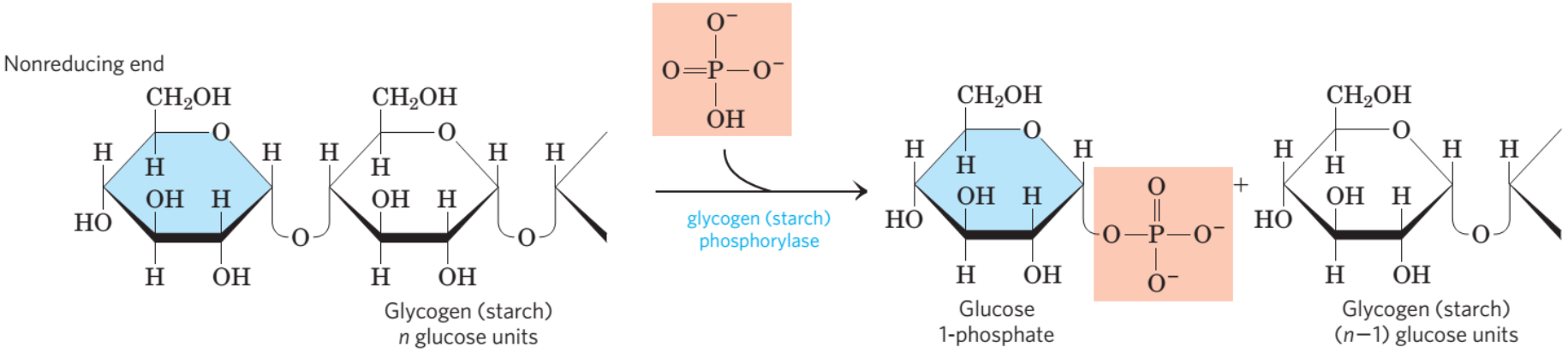
❖Dietary glycogen has essentially the same structure as starch, and its digestion proceeds by the same pathway.

❖Most animals cannot digest cellulose for lack of the enzyme cellulase, which attacks the (β1→4) glycosidic bonds of cellulose.

❖Glycogen stored in animal tissues (endogenous glycogen, primarily liver and skeletal muscle), in microorganisms, or in plant tissues can be mobilized for use within the same cell by a phosphorolytic reaction catalyzed by glycogen phosphorylase (starch phosphorylase in plants).

❖These enzymes catalyze an attack by Pi on the (α1→4) glycosidic linkage that joins the last two glucose residues at a nonreducing end, generating glucose 1-phosphate and a polymer one glucose unit shorter.

❖Phosphorolysis preserves some of the energy of the glycosidic bond in the phosphate ester glucose 1-phosphate.



❖Glycogen phosphorylase (or starch phosphorylase) acts repetitively until it approaches an (1→6) branch point, where its action stops and a debranching enzyme removes the branches.

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

$$\text{Glucose 1-phosphate} \rightleftharpoons \text{glucose 6-phosphate}$$

❖The general name mutase is given to enzymes that catalyze the transfer of a functional group from one position to another in the same molecule.

❖Mutases are a subclass of isomerases, enzymes that interconvert stereoisomers or structural or positional isomers.

❖The glucose 6-phosphate formed in the phosphoglucomutase reaction can enter glycolysis or another pathway such as the pentose phosphate pathway .

❖Disaccharides must be hydrolyzed to monosaccharides before entering cells.

❖Intestinal disaccharides and dextrins are hydrolyzed by enzymes attached to the outer surface of the intestinal epithelial cells:

$$\text{Dextrin} + n\text{H}_2\text{O} \xrightarrow[\text{dextrinase}]{} n \text{ D-glucose}$$

$$\text{Maltose} + \text{H}_2\text{O} \xrightarrow[\text{maltase}]{} 2 \text{ D-glucose}$$

$$\text{Lactose} + \text{H}_2\text{O} \xrightarrow[\text{lactase}]{} \text{D-galactose} + \text{D-glucose}$$

$$\text{Sucrose} + \text{H}_2\text{O} \xrightarrow[\text{sucrase}]{} \text{D-fructose} + \text{D-glucose}$$

$$\text{Trehalose} + \text{H}_2\text{O} \xrightarrow[\text{trehalase}]{} 2 \text{ D-glucose}$$

❖The monosaccharides so formed are actively transported into the epithelial cells, then passed into the blood to be carried to various tissues, where they are phosphorylated and funneled into the glycolytic sequence.

❖Other monosaccharides enter the glycolytic pathway at several points.

❖In most organisms, hexoses other than glucose can undergo glycolysis after conversion to a phosphorylated derivative.

❖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:

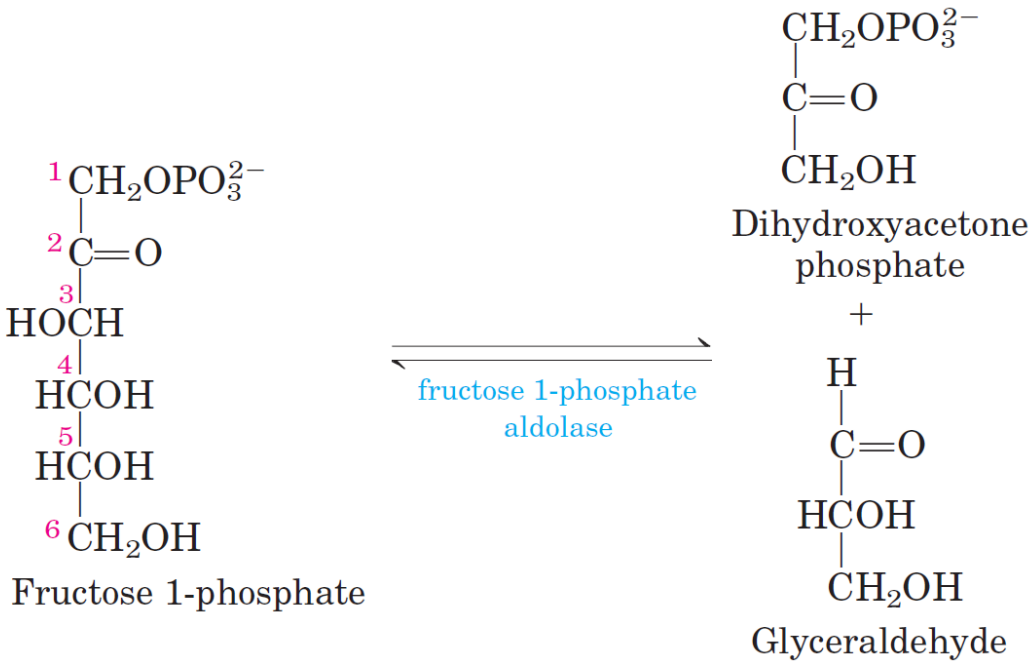
$$\text{Fructose} + \text{ATP} \xrightarrow{Mg^{2+}} \text{fructose 6-phosphate} + \text{ADP}$$

❖This is a major pathway of fructose entry into glycolysis in the muscles and kidney.

❖In the liver, fructose enters by a different pathway. The liver enzyme fructokinase catalyzes the phosphorylation of fructose at C-1 rather than C-6:

$$\text{Fructose} + \text{ATP} \xrightarrow{Mg^{2+}} \text{fructose 1-phosphate} + \text{ADP}$$

❖The fructose 1-phosphate is then cleaved to glyceraldehyde and dihydroxyacetone phosphate by fructose 1-phosphate aldolase:



❖Dihydroxyacetone phosphate is converted to glyceraldehyde 3-phosphate by the glycolytic enzyme triose phosphate isomerase.

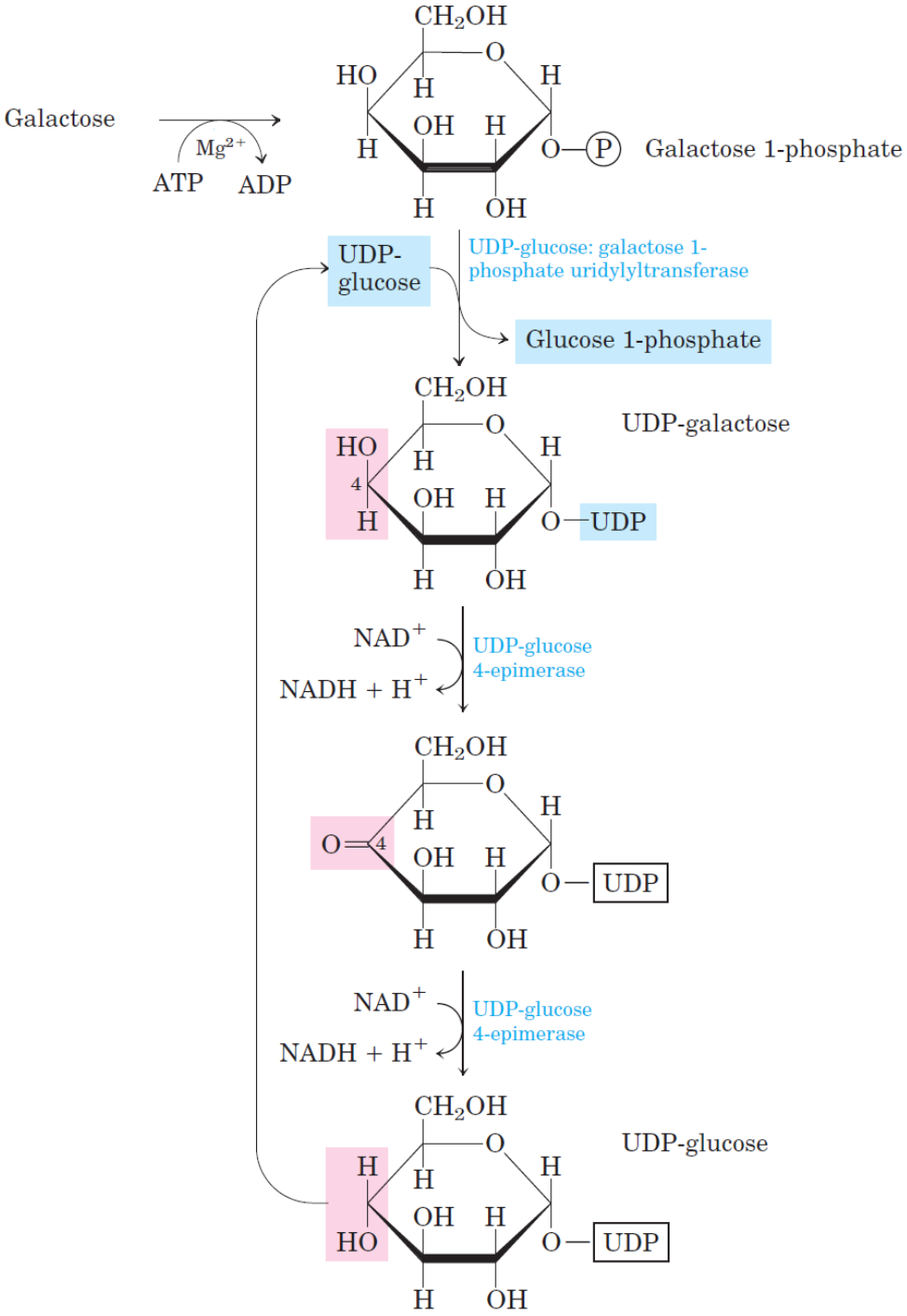
❖Glyceraldehyde is phosphorylated by ATP and triose kinase to glyceraldehyde 3-phosphate:

$$\text{Glyceraldehyde} + \text{ATP} \xrightarrow{Mg^{2+}} \text{glyceraldehyde 3-phosphate} + \text{ADP}$$

❖Thus both products of fructose 1-phosphate hydrolysis enter the glycolytic pathway as glyceraldehyde 3-phosphate.

❖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:

$$\text{Galactose} + \text{ATP} \xrightarrow{Mg^{2+}} \text{galactose 1-phosphate} + \text{ADP}$$



❖The galactose 1-phosphate is then converted to its epimer at C-4, glucose 1-phosphate, by a set of reactions in which **uridine diphosphate (UDP)** functions as a coenzyme-like carrier of hexose groups.

❖A defect in any of the three enzymes in this pathway causes **galactosemia** in humans.

❖D-Mannose, released in the digestion of various polysaccharides and glycoproteins of foods, can be phosphorylated at C-6 by hexokinase:

$$\text{Mannose} + \text{ATP} \xrightarrow{Mg^{2+}} \text{mannose 6-phosphate} + \text{ADP}$$

❖Mannose 6-phosphate is isomerized by phosphomannose isomerase to yield fructose 6-phosphate, an intermediate of glycolysis.

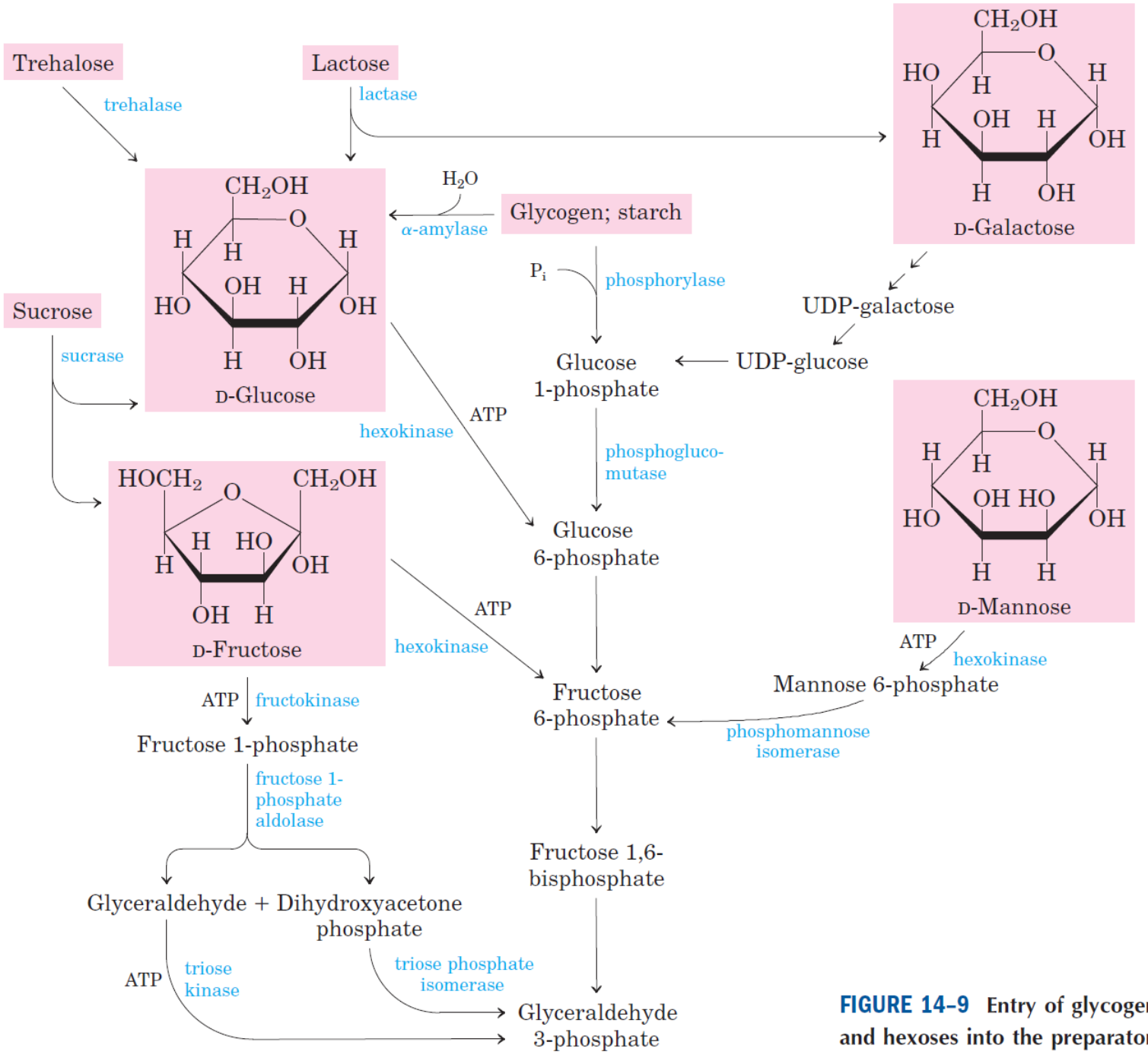


FIGURE 14–9 Entry of glycogen, starch, disaccharides, and hexoses into the preparatory stage of glycolysis.

Fates of Pyruvate under Anaerobic Conditions: Fermentation

❖Under aerobic conditions, the pyruvate formed in the final step of glycolysis is oxidized to acetate (acetylCoA), which enters the citric acid cycle and is oxidized to CO_2 and H_2O.

❖The NADH formed by dehydrogenation of glyceraldehyde 3-phosphate is ultimately reoxidized to NAD^+ by passage of its electrons to O_2 in mitochondrial respiration.

❖Under hypoxic (low-oxygen) conditions, however—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 O_2.

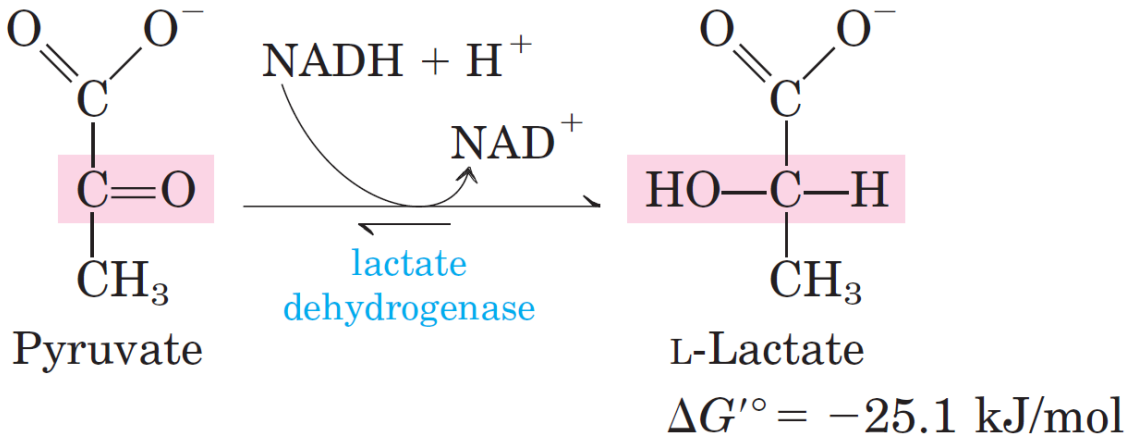
❖Failure to regenerate NAD^+ would leave the cell with no electron acceptor for the oxidation of glyceraldehyde 3-phosphate, and the energy-yielding reactions of glycolysis would stop.

❖NAD^+ must therefore be regenerated in some other way.

❖The earliest cells lived in an atmosphere almost devoid of oxygen and had to develop strategies for deriving energy from fuel molecules under anaerobic conditions.

❖Most modern organisms have retained the ability to continually regenerate NAD^+ during anaerobic glycolysis by transferring electrons from NADH to form a reduced end product such as lactate or ethanol.

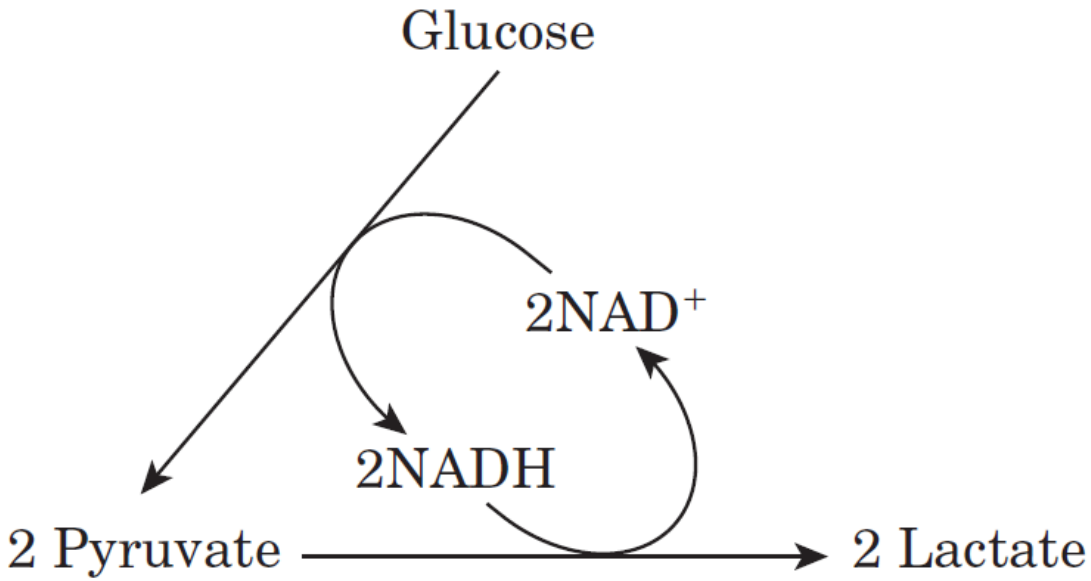
❖When animal tissues cannot be supplied with sufficient oxygen to support aerobic oxidation of the pyruvate and NADH produced in glycolysis, NAD^+ is regenerated from NADH by the reduction of pyruvate to lactate.



❖Some tissues and cell types (such as erythrocytes, which have no mitochondria and thus cannot oxidize pyruvate to CO2) produce lactate from glucose even under aerobic conditions.

❖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 NAD1, 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.

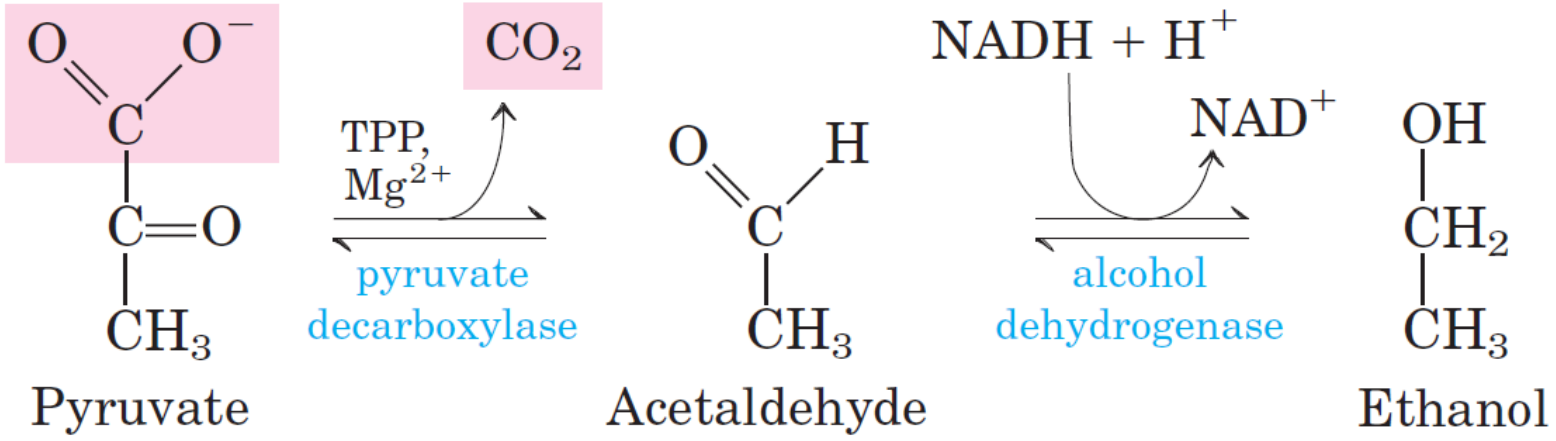
❖Although conversion of glucose to lactate includes two oxidation-reduction steps, there is no net change in the oxidation state of carbon; in glucose ($C_6H_{12}O_6$) and lactic acid ($C_3H_6O_3$), the H:C ratio is the same.

❖Nevertheless, some of the energy of the glucose molecule has been extracted by its conversion to lactate—enough to give a net yield of two molecules of ATP for every glucose molecule consumed.

❖Fermentation is the general term for such processes, which extract energy (as ATP) but do not consume oxygen or change the concentrations of NAD^+ or NADH.

❖Yeast and other microorganisms ferment glucose to ethanol and CO_2, rather than to lactate.

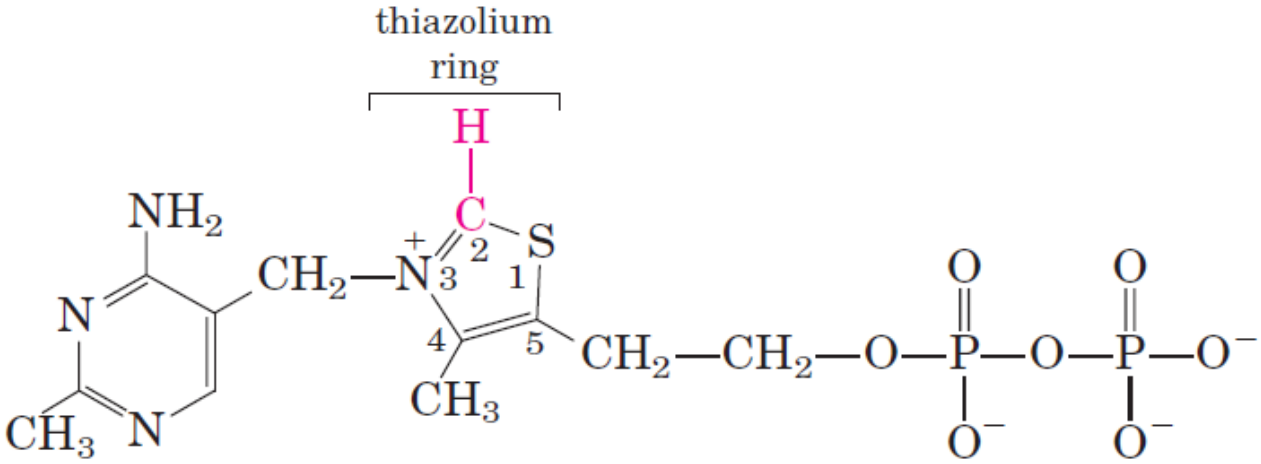
❖Glucose is converted to pyruvate by glycolysis, and the pyruvate is converted to ethanol and CO_2 in a two-step process:



❖In the first step, pyruvate is decarboxylated in an irreversible reaction catalyzed by pyruvate decarboxylase.

❖This reaction is a simple decarboxylation and does not involve the net oxidation of pyruvate.

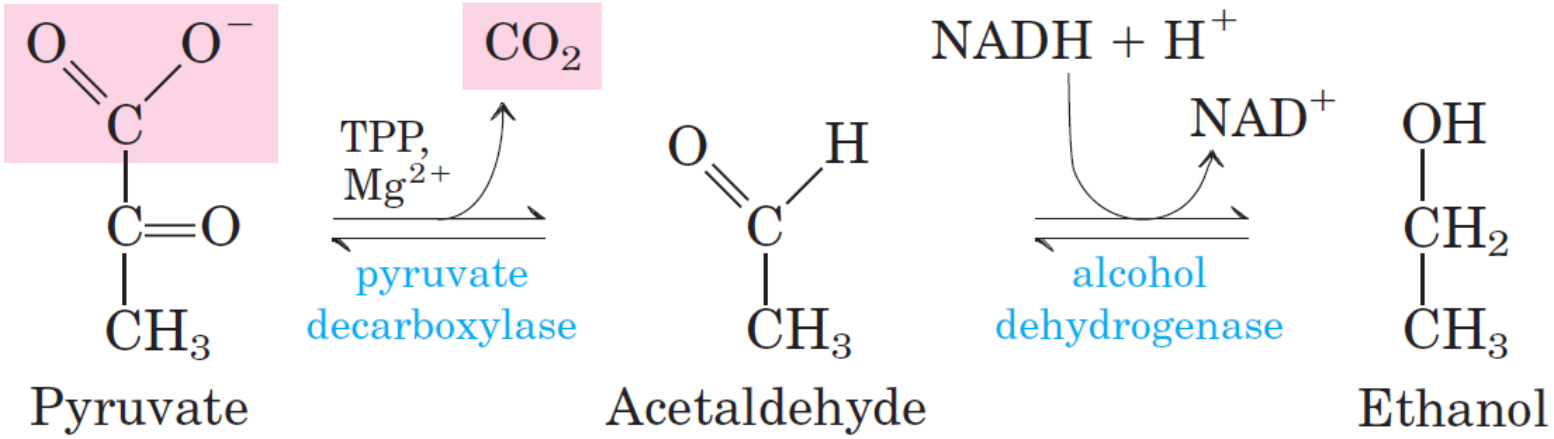
❖Pyruvate decarboxylase requires Mg^{2+} and has a tightly bound coenzyme, thiamine pyrophosphate.



Thiamine pyrophosphate (TPP)

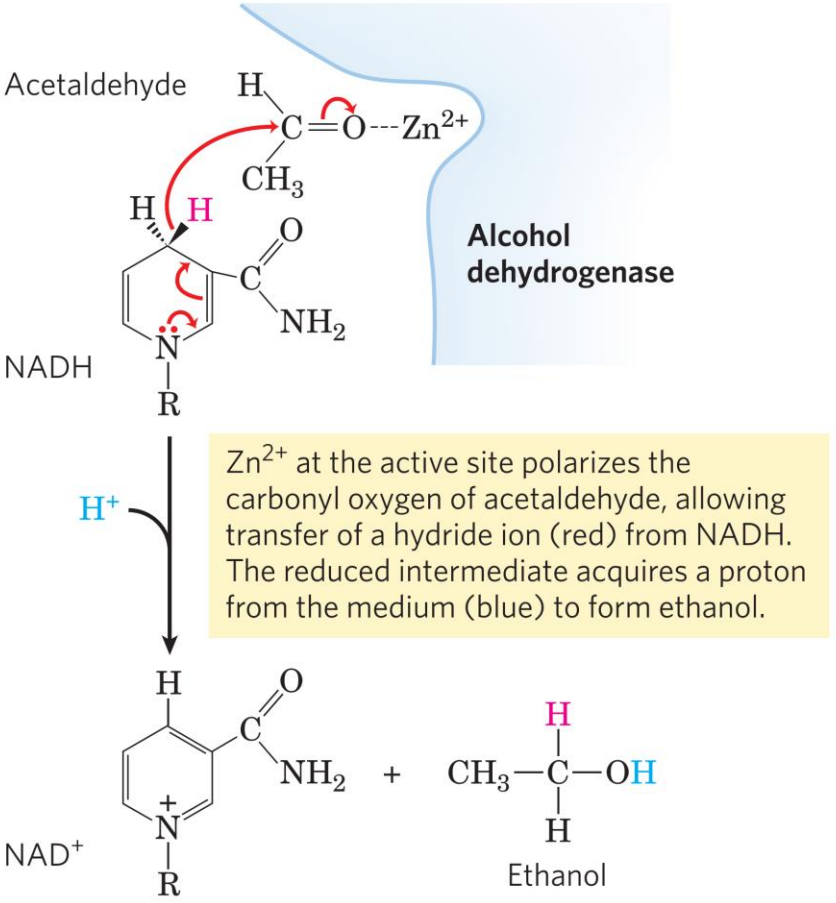
(a)

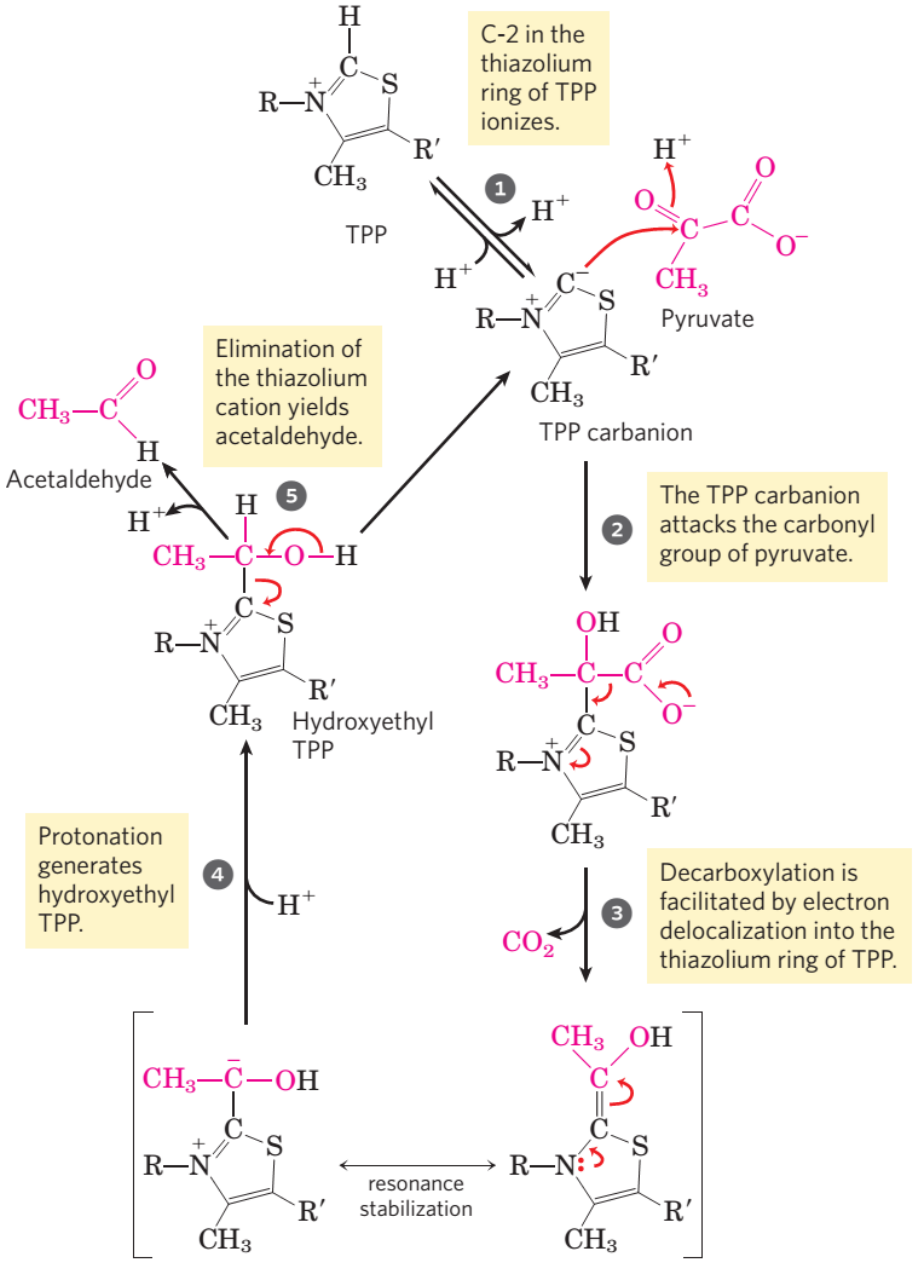
❖In the second step, acetaldehyde is reduced to ethanol through the action of alcohol dehydrogenase, with the reducing power furnished by NADH derived from the dehydrogenation of glyceraldehyde 3-phosphate.



❖Ethanol and CO_2 are thus the end products of ethanol fermentation, and the overall equation is:

$$\text{Glucose} + 2\text{ADP} + 2\text{P}_\text{i} \longrightarrow 2\ \text{ethanol} + 2\text{CO}_2 + 2\text{ATP} + 2\text{H}_2\text{O}$$


Acetaldehyde
Alcohol dehydrogenase
NADH
NAD+
Ethanol
H+
Zn2+ at the active site polarizes the carbonyl oxygen of acetaldehyde, allowing transfer of a hydride ion (red) from NADH. The reduced intermediate acquires a proton from the medium (blue) to form ethanol.


C-2 in the thiazolium ring of TPP ionizes.
TPP
Pyruvate
TPP carbanion
Elimination of the thiazolium cation yields acetaldehyde.
Acetaldehyde
The TPP carbanion attacks the carbonyl group of pyruvate.
Hydroxyethyl TPP
Protonation generates hydroxyethyl TPP.
Decarboxylation is facilitated by electron delocalization into the thiazolium ring of TPP.
resonance stabilization

❖Beer brewing was a science learned early in human history, and later refined for larger-scale production.

❖Brewers prepare beer by ethanol fermentation of the carbohydrates in cereal grains (seeds) such as barley, carried out by yeast glycolytic enzymes.

❖The carbohydrates, largely polysaccharides, must first be degraded to disaccharides and monosaccharides.

❖In a process called malting, the barley seeds are allowed to germinate until they form the hydrolytic enzymes required to break down their polysaccharides, at which point germination is stopped by controlled heating.

❖The product is malt, which contains enzymes that catalyze the hydrolysis of the β linkages of cellulose and other cell wall polysaccharides of the barley husks, and enzymes such as α-amylase and maltase.

❖ The brewer next prepares the wort, the nutrient medium required for fermentation by yeast cells.

❖The malt is mixed with water and then mashed or crushed.

❖This allows the enzymes formed in the malting process to act on the cereal polysaccharides to form maltose, glucose, and other simple sugars, which are soluble in the aqueous medium.

❖The remaining cell matter is then separated, and the liquid wort is boiled with hops to give flavor.

❖The wort is cooled and then aerated.

❖Now the yeast cells are added.

❖In the aerobic wort the yeast grows and reproduces very rapidly, using energy obtained from available sugars.

❖No ethanol forms during this stage, because the yeast, amply supplied with oxygen, oxidizes the pyruvate formed by glycolysis to CO_2 and H_2O via the citric acid cycle.

❖When all the dissolved oxygen in the vat of wort has been consumed, the yeast cells switch to anaerobic metabolism, and from this point they ferment the sugars into ethanol and CO_2.

❖The fermentation process is controlled in part by the concentration of the ethanol formed, by the pH, and by the amount of remaining sugar.

❖After fermentation has been stopped, the cells are removed and the “raw” beer is ready for final processing.

❖In the final steps of brewing, the amount of foam (or head) on the beer, which results from dissolved proteins, is adjusted.

❖Normally this is controlled by proteolytic enzymes that arise in the malting process.

❖If these enzymes act on the proteins too long, the beer will have very little head and will be flat; if they do not act long enough, the beer will not be clear when it is cold.

❖Sometimes proteolytic enzymes from other sources are added to control the head.

❖ Much of the technology developed for large-scale production of alcoholic beverages is now finding application to a wholly different problem: the production of ethanol as a renewable fuel.

❖With the continuing depletion of the known stores of fossil fuels and the rising cost of fuel for internal combustion engines, there is increased interest in the use of ethanol as a fuel substitute or extender.

❖The principal advantage of ethanol as a fuel is that it can be produced from relatively inexpensive and renewable resources rich in sucrose, starch, or cellulose—starch from corn or wheat, sucrose from beets or cane, and cellulose from straw, forest industry waste, or municipal solid waste.

❖Typically, the raw material (feedstock) is first converted chemically to monosaccharides, then fed to a hardy strain of yeast in an industrial-scale fermenter.

❖The fermentation can yield not only ethanol for fuel but also side products such as proteins that can be used as animal feed.

Gluconeogenesis

❖The central role of glucose in metabolism arose early in evolution, and this sugar remains the nearly universal fuel and building block in modern organisms, from microbes to humans.

❖In mammals, some tissues depend almost completely on glucose for their metabolic energy.

❖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.

❖However, the supply of glucose from these stores is not always sufficient; between meals and during longer fasts, or after vigorous exercise, glycogen is depleted.

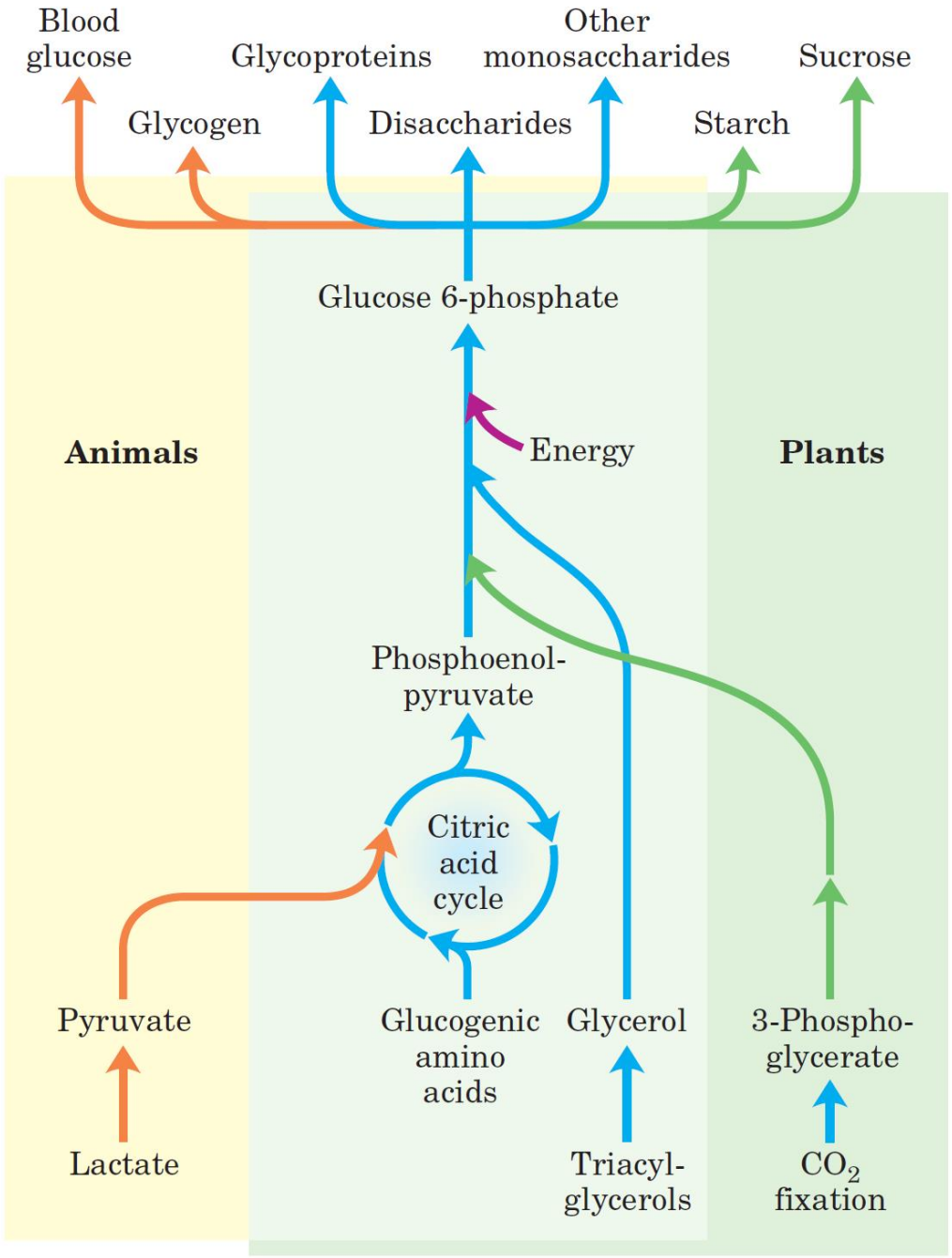
❖For these times, organisms need a method for synthesizing glucose from noncarbohydrate precursors.

❖This is accomplished by a pathway called gluconeogenesis (“new formation of sugar”), which converts pyruvate and related three- and four-carbon compounds to glucose.

❖Gluconeogenesis occurs in all animals, plants, fungi, and microorganisms.

❖The reactions are essentially the same in all tissues and all species.

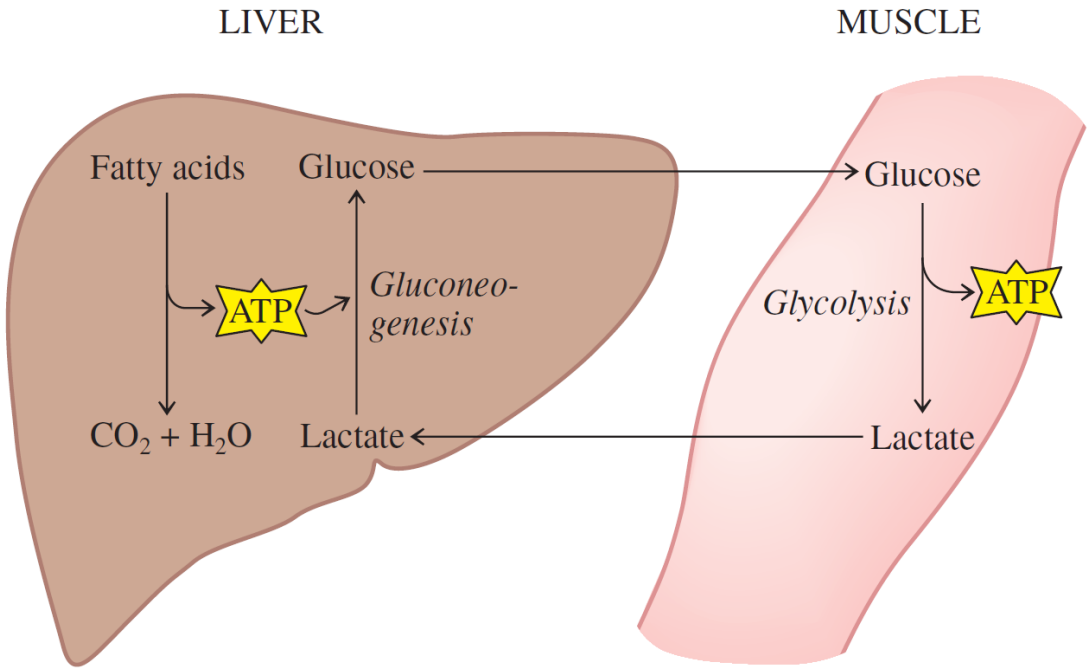
❖The important precursors of glucose in animals are three-carbon compounds such as lactate, pyruvate, and glycerol, as well as certain amino acids


Blood glucose
Glycoproteins
Other monosaccharides
Sucrose
Glycogen
Disaccharides
Starch
Glucose 6-phosphate
Animals
Energy
Plants
Phosphoenol-pyruvate
Citric acid cycle
Pyruvate
Glucogenic amino acids
Glycerol
3-Phospho-glycerate
Lactate
Triacyl-glycerols
CO_2 fixation

❖In mammals, gluconeogenesis takes place mainly in the liver, and to a lesser extent in renal cortex and in the epithelial cells that line the inside of the small intestine.

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

❖After vigorous exercise, lactate produced by anaerobic glycolysis in skeletal muscle returns to the liver and is converted to glucose, which moves back to muscle and is converted to glycogen—a circuit called the Cori cycle

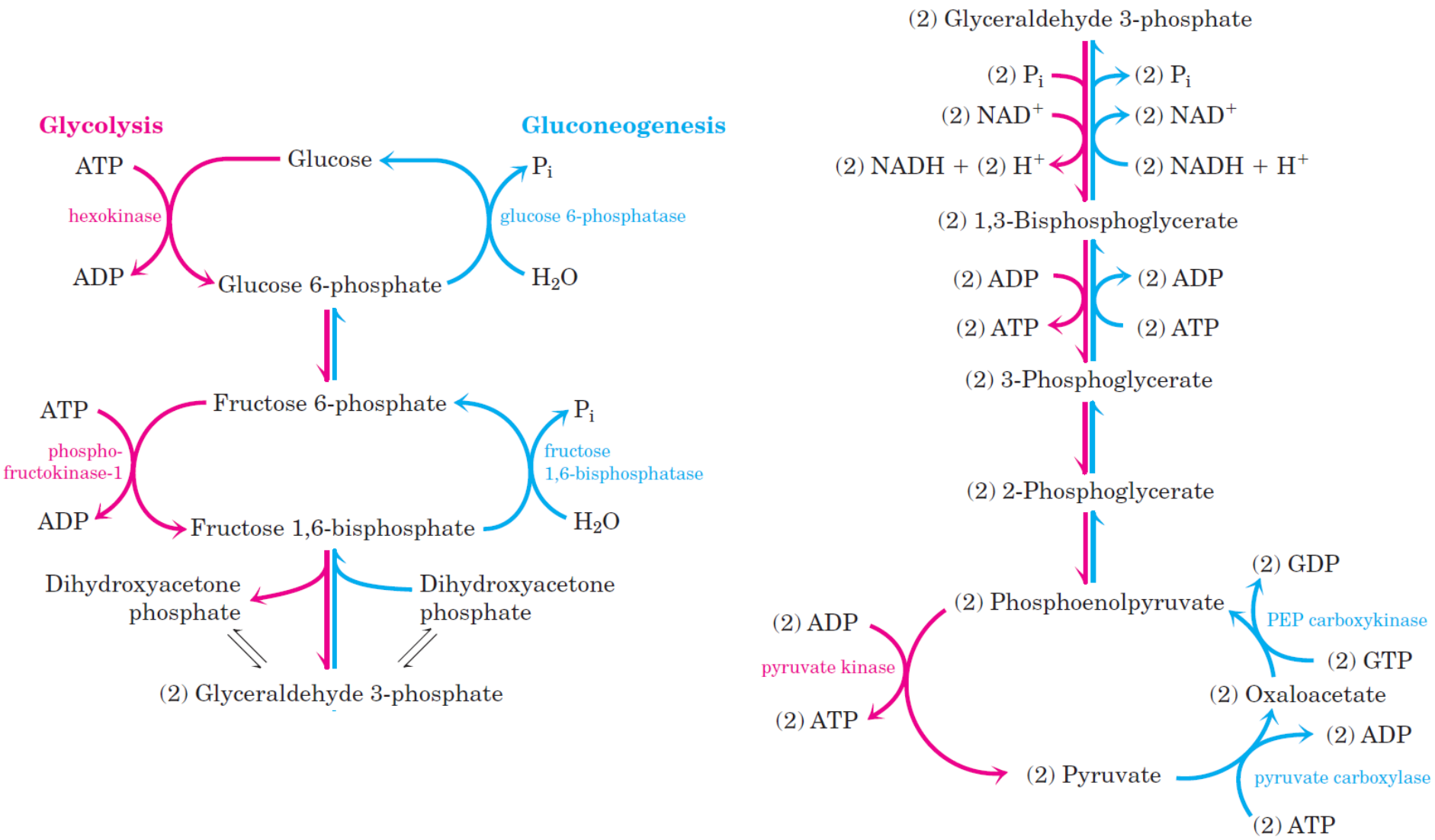


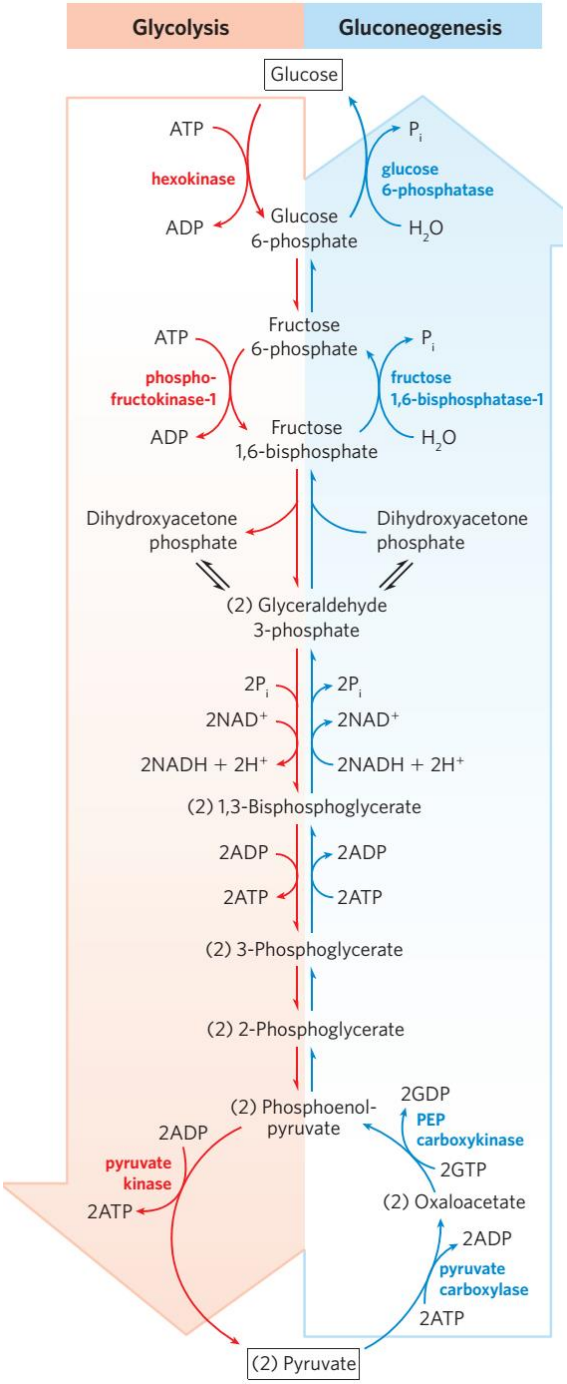
❖Gluconeogenesis and glycolysis are not identical pathways running in opposite directions, although they do share several steps.

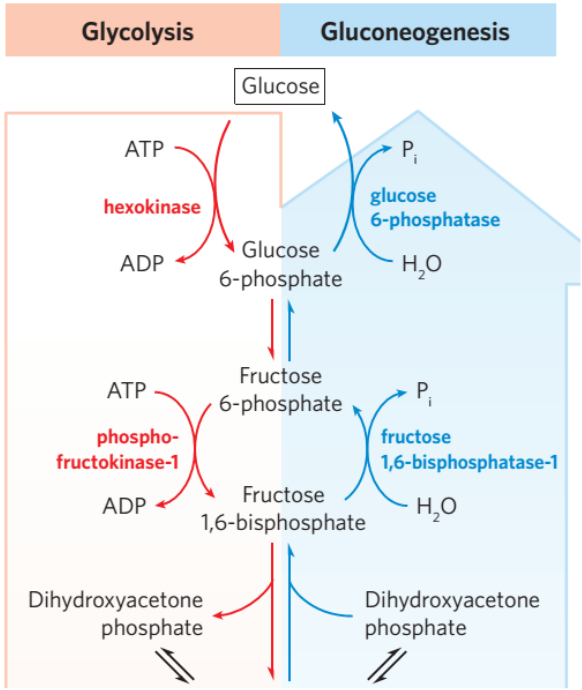
❖7 of the 10 enzymatic reactions of gluconeogenesis are the reverse of glycolytic reactions.

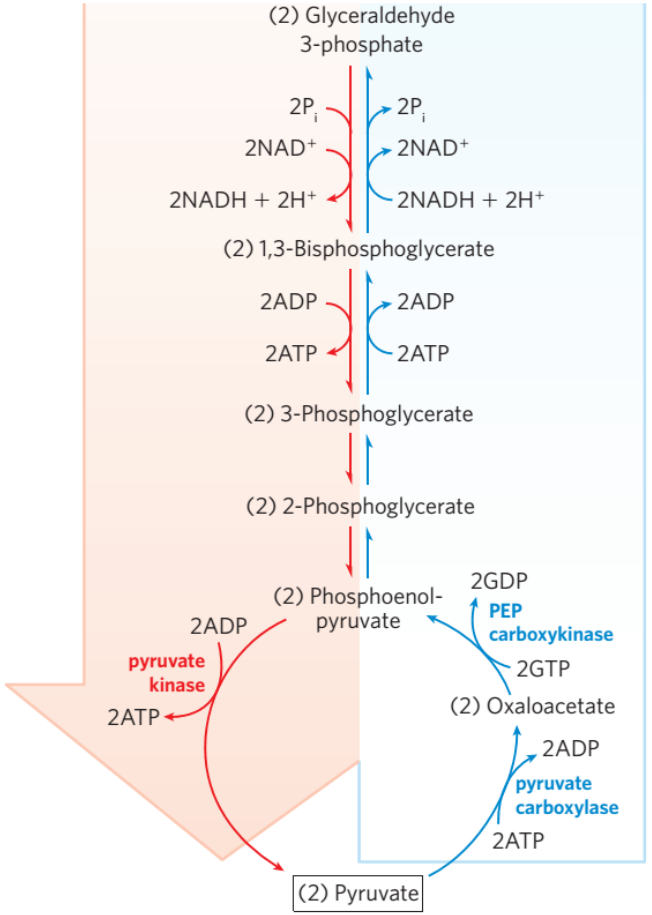
❖However, three reactions of glycolysis are essentially irreversible in vivo and cannot be used in gluconeogenesis:

- the conversion of glucose to glucose 6-phosphate by hexokinase,
- the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate by phosphofructokinase-1, and
- the conversion of phosphoenolpyruvate to pyruvate by pyruvate kinase.


Glycolysis
Gluconeogenesis
ATP
Glucose
P_i
hexokinase
glucose 6-phosphatase
ADP
Glucose 6-phosphate
H_2O
ATP
Fructose 6-phosphate
P_i
phospho-
fructokinase-1
fructose
1,6-bisphosphatase
ADP
Fructose 1,6-bisphosphate
H_2O
Dihydroxyacetone
phosphate
Dihydroxyacetone
phosphate
(2) Glyceraldehyde 3-phosphate
(2) Glyceraldehyde 3-phosphate
(2) P_i
(2) P_i
(2) NAD^+
(2) NAD^+
(2) NADH + (2) H^+
(2) NADH + H^+
(2) 1,3-Bisphosphoglycerate
(2) ADP
(2) ADP
(2) ATP
(2) ATP
(2) 3-Phosphoglycerate
(2) 2-Phosphoglycerate
(2) GDP
(2) Phosphoenolpyruvate
PEP carboxykinase
(2) ADP
(2) GTP
pyruvate kinase
(2) Oxaloacetate
(2) ATP
(2) ADP
(2) Pyruvate
pyruvate carboxylase
(2) ATP


Glycolysis
Gluconeogenesis
Glucose
ATP
hexokinase
ADP
Glucose 6-phosphate
P_i
glucose 6-phosphatase
H_2O
Fructose 6-phosphate
phospho-fructokinase-1
fructose 1,6-bisphosphatase-1
Fructose 1,6-bisphosphate
Dihydroxyacetone phosphate
(2) Glyceraldehyde 3-phosphate
$2P_i$
2NAD+
2NADH + 2H+
(2) 1,3-Bisphosphoglycerate
2ADP
2ATP
(2) 3-Phosphoglycerate
(2) 2-Phosphoglycerate
(2) Phosphoenol-pyruvate
2GDP
PEP carboxykinase
2GTP
(2) Oxaloacetate
pyruvate kinase
pyruvate carboxylase
(2) Pyruvate


Glycolysis
Gluconeogenesis
Glucose
ATP
hexokinase
ADP
Glucose 6-phosphate
P_i
glucose 6-phosphatase
H_2O
Fructose 6-phosphate
phospho-fructokinase-1
fructose 1,6-bisphosphatase-1
Fructose 1,6-bisphosphate
Dihydroxyacetone phosphate


(2) Glyceraldehyde 3-phosphate
$2P_i$
2NAD+
2NADH + 2H+
(2) 1,3-Bisphosphoglycerate
2ADP
2ATP
(2) 3-Phosphoglycerate
(2) 2-Phosphoglycerate
(2) Phosphoenol-pyruvate
2GDP
PEP carboxykinase
2GTP
(2) Oxaloacetate
pyruvate kinase
pyruvate carboxylase
(2) Pyruvate

❖In gluconeogenesis, the three irreversible steps are bypassed by a separate set of enzymes, catalyzing reactions that are sufficiently exergonic to be effectively irreversible in the direction of glucose synthesis.

❖Thus, both glycolysis and gluconeogenesis are irreversible processes in cells. In animals, both pathways occur largely in the cytosol, necessitating their reciprocal and coordinated regulation.

❖ Separate regulation of the two pathways is brought about through controls exerted on the enzymatic steps unique to each.

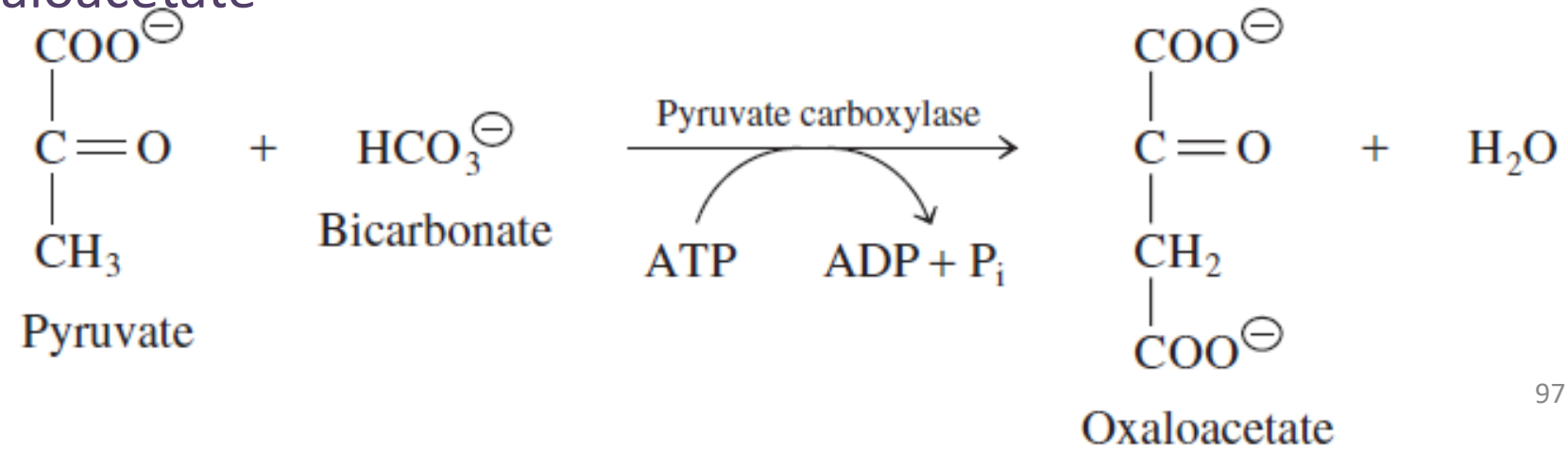
❖We begin by considering the three bypass reactions of gluconeogenesis.

Conversion of Pyruvate to Phosphoenolpyruvate

❖The first of the bypass reactions in gluconeogenesis is the conversion of pyruvate to phosphoenolpyruvate (PEP).

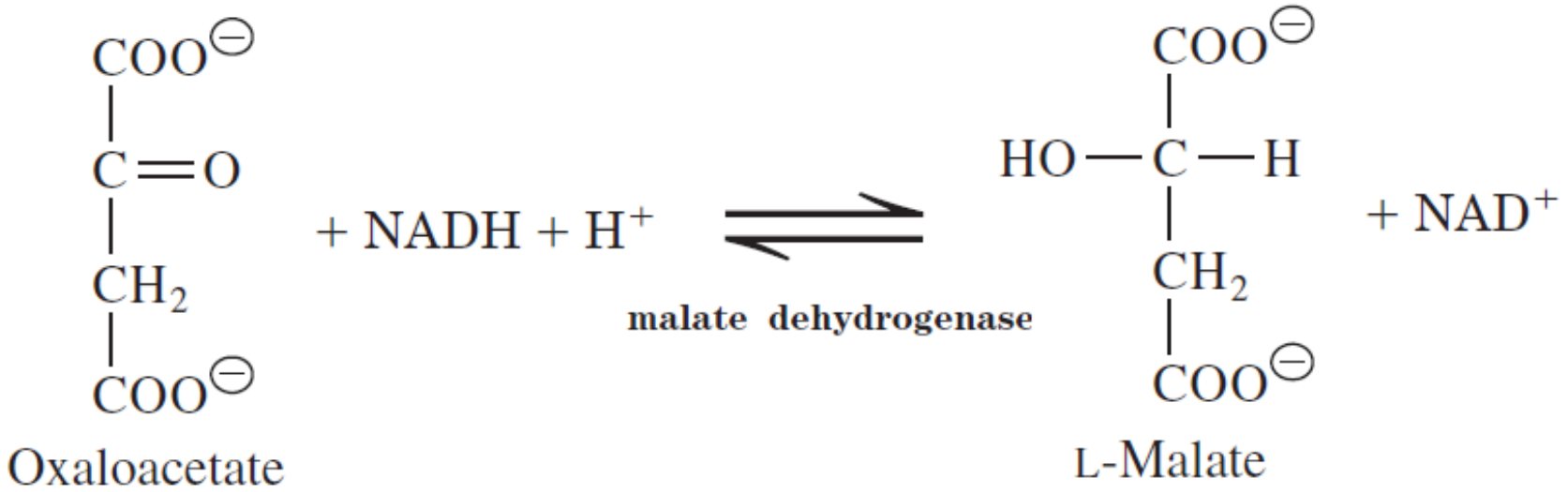
❖Pyruvate is first transported from the cytosol into mitochondria or is generated from alanine within mitochondria by transamination.

