Unit4: Carbohydrates II

METABOLISM OF CARBOHYDRATES

4.1- METABOLISM OF CARBOHYDRATES

Glucose occupies a crucial position in the metabolism of plants, animals, and many microorganisms being rich in potential energy, and thus a good fuel. 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 there is need of energy it can be released from these intracellular storage polymers and used to produce ATP (aerobically or anaerobically).

Glucose is also a remarkably versatile precursor, capable of supplying a huge array of metabolic intermediates for biosynthetic reactions (Escherichia coli can obtain from glucose the carbon skeletons for every amino acid, nucleotide, coenzyme, fatty acid, or other metabolic intermediate needed for growth). In animals and vascular plants, glucose has four major fates:

- Synthesis of complex polysaccharides for extracellular space
- It may be stored (as a polysaccharide or as sucrose)
- Oxidized to a three-carbon compound (pyruvate) via glycolysis to provide ATP and metabolic intermediates
- Oxidized via the pentose phosphate (phosphogluconate) pathway to yield ribose 5-phosphate for nucleic acid synthesis and NADPH for reductive biosynthetic processes.

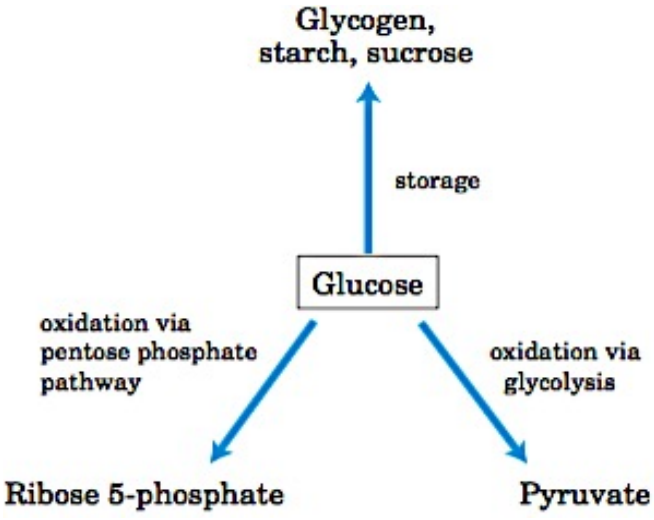


Figure 23 Pathways of glucose utilization.

Organisms that do not have access to glucose from other sources must make it.

Photosynthetic organisms- make glucose by first reducing atmospheric CO_2 to trioses, then converting the trioses to glucose).

Non-photosynthetic cells- make glucose from simpler three and four-carbon precursors by the process of gluconeogenesis.

In this section we will study the individual reactions of glycolysis, gluconeogenesis, and the pentose phosphate pathway and the functional significance of each pathway.

4.1.1- GLYCOLYSIS: OVERVIEW

Glycolysis (from the Greek glykys, meaning "sweet," and lysis, meaning "splitting"), involves the degradation of a molecule of glucose in a series of enzyme-catalyzed reactions to yield two molecules of the three-carbon compound pyruvate. Glycolytic pathway occupies a central position in glucose metabolism. This pathway is the biggest flux of carbon in almost every cell. During the sequential reactions of glycolysis, some of the free energy released from glucose is conserved in the form of ATP and NADH. The complete glycolytic pathway was elucidated by 1940, largely through the pioneering contributions of Gustav Embden, Otto Meyerhof, Carl Neuberg, Jacob Parnas, Otto Warburg, Gerty Cori, and Carl Cori. Glycolysis (figure 1) is also known as the ***Embden-Meyerhof pathway*.**

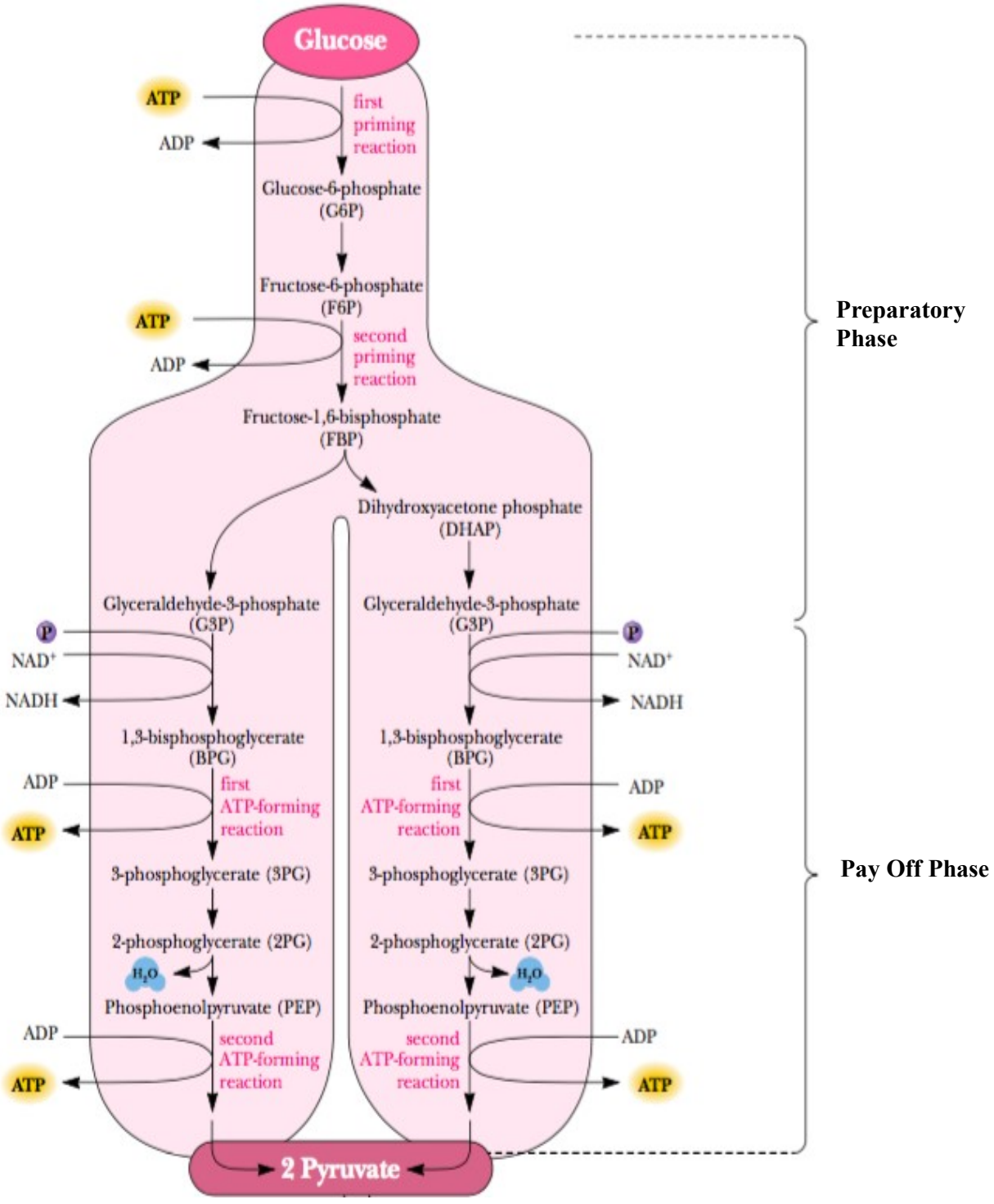


Figure 1 Glycolytic pathway.

The breakdown of the six-carbon glucose into two molecules of the three-carbon pyruvate

occurs in ten steps (fig. 24), the first five of which constitute the **preparatory phase** in which 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 into a common product, glyceraldehyde 3-phosphate.

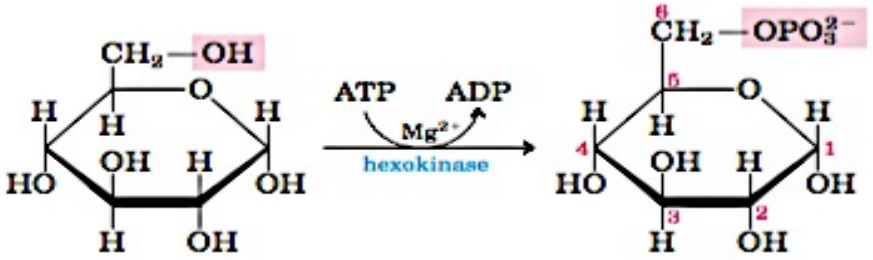
In the payoff phase, each of the two molecules of glyceraldehyde 3-phosphate derived from glucose undergoes oxidation; the energy of this oxidation reaction is conserved in the formation of one NADH and two ATP per triose phosphate oxidized. The net equation for the overall process is

$$\text{Glucose} + 2NAD^+ + 2ADP + 2P_i \rightarrow 2\text{pyruvate} + 2NADH + 2H^+ + 2ATP + 2H_2O$$

THE FIRST PHASE OF GLYCOLYSIS

Reaction 1: Phosphorylation of Glucose by Hexokinase or Glucokinase—The First Priming Reaction

The initial reaction of the glycolysis pathway involves phosphorylation of glucose at carbon atom 6 by either hexokinase or glucokinase. The formation of such a phosphoester is thermodynamically unfavorable and requires energy in form of ATP to operate in the forward direction. The hexokinase or glucokinase reaction is one of two **priming reactions** in the cycle that requires two priming ATP molecules to start the sequence of reactions and delivers four molecules of ATP in the end.



Glucose Glucose 6-phosphate $\Delta G'^{\circ}$=-16.7 kJ/mol

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

*NOTE *In most animal, plant, and microbial cells, the enzyme that phosphorylates glucose is* ***hexokinase.*** *Magnesium ion (Mg^{2+}) is required for this reaction, as for the other kinase enzymes in the glycolytic pathway. The true substrate for the hexokinase reaction is $MgATP^{2-}$ Liver contains an enzyme called* ***glucokinase,*** *which also carries out this reaction in but is highly specific for D-glucose, has a much higher Km for glucose (approximately 10.0 mM), and is not product-inhibited. With such a high Km for glucose, glucokinase becomes important metabolically only when liver glucose levels are high (for example, when the individual has consumed large amounts of sugar). When glucose levels are low, hexokinase is primarily responsible for phosphorylating glucose. However, when glucose levels are high, glucose is converted by glucokinase to glucose-6-phosphate and is eventually stored in the liver as glycogen.*

Importance of Phosphorylated Intermediates *Each of the nine glycolytic intermediates between glucose and pyruvate is phosphorylated. The phosphoryl groups appear to have three functions.*

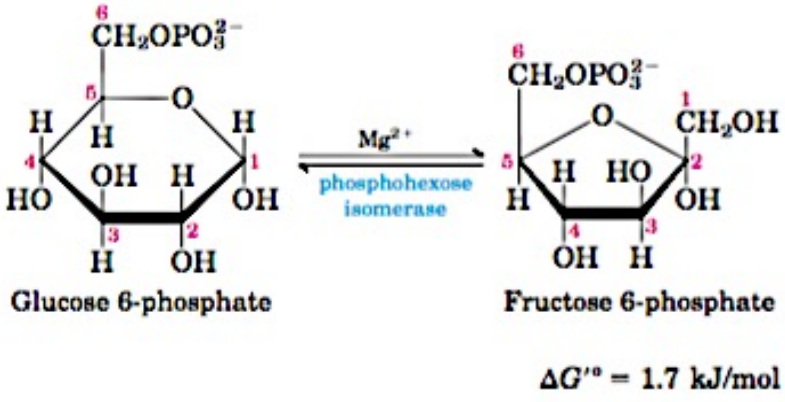
- *Phosphorylated glycolytic intermediates cannot leave the cell (plasma membrane generally lacks transporters for phosphorylated sugars). After the initial phosphorylation, no further energy is necessary to retain phosphorylated intermediates in the cell, despite the large difference in their intracellular and extracellular concentrations.*

- *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-bisphos- phoglycerate and phosphoenolpyruvate) donate phosphoryl groups to ADP to form ATP.*

- *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.*

Reaction 2: Phosphoglucoisomerase catalyzes the isomerization of glucose-6-phosphate

The second step in glycolysis is a common type of metabolic reaction: the isomerization of a sugar. In this particular case, the carbonyl oxygen of glucose- 6-phosphate is shifted from C-1 to C-2. This amounts to isomerization of an aldose (glucose-6-phosphate) to a ketose—fructose-6-phosphate. The reaction is necessary for two reasons. First, the next step in glycolysis is phosphorylation at C-1, and the hemiacetal -OH of glucose would be more difficult to phosphorylate than a simple primary hydroxyl. Second, the isomerization to fructose (with a carbonyl group at position 2 in the linear form) activates carbon C-3 for cleavage in the fourth step of glycolysis. The enzyme responsible for this isomerization is **phosphoglucoisomerase,** also known as **glucose phosphate isomerase. The reaction proceeds readily in either direction, as might be expected from the relatively small change in standard free energy.**



Reaction 3: Phosphofructokinase—The Second Priming Reaction

The action of phosphoglucoisomerase, "moving" the carbonyl group from C-1 to C-2, creates a new primary alcohol function at C-1. The next step in the glycolytic pathway is the phosphorylation of this group by **phosphofructokinase (PFK-1).**

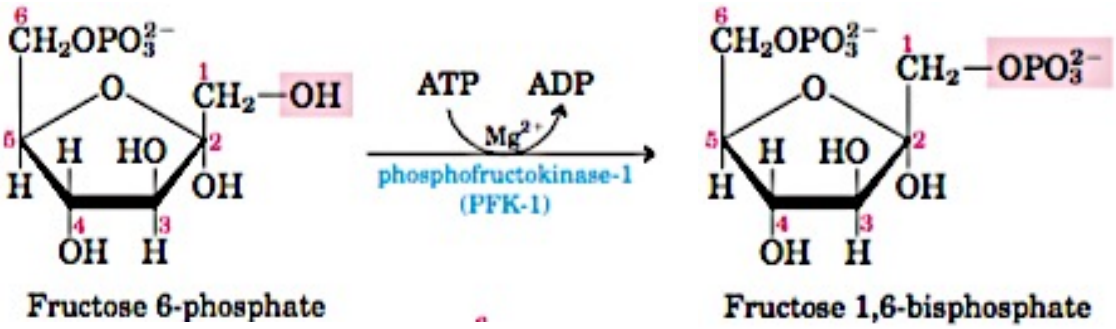
Note*- This enzyme is called PFK-1 to distinguish it from a second enzyme (PFK-2) that catalyzes the formation of fructose 2,6-bisphosphate from fructose 6-phosphate in a separate pathway.

Once again, the substrate that provides the phosphoryl group is ATP. Like the hexokinase, glucokinase reaction, the phosphorylation of fructose-6-phosphate is a priming reaction and is endergonic:

Fructose-6-P + P_i →fructose-1,6-bisphosphate $\Delta G'^{\circ}$= 16.3 kJ/mol

When coupled (by phosphofructokinase) with the hydrolysis of ATP, the overall reaction is strongly exergonic:

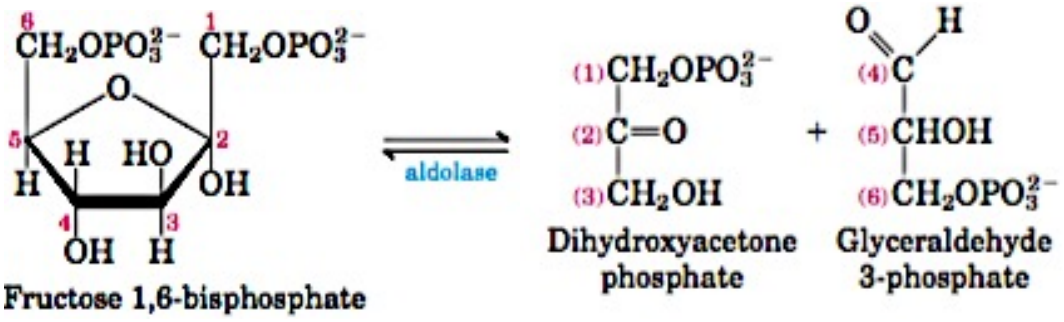
Fructose-6-P + ATP →fructose-1,6-bisphosphate ADP $\Delta G'^{\circ}$=-14.2 kJ/mol



This 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. Just as the hexokinase reaction commits the cell to take up glucose, *the phosphofructokinase reaction commits the cell to metabolize glucose* rather than converting it to another sugar or storing it. Similarly, just as the large free energy change of the hexokinase reaction makes it a likely candidate for regulation, so the phosphofructokinase reaction is an important site of regulation— indeed, the most important site in the glycolytic pathway. The activity of PFK-1 is increased whenever the cell's ATP supply is depleted or when the ATP breakdown products, ADP and AMP (particularly the latter), are in excess. 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.

Reaction 4: Cleavage of Fructose-1, 6-bisphosphate

Fructose bisphosphate aldolase catalyzes a reversible aldol condensation that involves thecleavage of fructose-1,6-bisphosphate between the C-3 and C-4 carbons to yield two triose phosphates. The products are dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate.

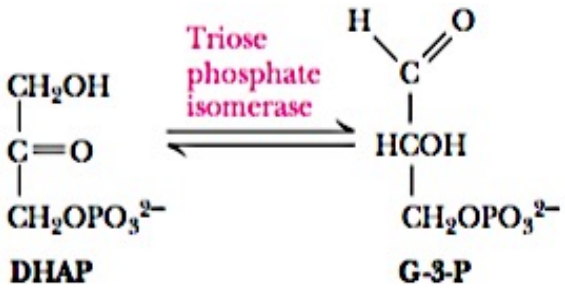


$\Delta G'^{\circ}$=23.8 kJ/mol

Reaction 5: Triose phosphate interconversion

Of the two products of the aldolase reaction, only glyceraldehyde-3-phosphate goes directly into the second phase of glycolysis. The other triose phosphate, dihydroxyacetone phosphate, gets converted to glyceraldehyde-3-phosphate by the enzyme **triose phosphate isomerase.** This reaction thus permits both products of the aldolase reaction to continue in the glycolytic pathway, and in essence makes the C-1, C-2, and C-3 carbons of the starting glucose molecule equivalent to the C-6, C-5, and C-4 carbons, respectively.

The triose phosphate isomerase reaction completes the first phase of glycolysis, each glucose that passes through being converted to two molecules of glyceraldehyde-3-phosphate. Although the last two steps of the pathway are energetically unfavorable, the overall five-step reaction sequence has a net $\Delta G'^{\circ}$ of +2.2 kJ/mol. It is the free energy of hydrolysis from the two priming molecules of ATP that brings the overall equilibrium constant close to 1 under standard-state conditions. The net ΔG under cellular conditions is quite negative.

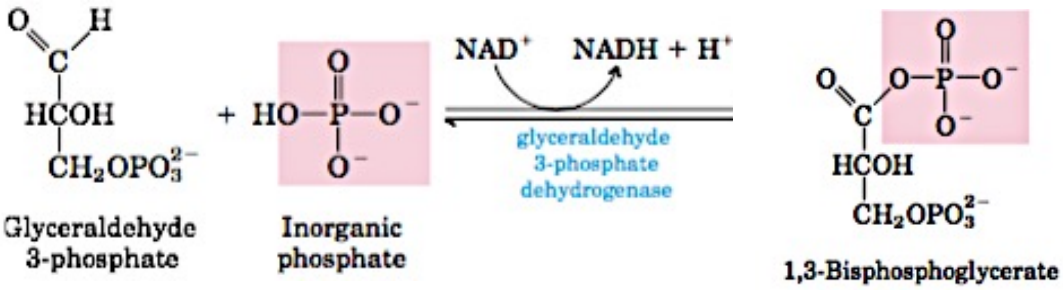


THE PAYOFF PHASE

The payoff phase of glycolysis includes the energy-conserving phosphorylation steps in which some of the free energy of the glucose molecule is conserved in the form of ATP. One molecule of glucose yields two molecules of glyceraldehyde 3-phosphate; 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 devoted in the preparatory phase of glycolysis to phosphorylate the two ends of the hexose molecule.

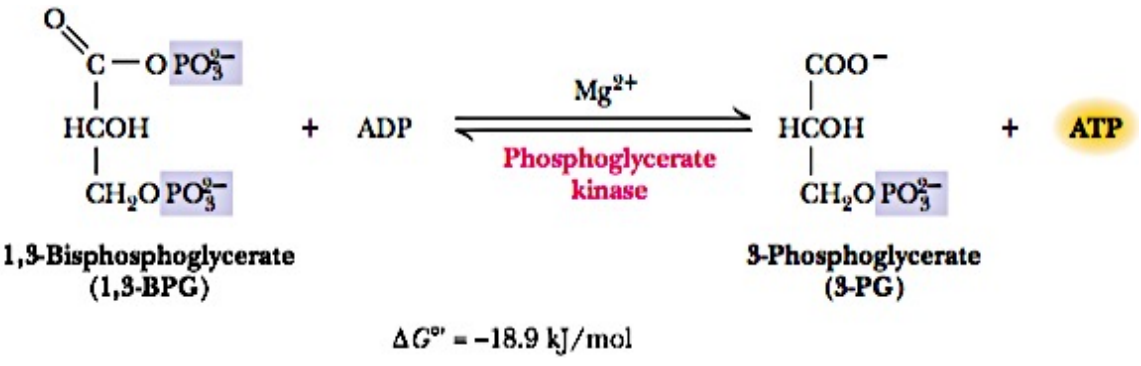
Reaction 6: Formation of 1,3 Bisphosphoglycerate by oxidation

In the first glycolytic reaction to involve oxidation–reduction, glyceraldehyde- 3-phosphate is oxidized to 1,3-bisphosphoglycerate by **glyceraldehyde-3-phosphate dehydrogenase.** Although the oxidation of an aldehyde to a carboxylic acid is a highly exergonic reaction, the overall reaction involves both formation of a carboxylic-phosphoric anhydride and the reduction of NAD^+ to NADH and is therefore slightly endergonic at standard state, with a $\Delta G'^\circ$ of 6.30 kJ/mol.



Reaction 7: Exergonic reaction leading to formation of ATP

The glycolytic pathway breaks even in terms of ATPs consumed and produced with this reaction. The enzyme **phosphoglycerate kinase** transfers a phosphoryl group from 1,3-bisphosphoglycerate to ADP to form an ATP. Because each glucose molecule sends two molecules of glyceraldehyde-3-phosphate into the second phase of glycolysis and because two ATPs were consumed per glucose in the first-phase reactions, the phosphoglycerate kinase reaction "pays off" the ATP debt created by the priming reactions. As might be expected for a phosphoryl transfer enzyme, Mg^{2+} ion is required for activity, and the true nucleotide substrate for the reaction is $MgADP^-$. It is appropriate to view the sixth and seventh reactions of glycolysis as a coupled pair, with 1, 3-bis-phosphoglycerate as an intermediate. The phosphoglycerate kinase reaction is sufficiently exergonic at standard state to pull the G-3-P dehydrogenase reaction along. (In fact, the aldolase and triose phosphate isomerase are also pulled forward by phosphoglycerate kinase.) The net result of these coupled reactions is



Glyceraldehyde 3-phosphate +ADP +P_i +NAD^+ ⟺3-phosphoglycerate + ATP +NADH +H^+

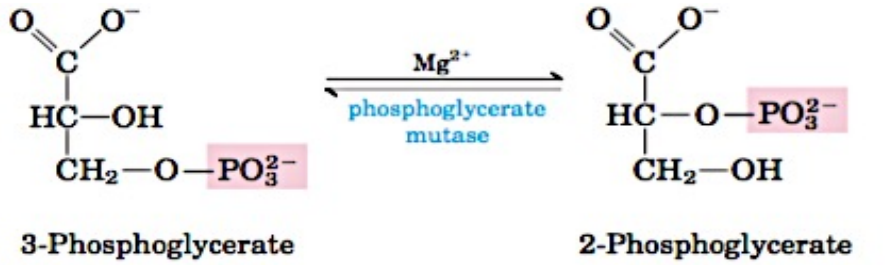
$\Delta G'^\circ$= - 12.5 kJ/mol

Thus the overall reaction is exergonic. 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.***

Reaction 8: Conversion of 3-Phosphoglycerate to 2-Phosphoglycerate

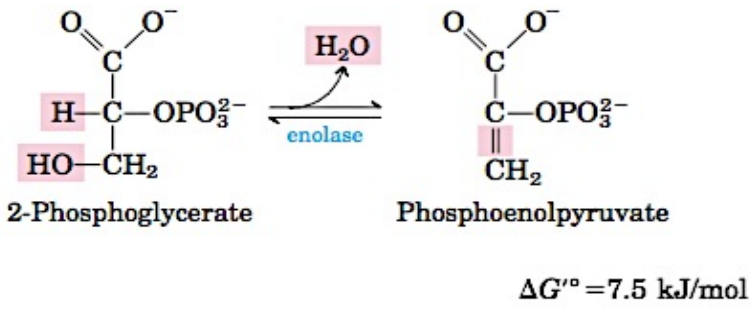
The remaining steps in the glycolytic pathway prepare for synthesis of the second ATP equivalent. This begins with the **phosphoglycerate mutase** reaction, in which the phosphoryl group of 3-phosphoglycerate is moved from C-3 to C-2. (The term *mutase* is applied to enzymes that catalyze migration of a functional group within a substrate molecule.) The free energy change for this reaction is very small under cellular conditions ($\Delta G'^{\circ}$= 0.83 kJ/mol in erythrocytes).

The reaction occurs in two steps : A phosphoryl group initially attached to a His residue of the mutase is transferred to the hydroxyl group at C-2 of 3-phosphoglycerate, forming 2,3-bisphosphoglycerate (2,3-BPG). The phosphoryl group at C-3 of 2,3-BPG is then transferred to the same His residue, producing 2-phosphoglycerate and regenerating the phosphorylated enzyme. Phosphoglycerate mutase is initially phosphorylated by phosphoryl transfer from 2,3-BPG, which is required in small quantities to initiate the catalytic cycle and is continuously regenerated by that cycle. Although in most cells 2,3-BPG is present in only trace amounts, it is a major component of erythrocytes, where it regulates the affinity of hemoglobin for oxygen.



9: Dehydration of 2-Phosphoglycerate to Phosphoenolpyruvate

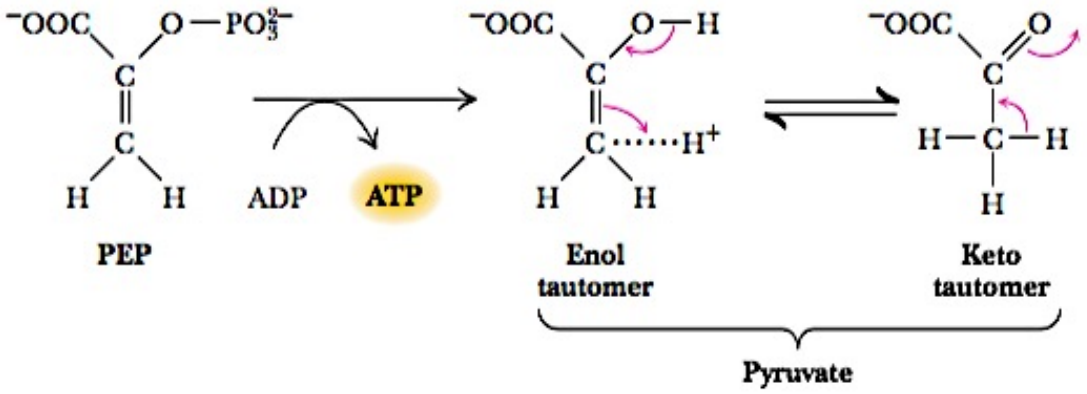
Enolase catalyzes the formation of phosphoenolpyruvate from 2-phosphoglycerate. The reaction in essence involves dehydration—the removal of a water molecule—to form the enol structure of PEP.



The enzyme is strongly inhibited by fluoride ion in the presence of phosphate. Inhibition arises from the formation of *fluorophosphate (FPO_3^{2-}),* which forms a complex with Mg^{2+} at the active site of the enzyme.

Reaction 10: Formation of Pyruvate

The second ATP-synthesizing reaction of glycolysis is catalyzed by **pyruvate kinase,** which brings the pathway at last to its pyruvate branch point. Pyruvate kinase mediates the transfer of a phosphoryl group from phosphoenolpyruvate to ADP to make ATP and pyruvate. The reaction requires Mg^{2+} ion and is stimulated by K^+ and certain other monovalent cations. The high free energy change for the conversion of PEP to pyruvate is due largely to the highly favorable and spontaneous conversion of the enol tautomer of pyruvate to the more stable keto form following the phosphoryl group transfer step. The overall reaction has a large, negative standard free- energy change, due in large part to the spontaneous con- version of the enol form of pyruvate to the keto form. The $\Delta G'^{\circ}$ of phosphoenolpyruvate hydrolysis is -61.9 kJ/mol; about half of this energy is conserved in the formation of the phosphoanhydride bond of ATP ($\Delta G'^{\circ}$= -30.5 kJ/mol), and the rest ($\Delta G'^{\circ}$= -31.4 kJ/mol) constitutes a large driving force pushing the reaction toward ATP synthesis. The pyruvate kinase reaction is essentially irreversible under intra- cellular conditions and is an important site of regulation.