❖Then pyruvate carboxylase, a mitochondrial enzyme that requires the coenzyme biotin, converts the pyruvate to oxaloacetate

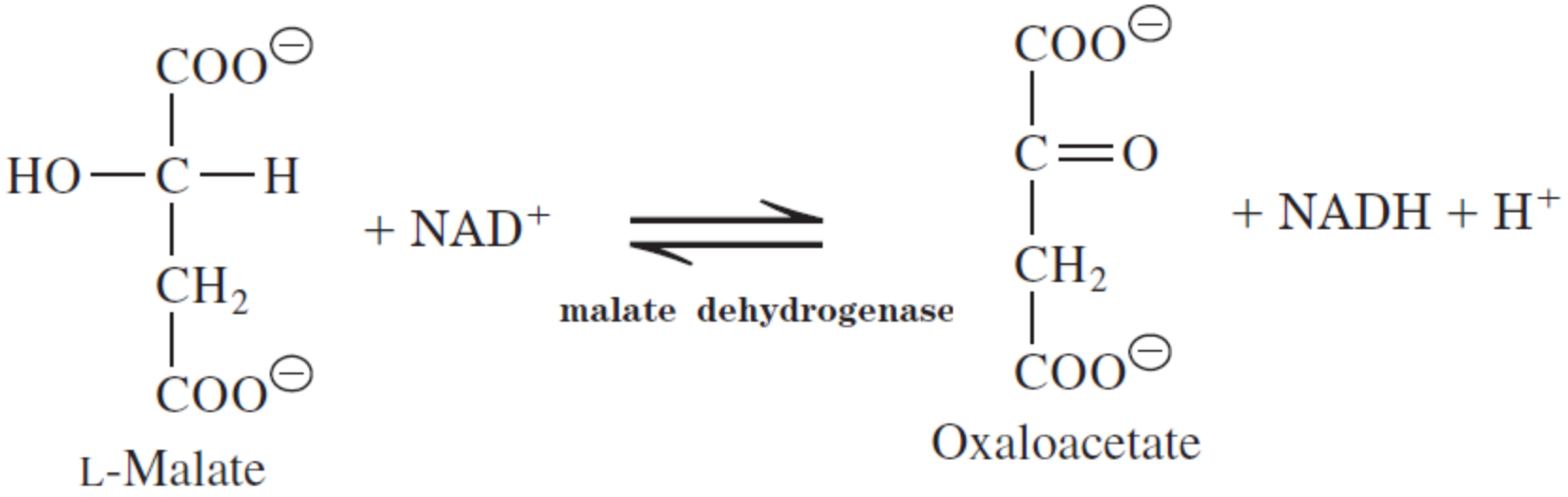


❖Pyruvate carboxylase is the first regulatory enzyme in the gluconeogenic pathway, requiring acetyl-CoA as a positive effector.

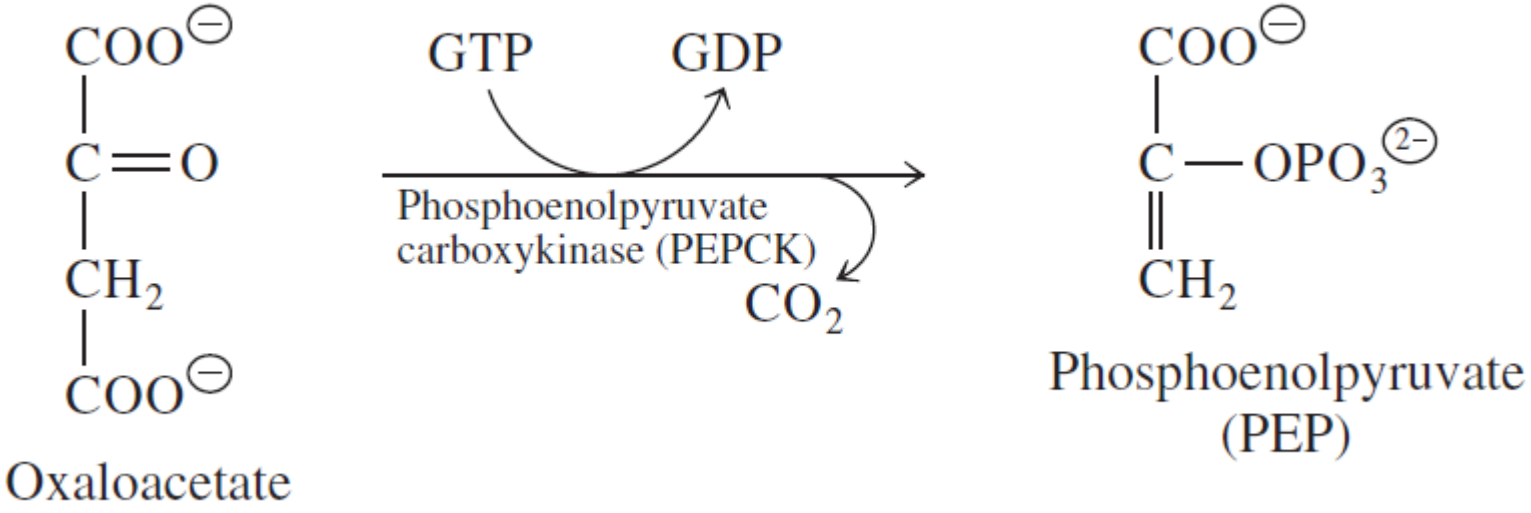
❖Because the mitochondrial membrane has no transporter for oxaloacetate, before export to the cytosol the oxaloacetate formed from pyruvate must be reduced to malate by mitochondrial malate dehydrogenase, at the expense of NADH:



❖Malate leaves the mitochondrion through aspecific transporter in the inner mitochondrial membrane, and in the cytosol it is reoxidized to oxaloacetate, with the production of cytosolic NADH:



❖The oxaloacetate is then converted to PEP by phosphoenolpyruvate carboxykinase.



❖This Mg^{2+}-dependent reaction requires GTP as the phosphoryl group donor:

❖The reaction is reversible under intracellular conditions; the formation of one high-energy phosphate compound (PEP) is balanced by the hydrolysis of another (GTP).

❖The overall equation for this set of bypass reactions, the sum of Equations is;

$$\text{Pyruvate} + \text{ATP} + \text{GTP} + \text{HCO}_3^- \longrightarrow \text{PEP} + \text{ADP} + \text{GDP} + \text{P}_\text{i} + \text{CO}_2$$
$$\Delta G'^\circ = 0.9 \text{ kJ/mol}$$

❖There is a logic to the route of these reactions through the mitochondrion.

❖The [NADH]/[NAD^+] ratio is about 10^5 times lower than in mitochondria.

❖Because cytosolic NADH is consumed in gluconeogenesis, 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 reducing equivalents to the cytosol, where they are scarce.

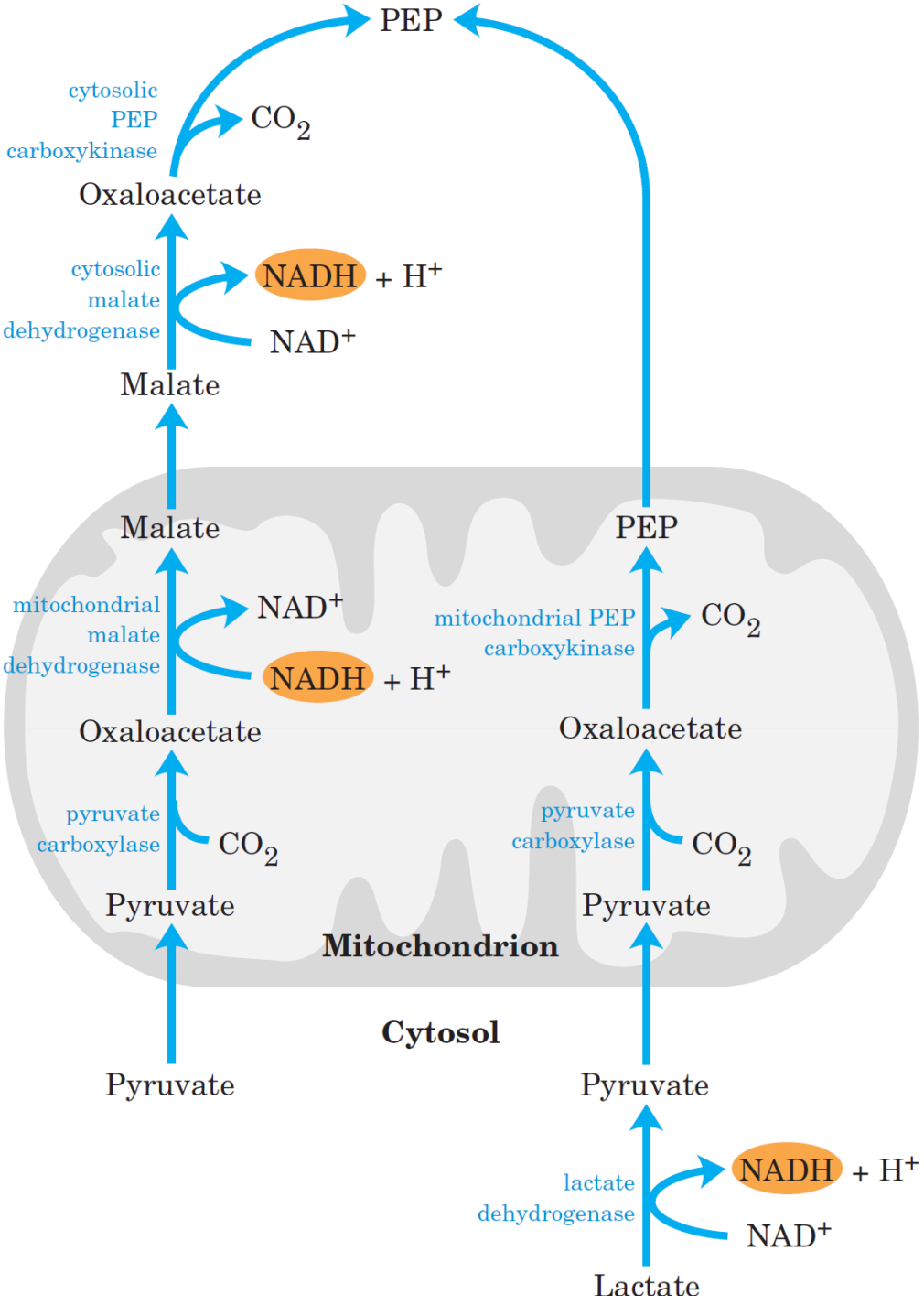
❖A second pyruvate → PEP bypass predominates when lactate is the glucogenic precursor.

❖This pathway makes use of lactate produced by glycolysis in erythrocytes or anaerobic muscle, for example, and it is particularly important in large vertebrates after vigorous exercise.

❖The conversion of lactate to pyruvate in the cytosol of hepatocytes yields NADH, and the export of reducing equivalents (as malate) from mitochondria is therefore unnecessary.

❖After the pyruvate produced by the lactate dehydrogenase reaction is transported into the mitochondrion, it is converted to oxaloacetate by pyruvate carboxylase, as described above.

❖This oxaloacetate, however, is converted directly to PEP by a mitochondrial isozyme of PEP carboxykinase, and the PEP is transported out of the mitochondrion to continue on the gluconeogenic path.


PEP
cytosolic PEP carboxykinase
CO_2
Oxaloacetate
cytosolic malate dehydrogenase
NADH + H^+
NAD^+
Malate
Malate
PEP
mitochondrial malate dehydrogenase
NAD^+
NADH + H^+
mitochondrial PEP carboxykinase
CO_2
Oxaloacetate
Oxaloacetate
pyruvate carboxylase
CO_2
pyruvate carboxylase
CO_2
Pyruvate
Pyruvate
Mitochondrion
Cytosol
Pyruvate
Pyruvate
lactate dehydrogenase
NADH + H^+
NAD^+
Lactate

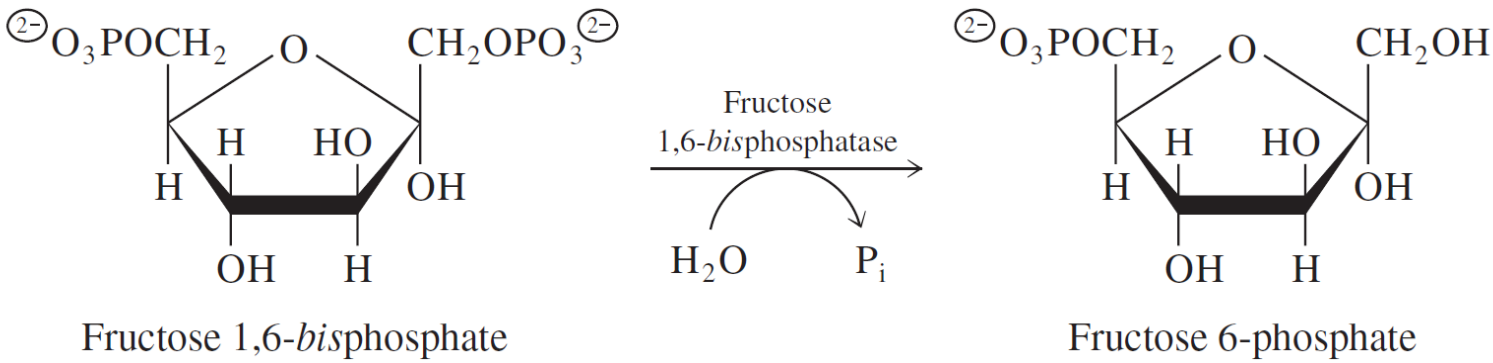
Conversion of Fructose 1,6-Bisphosphate to Fructose 6-Phosphate

❖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 in intact cells, the generation of fructose 6-phosphate from fructose 1,6-bisphosphate is catalyzed by a different enzyme, Mg^{2+}-dependent fructose 1,6-bisphosphatase (FBPase-1).

Fructose 1,6-bisphosphate + H_2O ⟶ fructose 6-phosphate + P_i

$\Delta G'^{\circ} = -16.3$ kJ/mol



Fructose 1,6-*bis*phosphate

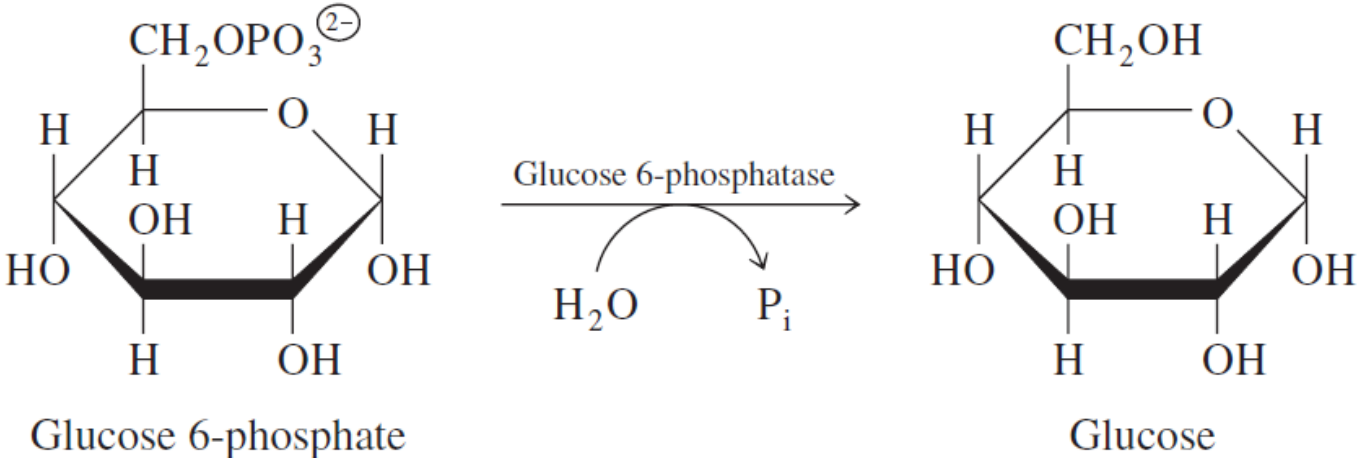
Fructose 6-phosphate

Conversion of Glucose 6-Phosphate to Glucose

❖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:



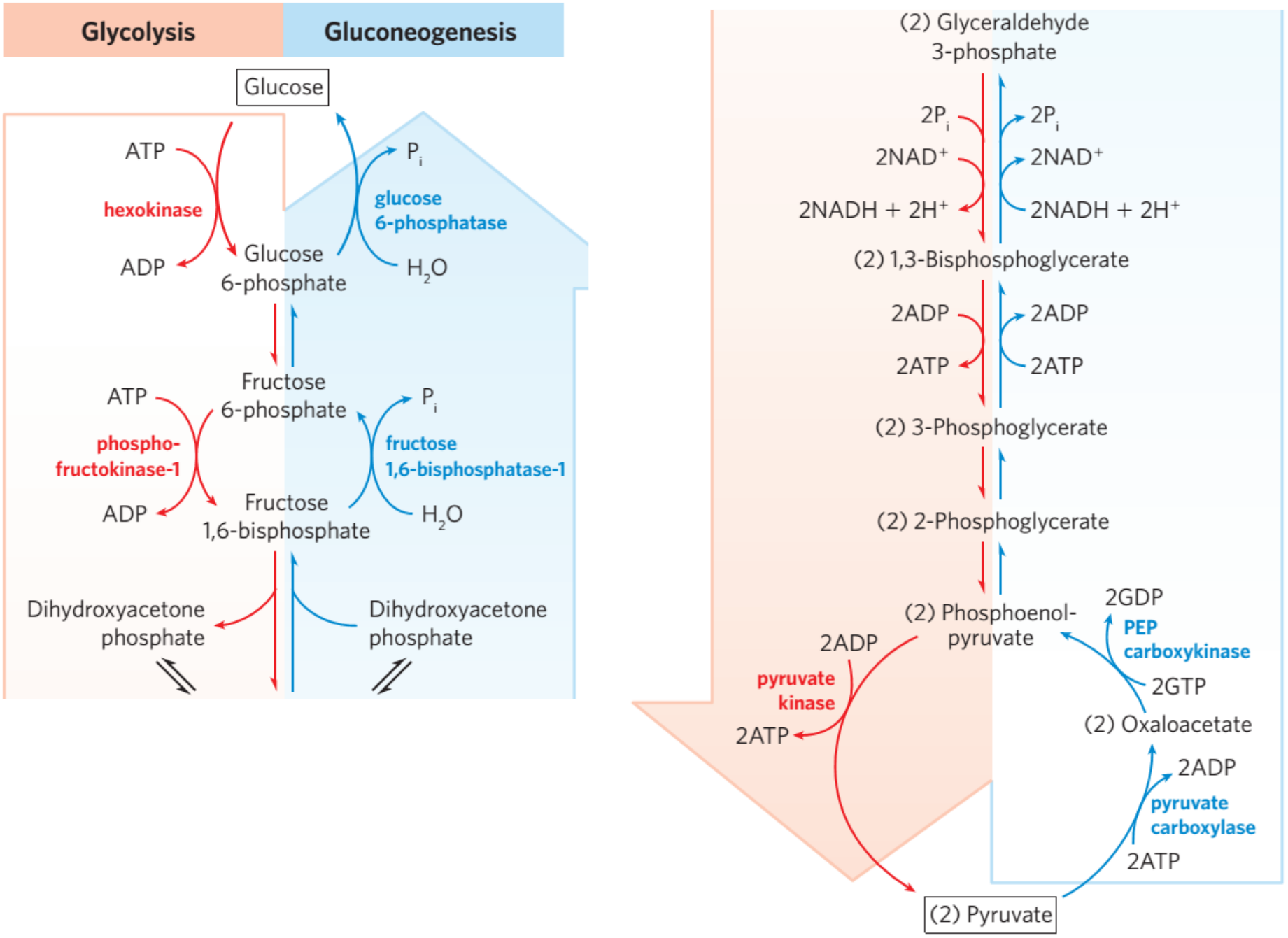
Glucose 6-phosphate

Glucose

❖This Mg^{2+}-activated enzyme is found on the lumenal side of the endoplasmic reticulum of hepatocytes, renal cells, and epithelial cells of the small intestine, but not in other tissues, which are therefore 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.


Glycolysis
Gluconeogenesis
Glucose
ATP
hexokinase
ADP
Glucose 6-phosphate
P_i
glucose 6-phosphatase
H_2O
Fructose 6-phosphate
ATP
phospho-fructokinase-1
ADP
Fructose 1,6-bisphosphate
P_i
fructose 1,6-bisphosphatase-1
H_2O
Dihydroxyacetone phosphate
Dihydroxyacetone phosphate
(2) Glyceraldehyde 3-phosphate
$2P_i$
$2NAD^+$
$2NADH + 2H^+$
$2P_i$
$2NAD^+$
$2NADH + 2H^+$
(2) 1,3-Bisphosphoglycerate
2ADP
2ATP
2ADP
2ATP
(2) 3-Phosphoglycerate
(2) 2-Phosphoglycerate
(2) Phosphoenol-pyruvate
2GDP
PEP carboxykinase
2GTP
2ADP
pyruvate kinase
2ATP
(2) Oxaloacetate
2ADP
pyruvate carboxylase
2ATP
(2) Pyruvate

Gluconeogenesis Is Energetically Expensive, but Essential

❖The sum of the biosynthetic reactions leading from pyruvate to free blood glucose is

$$2\ \text{Pyruvate} + 4\text{ATP} + 2\text{GTP} + 2\text{NADH} + 2\text{H}^+ + 4\text{H}_2\text{O} \longrightarrow \text{glucose} + 4\text{ADP} + 2\text{GDP} + 6\text{P}_\text{i} + 2\text{NAD}^+$$

❖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, this is not simply the reverse of the equation for conversion of glucose to pyruvate by glycolysis, which would require only two molecules of ATP:

$$\text{Glucose} + 2\text{ADP} + 2\text{P}_\text{i} + 2\text{NAD}^+ \longrightarrow 2\ \text{pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}^+ + 2\text{H}_2\text{O}$$

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_2O ⇌ 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_2O ⟶ glucose + P_i	
Sum: 2 Pyruvate + 4ATP + 2GTP + 2NADH + $2H^+$ + $4H_2O$ ⟶ glucose + 4ADP + 2GDP + $6P_i$ + $2NAD^+$	

Note: 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 (p. 517).

Citric Acid Cycle Intermediates and Some Amino Acids Are Glucogenic

❖The biosynthetic pathway to glucose described above allows the net synthesis of glucose not only from pyruvate but also from the four-, five-, and six-carbon intermediates of the citric acid cycle.

❖Citrate, isocitrate, α-ketoglutarate, succinyl-CoA, succinate, fumarate, and malate—all are citric acid cycle intermediates that can undergo oxidation to oxaloacetate.

❖Some or all of the carbon atoms of most amino acids derived from proteins are ultimately catabolized to pyruvate or to intermediates of the citric acid cycle.

❖Such amino acids can therefore undergo net conversion to glucose and are said to be glucogenic.

❖No net conversion of fatty acids to glucose occurs in mammals.

❖The catabolism of most fatty acids yields only acetyl-CoA.

❖Mammals cannot use acetyl-CoA as a precursor of glucose, because the pyruvate dehydrogenase reaction is irreversible and cells have no other pathway to convert acetyl-CoA to pyruvate.

❖Plants, yeast, and many bacteria do have a pathway (the glyoxylate cycle) for converting acetyl-CoA to oxaloacetate, so these organisms can use fatty acids as the starting material for gluconeogenesis.

❖Although mammals cannot convert fatty acids to carbohydrate, they can use the small amount of glycerol produced from the breakdown of fats (triacylglycerols) for gluconeogenesis.

❖If glycolysis (the conversion of glucose to pyruvate) and gluconeogenesis (the conversion of pyruvate to glucose) were allowed to proceed simultaneously at high rates, the result would be the consumption of ATP and the production of heat.

❖PFK-1 and FBPase-1 enzymatic reactions, and several others in the two pathways, are regulated allosterically and by covalent modification (phosphorylation).

❖The pathways are regulated so that when the flux of glucose through glycolysis goes up, the flux of pyruvate toward glucose goes down, and vice versa

Pentose Phosphate Pathway of Glucose Oxidation

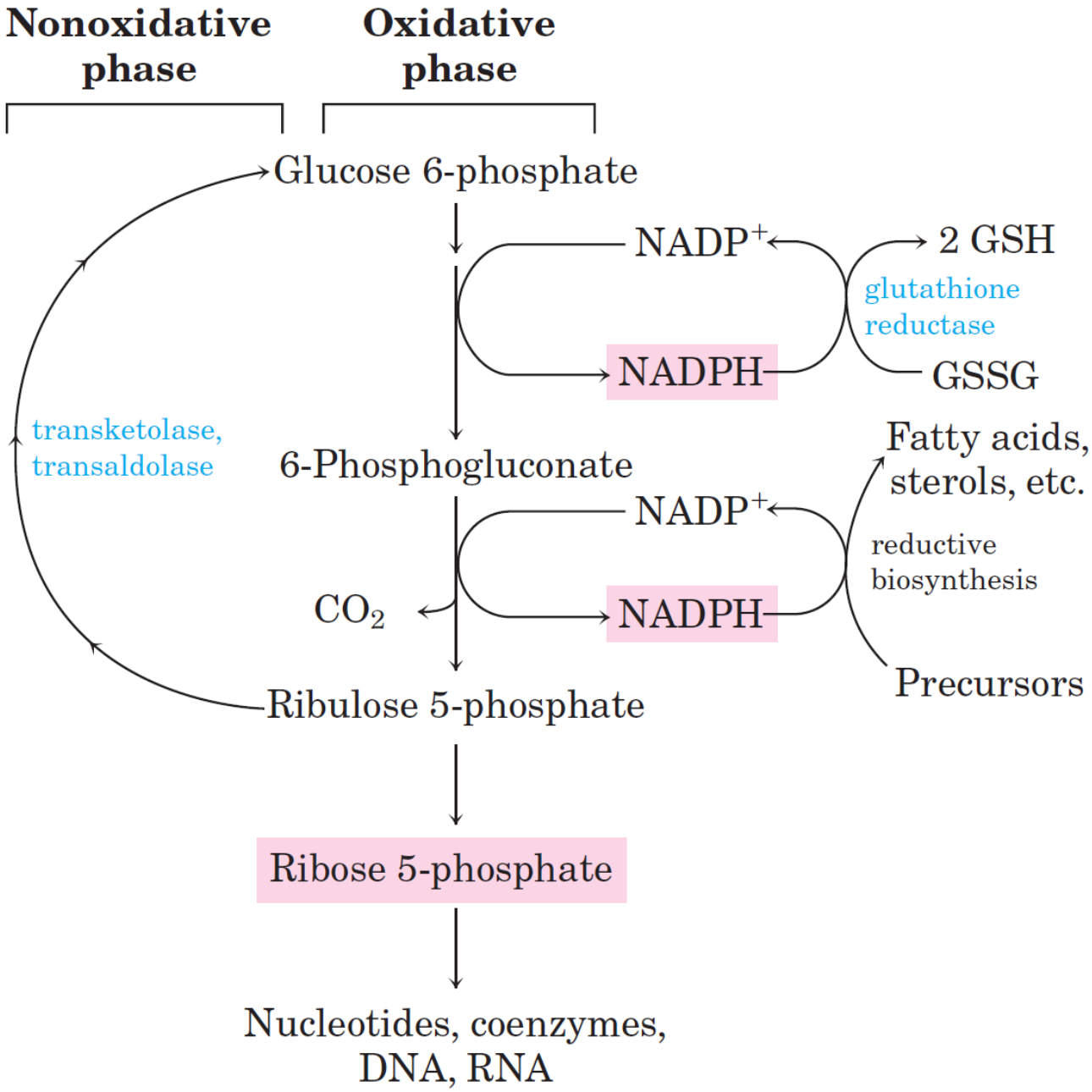
❖In most animal tissues, the major catabolic fate of glucose 6-phosphate is glycolytic breakdown to pyruvate, much of which is then oxidized via the citric acid cycle, ultimately leading to the formation of ATP.

❖Glucose 6-phosphate does have other catabolic fates, however, which lead to specialized products needed by the cell.

❖Of particular importance in some tissues is the oxidation of glucose 6-phosphate to pentose phosphates by the **pentose phosphate pathway**.

❖In this oxidative pathway, $NADP^+$ is the electron acceptor, yielding NADPH.

❖Rapidly dividing cells, such as those of bone marrow, skin, and intestinal mucosa, and those of tumors, use the pentose ribose 5-phosphate to make RNA, DNA, and such coenzymes as ATP, NADH, $FADH_2$, and coenzyme A.


Nonoxidative phase
Oxidative phase
Glucose 6-phosphate
NADP+
2 GSH
glutathione reductase
NADPH
GSSG
transketolase, transaldolase
6-Phosphogluconate
Fatty acids, sterols, etc.
NADP+
reductive biosynthesis
CO2
NADPH
Precursors
Ribulose 5-phosphate
Ribose 5-phosphate
Nucleotides, coenzymes, DNA, RNA

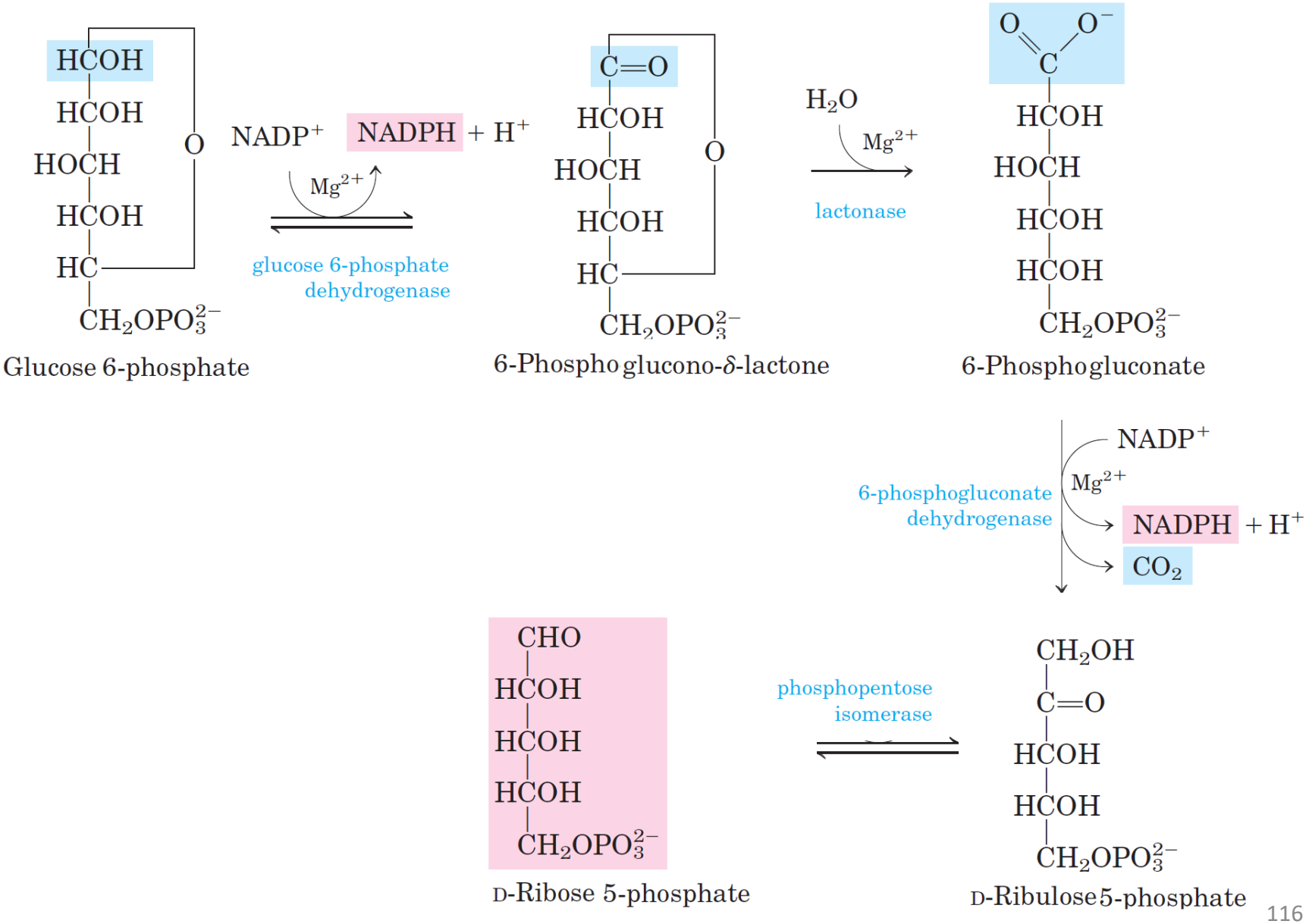
❖In other tissues, the essential product of the pentose phosphate pathway is not the pentoses but the electron donor NADPH, needed for reductive biosynthesis or to counter the damaging effects of oxygen radicals.

❖Tissues that carry out extensive fatty acid synthesis (liver, adipose, lactating mammary gland) or very active synthesis of cholesterol and steroid hormones (liver, adrenal glands, gonads) require the NADPH provided by this pathway.

❖Erythrocytes and the cells of the lens and cornea are directly exposed to oxygen and thus to the damaging free radicals generated by oxygen.

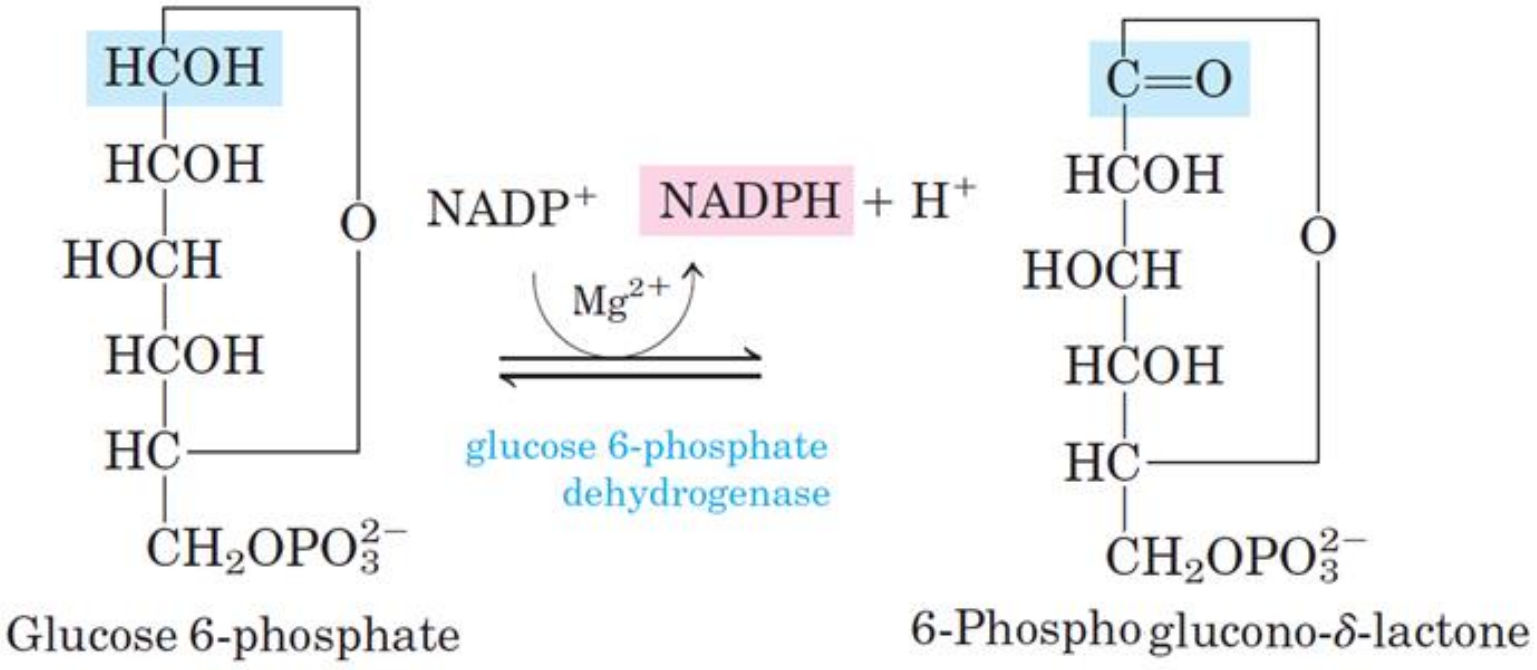
❖By maintaining a reducing atmosphere (a high ratio of NADPH to $NADP^+$ and a high ratio of reduced to oxidized glutathione), such cells can prevent or undo oxidative damage to proteins, lipids, and other sensitive molecules.

The Oxidative Phase

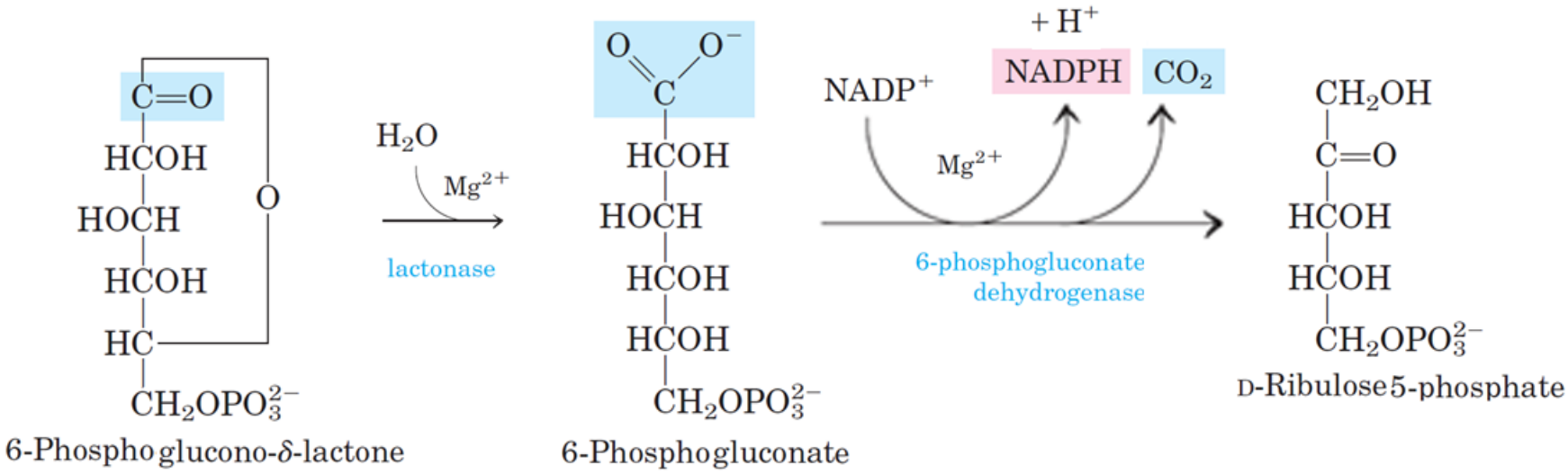


❖The first reaction of the pentose phosphate pathway is the oxidation of glucose 6-phosphate by glucose 6-phosphate dehydrogenase (G6PD) to form 6-phosphoglucono-d-lactone, an intramolecular ester.

❖$NADP^+$ is the electron acceptor, and the overall equilibrium lies far in the direction of NADPH formation.

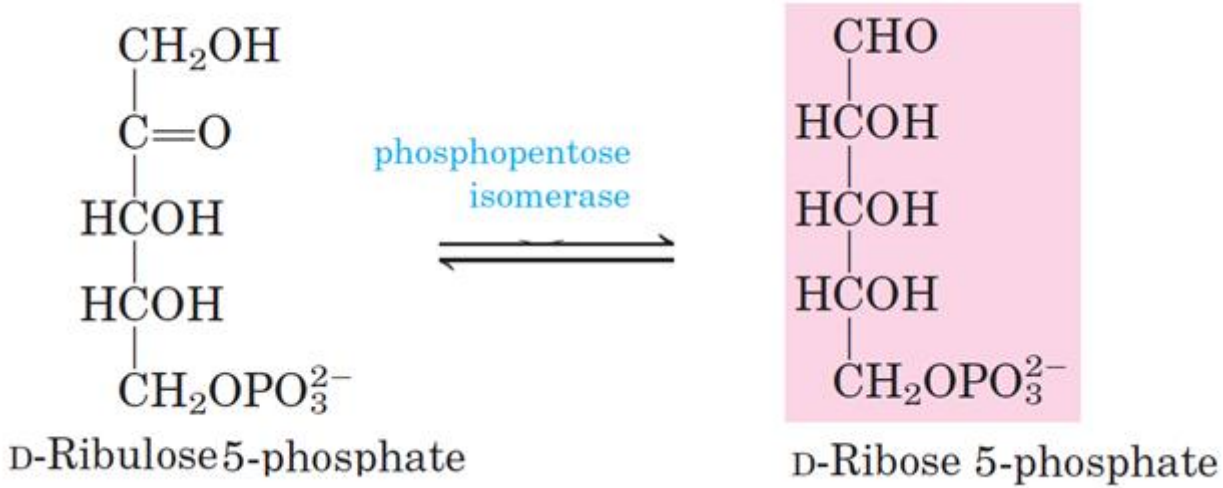


❖The lactone is hydrolyzed to the free acid 6-phosphogluconate by a specific lactonase, then 6-phosphogluconate undergoes oxidation and decarboxylation by 6-phosphogluconate dehydrogenase to form the ketopentose ribulose 5-phosphate.



❖ The reaction generates a second molecule of NADPH.

❖**Phosphopentose isomerase** converts ribulose 5-phosphate to its aldose isomer, ribose 5-phosphate.



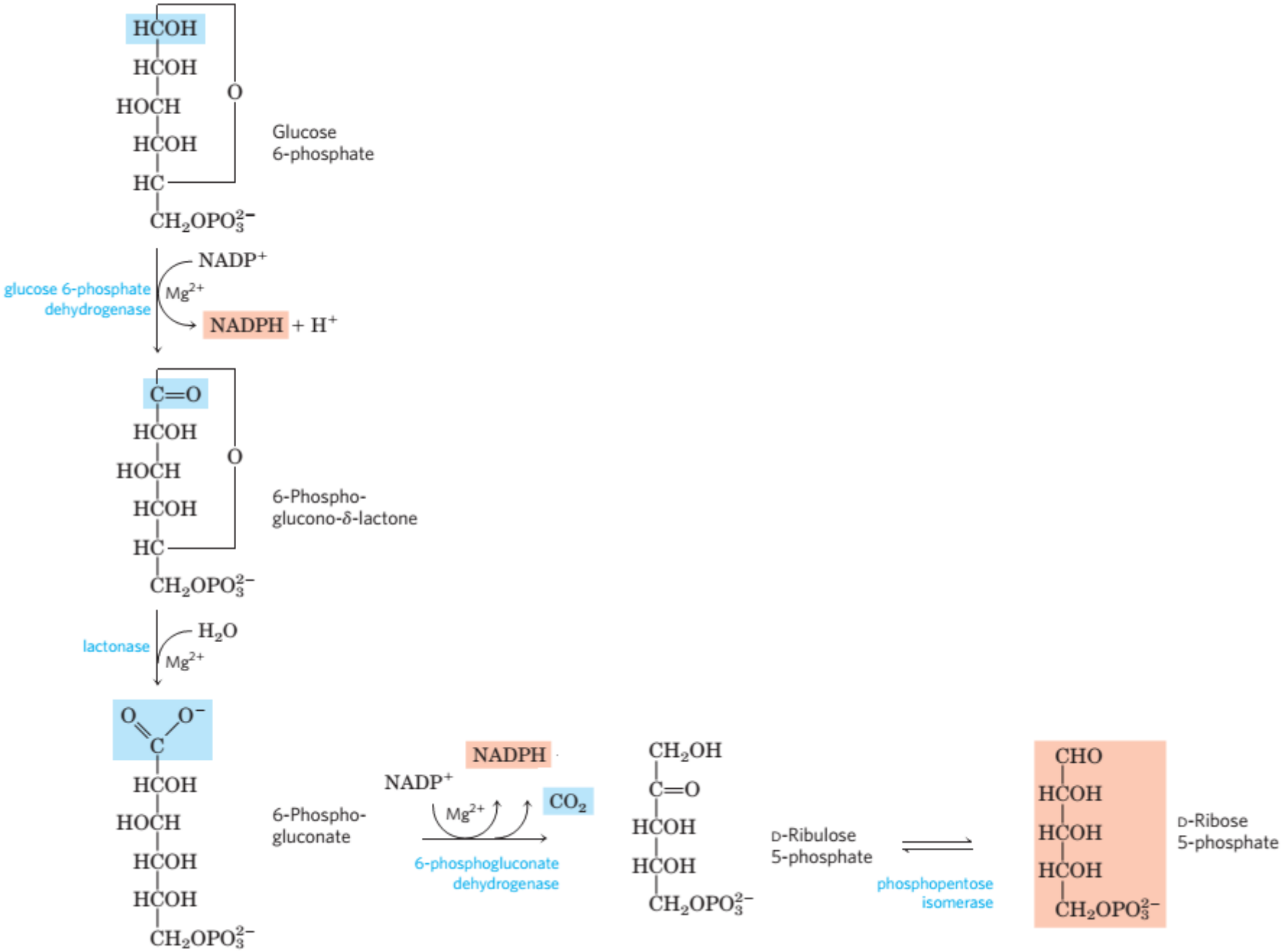
D-Ribulose 5-phosphate

D-Ribose 5-phosphate

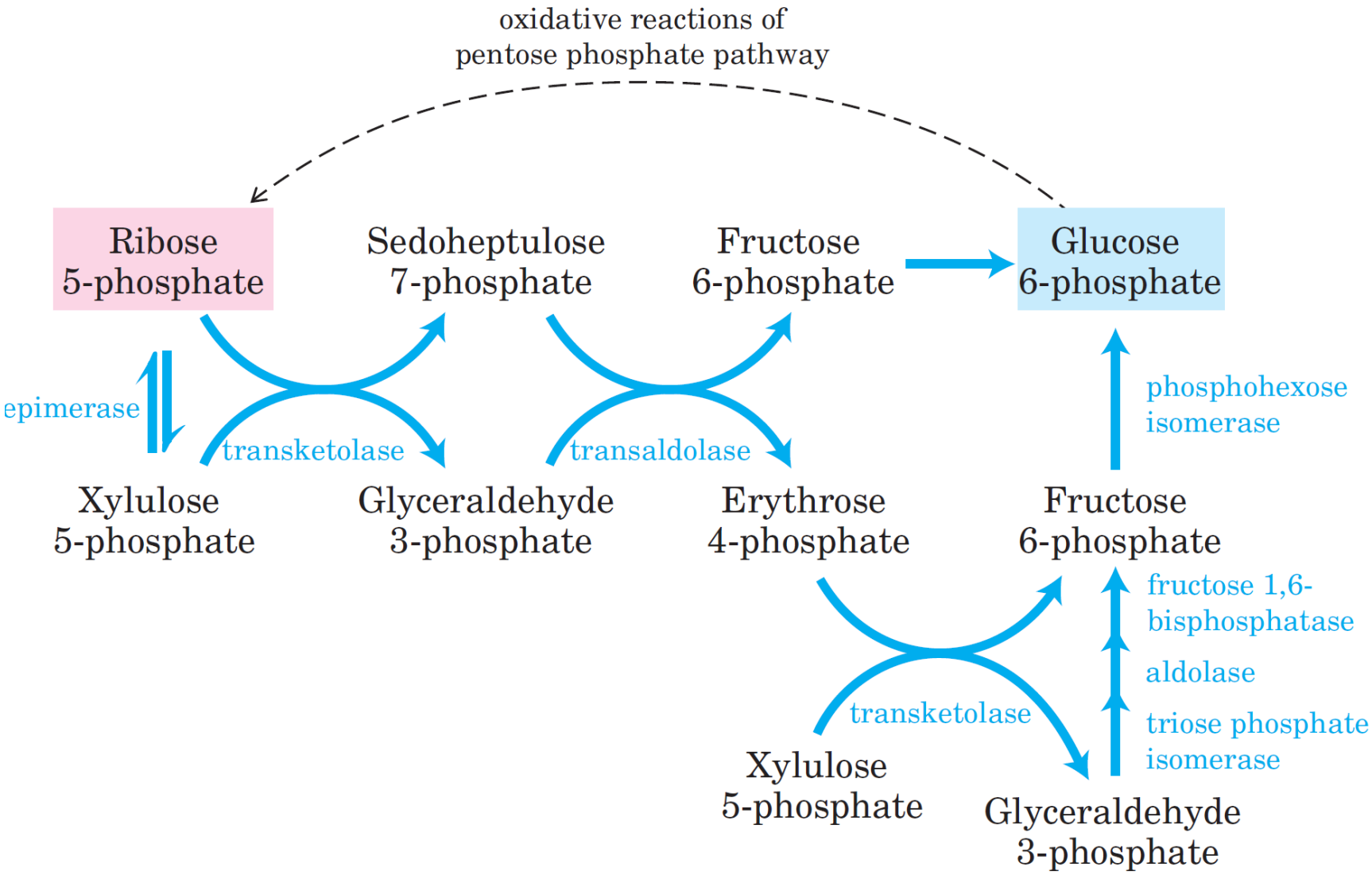
❖In some tissues, the pentose phosphate pathway ends at this point, and its overall equation is

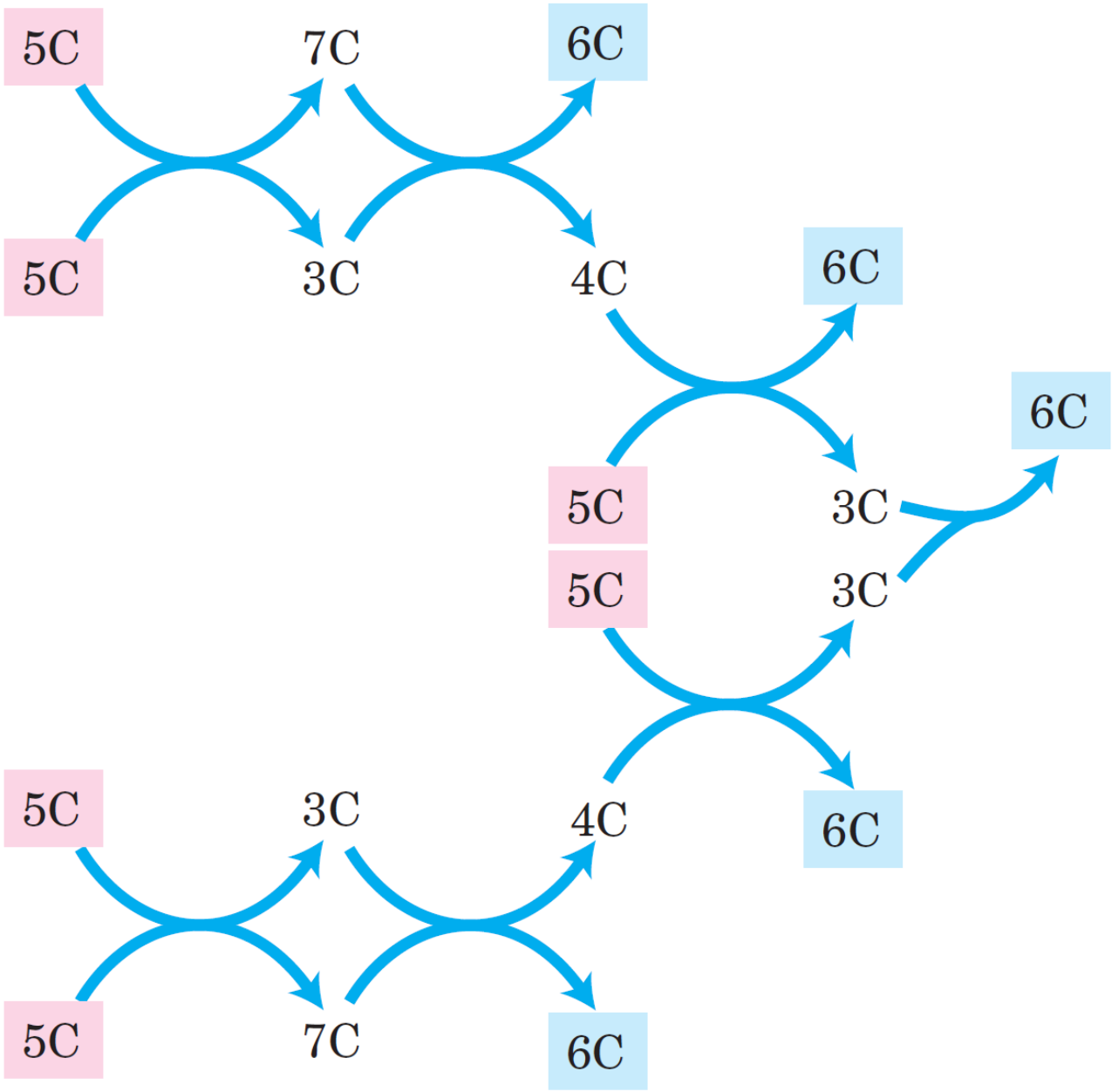
$$\text{Glucose 6-phosphate} + 2\text{NADP}^+ + \text{H}_2\text{O} \longrightarrow \text{ribose 5-phosphate} + \text{CO}_2 + 2\text{NADPH} + 2\text{H}^+$$

❖ The net result is the production of NADPH, a reductant for biosynthetic reactions, and ribose 5-phosphate, a precursor for nucleotide synthesis.


Glucose 6-phosphate
glucose 6-phosphate dehydrogenase
NADP+
Mg2+
NADPH + H+
6-Phospho-glucono-δ-lactone
lactonase
H2O
Mg2+
6-Phospho-gluconate
NADPH
NADP+
Mg2+
CO2
6-phosphogluconate dehydrogenase
D-Ribulose 5-phosphate
phosphopentose isomerase
D-Ribose 5-phosphate

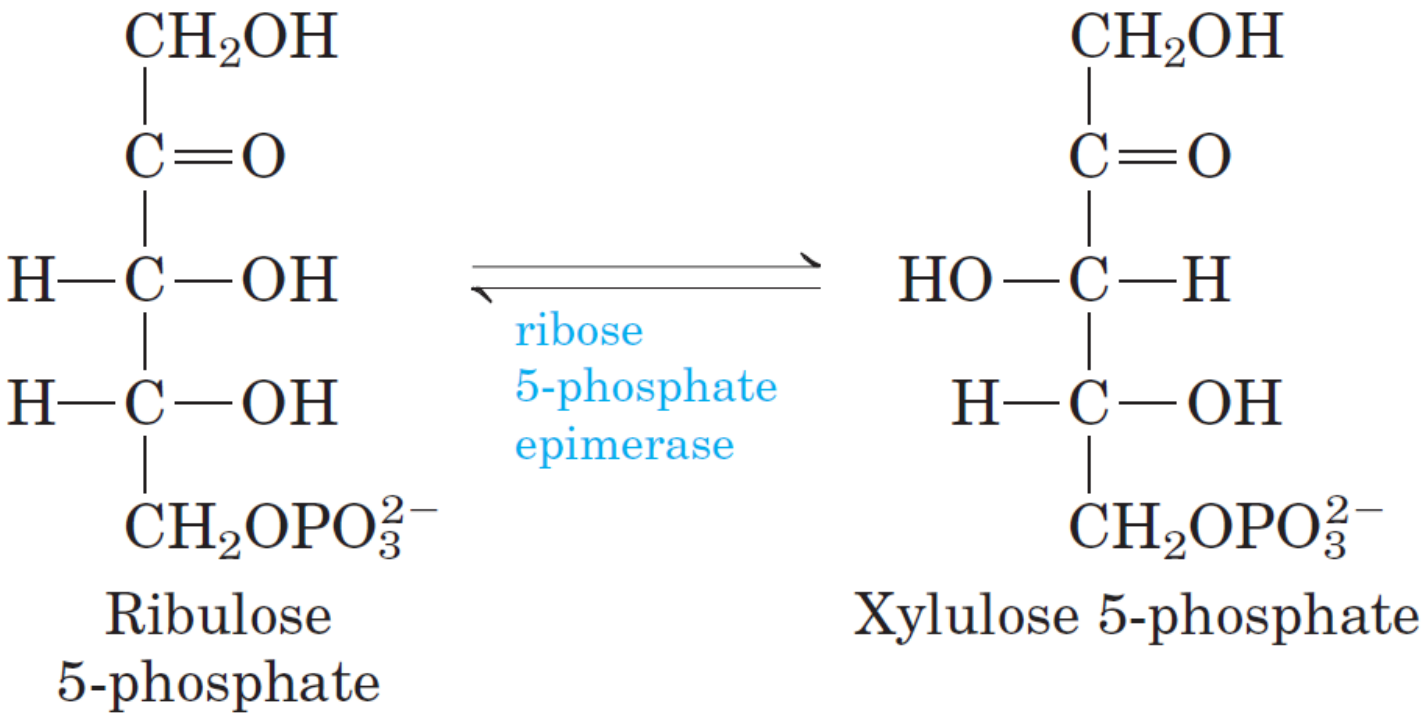
The Nonoxidative Phase Recycles Pentose Phosphates to Glucose 6-Phosphate




5C
7C
6C
5C
3C
4C
6C
6C
5C
3C
5C
3C
5C
3C
4C
6C
5C
7C
6C

❖In tissues that require primarily NADPH, the pentose phosphates produced in the oxidative phase of the pathway are recycled into glucose 6-phosphate.

❖In this nonoxidative phase, ribulose 5-phosphate is first epimerized to xylulose 5-phosphate:



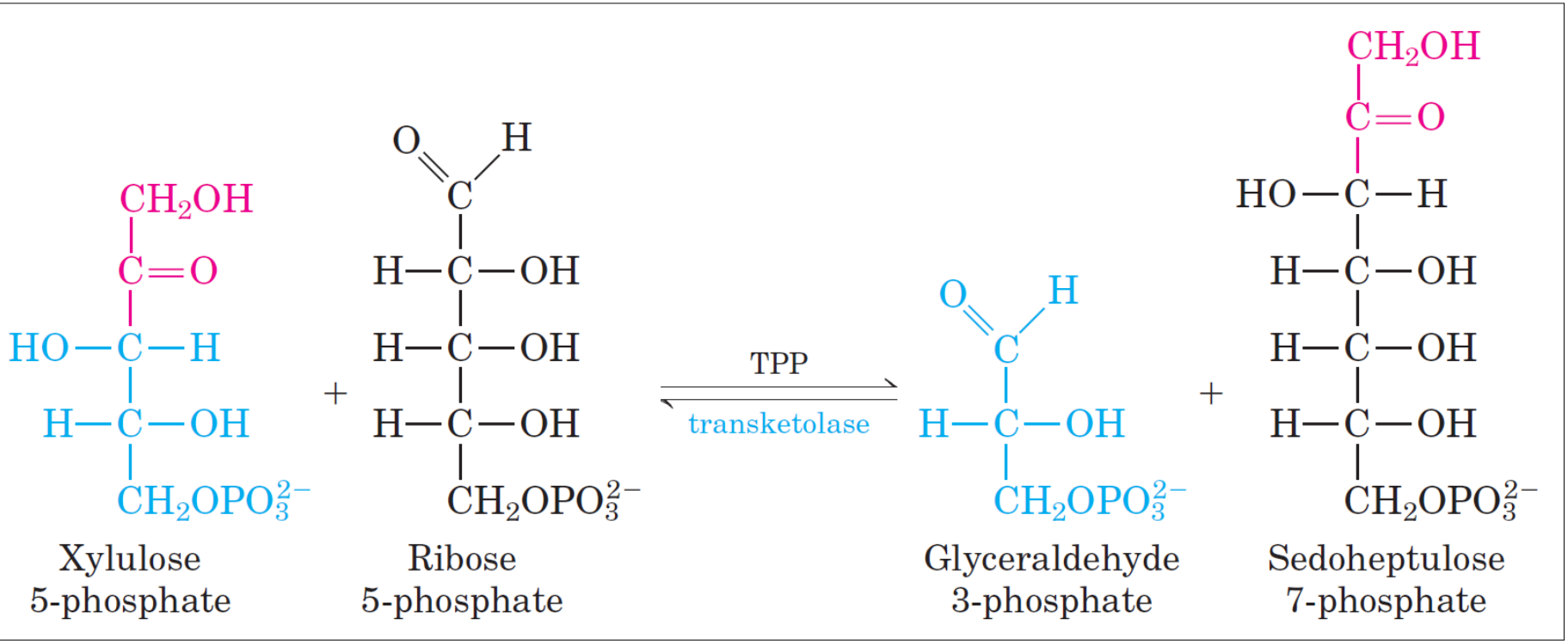
❖Then, in a series of rearrangements of the carbon skeletons, six five-carbon sugar phosphates are converted to five six-carbon sugar phosphates, completing the cycle and allowing continued oxidation of glucose 6-phosphate with production of NADPH.

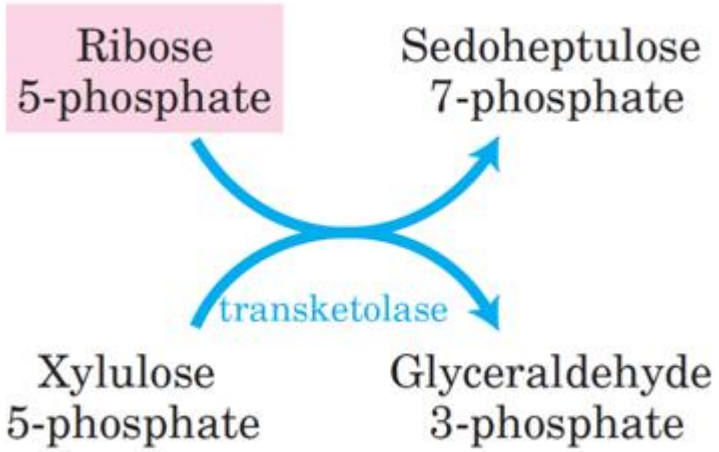
❖Continued recycling leads ultimately to the conversion of glucose 6-phosphate to six CO_2.

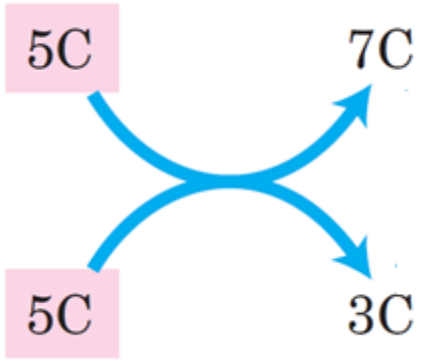
❖Two enzymes unique to the pentose phosphate pathway act in these interconversions of sugars: transketolase and transaldolase.

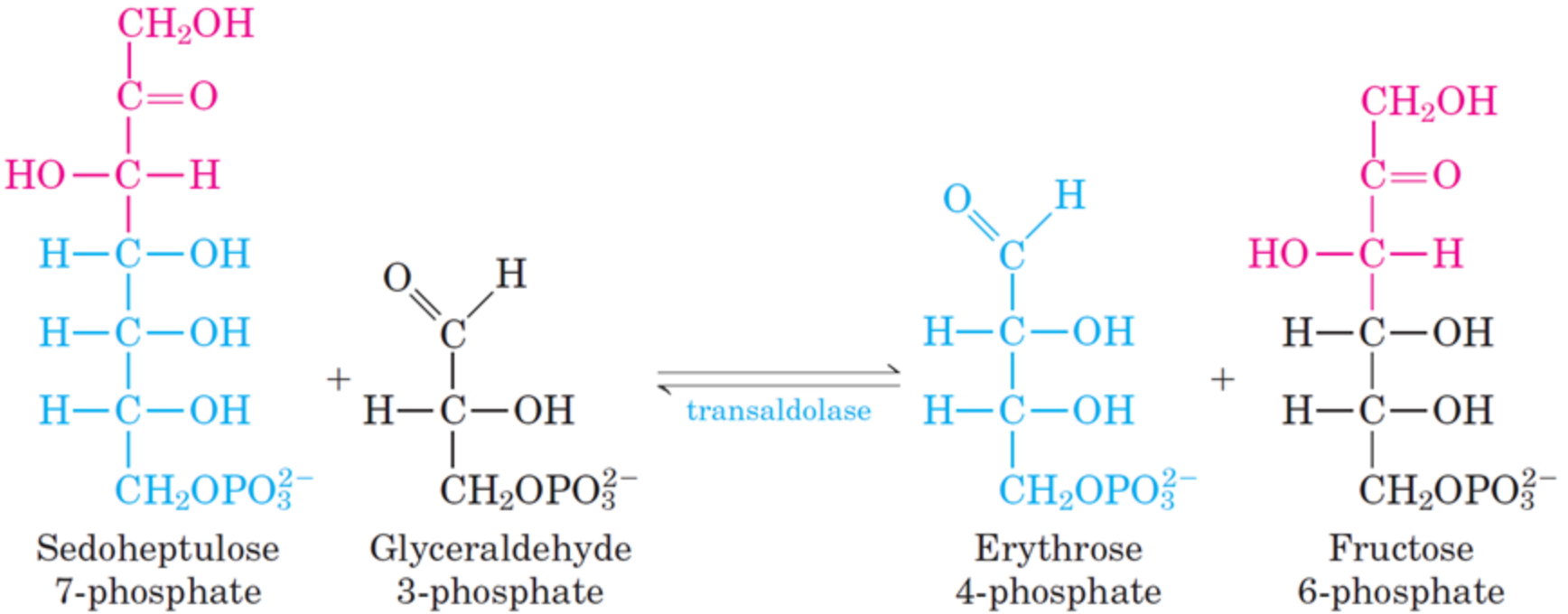
❖Transketolase catalyzes the transfer of a twocarbon fragment from a ketose donor to an aldose acceptor.