THE OVERALL BALANCE SHEET OF GLYCOLYSIS

The left-hand side of the following equation shows all the inputs of ATP, NAD^+, ADP, and Pi and the right-hand side shows all the outputs (Note*- each molecule of glucose yields two molecules of pyruvate):

Glucose +2ATP+ 2NAD^++4ADP +2Pi $\rightarrow$2 pyruvate+ 2ADP+ 2NADH +2H^+ +4ATP+ 2H_2O

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

Glucose + 2NAD^+ + 2ADP +2Pi $\rightarrow$2 pyruvate+ 2NADH +2H^+ + 2ATP+ 2H_2O

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 electron- transfer chain, which in eukaryotic cells is located in the mitochondria. The electron-transfer chain passes these

electrons to their ultimate destination, O_2:

$2NADH + 2H^+ + O_2 \rightarrow 2NAD^+ + 2H_2O$

Electron transfer from NADH to O_2 in mitochondria provides the energy for synthesis of ATP by respiration- linked 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. Four electrons, as two hydride ions, are transferred from two molecules of glyceraldehyde 3-phosphate to two of NAD^+ (the pathway of electrons).

4.1.2 METABOLIC FATES OF THE PRODUCTS OF GLYCOLYSIS: NADH AND PYRUVATE

In addition to ATP, the products of glycolysis are NADH and pyruvate. Their processing depends upon other cellular pathways. NADH must be recycled to NAD^+, lest NAD^+become limiting in glycolysis. What a given cell does with the pyruvate produced in glycolysis depends in part on the availability of oxygen. The pyruvate formed by glycolysis is further metabolized via one of two catabolic routes.

AEROBIC PATHWAY

Under aerobic conditions, pyruvate can be sent into the citric acid cycle (also known as the tricarboxylic acid cycle), where it is oxidized to CO_2 with the production of additional NADH (and $FADH_2$). Under aerobic conditions, the NADH produced in glycolysis and the citric acid cycle is reoxidized to NAD^+in the mitochondrial electron transport chain.

ANAEROBIC PATHWAY

Under anaerobic conditions, the pyruvate produced in glycolysis is processed differently. In yeast, it is reduced to ethanol; in other microorganisms and in animals, it is reduced to lactate. These processes are examples of **fermentation**—the production of ATP energy by reaction pathways in which organic molecules function as donors and acceptors of electrons. In either case, reduction of pyruvate provides a means of reoxidizing the NADH produced in the glyceraldehyde-3-phosphate dehydrogenase reaction of glycolysis.

LACTIC ACID FERMENTATION

In animal tissues experiencing anaerobic conditions, pyruvate is reduced to lactate. Pyruvate reduction occurs in tissues that normally experience minimal access to blood flow (e.g., the cornea of the eye) and also in rapidly contracting skeletal muscle. When skeletal muscles are exercised strenuously, the available tissue oxygen is consumed, and the pyruvate generated by glycolysis can no longer be oxidized in the TCA cycle and 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 NADnecessary for the continuity of glycolysis. Instead, excess pyruvate is reduced to lactate by lactate dehydrogenase (Figure 2). In muscle tissue under anaerobic conditions, lactate represents the end product of glycolysis. Most of this lactate must be carried out of the muscle by the blood and transported to the liver, where it can be

resynthesized into glucose via gluconeogenesis.

Lactate produced by anaerobic microorganisms during **lactic acid fermentation is** responsible for the taste of sour milk and for the characteristic taste and fragrance of sauerkraut, which in reality is fermented cabbage.

ETHANOL (ALCOHOL) FERMENTATION

In some plant tissues and in certain invertebrates, protists, and microorganisms such as brewer's yeast, pyruvate is converted under hypoxic or anaerobic conditions into ethanol and CO_2, a process called ethanol (alcohol) fermentation (Fig. 3). In yeast, **alcoholic fermentation** is a two-step process. Pyruvate is decarboxylated to acetaldehyde by **pyruvate decarboxylase** in an essentially irreversible reaction. Thiamine pyrophosphate is a required cofactor for this enzyme. The second step, the reduction of acetaldehyde to ethanol by NADH, is catalyzed by **alcohol dehydrogenase**. At pH 7, the reaction equilibrium strongly favors ethanol. The end products of alcoholic fermentation are thus ethanol and carbon dioxide. Alcoholic fermentations are the basis for the brewing of beers and the fermentation of grape sugar in wine making

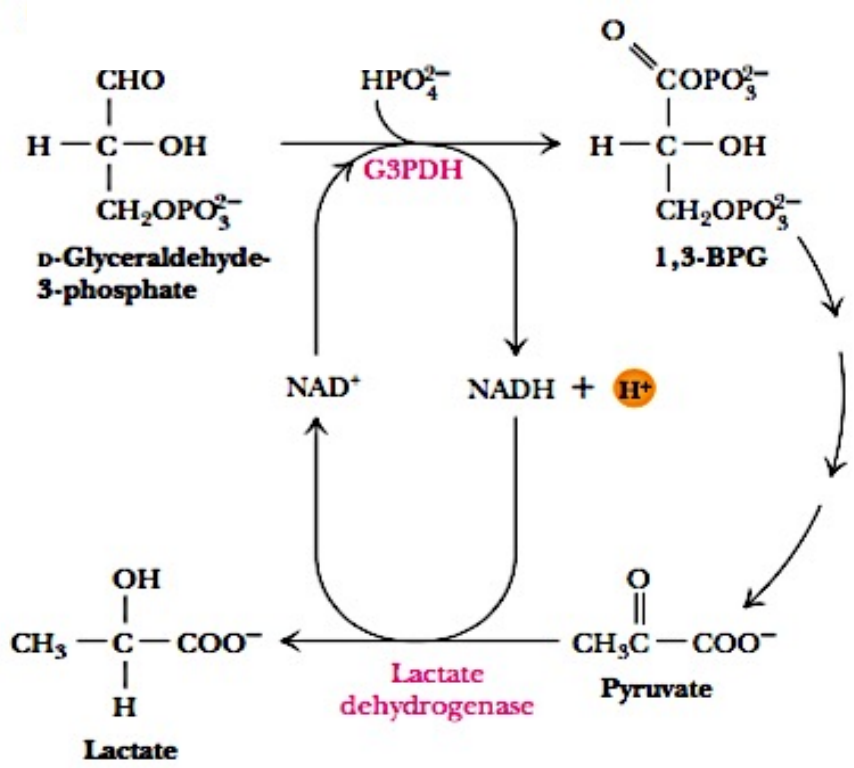


Figure 2 Lactic acid fermentation

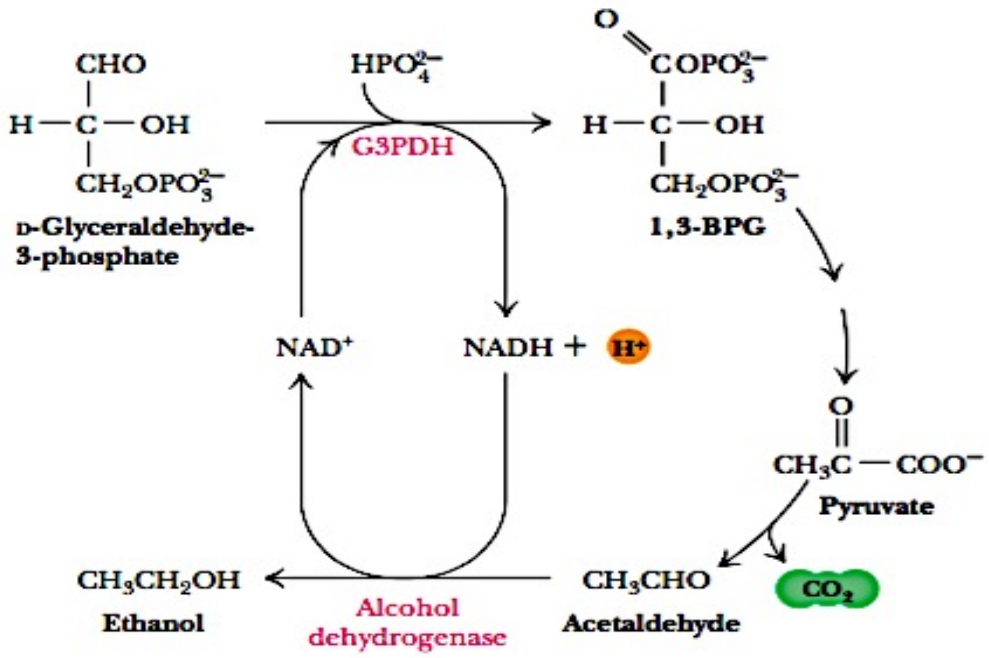


Figure 3 Alcoholic fermentation

4.1.3 UTILIZATION OF OTHER SUBSTRATES IN GLYCOLYSIS

Sugars other than glucose (both simple and complex), can enter the cycle and become one of the intermediates of glycolysis. The most significant are the storage polysaccharides glycogen and starch; the disaccharides maltose, lactose, trehalose, and sucrose; and the monosaccharides fructose, mannose, and galactose (figure 4).

Entry of glycogen and starch

Glycogen in animal tissues and in microorganisms (and starch in plants) is 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 ($\alpha 1 \rightarrow 4$) glycosidic linkage that joins the last two glucose residues at a nonreducing end, generating glucose 1-phosphate and a polymer one glucose unit shorter. Glycogen phosphorylase (or starch phosphorylase) acts repetitively until it approaches a ($\alpha 1 \rightarrow 6$) branch point where its action stops. 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

glucose 1-phosphate $\Leftrightarrow$glucose 6-phosphate

The glucose 6-phosphate thus formed can enter glycolysis or another pathway such as the pentose phosphate pathway.

Entry of fructose

Fructose produced by breakdown of sucrose, may participate in glycolysis by at least two different routes. In the liver, fructose is phosphorylated at C-1 by the enzyme fructokinase:

D-Fructose + ATP^{4-} → D-fructose-1-phosphate^{2-} + ADP^{3-} + H^{+}

Subsequent action by fructose-1-phosphate aldolase cleaves fructose-1-P in a manner like the fructose bisphosphate aldolase reaction to produce dihydroxyacetone phosphate and D-glyceraldehyde:

D-Fructose-1-P^{2-} → D-glyceraldehyde + dihydroxyacetone phosphate^{2-}

Dihydroxyacetone phosphate is an intermediate in glycolysis. D-Glyceraldehyde can be phosphorylated by triose kinase in the presence of ATP to form D-glyceraldehyde-3-phosphate, another glycolytic intermediate.

In the kidney and in muscle tissues, fructose is readily phosphorylated by hexokinase, which, as pointed out above, can utilize several different hexose substrates. The free energy of hydrolysis of ATP drives the reaction forward:

D-Fructose + ATP^{4-} → D-fructose-1-phosphate^{2-} + ADP^{3-} + H^{+}

Fructose-6-phosphate generated in this way enters the glycolytic pathway directly in step 3, the second priming reaction. This is the principal means for channeling fructose into glycolysis in adipose tissue, which contains high levels of fructose.

Entry of Galactose

A somewhat more complicated route into glycolysis is followed by galactose, another simple hexose sugar. The process, called the Leloir pathway after Luis Leloir, its discoverer, begins with phosphorylation from ATP at the C-1 position by enzyme galactokinase:

D-Galactose + ATP^{4-} → D-galactose-1-phosphate^{2-} + ADP^{3-} + H^{+}

Galactose-1-phosphate is then converted into *UDP-galactose* (a sugar nucleotide) by galactose-1-phosphate uridylyltransferase, with concurrent production of glucose-1-phosphate and consumption of a molecule of UDP-glucose (figure 5). The glucose-1-phosphate produced by the transferase reaction is a substrate for the phosphoglucomutase reaction, which produces glucose-6-phos-phate, a glycolytic substrate. The other transferase product, UDP-galactose, is converted to UDP-glucose by UDP-glucose-4-epimerase. The combined action of the uridylyltransferase and epimerase thus produces glucose-1-P from galactose-1-P, with regeneration of UDP-glucose.

A rare hereditary condition known as galactosemia involves defects in galactose-1-P uridylyltransferase that render the enzyme inactive. Toxic levels of galactose accumulate in afflicted individuals, causing cataracts and permanent neurological disorders.

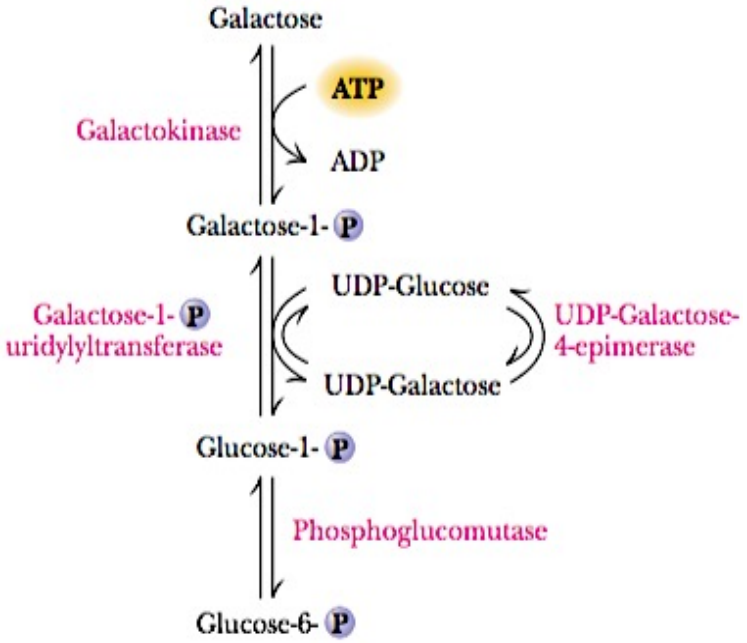


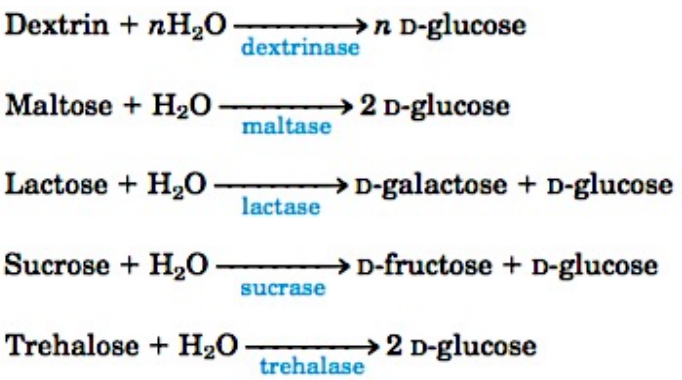
Figure 5 Galactose metabolism via the Leloir pathway.