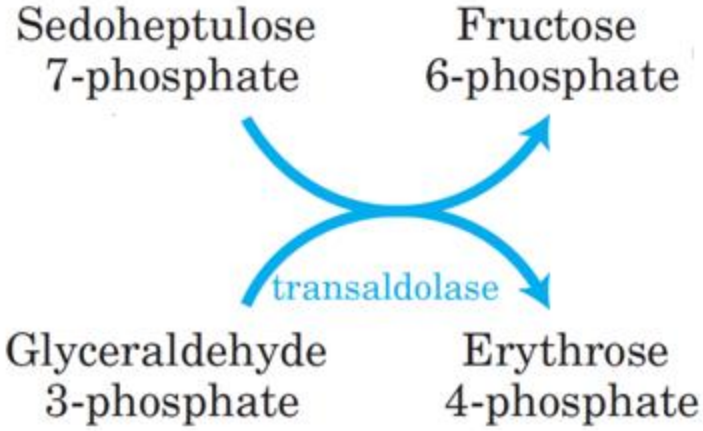
❖Transaldolase catalyzes a reaction similar to the aldolase reaction of glycolysis.

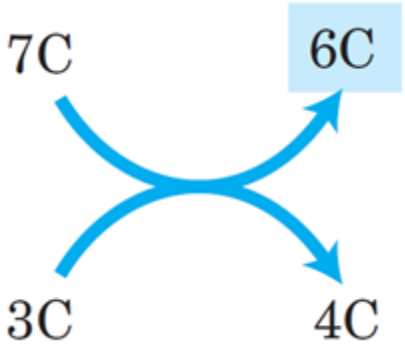

Xylulose 5-phosphate
Ribose 5-phosphate
TPP
transketolase
Glyceraldehyde 3-phosphate
Sedoheptulose 7-phosphate

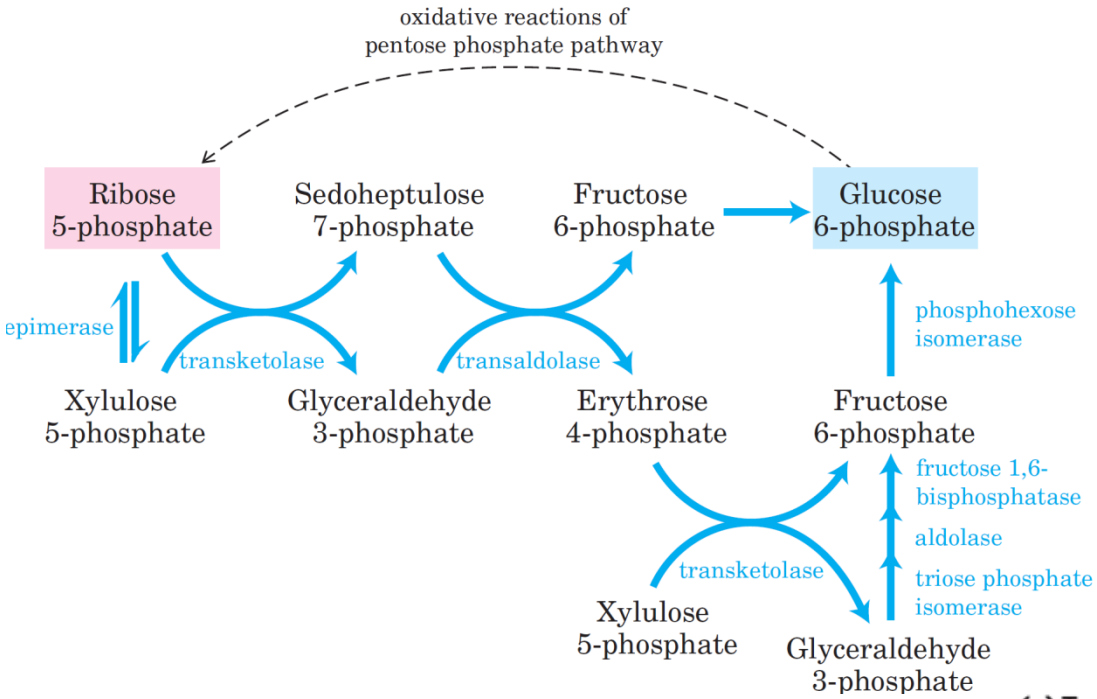

Ribose 5-phosphate
Sedoheptulose 7-phosphate
transketolase
Xylulose 5-phosphate
Glyceraldehyde 3-phosphate

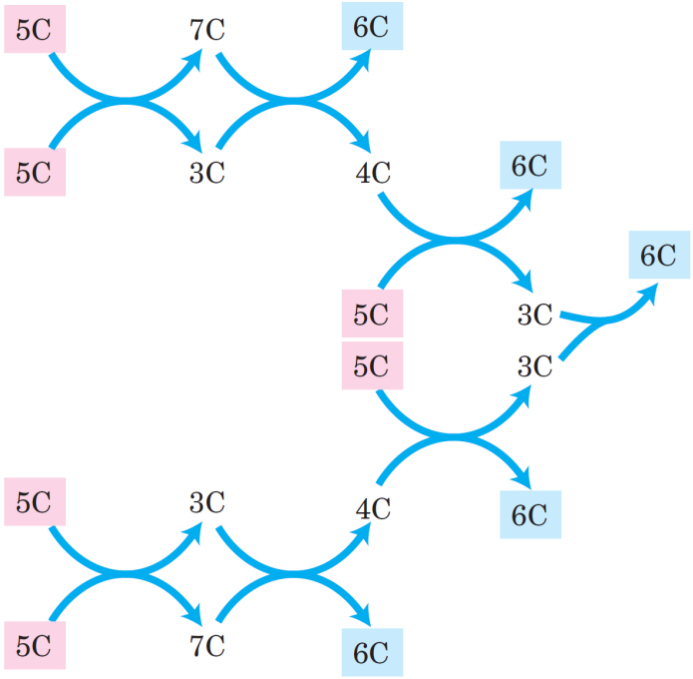

5C
7C
5C
3C

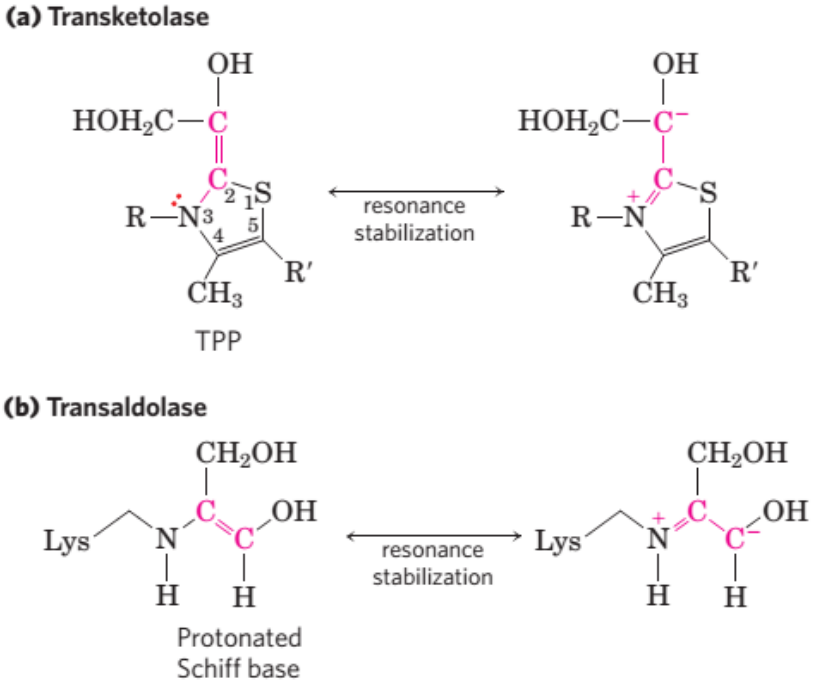

Sedoheptulose 7-phosphate
Glyceraldehyde 3-phosphate
transaldolase
Erythrose 4-phosphate
Fructose 6-phosphate


Sedoheptulose 7-phosphate
Fructose 6-phosphate
transaldolase
Glyceraldehyde 3-phosphate
Erythrose 4-phosphate


7C
6C
3C
4C


oxidative reactions of
pentose phosphate pathway
Ribose
5-phosphate
Sedoheptulose
7-phosphate
Fructose
6-phosphate
Glucose
6-phosphate
epimerase
transketolase
transaldolase
phosphohexose
isomerase
Xylulose
5-phosphate
Glyceraldehyde
3-phosphate
Erythrose
4-phosphate
Fructose
6-phosphate
fructose 1,6-
bisphosphatase
aldolase
triose phosphate
isomerase
transketolase
Xylulose
5-phosphate
Glyceraldehyde
3-phosphate


5C
7C
6C
5C
3C
4C
6C
5C
3C
6C
5C
3C
3C
5C
3C
4C
6C
5C
7C
6C


(a) Transketolase
OH
HOH2C
C
C
2
1
S
R
N
3
4
5
R′
CH3
TPP
resonance
stabilization
OH
HOH2C
C−
C
S
R
N
R′
CH3
(b) Transaldolase
CH2OH
C
OH
Lys
N
C
H
H
Protonated
Schiff base
resonance
stabilization
CH2OH
C
OH
Lys
N
C−
H
H

❖All the enzymes in the pentose phosphate pathway are located in the cytosol, like those of glycolysis and most of those of gluconeogenesis.

❖In fact, these three pathways are connected through several shared intermediates and enzymes.

❖The glyceraldehyde 3-phosphate formed by the action of transketolase is readily converted to dihydroxyacetone phosphate by the glycolytic enzyme triose phosphate isomerase, and these two trioses can be joined by the aldolase as in gluconeogenesis, forming fructose 1,6-bisphosphate.

❖Alternatively, the triose phosphates can be oxidized to pyruvate by the glycolytic reactions.

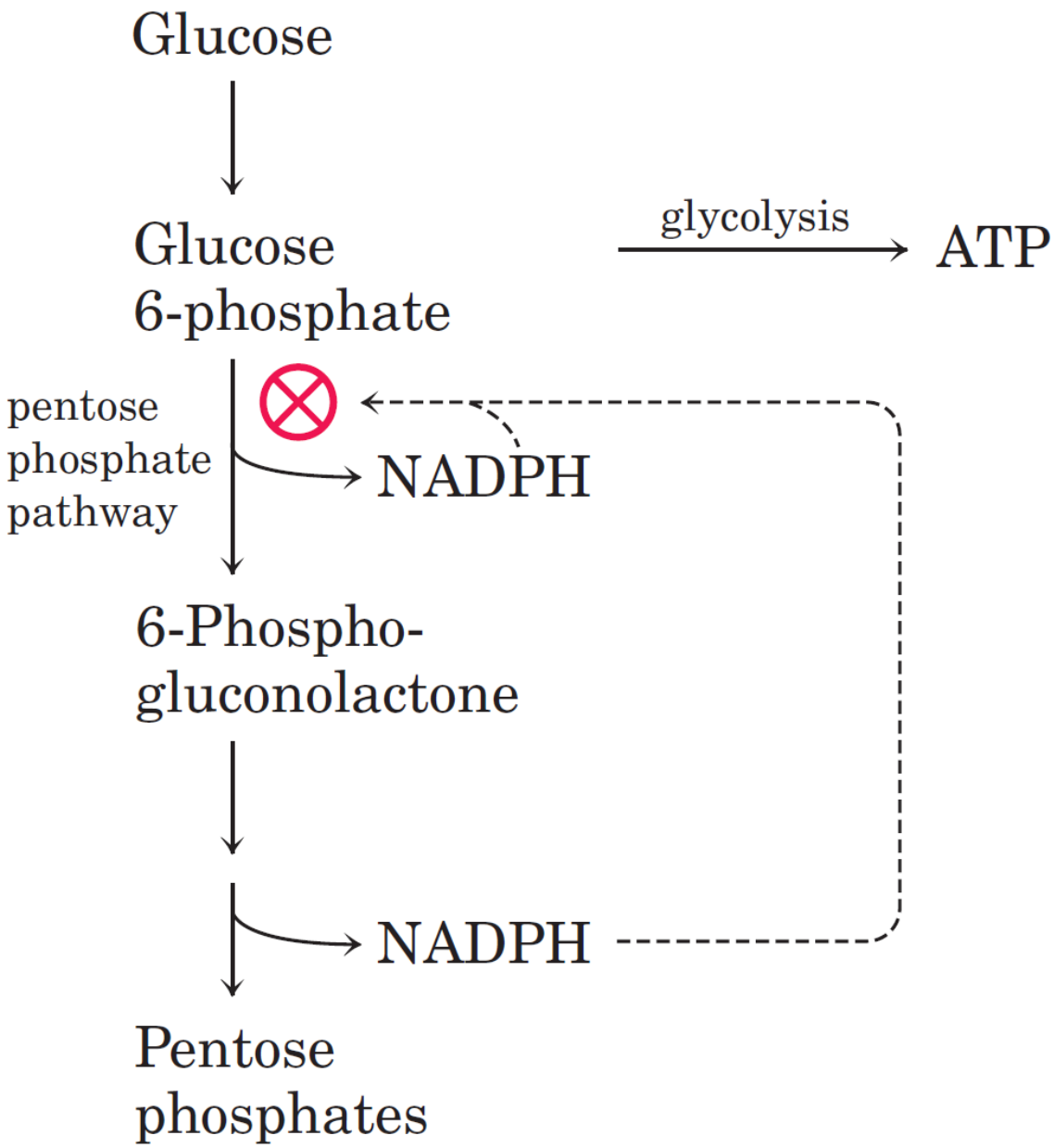
❖The fate of the trioses is determined by the cell's relative needs for pentose phosphates, NADPH, and ATP.

❖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 thereby 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.


Glucose
Glucose
6-phosphate
glycolysis
ATP
pentose
phosphate
pathway
NADPH
6-Phospho-
gluconolactone
NADPH
Pentose
phosphates

The Metabolism of Glycogen

❖In organisms from bacteria to plants to vertebrates, excess glucose is converted to polymeric forms for storage—glycogen in vertebrates and many microorganisms, starch in plants.

❖In vertebrates, glycogen is found primarily in the liver and skeletal muscle; it may represent up to 10% of the weight of liver and 1% to 2% of the weight of muscle.

❖If this much glucose were dissolved in the cytosol of a hepatocyte, its concentration would be about 0.4 M, enough to dominate the osmotic properties of the cell.

❖When stored as a large polymer (glycogen), however, the same mass of glucose has a concentration of only 0.01 µM.

❖The glycogen in muscle is there to provide a quick source of energy for either aerobic or anaerobic metabolism.

❖Muscle glycogen can be exhausted in less than an hour during vigorous activity.

❖Liver glycogen serves as a reservoir of glucose for other tissues when dietary glucose is not available (between meals or during a fast); this is especially important for the neurons of the brain, which cannot use fatty acids as fuel.

❖Liver glycogen can be depleted in 12 to 24 hours.

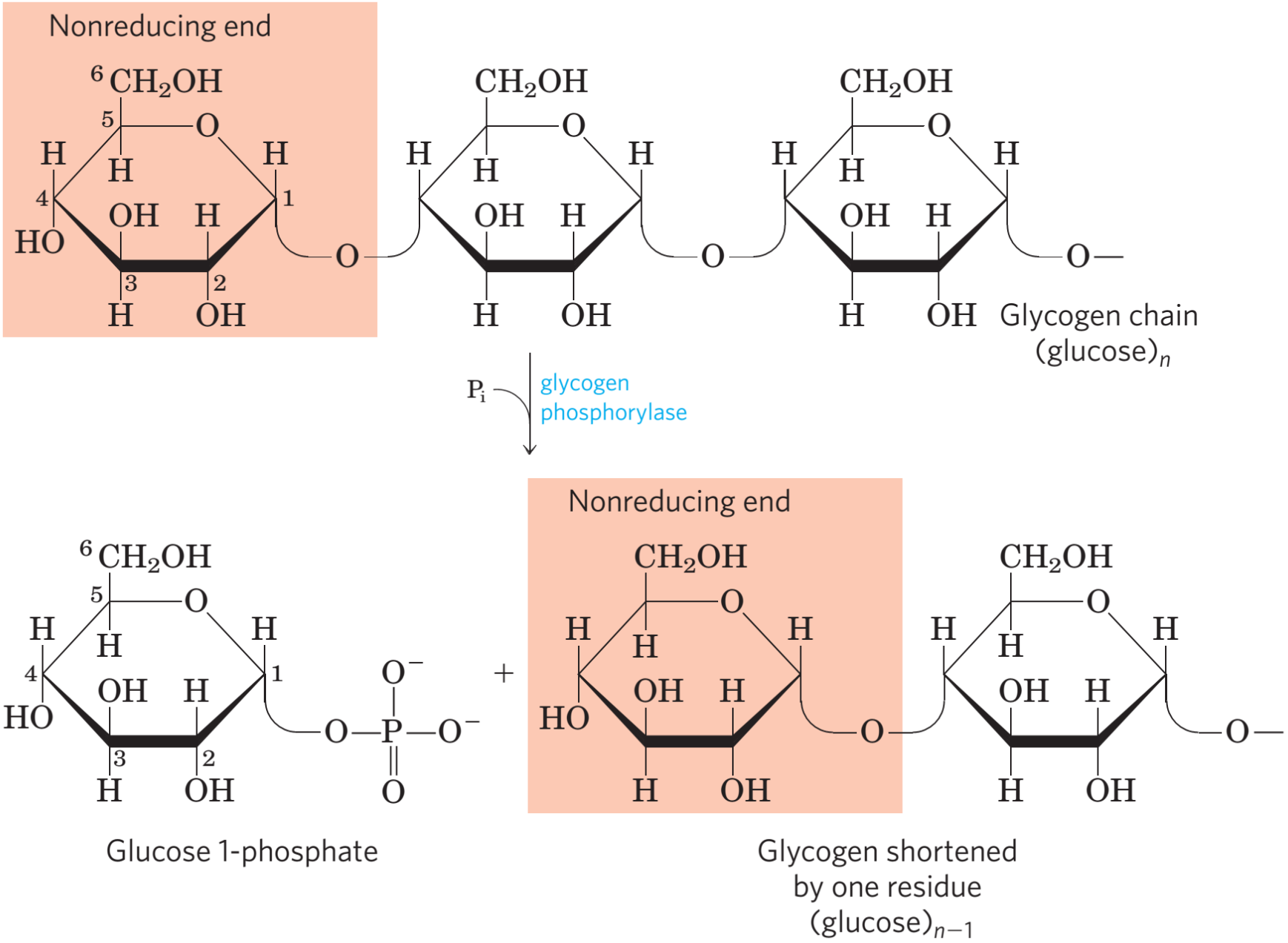
❖The breakdown of glycogen to glucose 1-phosphate is called as glycogenolysis, while synthesis of glycogen is called as glycogenesis.

Glycogen Breakdown, Glycogenolysis

❖In skeletal muscle and liver, the glucose units of the outer branches of glycogen enter the glycolytic pathway through the action of three enzymes: glycogen phosphorylase, glycogen debranching enzyme, and phosphoglucomutase.

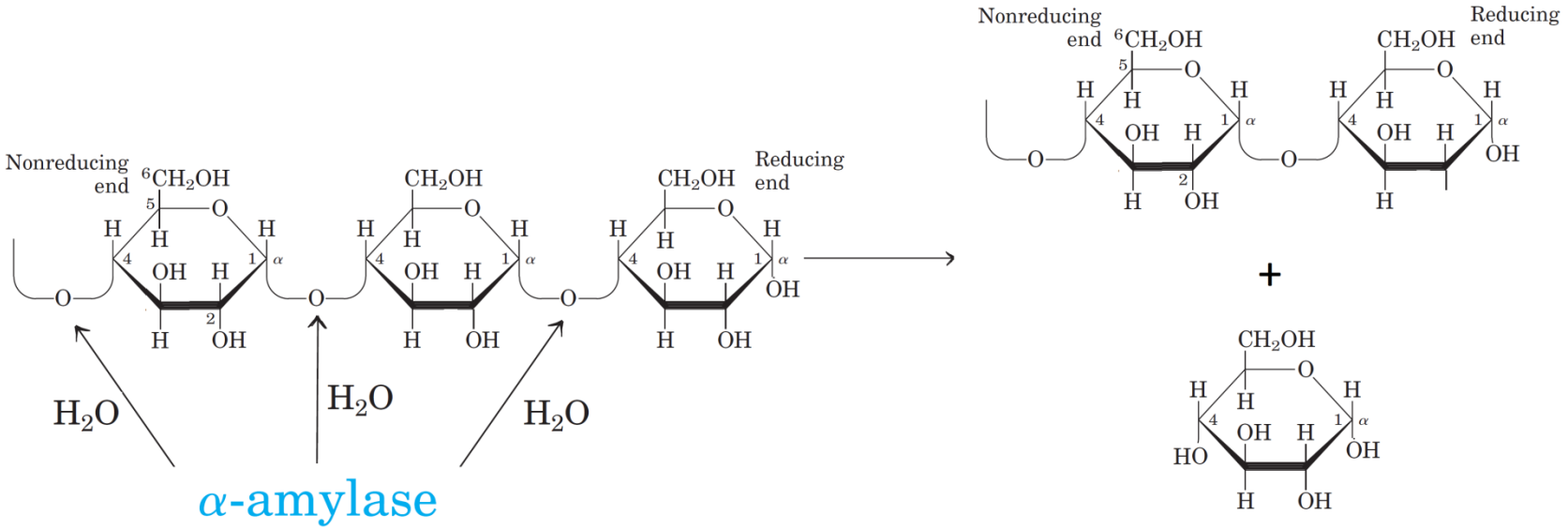
❖Glycogen phosphorylase catalyzes the reaction in which an (1→4) glycosidic linkage between two glucose residues at a nonreducing end of glycogen undergoes attack by inorganic phosphate (Pi), removing the terminal glucose residue as α-D-glucose 1-phosphate.

❖Pyridoxal phosphate is an essential cofactor in the glycogen phosphorylase reaction


Nonreducing end
Glycogen chain
(glucose)$_n$
P_i
glycogen
phosphorylase
Nonreducing end
Glucose 1-phosphate
Glycogen shortened
by one residue
(glucose)$_{n-1}$

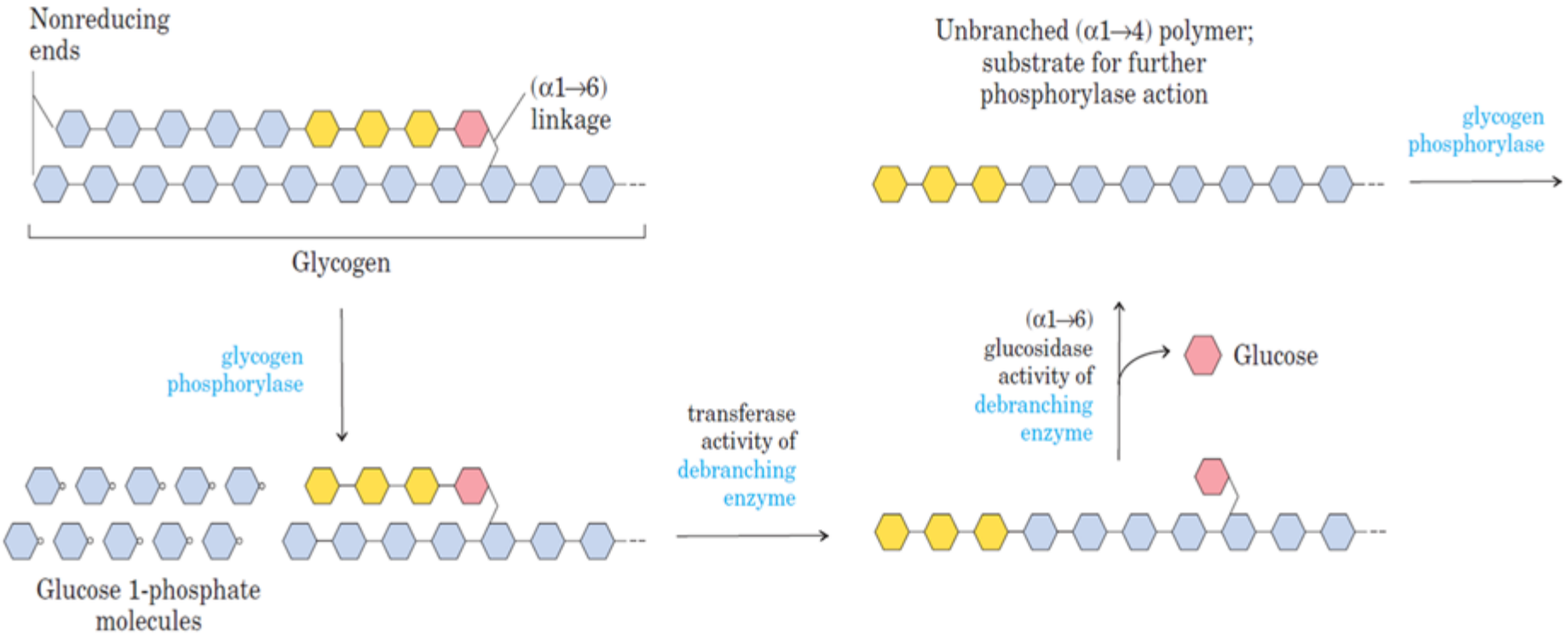
❖This phosphorolysis reaction is different from the hydrolysis of glycosidic bonds by amylase during intestinal degradation of dietary glycogen and starch.

❖In phosphorolysis, some of the energy of the glycosidic bond is preserved in the formation of the phosphate ester, glucose 1-phosphate.



❖Glycogen phosphorylase acts repetitively on the nonreducing ends of glycogen branches until it reaches a point four glucose residues away from an (1→6) branch point, where its action stops.

❖Further degradation by glycogen phosphorylase can occur only after the debranching enzyme catalyzes two successive reactions that transfer branches.



❖Once these branches are transferred and the glucosyl residue at C-6 is hydrolyzed, glycogen phosphorylase activity can continue

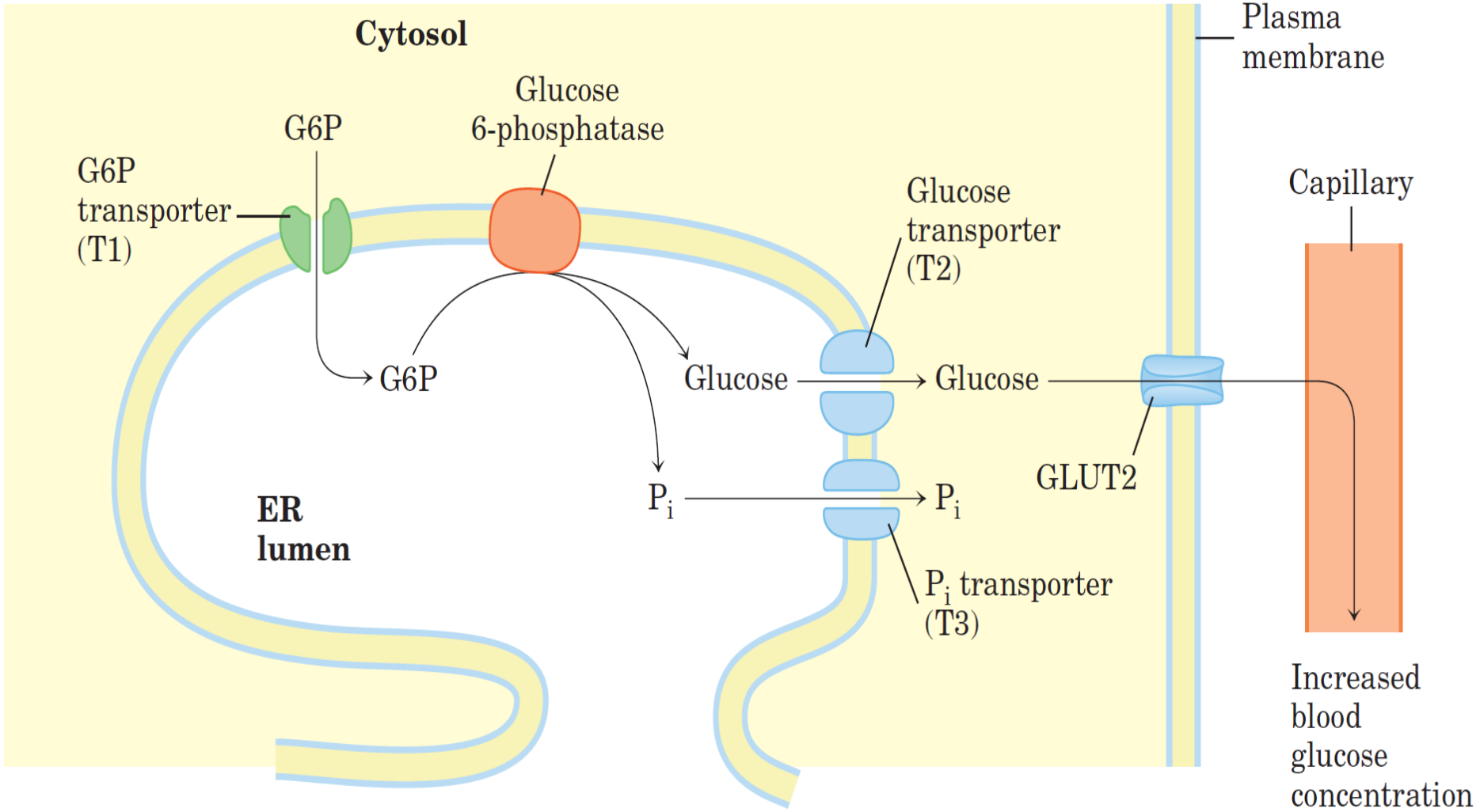
❖Glucose 1-phosphate, the end product of the glycogen phosphorylase reaction, is converted to glucose 6-phosphate by phosphoglucomutase, which catalyzes the reversible reaction

$$\text{Glucose 1-phosphate} \rightleftharpoons \text{glucose 6-phosphate}$$

❖The glucose 6-phosphate formed from glycogen in skeletal muscle can enter glycolysis and serve as an energy source to support muscle contraction.

❖In liver, glycogen breakdown serves a different purpose: to release glucose into the blood when the blood glucose level drops, as it does between meals.

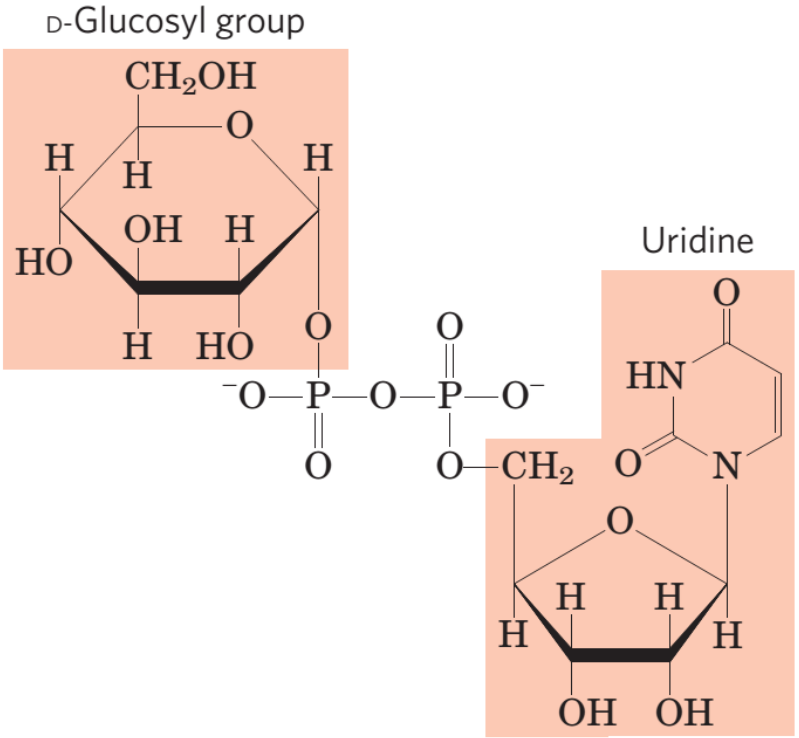
❖This requires the enzyme glucose 6-phosphatase, present in liver and kidney but not in other tissues.



❖Glucose 6-phosphatase is an integral membrane protein of the endoplasmic reticulum, with its active site on the lumenal side of the ER, thus it is separated from glycolysis.

Glycogen Synthesis- Glycogenesis

❖Many of the reactions in which hexoses are transformed or polymerized involve sugar nucleotides, compounds in which the anomeric carbon of a sugar is activated by attachment to a nucleotide through a phosphate ester linkage.



❖Sugar nucleotides are the substrates for polymerization of monosaccharides into disaccharides, glycogen, starch, cellulose, and more complex extracellular polysaccharides.

❖Glycogen synthesis takes place in virtually all animal tissues but is especially prominent in the liver and skeletal muscles.

❖The starting point for synthesis of glycogen is glucose 6-phosphate.

$$\text{D-Glucose} + \text{ATP} \longrightarrow \text{D-glucose 6-phosphate} + \text{ADP}$$

❖This can be derived from free glucose in a reaction catalyzed by the isozymes hexokinase I and hexokinase II in muscle and hexokinase IV (glucokinase) in liver.

❖However, some ingested glucose takes a more roundabout path to glycogen.

❖It is first taken up by erythrocytes and converted to lactate glycolytically; the lactate is then taken up by the liver and converted to glucose 6-phosphate by gluconeogenesis.

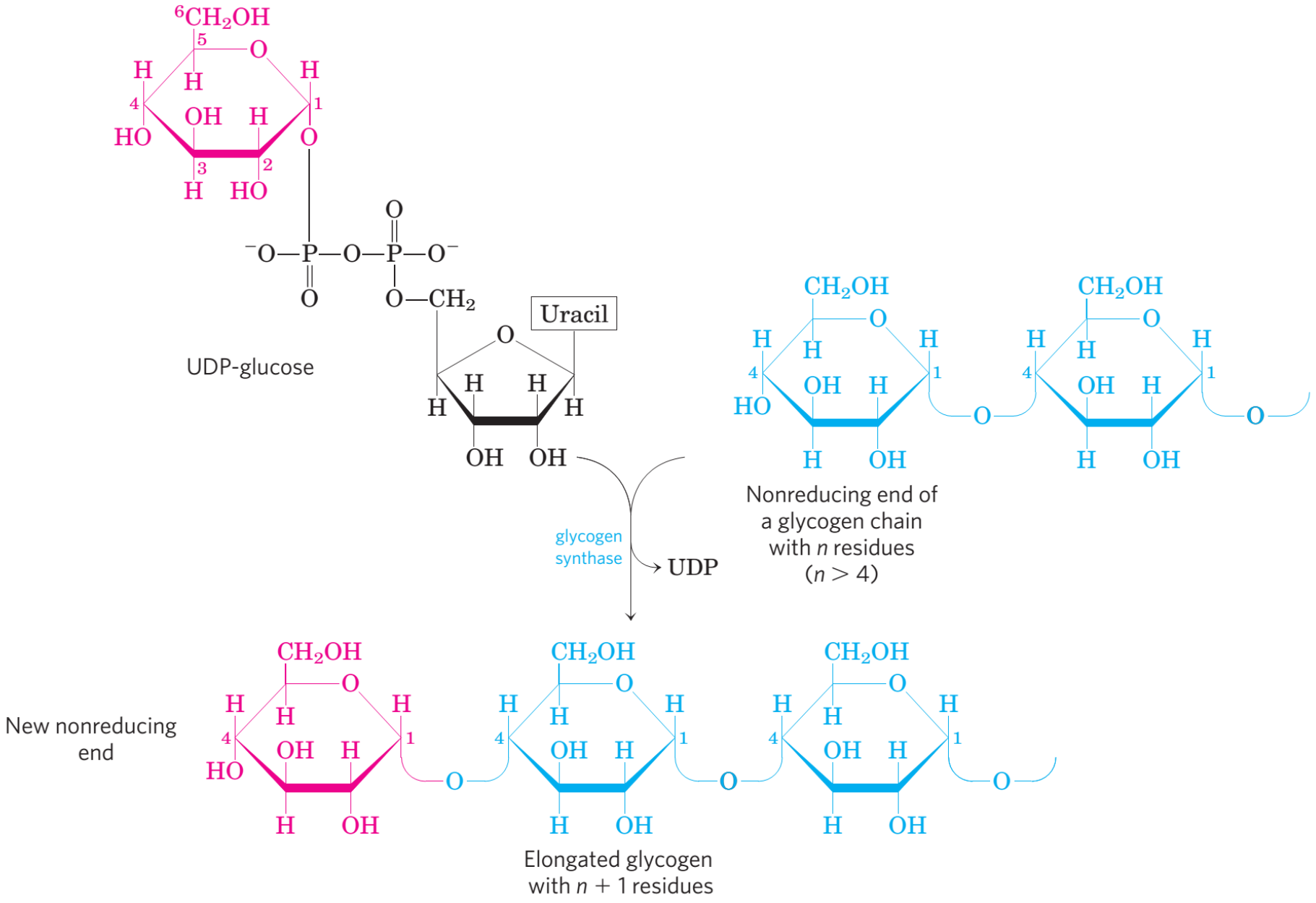
❖To initiate glycogen synthesis, the glucose 6-phosphate is converted to glucose 1-phosphate in the phosphoglucomutase reaction:

$$\text{Glucose 6-phosphate} \rightleftharpoons \text{glucose 1-phosphate}$$

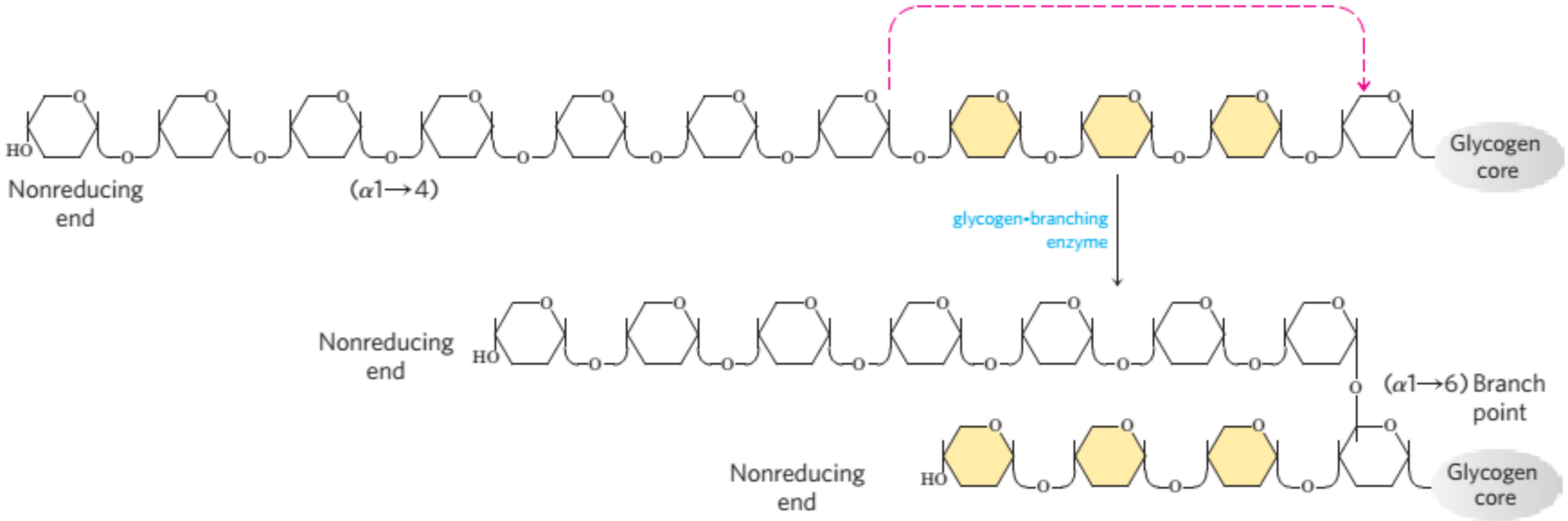
❖The product of this reaction is converted to UDP-glucose by the action of UDP-glucose pyrophosphorylase, in a key step of glycogen biosynthesis.

$$\text{Glucose 1-phosphate} + \text{UTP} \longrightarrow \text{UDP-glucose} + \text{PP}_\text{i}$$

❖UDP-glucose is the immediate donor of glucose residues in the reaction catalyzed by glycogen synthase, which promotes the transfer of the glucose residue from UDP-glucose to a nonreducing end of a branched glycogen molecule.


UDP-glucose
Uracil
glycogen synthase
UDP
Nonreducing end of a glycogen chain with n residues (n > 4)
New nonreducing end
Elongated glycogen with n + 1 residues

❖Glycogen synthase cannot make the (α1→6) bonds found at the branch points of glycogen; these are formed by the glycogen-branching enzyme.



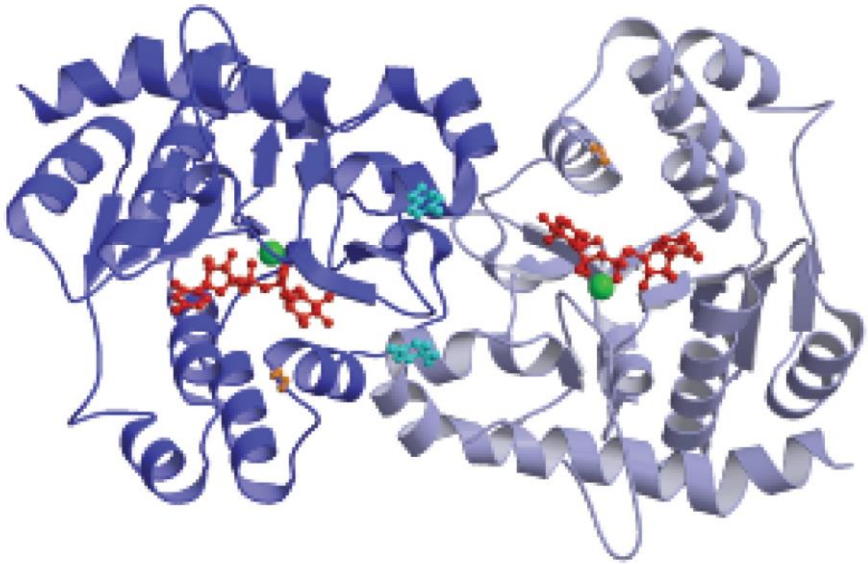
❖The biological effect of branching is to make the glycogen molecule more soluble and to increase the number of nonreducing ends.

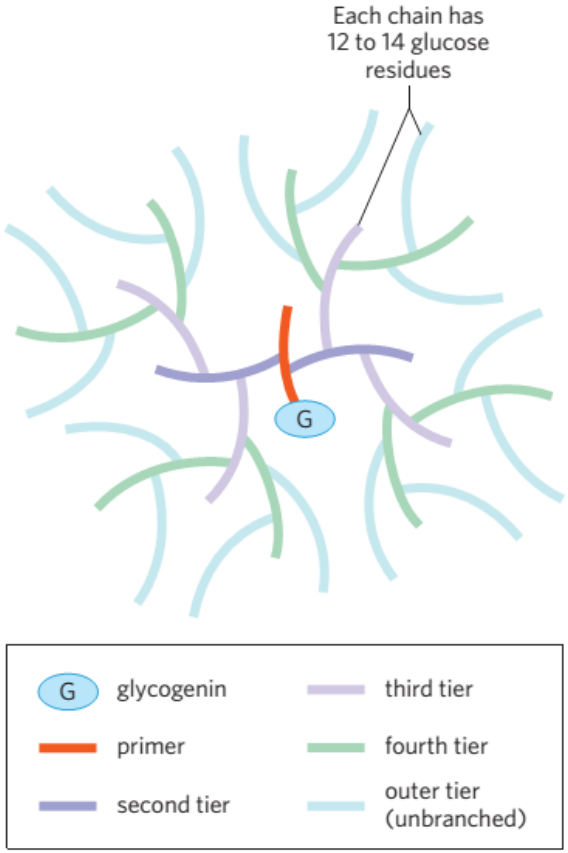
❖This increases the number of sites accessible to glycogen phosphorylase and glycogen synthase, both of which act only at nonreducing ends.

❖Glycogen synthase cannot initiate a new glycogen chain de novo.

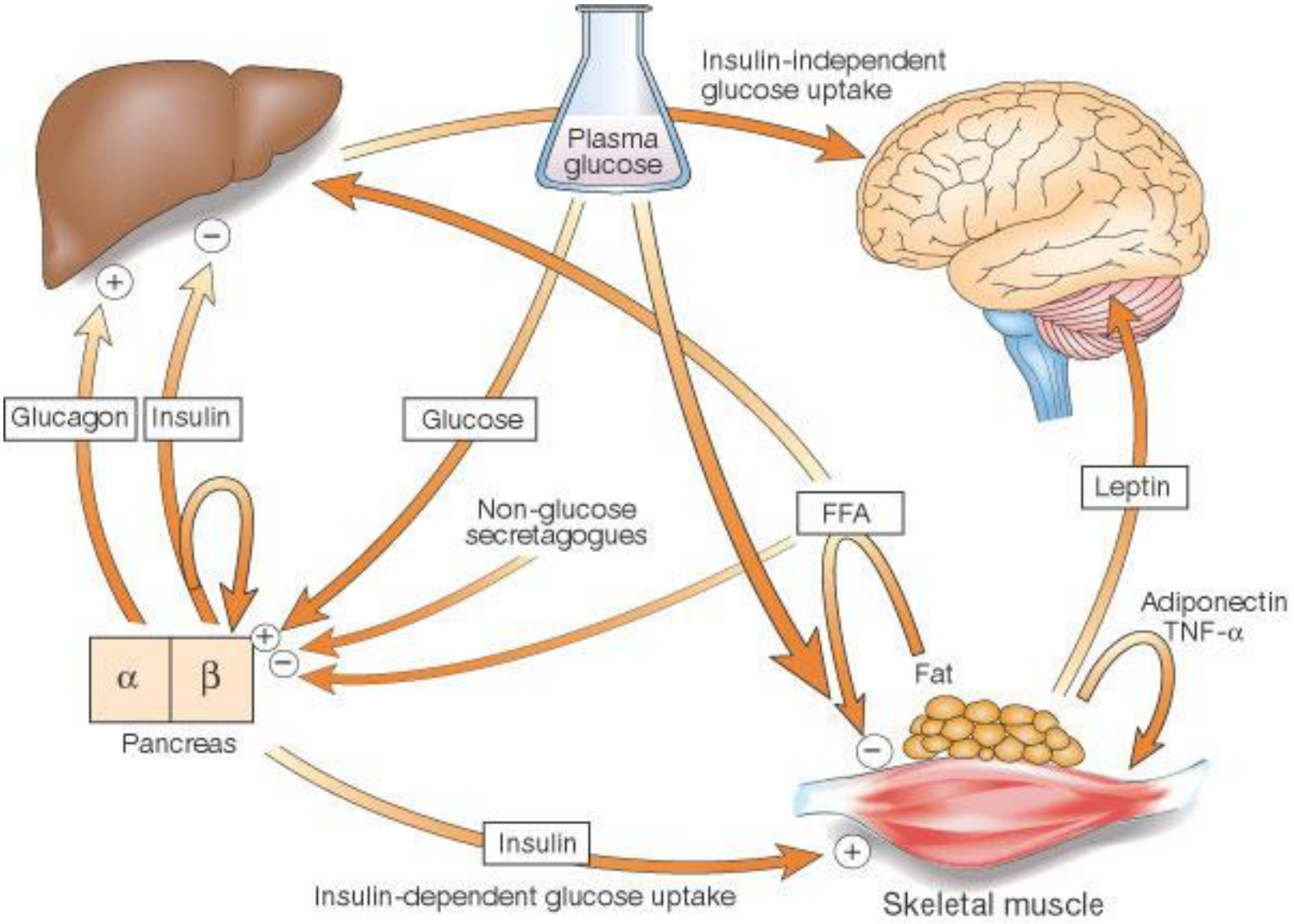
❖It requires a primer, usually a preformed (α1→4) polyglucose chain or branch having at least eight glucose residues.

❖The protein glycogenin is both the primer on which new chains are assembled and the enzyme that catalyzes their assembly.





Principles of Metabolic Regulation



❖Metabolic regulation, a central theme in biochemistry, is one of the most remarkable features of living organisms.

❖Of the thousands of enzyme catalyzed reactions that can take place in a cell, there is probably not one that escapes some form of regulation.

❖Although it is convenient for the student of biochemistry to divide metabolic processes into “pathways” that play discrete roles in the cell’s economy, no such separation exists in the living cell.

❖Rather, every pathway we discuss in this book is inextricably intertwined with all the other cellular pathways in a multidimensional network of reactions.

❖For example, there are four possible fates for glucose 6-phosphate in a hepatocyte:

- breakdown by glycolysis for the production of ATP,
- breakdown in the pentose phosphate pathway for the production of NADPH and pentose phosphates,
- use in the synthesis of complex polysaccharides of the extracellular matrix, or
- hydrolysis to glucose and phosphate to replenish blood glucose.

❖In fact, glucose 6-phosphate has other possible fates in hepatocytes, too; it may, for example, be used to synthesize other sugars, such as glucosamine, galactose and neuraminic acid, for use in protein glycosylation, or it may be partially degraded to provide acetyl-CoA for fatty acid and sterol synthesis.

❖When any cell uses glucose 6-phosphate for one purpose, that "decision" affects all the other pathways for which glucose 6-phosphate is a precursor or intermediate.

❖Any change in the allocation of glucose 6-phosphate to one pathway affects, directly or indirectly, the flow of metabolites through all the others.

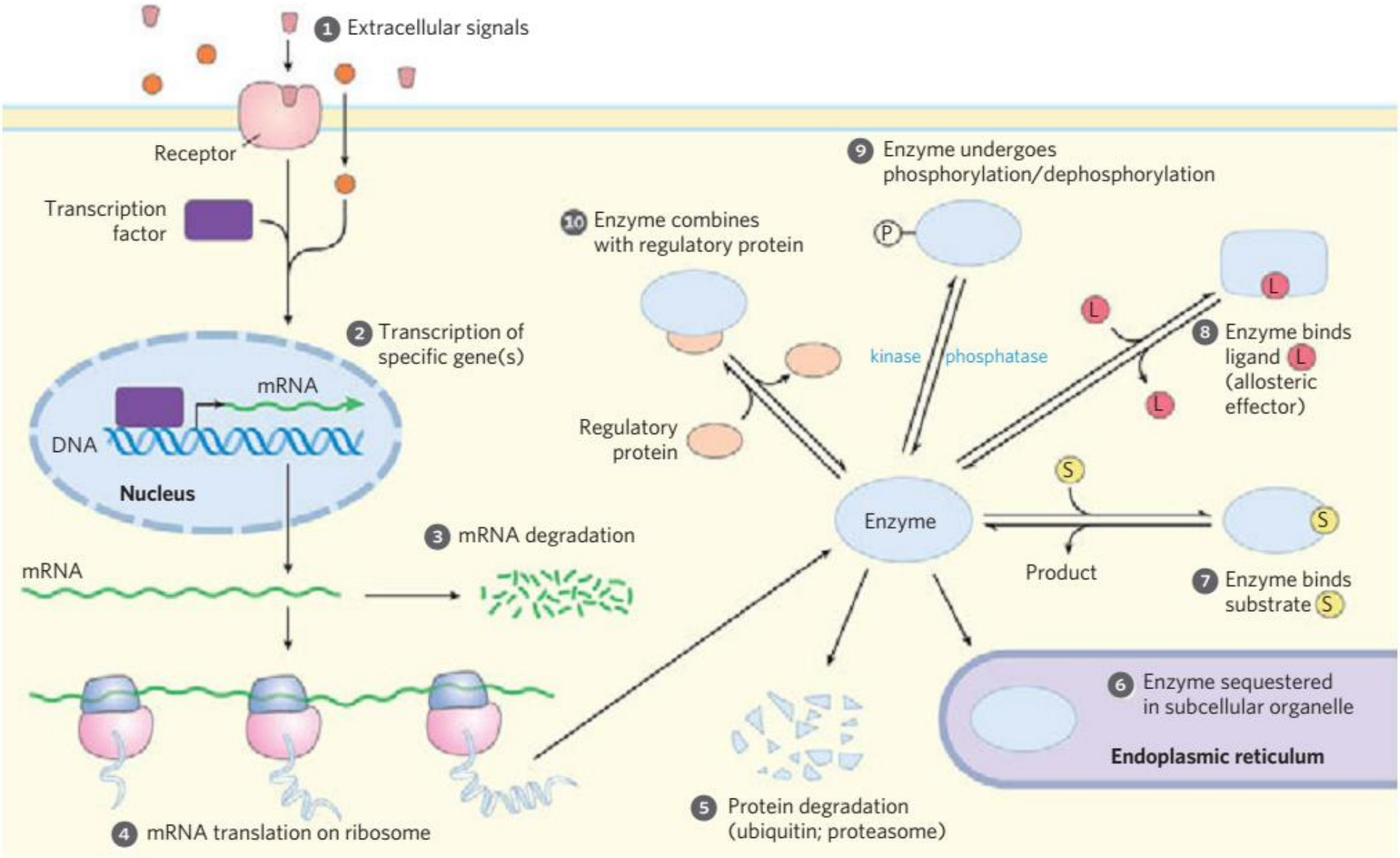
❖The ability of a cell to carry out all these interlocking metabolic processes simultaneously—obtaining every product in the amount needed and at the right time, in the face of major perturbations from outside, and without generating leftovers—is an astounding accomplishment.

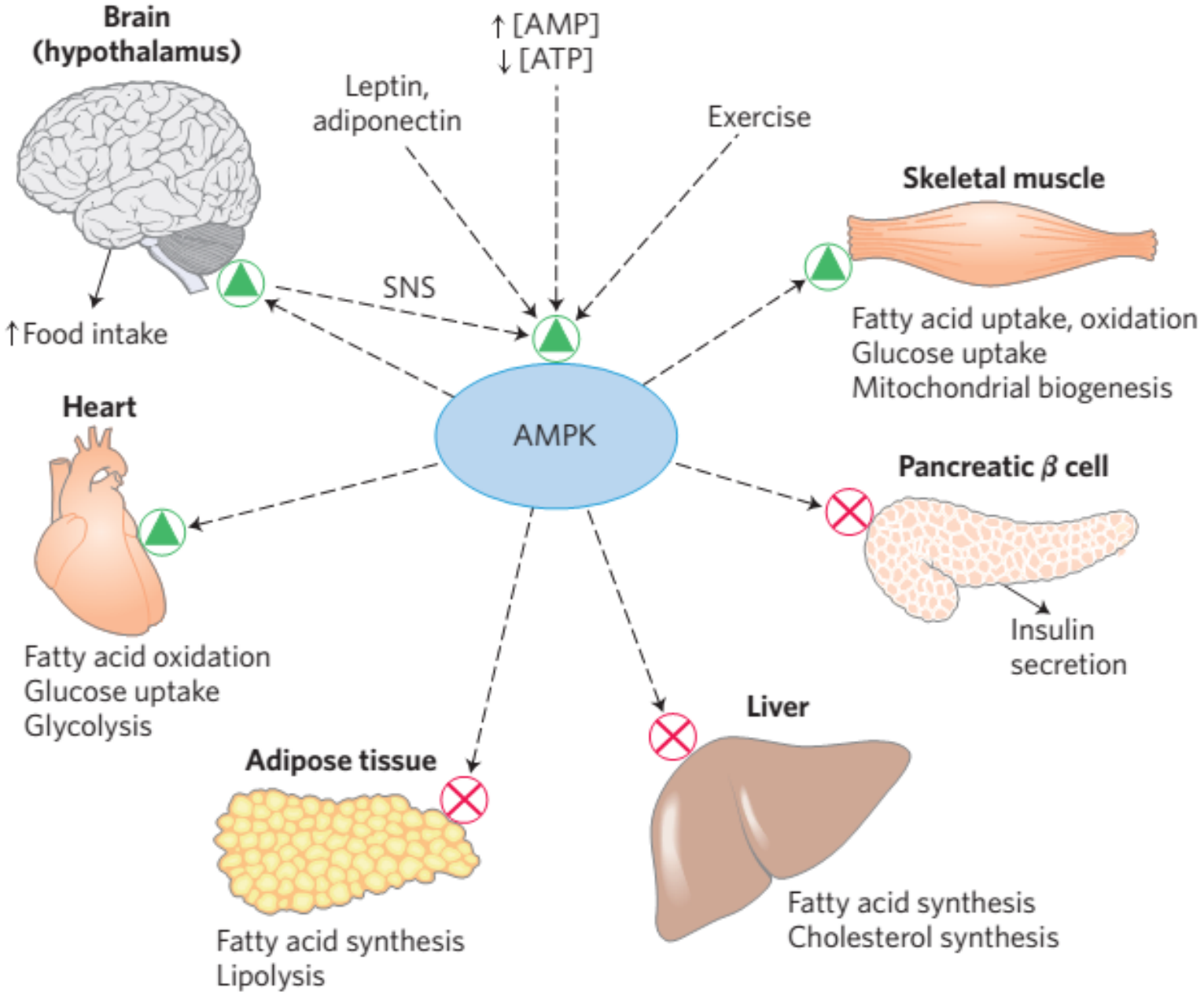
❖The pathways of glucose metabolism provide, in the catabolic direction, the energy essential to oppose the forces of entropy and, in the anabolic direction, biosynthetic precursors and a storage form of metabolic energy.

❖These reactions are so important to survival that very complex regulatory mechanisms have evolved to ensure that metabolites move through each pathway in the correct direction and at the correct rate to match exactly the cell's or the organism's changing circumstances.

❖In the course of evolution, organisms have acquired a remarkable collection of regulatory mechanisms for maintaining homeostasis at the molecular, cellular, and organismal levels, as reflected in the proportion of genes that encode regulatory machinery.

❖In humans, about 4,000 genes (12% of all genes) encode regulatory proteins, including a variety of receptors, regulators of gene expression, and more than 500 different protein kinases!


1 Extracellular signals
Receptor
Transcription factor
2 Transcription of specific gene(s)
mRNA
DNA
Nucleus
3 mRNA degradation
mRNA
4 mRNA translation on ribosome
5 Protein degradation (ubiquitin; proteasome)
6 Enzyme sequestered in subcellular organelle
Endoplasmic reticulum
7 Enzyme binds substrate S
Product
Enzyme
8 Enzyme binds ligand L (allosteric effector)
9 Enzyme undergoes phosphorylation/dephosphorylation
kinase
phosphatase
10 Enzyme combines with regulatory protein
Regulatory protein


Brain
(hypothalamus)
↑ [AMP]
↓ [ATP]
Leptin,
adiponectin
Exercise
Skeletal muscle
SNS
↑ Food intake
Fatty acid uptake, oxidation
Glucose uptake
Mitochondrial biogenesis
AMPK
Heart
Pancreatic β cell
Fatty acid oxidation
Glucose uptake
Glycolysis
Insulin
secretion
Liver
Adipose tissue
Fatty acid synthesis
Cholesterol synthesis
Fatty acid synthesis
Lipolysis

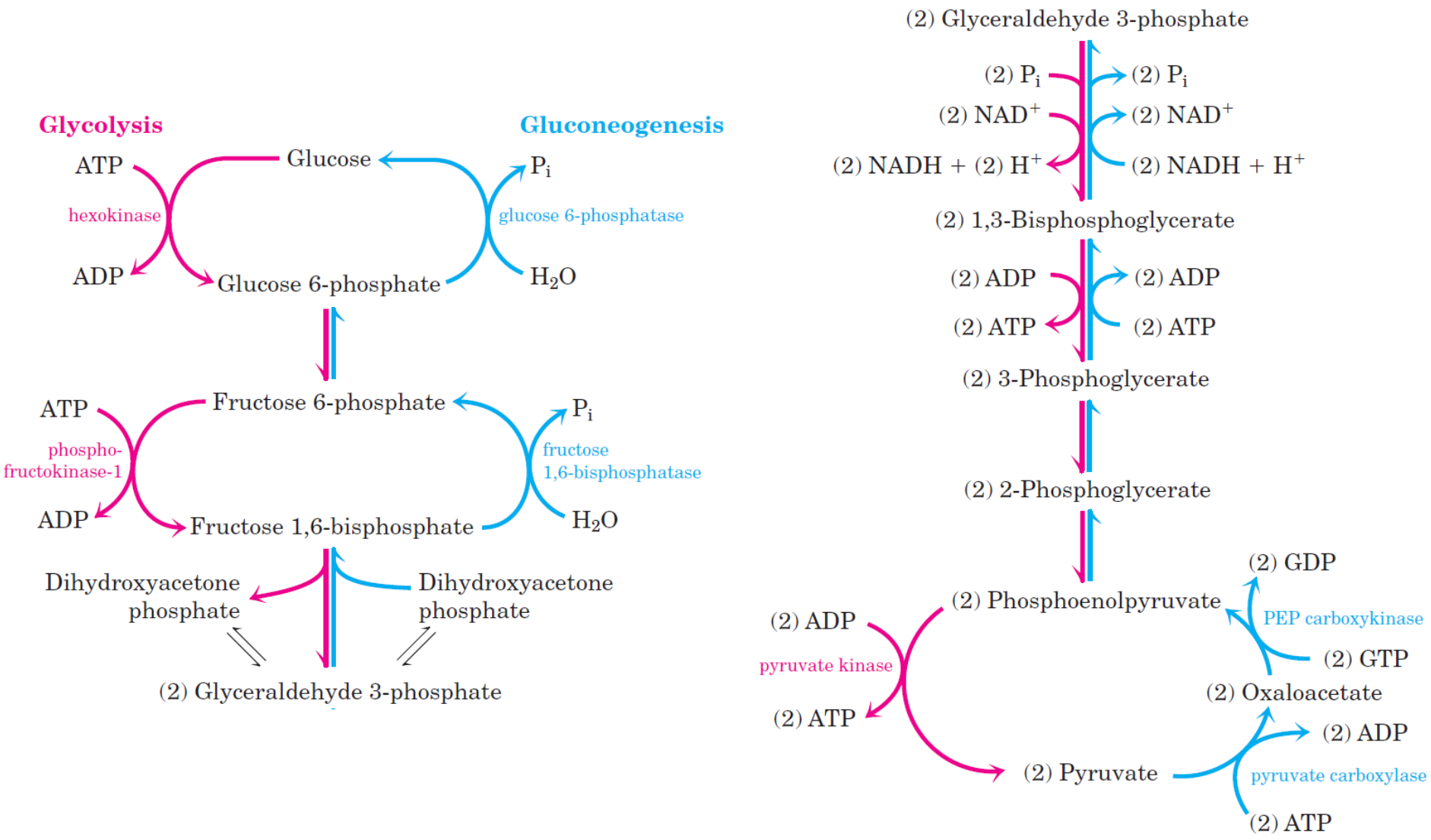
Coordinated Regulation of Glycolysis and Gluconeogenesis

❖In mammals, gluconeogenesis occurs primarily in the liver, where its role is to provide glucose for export to other tissues when glycogen stores are exhausted and when no dietary glucose is available.

❖Gluconeogenesis employs several of the enzymes that act in glycolysis, but it is not simply the reversal of glycolysis.

❖Seven of the glycolytic reactions are freely reversible, and the enzymes that catalyze these reactions also function in gluconeogenesis.

❖Three reactions of glycolysis are so exergonic as to be essentially irreversible: those catalyzed by hexokinase, PFK-1, and pyruvate kinase.


Glycolysis
Gluconeogenesis
ATP
Glucose
P_i
hexokinase
glucose 6-phosphatase
ADP
Glucose 6-phosphate
H_2O
ATP
Fructose 6-phosphate
P_i
phospho-
fructokinase-1
fructose
1,6-bisphosphatase
ADP
Fructose 1,6-bisphosphate
H_2O
Dihydroxyacetone
phosphate
Dihydroxyacetone
phosphate
(2) Glyceraldehyde 3-phosphate
(2) Glyceraldehyde 3-phosphate
(2) P_i
(2) P_i
(2) NAD^+
(2) NAD^+
(2) NADH + (2) H^+
(2) NADH + H^+
(2) 1,3-Bisphosphoglycerate
(2) ADP
(2) ADP
(2) ATP
(2) ATP
(2) 3-Phosphoglycerate
(2) 2-Phosphoglycerate
(2) GDP
(2) Phosphoenolpyruvate
PEP carboxykinase
(2) ADP
(2) GTP
pyruvate kinase
(2) Oxaloacetate
(2) ATP
(2) ADP
(2) Pyruvate
pyruvate carboxylase
(2) ATP

❖Gluconeogenesis uses detours around each of these irreversible steps.

❖At each of the three points where glycolytic reactions are bypassed by alternative, gluconeogenic reactions, simultaneous operation of both pathways would consume ATP without accomplishing any chemical or biological work.