Lactose Intolerance

A much more common metabolic disorder, lactose intolerance, occurs commonly in most parts of the world. Lactose intolerance is an inability to digest lactose because of the absence of the enzyme lactase in the intestines of adults. Lactose cannot be completely digested and absorbed in the small intestine and passes into the large intestine, where bacteria convert it to toxic products that cause abdominal cramps and diarrhea. The problem is further complicated because undigested lactose and its metabolites increase the osmolarity of the intestinal contents, favoring the retention of water in the intestine. In most parts of the world where lactose intolerance is prevalent, adults do not use milk as a food, although milk products predigested with lactase are commercially available in some countries.

Entry of Disaccharides

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.



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.

Entry of glycerol

Glycerol is the last important simple metabolite, which is produced in substantial amounts by the decomposition of *triacylglycerols.* It gets converted to glycerol-3-phosphate by the action of glycerol kinase and then oxidized to dihydroxyacetone phosphate by the action of glycerol phosphate dehydrogenase, with NAD^+ as the required coenzyme. The dihydroxyacetone phosphate thereby produced enters the glycolytic pathway as a substrate for triose phosphate isomerase.

The glycerol kinase reaction -

Glycerol +ATP → Glycerol-3-phosphate + ADP

The glycerol phosphate dehydrogenase reaction -

Glycerol-3-phosphate + NAD^+ → Dihydroxyacetone phosphate + NADH + H^+

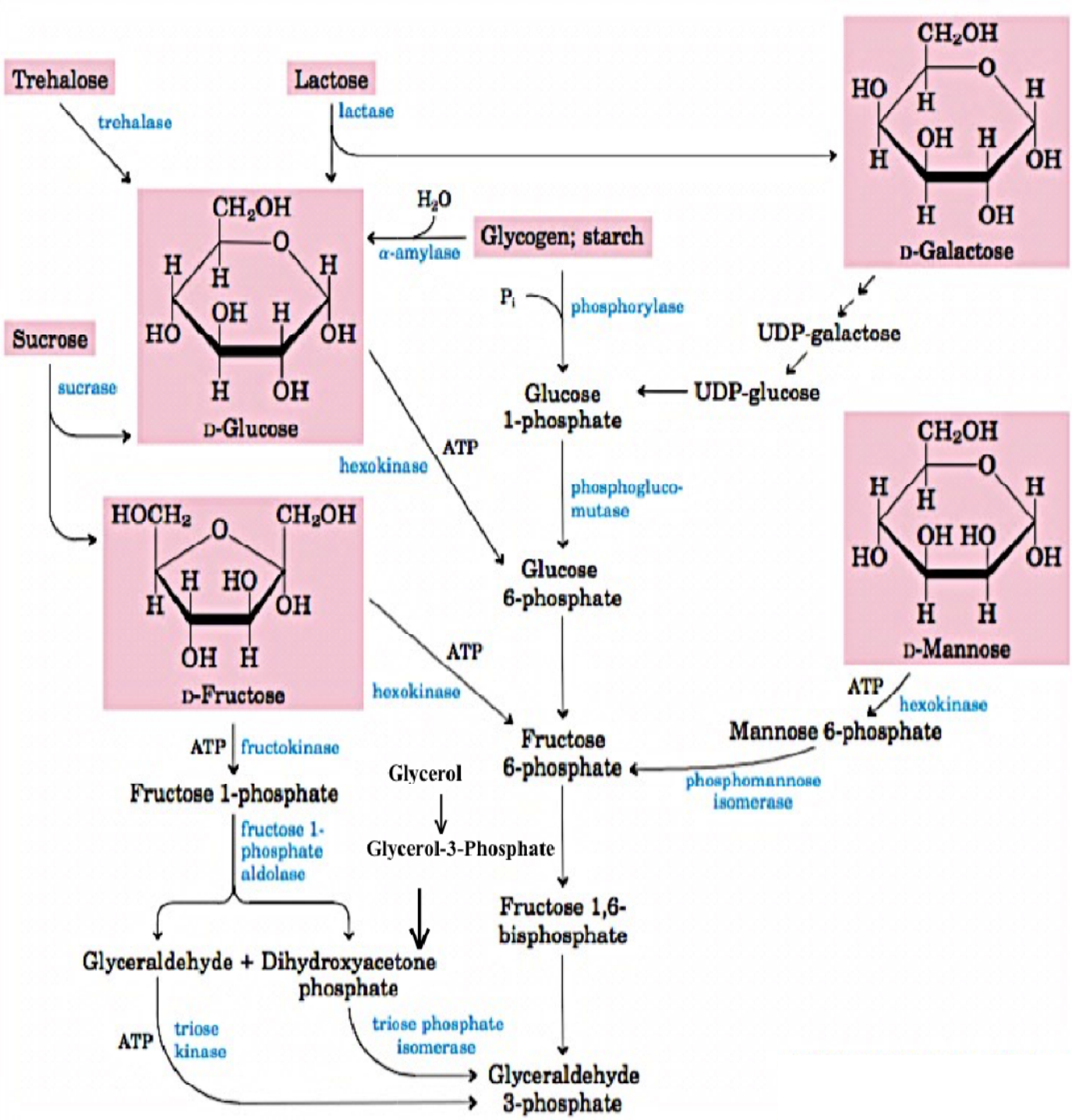


Figure 6 Entry of glycogen, starch, disaccharides, hexoses and glycerol into the preparatory stage of glycolysis.

4.2. GLUCONEOGENESIS

The human body carries only a little more than a one-day supply of glucose. If glucose is not obtained in the diet, the body must produce new glucose from noncarbohydrate precursors. It can be accomplished by **gluconeogenesis,** which means the generation (*genesis*) of new (*neo*) glucose. Additionally, muscles consume large amounts of glucose via glycolysis, producing large amounts of pyruvate. In vigorous exercise, muscle cells become anaerobic and pyruvate is converted to lactate. Gluconeogenesis salvages this pyruvate and lactate and reconverts it to glucose.

The substrates of gluconeogenesis

In addition to pyruvate and lactate, other noncarbohydrate precursors are used as substrates for gluconeogenesis in animals including amino acids, glycerol and all the TCA cycle intermediates. On the other hand, fatty acids are not substrates for gluconeogenesis in animals, because most fatty acids yield only acetyl-CoA upon degradation and animals do not carry out net synthesis of sugars from acetyl-CoA. Lysine and leucine are the only amino acids that are not substrates for gluconeogenesis. These amino acids produce only acetyl-CoA upon degradation.

Note: Acetyl-CoA is a substrate for gluconeogenesis when the glyoxylate cycle is operating.

Site of Gluconeogenesis

The major sites of gluconeogenesis are the liver and kidneys, which account for about 90% and 10% of the body's gluconeogenic activity, respectively. Glucose produced by gluconeogenesis in the liver and kidney is released into the blood and is subsequently absorbed by brain, heart, muscle, and red blood cells to meet their metabolic needs. In turn, pyruvate and lactate produced in these tissues are returned to the liver and kidney to be used as gluconeogenic substrates.

The Pathway

Gluconeogenesis and glycolysis are not identical pathways running in opposite directions, although they do share several steps, seven of the ten 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.***In cells, these three reactions are characterized by a large negative free-energy change, ΔG, whereas other glycolytic reactions have a ΔG near 0. 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.The processes of glycolysis and gluconeogenesis must be regulated in a reciprocal fashion so that when glycolysis is active, gluconeogenesis is inhibited, and vice-versa. Separate regulation of the two pathways is brought about through controls exerted on the enzymatic steps unique to each.

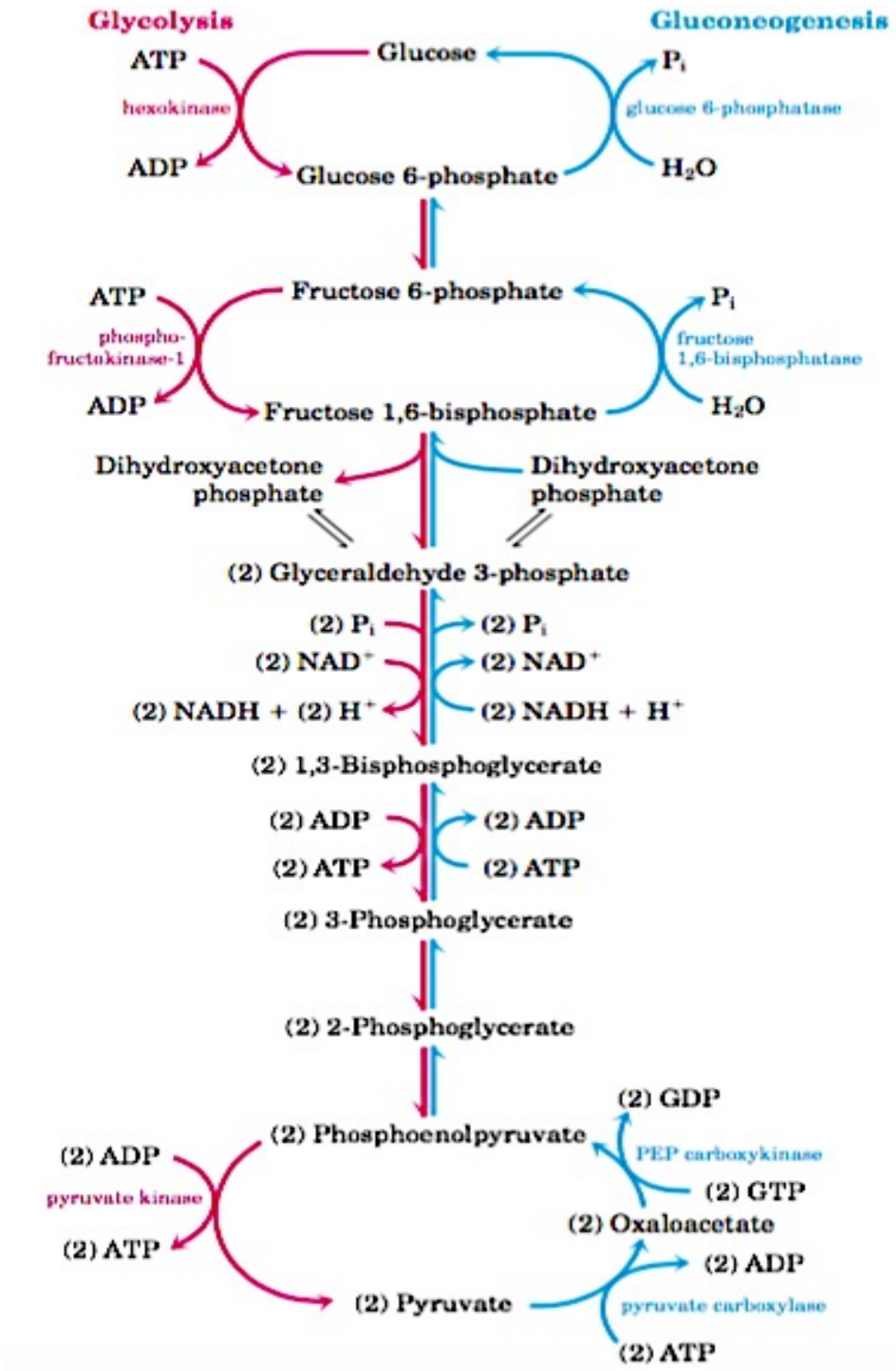


Figure 7 Opposing pathways of glycolysis and gluconeogenesis

The Unique Reactions of Gluconeogenesis

(1) Formation of PEP from pyruvate by Pyruvate Carboxylase

Initiation of gluconeogenesis occurs by the pyruvate carboxylase reaction —**the conversion of pyruvate to oxaloacetate.**Route from pyruvate to PEP is the predominant path when pyruvate or alanine is the glucogenic precursor. A second pathway, described later, predominates when lactate is the glucogenic precursor.

The reaction takes place in two discrete steps, involves ATP and bicarbonate as substrates. Pyruvate is first transported from the cytosol into mitochondria or is generated from alanine within mitochondria by transamination, in which the α-amino group is removed from alanine (leaving pyruvate) and added to anα-keto carboxylic acid. Then pyruvate carboxylase, a mitochondrial enzyme converts the pyruvate to oxaloacetate:

$$\text{Pyruvate} + HCO^-_3 + \text{ATP} \rightarrow \text{oxaloacetate} + \text{ADP} + P_i$$

Pyruvate carboxylase a tetrameric enzyme (500 kD)is the first regulatory enzyme in the gluconeogenic pathway, utilizes biotin as a coenzyme and acetyl-coenzyme A as an allosteric activator. The pyruvate carboxylase reaction can replenish intermediates in another central metabolic pathway, the citric acid cycle.

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:

$$\text{Oxaloacetate} + \text{NADH} + H^+ \Leftrightarrow \text{L-malate} + NAD^+$$

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

$$\text{Malate} + NAD^+ \rightarrow \text{oxaloacetate} + \text{NADH} + H^+$$

The oxaloacetate is then converted to PEP by phosphoenolpyruvate carboxykinase. This Mg^{2+}dependent reaction requires GTP as the phosphoryl group donor:

$$\text{Oxaloacetate} + \text{GTP} \Leftrightarrow \text{PEP} + CO_2 + \text{GDP}$$

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

A second pyruvate→PEP pathway predominates when lactate is the glucogenic precursor. This pathway makes use of lactate produced by glycolysis in erythrocytes or anaerobic muscle, (after vigorous exercise). The lactate thus produced can be transported from muscle to the liver, where it is reoxidized by liver lactate dehydrogenase to yield pyruvate, which is converted eventually to glucose. In this way, the liver shares in the metabolic stress created by vigorous exercise. It exports glucose to muscle, which produces lactate, which can be processed by the liver into new glucose. This is referred to as the **Cori cycle.** 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.

(2) Conversion of Fructose 1,6 Bisphosphate to Fructose-6- phosphate by Fructose-1, 6-Bisphosphatase

The second glycolytic reaction that cannot participate in gluconeogenesis is the hydrolysis of fructose-1, 6-bisphosphate to fructose-6-phosphate, like all phosphate ester hydrolyses, is a thermodynamically favorable (exergonic) reaction under standard-state conditions. Under physiological conditions in the liver, the reaction is also exergonic. **Fructose-1, 6-bisphosphatase** is an allosterically regulated enzyme. Citrate stimulates bisphosphatase activity, but *fructose-2, 6-bisphosphate* is a potent allosteric inhibitor. AMP also inhibits the bisphosphatase; the inhibition by AMP is enhanced by fructose-2, 6-bisphosphate.