❖For example, PFK-1 and FBPase-1 catalyze opposing reactions:

$$\text{ATP} + \text{fructose 6-phosphate} \xrightarrow{\text{PFK-1}} \text{ADP} + \text{fructose 1,6-bisphosphate}$$

$$\text{Fructose 1,6-bisphosphate} + \text{H}_2\text{O} \xrightarrow{\text{FBPase-1}} \text{fructose 6-phosphate} + \text{P}_\text{i}$$

The sum of these two reactions is:

$$\text{ATP} + \text{H}_2\text{O} \longrightarrow \text{ADP} + \text{P}_\text{i} + \text{heat}$$

❖The sum of these two reactions is, hydrolysis of ATP without any useful metabolic work being done.

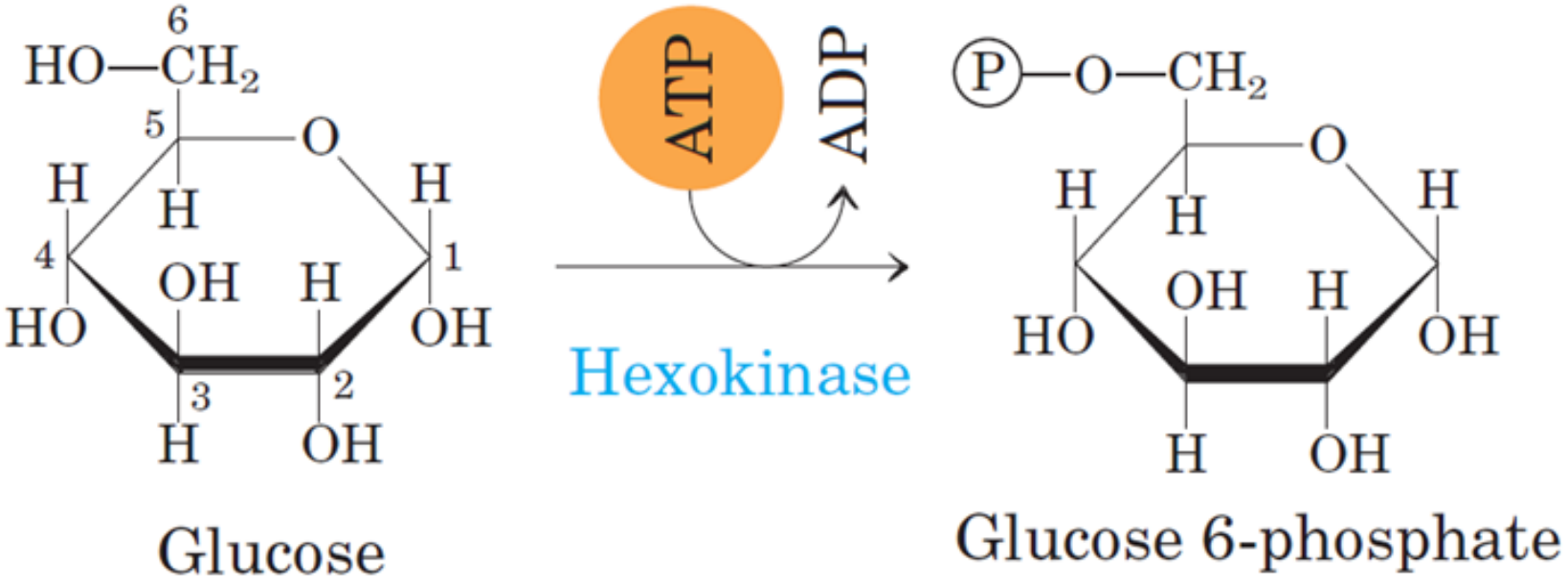
❖Clearly, if these two reactions were allowed to proceed simultaneously at a high rate in the same cell, a large amount of chemical energy would be dissipated as heat.

❖This uneconomical process has been called a **futile cycle**.

❖However, as we shall see later, such cycles may provide advantages for controlling pathways, and the term **substrate cycle** is a better description.

Regulation of Hexokinase

❖Hexokinase, which catalyzes the entry of glucose into the glycolytic pathway, is a regulatory enzyme.



❖The predominant hexokinase isozyme of myocytes (hexokinase II) has a high affinity for glucose—it is half saturated at about 0.1 mM.

❖Because glucose entering myocytes from the blood (where the glucose concentration is 4 to 5 mM) produces an intracellular glucose concentration high enough to saturate hexokinase II, the enzyme normally acts at or near its maximal rate.

❖Muscle hexokinase I and hexokinase II are allosterically inhibited by their product, glucose 6-phosphate.

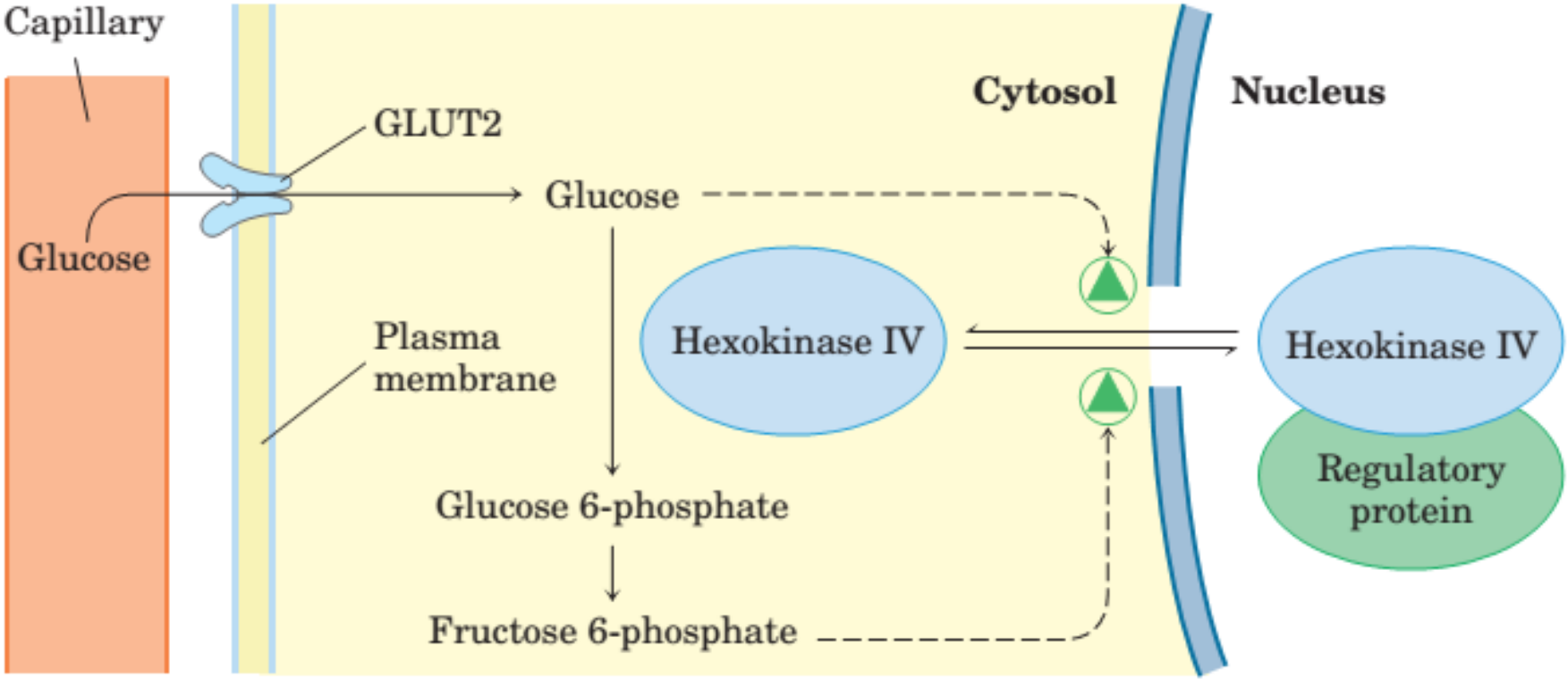
❖The predominant hexokinase isozyme of liver is hexokinase IV (glucokinase), which differs in three important respects from hexokinases I–III of muscle.

❖First, the glucose concentration at which hexokinase IV is half-saturated (about 10 mM) is higher than the usual concentration of glucose in the blood.

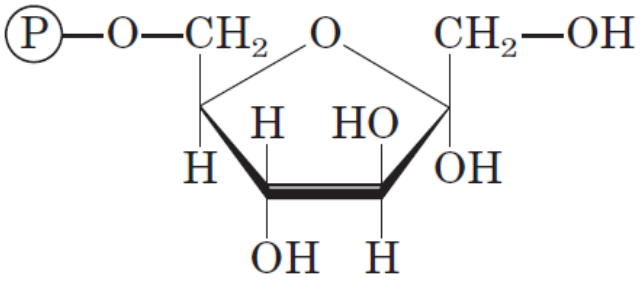
❖The high Km of hexokinase IV allows its direct regulation by the level of blood glucose.

❖Second, hexokinase IV is not inhibited by glucose 6-phosphate, and it can therefore continue to operate when the accumulation of glucose 6-phosphate completely inhibits hexokinases I–III.

❖Finally, hexokinase IV is subject to inhibition by the reversible binding of a regulatory protein specific to liver so the liver does not compete with other organs for the scarce glucose.


Capillary
GLUT2
Glucose
Glucose
Cytosol
Nucleus
Plasma membrane
Hexokinase IV
Hexokinase IV
Regulatory protein
Glucose 6-phosphate
Fructose 6-phosphate

Regulation of PFK-1



Fructose 6-phosphate

second priming reaction

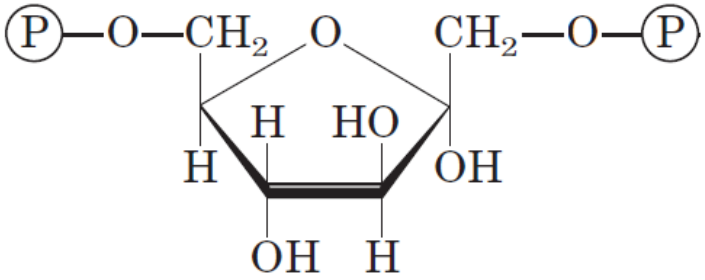
3

ATP

ADP

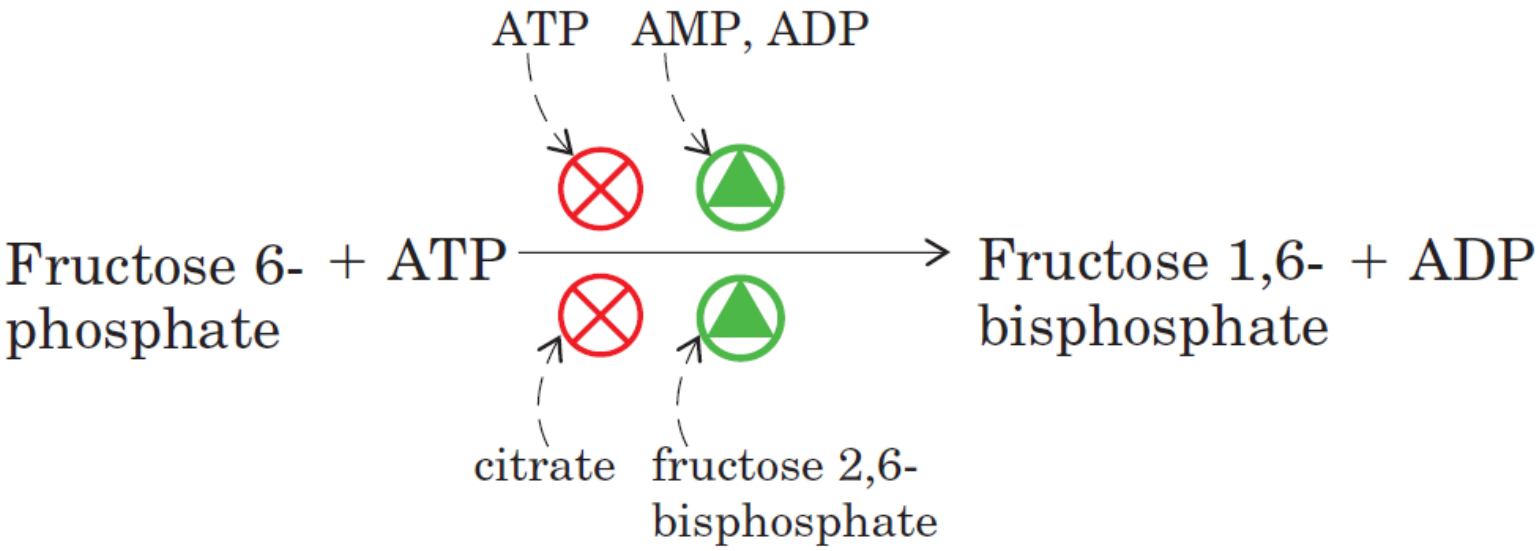
Phosphofructokinase-1

Fructose 1,6-bisphosphate



❖PFK-1, in addition to its substrate-binding sites, has several regulatory sites at which allosteric activators or inhibitors bind.

❖When high cellular [ATP] signals that ATP is being produced faster than it is being consumed, ATP inhibits PFK-1 by binding to an allosteric site and lowering the affinity of the enzyme for its substrate fructose 6-phosphate.



❖ADP and AMP, which increase in concentration as consumption of ATP outpaces production, act allosterically to relieve this inhibition by ATP.

❖These effects combine to produce higher enzyme activity when ADP or AMP accumulates and lower activity when ATP accumulates.

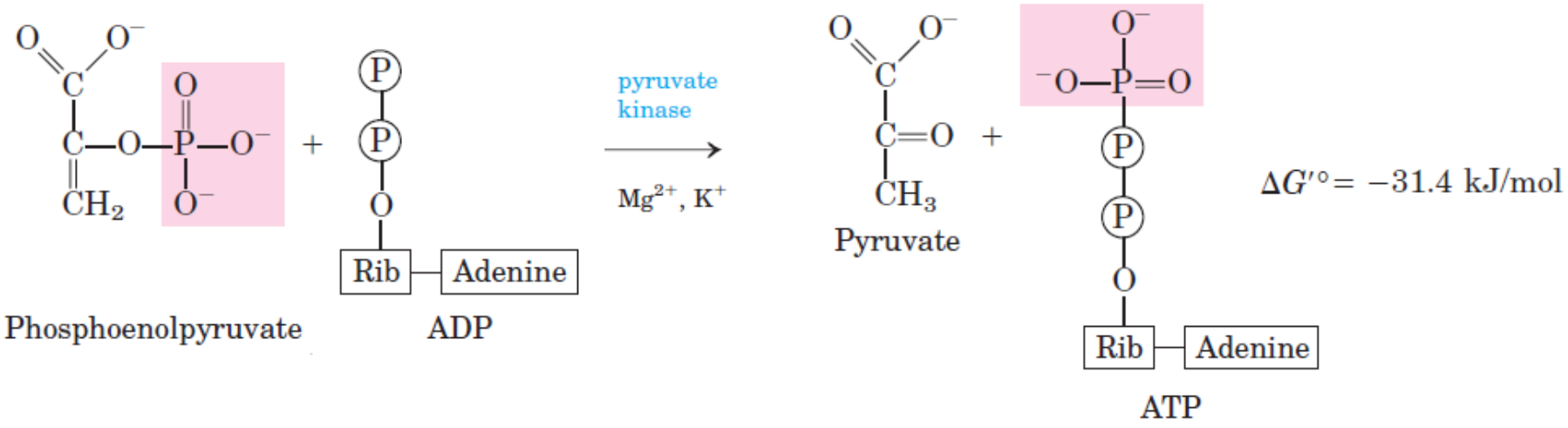
❖Citrate (the ionized form of citric acid), a key intermediate in the aerobic oxidation of pyruvate, fatty acids, and amino acids, is also an allosteric regulator of PFK-1; high citrate concentration increases the inhibitory effect of ATP, further reducing the flow of glucose through glycolysis.

❖In this case, as in several others encountered later, citrate serves as an intracellular signal that the cell is meeting its current needs for energy-yielding metabolism by the oxidation of fats and proteins

❖Fructose 2,6-bisphosphate is a potent allosteric activator of PFK-1

Regulation of Pyruvate Kinase

❖At least three isozymes of pyruvate kinase are found in vertebrates, differing in their tissue distribution and their response to modulators.



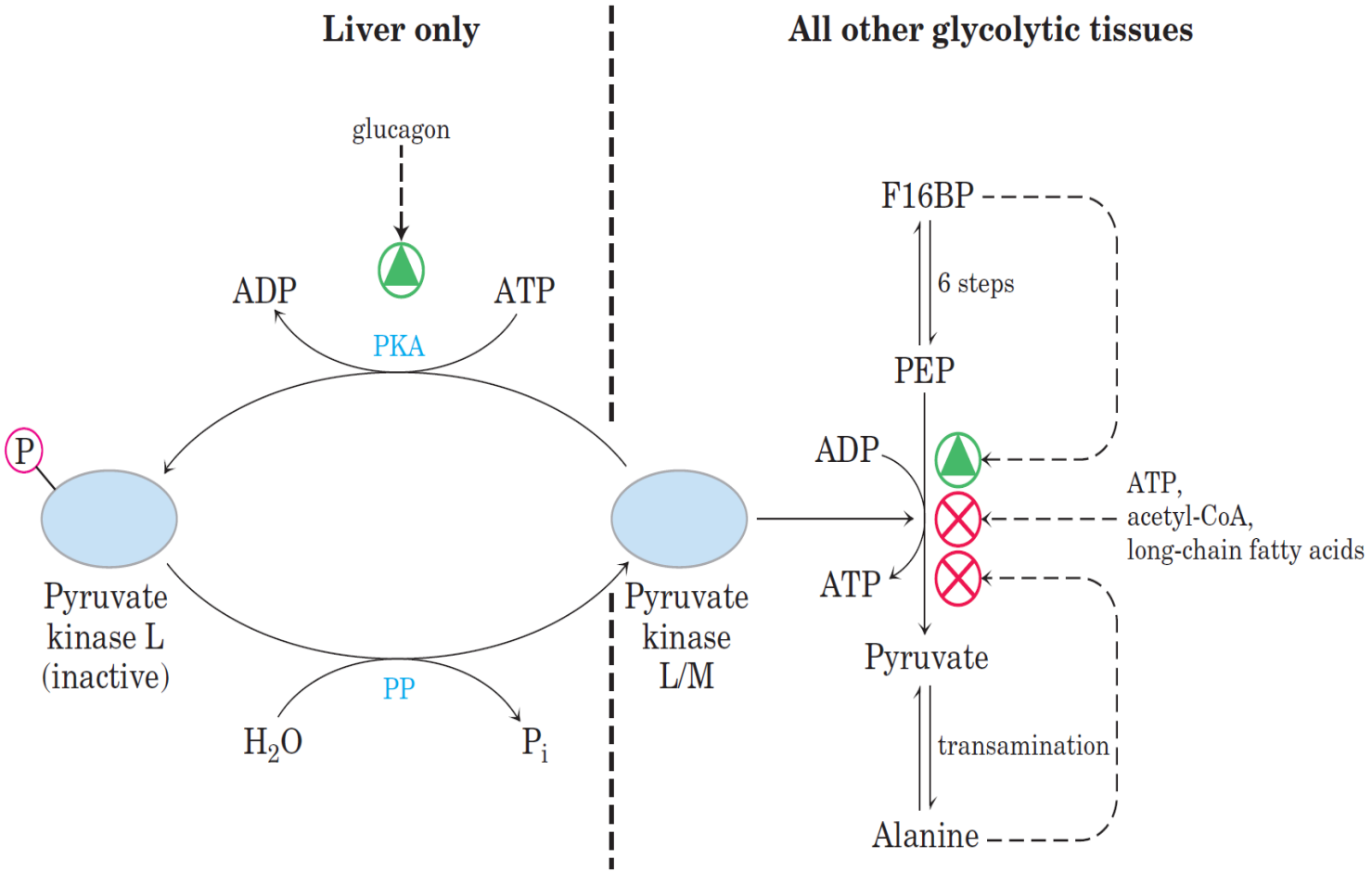
❖High concentrations of ATP, acetyl-CoA, and long-chain fatty acids (signs ofabundant energy supply) allosterically inhibit all isozymes of pyruvate kinase.

❖The liver isozyme (L form), but not the muscle isozyme (M form), is subject to further regulation by phosphorylation.

❖When low blood glucose causes glucagon release, cAMPdependent protein kinase phosphorylates the L isozyme of pyruvate kinase, inactivating it.

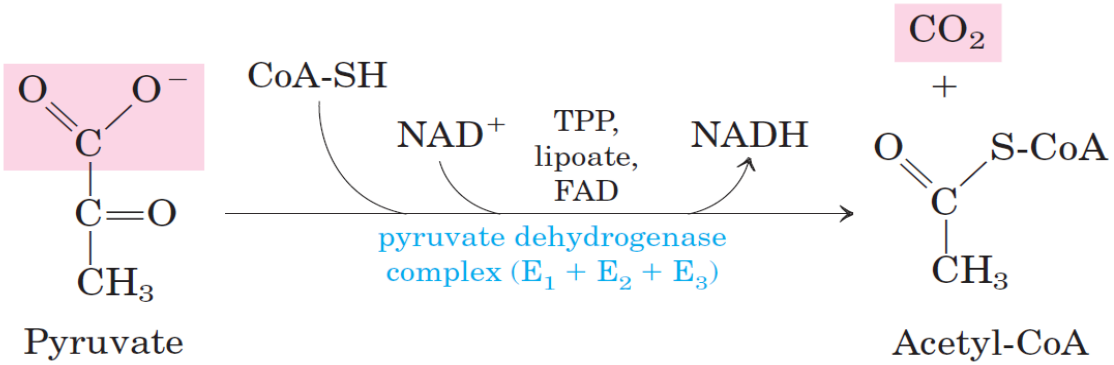
❖This slows the use ofglucose as a fuel in liver, sparing it for export to the brain and other organs.

❖ In muscle, the effect of increased [cAMP] is quite different. In response to epinephrine, cAMP activates glycogen breakdown and glycolysis and provides the fuel needed for the fight-or-flight response.

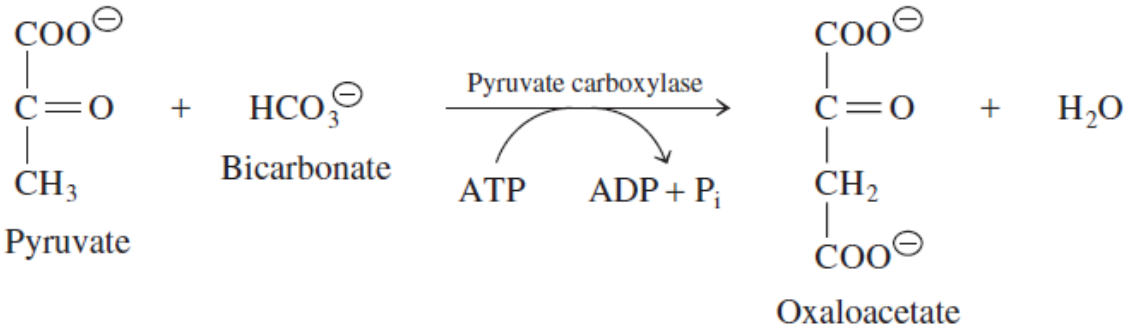

Liver only
All other glycolytic tissues
glucagon
ADP
ATP
PKA
P
Pyruvate kinase L (inactive)
PP
H_2O
P_i
Pyruvate kinase L/M
F16BP
6 steps
PEP
ADP
ATP
ATP, acetyl-CoA, long-chain fatty acids
Pyruvate
transamination
Alanine

Regulation of Gluconeogenesis

❖In the pathway leading from pyruvate to glucose, the first control point determines the fate of pyruvate in the mitochondrion: its conversion either to acetyl-CoA (by the pyruvate dehydrogenase complex) to fuel the citric acid cycle or to oxaloacetate (by pyruvate carboxylase) to start the process of gluconeogenesis.



$\Delta G'^{\circ} = -33.4$ kJ/mol



❖When fatty acids are readily available as fuels, their breakdown in liver mitochondria yields acetyl-CoA, a signal that further oxidation of glucose for fuel is not necessary.

❖Acetyl-CoA is a positive allosteric modulator of pyruvate carboxylase and a negative modulator of pyruvate dehydrogenase, through stimulation of a protein kinase that inactivates the dehydrogenase.

❖When the cell's energy needs are being met, oxidative phosphorylation slows, [NADH] rises relative to [NAD+] and inhibits the citric acid cycle, and acetyl-CoA accumulates.

❖The increased concentration of acetylCoA inhibits the pyruvate dehydrogenase complex, slowing the formation of acetyl-CoA from pyruvate, and stimulates gluconeogenesis by activating pyruvate carboxylase.

❖Oxaloacetate formed in this way is converted to phosphoenolpyruvate (PEP) in the reaction catalyzed by PEP carboxykinase.

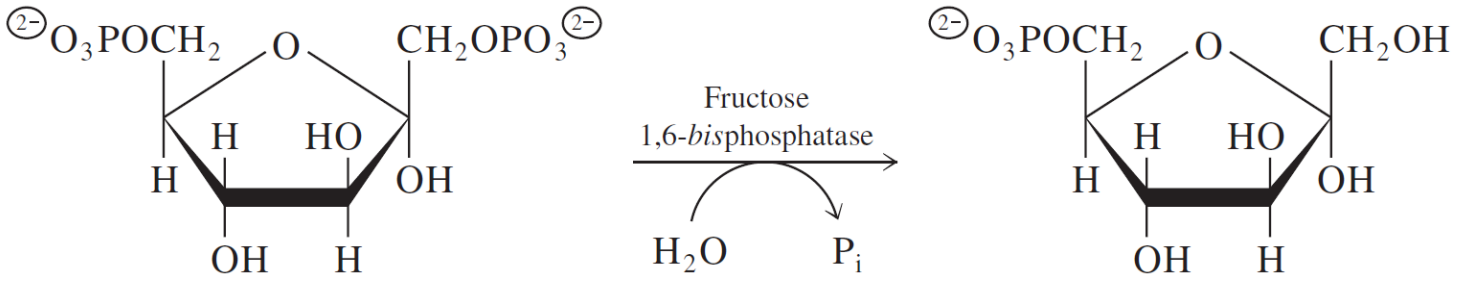
❖In mammals, the regulation of this key enzyme occurs primarily at the level of its synthesis and breakdown, in response to dietary and hormonal signals.

❖Fasting or high glucagon levels act through cAMP to increase the rate of transcription and to stabilize the mRNA.

❖Insulin, or high blood glucose, has the opposite effects.

❖Generally triggered by a signal from outside the cell (diet, hormones), these changes take place on a time scale of minutes to hours.

❖FBPase-1, is strongly inhibited (allosterically) by AMP; when the cell's supply of ATP is low, the ATP-requiring synthesis of glucose slows.



Fructose 1,6-*bis*phosphate

Fructose 6-phosphate

❖ Thus these opposing steps in the glycolytic and gluconeogenic pathways—those catalyzed by PFK-1 and FBPase-1—are regulated in a coordinated and reciprocal manner.

❖In general, when sufficient concentrations of acetyl-CoA or citrate (the product of acetylCoA condensation with oxaloacetate) are present, or when a high proportion of the cell's adenylate is in the form of ATP, gluconeogenesis is favored.

❖When the level of AMP increases, it promotes glycolysis by stimulating PFK-1

❖The special role of the liver in maintaining a constant blood glucose level requires additional regulatory mechanisms to coordinate glucose production and consumption.

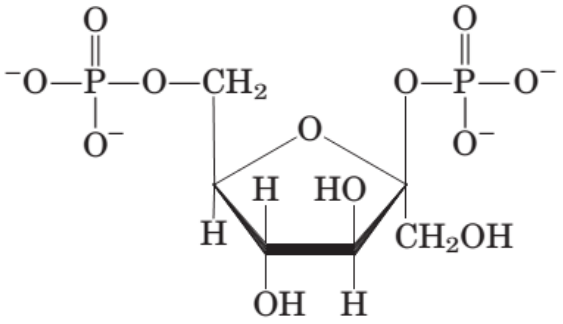
❖When the blood glucose level decreases, the hormone glucagon signals the liver to produce and release more glucose and to stop consuming it for its own needs.

❖One source of glucose is glycogen stored in the liver; another source is gluconeogenesis, using pyruvate, lactate, glycerol, or certain amino acids as starting material.

❖When blood glucose is high, insulin signals the liver to use glucose as a fuel and as a precursor for the synthesis and storage of glycogen and triacylglycerol.

❖

❖The rapid hormonal regulation of glycolysis and gluconeogenesis is mediated by fructose 2,6-bisphosphate, an allosteric effector for the enzymes PFK-1 and FBPase-1.

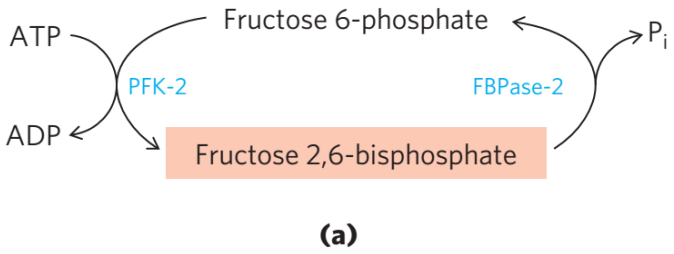


Fructose 2,6-bisphosphate

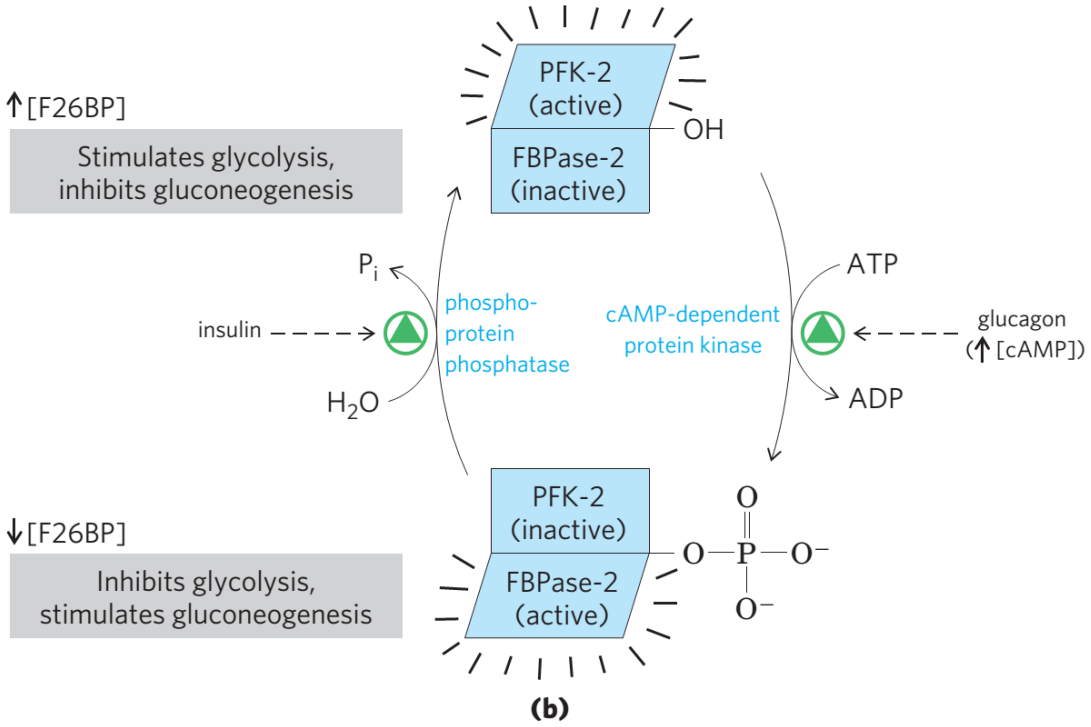
❖When fructose 2,6-bisphosphate binds to its allosteric site on PFK-1, it increases the enzyme's affinity for its substrate fructose 6-phosphate and reduces its affinity for the allosteric inhibitors ATP and citrate.

❖At the physiological concentrations of its substrates, ATP and fructose 6-phosphate, and of its other positive and negative effectors (ATP, AMP, citrate), PFK-1 is virtually inactive in the absence of fructose 2,6-bisphosphate.

❖Fructose 2,6-bisphosphate has the opposite effect on FBPase-1: it reduces its affinity for its substrate, thereby slowing gluconeogenesis.


ATP
Fructose 6-phosphate
P_i
PFK-2
FBPase-2
ADP
Fructose 2,6-bisphosphate

(a)


↑[F26BP]
Stimulates glycolysis, inhibits gluconeogenesis
PFK-2 (active)
FBPase-2 (inactive)
OH
P_i
insulin
H_2O
phospho-protein phosphatase
cAMP-dependent protein kinase
ATP
glucagon (↑[cAMP])
ADP
↓[F26BP]
Inhibits glycolysis, stimulates gluconeogenesis
PFK-2 (inactive)
FBPase-2 (active)
O
O—P—O⁻
O⁻

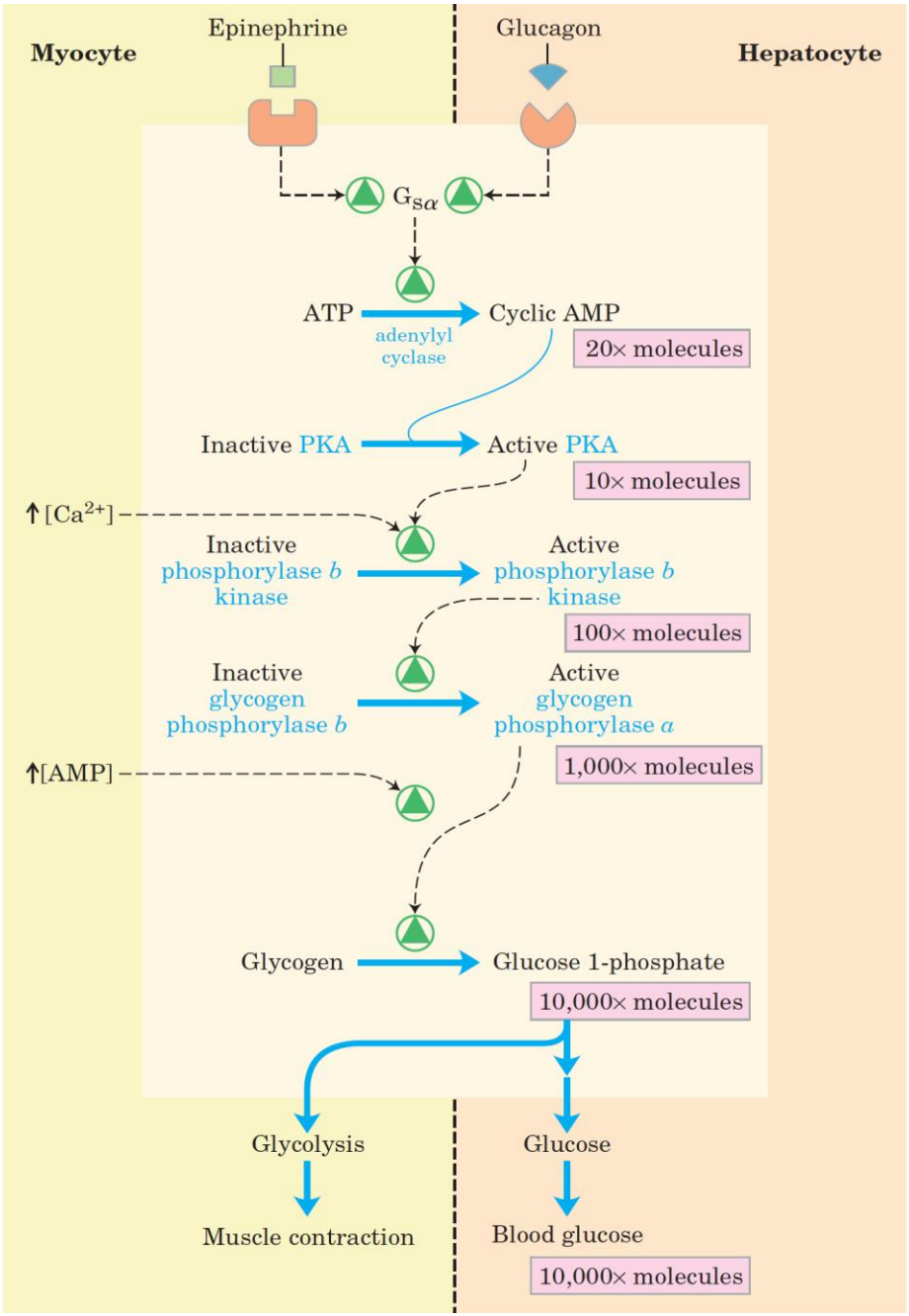
(b)

Coordinated Regulation of Glycogen Synthesis and Breakdown

❖The mobilization of stored glycogen is brought about by glycogen phosphorylase, which degrades glycogen to glucose 1-phosphate.

❖The glycogen phosphorylase of skeletal muscle exists in two interconvertible forms: glycogen phosphorylase a, which is catalytically active, and glycogen phosphorylase b, which is less active .

❖The enzyme (phosphorylase b kinase) responsible for activating phosphorylase by transferring a phosphoryl group to its Ser residue is itself activated by epinephrine or glucagon.



❖By binding to specific surface receptors, either epinephrine acting on a myocyte (left) or glucagon acting on a hepatocyte (right) activates a GTP-binding protein which triggers a rise in [cAMP], activating PKA.

❖This sets off a cascade of phosphorylations; PKA activates phosphorylase b kinase, which then activates glycogen phosphorylase.

❖Such cascades effect a large amplification of the initial signal.

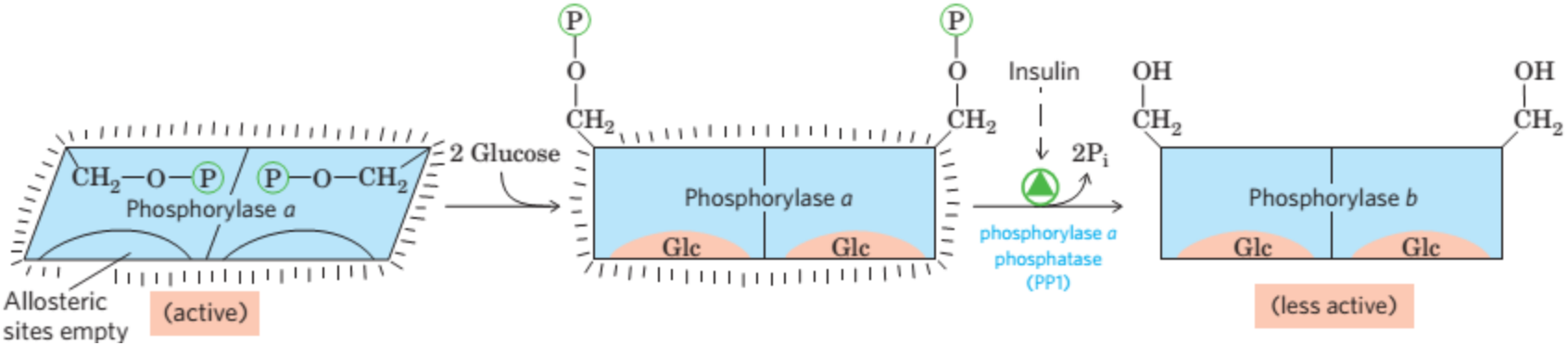
❖The resulting breakdown of glycogen provides glucose, which in the myocyte can supply ATP (via glycolysis) for muscle contraction and in the hepatocyte is released into the blood to counter the low blood glucose.

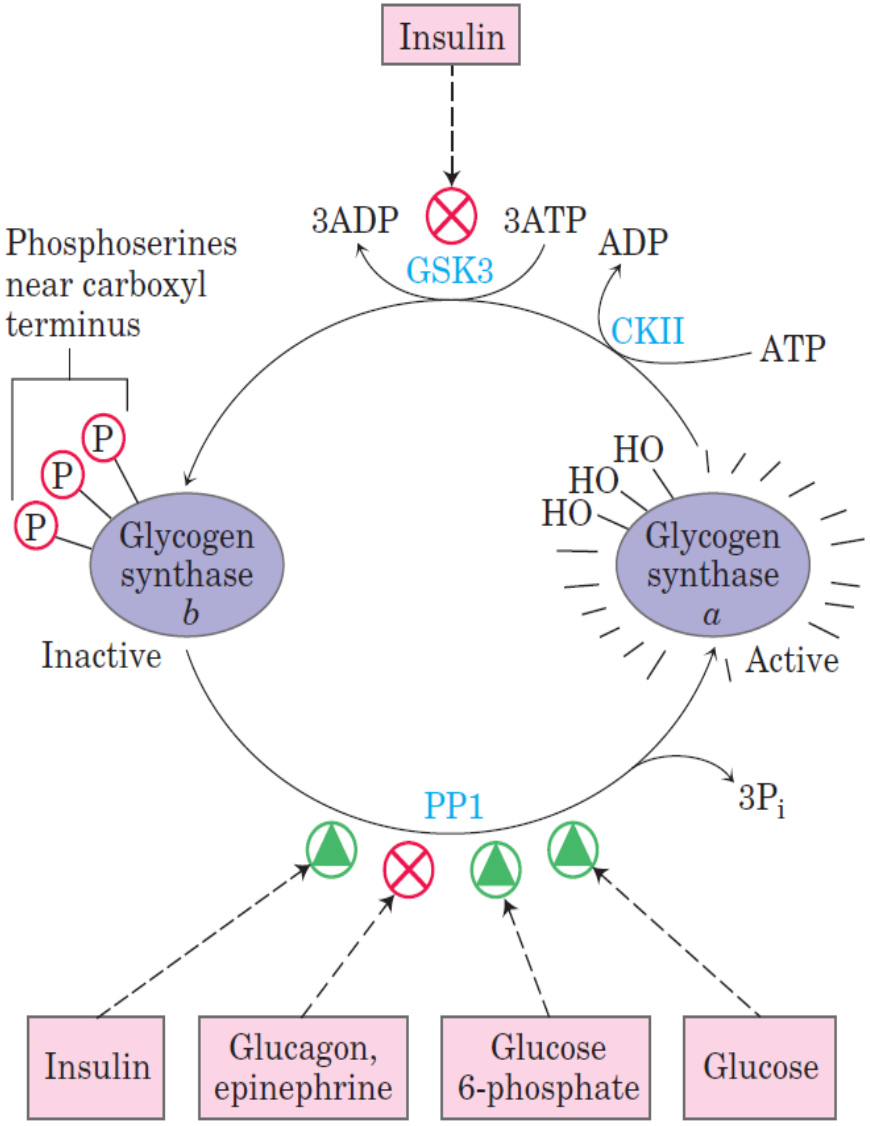
❖When the blood glucose level is too low, glucagon activates phosphorylase b kinase, which in turn converts phosphorylase b to its active a form, initiating the release of glucose into the blood.

❖When blood glucose levels return to normal, glucose enters hepatocytes and binds to an inhibitory allosteric site on phosphorylase a.

❖This binding also produces a conformational change that exposes the phosphorylated Ser residues to PP1, which catalyzes their dephosphorylation and inactivates the phosphorylase.

❖The allosteric site for glucose allows liver glycogen phosphorylase to act as its own glucose sensor and to respond appropriately to changes in blood glucose.





❖Like glycogen phosphorylase, glycogen synthase can exist in phosphorylated and dephosphorylated forms.

❖Its active form, glycogen synthase a, is unphosphorylated.

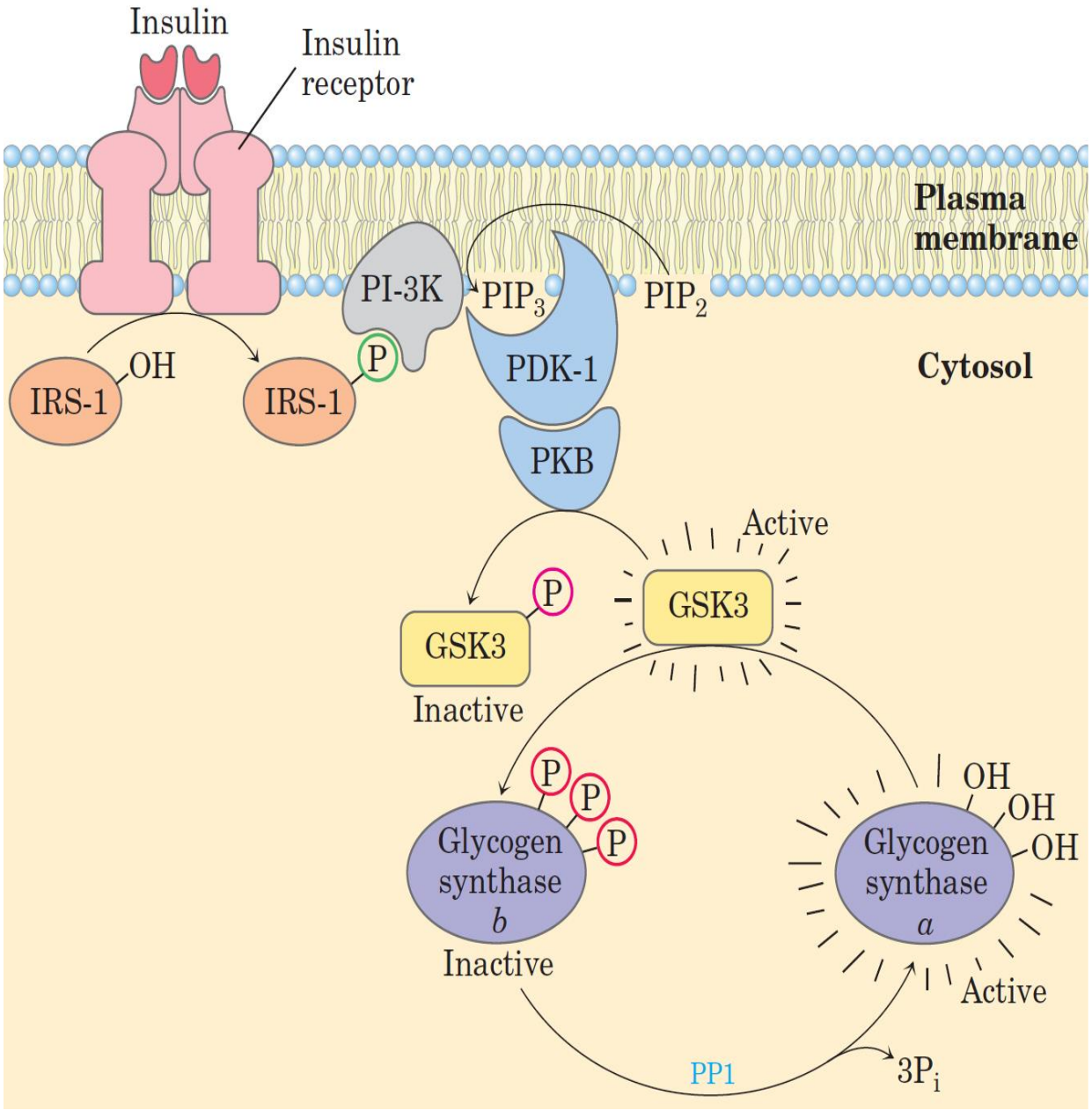
❖The most important regulatory kinase is glycogen synthase kinase 3 (GSK3).

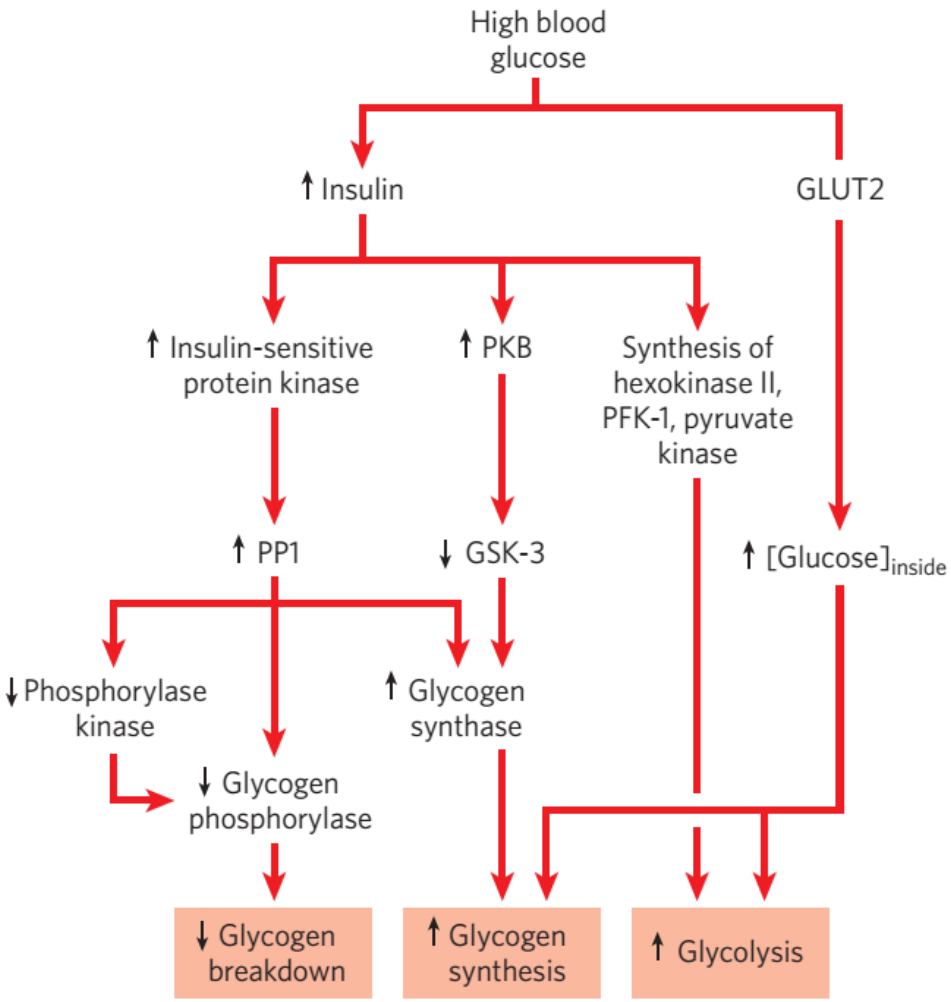
❖One way in which insulin triggers intracellular changes is by activating a protein kinase (PKB) that in turn phosphorylates and inactivates GSK3.

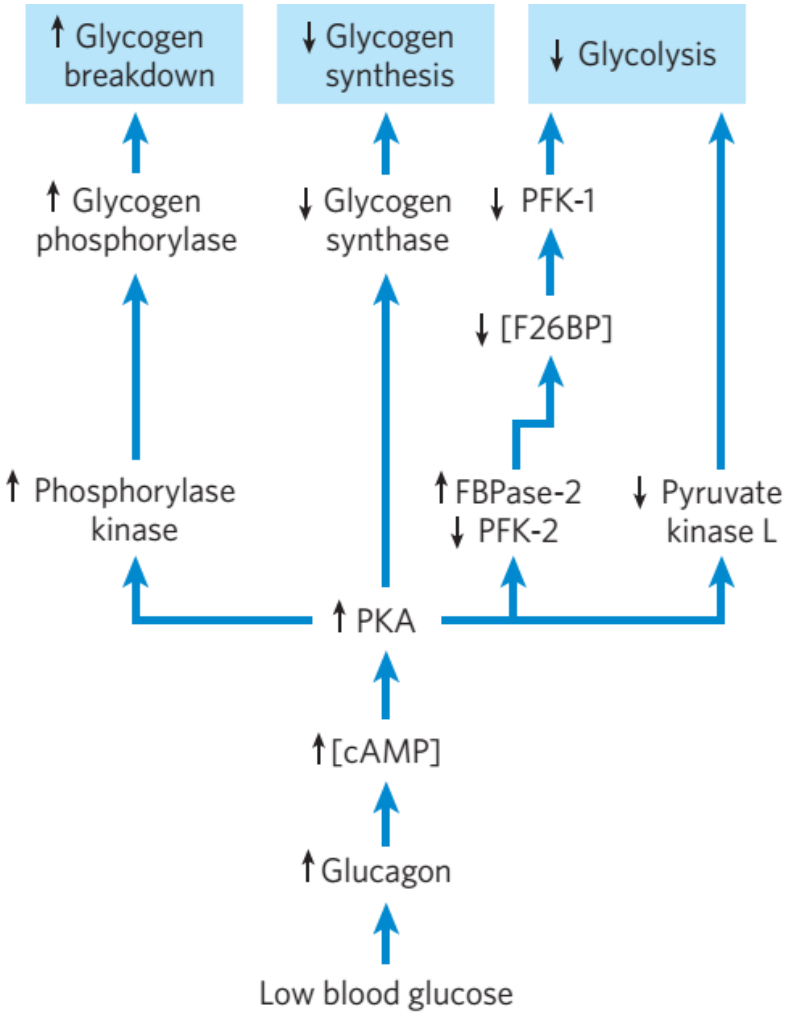
❖A single enzyme, phosphoprotein phosphatase 1, PP1, can remove phosphoryl groups from all three of the enzymes phosphorylated in response to glucagon (liver) and epinephrine (liver and muscle):

- phosphorylase kinase,
- glycogen phosphorylase, and
- glycogen synthase.

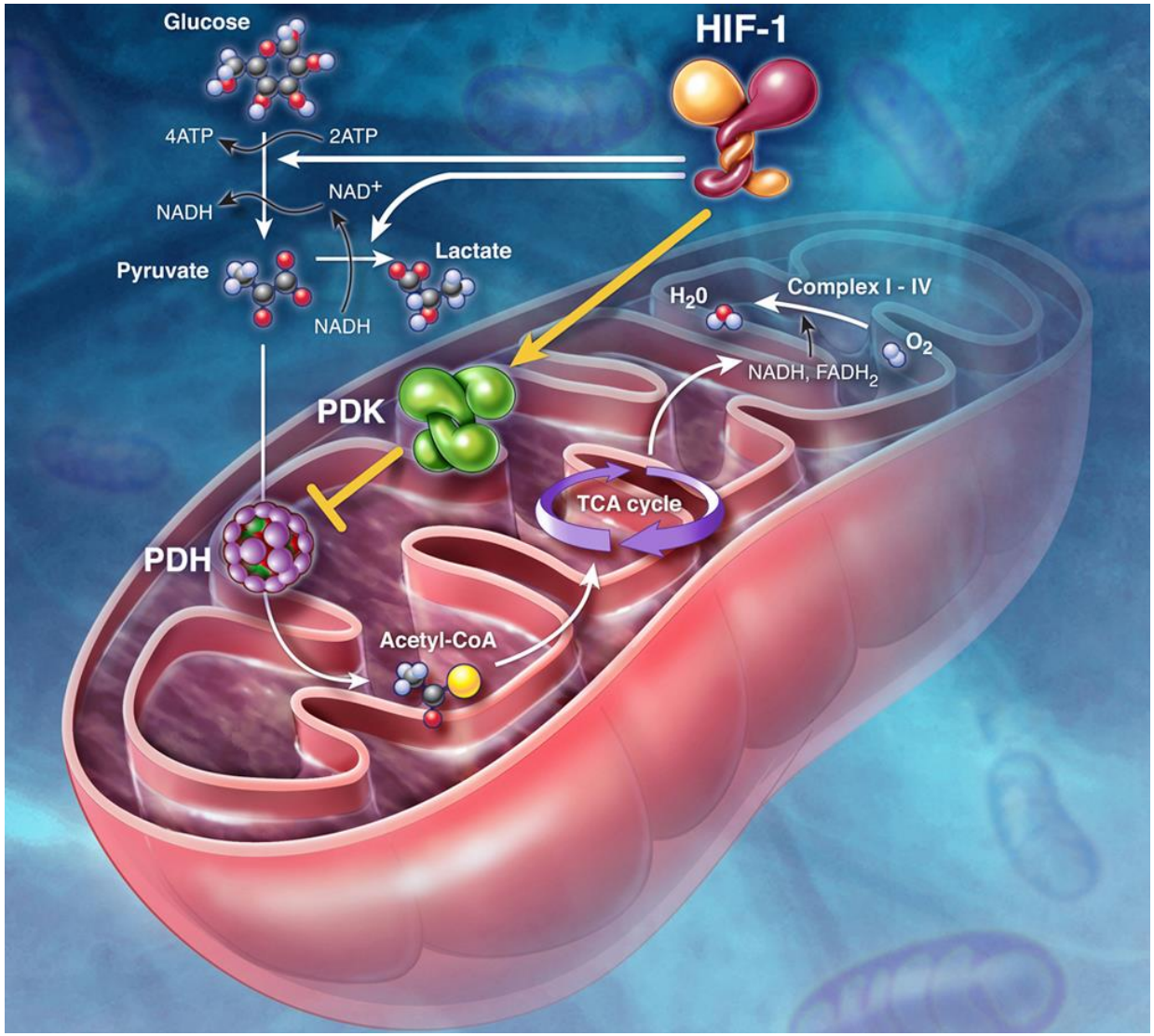
❖Insulin stimulates glycogen synthesis by activating PP1 and by inactivating GSK3.

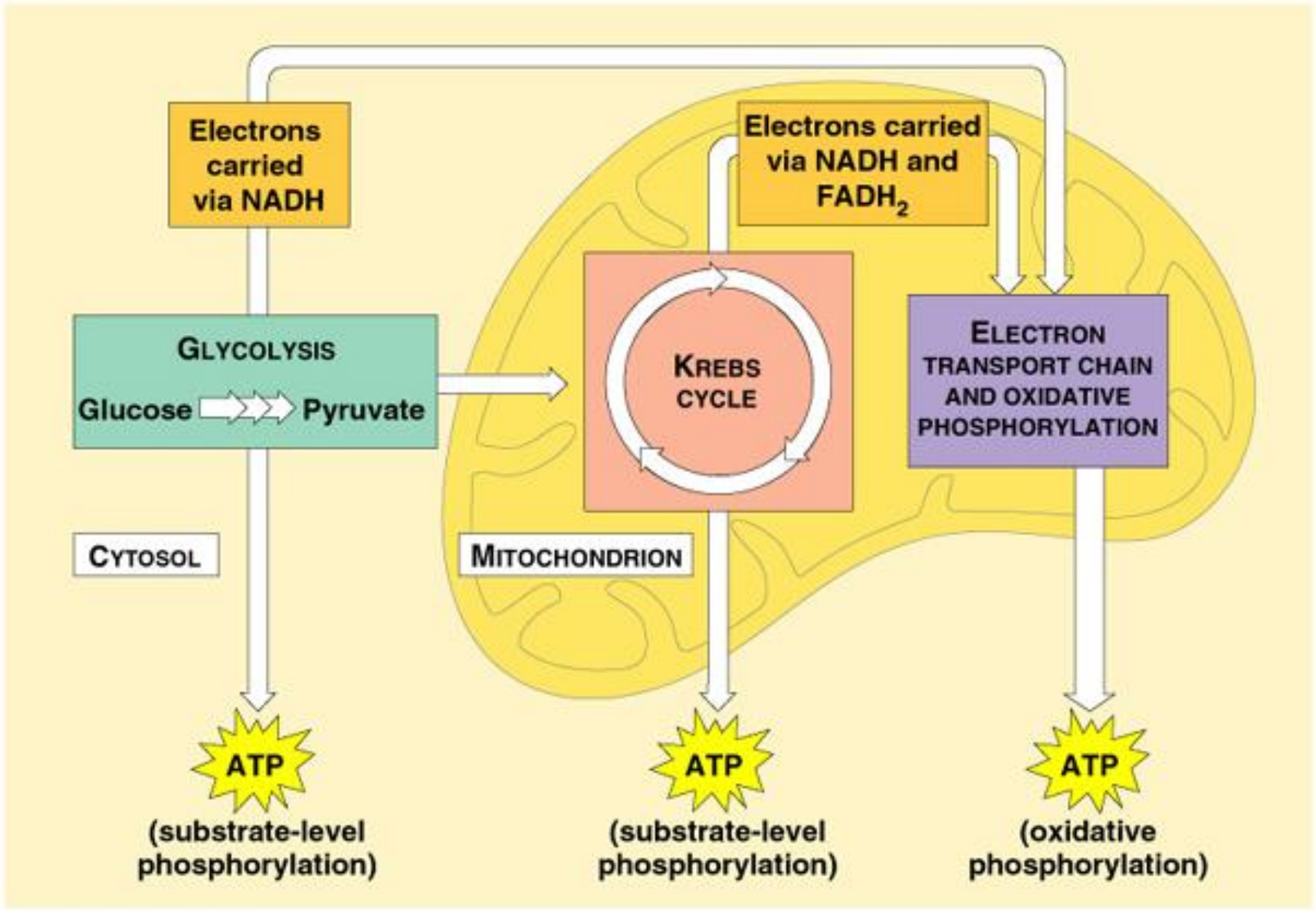

Insulin
Insulin receptor
Plasma membrane
PI-3K
PIP3
PIP2
PDK-1
PKB
Cytosol
IRS-1
OH
IRS-1
P
Active
GSK3
P
GSK3
Inactive
P
P
P
Glycogen synthase b
Inactive
OH
OH
OH
Glycogen synthase a
Active
PP1
3Pi


High blood glucose
↑ Insulin
GLUT2
↑ Insulin-sensitive protein kinase
↑ PKB
Synthesis of hexokinase II, PFK-1, pyruvate kinase
↑ PP1
↓ GSK-3
↑ [Glucose]inside
↓ Phosphorylase kinase
↑ Glycogen synthase
↓ Glycogen phosphorylase
↓ Glycogen breakdown
↑ Glycogen synthase
↑ Glycolysis


↑ Glycogen breakdown
↓ Glycogen synthesis
↓ Glycolysis
↑ Glycogen phosphorylase
↓ Glycogen synthase
↓ PFK-1
↓ [F26BP]
↑ Phosphorylase kinase
↑ FBPase-2
↓ PFK-2
↓ Pyruvate kinase L
↑ PKA
↑ [cAMP]
↑ Glucagon
Low blood glucose

CARBOHYDRATE METABOLISM III: CITRIC ACID CYCLE





Cellular Respiration

❖Some cells obtain energy (ATP) by fermentation, breaking down glucose in the absence of oxygen.

❖For most eukaryotic cells and many bacteria, which live under aerobic conditions and oxidize their organic fuels to carbon dioxide and water, glycolysis is but the first stage in the complete oxidation of glucose.

❖Rather than being reduced to lactate, ethanol, or some other fermentation product, the pyruvate produced by glycolysis is further oxidized to H_2O and CO_2.

❖This aerobic phase of catabolism is called **respiration**.

❖Cellular respiration occurs in three major stages.

❖In the first, organic fuel molecules—glucose, fatty acids, and some amino acids—are oxidized to yield two-carbon fragments in the form of the acetyl-coenzyme A.

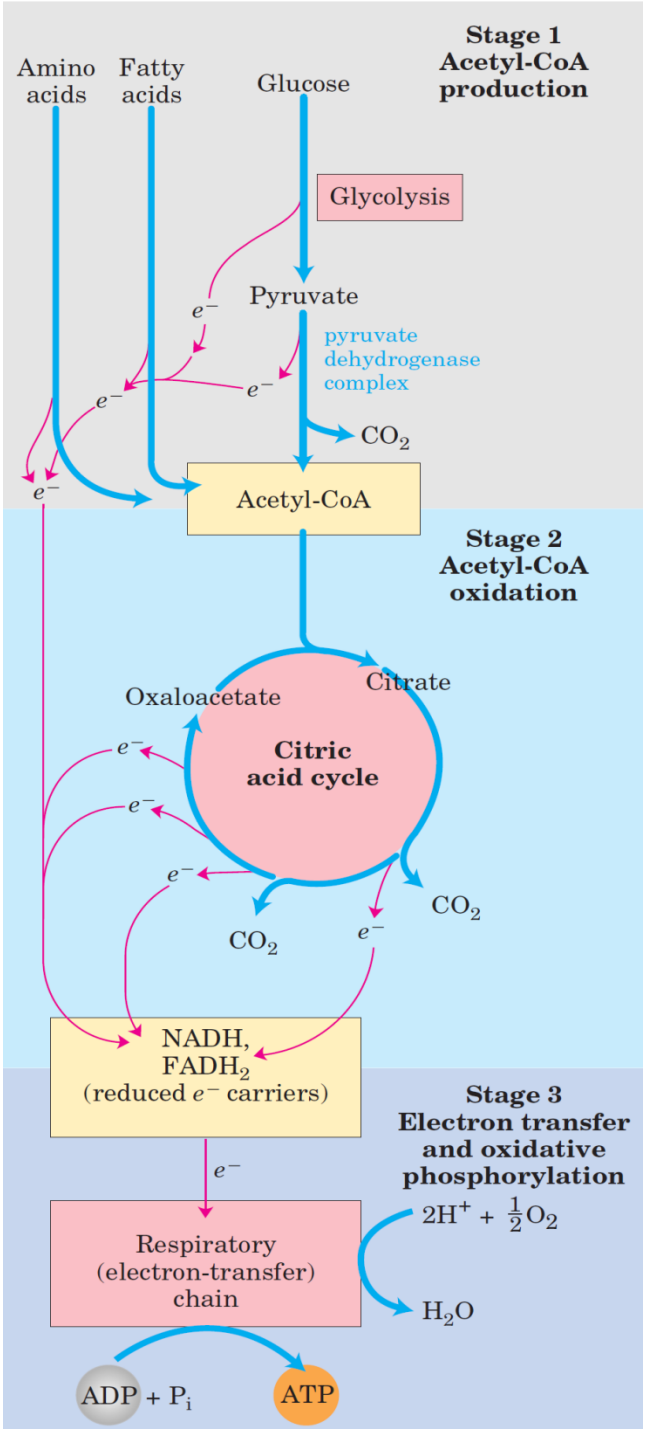
❖In the second stage, the acetyl groups are fed into the citric acid cycle, which enzymatically oxidizes them to CO_2; the energy released is conserved in the reduced electron carriers NADH and $FADH_2$.