Fructose 1, 6-bisphosphate + $H_2O \rightarrow$fructose- 6-phosphate + P_i

(3) Generation of Glucose

The final step in the gluconeogenesis pathway is the dephosphorylation of glucose-6-phosphate to glucose by the action of **glucose-6-phosphatase.** The reaction is catalyzed by glucose 6-phosphatase and it does not require synthesis of ATP; it is a simple hydrolysis of a phosphate ester:

Glucose 6-phosphate + $H_2O \rightarrow$ glucose + Pi

This enzyme is present in the lumenal side of the membranes of the endoplasmic reticulum of liver and kidney cells, but is absent in muscle and brain. For this reason, **gluconeogenesis is not carried out in muscle and brain.** Its membrane association is important to its function because the substrate is hydrolyzed as it passes into the endoplasmic reticulum itself. Vesicles form from the endoplasmic reticulum membrane and diffuse to the plasma membrane and fuse with it, releasing their glucose contents into the bloodstream.

4.2.1 COORDINATED REGULATION OF GLYCOLYSIS AND GLUCONEOGENESIS

Hexokinase, which catalyzes the entry of free glucose into the glycolytic pathway, is a regulatory enzyme. There are four isozymes (designated I to IV), encoded by four different genes. Muscle hexokinases I and II are allosterically inhibited by their product, glucose 6-phosphate, so whenever the cellular concentration of glucose 6-phosphate rises above its normal level, these isozymes are temporarily and reversibly inhibited, bringing the rate of glucose 6-phosphate formation into balance with the rate of its utilization and reestablishing the steady state.

The different hexokinase isozymes of liver and muscle reflect the different roles of these organs in carbohydrate metabolism: muscle consumes glucose, using it for energy production, whereas liver maintains blood glucose homeostasis by removing or producing glucose, depending on the prevailing glucose concentration.

Phosphofructokinase-1 Is under complex allosteric regulation

The metabolically irreversible reaction catalyzed by PFK-1 is the step that commits glucose to glycolysis. In addition to its substrate-binding sites, this complex enzyme has several regulatory sites at which allosteric activators or inhibitors bind. ATP is not only a substrate for PFK-1 but also an end product of the glycolytic pathway. 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 lowers the affinity of the enzyme for 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, also serves as 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.

The most significant allosteric regulator of PFK-1 is fructose 2,6-bisphosphate, which strongly activates the enzyme.

Pyruvate Kinase Is allosterically inhibited by ATP

High concentrations of ATP, acetyl-CoA, and long-chain fatty acids (signs of abundant 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, cAMP-dependent protein kinase phosphorylates the L isozyme of pyruvate kinase, inactivating it. This slows the use of glucose 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.

Gluconeogenesis is regulated at several steps

In the pathway leading from pyruvate to glucose, the first control point determines the fate of pyruvate in the mitochondrion. Pyruvate can be converted either to acetyl-CoA to fuel the citric acid cycle, or to oxaloacetate (by pyruvate carboxylase) to start the process of gluconeogenesis. 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 energetic needs are being met, oxidative phosphorylation slows, NADH rises relative to NAD and inhibits the citric acid cycle, and acetyl-CoA gets accumulated. The increased concentration of acetyl-CoA inhibits the pyruvate dehydrogenase complex, slowing the formation of acetyl-CoA from pyruvate, and stimulates gluconeogenesis by activating pyruvate carboxylase, allowing excess pyruvate to be converted to glucose.

The second control point in gluconeogenesis is the reaction catalyzed by FBPase-1, which is strongly inhibited by AMP. The corresponding glycolytic enzyme, PFK-1, is stimulated by AMP and ADP but inhibited by citrate and ATP. Thus these opposing steps in the two

pathways are regulated in a coordinated and reciprocal manner. In general, when sufficient concentrations of acetyl-CoA or citrate (the product of acetyl- CoA condensation with oxaloacetate) are present, or when a high proportion of the cell's adenylate is in the form of ATP, gluconeogenesis is favored. AMP promotes glycogen degradation and glycolysis by activating glycogen phosphorylase (via activation of phosphorylase kinase) and stimulating the activity of PFK-1. Another set of regulatory processes is triggered from outside the cell by the hormones insulin and glucagon, which signal too much or too little glucose in the blood, respectively, or by epinephrine, which signals the impending need for fuel for a fight-or-flight response. These hormonal signals bring about covalent modification (phosphorylation or dephosphorylation) of target proteins inside the cell; this takes place on a somewhat longer time scale than the internally driven allosteric mechanisms—seconds or minutes, rather than milliseconds.

Fructose 2,6-Bisphosphate Is a Potent Regulator of Glycolysis and Gluconeogenesis

The hormonal regulation of glycolysis and gluconeogenesis is mediated by fructose 2,6-bisphosphate, an allosteric effector for the enzymes PFK-1 and FBPase-1.When fructose 2,6-bisphosphate binds to its allosteric site on PFK-1, it increases that 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 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 activates PFK-1 and stimulates glycolysis in liver and, at the same time, inhibits FBPase-1, thereby slowing gluconeogenesis.

The cellular concentration of fructose 2,6-bisphosphate is set by the relative rates of its formation and breakdown. It is formed by phosphorylation of fructose 6-phosphate, catalyzed by phosphofructokinase-2 (PFK-2), and is broken down by fructose 2, 6- bisphosphatase (FBPase-2). (PFK-2 and FBPase-2 are two distinct enzymatic activities of a single, bifunctional protein. The balance of these two activities in the liver, which determines the cellular level of fructose 2,6- bisphosphate, is regulated by hormones glucagon and insulin. Glucagon stimulates the adenylyl cyclase of liver to synthesize 3, 5 -cyclic AMP (cAMP) from ATP. Then cAMP activates cAMP-dependent protein kinase that transfers a phosphoryl group from ATP to the bifunctional protein PFK-2/FBPase-2. Phosphorylation of this protein enhances its FBPase-2 activity and inhibits its PFK-2 activity. Glucagon thereby lowers the cellular level of fructose 2, 6-bisphosphate, inhibiting glycolytic pathway and stimulating gluconeogenesis. The resulting production of more glucose enables the liver to replenish blood glucose in response to glucagon. Insulin has the opposite effect. It stimulates the activity of a phosphoprotein phosphatase that catalyzes removal of the phosphoryl group from the bifunctional protein PFK-2/FBPase-2, activating its PFK-2 activity, increasing the level of fructose 2, 6-bisphosphate thereby stimulating glycolysis, and inhibiting gluconeogenesis pathway.

Xylulose 5-Phosphate, a Key Regulator of Carbohydrate

Another regulatory mechanism also acts by controlling the level of fructose 2,6-bisphosphate. In the mammalian liver, xylulose 5-phosphate, a product of the hexose monophosphate pathway, mediates the increase in glycolysis that follows ingestion of a high-carbohydrate meal. The xylulose 5-phosphate concentration rises as glucose entering the liver is converted to glucose 6-phosphate and enters both the glycolytic and hexose monophosphate pathways. Xylulose 5-phosphate activates a phosphoprotein phosphatase, PP2A that dephosphorylates the

bifunctional PFK-2/FBPase-2 enzyme. Dephosphorylation activates PFK-2 and inhibits FBPase-2, and the resulting rise in fructose 2, 6-bisphosphate stimulates glycolysis and inhibits gluconeogenesis. The increased glycolysis boosts the production of acetyl-CoA, while the increased flow of hexose through the hexose monophosphate pathway generates NADPH. Acetyl-CoA and NADPH are the starting materials for fatty acid synthesis, which has long been known to increase dramatically in response to intake of a high-carbohydrate meal.

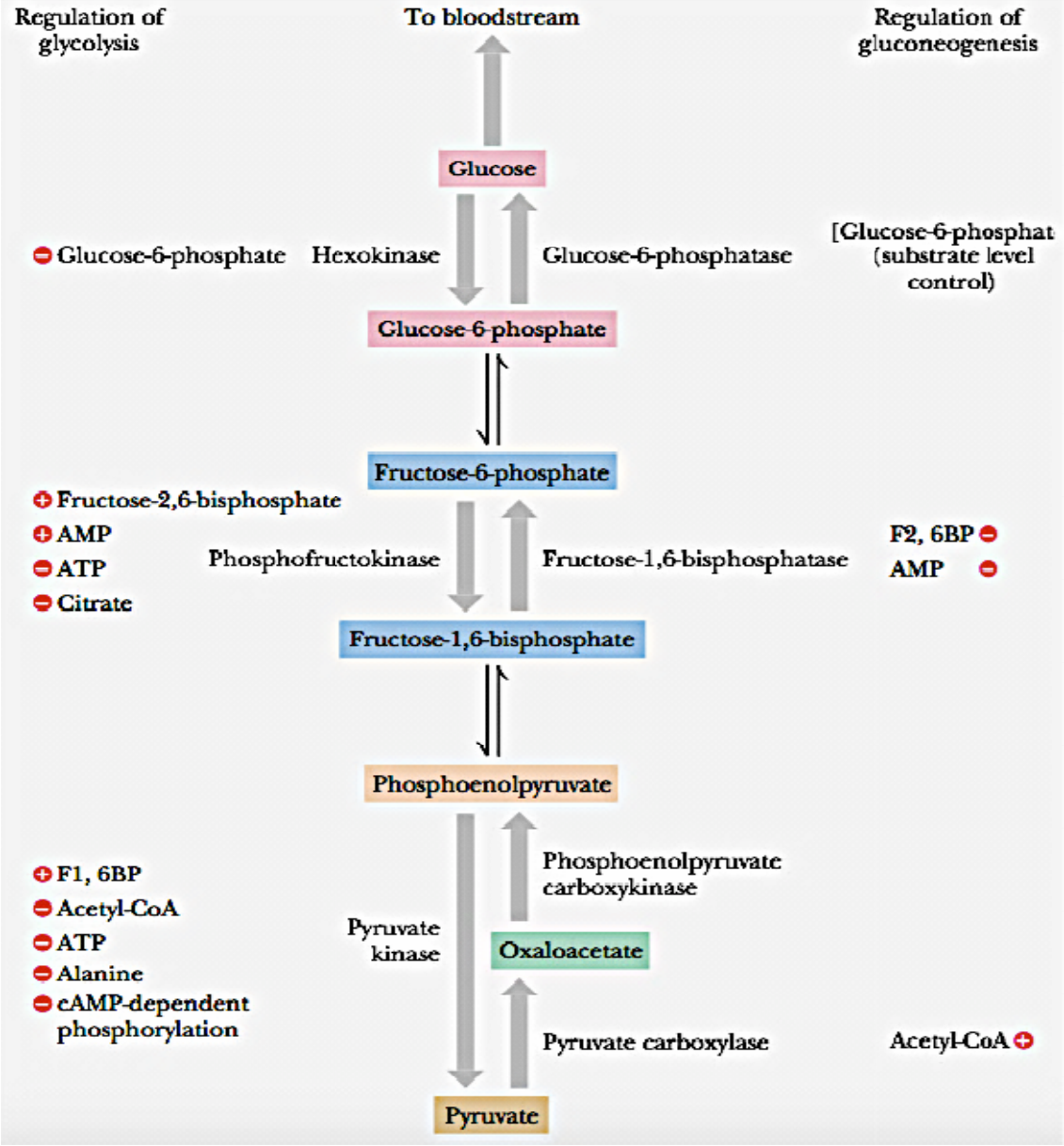


Figure 8 Regulation mechanisms in glycolysis and gluconeogenesis. Acitvators are depicted by plus sign and inhibitors by minus sign

4.2.2 PENTOSE PHOSPHATE PATHWAY

Cells require a constant supply of NADPH for oxidation-reduction reactions vital for biosynthetic purposes. Much of this requirement is met by a glucose-based metabolic sequence called **the pentose phosphate pathway, the hexose mono-phosphate shunt, or the phosphogluconate pathway**. In addition to providing NADPH for biosynthetic processes, this pathway produces *ribose-5-phosphate,* which is essential for nucleic acid synthesis. Several metabolites of the pentose phosphate pathway can also be shuttled into glycolysis. Rapidly dividing cells, such as those of bone marrow, skin and intestinal mucosa, use the pentoses to make RNA, DNA, and such coenzymes as ATP, NADH, $FADH_2$, and coenzyme A.

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

An Overview

The pentose phosphate pathway begins with glucose-6-phosphate, a six-carbon sugar, and produces three-, four-, five-, six-, and seven-carbon sugars (figure 9). Two successive oxidations lead to the reduction of $NADP^+$to NADPH and the release of CO_2. Five subsequent non-oxidative steps produce a variety of carbohydrates, some of which may enter the glycolytic pathway. The enzymes of the pentose phosphate pathway are particularly abundant in the cytoplasm of liver and adipose cells. **These enzymes are largely absent in muscle**, where glucose-6-phosphate is utilized primarily for energy production via glycolysis and the TCA cycle. These pentose phosphate pathway enzymes are located in the cytosol, which is the site of fatty acid synthesis, a pathway heavily dependent on NADPH for reductive reactions.

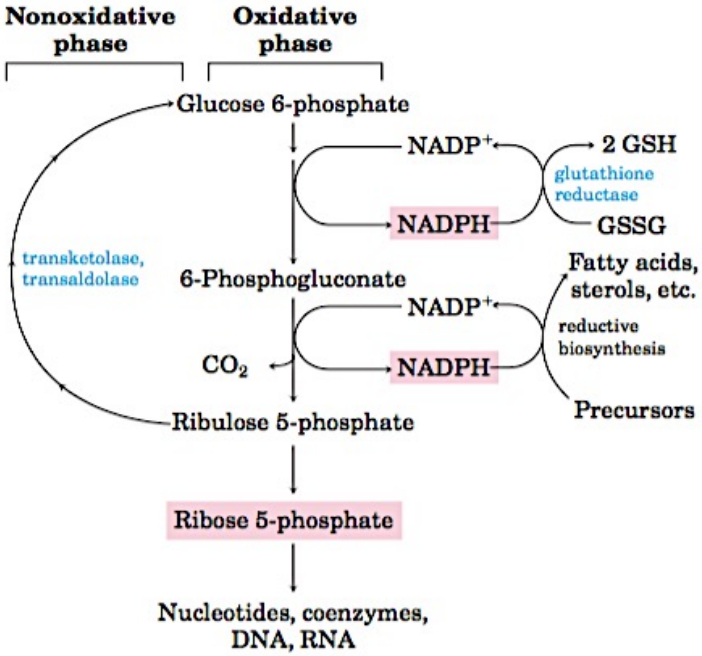


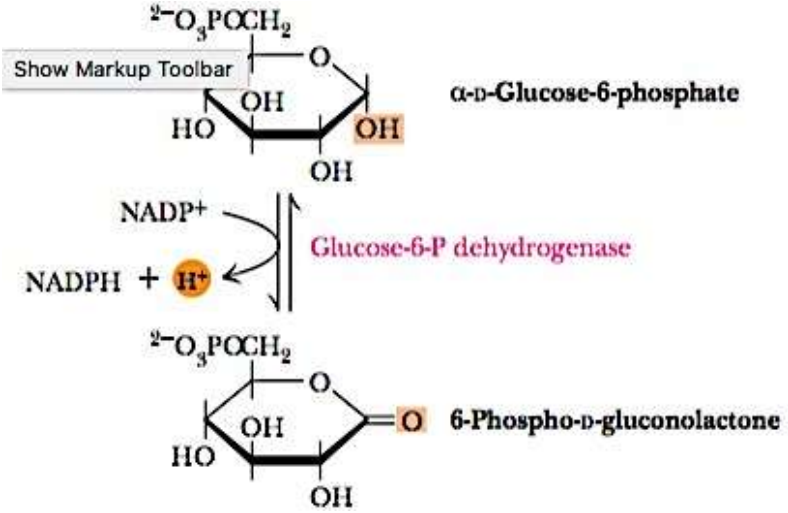
Figure 9 Oxidative Steps of the Pentose Phosphate Pathway

OXIDATIVE PHASE

(1) Oxidation of glucose-6-phosphate

The pentose phosphate pathway begins with the oxidation of glucose-6-phosphate. The products of the reaction are**6-Phospho-D-gluconolactone (**cyclic ester) and NADPH (Step 1). Glucose-6-phosphate dehydrogenase, which catalyzes this reaction, is highly specific for $NADP^+$. As the first step of a major pathway, the reaction is irreversible and highly regulated. Glucose-6-phosphate dehydrogenase is strongly inhibited by the product coenzyme, NADPH, and also by fatty acid esters of coenzyme A (which are intermediates of fatty acid biosynthesis).