❖In the third stage of respiration, these reduced coenzymes are themselves oxidized, giving up protons (H^+) and electrons.

❖The electrons are transferred to O_2—the final electron acceptor—via a chain of electron-carrying molecules known as the respiratory chain.

❖In the course of electron transfer, the large amount of energy released is conserved in the form of ATP, by a process called oxidative phosphorylation.

❖In aerobic organisms, glucose and other sugars, fatty acids, and most amino acids are ultimately oxidized to CO_2 and H_2O via the citric acid cycle and the respiratory chain.

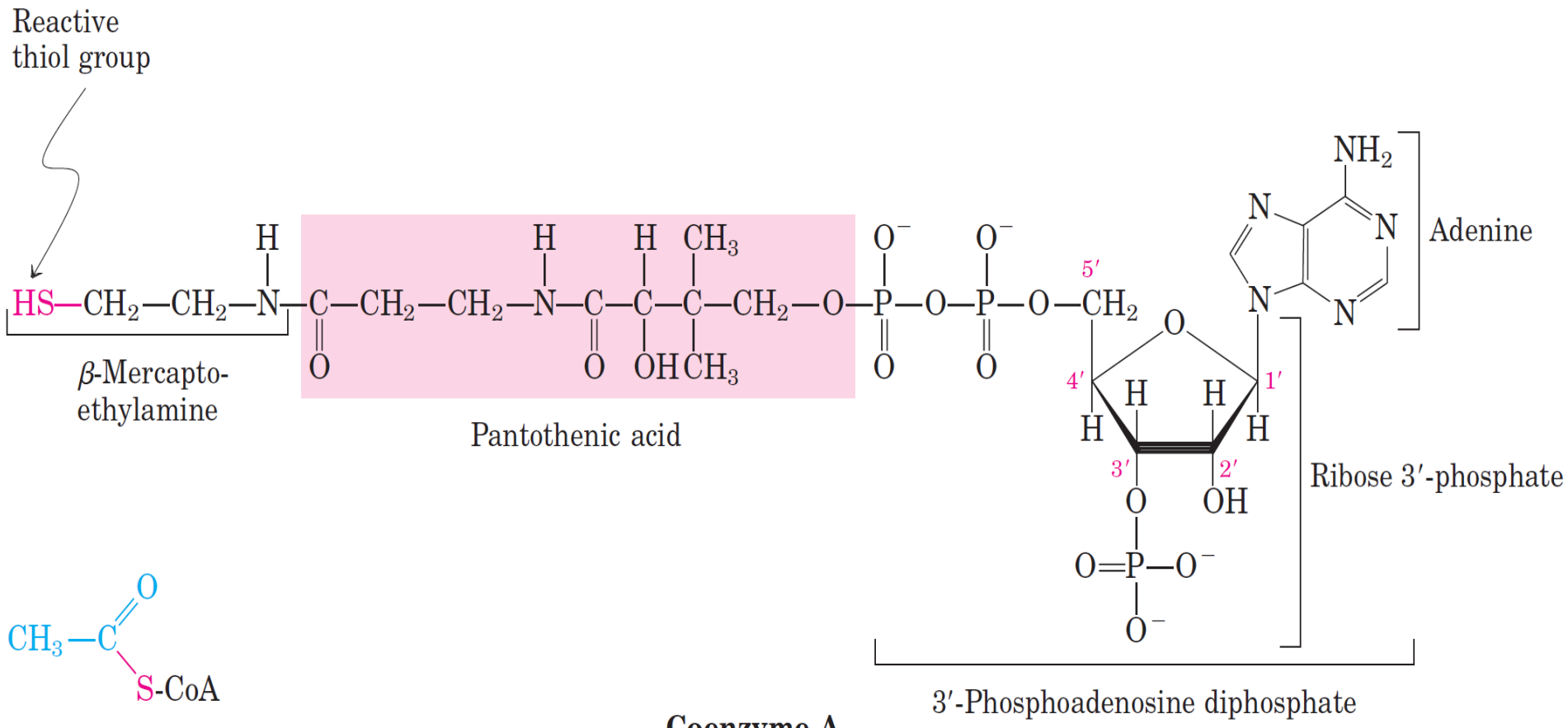


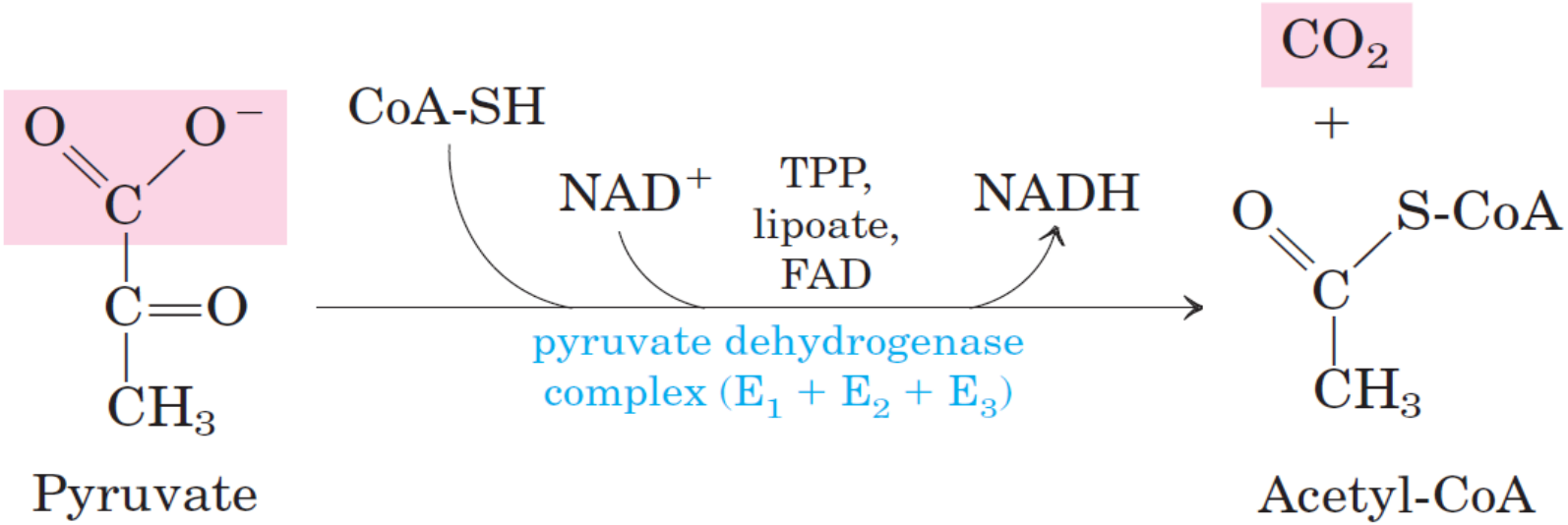
❖Before entering the citric acid cycle, the carbon skeletons of sugars, fatty acids and some amino acids are degraded to the acetyl group of acetyl-CoA, the form in which the cycle accepts most of its fuel input.

❖Pyruvate, derived from glucose and other sugars by glycolysis, is oxidized to acetyl-CoA and CO_2 by the pyruvate dehydrogenase (PDH) complex, located in the mitochondria of eukaryotic cells and in the cytosol of bacteria.

❖The overall reaction catalyzed by the pyruvate dehydrogenase complex is an oxidative decarboxylation.

❖This is an irreversible oxidation process in which the carboxyl group is removed from pyruvate as a molecule of CO_2 and the two remaining carbons become the acetyl group of acetyl-CoA


Reactive
thiol group
β-Mercapto-
ethylamine
Pantothenic acid
Adenine
Ribose 3′-phosphate
3′-Phosphoadenosine diphosphate
Acetyl-CoA
Coenzyme A



$$\Delta G'^{\circ} = -33.4 \text{ kJ/mol}$$

❖The NADH formed in this reaction gives up a hydride ion ($:H^-$) to the respiratory chain, which carries the two electrons to oxygen or, in anaerobic microorganisms, to an alternative electron acceptor such as nitrate or sulfate.

❖The transfer of electrons from NADH to oxygen ultimately generates 2.5 molecules of ATP per pair of electrons.

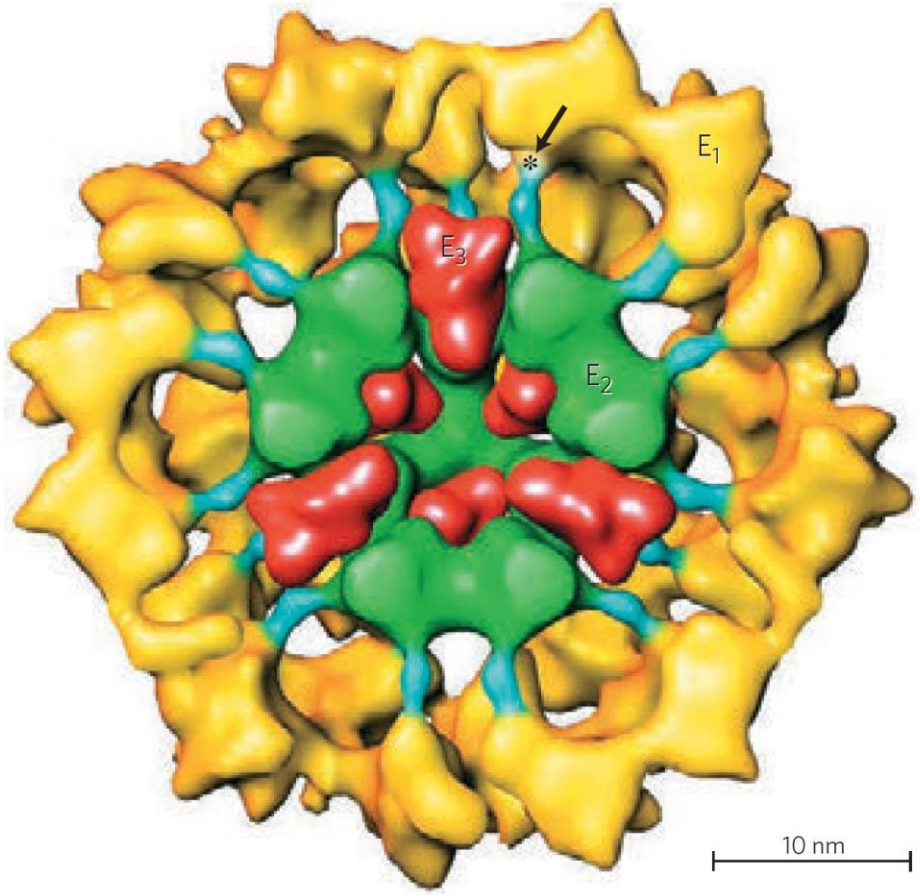
❖The combined dehydrogenation and decarboxylation of pyruvate to the acetyl group of acetyl-CoA requires the sequential action of three different enzymes and five different coenzymes or prosthetic groups—thiamine pyrophosphate (TPP), flavin adenine dinucleotide (FAD), coenzyme A (CoA), nicotinamide adenine dinucleotide (NAD), and lipoate.

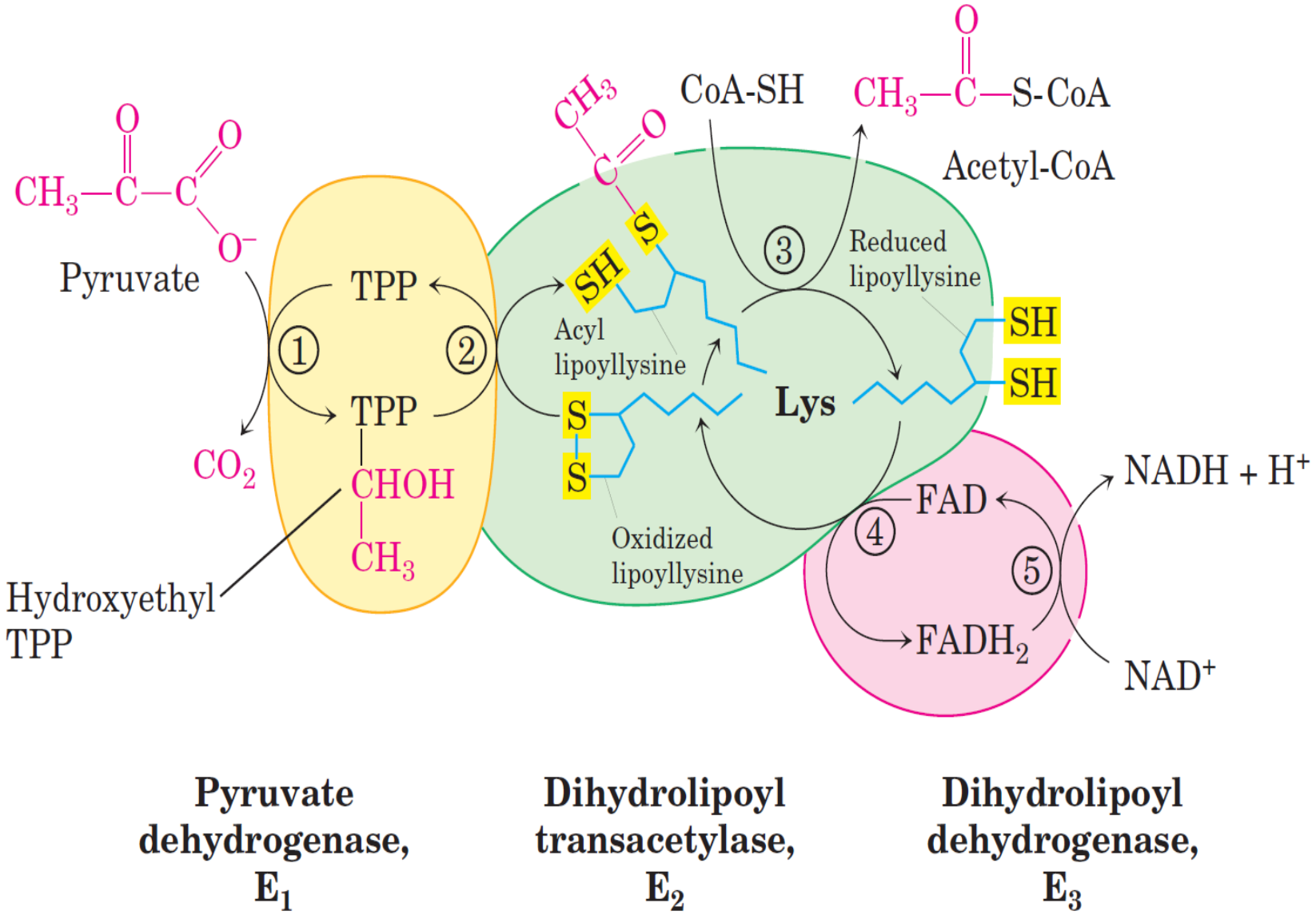
❖Four different vitamins required in human nutrition are vital components of this system: thiamine (in TPP), riboflavin (in FAD), niacin (in NAD), and pantothenate (in CoA).

❖Coenzyme A has a reactive thiol (—SH) group that is critical to the role of CoA as an acyl carrier in a number of metabolic reactions.

❖Acyl groups are covalently linked to the thiol group, forming thioesters and because of their relatively high standard free energies of hydrolysis, thioesters, have a high acyl group transfer potential and can donate their acyl groups to a variety of acceptor molecules.

❖The PDH complex contains three enzymes—pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3)—each present in multiple copies.




Pyruvate
TPP
TPP
CHOH
CH3
CO2
Hydroxyethyl TPP
1
2
CoA-SH
Acetyl-CoA
Acyl lipoyllysine
Oxidized lipoyllysine
Lys
Reduced lipoyllysine
3
SH
SH
SH
S
S
S
FAD
FADH2
4
5
NADH + H+
NAD+
Pyruvate dehydrogenase, E1
Dihydrolipoyl transacetylase, E2
Dihydrolipoyl dehydrogenase, E3

❖Pyruvate dehydrogenase complex carries out the five consecutive reactions in the decarboxylation and dehydrogenation of pyruvate.

❖Step 1 is essentially identical to the reaction catalyzed by pyruvate decarboxylase; C-1 of pyruvate is released as CO_2, and C-2, which in pyruvate has the oxidation state of an aldehyde, is attached to TPP as a hydroxyethyl group.

❖This first step is the slowest and therefore limits the rate of the overall reaction.

❖In step 2 the hydroxyethyl group is oxidized to the level of a carboxylic acid (acetate).

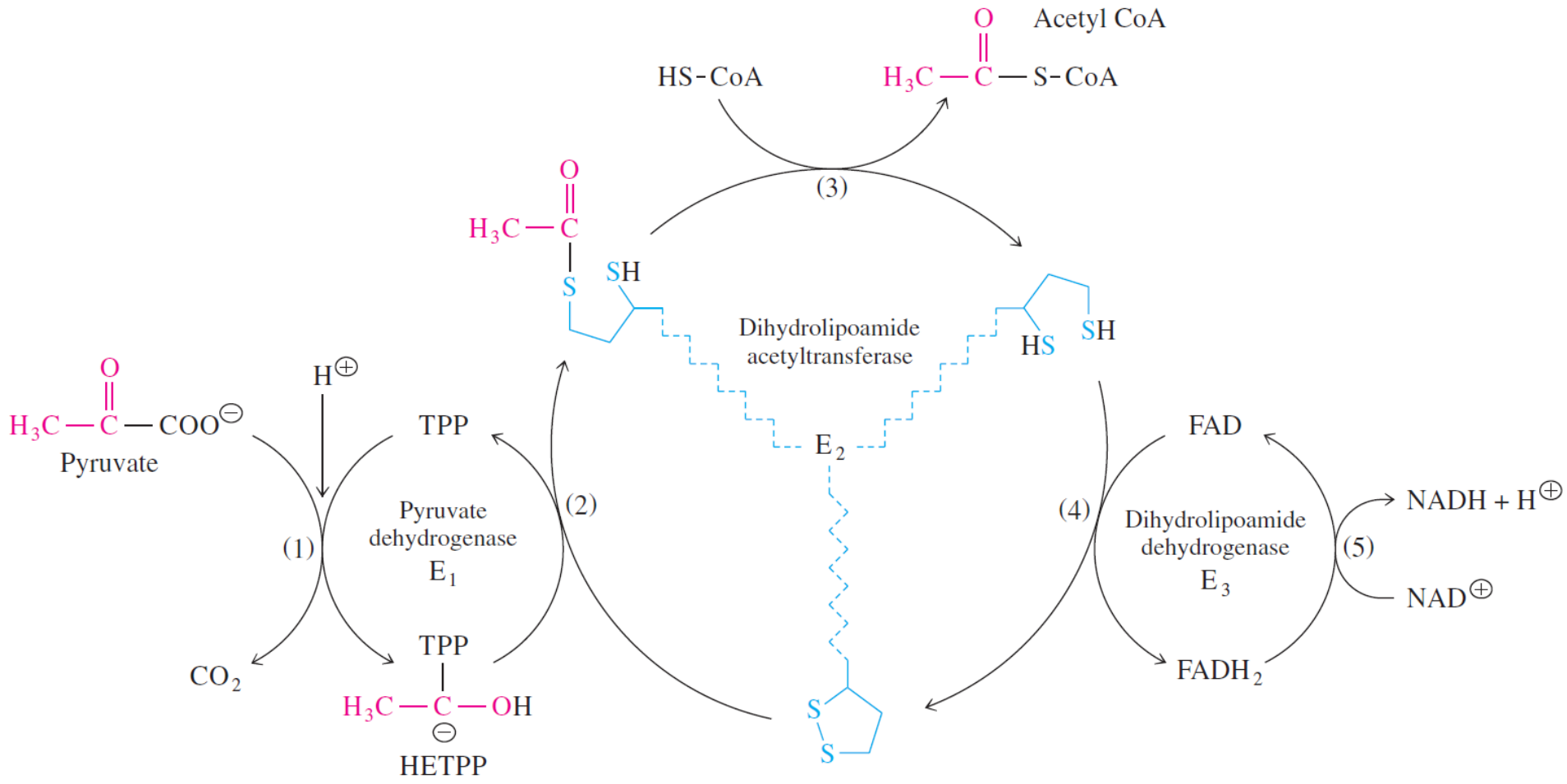
❖The two electrons removed in this reaction reduce the S-S of a lipoyl group on E2 to two thiol (—SH) groups.

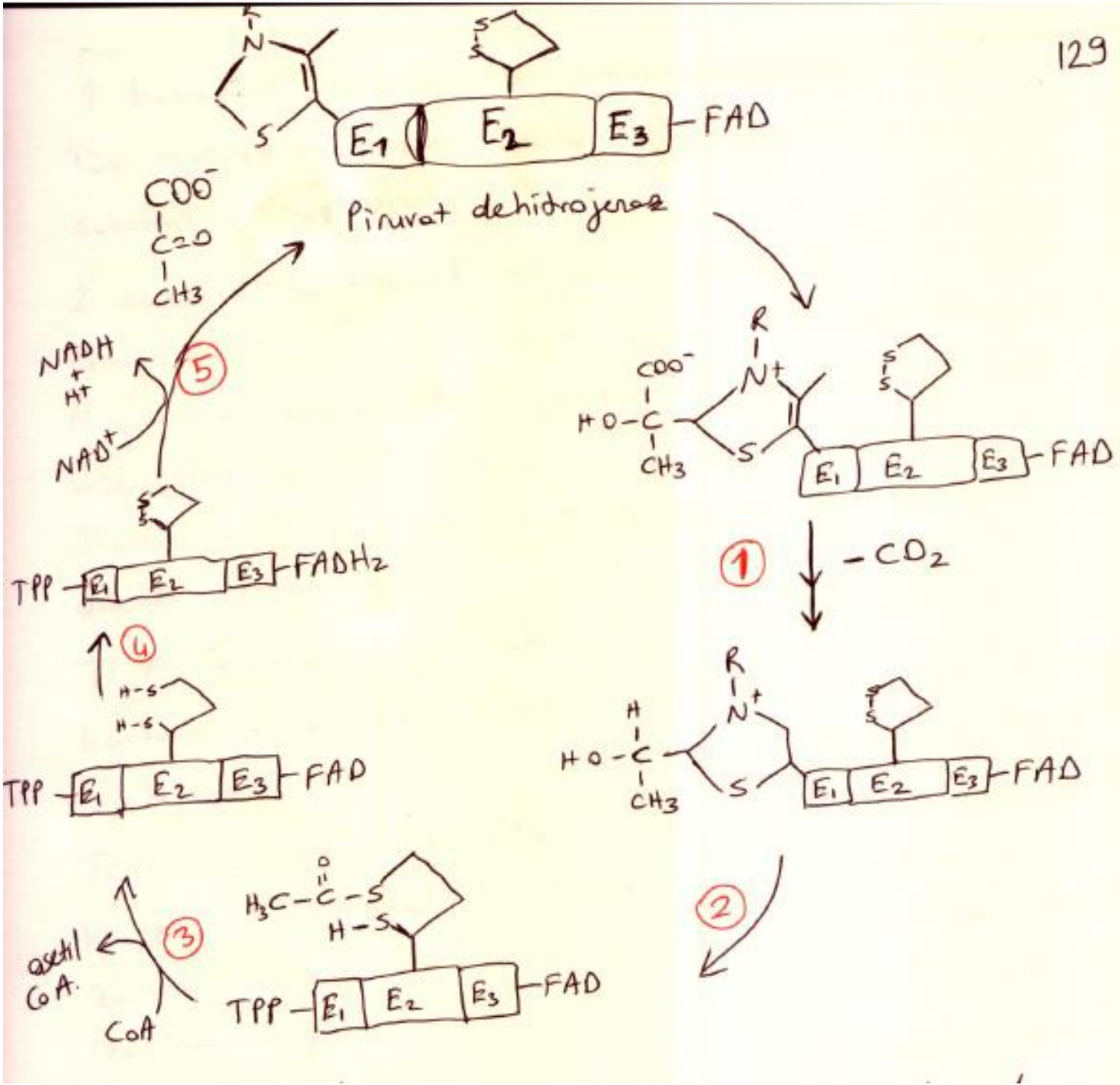
❖The acetyl moiety produced in this oxidation-reduction reaction is first esterified to one of the lipoyl —SH groups, then transesterified to CoA to form acetyl-CoA (step 3).

❖Thus the energy of oxidation drives the formation of a high-energy thioester of acetate.

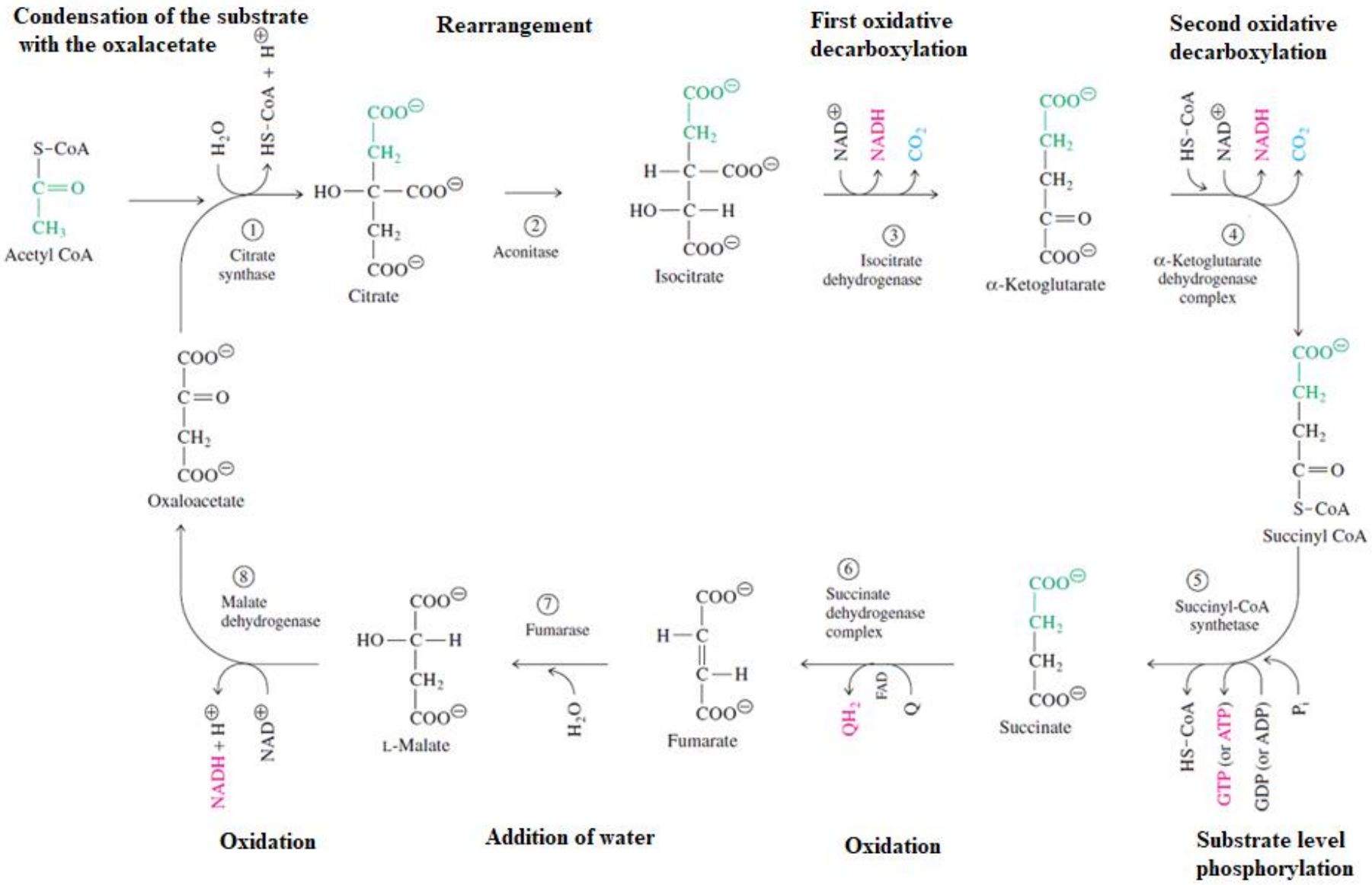
❖The remaining reactions catalyzed by the PDH complex (by E3, in steps 4 and 5) are electron transfers necessary to regenerate the oxidized (disulfide) form of the lipoyl group of E2 to prepare the enzyme complex for another round of oxidation.

❖The electrons removed from the hydroxyethyl group derived from pyruvate pass through FAD to NAD^+.


Acetyl CoA
HS-CoA
(3)
Dihydrolipoamide
acetyltransferase
E2
Pyruvate
H⊕
TPP
(1)
Pyruvate
dehydrogenase
E1
(2)
CO2
TPP
HETPP
FAD
(4)
Dihydrolipoamide
dehydrogenase
E3
(5)
NADH + H⊕
NAD⊕
FADH2


E1
E2
E3
FAD
Piruvat dehidrojenaz
COO⁻
C=O
CH3
NADH + H+
NAD+
5
TPP
FADH2
4
H-S
1
-CO2
HO-C
2
3
acetil CoA.
CoA
H3C-C
O

Reactions of the Citric Acid Cycle



❖To begin a turn of the cycle, acetyl-CoA donates its acetyl group to the four-carbon compound oxaloacetate to form the six-carbon citrate.

❖Citrate is then transformed into isocitrate, also a six-carbon molecule, which is dehydrogenated with loss of CO_2 to yield the five-carbon compound α -ketoglutarate.

❖α-Ketoglutarate undergoes loss of a second molecule of CO_2 and ultimately yields the four-carbon compound succinate.

❖Succinate is then enzymatically converted in three steps into the four-carbon oxaloacetate—which is then ready to react with another molecule of acetyl-CoA.

❖In each turn of the cycle, one acetyl group (two carbons) enters as acetyl-CoA and two molecules of CO_2 leave; one molecule of oxaloacetate is used to form citrate and one molecule of oxaloacetate is regenerated.

❖No net removal of oxaloacetate occurs; one molecule of oxaloacetate can theoretically bring about oxidation of an infinite number of acetyl groups, and, in fact, oxaloacetate is present in cells in very low concentrations.

❖Four of the eight steps in this process are oxidations, in which the energy of oxidation is very efficiently conserved in the form of the reduced coenzymes NADH and $FADH_2$.

❖Although the citric acid cycle is central to energy-yielding metabolism its role is not limited to energy conservation.

❖Four- and five-carbon intermediates of the cycle serve as precursors for a wide variety of products.

❖To replace intermediates removed for this purpose, cells employ anaplerotic (replenishing) reactions.

❖The entire set of reactions of the citric acid cycle takes place in mitochondria.

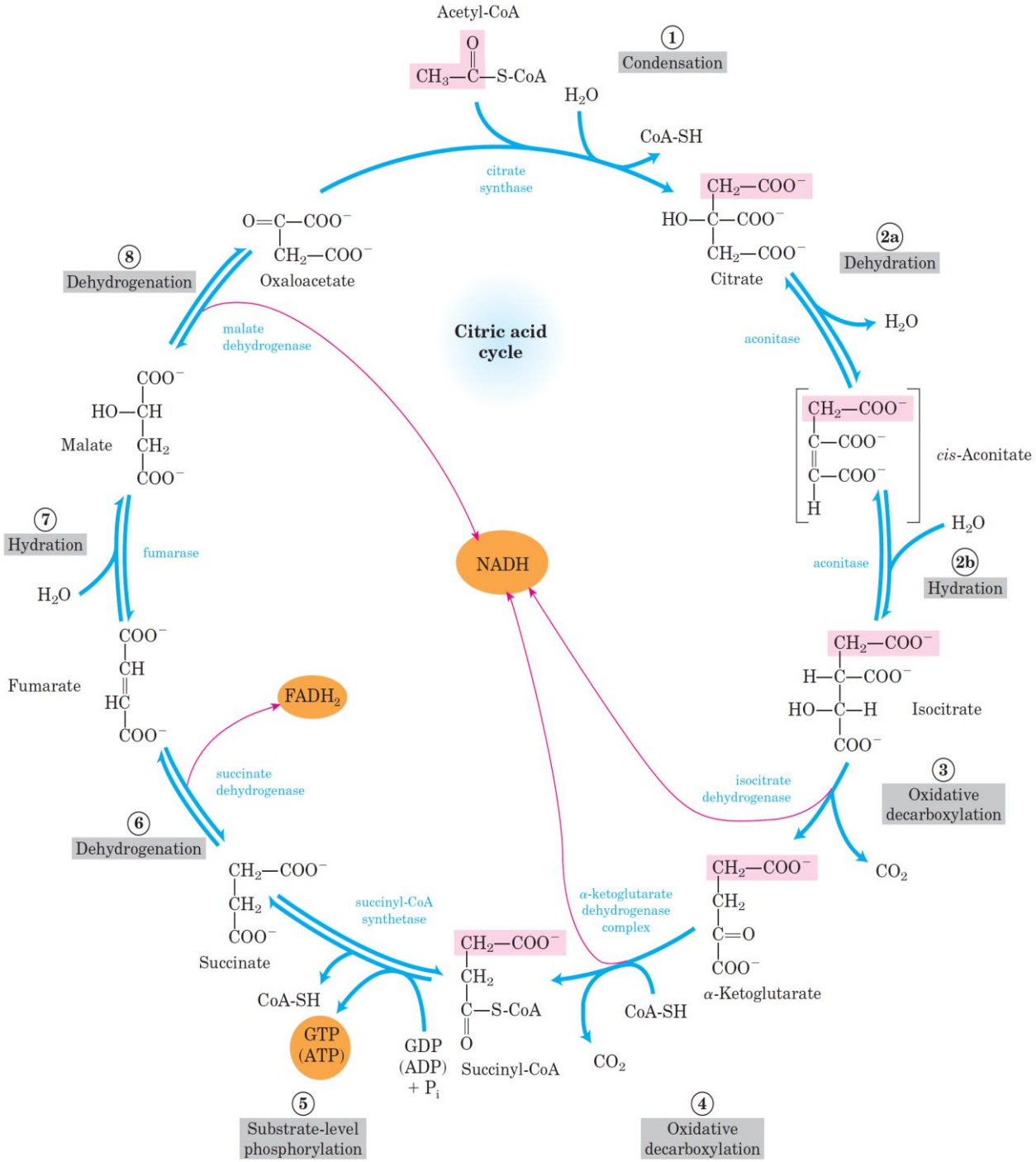
❖Isolated mitochondria were found to contain not only all the enzymes and coenzymes required for the citric acid cycle, but also all the enzymes and proteins necessary for the last stage of respiration—electron transfer and ATP synthesis by oxidative phosphorylation.

❖Mitochondria also contain the enzymes for the oxidation of fatty acids and some amino acids to acetyl-CoA, and the oxidative degradation of other amino acids to α-ketoglutarate, succinyl-CoA, or oxaloacetate.

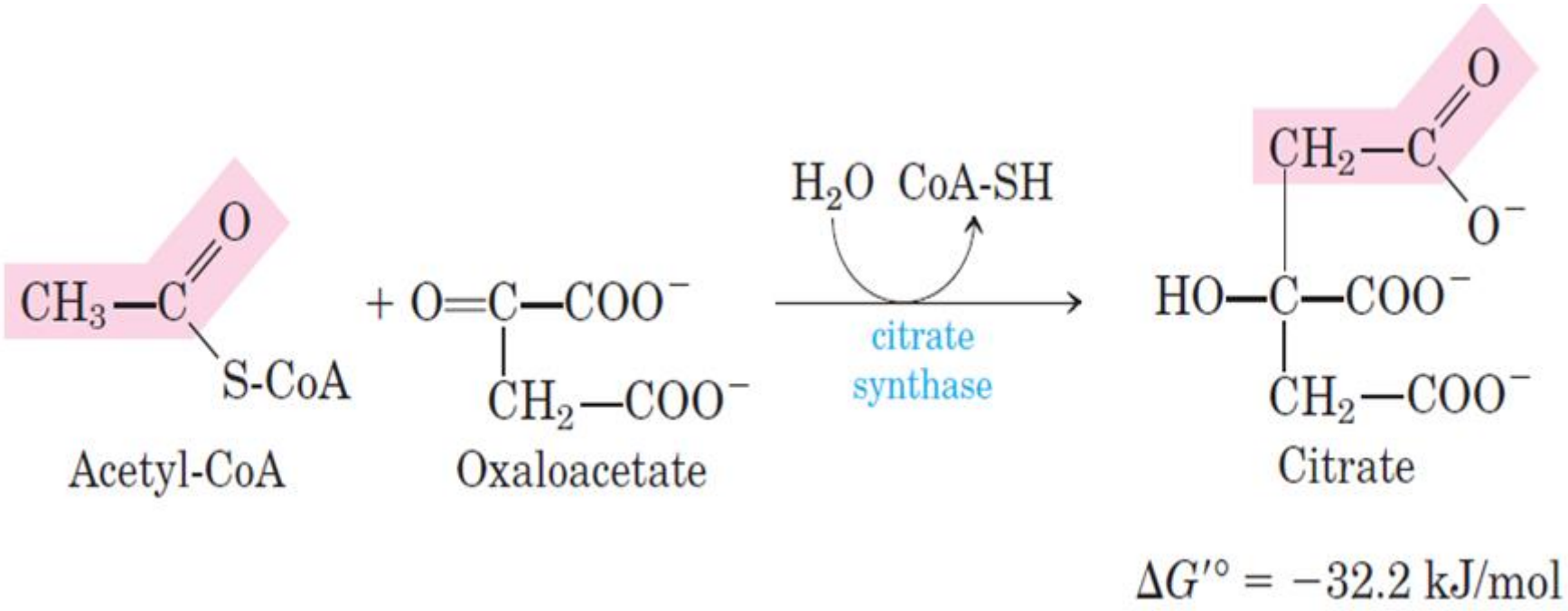
❖Thus, in nonphotosynthetic eukaryotes, the mitochondrion is the site of most energy-yielding oxidative reactions and of the coupled synthesis of ATP.

❖In photosynthetic eukaryotes, mitochondria are the major site of ATP production in the dark, but in daylight chloroplasts produce most of the organism's ATP.

❖In most bacteria, the enzymes of the citric acid cycle are in the cytosol, and the plasma membrane plays a role analogous to that of the inner mitochondrial membrane in ATP synthesis

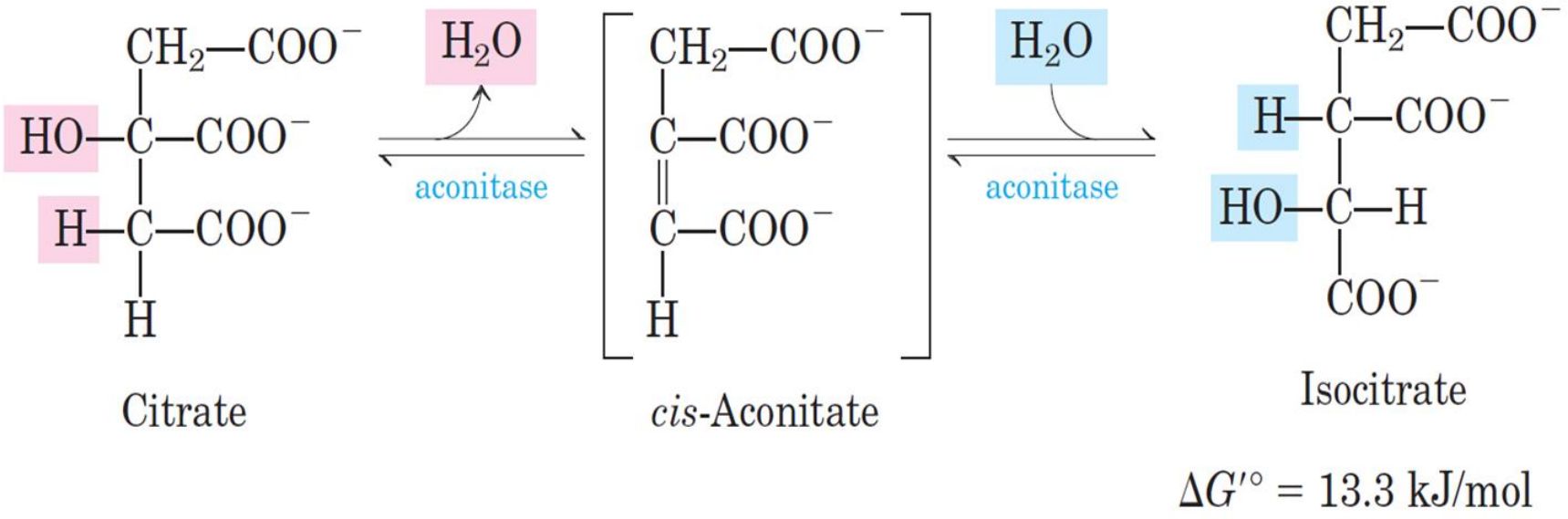

Acetyl-CoA
CH3—C—S-CoA
1
Condensation
H2O
CoA-SH
citrate synthase
CH2—COO⁻
HO—C—COO⁻
CH2—COO⁻
Citrate
2a
Dehydration
H2O
aconitase
cis-Aconitate
aconitase
H2O
2b
Hydration
CH2—COO⁻
H—C—COO⁻
HO—C—H
COO⁻
Isocitrate
3
Oxidative decarboxylation
CO2
isocitrate dehydrogenase
CH2—COO⁻
CH2
C=O
COO⁻
α-Ketoglutarate
α-ketoglutarate dehydrogenase complex
CH2—COO⁻
CH2
C—S-CoA
O
Succinyl-CoA
CO2
CoA-SH
succinyl-CoA synthetase
GTP (ATP)
GDP (ADP) + Pi
5
Substrate-level phosphorylation
CH2—COO⁻
CH2
COO⁻
Succinate
6
Dehydrogenation
succinate dehydrogenase
FADH2
COO⁻
CH
HC
COO⁻
Fumarate
7
Hydration
H2O
fumarase
COO⁻
HO—CH
CH2
COO⁻
Malate
8
Dehydrogenation
malate dehydrogenase
O=C—COO⁻
CH2—COO⁻
Oxaloacetate
NADH
Citric acid cycle
4
Oxidative decarboxylation

❖The first step of the citric acid cycle catalyzed by citrate synthase.



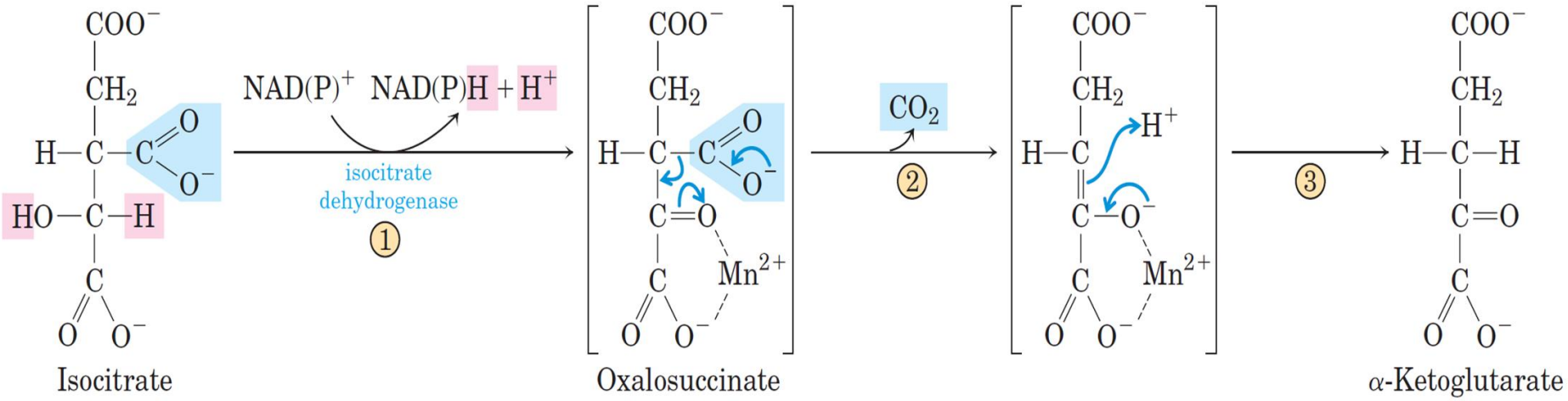
❖The carbonyl of oxaloacetate acts as an electrophilic center, which is attacked by the methyl carbon of acetyl-CoA in a Claisen condensation (reaction between a thioester (acetyl-CoA) and a ketone (oxaloacetate)) to form citrate.

❖The enzyme aconitase (more formally, aconitate hydratase) catalyzes the reversible transformation of citrate to isocitrate.



❖Aconitase contains an iron-sulfur center and promotes the reversible addition of H_2O to the double bond of enzyme-bound cis-aconitate.

❖In the next step, isocitrate dehydrogenase catalyzes oxidative decarboxylation of isocitrate to form α-ketoglutarate.

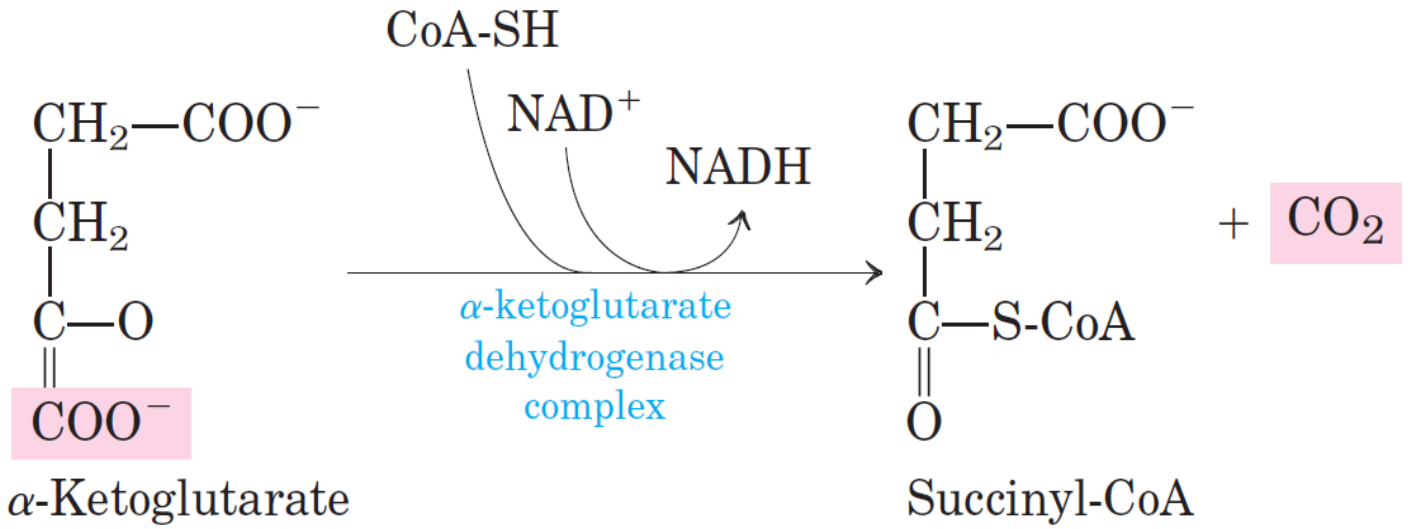


❖Mn^{2+} in the active site interacts with the carbonyl group of the intermediate oxalosuccinate, which is formed transiently but does not leave the binding site until decarboxylation converts it to α-ketoglutarate.

❖There are two different forms of isocitrate dehydrogenase in all cells, one requiring NAD^+ as electron acceptor and the other requiring $NADP^+$.

❖The next step is another oxidative decarboxylation, in which α-ketoglutarate is converted to succinyl-CoA and CO_2 by the action of the a-ketoglutarate dehydrogenase complex; NAD+ serves as electron acceptor and CoA as the carrier of the succinyl group.

❖The energy of oxidation of α-ketoglutarate is conserved in the formation of the thioester bond of succinyl-CoA.



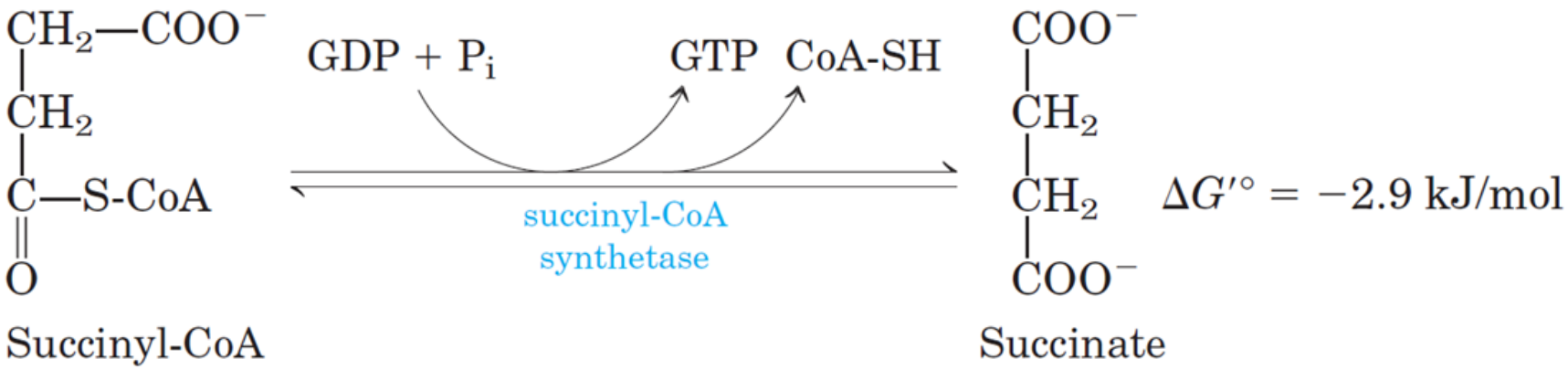
$$\Delta G'^{\circ} = -33.5 \text{ kJ/mol}$$

❖This reaction is virtually identical to the pyruvate dehydrogenase reaction.

❖Succinyl-CoA, like acetyl-CoA, has a thioester bond with a strongly negative standard free energy of hydrolysis.

❖In the next step of the citric acid cycle, energy released in the breakage of this bond is used to drive the synthesis of a phosphoanhydride bond in either GTP or ATP.

❖The enzyme that catalyzes this reversible reaction is called succinyl-CoA synthetase and this name indicates the participation of a nucleoside triphosphate in the reaction



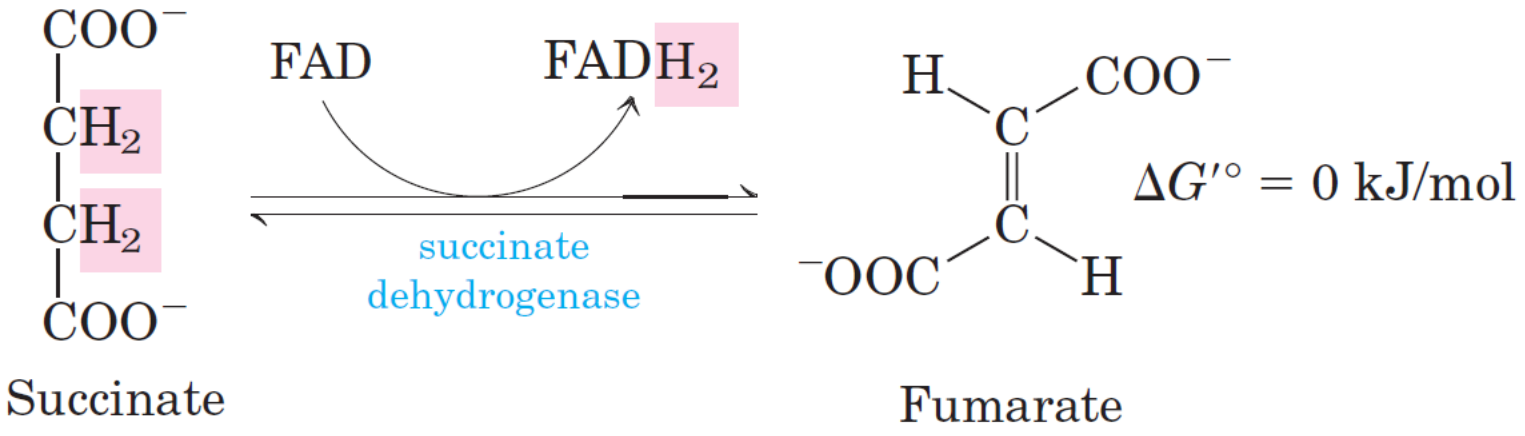
❖The formation of ATP (or GTP) at the expense of the energy released by the oxidative decarboxylation of α-ketoglutarate is a substrate-level phosphorylation, like the synthesis of ATP in the glycolytic reactions catalyzed by glyceraldehyde 3-phosphate dehydrogenase and pyruvate kinase.

❖The GTP formed by succinyl-CoA synthetase can donate its terminal phosphoryl group to ADP to form ATP, in a reversible reaction catalyzed by **nucleoside diphosphate kinase**.

$$\text{GTP} + \text{ADP} \longrightarrow \text{GDP} + \text{ATP} \qquad \Delta G'^{\circ} = 0 \text{ kJ/mol}$$

❖Thus the net result of the activity of either isozyme of succinyl-CoA synthetase is the conservation of energy as ATP.

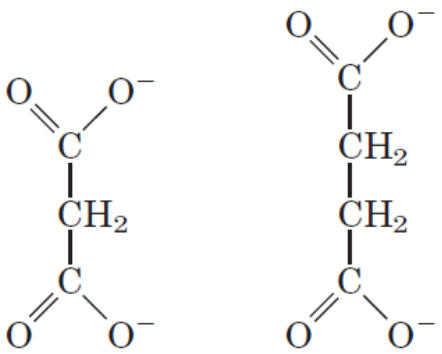
❖The succinate formed from succinyl-CoA is oxidized to fumarate by the flavoprotein succinate dehydrogenase:



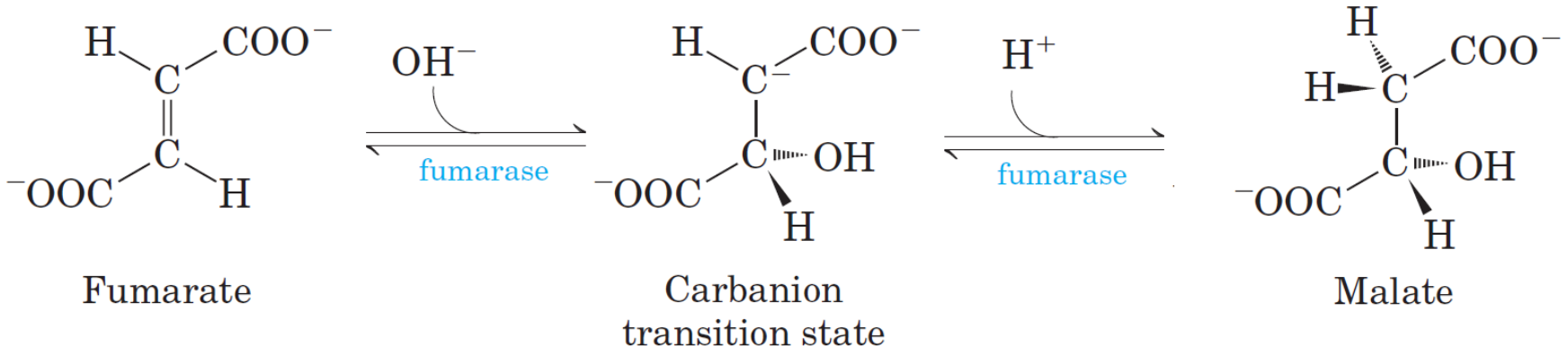
❖In eukaryotes, succinate dehydrogenase is tightly bound to the mitochondrial inner membrane and part of the electron transport system.

❖Electron flow from succinate through these carriers to the final electron acceptor, O2, is coupled to the synthesis of about 1.5 ATP molecules per pair of electrons (respiration-linked phosphorylation).

❖Malonate, an analog of succinate not normally present in cells, is a strong competitive inhibitor of succinate dehydrogenase, and its addition to mitochondria blocks the activity of the citric acid cycle.

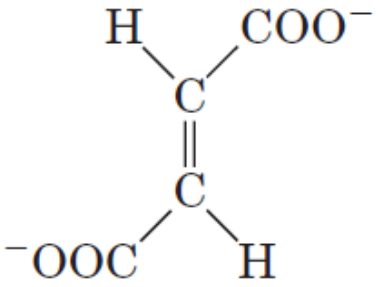


❖The reversible hydration of fumarate to L-malate is catalyzed by fumarase (formally, fumarate hydratase).

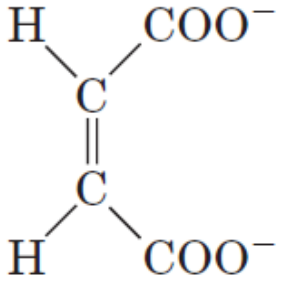


❖This enzyme is highly stereospecific; it catalyzes hydration of the trans double bond of fumarate but not the cis double bond of maleate (the cis isomer of fumarate).

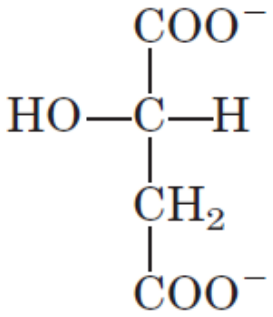
❖In the reverse direction (from L-malate to fumarate), fumarase is equally stereospecific: D-malate is not a substrate.



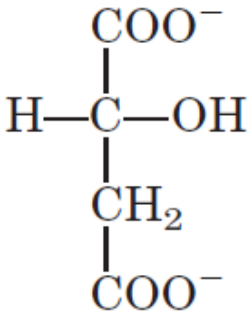
Fumarate



Maleate

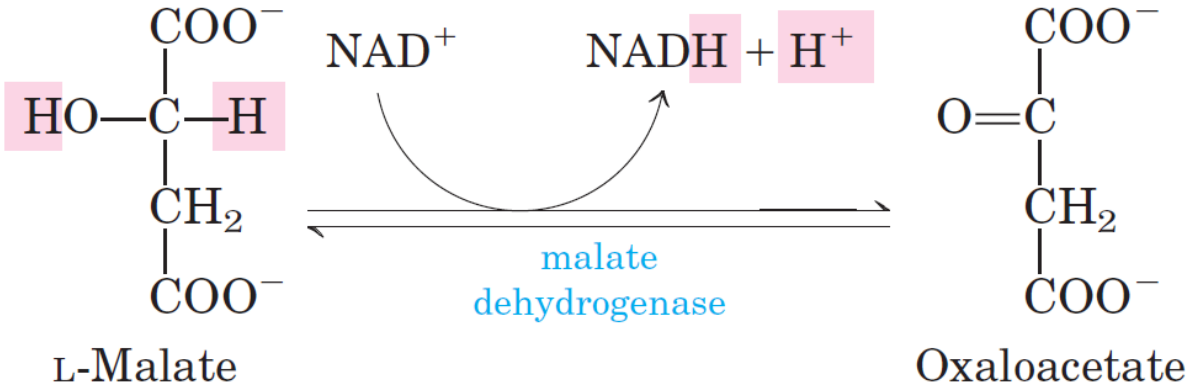


L-Malate



D-Malate

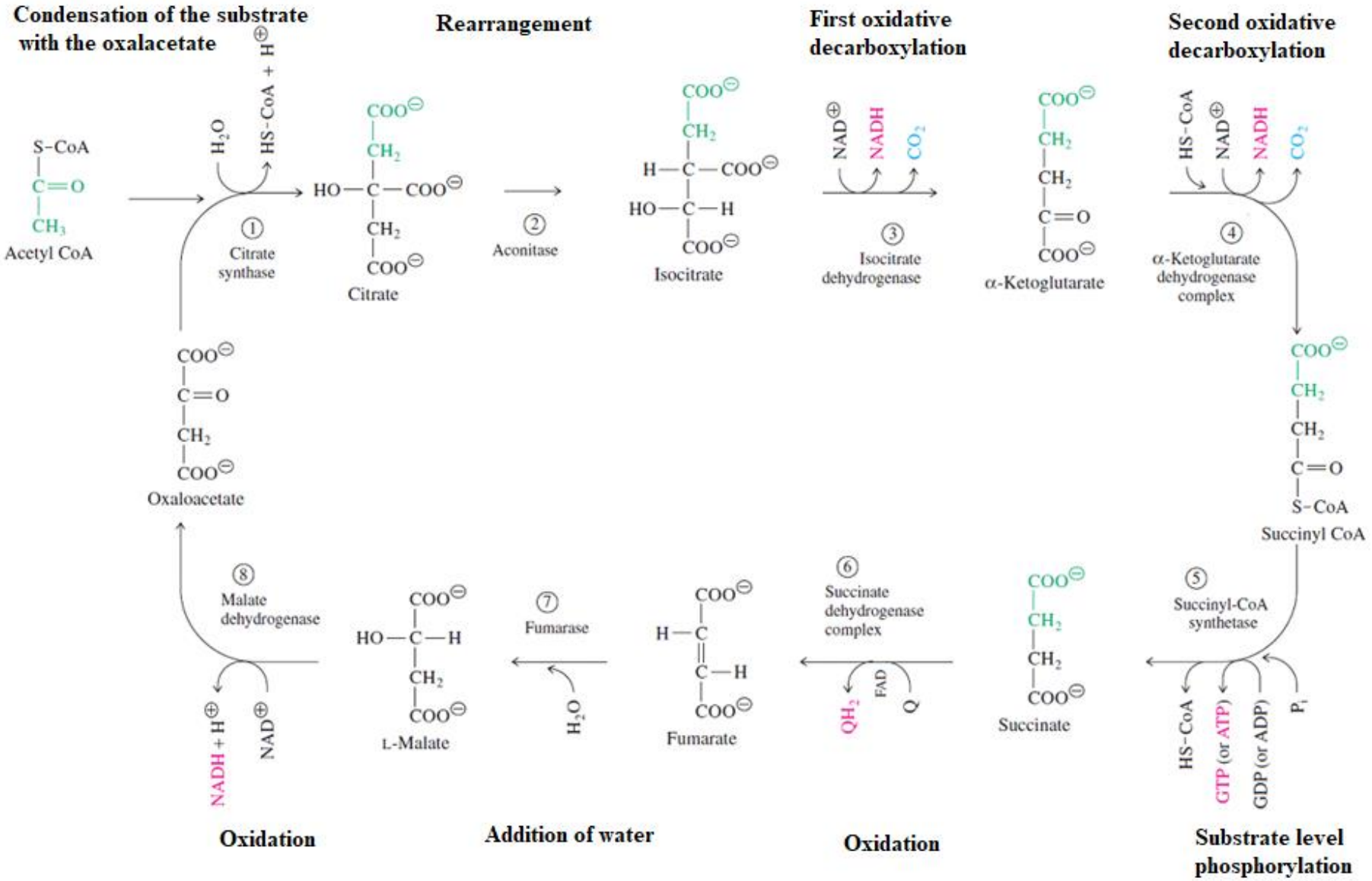
❖In the last reaction of the citric acid cycle, NAD-linked L-malate dehydrogenase catalyzes the oxidation of L-malate to oxaloacetate.

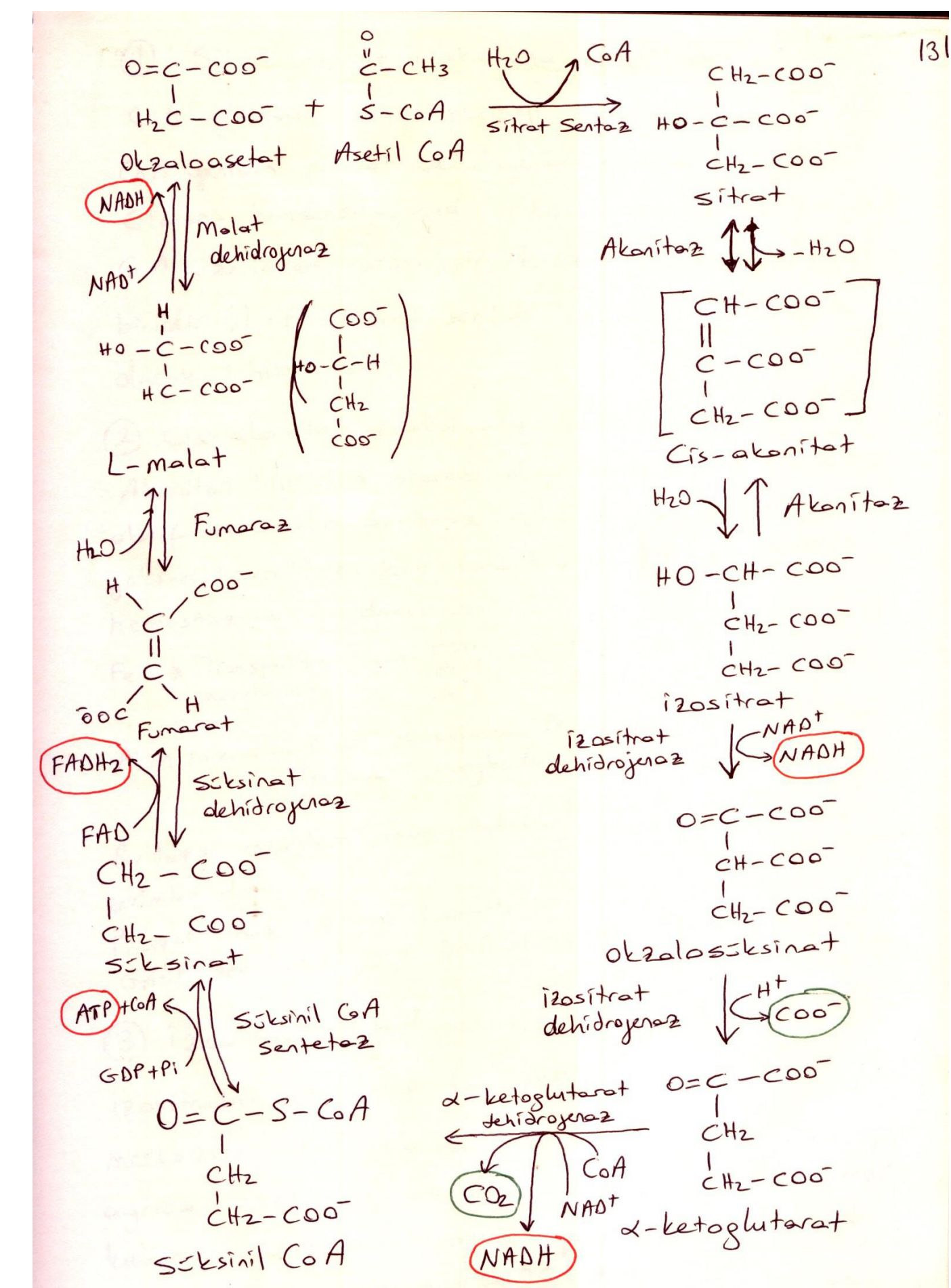


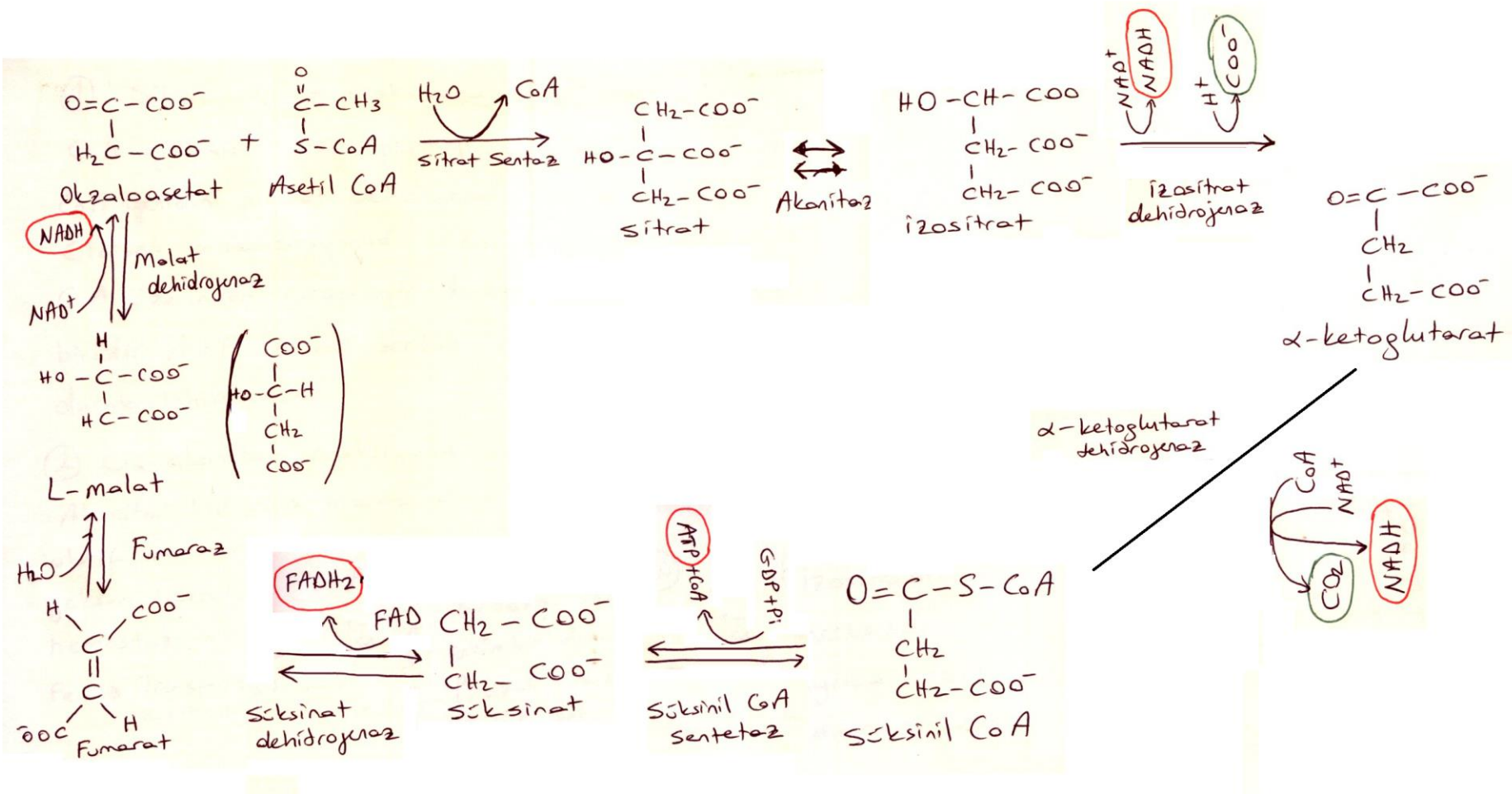
L-Malate Oxaloacetate

$\Delta G'^{\circ} = 29.7$ kJ/mol

❖The equilibrium of this reaction lies far to the left under standard thermodynamic conditions, but in intact cells oxaloacetate is continually removed by the highly exergonic citrate synthase reaction and this keeps the concentration of oxaloacetate in the cell extremely low ($<10^{-6}$ M), pulling the malate dehydrogenase reaction toward the formation of oxaloacetate.


Condensation of the substrate with the oxalacetate
Rearrangement
First oxidative decarboxylation
Second oxidative decarboxylation
Acetyl CoA
Citrate synthase
Citrate
Aconitase
Isocitrate
Isocitrate dehydrogenase
α-Ketoglutarate
α-Ketoglutarate dehydrogenase complex
Succinyl CoA
Succinyl-CoA synthetase
Succinate
Succinate dehydrogenase complex
Fumarate
Fumarase
L-Malate
Malate dehydrogenase
Oxaloacetate
Oxidation
Addition of water
Oxidation
Substrate level phosphorylation


Okzaloasetat
Asetil CoA
H2O
CoA
sitrat sentaz
sitrat
NADH
NAD+
Malat dehidrojenaz
Akonitaz
-H2O
L-malat
Cis-akonitat
H2O
Akonitaz
Fumaraz
izositrat
Fumarat
izositrat dehidrojenaz
FADH2
FAD
Süksinat dehidrojenaz
okzalosüksinat
Süksinat
izositrat dehidrojenaz
ATP
+CoA
GDP+Pi
Süksinil CoA sentetaz
α-ketoglutarat dehidrojenaz
CO2
Süksinil CoA
α-ketoglutarat


Okzaloasetat
Asetil CoA
Sitrat Sentaz
Sitrat
Akonitaz
İzositrat
İzositrat dehidrojenaz
α-ketoglutarat
α-ketoglutarat dehidrojenaz
Süksinil CoA
Süksinil CoA sentetaz
Süksinat
Süksinat dehidrojenaz
Fumarat
Fumaraz
L-malat
Malat dehidrojenaz

The Energy of Oxidations in the Cycle Is Efficiently Conserved

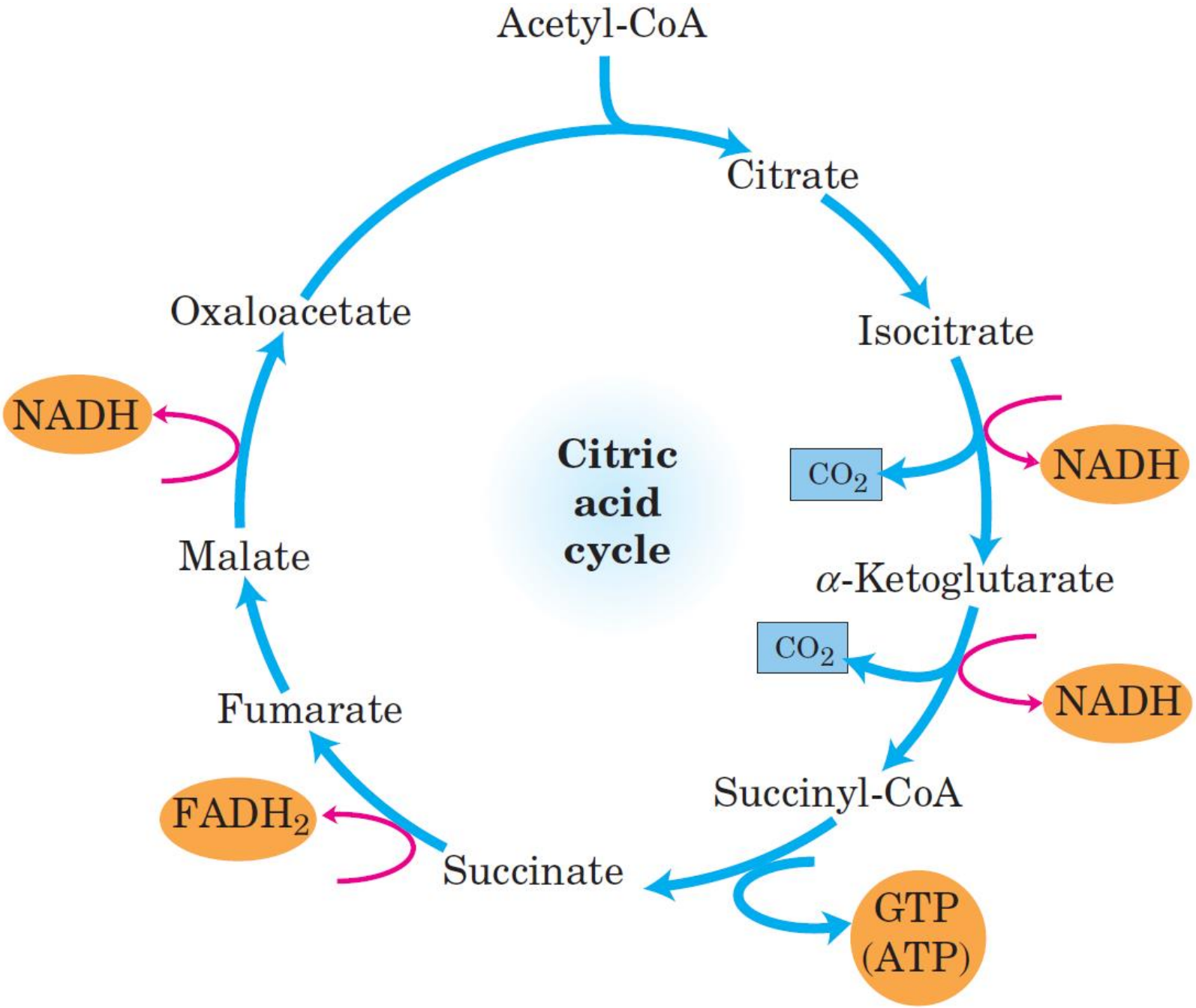
❖A two-carbon acetyl group entered the cycle by combining with oxaloacetate.

❖Two carbon atoms emerged from the cycle as CO_2 from the oxidation of isocitrate and α-ketoglutarate.

❖The energy released by these oxidations was conserved in the reduction of three NAD^+ and one FAD and the production of one ATP or GTP.

❖At the end of the cycle a molecule of oxaloacetate was regenerated.

❖Note that the two carbon atoms appearing as CO_2 are not the same two carbons that entered in the form of the acetyl group; additional turns around the cycle are required to release these carbons as CO_2


Acetyl-CoA
Citrate
Isocitrate
NADH
CO_2
α-Ketoglutarate
NADH
CO_2
Succinyl-CoA
GTP
(ATP)
Succinate
$FADH_2$
Fumarate
Malate
NADH
Oxaloacetate
Citric
acid
cycle

❖Although the citric acid cycle directly generates only one ATP per turn (in the conversion of succinyl-CoA to succinate), the four oxidation steps in the cycle provide a large flow of electrons into the respiratory chain via NADH and $FADH_2$ and thus lead to formation of a large number of ATP molecules during oxidative phosphorylation.

❖We saw in glycolysis pathway that the energy yield from the production of two molecules of pyruvate from one molecule of glucose is 2 ATP and 2 NADH.

❖In oxidative phosphorylation, passage of two electrons from NADH to O^2 drives the formation of about 2.5 ATP, and passage of two electrons from $FADH_2$ to O^2 yields about 1.5 ATP.

❖This stoichiometry allows us to calculate the overall yield of ATP from the complete oxidation of glucose.

❖When both pyruvate molecules are oxidized to 6 CO_2 via the pyruvate dehydrogenase complex and the citric acid cycle, and the electrons are transferred to O_2 via oxidative phosphorylation, as many as 32 ATP are obtained per glucose.

❖In round numbers, this represents the conservation of 32 x 30.5 kJ/mol = 976 kJ/mol or 34% of the theoretical maximum of about 2,840 kJ/mol available from the complete oxidation of glucose.

❖These calculations employ the standard free-energy changes; when corrected for the actual free energy required to form ATP within cells, the calculated efficiency of the process is closer to 65%.

TABLE 16–1 Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Complex Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation

Reaction	Number of ATP or reduced coenzyme directly formed	Number of ATP ultimately formed*
Glucose ⟶ glucose 6-phosphate	−1 ATP	−1
Fructose 6-phosphate ⟶ fructose 1,6-bisphosphate	−1 ATP	−1
2 Glyceraldehyde 3-phosphate ⟶ 2 1,3-bisphosphoglycerate	2 NADH	3 or 5†
2 1,3-Bisphosphoglycerate ⟶ 2 3-phosphoglycerate	2 ATP	2
2 Phosphoenolpyruvate ⟶ 2 pyruvate	2 ATP	2
2 Pyruvate ⟶ 2 acetyl-CoA	2 NADH	5
2 Isocitrate ⟶ 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate ⟶ 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA ⟶ 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate ⟶ 2 fumarate	2 $FADH_2$	3
2 Malate ⟶ 2 oxaloacetate	2 NADH	5
Total		30–32

*This is calculated as 2.5 ATP per NADH and 1.5 ATP per $FADH_2$. A negative value indicates consumption.

†This number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix; see Figures 19–30 and 19–31.

❖The eight-step cyclic process for oxidation of simple two-carbon acetyl groups to CO_2 may seem unnecessarily cumbersome and not in keeping with the biological principle of maximum economy.

❖The role of the citric acid cycle is not confined to the oxidation of acetate, however.

❖This pathway is the hub of intermediary metabolism.

❖Four- and five-carbon end products of many catabolic processes feed into the cycle to serve as fuels.

❖Oxaloacetate and -ketoglutarate, for example, are produced from aspartate and glutamate, respectively, when proteins are degraded.

❖ Under some metabolic circumstances, intermediates are drawn out of the cycle to be used as precursors in a variety of biosynthetic pathways.

❖In aerobic organisms, the citric acid cycle is an amphibolic pathway, one that serves in both catabolic and anabolic processes.

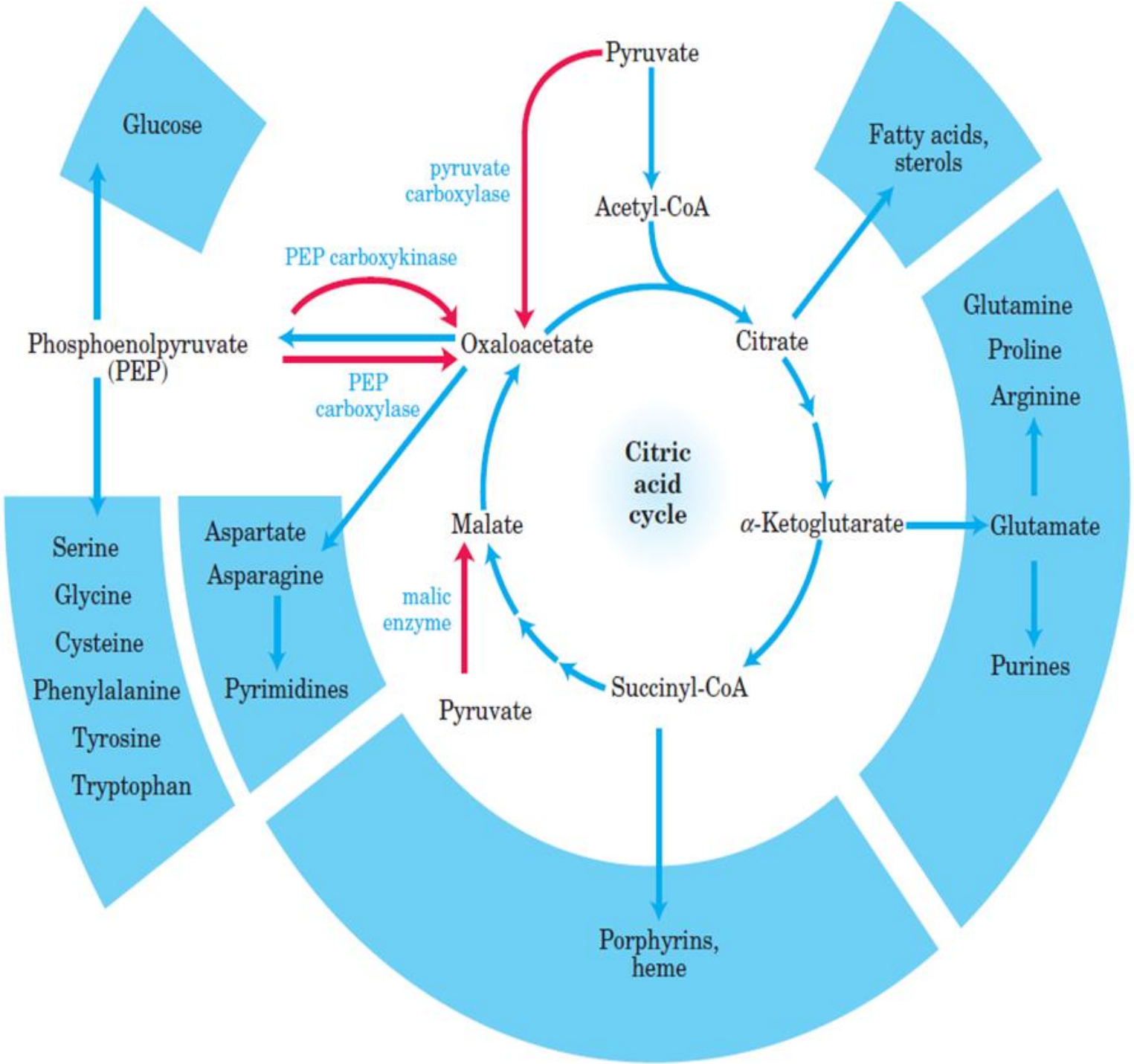
❖Besides its role in the oxidative catabolism of carbohydrates, fatty acids, and amino acids, the cycle provides precursors for many biosynthetic pathways, through reactions that served the same purpose in anaerobic ancestors.

❖α-Ketoglutarate and oxaloacetate can, for example, serve as precursors of the amino acids aspartate and glutamate by simple transamination (Chapter 22).

❖Through aspartate and glutamate, the carbons of oxaloacetate and α-ketoglutarate are then used to build other amino acids, as well as purine and pyrimidine nucleotides.

❖Oxaloacetate is converted to glucose in gluconeogenesis.

❖Succinyl-CoA is a central intermediate in the synthesis of the


Glucose
Pyruvate
pyruvate carboxylase
Acetyl-CoA
Fatty acids, sterols
PEP carboxykinase
Phosphoenolpyruvate (PEP)
Oxaloacetate
Citrate
PEP carboxylase
Glutamine
Proline
Arginine
Citric acid cycle
Serine
Glycine
Cysteine
Phenylalanine
Tyrosine
Tryptophan
Aspartate
Asparagine
Pyrimidines
Malate
α-Ketoglutarate
Glutamate
malic enzyme
Pyruvate
Succinyl-CoA
Purines
Porphyrins, heme

❖As intermediates of the citric acid cycle are removed to serve as biosynthetic precursors, they are replenished by anaplerotic reactions.

❖Under normal circumstances, the reactions by which cycle intermediates are siphoned off into other pathways and those by which they are replenished are in dynamic balance, so that the concentrations of the citric acid cycle intermediates remain almost constant.

TABLE 16–2 Anaplerotic Reactions

Reaction	Tissue(s)/organism(s)
$\text{Pyruvate} + HCO_3^- + \text{ATP} \xrightleftharpoons{\text{pyruvate carboxylase}} \text{oxaloacetate} + \text{ADP} + P_i$	Liver, kidney
$\text{Phosphoenolpyruvate} + CO_2 + \text{GDP} \xrightleftharpoons{\text{PEP carboxykinase}} \text{oxaloacetate} + \text{GTP}$	Heart, skeletal muscle
$\text{Phosphoenolpyruvate} + HCO_3^- \xrightleftharpoons{\text{PEP carboxylase}} \text{oxaloacetate} + P_i$	Higher plants, yeast, bacteria
$\text{Pyruvate} + HCO_3^- + \text{NAD(P)H} \xrightleftharpoons{\text{malic enzyme}} \text{malate} + \text{NAD(P)}^+$	Widely distributed in eukaryotes and bacteria

Regulation of the Citric Acid Cycle

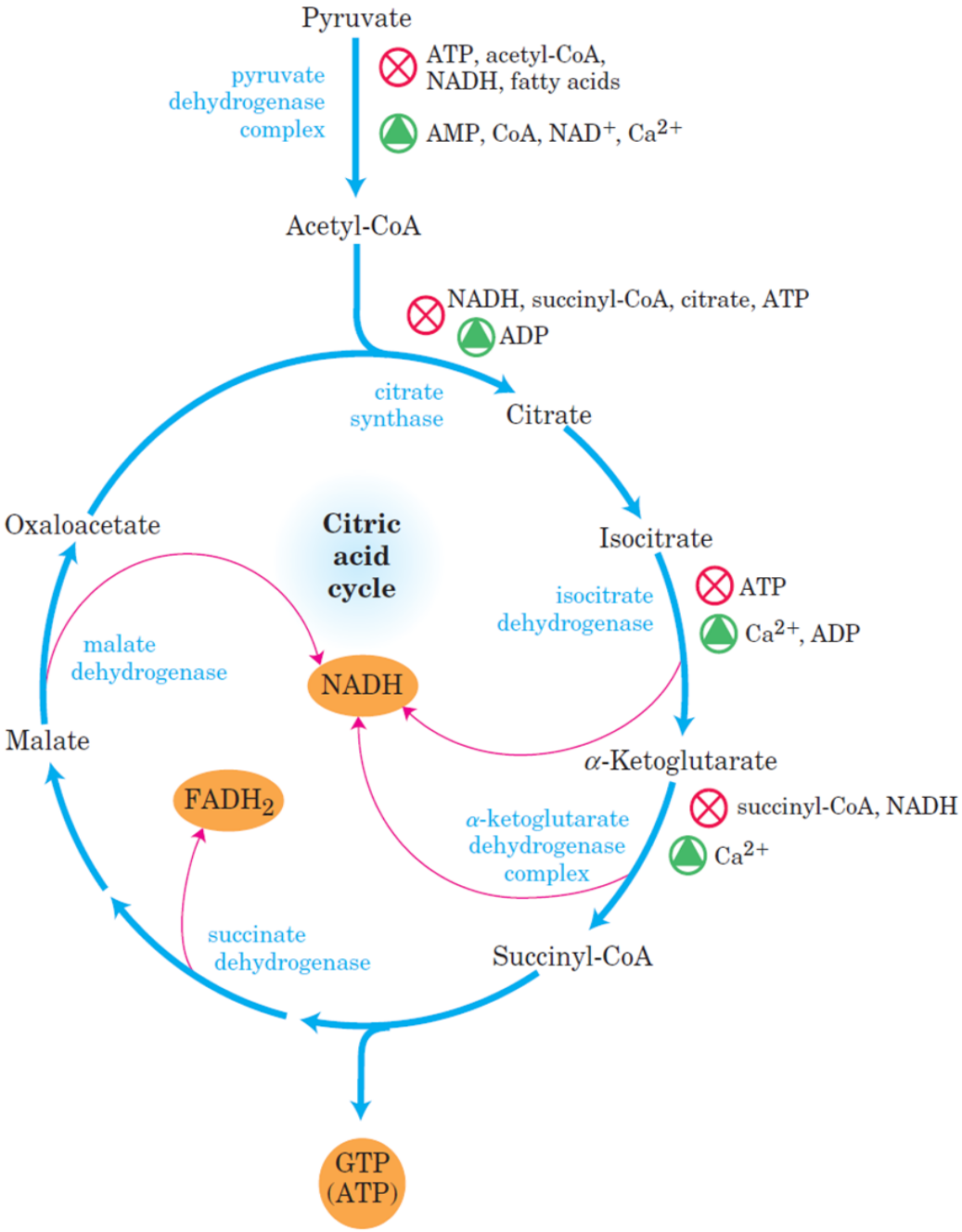
- The flow of carbon atoms from pyruvate into and through the citric acid cycle is under tight regulation at two levels:

  - Conversion of pyruvate to acetyl-CoA, (the PDH complex reaction)

  - and the entry of acetyl-CoA into the cycle (the citrate synthase reaction).

- Acetyl-CoA is also produced by pathways other than the PDH complex reaction—most cells produce acetyl-CoA from the oxidation of fatty acids and certain amino acids—and the availability of intermediates from these other pathways is important in the regulation of pyruvate oxidation and of the citric acid cycle.

- The cycle is also regulated at the isocitrate dehydrogenase and α-ketoglutarate dehydrogenase reactions.


Pyruvate
pyruvate dehydrogenase complex
ATP, acetyl-CoA, NADH, fatty acids
AMP, CoA, NAD+, Ca2+
Acetyl-CoA
NADH, succinyl-CoA, citrate, ATP
ADP
citrate synthase
Citrate
Isocitrate
ATP
isocitrate dehydrogenase
Ca2+, ADP
Citric acid cycle
Oxaloacetate
malate dehydrogenase
NADH
Malate
α-Ketoglutarate
succinyl-CoA, NADH
α-ketoglutarate dehydrogenase complex
Ca2+
FADH2
succinate dehydrogenase
Succinyl-CoA
GTP (ATP)

❖Three factors govern the rate of flux through the cycle: substrate availability, inhibition by accumulating products, and allosteric feedback inhibition of the enzymes that catalyze early steps in the cycle.

❖Each of the three strongly exergonic steps in the cycle—those catalyzed by citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase —can become the rate-limiting step under some circumstances.

❖Under normal conditions, the rates of glycolysis and of the citric acid cycle are integrated so that only as much glucose is metabolized to pyruvate as is needed to supply the citric acid cycle with its fuel, the acetyl groups of acetyl-CoA.

❖Citrate, the product of the first step of the citric acid cycle, is an important allosteric inhibitor of phosphofructokinase-1 in the glycolytic pathway.

The Glyoxylate Cycle

❖Vertebrates cannot convert fatty acids, or the acetate derived from them, to carbohydrates.

❖Conversion of phosphoenolpyruvate to pyruvate and of pyruvate to acetyl-CoA are so exergonic as to be essentially irreversible.

❖If a cell cannot convert acetate into phosphoenolpyruvate, acetate cannot serve as the starting material for the gluconeogenic pathway, which leads from phosphoenolpyruvate to glucose.

❖Without this capacity, then, a cell or organism is unable to convert fuels or metabolites that are degraded to acetate (fatty acids and certain amino acids) into carbohydrates.

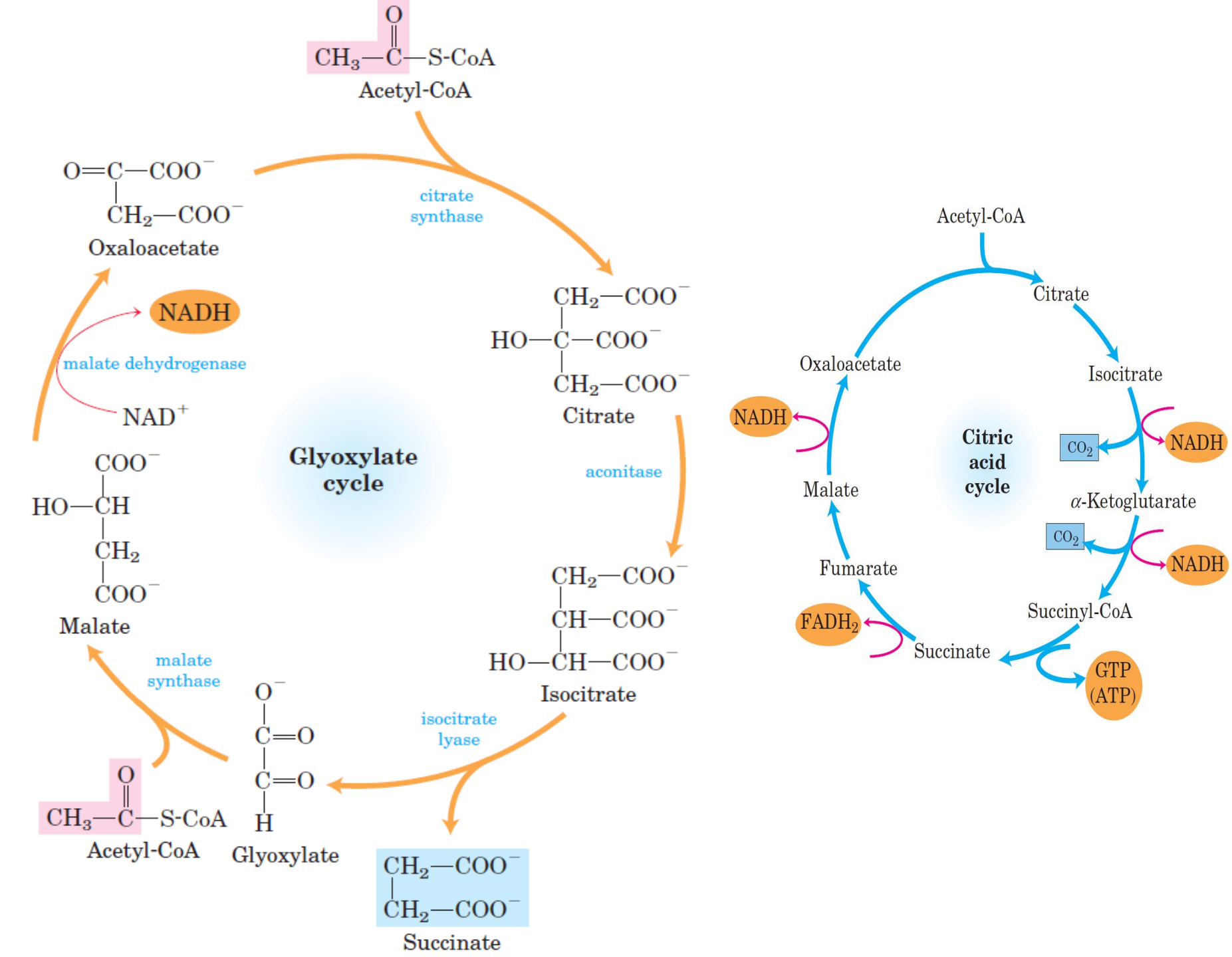
❖As noted in the discussion of anaplerotic reactions, phosphoenolpyruvate can be synthesized from oxaloacetate in the reversible reaction catalyzed by PEP carboxykinase:

$$\text{Oxaloacetate} + \text{GTP} \rightleftharpoons \text{phosphoenolpyruvate} + CO_2 + \text{GDP}$$

❖Because the carbon atoms of acetate molecules that enter the citric acid cycle appear eight steps later in oxaloacetate, it might seem that this pathway could generate oxaloacetate from acetate and thus generate phosphoenolpyruvate for gluconeogenesis.

❖However, as an examination of the stoichiometry of the citric acid cycle shows, there is no net conversion of acetate to oxaloacetate; in vertebrates, for every two carbons that enter the cycle as acetyl-CoA, two leave as CO2.

❖In many organisms other than vertebrates, the glyoxylate cycle serves as a mechanism for converting acetate to carbohydrate.


Acetyl-CoA
citrate synthase
Oxaloacetate
NADH
malate dehydrogenase
NAD+
Citrate
aconitase
Glyoxylate cycle
Malate
Isocitrate
malate synthase
isocitrate lyase
Acetyl-CoA
Glyoxylate
Succinate
Acetyl-CoA
Citrate
Isocitrate
NADH
CO2
NADH
Oxaloacetate
Citric acid cycle
α-Ketoglutarate
CO2
NADH
Malate
Fumarate
Succinyl-CoA
FADH2
Succinate
GTP (ATP)

❖In plants, certain invertebrates, and some microorganisms (including *E. coli* and yeast) acetate can serve both as an energy-rich fuel and as a source of phosphoenolpyruvate for carbohydrate synthesis.

❖Each turn of the glyoxylate cycle consumes two molecules of acetyl-CoA and produces one molecule of succinate, which is then available for biosynthetic purposes.

❖The succinate may be converted through fumarate and malate into oxaloacetate, which can then be converted to phosphoenolpyruvate by PEP carboxykinase, and thus to glucose by gluconeogenesis.

❖Vertebrates do not have the enzymes specific to the glyoxylate cycle (isocitrate lyase and malate synthase) and therefore cannot bring about the net synthesis of glucose from fatty acids.

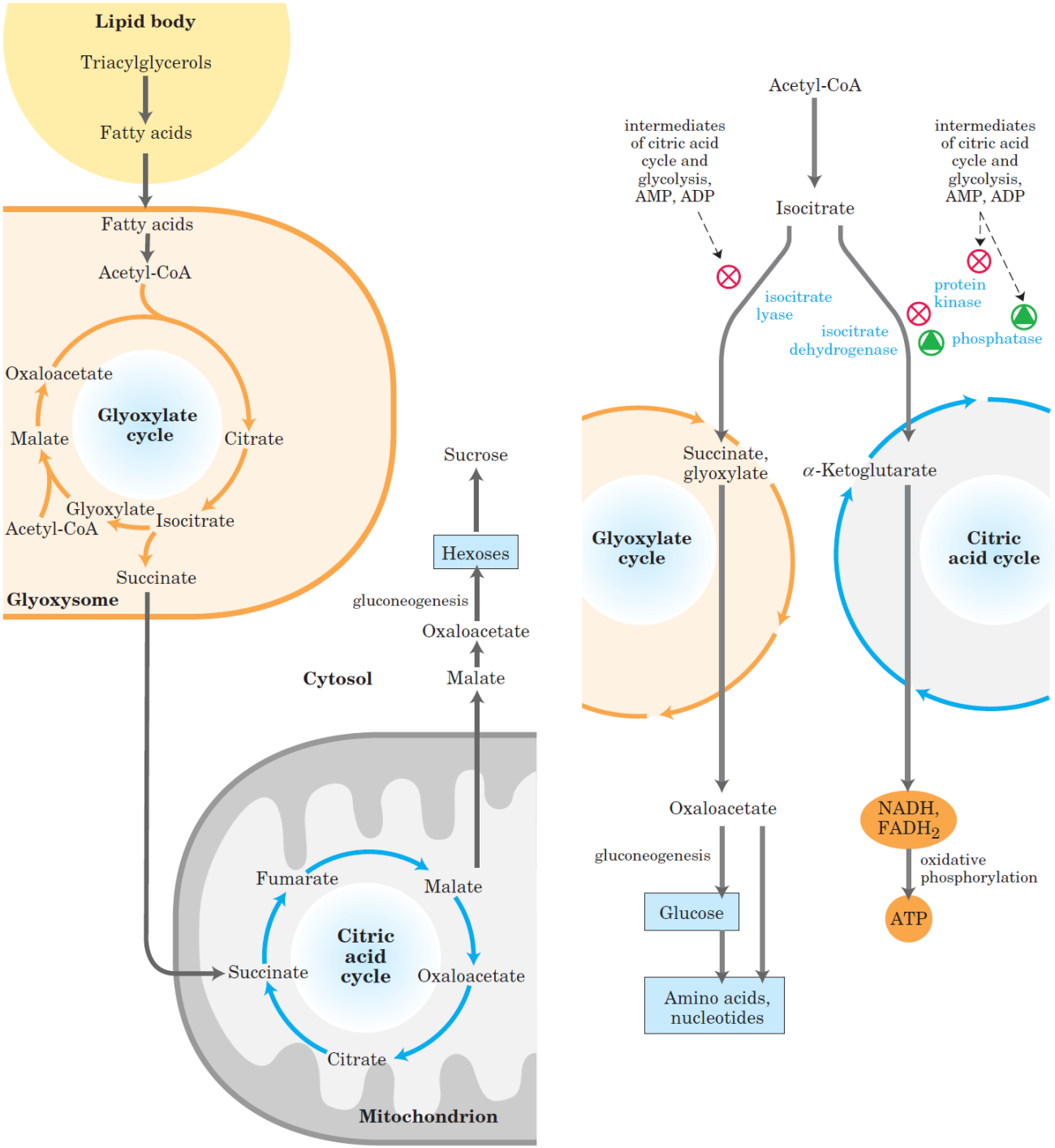
❖Isocitrate is a crucial intermediate, at the branch point between the glyoxylate and citric acid cycles.

❖Isocitrate dehydrogenase is regulated by covalent modification: a specific protein kinase phosphorylates and thereby inactivates the dehydrogenase.

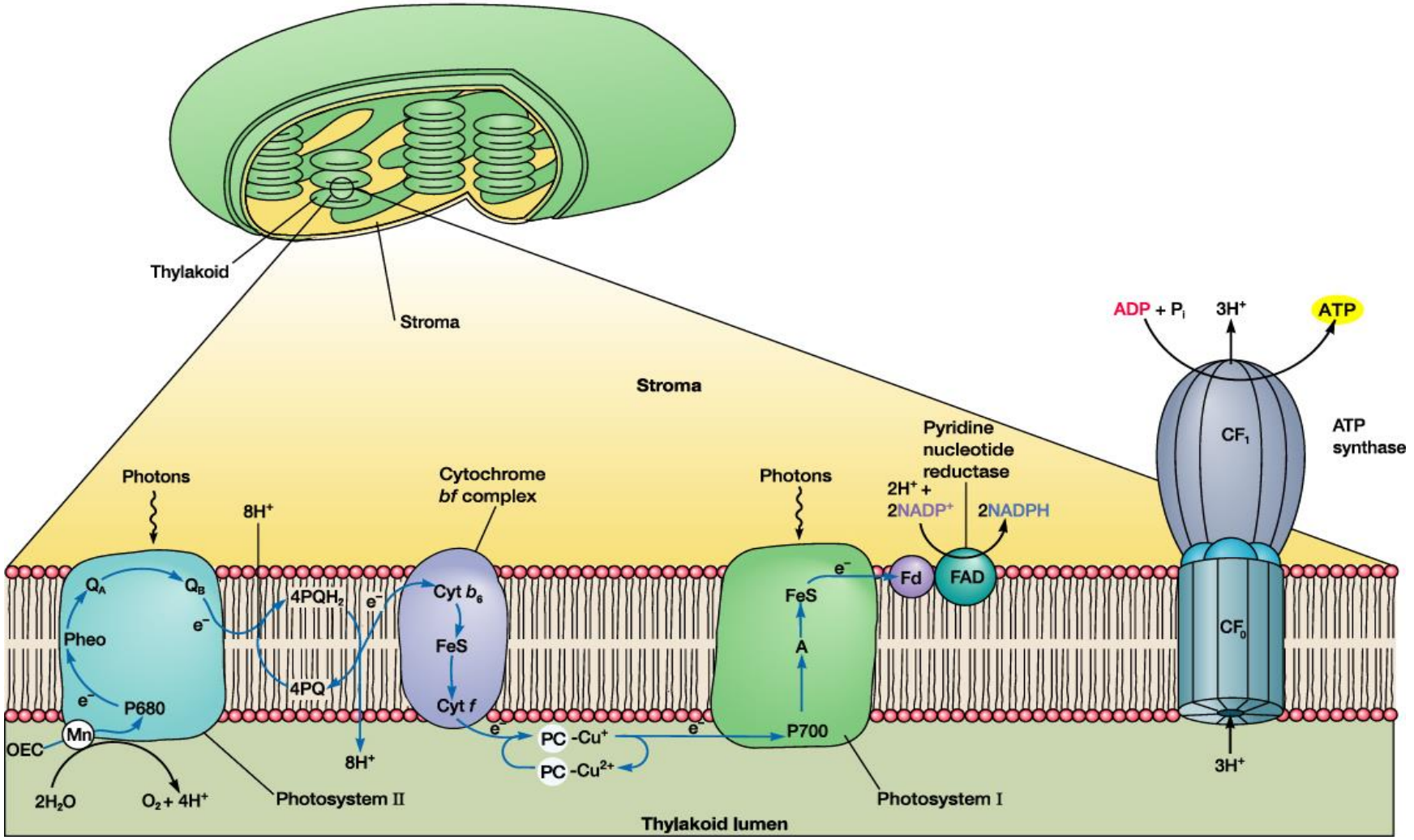
❖This inactivation shunts isocitrate to the glyoxylate cycle, where it begins the synthetic route toward glucose.

❖A phosphoprotein phosphatase removes the phosphoryl group from isocitrate dehydrogenase, reactivating the enzyme and sending more isocitrate through the energy-yielding citric acid cycle.

❖The regulatory protein kinase and phosphoprotein phosphatase are separate enzymatic activities of a single polypeptide.


Lipid body
Triacylglycerols
Fatty acids
Fatty acids
Acetyl-CoA
Oxaloacetate
Glyoxylate cycle
Malate
Citrate
Glyoxylate
Acetyl-CoA
Isocitrate
Succinate
Glyoxysome
Sucrose
Hexoses
gluconeogenesis
Oxaloacetate
Cytosol
Malate
Fumarate
Malate
Citric acid cycle
Succinate
Oxaloacetate
Citrate
Mitochondrion
Acetyl-CoA
intermediates of citric acid cycle and glycolysis, AMP, ADP
Isocitrate
intermediates of citric acid cycle and glycolysis, AMP, ADP
isocitrate lyase
protein kinase
isocitrate dehydrogenase
phosphatase
Succinate, glyoxylate
α-Ketoglutarate
Glyoxylate cycle
Citric acid cycle
Oxaloacetate
NADH, FADH2
gluconeogenesis
oxidative phosphorylation
Glucose
ATP
Amino acids, nucleotides

Carbohydrate Metabolism IV
Oxidative Phosphorylation and Photophosphorylation



Oxidative Phosphorylation and Photophosphorylation

❖Oxidative phosphorylation is the culmination of energy-yielding metabolism in aerobic organisms.

❖All oxidative steps in the degradation of carbohydrates, fats, and amino acids converge at this final stage of cellular respiration, in which the energy of oxidation drives the synthesis of ATP.

❖Photophosphorylation is the means by which photosynthetic organisms capture the energy of sunlight—the ultimate source of energy in the biosphere— and harness it to make ATP.

❖Together, oxidative phosphorylation and photophosphorylation account for most of the ATP synthesized by most organisms most of the time.

❖In eukaryotes, oxidative phosphorylation occurs in mitochondria, photophosphorylation in chloroplasts.

❖The pathways to ATP synthesis in mitochondria and chloroplasts have challenged and fascinated biochemists for more than half a century, and the fascination has grown with our deepening appreciation of these fundamental mechanisms in living organisms, their conservation in evolution, and their structural bases.

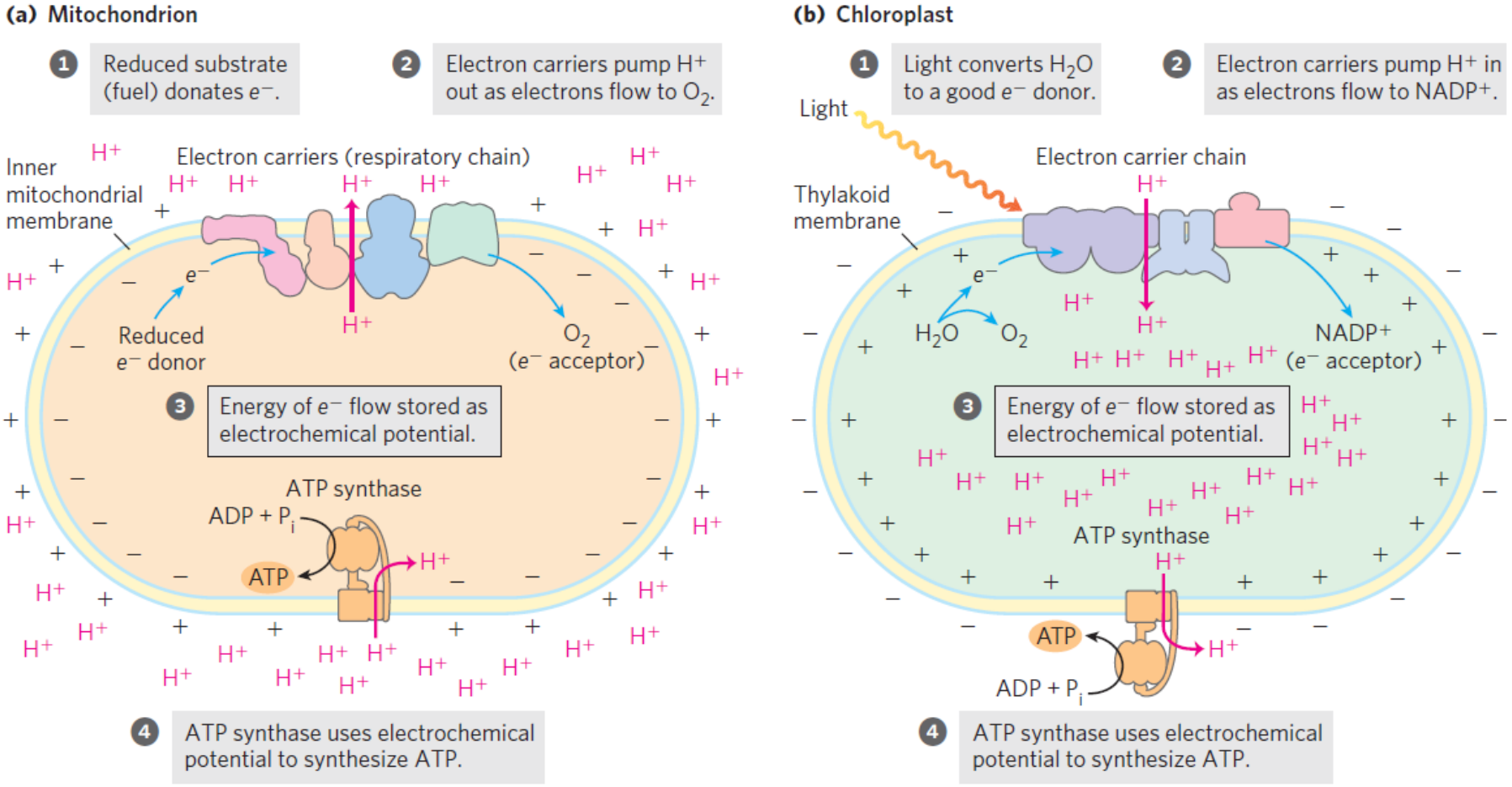
❖Our current understanding of ATP synthesis in mitochondria and chloroplasts is based on the hypothesis, introduced by Peter Mitchell in 1961, that transmembrane differences in proton concentration are the reservoir for the energy extracted from biological oxidation reactions which called as chemiosmotic theory.

❖Oxidative phosphorylation and photophosphorylation are mechanistically similar in three respects.

❖(1) Both processes involve the flow of electrons through a chain of membrane-bound carriers.

❖(2) The free energy made available by this “downhill” (exergonic) electron flow is coupled to the “uphill” transport of protons across a proton-impermeable membrane, conserving the free energy of fuel oxidation as a transmembrane electrochemical potential.

❖(3) The transmembrane flow of protons back down their concentration gradient through specific protein channels provides the free energy for synthesis of ATP, catalyzed by a membrane protein complex (ATP synthase) that couples proton flow to phosphorylation of ADP.


(a) Mitochondrion
1 Reduced substrate (fuel) donates e⁻.
2 Electron carriers pump H⁺ out as electrons flow to O2.
Inner mitochondrial membrane
Electron carriers (respiratory chain)
e⁻
Reduced e⁻ donor
O2 (e⁻ acceptor)
3 Energy of e⁻ flow stored as electrochemical potential.
ATP synthase
ADP + Pi
ATP
4 ATP synthase uses electrochemical potential to synthesize ATP.
(b) Chloroplast
1 Light converts H2O to a good e⁻ donor.
2 Electron carriers pump H⁺ in as electrons flow to NADP⁺.
Light
Thylakoid membrane
Electron carrier chain
e⁻
H2O
O2
NADP+ (e⁻ acceptor)
3 Energy of e⁻ flow stored as electrochemical potential.
ATP synthase
ATP
ADP + Pi
4 ATP synthase uses electrochemical potential to synthesize ATP.

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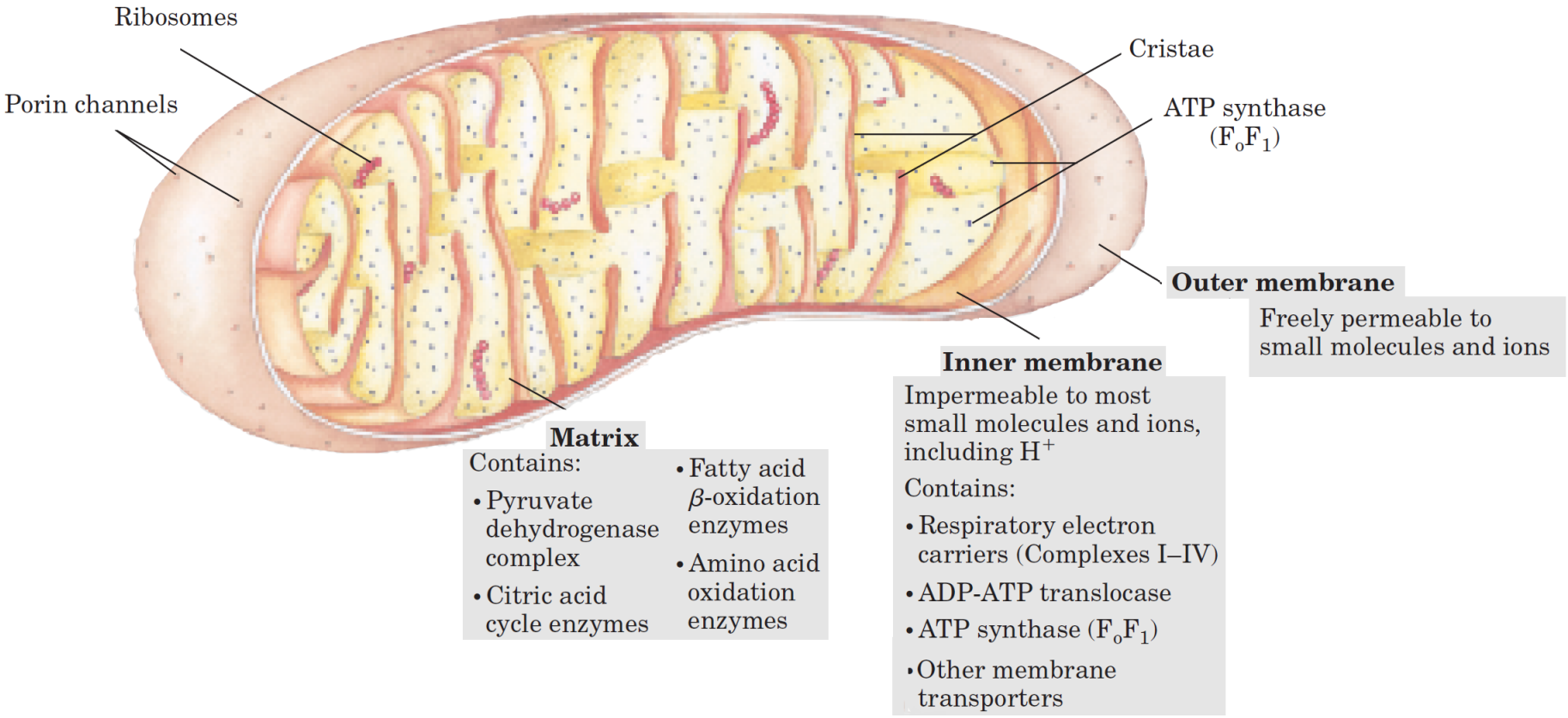
❖Mitochondria are the site of oxidative phosphorylation in eukaryotes.

❖Mitochondria, like gramnegative bacteria, have two membranes.



❖The outer mitochondrial membrane is readily permeable to small molecules (Mr, 5,000) and ions, which move freely through transmembrane channels formed by a family of integral membrane proteins called porins.

❖The inner membrane is impermeable to most small molecules and ions, including protons; the only species that cross this membrane do so through specific transporters.

❖The inner membrane bears the components of the respiratory chain and the ATP synthase.

❖The mitochondrial matrix, enclosed by the inner membrane, contains the pyruvate dehydrogenase complex and the enzymes of the citric acid cycle, the fatty acid β-oxidation pathway, and the pathways of amino acid oxidation—all the pathways of fuel oxidation except glycolysis, which takes place in the cytosol.

❖The selectively permeable inner membrane segregates the intermediates and enzymes of cytosolic metabolic pathways from those of metabolic processes occurring in the matrix.

❖However, specific transporters carry pyruvate, fatty acids, and amino acids or their -keto derivatives into the matrix for access to the machinery of the citric acid cycle.

❖ADP and Pi are specifically transported into the matrix as newly synthesized ATP is transported out.

❖Oxidative phosphorylation begins with the entry of electrons into the chain of electron carriers called the respiratory chain.

❖Most of these electrons arise from the action of dehydrogenases that collect electrons from catabolic pathways and funnel them into universal electron acceptors—nicotinamide nucleotides (NAD^+ or $NADP^+$) or flavin nucleotides (FMN or FAD).

❖Nicotinamide nucleotide–linked dehydrogenases catalyze reversible reactions of the following general types:

$$\text{Reduced substrate} + \text{NAD(P)}^+ \rightleftharpoons \text{oxidized substrate} + \text{NAD(P)H} + \text{H}^+$$

❖NAD-linked dehydrogenases remove two hydrogen atoms from their substrates. One of these is transferred as a hydride ion ($:H^+$) to NAD^+, the other is released as H in the medium.

❖NADH and NADPH are water-soluble electron carriers that associate reversibly with dehydrogenases.

❖Flavoproteins contain a very tightly, sometimes covalently, bound flavin nucleotide, either FMN or FAD.

❖The oxidized flavin nucleotide can accept either one electron (yielding the semiquinone form) or two (yielding $FADH_2$ or $FMNH_2$).

❖The mitochondrial respiratory chain consists of a series of sequentially acting electron carriers, most of which are integral proteins with prosthetic groups capable of accepting and donating either one or two electrons.

❖Three types of electron transfers occur in oxidative phosphorylation:

❖(1) direct transfer of electrons, as in the reduction of Fe^{3+} to Fe^{2+},

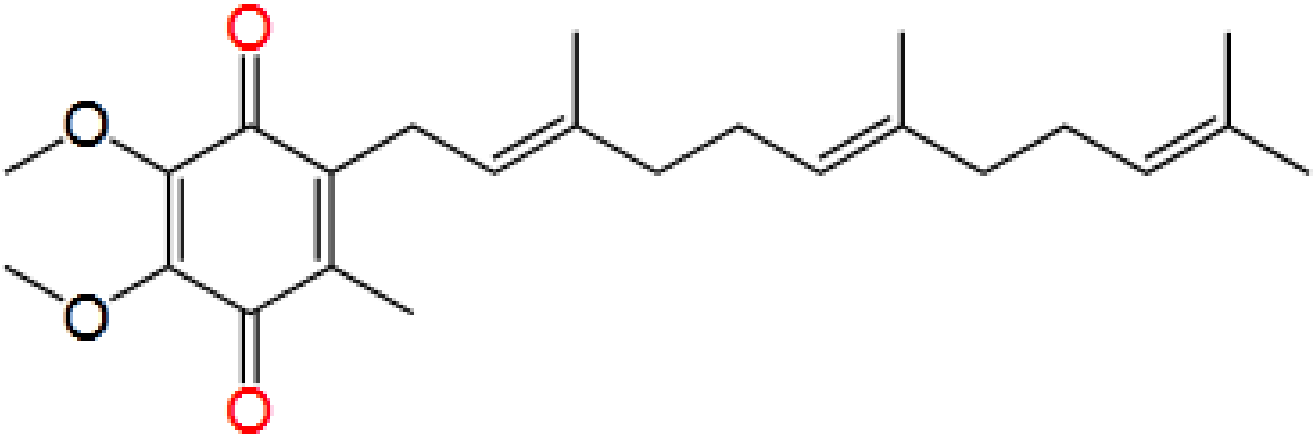
❖(2) transfer as a hydrogen atom ($H^+ + e^-$), and

❖(3) transfer as a hydride ion ($:H^-$), which bears two electrons.

❖The term reducing equivalent is used to designate a single electron equivalent transferred in an oxidation-reduction reaction.

❖In addition to NAD and flavoproteins, three other types of electron-carrying molecules function in the respiratory chain: a hydrophobic quinone (**ubiquinone**) and two different types of iron-containing proteins (**cytochromes** and **iron-sulfur proteins**).

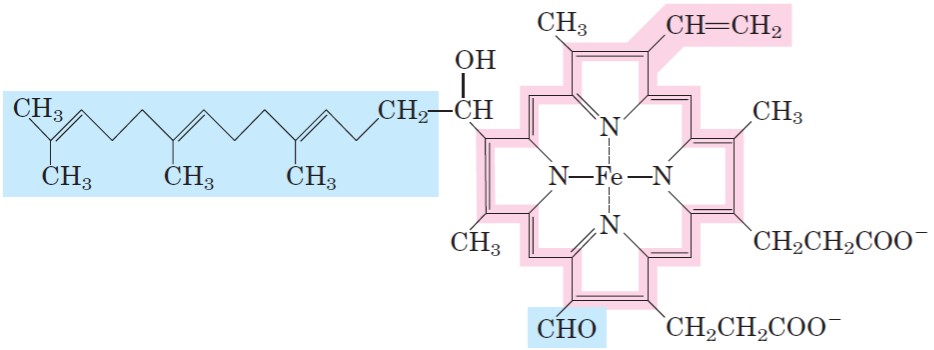
❖Ubiquinone (also called coenzyme Q, or simply Q) is a lipid-soluble benzoquinone with a long isoprenoid side chain.



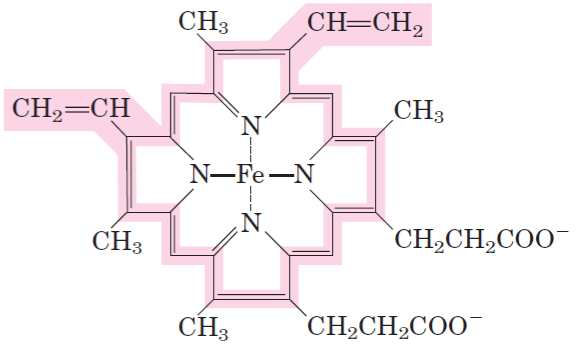
❖The closely related compounds plastoquinone (of plant chloroplasts) and menaquinone (of bacteria) play roles analogous to that of ubiquinone, carrying electrons in membrane-associated electron-transfer chains.

❖The cytochromes are proteins with characteristic strong absorption of visible light, due to their iron-containing heme prosthetic groups.

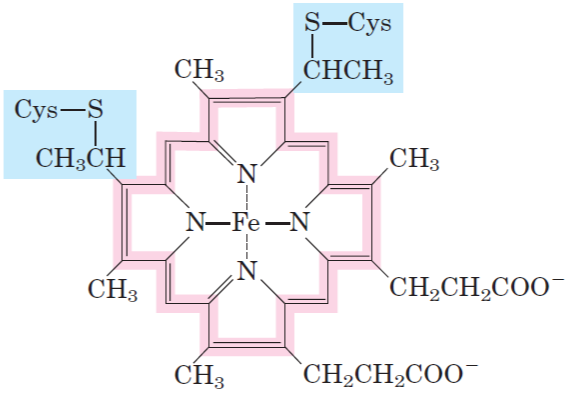
❖Mitochondria contain three classes of cytochromes, designated a, b, and c, which are distinguished by differences in their light-absorption spectra.



Heme A
(in *a*-type cytochromes)

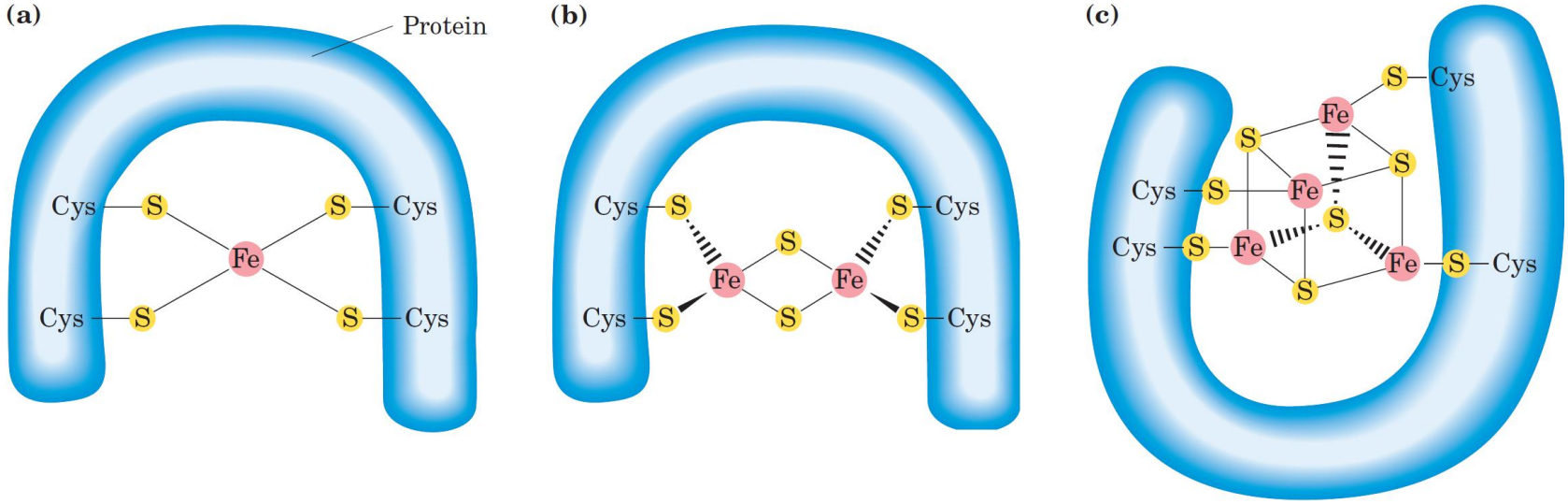


Iron protoporphyrin IX
(in *b*-type cytochromes)



Heme C
(in *c*-type cytochromes)

❖In iron-sulfur proteins, the iron is present not in heme but in association with inorganic sulfur atoms or with the sulfur atoms of Cys residues in the protein, or both.



❖Rieske iron-sulfur proteins are a variation on this theme, in which one Fe atom is coordinated to two His residues rather than two Cys residues.

❖In the overall reaction catalyzed by the mitochondrial respiratory chain, electrons move from NADH, succinate, or some other primary electron donor through flavoproteins, ubiquinone, iron-sulfur proteins, and cytochromes, and finally to O2.