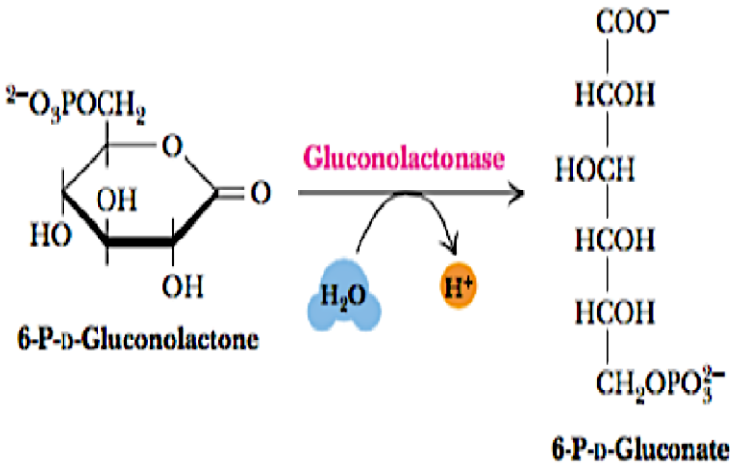
(Step 1)



(2) Hydrolysis fo Phosphogluconolactone

The **6-Phospho-D-gluconolactone** produced in step 1 is hydrolytically unstable and readily undergoes a spontaneous ring-opening hydrolysis, although an enzyme, gluconolactonase, accelerates this reaction. The linear product, the sugar acid 6-phospho-D-gluconate, is further oxidized in step 3.

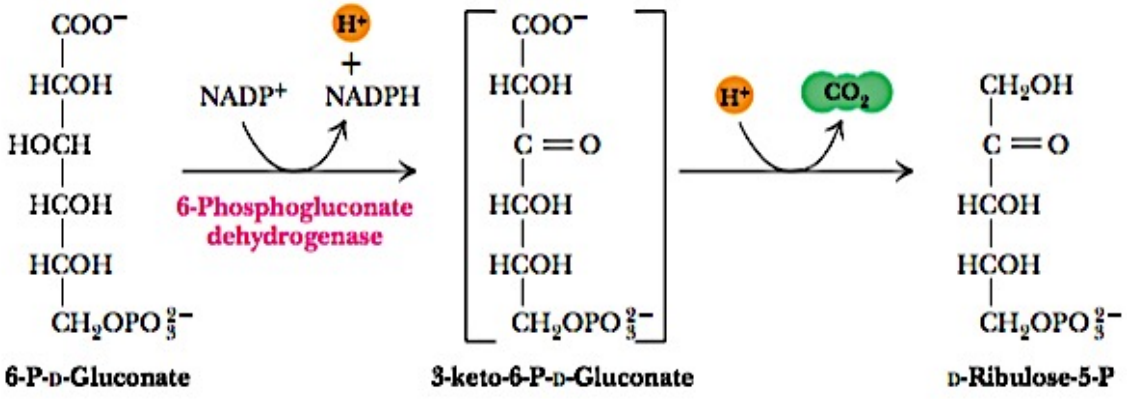
(Step 2)



(3) Oxidation of 6-phospho-D-gluconate

The oxidative decarboxylation of 6-phosphogluconate by 6-phosphogluconate dehydrogenase yields D-ribulose-5-phosphate and another equivalent of NADPH. There are two distinct steps in this reaction: the initial NADP -dependent dehydrogenation yields a -keto acid, 3-keto-6-phosphogluconate, which is very susceptible to decarboxylation (the second step). The resulting product, D-ribulose-5-P, is the substrate for the nonoxidative reactions composing the rest of this pathway.

(Step 3)



The Nonoxidative Steps of the Pentose Phosphate Pathway

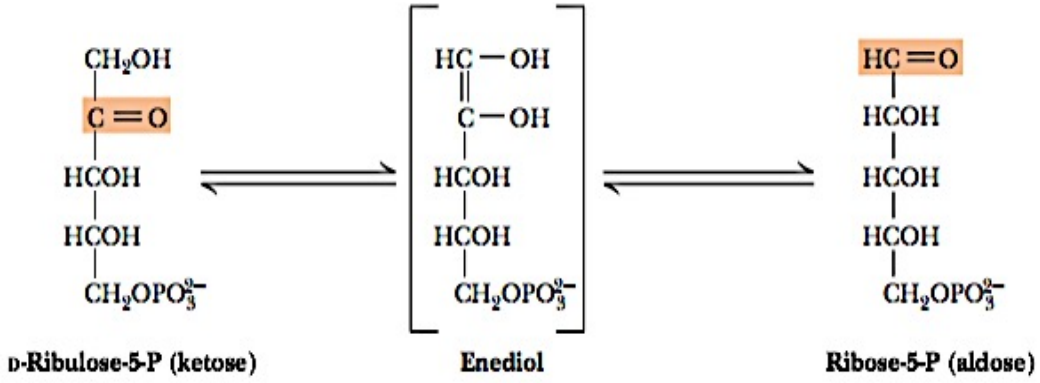
This portion of the pathway begins with an isomerization and an epimerization, and it leads to the formation of either D-ribose-5-phosphate or D-xylulose- 5-phosphate. These intermediates can then be converted into glycolytic intermediates or directed to biosynthetic processes.

(4) Formation of Ribose-5-phosphate

This enzyme interconverts ribulose-5-P and ribose-5-P via an enediol intermediate. The reaction (and mechanism) is quite similar to the phosphoglucoisomerase reaction of glycolysis, which interconverts glucose-6-P and fructose-6-P. The ribose-5-P produced in this reaction is utilized in the biosynthesis of coenzymes (including NADH, NADPH, FAD, and B_{12}), nucleotides, and nucleic acids (DNA and RNA). The net reaction for the first four steps of the pentose phosphate pathway is

Glucose-6-P + 2 $NADP^+ \rightarrow$ ribose-5-P +2 NADPH + $2H^+$+ CO_2

(Step 4)



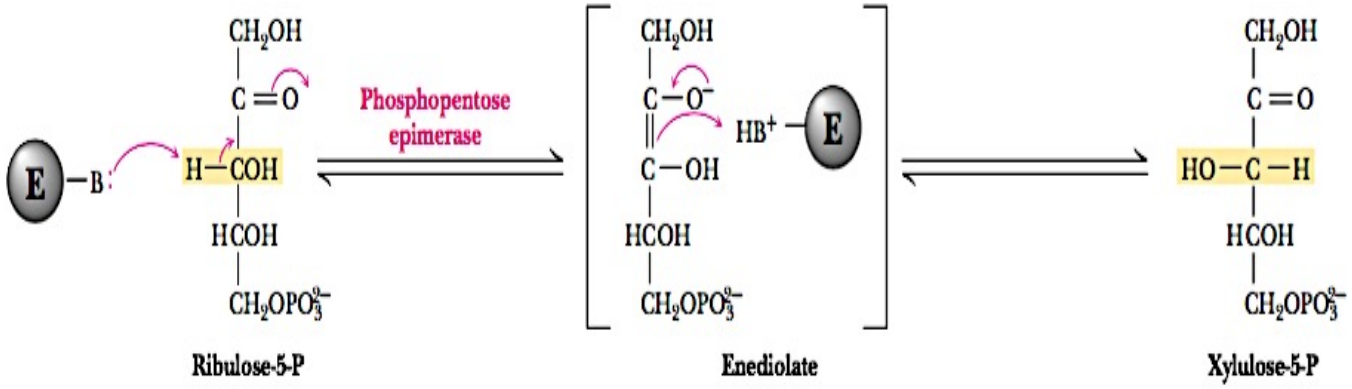
(5) Formation of Xylulose-5-phosphate

This reaction converts ribulose-5-P to another ketose, namely, xylulose-5-P. This reaction also proceeds by an enediol intermediate, but involves an inversion at C-3. In the reaction, an acidic proton located α- to a carbonyl carbon is removed to generate the enediolate, but the proton is added back to the same carbon from the opposite side.

Note* -Interchange of groups on a single carbon is an epimerization, and interchange of groups between carbons is referred to as an isomerization.

To this point, the pathway has generated a pool of pentose phosphates. The $\Delta G'^{\circ}$ for each of the last two reactions is small, and the three pentose-5-phosphates coexist at equilibrium. The pathway also produces two molecules of NADPH for each glucose-6-P converted to pentose-5-phosphate. The next three steps rearrange the five-carbon skeletons of the pentoses to produce three-, four-, six-, and seven-carbon units, which can be used for various metabolic purposes.

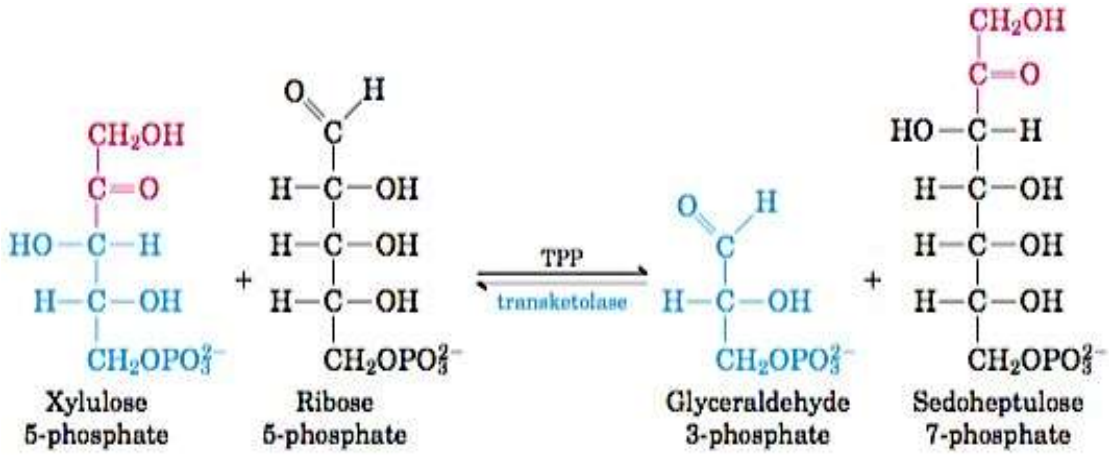
(Step 5)



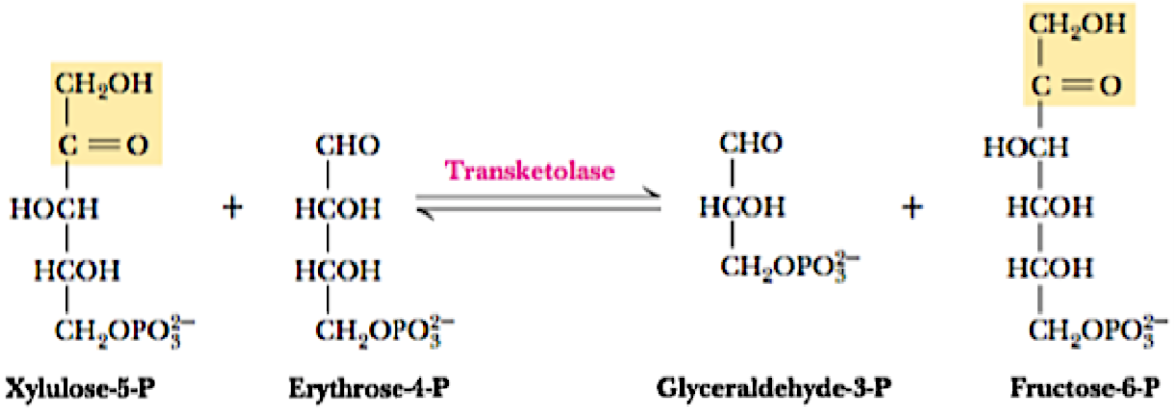
(6) and (8) reactions catalysed by Transketolase

The transketolase enzyme acts at both steps 6 and 8 of the pentose phosphate pathway. In both cases, the enzyme catalyzes the transfer of two-carbon units. In these reactions (and also in step 7, the transaldolase reaction, which transfers three-carbon units), the donor molecule is a ketose and the recipient is an aldose. In step 6, xylulose-5-phosphate transfers a two-carbon unit to ribose-5-phosphate to form glyceraldehyde-3-phosphate and sedoheptulose-7-phosphate. Step 8 involves a two-carbon transfer from xylulose-5-phosphate to erythrose 4-phosphate to produce another glyceraldehyde- 3-phosphate and a fructose-6-phosphate. Three of these products enter directly into the glycolytic pathway. Transketolase is a thiamine pyrophosphate–dependent enzyme, and the mechanism involves abstraction of the acidic thiazole proton of TPP, attack by the resulting carbanion at the carbonyl carbon of the ketose phosphate substrate, expulsion of the glyceraldehyde-3-phosphate product, and transfer of the two-carbon unit. Transketolase can process a variety of 2-keto sugar phosphates in a similar manner. It is specific for ketose substrates with the configuration shown, but can accept a variety of aldose phosphate substrates.