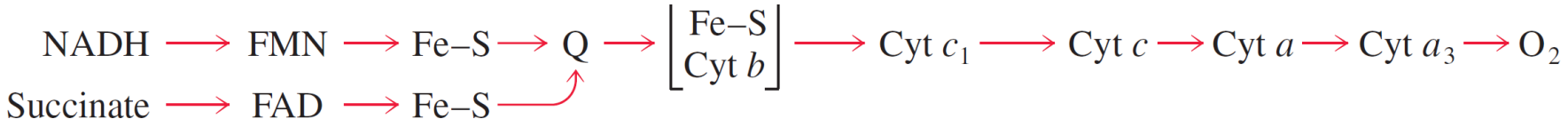
❖The electron carriers of the respiratory chain are organized into membrane-embedded supramolecular complexes that can be physically separated.

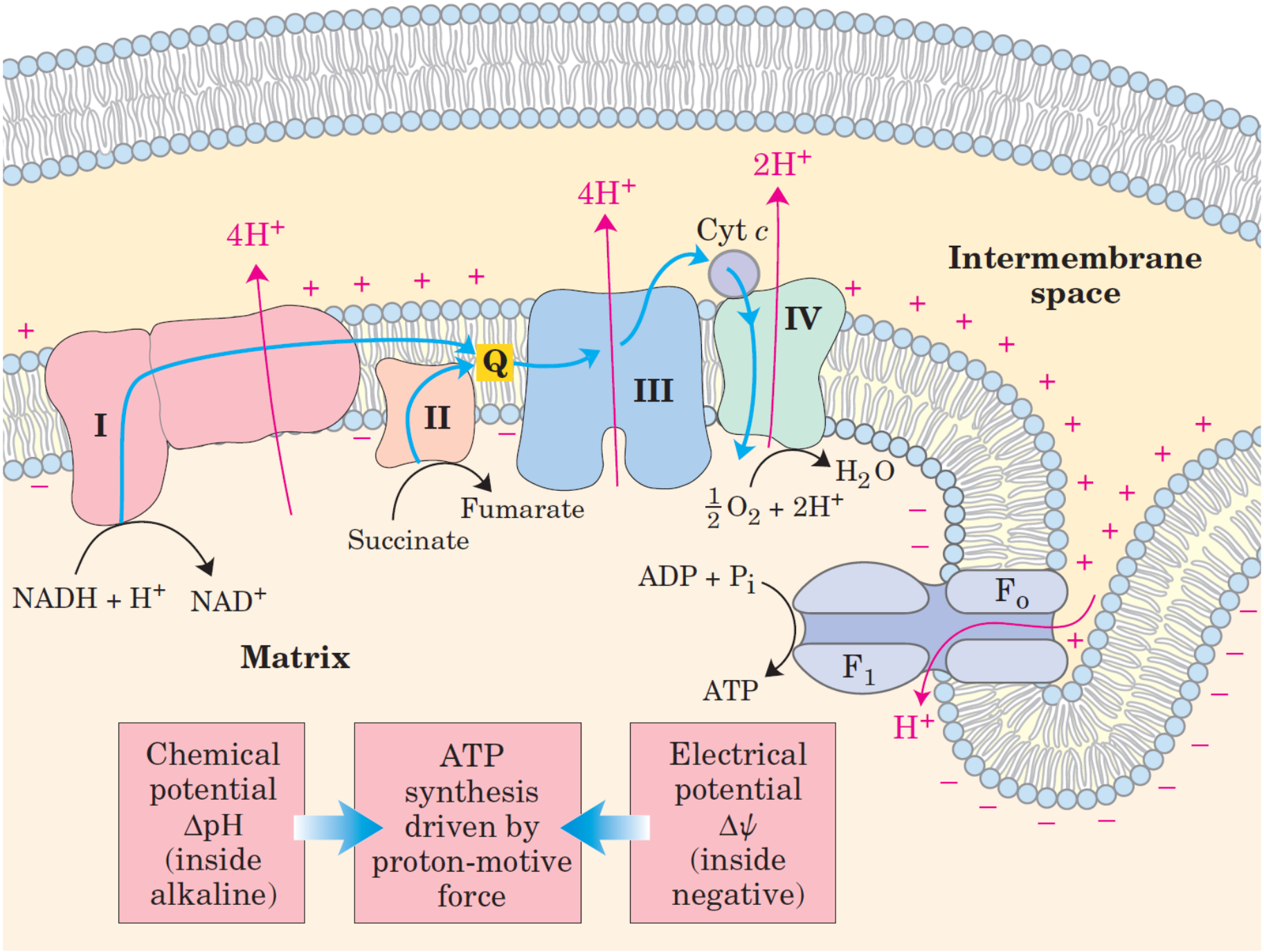
❖Gentle treatment of the inner mitochondrial membrane with detergents allows the resolution of four unique electron-carrier complexes, each capable of catalyzing electron transfer through a portion of the chain.

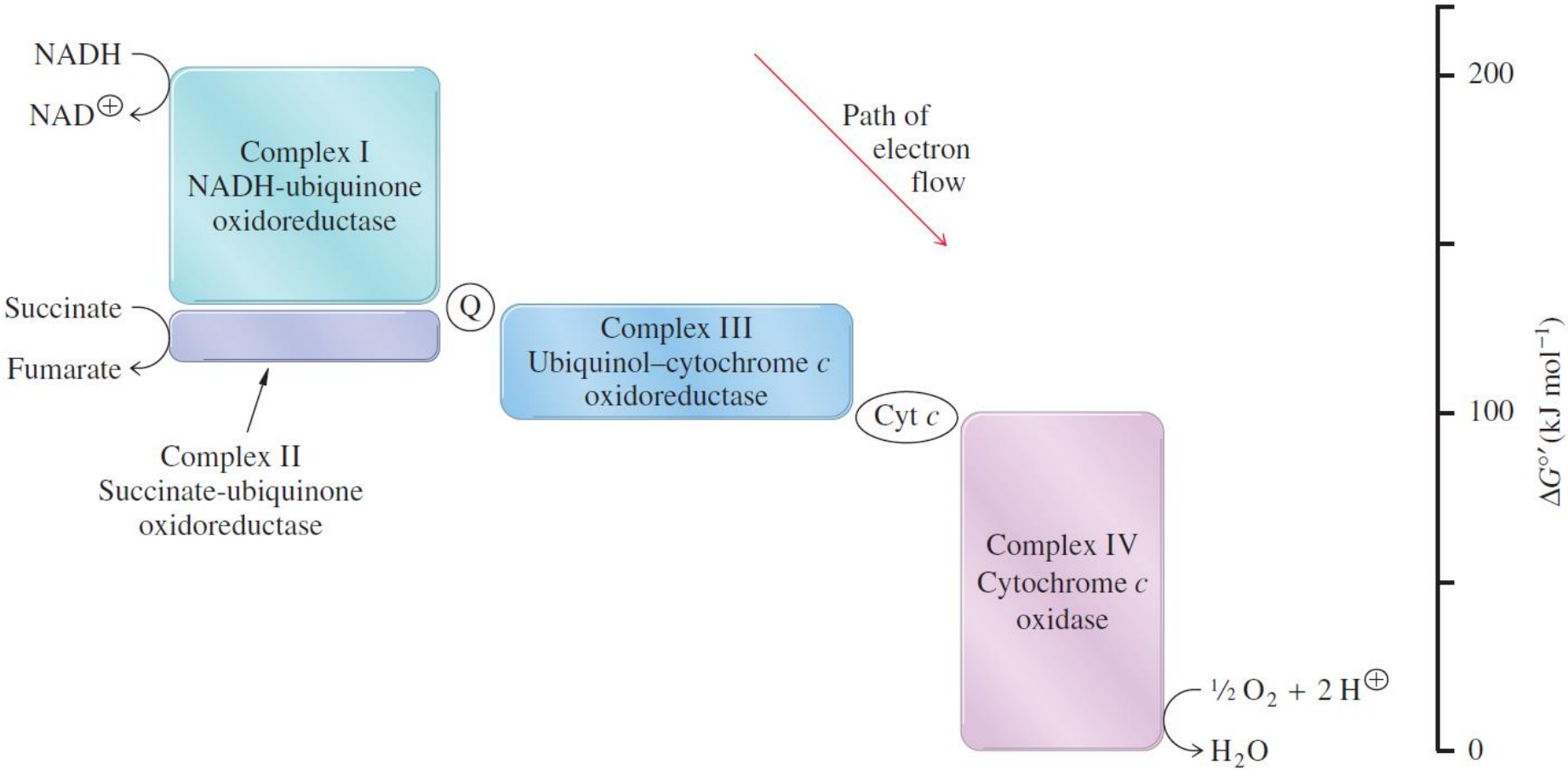
❖Complexes I and II catalyze electron transfer to ubiquinone from two different electron donors: NADH (Complex I) and succinate (Complex II).

❖Complex III carries electrons from reduced ubiquinone to cytochrome c, and Complex IV completes the sequence by transferring electrons from cytochrome c to O_2.

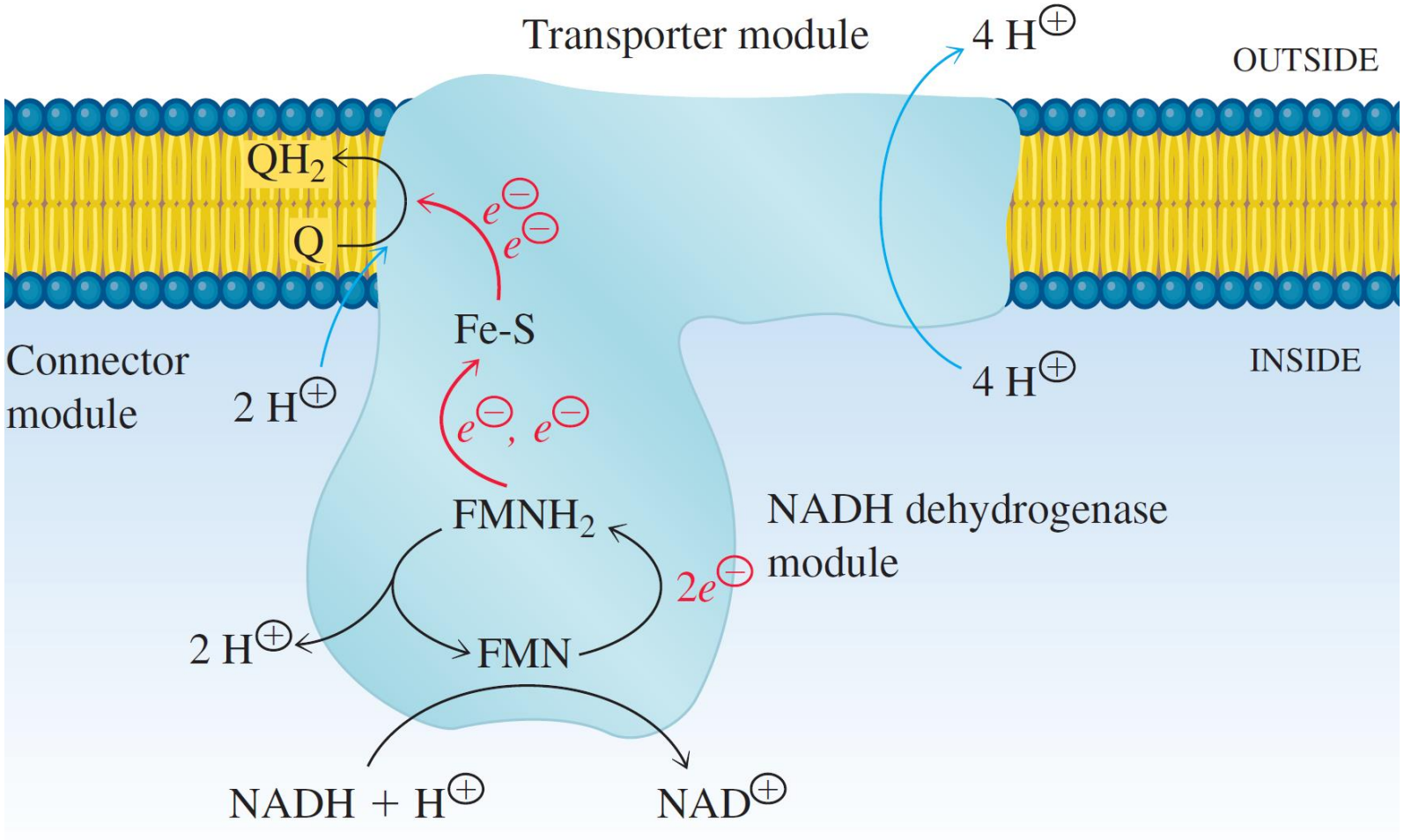
❖Complex V is an ATP synthase.



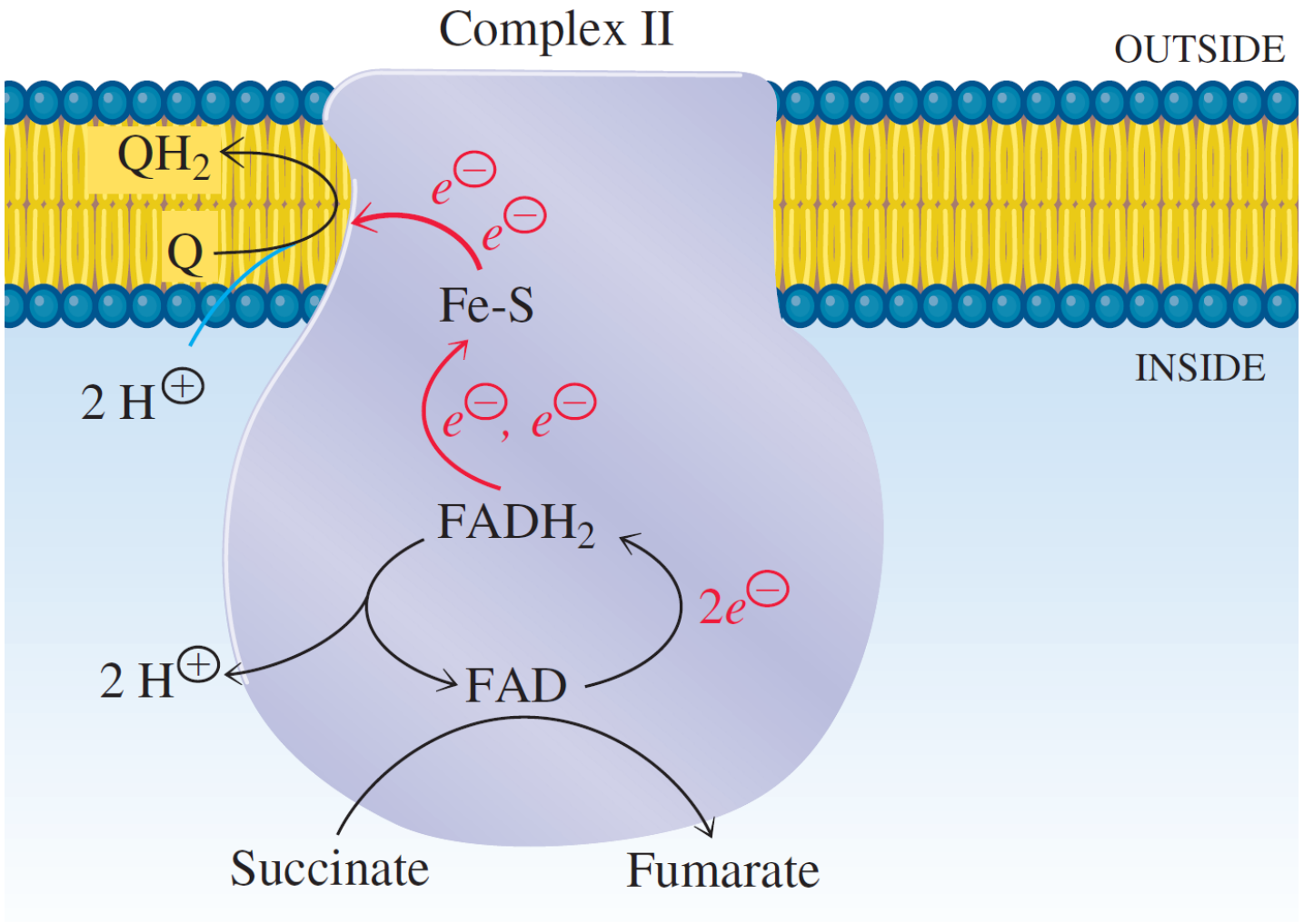

4H+
4H+
2H+
Cyt c
Intermembrane space
I
II
Q
III
IV
Succinate
Fumarate
H2O
½ O2 + 2H+
NADH + H+
NAD+
Matrix
ADP + Pi
ATP
Fo
F1
H+
Chemical potential ΔpH (inside alkaline)
ATP synthesis driven by proton-motive force
Electrical potential Δψ (inside negative)


NADH
NAD⊕
Complex I
NADH-ubiquinone
oxidoreductase
Succinate
Fumarate
Complex II
Succinate-ubiquinone
oxidoreductase
Q
Complex III
Ubiquinol–cytochrome c
oxidoreductase
Cyt c
Complex IV
Cytochrome c
oxidase
½ O2 + 2 H⊕
H2O
Path of
electron
flow
200
100
0
ΔG°′ (kJ mol−1)

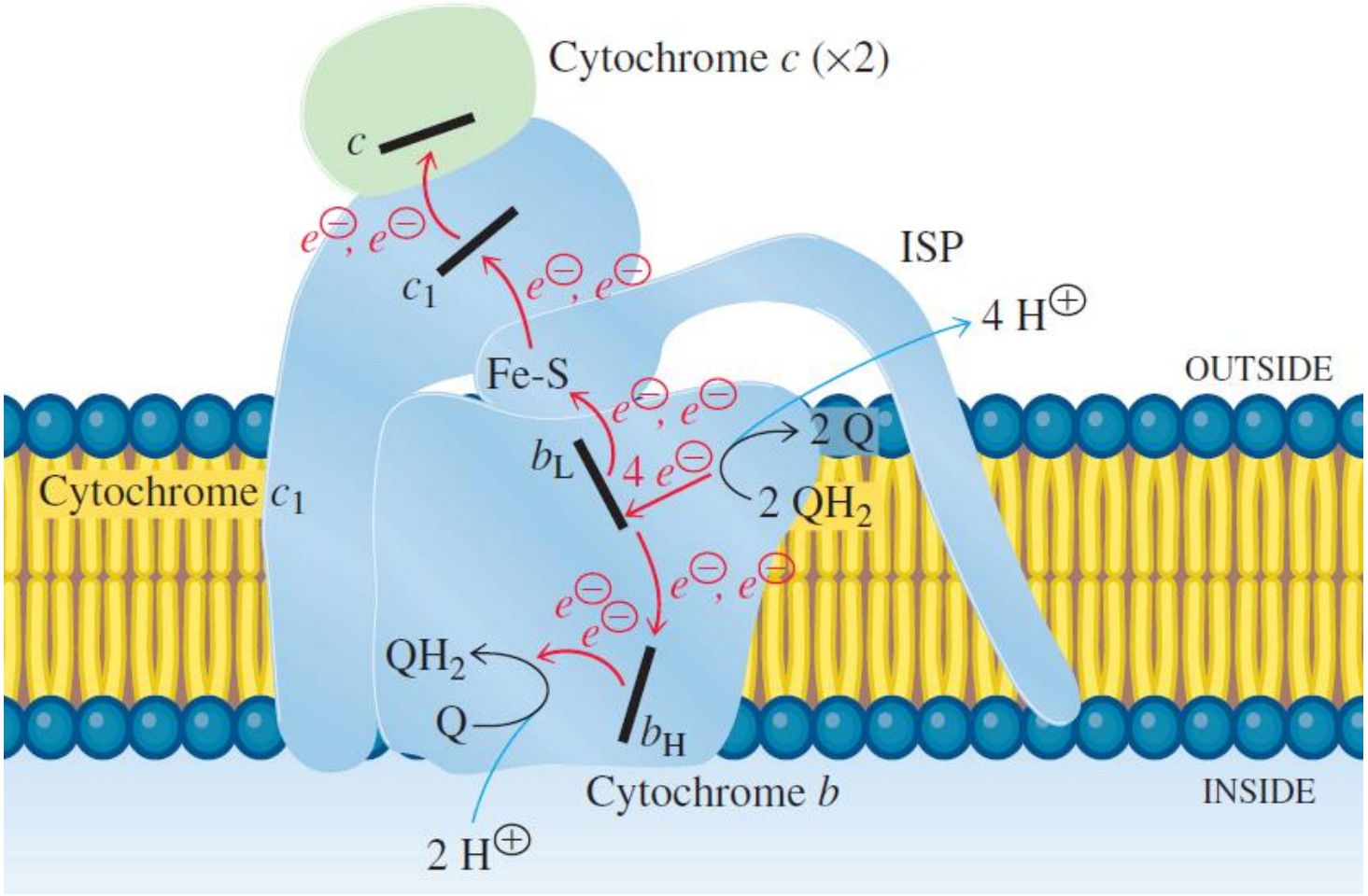
❖Complex I, also called NADH:ubiquinone oxidoreductase or NADH dehydrogenase catalyzes two simultaneous and obligately coupled processes: (1) the exergonic transfer a hydride ion from NADH and a proton from the matrix to ubiquinone, and (2) the endergonic transfer of four protons from the matrix to the intermembrane space.



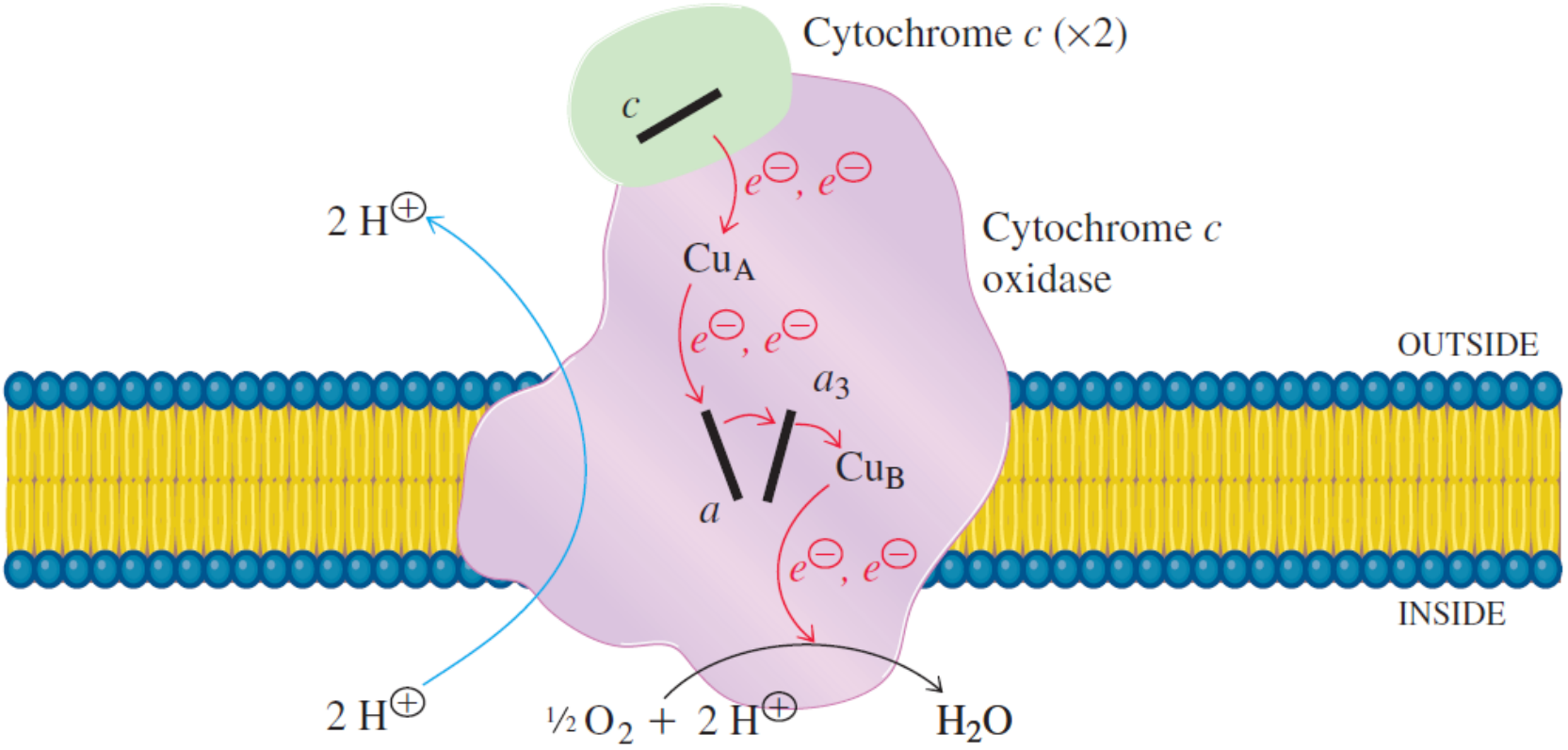
❖Complex II succinate:ubiquinon oxidoreductase or succinate dehydrogenase is an in the citric acid and transfer elestrons from succinate to ubiquinon.



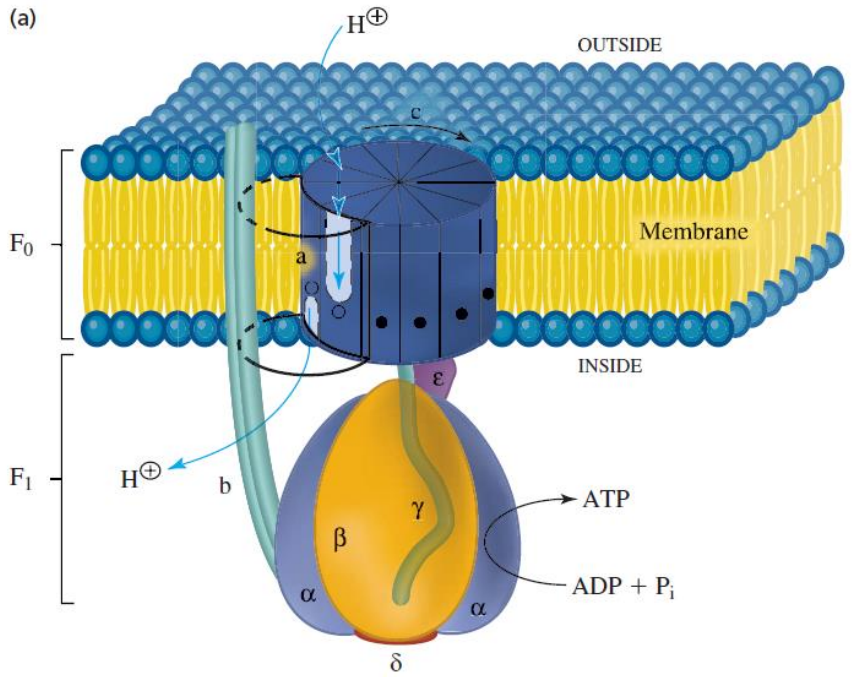
❖Complex III, also called cytochrome bc1 complex or ubiquinone : cytochrome c oxidoreductase, couples the transfer of electrons from ubiquinol (QH2) to cytochrome c with the vectorial transport of protons from the matrix to the intermembrane space.

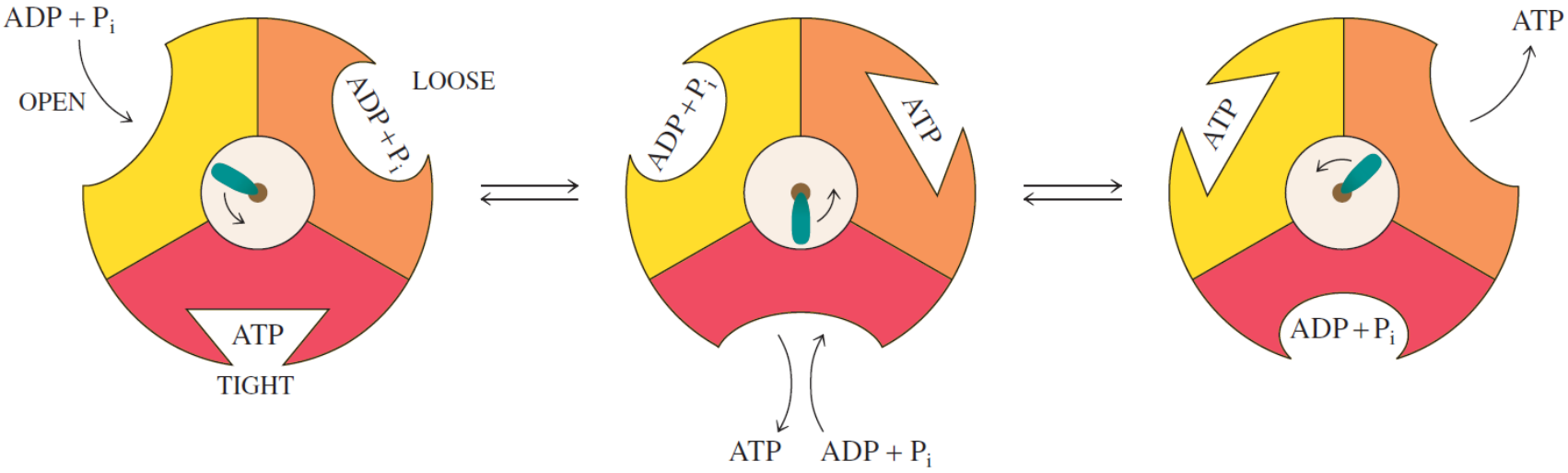


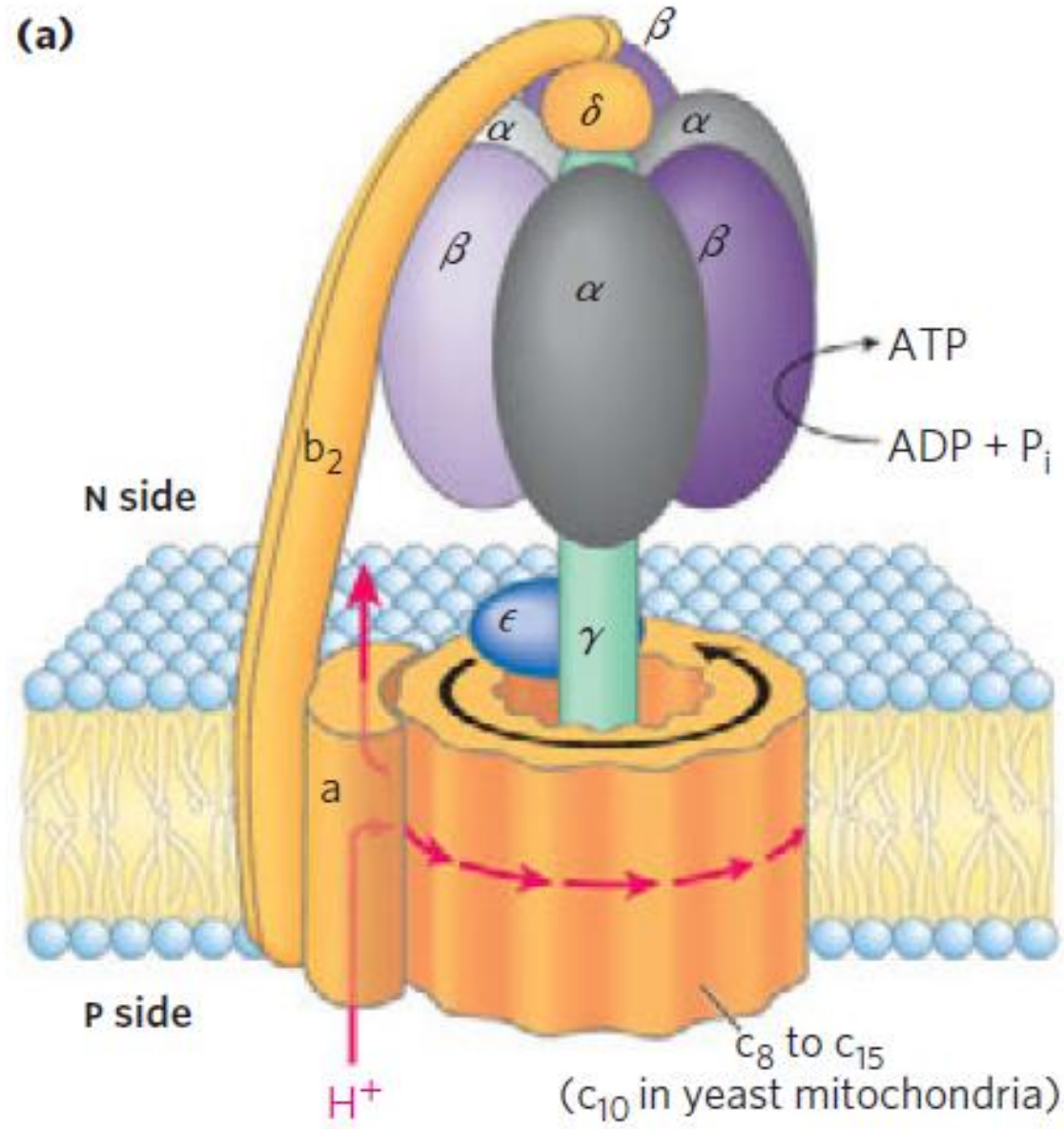
❖In the final step of the respiratory chain, Complex IV, also called cytochrome oxidase, carries electrons from cytochrome c to molecular oxygen, reducing it to H_2O.

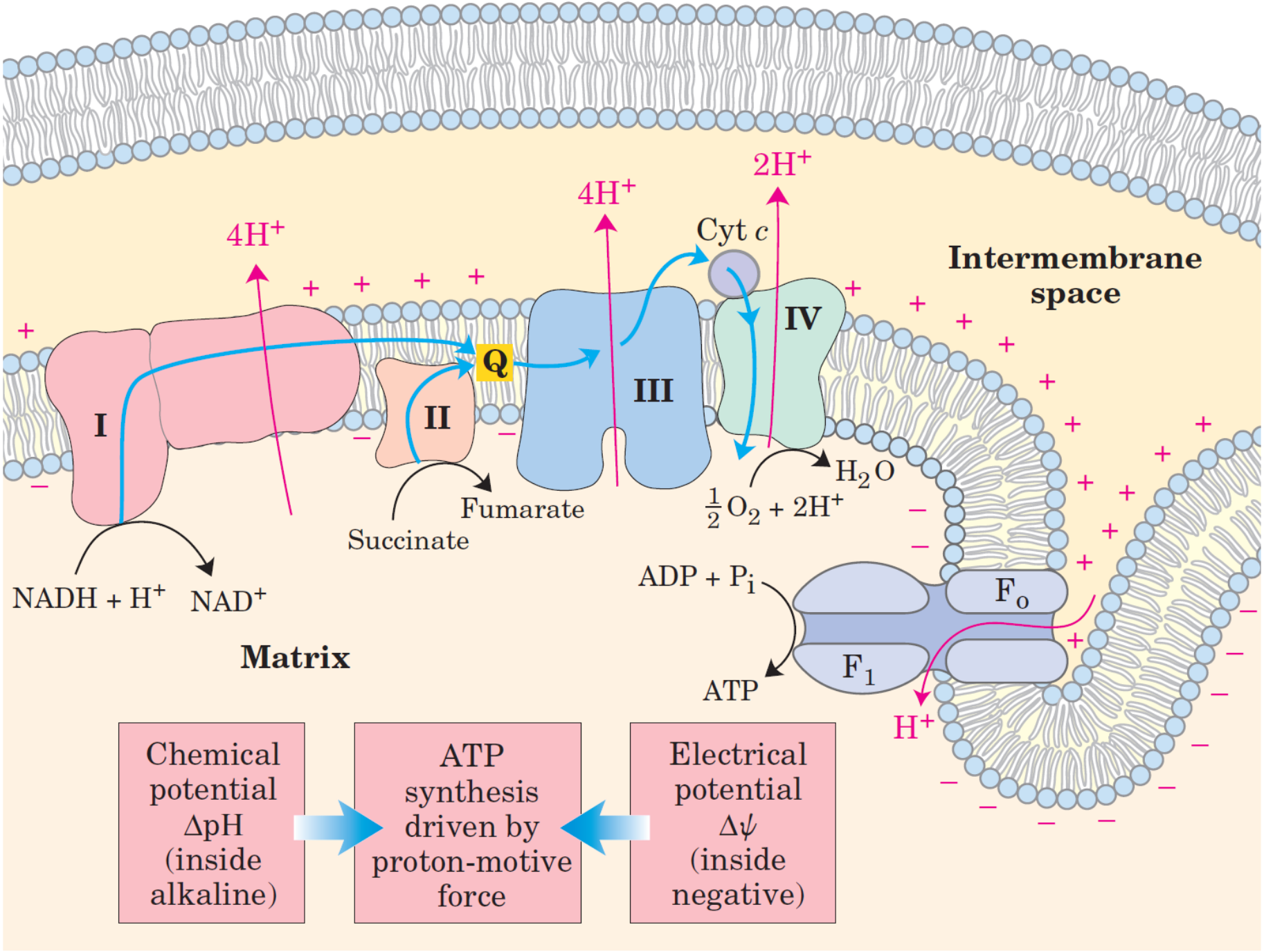


❖Complex V is an ATP synthase and using proton gradient generated through membran bounded electron transport synthesize the production of ATP from ADP and Pi.



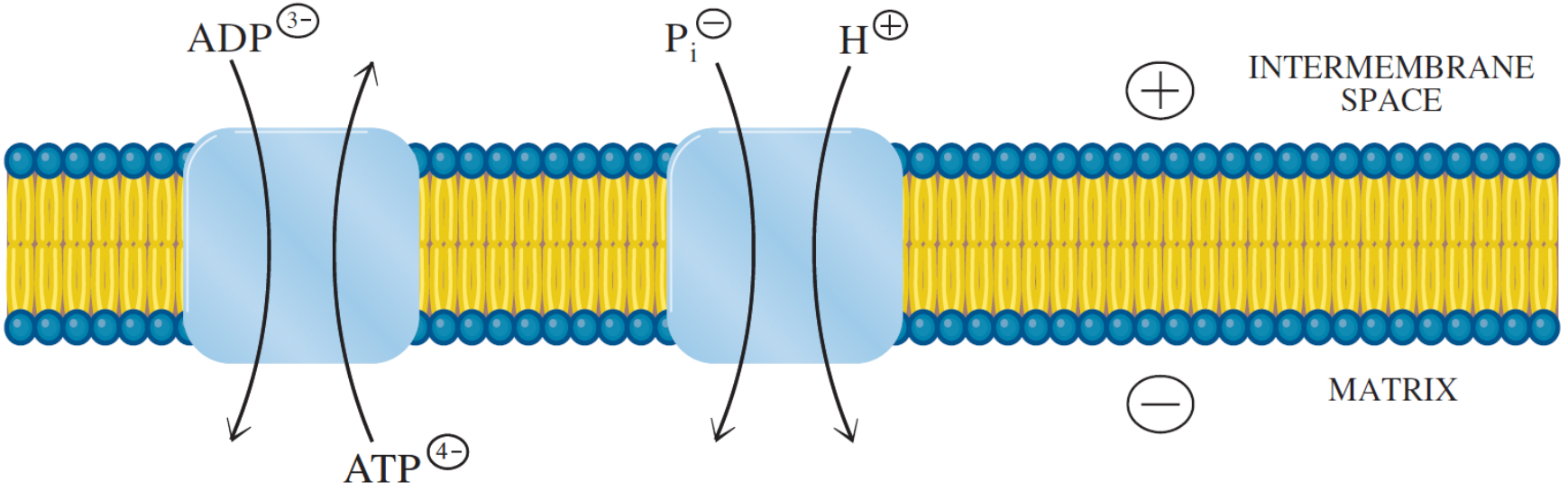



(a)
β
δ
α
α
β
α
β
ATP
ADP + Pi
b2
N side
ε
γ
a
P side
H+
c8 to c15
(c10 in yeast mitochondria)


4H+
4H+
2H+
Cyt c
Intermembrane space
I
II
Q
III
IV
Succinate
Fumarate
H2O
½ O2 + 2H+
NADH + H+
NAD+
Matrix
ADP + Pi
ATP
Fo
F1
H+
Chemical potential ΔpH (inside alkaline)
ATP synthesis driven by proton-motive force
Electrical potential Δψ (inside negative)

❖Although the primary role of the proton gradient in mitochondria is to furnish energy for the synthesis of ATP, the proton-motive force also drives several transport processes essential to oxidative phosphorylation.

❖The inner mitochondrial membrane is generally impermeable to charged species, but two specific systems transport ADP and Pi into the matrix and ATP out to the cytosol; adenine nucleotide translocase and phosphate translocase.



❖The NADH dehydrogenase of the inner mitochondrial membrane of animal cells can accept electrons only from NADH in the matrix.

❖Given that the inner membrane is not permeable to NADH, how can the NADH generated by glycolysis in the cytosol be reoxidized to NAD^+ by O2 via the respiratory chain?

❖Special shuttle systems carry reducing equivalents from cytosolic NADH into mitochondria by an indirect route.

❖The most active NADH shuttle, which functions in liver, kidney, and heart mitochondria, is the malate-aspartate shuttle.

❖The reducing equivalents of cytosolic NADH are first transferred to cytosolic oxaloacetate to yield malate, catalyzed by cytosolic malate dehydrogenase.

❖The malate thus formed passes through the inner membrane via the malate–α-ketoglutarate transporter.

❖Within the matrix the reducing equivalents are passed to NAD^+ by the action of matrix malate dehydrogenase, forming NADH; this NADH can pass electrons directly to the respiratory chain.

❖About 2.5 molecules of ATP are generated as this pair of electrons passes to O_2.

❖Cytosolic oxaloacetate must be regenerated by transamination reactions and the activity of membrane transporters to start another cycle of the shuttle.

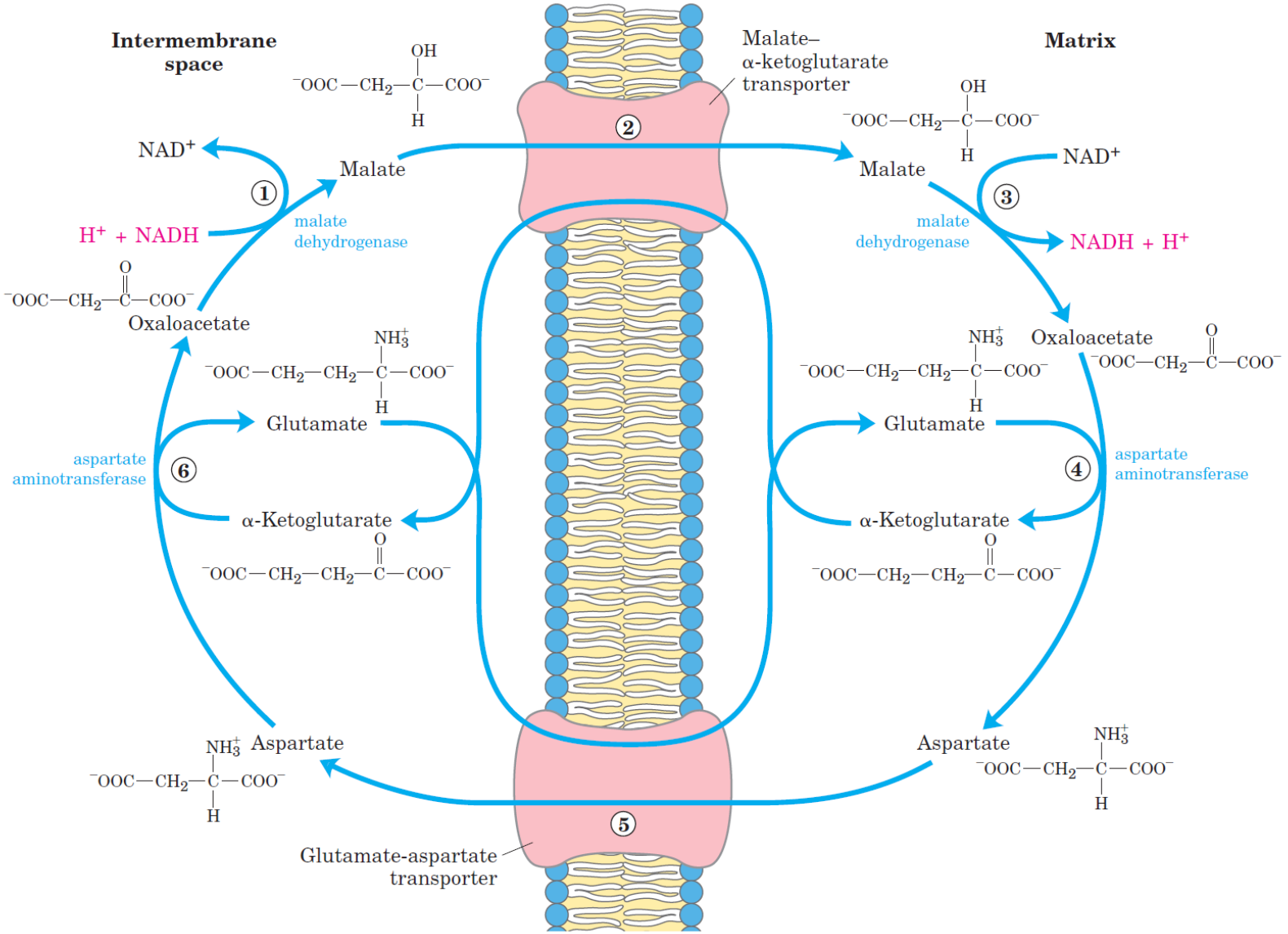
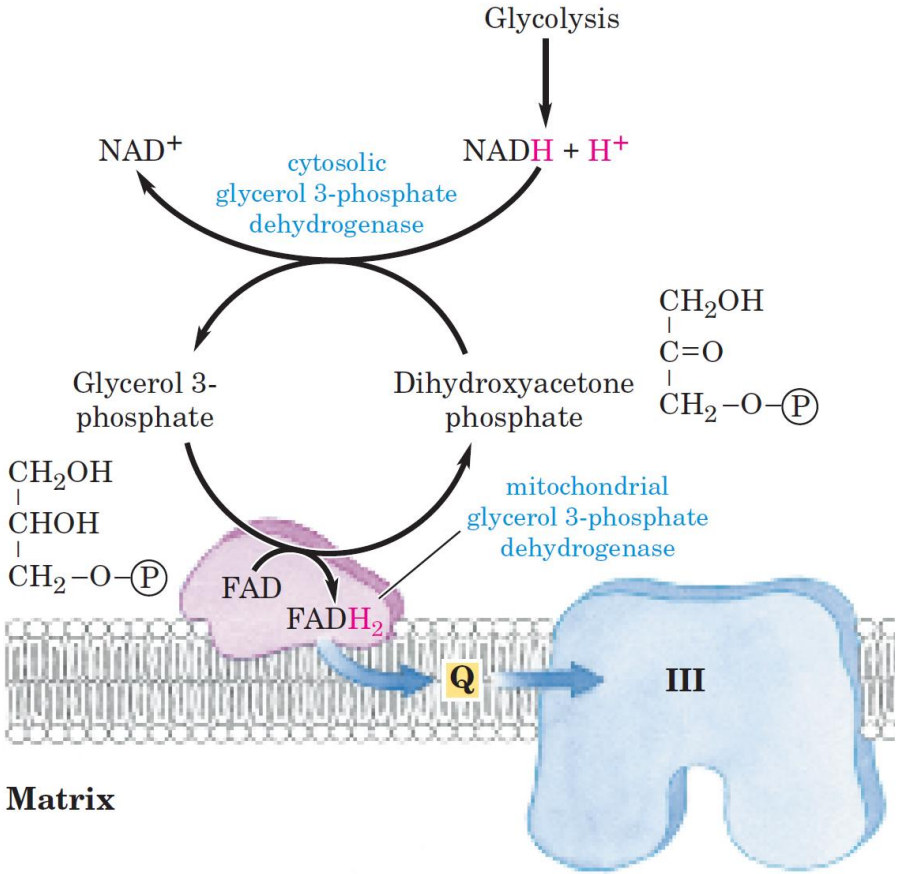
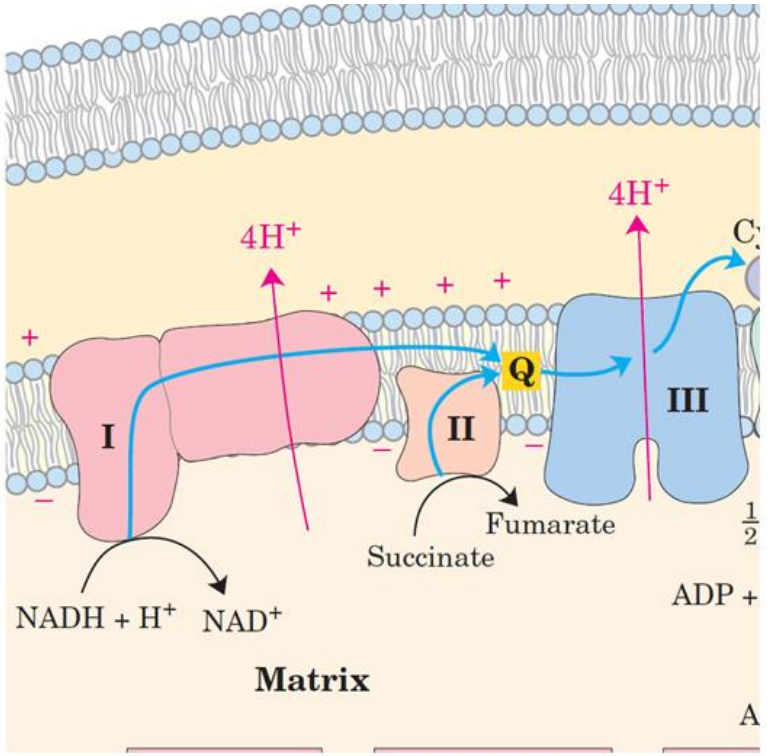


FIGURE 19–27 Malate-aspartate shuttle. This shuttle for transporting reducing equivalents from cytosolic NADH into the mitochondrial matrix is used in liver, kidney, and heart. (1) NADH in the cytosol (intermembrane space) passes two reducing equivalents to oxaloacetate, producing malate. (2) Malate crosses the inner membrane via the malate–α-ketoglutarate transporter. (3) In the matrix, malate passes two reducing equivalents to NAD^+, and the resulting NADH is oxidized by the respiratory chain. The oxaloacetate formed from malate cannot pass directly into the cytosol. (4) It is first transaminated to aspartate, which (5) can leave via the glutamate-aspartate transporter. (6) Oxaloacetate is regenerated in the cytosol, completing the cycle.

❖Skeletal muscle and brain use a different NADH shuttle, the glycerol 3-phosphate shuttle.

❖It differs from the malate-aspartate shuttle in that it delivers the reducing equivalents from NADH to ubiquinone and thus into Complex III, not Complex I, providing only enough energy to synthesize 1.5 ATP molecules per pair of electrons.





Regulation of Oxidative Phosphorylation

❖Complete oxidation of a molecule of glucose to CO2 yields 30 or 32 ATP

TABLE 19–5 ATP Yield from Complete Oxidation of Glucose

Process	*Direct product*	*Final ATP*
Glycolysis	2 NADH (cytosolic)	3 or 5*
	2 ATP	2
Pyruvate oxidation (two per glucose)	2 NADH (mitochondrial matrix)	5
Acetyl-CoA oxidation in citric acid cycle (two per glucose)	6 NADH (mitochondrial matrix)	15
	2 $FADH_2$	3
	2 ATP or 2 GTP	2
Total yield per glucose		30 or 32

*The number depends on which shuttle system transfers reducing equivalents into the mitochondrion.

❖By comparison, glycolysis under anaerobic conditions (lactate fermentation) yields only 2 ATP per glucose.

❖Aerobic oxidative pathways that result in electron transfer to O_2 accompanied by oxidative phosphorylation therefore account for the vast majority of the ATP produced in catabolism.

❖Therefore the regulation of ATP production by oxidative phosphorylation to match the cell's fluctuating needs for ATP is absolutely essential.

❖The rate of respiration (O2 consumption) in mitochondria is tightly regulated; it is generally limited by the availability of ADP as a substrate for phosphorylation.

❖The intracellular concentration of ADP is one measure of the energy status of cells.

❖Another, related measure is the mass-action ratio of the ATP-ADP system.

❖Usually this ratio is very high, so the ATP-ADP system is almost fully phosphorylated.

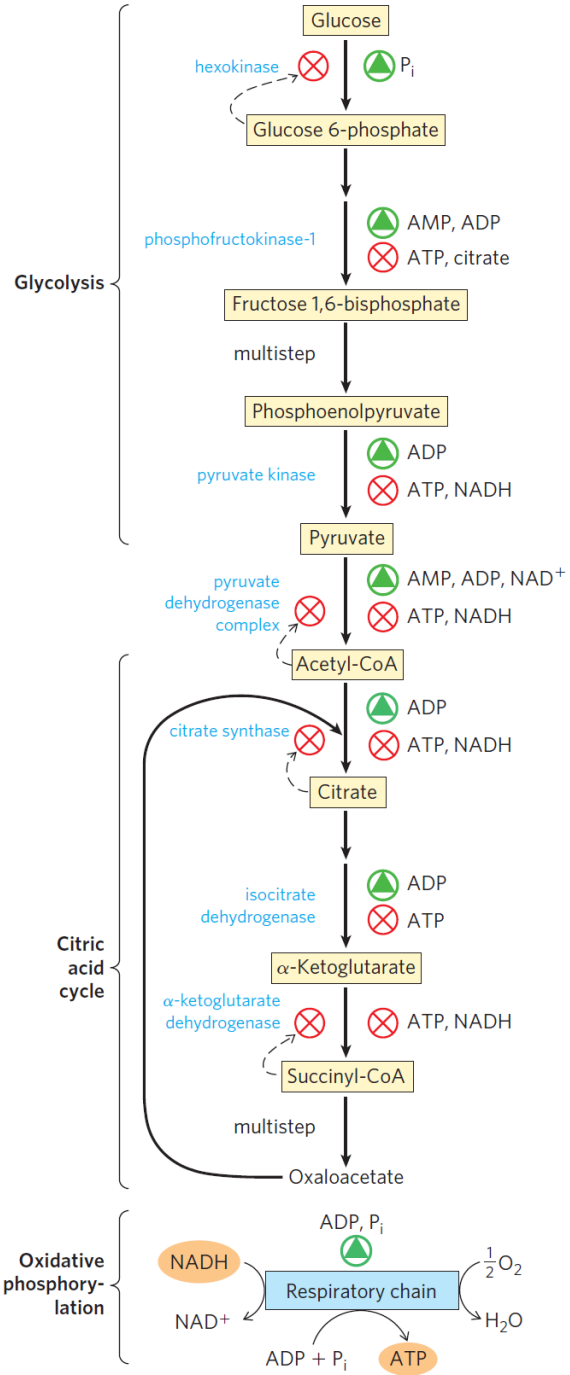
❖When the rate of some energy-requiring process (protein synthesis, for example) increases, the rate of breakdown of ATP to ADP and Pi increases, lowering the mass-action ratio.

❖With more ADP available for oxidative phosphorylation, the rate of respiration increases, causing regeneration of ATP.

❖This continues until the mass-action ratio returns to its normal high level, at which point respiration slows again.

❖The rate of oxidation of cellular fuels is regulated with such sensitivity and precision that the [ATP]/([ADP][Pi]) ratio fluctuates only slightly in most tissues, even during extreme variations in energy demand.

❖In short, ATP is formed only as fast as it is used in energy-requiring cellular activities.


Glucose
hexokinase
Pi
Glucose 6-phosphate
phosphofructokinase-1
AMP, ADP
ATP, citrate
Fructose 1,6-bisphosphate
multistep
Phosphoenolpyruvate
pyruvate kinase
ADP
ATP, NADH
Pyruvate
pyruvate dehydrogenase complex
AMP, ADP, NAD+
ATP, NADH
Acetyl-CoA
citrate synthase
ADP
ATP, NADH
Citrate
isocitrate dehydrogenase
ADP
ATP
α-Ketoglutarate
α-ketoglutarate dehydrogenase
ATP, NADH
Succinyl-CoA
multistep
Oxaloacetate
Glycolysis
Citric acid cycle
Oxidative phosphory-lation
ADP, Pi
NADH
Respiratory chain
½O2
H2O
NAD+
ADP + Pi
ATP

PHOTOSYNTHESIS

❖The capture of solar energy by photosynthetic organisms and its conversion to the chemical energy of reduced organic compounds is the ultimate source of nearly all biological energy on Earth.

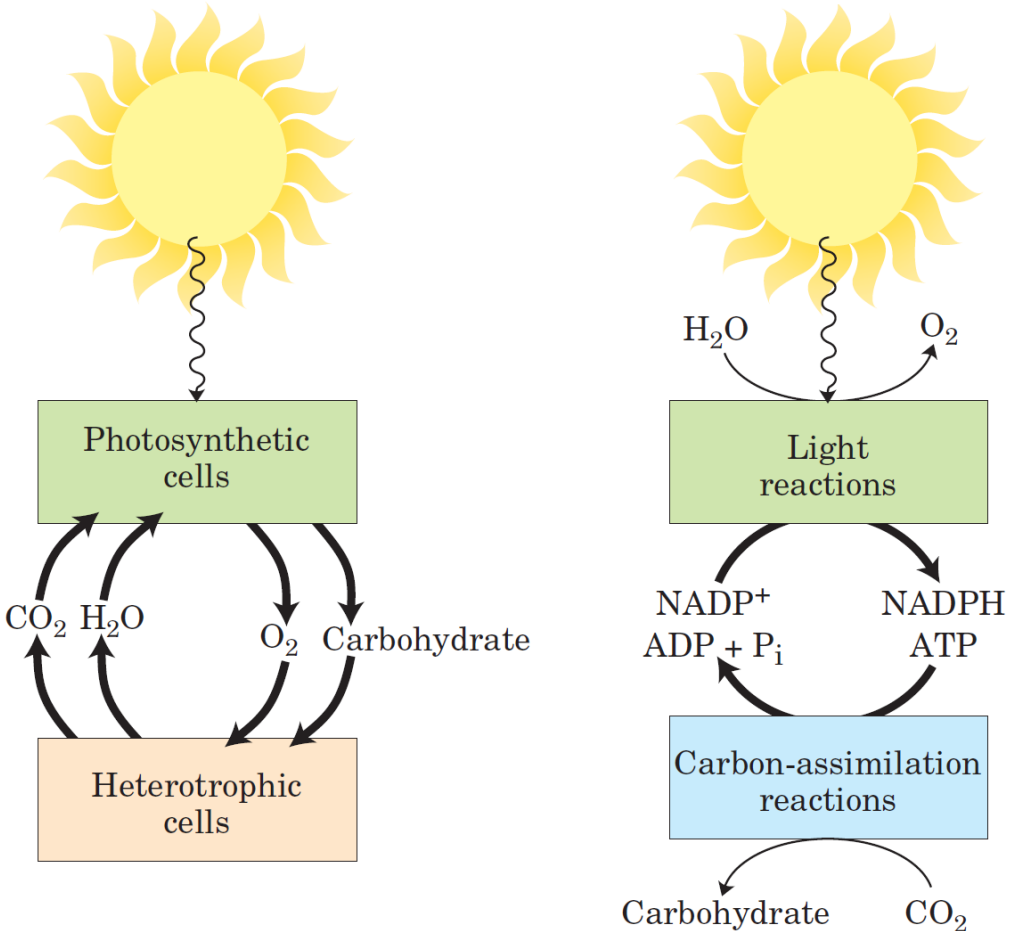
❖Photosynthetic and heterotrophic organisms live in a balanced steady state in the biosphere.

❖Photosynthetic organisms trap solar energy and form ATP and NADPH, which they use as energy sources to make carbohydrates and other organic compounds from CO_2 and H_2O; simultaneously, they release O_2 into the atmosphere.

❖Aerobic heterotrophs (humans, for example, as well as plants during dark periods) use the O_2 so formed to degrade the energy-rich organic products of photosynthesis to CO_2 and H_2O, generating ATP.

❖The CO_2 returns to the atmosphere, to be used again by photosynthetic organisms.

❖Solar energy thus provides the driving force for the continuous cycling of CO_2 and O_2 through the biosphere and provides the reduced substrates on which nonphotosynthetic organisms depend.



❖Photosynthesis occurs in a variety of bacteria and in unicellular eukaryotes (algae) as well as in plants.

❖Although the process in these organisms differs in detail, the underlying mechanisms are remarkably similar, and much of our understanding of photosynthesis in vascular plants is derived from studies of simpler organisms.

❖The overall equation for photosynthesis in plants describes an oxidation-reduction reaction in which H_2O donates electrons (as hydrogen) for the reduction of CO_2 to carbohydrate (CH_2O):

$$CO_2 + H_2O \xrightarrow{\text{light}} O_2 + (CH_2O)_n$$

❖Unlike NADH (the major electron donor in oxidative phosphorylation), H2O is a poor donor of electrons; its standard reduction potential is 0.816 V, compared with -0.320 V for NADH.

❖Photophosphorylation differs from oxidative phosphorylation in requiring the input of energy in the form of light to create a good electron donor and a good electron acceptor.

❖In photophosphorylation, electrons flow through a series of membrane-bound carriers including cytochromes, quinones, and iron-sulfur proteins, while protons are pumped across a membrane to create an electrochemical potential.

❖Electron transfer and proton pumping are catalyzed by membrane complexes homologous in structure and function to Complex III of mitochondria.

❖The electrochemical potential they produce is the driving force for ATP synthesis from ADP and Pi, catalyzed by a membrane-bound ATP synthase complex closely similar to that of mitochondria and bacteria.

❖Photosynthesis in plants encompasses two processes:

- the light-dependent reactions, or light reactions, which occur only when plants are illuminated,

- and the carbon-assimilation reactions (or carbon fixation reactions), sometimes misleadingly called the dark reactions, which are driven by products of the light reactions.

❖In the light reactions, chlorophyll and other pigments of photosynthetic cells absorb light energy and conserve it as ATP and NADPH; simultaneously, O_2 is evolved.

❖In the carbon-assimilation reactions, ATP and NADPH are used to reduce CO_2 to form triose phosphates, starch, and sucrose, and other products derived from them.

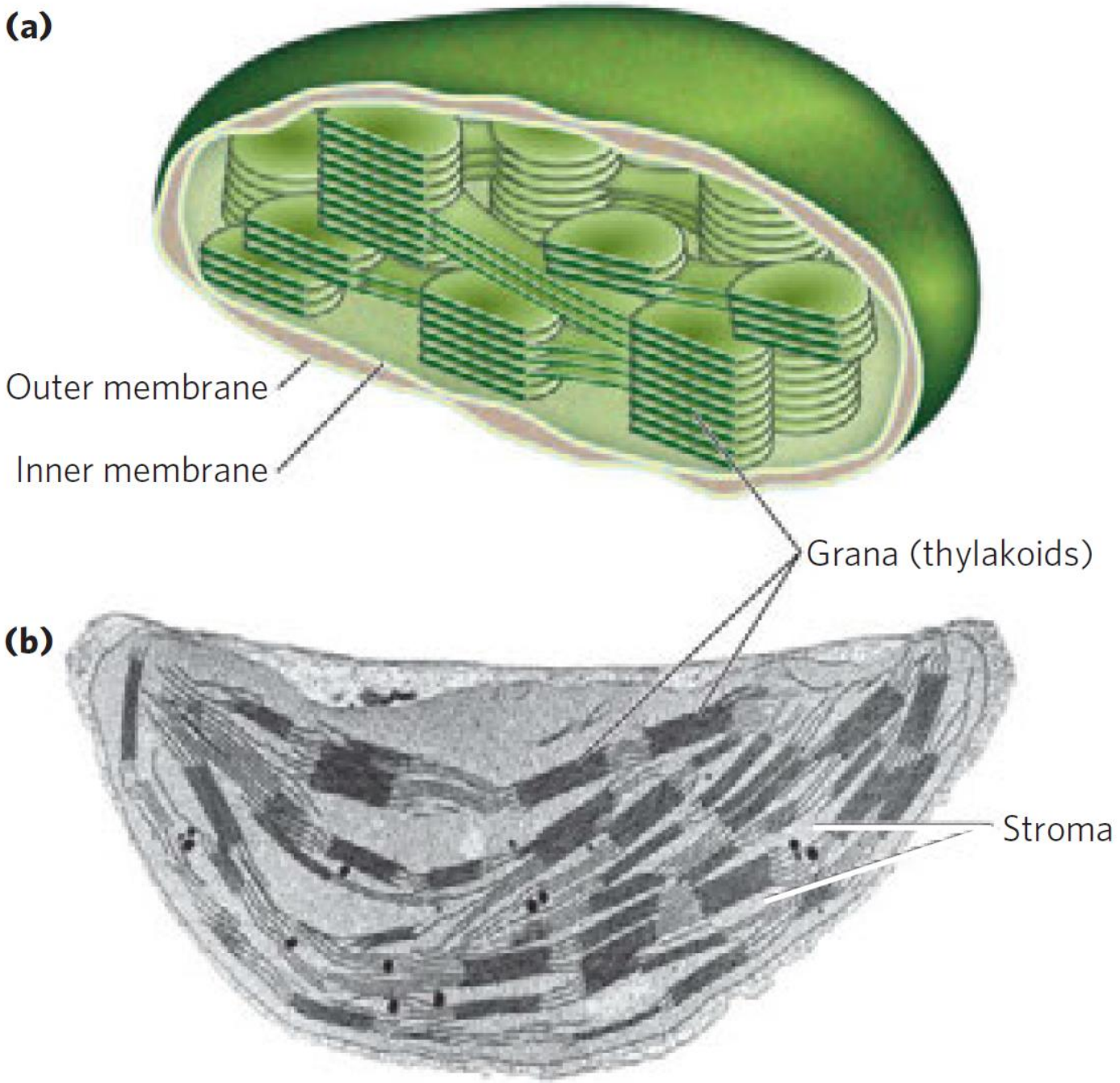
❖In photosynthetic eukaryotic cells, both the light dependent and the carbon-assimilation reactions take place in the chloroplasts.

❖Like mitochondria, they are surrounded by two membranes, an outer membrane that is permeable to small molecules and ions, and an inner membrane that encloses the internal compartment.

❖This compartment contains many flattened, membrane-surrounded vesicles or sacs, the thylakoids, usually arranged in stacks called grana.

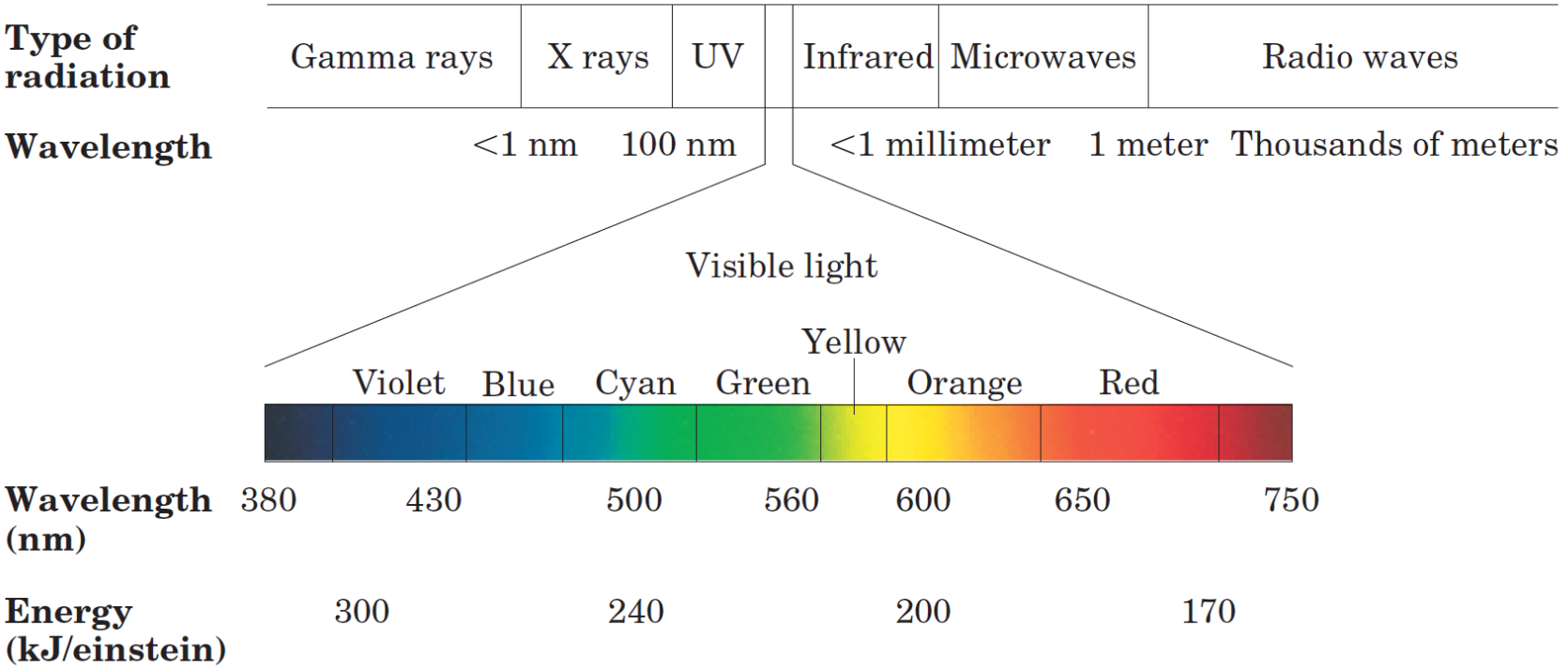
❖Embedded in the thylakoid membranes (commonly called lamellae) are the photosynthetic pigments and the enzyme complexes that carry out the light reactions and ATP synthesis.

❖The stroma (the aqueous phase enclosed by the inner membrane) contains most of the enzymes required for the carbon-assimilation reactions.


(a)
Outer membrane
Inner membrane
Grana (thylakoids)
(b)
Stroma

❖Visible light is electromagnetic radiation of wavelengths 400 to 700 nm, a small part of the electromagnetic spectrum, ranging from violet to red.

❖The energy of a single photon (a quantum of light) is greater at the violet end of the spectrum than at the red end; shorter wavelength (and higher frequency) corresponds to higher energy.



❖When a photon is absorbed, an electron in the absorbing molecule (chromophore) is lifted to a higher energy level.

❖This is an all-or-nothing event: to be absorbed, the photon must contain a quantity of energy (a quantum) that exactly matches the energy of the electronic transition.

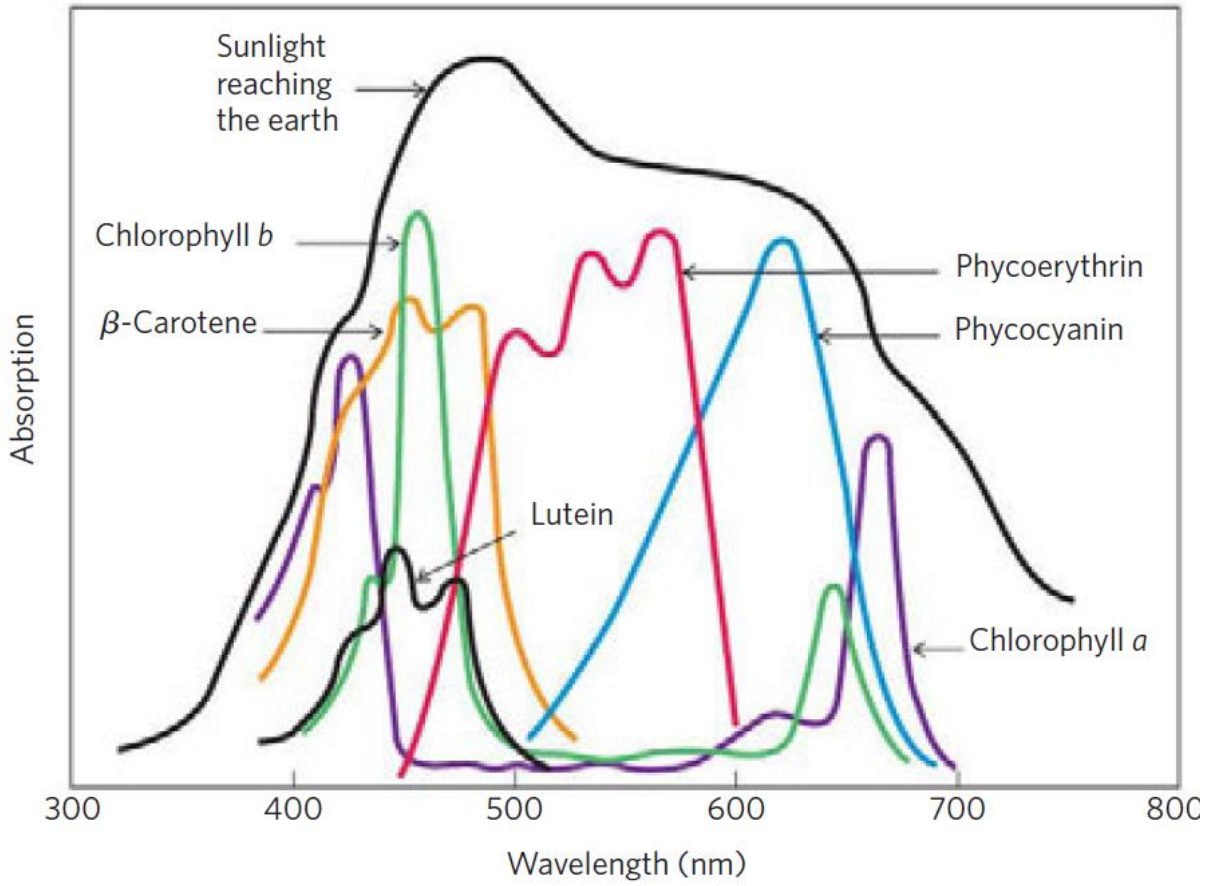
❖A molecule that has absorbed a photon is in an excited state, which is generally unstable.

❖An electron lifted into a higher-energy orbital usually returns rapidly to its lower-energy orbital; the excited molecule decays to the stable ground state, giving up the absorbed quantum as light or heat or using it to do chemical work.

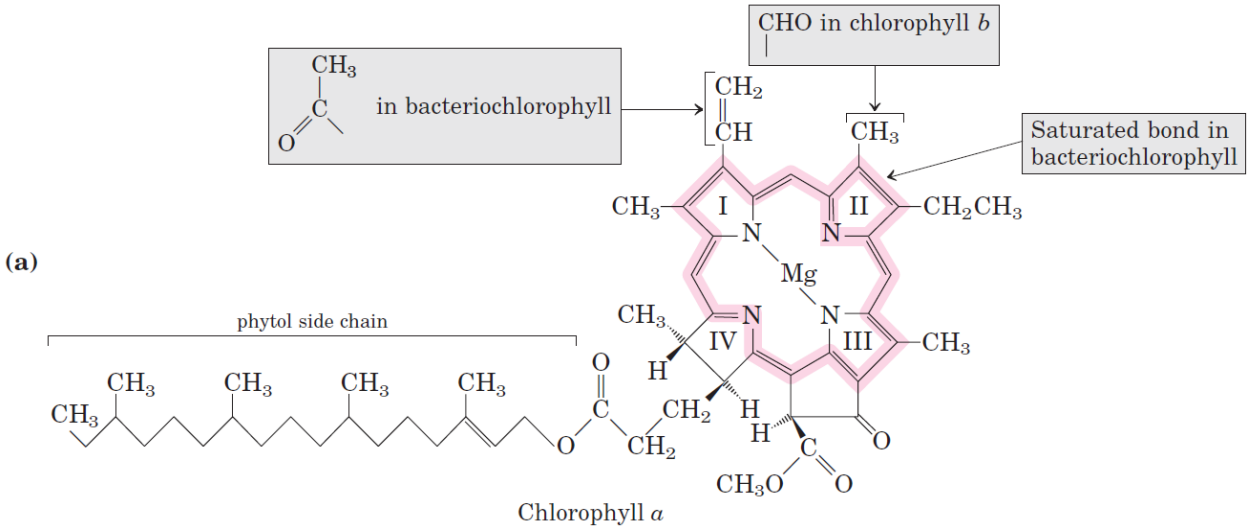
❖Light emission accompanying decay of excited molecules (called fluorescence) is always at a longer wavelength (lower energy) than that of the absorbed light.

❖An alternative mode of decay important in photosynthesis involves direct transfer of excitation energy from an excited molecule to a neighboring molecule.

❖Just as the photon is a quantum of light energy, so the exciton is a quantum of energy passed from an excited molecule to another molecule in a process called exciton transfer.

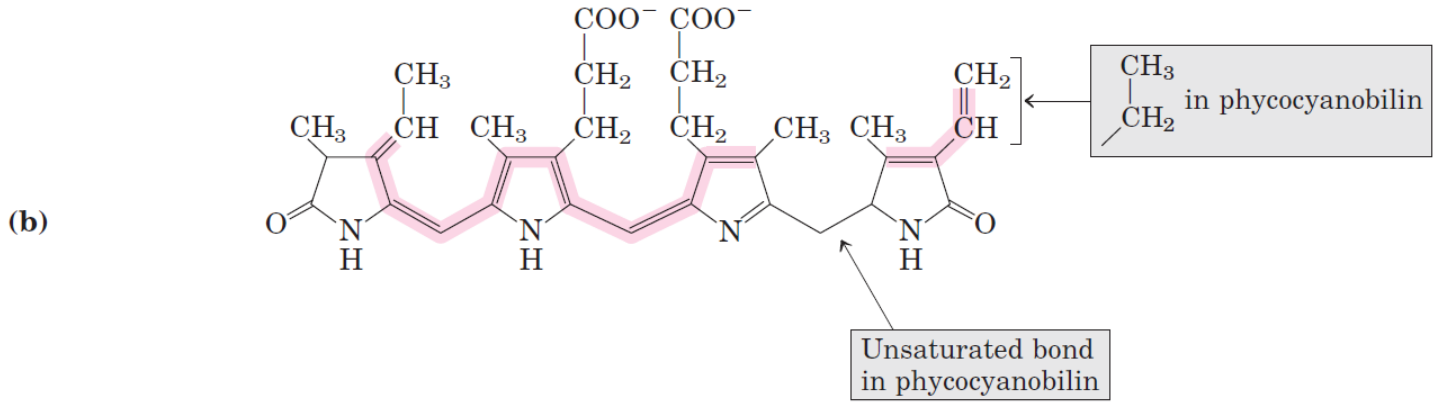


❖The most important light-absorbing pigments in the thylakoid membranes are the chlorophylls, green pigments with polycyclic, planar structures resembling the protoporphyrin of hemoglobin



Chlorophyll *a*

❖Cyanobacteria and red algae employ phycobilins such as phycoerythrobilin and phycocyanobilin as their light-harvesting pigments.

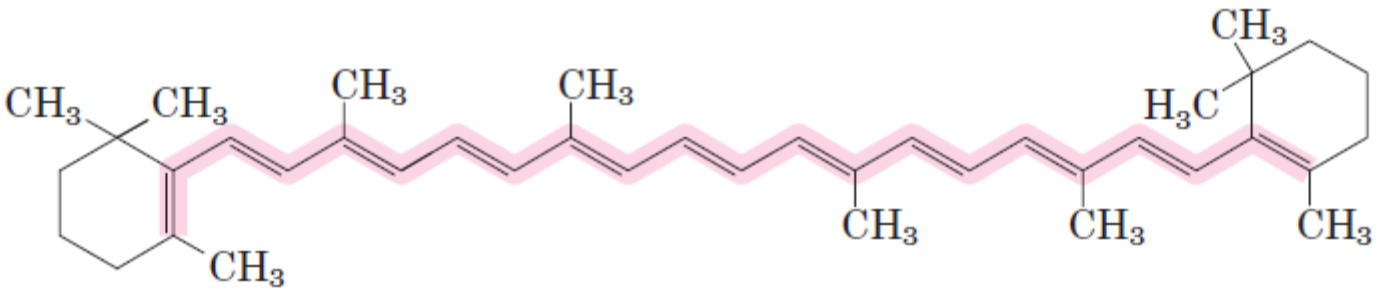


Phycoerythrobilin

❖In addition to chlorophylls, thylakoid membranes contain secondary light-absorbing pigments, or accessory pigments, called carotenoids.

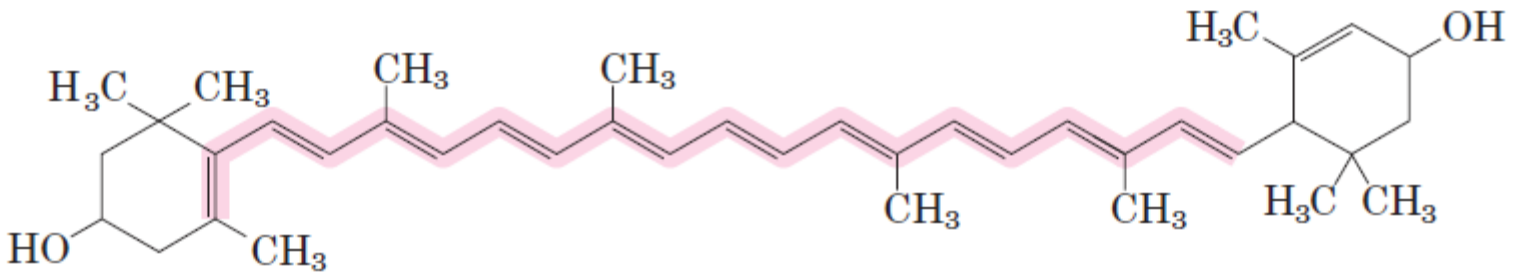
❖The carotenoid pigments absorb light at wavelengths not absorbed by the chlorophylls and thus are supplementary light receptors.

(c)



β-Carotene

(d)

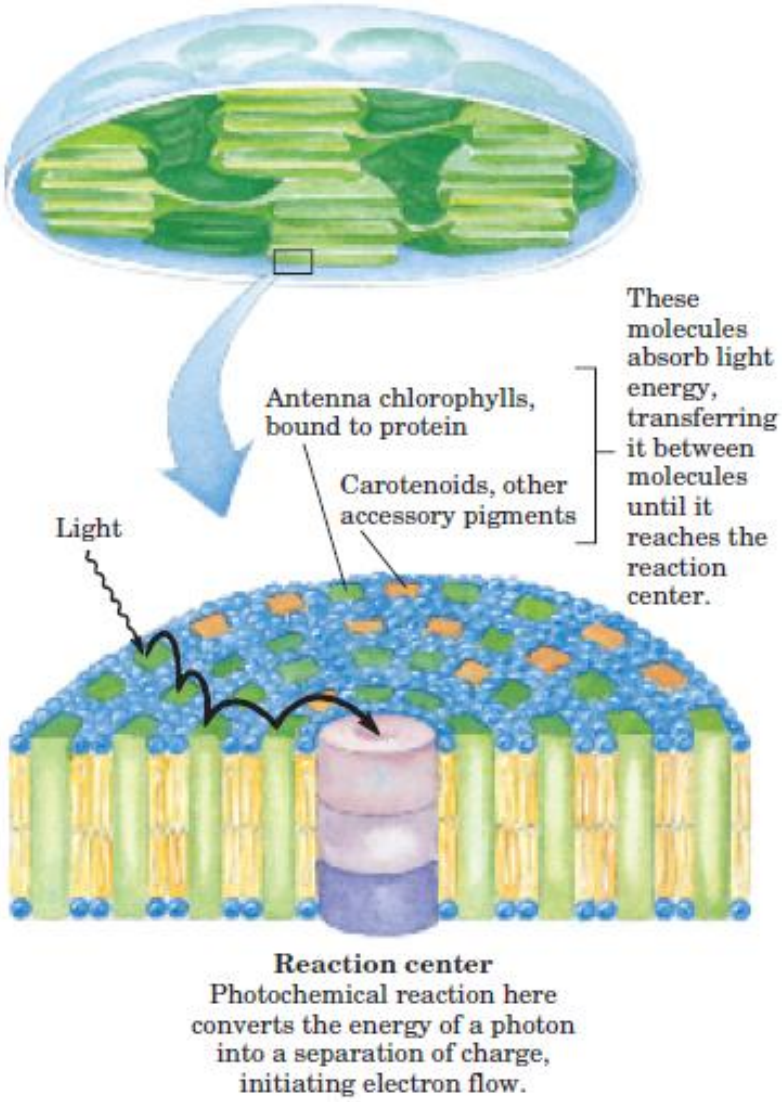


Lutein (xanthophyll)

❖The light-absorbing pigments of thylakoid or bacterial membranes are arranged in functional arrays called photosystems.

❖All the pigment molecules in a photosystem can absorb photons, but only a few chlorophyll molecules associated with the photochemical reaction center are specialized to transduce light into chemical energy.

❖The other pigment molecules in a photosystem are called light-harvesting or antenna molecules and they absorb light energy and transmit it rapidly and efficiently to the reaction center



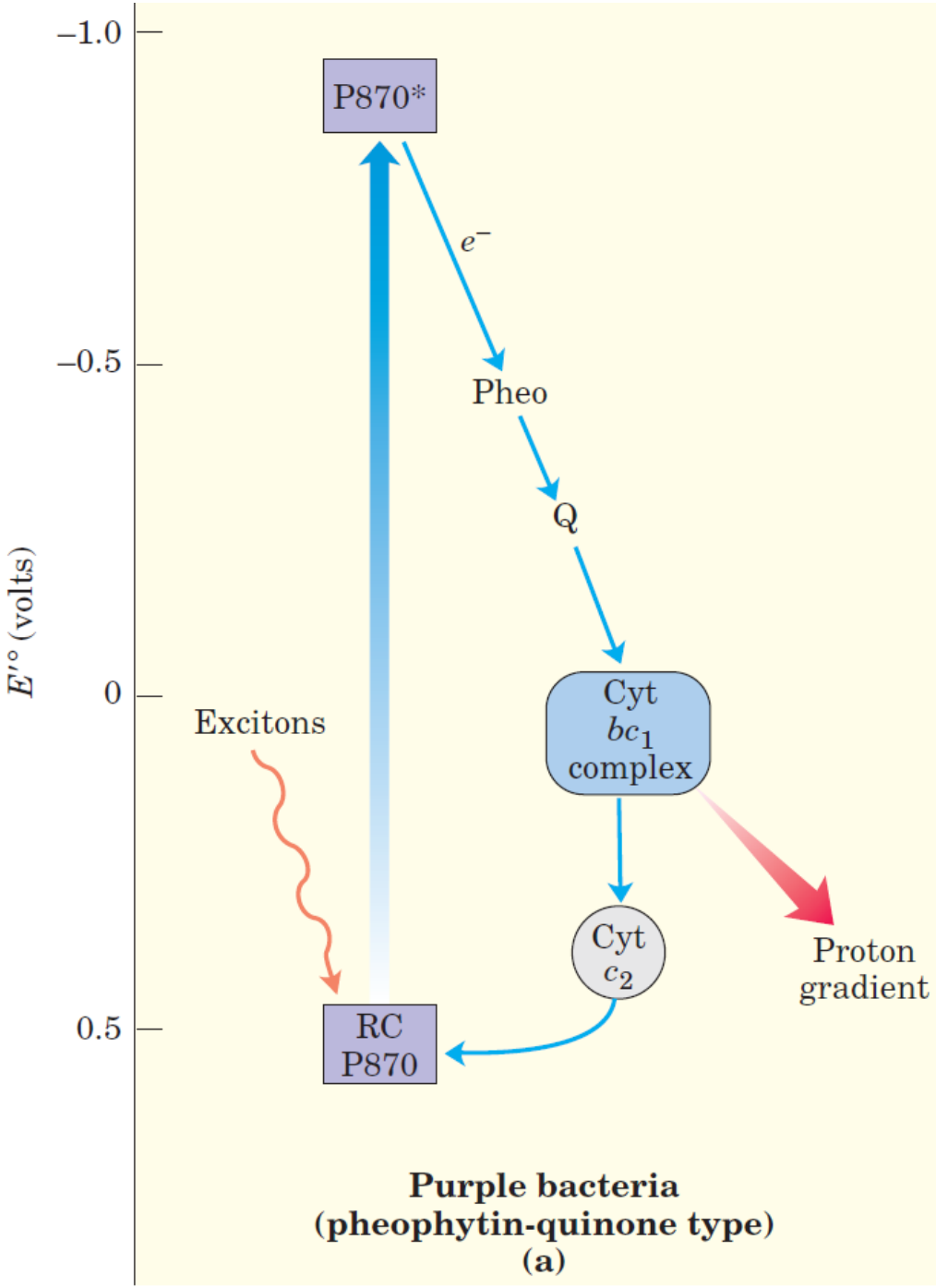
❖The photosynthetic machinery in purple bacteria consists of three basic modules:

- a single reaction center (P870),
- a cytochrome bc1 electron-transfer complex similar to Complex III of the mitochondrial electron-transfer chain,
- and an ATP synthase, also similar to that of mitochondria.

❖Illumination drives electrons through pheophytin and a quinone to the cytochrome bc1 complex; after passing through the complex, electrons flow through cytochrome c2 back to the reaction center, restoring its pre-illumination state.

❖This light-driven cyclic flow of electrons provides the energy for proton pumping by the cytochrome bc1 complex.

❖Powered by the resulting proton gradient, ATP synthase produces ATP, exactly as in mitochondria.


–1.0
–0.5
0
0.5
E'° (volts)
P870*
e⁻
Pheo
Q
Cyt
bc1
complex
Excitons
Cyt
c2
Proton
gradient
RC
P870
Purple bacteria
(pheophytin-quinone type)
(a)

❖Photosynthesis in green sulfur bacteria involves the same three modules as in purple bacteria, but the process differs in several respects and involves additional enzymatic reactions.

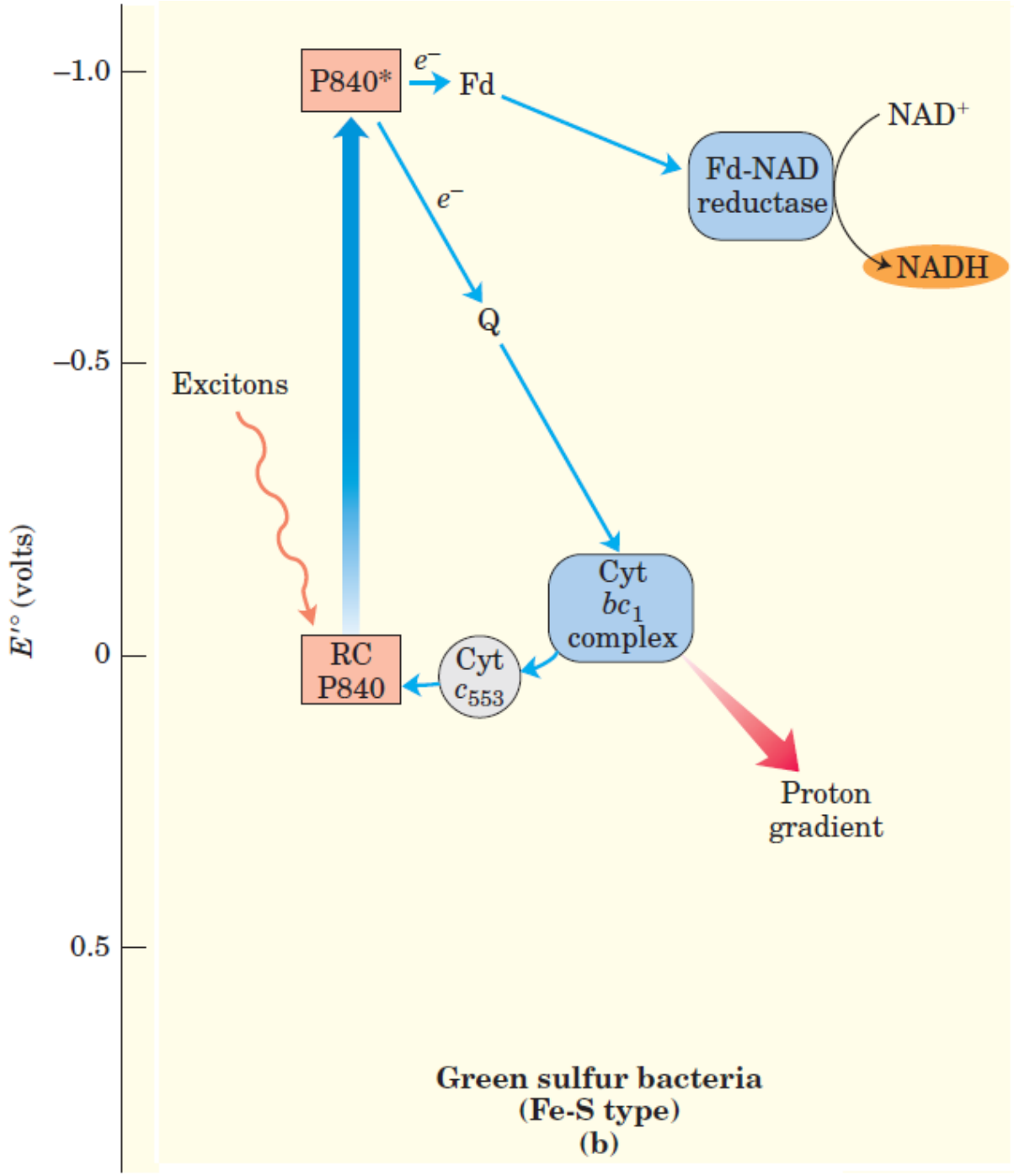
❖Excitation causes an electron to move from the reaction center to the cytochrome bc1 complex via a quinone carrier.

❖Electron transfer through this complex used for ATP synthesis.

❖However, in contrast to the cyclic flow of electrons in purple bacteria, some electrons flow from the reaction center to an iron-sulfur protein, ferredoxin, which then passes electrons via ferredoxin:NAD reductase to NAD^+, producing NADH.

❖The electrons taken from the reaction center to reduce NAD^+ are replaced by the oxidation of H_2S to elemental S, then to SO_4^{2-}, in the reaction that defines the green sulfur bacteria.

❖This oxidation of H_2S by bacteria is chemically analogous to the oxidation of H_2O by oxygenic plants.


–1.0
–0.5
0
0.5
E'° (volts)
P840*
e⁻
Fd
NAD⁺
Fd-NAD reductase
NADH
e⁻
Q
Excitons
Cyt bc1 complex
RC P840
Cyt c553
Proton gradient
Green sulfur bacteria (Fe-S type) (b)

❖The photosynthetic apparatus of modern cyanobacteria, algae, and vascular plants is more complex than the one-center bacterial systems, and it seems to have evolved through the combination of two simpler bacterial photocenters.

❖The thylakoid membranes of chloroplasts have two different kinds of photosystems, each with its own type of photochemical reaction center and set of antenna molecules.

❖The two systems have distinct and complementary functions.

❖Photosystem II (PSII) is a pheophytin-quinone type of system (like the single photosystem of purple bacteria) containing roughly equal amounts of chlorophylls a and b.

❖Excitation of its reaction-center P680 drives electrons through the cytochrome b6 f complex with concomitant movement of protons across the thylakoid membrane.

❖Photosystem I (PSI) is structurally and functionally related to the type I reaction center of green sulfur bacteria. It has a reaction center designated P700 and a high ratio of chlorophyll a to chlorophyll b.

❖Excited P700 passes electrons to the Fe-S protein ferredoxin, then to $NADP^+$, producing NADPH.

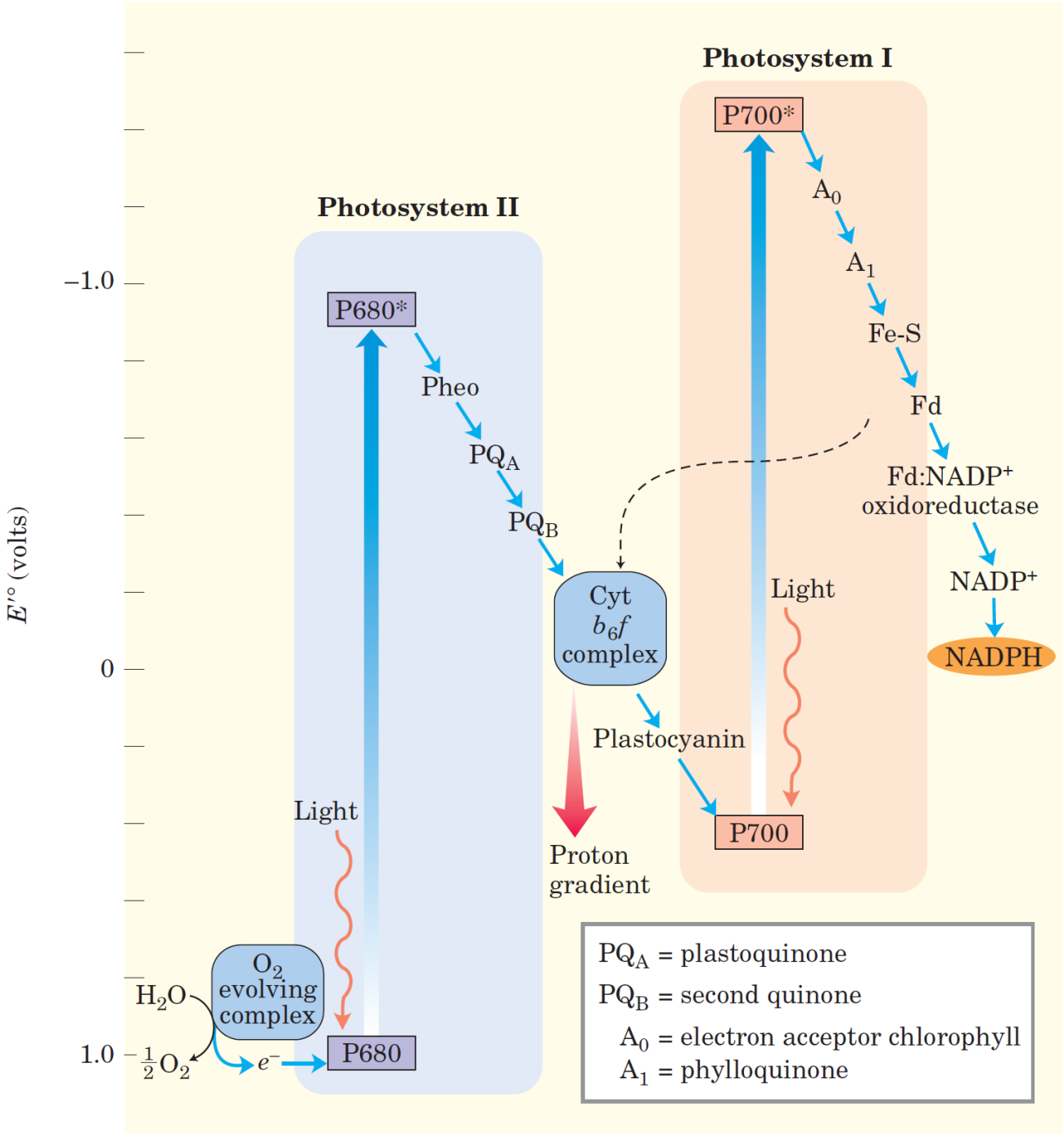
❖These two reaction centers in plants act in tandem to catalyze the light-driven movement of electrons from H_2O to $NADP^+$.

❖Electrons are carried between the two photosystems by the soluble protein plastocyanin, a one-electron carrier functionally similar to cytochrome c of mitochondria.

❖To replace the electrons that move from PSII through PSI to $NADP^+$, cyanobacteria and plants oxidize H_2O (as green sulfur bacteria oxidize H2S), producing O_2.

❖This process is called oxygenic photosynthesis to distinguish it from the anoxygenic photosynthesis of purple and green sulfur bacteria.

❖All O_2-evolving photosynthetic cells—those of plants, algae, and cyanobacteria— contain both PSI and PSII; organisms with only one photosystem do not evolve O_2.


Photosystem I
Photosystem II
P700*
A0
A1
Fe-S
Fd
Fd:NADP+ oxidoreductase
NADP+
NADPH
P680*
Pheo
PQA
PQB
Cyt b6f complex
Light
Plastocyanin
P700
Proton gradient
Light
O2 evolving complex
H2O
½ O2
e−
P680
E'° (volts)
−1.0
0
1.0
PQA = plastoquinone
PQB = second quinone
A0 = electron acceptor chlorophyll
A1 = phylloquinone

❖This diagram, often called the Z scheme because of its overall form, outlines the pathway of electron flow between the two photosystems and the energy relationships in the light reactions.

❖The Z scheme thus describes the complete route by which electrons flow from H2O to $NADP^+$, according to the equation.

$$2H_2O + 2NADP^+ + 8 \text{ photons} \longrightarrow O_2 + 2NADPH + 2H^+$$

❖For every two photons absorbed (one by each photosystem), one electron is transferred from H2O to $NADP^+$.

❖To form one molecule of O_2, which requires transfer of four electrons from two H_2O to two $NADP^+$, a total of eight photons must be absorbed, four by each photosystem.

ATP Synthesis by Photophosphorylation

❖The combined activities of the two plant photosystems move electrons from water to NADP+, conserving some of the energy of absorbed light as NADPH.

❖Simultaneously, protons are pumped across the thylakoid thylakoid membrane and energy is conserved as an electrochemical potential.

❖This proton gradient drives the synthesis of ATP, the other energy conserving product of the light-dependent reactions.

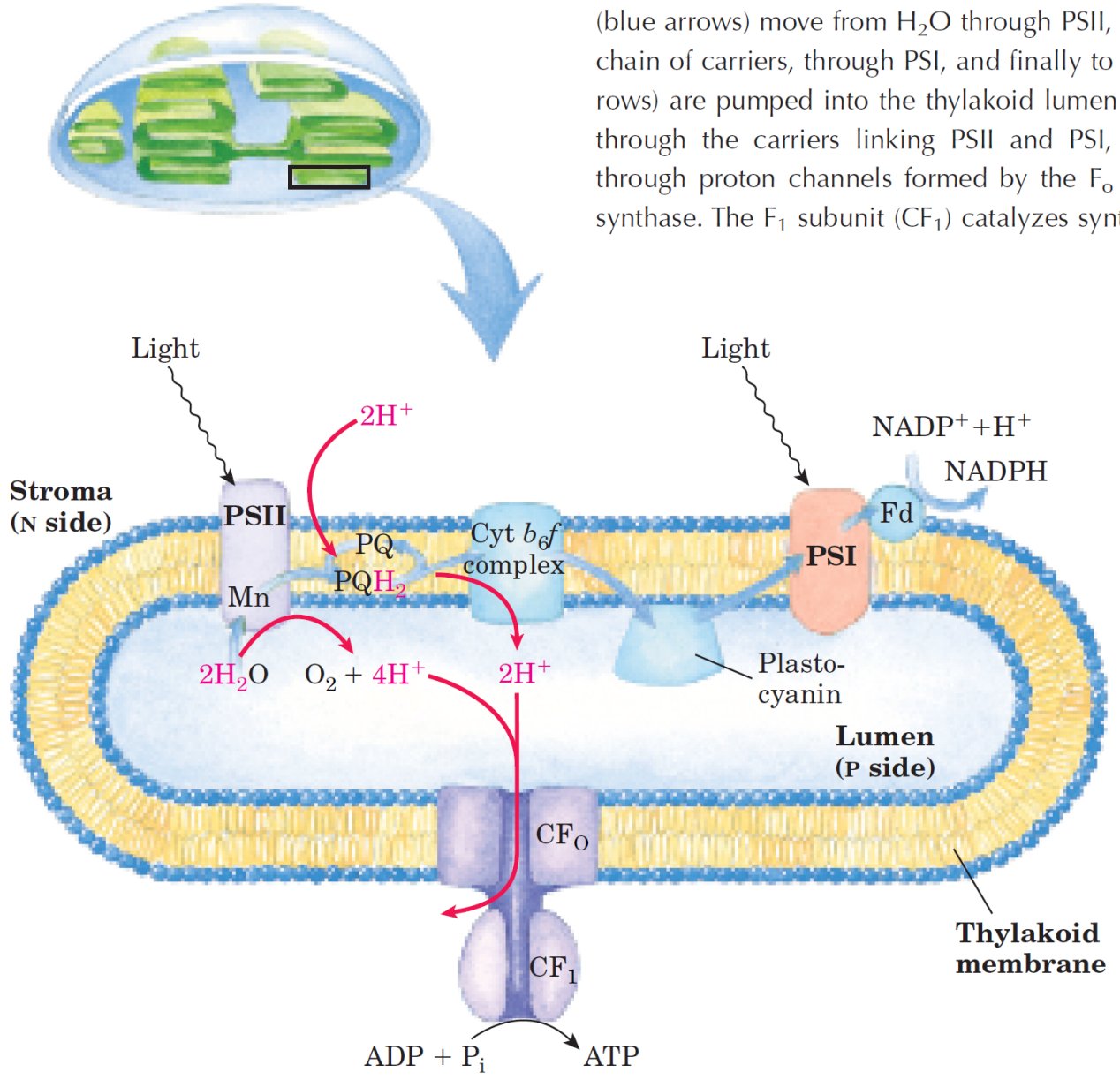


FIGURE 19–57 Proton and electron circuits in thylakoids. Electrons (blue arrows) move from H_2O through PSII, through the intermediate chain of carriers, through PSI, and finally to $NADP^+$. Protons (red arrows) are pumped into the thylakoid lumen by the flow of electrons through the carriers linking PSII and PSI, and reenter the stroma through proton channels formed by the F_o (designated CF_o) of ATP synthase. The F_1 subunit (CF_1) catalyzes synthesis of ATP.

Carbohydrate Biosynthesis in Plants and Bacteria

❖The synthesis of carbohydrates in animal cells always employs precursors having at least three carbons, all of which are less oxidized than the carbon in CO2.

❖Plants and photosynthetic microorganisms, by contrast, can synthesize carbohydrates from CO2 and water, reducing CO2 at the expense of the energy and reducing power furnished by the ATP and NADPH that are generated by the light-dependent reactions of photosynthesis.

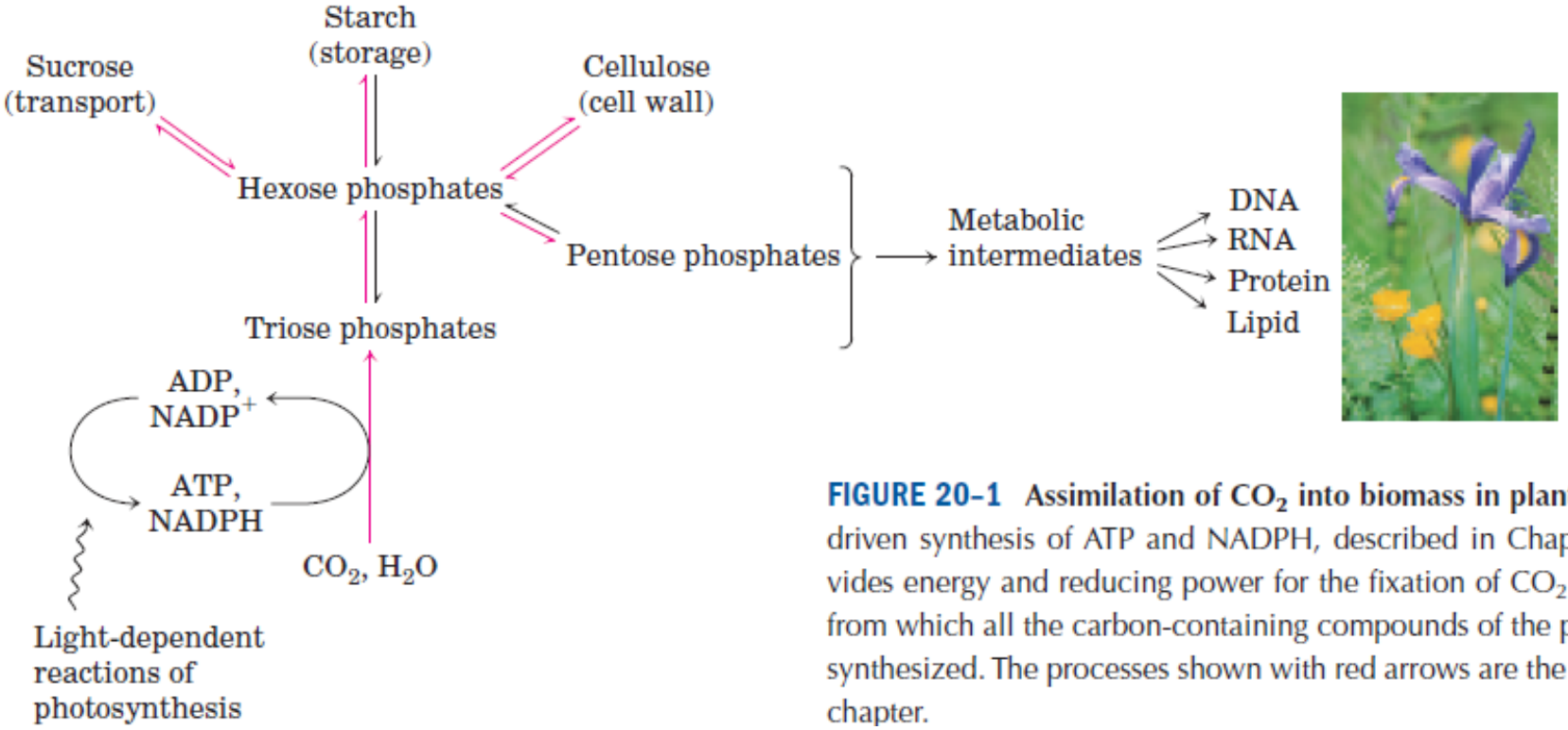


FIGURE 20–1 Assimilation of CO_2 into biomass in plants. The light-driven synthesis of ATP and NADPH, described in Chapter 19, provides energy and reducing power for the fixation of CO_2 into trioses, from which all the carbon-containing compounds of the plant cell are synthesized. The processes shown with red arrows are the focus of this chapter.

❖Most of the biosynthetic activities in plants (including CO_2 assimilation) occur in plastids, a family of self-reproducing organelles bounded by a double membrane and containing a small genome that encodes some of their proteins.

❖Chloroplasts are the sites of CO_2 assimilation.

❖The first stage in the assimilation of CO_2 into biomolecules is the carbon-fixation reaction: condensation of CO_2 with a five-carbon acceptor, ribulose 1,5-bisphosphate, to form two molecules of 3-phosphoglycerate.

❖In the second stage, the 3-phosphoglycerate is reduced to triose phosphates.

❖Overall, three molecules of CO_2 are fixed to three molecules of ribulose 1,5-bisphosphate to form six molecules of glyceraldehyde 3-phosphate (18 carbons) in equilibrium with dihydroxyacetone phosphate.

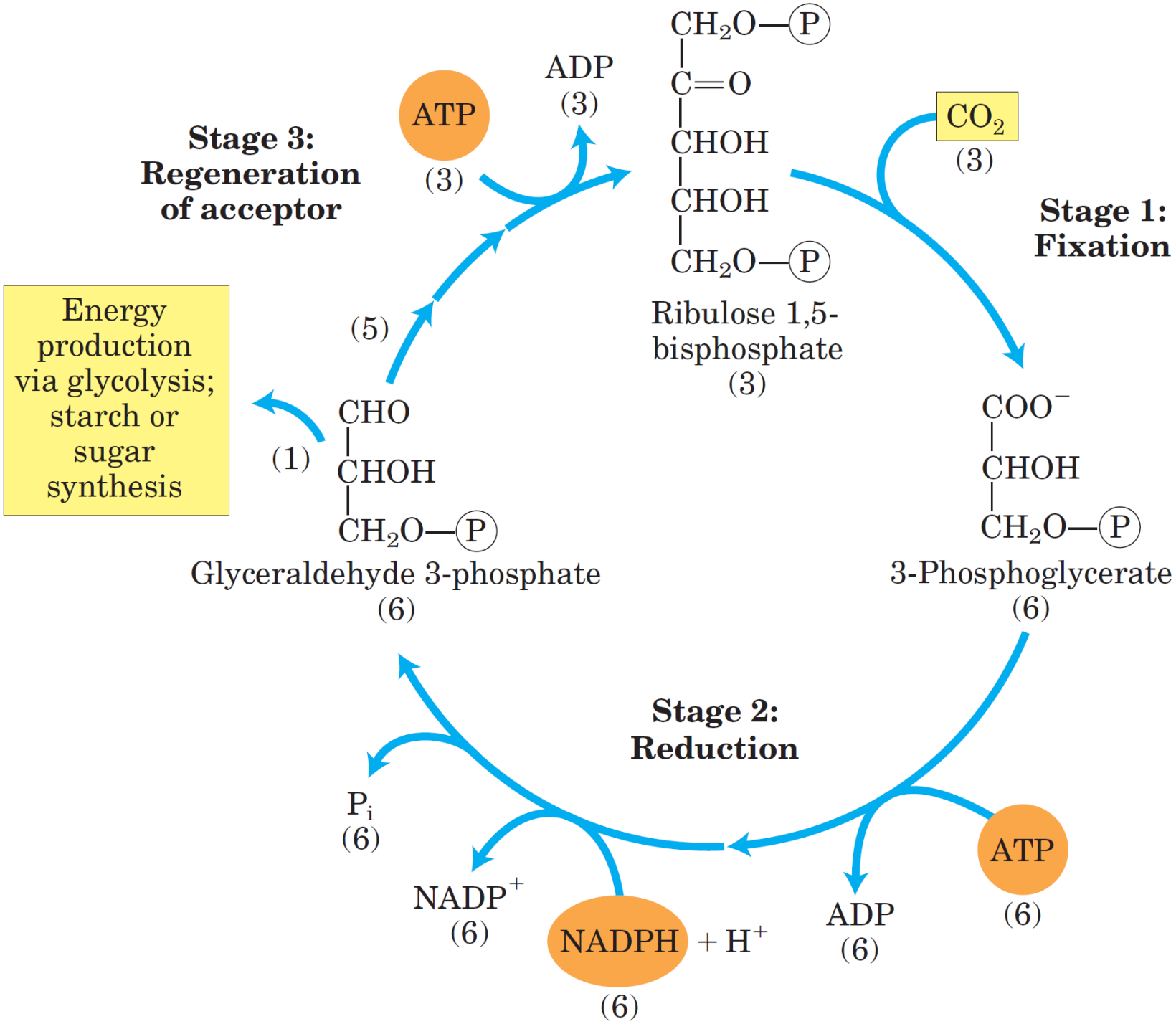
❖In the third stage, five of the six molecules of triose phosphate (15 carbons) are used to regenerate three molecules of ribulose 1,5-bisphosphate (15 carbons), the starting material.

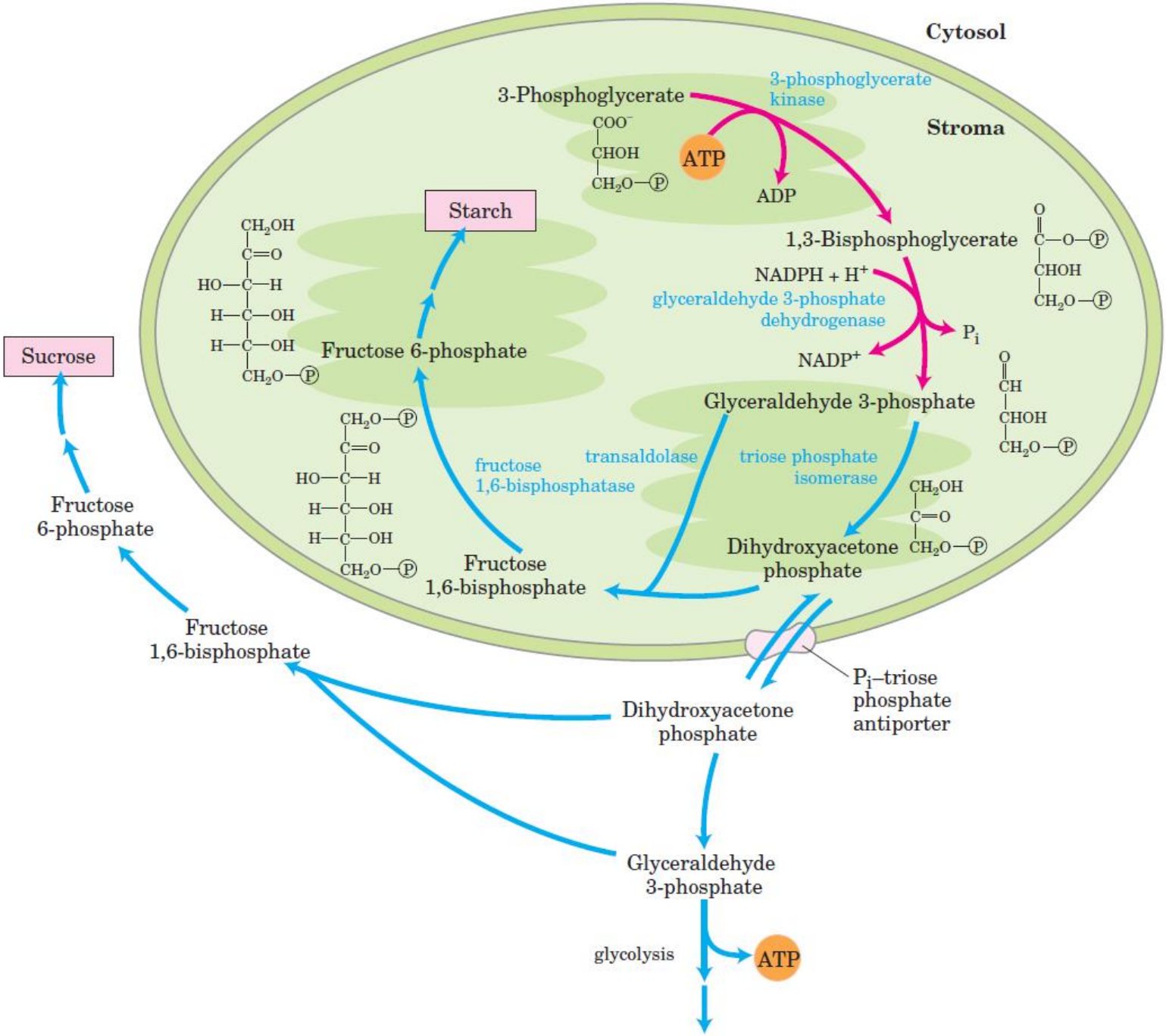
❖The sixth molecule of triose phosphate, the net product of photosynthesis, can be used to make hexoses for fuel and building materials, sucrose for transport to non-photosynthetic tissues, or starch for storage.

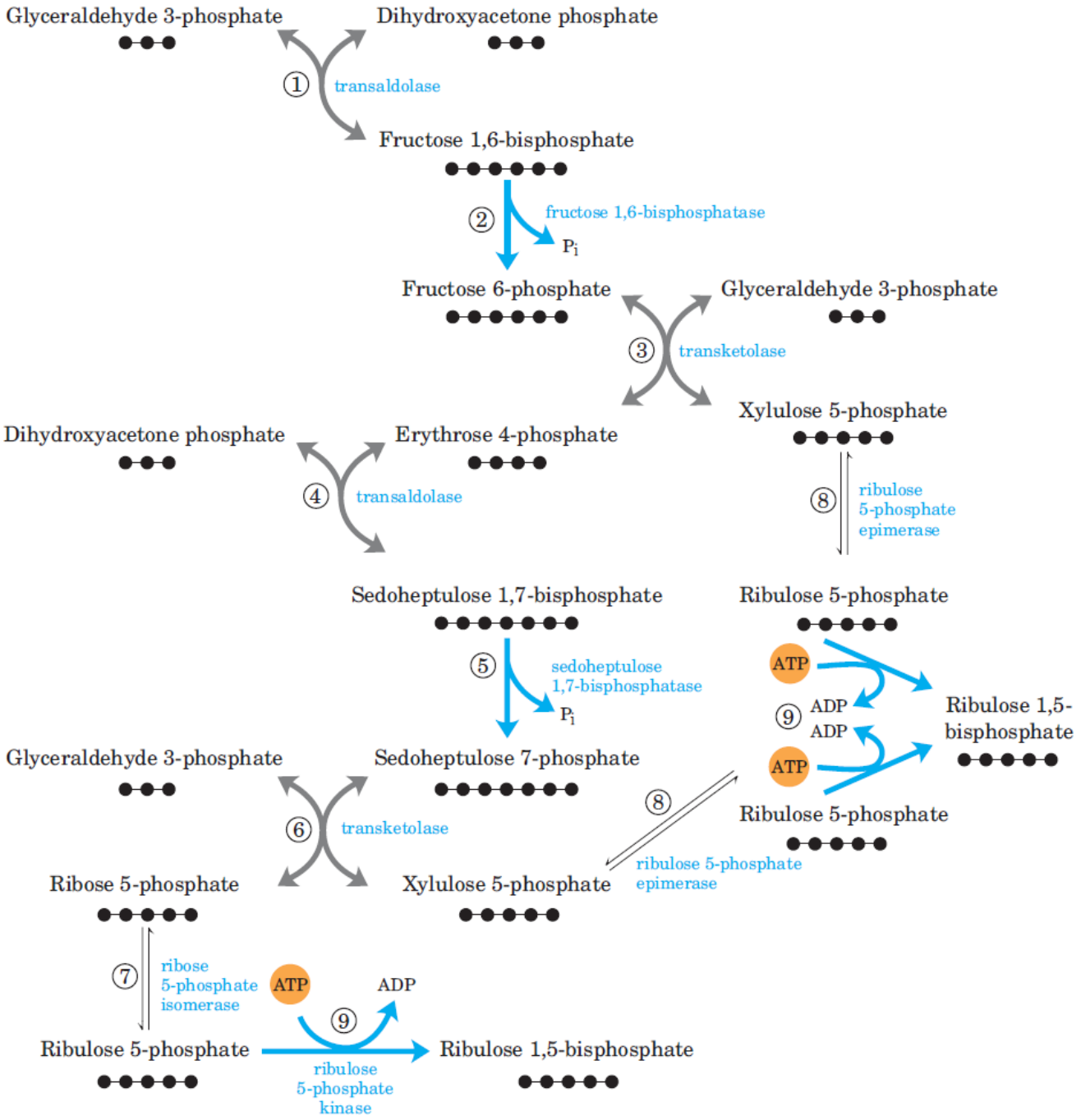
❖Thus the overall process is cyclical, with the continuous conversion of CO_2 to triose and hexose phosphates.

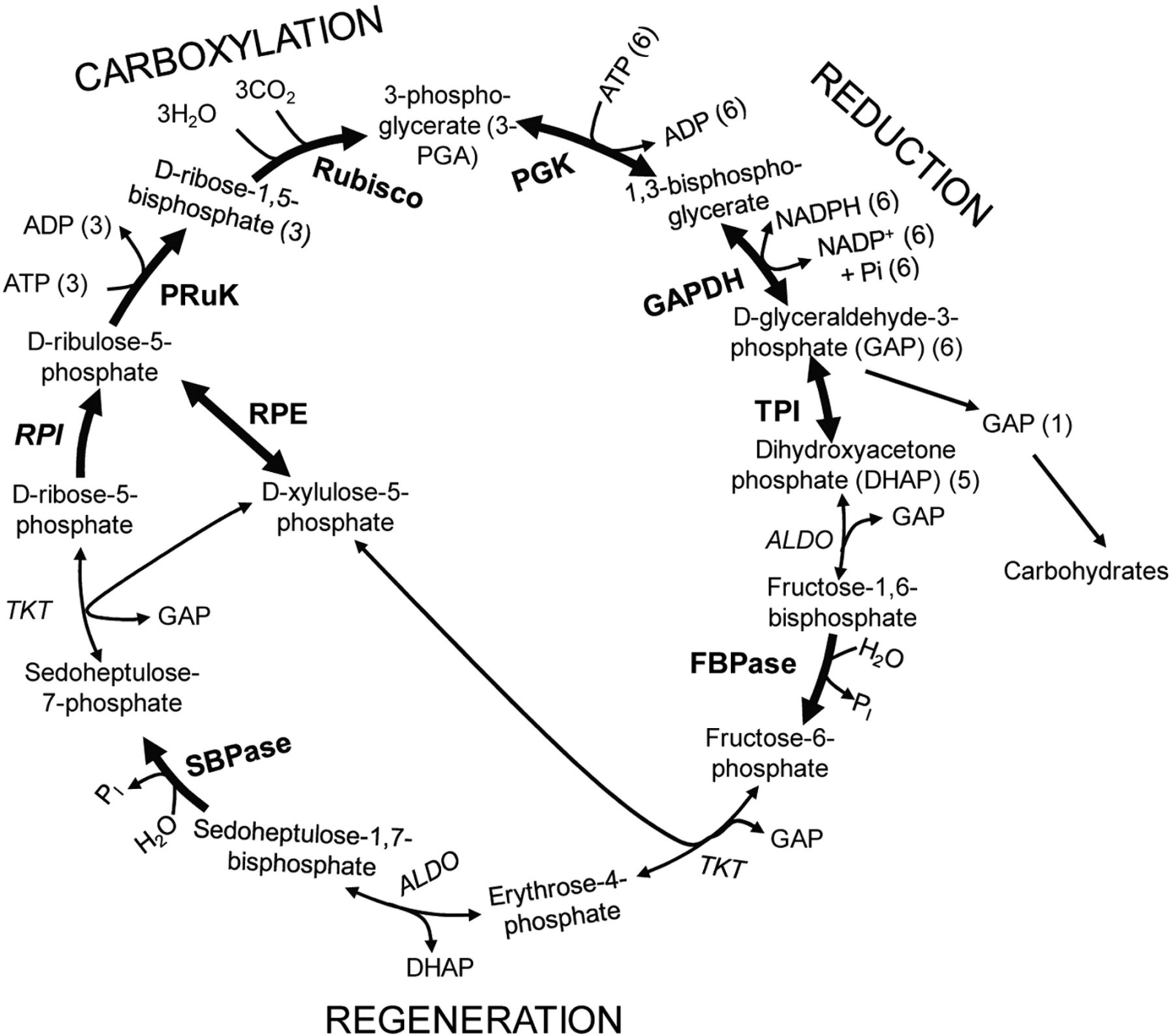
❖Fructose 6-phosphate is a key intermediate in stage 3 of CO_2 assimilation; it stands at a branch point, leading either to regeneration of ribulose 1,5-bisphosphate or to synthesis of starch.

❖The pathway from hexose phosphate to pentose bisphosphate involves many of the same reactions used in animal cells for the conversion of pentose phosphates to hexose phosphates during the nonoxidative phase of the pentose phosphate pathway


Stage 3:
Regeneration
of acceptor
ATP
(3)
ADP
(3)
CH2O—P
C=O
CHOH
CHOH
CH2O—P
Ribulose 1,5-
bisphosphate
(3)
CO2
(3)
Stage 1:
Fixation
Energy
production
via glycolysis;
starch or
sugar
synthesis
(5)
(1)
CHO
CHOH
CH2O—P
Glyceraldehyde 3-phosphate
(6)
COO−
CHOH
CH2O—P
3-Phosphoglycerate
(6)
Stage 2:
Reduction
Pi
(6)
NADP+
(6)
NADPH + H+
(6)
ADP
(6)
ATP
(6)


Cytosol
Stroma
3-Phosphoglycerate
3-phosphoglycerate kinase
ATP
ADP
1,3-Bisphosphoglycerate
NADPH + H+
glyceraldehyde 3-phosphate dehydrogenase
Pi
NADP+
Glyceraldehyde 3-phosphate
transaldolase
triose phosphate isomerase
Dihydroxyacetone phosphate
fructose 1,6-bisphosphatase
Fructose 1,6-bisphosphate
Fructose 6-phosphate
Starch
Sucrose
Fructose 6-phosphate
Fructose 1,6-bisphosphate
Dihydroxyacetone phosphate
Pi–triose phosphate antiporter
Glyceraldehyde 3-phosphate
glycolysis
ATP


Glyceraldehyde 3-phosphate
Dihydroxyacetone phosphate
① transaldolase
Fructose 1,6-bisphosphate
② fructose 1,6-bisphosphatase
Pi
Fructose 6-phosphate
Glyceraldehyde 3-phosphate
③ transketolase
Xylulose 5-phosphate
Dihydroxyacetone phosphate
Erythrose 4-phosphate
④ transaldolase
⑧ ribulose 5-phosphate epimerase
Sedoheptulose 1,7-bisphosphate
Ribulose 5-phosphate
⑤ sedoheptulose 1,7-bisphosphatase
Pi
ATP
⑨ ADP
ADP
ATP
Ribulose 1,5-bisphosphate
Glyceraldehyde 3-phosphate
Sedoheptulose 7-phosphate
⑥ transketolase
⑧
Ribulose 5-phosphate
ribulose 5-phosphate epimerase
Ribose 5-phosphate
Xylulose 5-phosphate
⑦ ribose 5-phosphate isomerase
ATP
ADP
⑨
Ribulose 5-phosphate
Ribulose 1,5-bisphosphate
ribulose 5-phosphate kinase


CARBOXYLATION
REDUCTION
REGENERATION
3CO2
3H2O
Rubisco
D-ribose-1,5-bisphosphate (3)
3-phospho-glycerate (3-PGA)
ATP (6)
ADP (6)
PGK
1,3-bisphospho-glycerate
NADPH (6)
NADP+ (6) + Pi (6)
GAPDH
D-glyceraldehyde-3-phosphate (GAP) (6)
ADP (3)
ATP (3)
PRuK
D-ribulose-5-phosphate
RPI
RPE
D-ribose-5-phosphate
D-xylulose-5-phosphate
TPI
Dihydroxyacetone phosphate (DHAP) (5)
GAP (1)
Carbohydrates
ALDO
GAP
Fructose-1,6-bisphosphate
TKT
GAP
Sedoheptulose-7-phosphate
FBPase
H2O
Pi
Fructose-6-phosphate
SBPase
Pi
H2O
Sedoheptulose-1,7-bisphosphate
ALDO
Erythrose-4-phosphate
TKT
GAP
DHAP

❖The net result of three turns of the Calvin cycle is the conversion of three molecules of CO2 and one molecule of phosphate to a molecule of triose phosphate.