(Step 6)



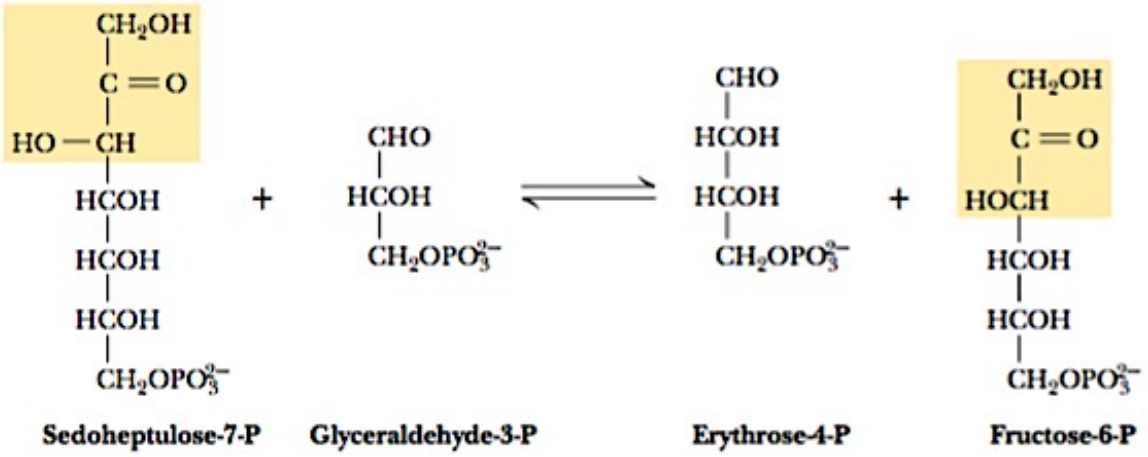
(Step 8)



(7) Reactions catalysed by Transaldolase

The transaldolase functions primarily to make a useful glycolytic substrate from the sedoheptulose-7-phosphate produced by the first transketolase reaction. This reaction is quite similar to the aldolase reaction of glycolysis, involving formation of a Schiff base intermediate between the sedoheptulose-7-phosphate and an active-site lysine residue. Elimination of the erythrose-4-phosphate product leaves an enamine of dihydroxyacetone, which remains stable at the active site (without imine hydrolysis) until the other substrate comes into position. Attack of the enamine carbanion at the carbonyl carbon of glyceraldehyde-3-phosphate is followed by hydrolysis of the Schiff base (imine) to yield the product fructose-6-phosphate.

(Step 7)



4.2.3 REGULATION OF PENTOSE PHOSPHATE PATHWAY

It is clear that glucose-6-phosphate can be used as a substrate either for glycolysis or for the pentose phosphate pathway. The cell makes this choice on the basis of its relative needs for biosynthesis and for energy from metabolism. ATP can be produced in abundance if glucose-6-phosphate is channeled into glycolysis. On the other hand, if NADPH or ribose-5-phosphate is needed, glucose-6-phosphate can be directed to the pentose phosphate pathway. The molecular basis for this regulatory decision depends on the enzymes that metabolize glucose-6-phosphate in glycolysis and the pentose phosphate pathway. In glycolysis, phosphoglucoisomerase converts glucose-6-phosphate to fructose-6-phosphate, which is utilized by phosphofructokinase (a highly regulated enzyme) to produce fructose-1, 6-bisphosphate. In the pentose phosphate pathway, glucose-6-phosphate dehydrogenase (also highly regulated) produces gluconolactone from glucose-6-phosphate. Thus, the fate of glucose-6-phosphate is determined to a large extent by the relative activities of phosphofructokinase and glucose-6-P dehydrogenase. Recall that PFK is inhibited when the ATP/AMP ratio increases, and that it is inhibited by citrate but activated by fructose-2, 6-bisphosphate. Thus, when the energy charge is high, glycolytic flux decreases. Glucose-6-P dehydrogenase, on the other hand, is inhibited by high levels of NADPH and also by the intermediates of fatty acid biosynthesis. Both of these are indicators that biosynthetic demands have been satisfied. If that is the case, glucose-6-phosphate dehydrogenase and the pentose phosphate pathway are inhibited. If NADPH levels drop, the pentose phosphate pathway turns on, and NADPH and ribose-5-phosphate are made for biosynthetic purposes.

Even when the latter choice has been made, however, the cell must still be “aware” of the relative needs for ribose-5-phosphate and NADPH (as well as ATP). Depending on these relative needs, the reactions of glycolysis and the pentose phosphate pathway can be combined in novel ways to emphasize the synthesis of needed metabolites.

4.4.4 GLYCOGEN METABOLISM

In a wide range of organisms, 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. The glycogen in muscle is there to provide a

quick source of energy for either aerobic or anaerobic metabolism. 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. In humans, the total amount of energy stored as glycogen is far less than the amount stored as fat (triacylglycerol), but fats cannot be converted to glucose in mammals and cannot be catabolized anaerobically.

Glycogen granules are complex aggregates of glycogen. The general mechanisms for storing and mobilizing glycogen are the same in muscle and liver, but the enzymes differ in subtle yet important ways that reflect the different roles of glycogen in the two tissues. Glycogen is also obtained in the diet and broken down in the gut, and this involves a separate set of hydrolytic enzymes that convert glycogen (and starch) to free glucose.

4.4.4.1 GLYCOGENOLYSIS (GLYCOGEN BREAKDOWN)

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$\alpha(1\rightarrow4)$ 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 (figure 10). The cleavage of a bond by the addition of orthophosphate is referred to as *phosphorolysis*. 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.

NOTE*- ***The phosphorolytic cleavage of glycogen is energetically advantageous because the released sugar is already phosphorylated. In contrast, a hydrolytic cleavage would yield glucose, which would then have to be phosphorylated at the expense of the hydrolysis of a molecule of ATP to enter the glycolytic pathway. An additional advantage of phosphorolytic cleavage for muscle cells is that glucose 1-phosphate, negatively charged under physiological conditions, cannot diffuse out of the cell. Pyridoxal phosphate is an essential cofactor in the glycogen phosphorylase reaction***.

Glycogen phosphorylase acts repetitively on the nonreducing ends of glycogen branches until it reaches a point four glucose residues away from an $\alpha(1\rightarrow6)$ branch point, where its action stops. How can the remainder of the glycogen molecule be mobilized for use as a fuel? Two additional enzymes, a *transferase* and α-*1, 6-glucosidase,* remodel the glycogen for continued degradation by the phosphorylase (Figure 11). *The transferase shifts a block of three glycosyl residues from one outer branch to the other.* This transfer exposes a single glucose residue joined by an $\alpha(1\rightarrow6)$-glycosidic linkage. $\alpha(1\rightarrow6)$-Glucosidase, also known as the debranching enzyme, hydrolyzes the $\alpha(1\rightarrow6)$ glycosidic bond, resulting in the release of a free glucose molecule.

This free glucose molecule is phosphorylated by the glycolytic enzyme hexokinase. Thus, the transferase and $\alpha(1\rightarrow6)$ glucosidase convert the branched structure into a linear one, which paves the way for further cleavage by phosphorylase. It is noteworthy that, in eukaryotes, thetransferase and the $\alpha(1\rightarrow6)$ glucosidase activities are present in a single 160-kd polypeptidechain, providing yet another example of a bifunctional enzyme.

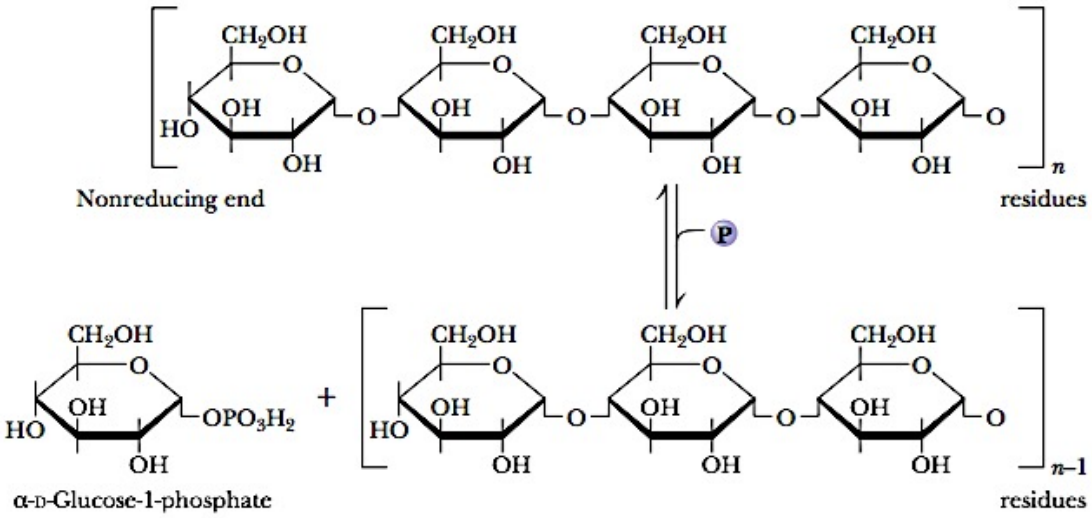


Figure 10 The glycogen phosphorylase reaction.

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

Glucose 1-phosphate ⇔glucose 6-phosphate

The catalytic site of an active mutase molecule contains a phosphorylated serine residue. The phosphoryl group is transferred from the serine residue to the C-6 hydroxyl group of glucose 1-phosphate to form glucose 1, 6-bisphosphate. The C-1 phosphoryl group of this intermediate is then shuttled to the same serine residue, resulting in the formation of glucose 6-phosphate and the regeneration of the phosphoenzyme.

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 an enzyme, glucose 6-phosphatase, that is present in liver and kidney but not in other tissues.** The enzyme is an integral membrane protein of the endoplasmic reticulum, with its active site on the lumenal side of the ER. Glucose 6-phosphate formed in the cytosol is transported into the ER lumen by a specific transporter (T1) and hydrolyzed at the lumenal surface by the glucose 6-phosphatase. The resulting Pi and glucose are thought to be carried back into the cytosol by two different transporters (T2 and T3), and the glucose leaves the hepatocyte via yet another transporter in the plasma membrane (GLUT2). **Note* that by having the active site of glucose 6-phosphatase inside the ER lumen, the cell separates this reaction from the process of glycolysis, which takes place in the cytosoland would be aborted by the action of glucose 6-phosphatase**.

Genetic defects in either glucose 6-phosphatase or T1 lead to serious derangement of glycogen metabolism, resulting in type Ia glycogen storage disease.

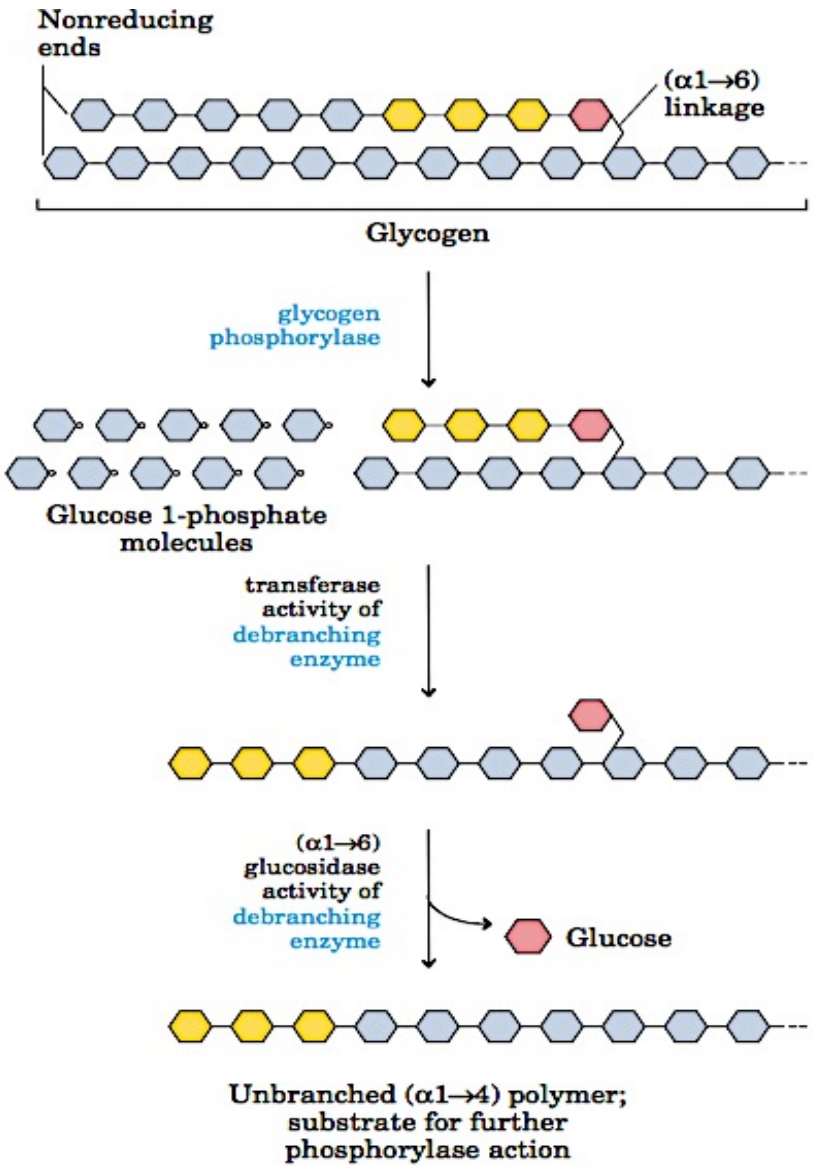


Figure 11 The reaction of the glycogen debranching enzyme.

Because muscle and adipose tissue lack glucose 6-phosphatase, they cannot convert the glucose 6-phosphate formed by glycogen breakdown to glucose, and these tissues therefore do not contribute glucose to the blood.

4.4.4.2- GLYCOGEN SYNTHESIS (GLYCOGENESIS)

Animals synthesize and store glycogen when glucose levels are high, but the synthetic pathway is not merely a reversal of the glycogen phosphorylase reaction. High levels of phosphate in the cell favor glycogen breakdown and prevent the phosphorylase reaction from synthesizing glycogen *in vivo,* in spite of the fact that $\Delta G'^{\circ}$ for the phosphorylase reaction actually favors glycogen synthesis. Hence, another reaction pathway must be employed in the cell for the net

synthesis of glycogen. In essence, this pathway must activate glucose units for transfer to glycogen chains.

Glucose Units are activated for transfer by formation of sugar nucleotides

Glycogen synthesis depended upon **sugar nucleotides,** which may be thought of as activated forms of sugar units. For example, formation of an ester linkage between the C-1 hydroxyl group and the β-phosphate of UDP activates the glucose moiety of **UDP-glucose.**

UDP-Glucose Synthesis is driven by pyrophosphate hydrolysis

Sugar nucleotides are formed from sugar-1-phosphates and nucleoside triphosphates by specific **pyrophosphorylase** enzymes. For example, **UDP-glucose pyrophosphorylase** catalyzes the formation of UDP-glucose from glucose-1-phosphate and uridine 5 –triphosphate (Figure 12):

Glucose-1-P + UTP → UDP-glucose pyrophosphate

The reaction proceeds via attack by a phosphate oxygen of glucose-1-phosphate on the α-phosphorus of UTP, with departure of the pyrophosphate anion. The reaction is a reversible one, but it is driven forward by subsequent hydrolysis of pyrophosphate:

Pyrophosphate + H_2O→2 Pi

The net reaction for sugar nucleotide formation (combining the preceding two equations) is thus Glucose-1-P + UTP + H_2O→ UDP-glucose +2 Pi

Sugar nucleotides of this type act as donors of sugar units in the biosynthesis of oligo- and polysaccharides. In animals, UDP-glucose is the donor of glucose units for glycogen synthesis, but ADP-glucose is the glucose source for starch synthesis in plants.

Glycogen Synthase catalyzes formation of α- (1→4) glycosidic bonds in glycogen

The very large glycogen polymer is built around a tiny protein core. The first glucose residue is covalently joined to the protein **glycogenin** via an acetal linkage to a tyrosine–OH group on the protein. Sugar units are added to the glycogen polymer by the action of **glycogen synthase.** The reaction involves transfer of a glucosyl unit from UDP-glucose to the C-4 hydroxyl group at a nonreducing end of a glycogen strand. The mechanism proceeds by cleavage of the C-O bond between the glucose moiety and the β-phosphate of UDP-glucose, leaving an oxonium ion intermediate, which is rapidly attacked by the C-4 hydroxyl oxygen of a terminal glucose unit on glycogen (Figure 13).

Glycogen branching occurs by transfer of terminal chain segments

Glycogen is a branched polymer of glucose units. The branches arise from α-(1→6) linkages which occur every 8 to 12 residues. The branches provide multiple sites for rapid degradation or elongation of the polymer and also increase its solubility. Glycogen branches are formed by amylo-(1→4) to (1→6) transglycosylase or glycosyl- (4→6) transferasealso known as *branching enzyme.* The reaction involves the transfer of a six- or seven-residue segment from the nonreducing end of a linear chain at least 11 residues in length to the C-6 hydroxyl of a glucose residue of the same chain or another chain (Figure 14). For each branching reaction, the resulting polymer has gained a new terminus at which growth can occur. Further glucose residues may be added to the new branch by glycogen synthase.

Note*-.*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.*

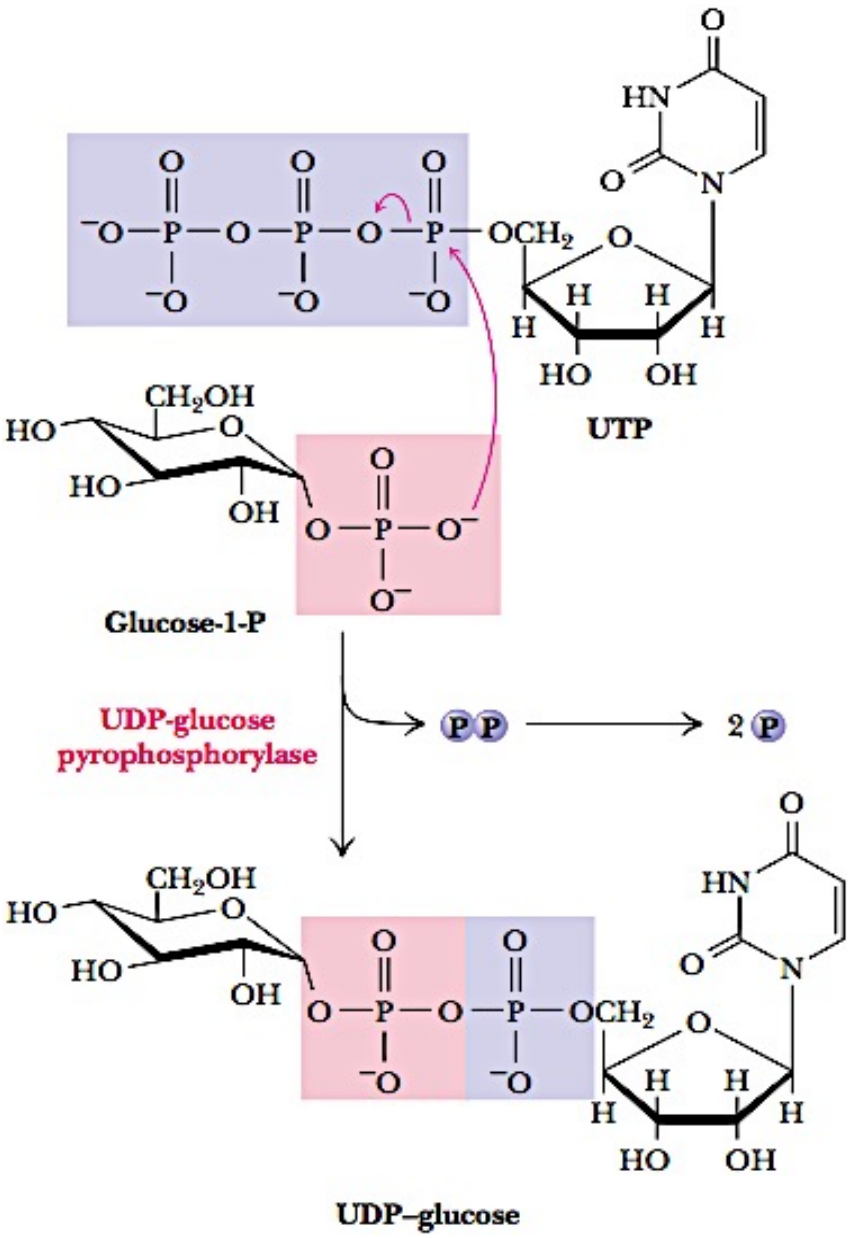


Figure 12 UDP-glucose pyrophosphorylase reaction

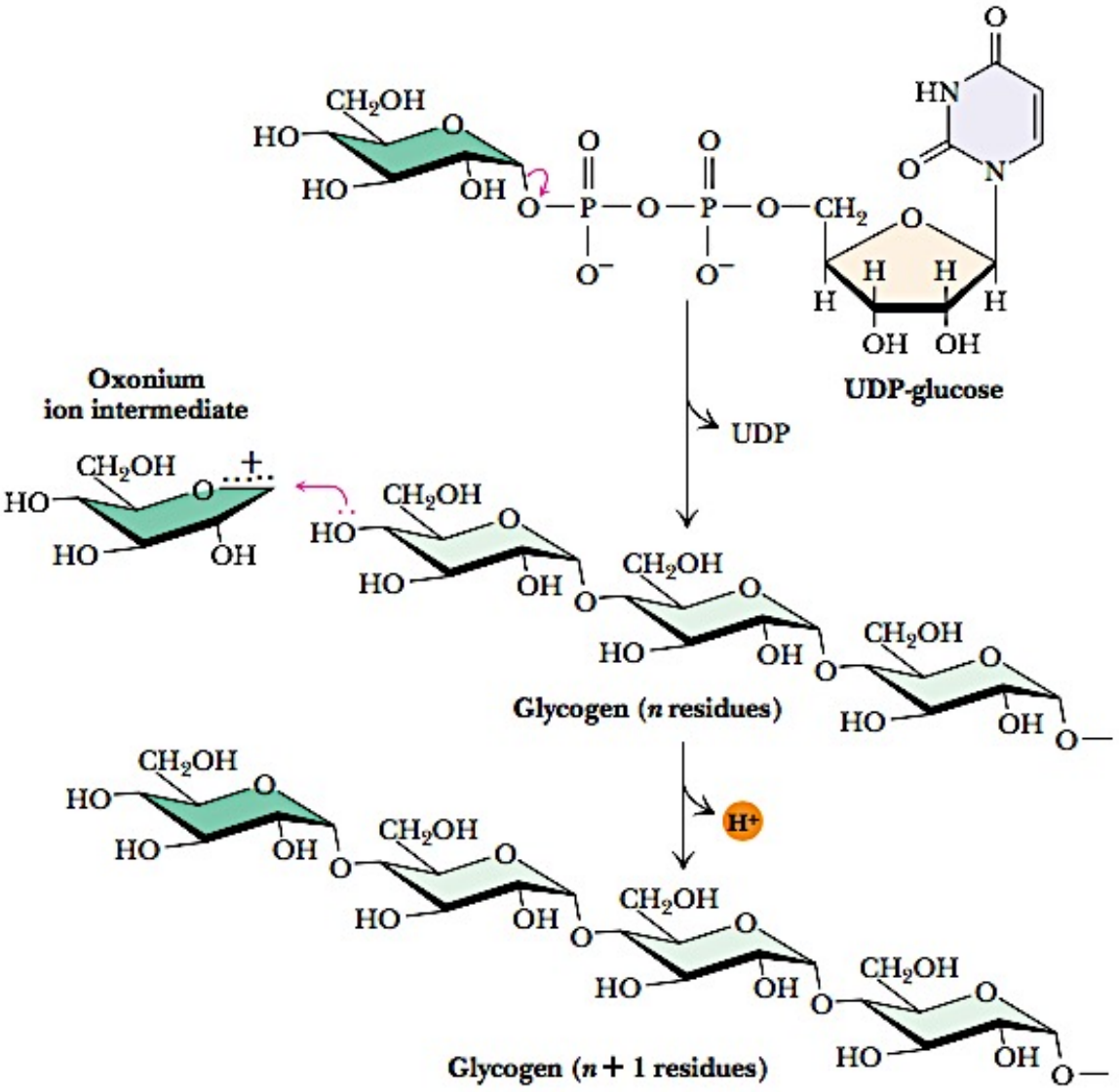


Figure 13 The glycogen synthase reaction

4.4.4.3 REGULATION OF GLYCOGEN METABOLISM

Control of glycogen metabolism is effected via reciprocal regulation of glycogen phosphorylase and glycogen synthase. Thus, activation of glycogen phosphorylase is tightly linked to inhibition of glycogen synthase, and vice versa. Regulation involves both allosteric control and covalent modification, with the latter being under hormonal control.

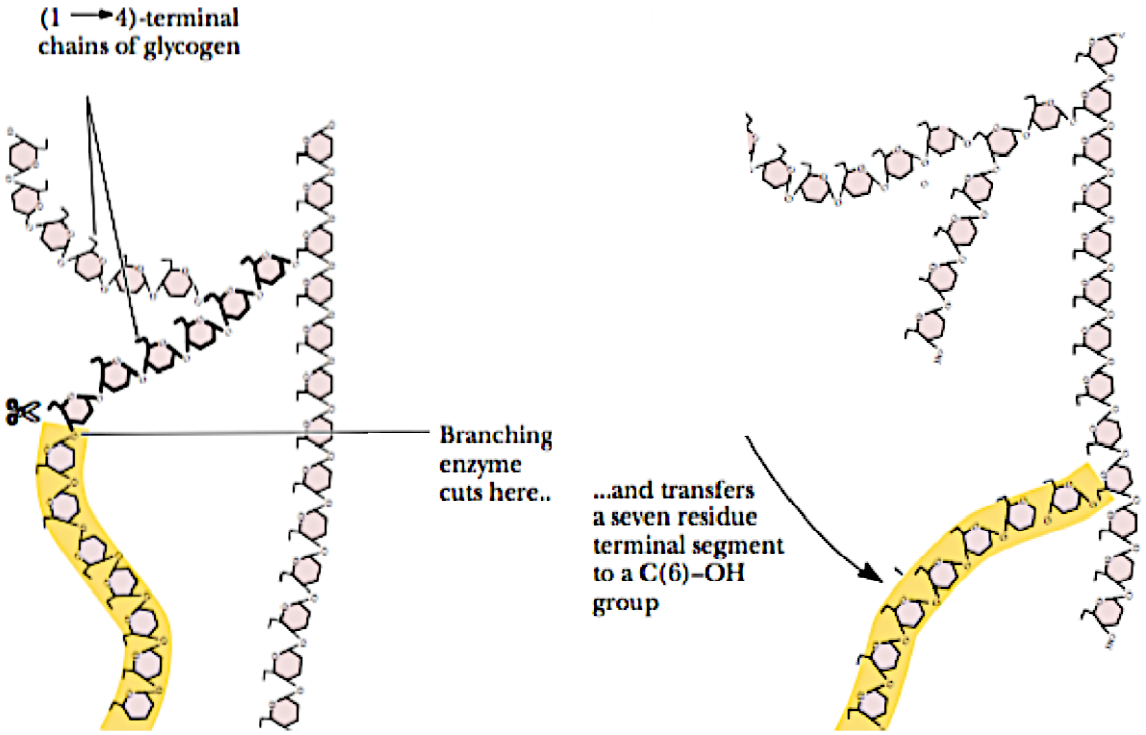


Figure 14 Formation of glycogen branches

Glycogen Phosphorylase

Glycogen metabolism is precisely controlled by multiple interlocking mechanisms, and the focus of this control is glycogen phosphorylase. *Phosphorylase is regulated by several allosteric effectors that signal the energy state of the cell as well as by reversible phosphorylation, which is responsive to hormones such as insulin, epinephrine, and glucagon.* There are differences in the control of glycogen metabolism in two tissues: skeletal muscle and liver. These differences are due to the fact that *the muscle uses glucose to produce energy for itself, whereas the liver maintains glucose homeostasis of the organism as a whole.*

The binding of the substrate *inorganic phosphate* (Pi) to muscle glycogen phosphorylase is highly cooperative, which allows the enzyme activity to increase markedly over a rather narrow range of substrate concentration. Pi is a *positive homotropic effector* with regard to its interaction with glycogen phosphorylase.

Feedback inhibition of glycogen phosphorylase by ATP and glucose-6-P provides a very effective way to regulate glycogen breakdown. Both ATP and glucose-6-P act by decreasing the affinity of glycogen phosphorylase for its substrate Pi. Because the binding of ATP or glucose-6-P has a negative effect on substrate binding, these substances act as *negative heterotropic effectors. When concentrations of ATP or glucose-6-P accumulate to high levels, glycogen phosphorylase is inhibited; when [ATP] and [glucose- 6-P] are low, the activity of glycogen phosphorylase is regulated by availability of its substrate, Pi.*

AMP also provides a regulatory signal to glycogen phosphorylase. It binds to the same site as ATP, but it stimulates glycogen phosphorylase rather than inhibiting it. AMP acts as a *positive heterotropic effector,* meaning that it enhances the binding of substrate to glycogen phosphorylase. Significant levels of AMP indicate that the energy status of the cell is low and that more energy (ATP) should be produced. Reciprocal changes in the cellular concentrations of ATP and AMP and their competition for binding to the same site (the *allosteric site*) on glycogen phosphorylase, with opposite effects, allow these two nucleotides to exert *rapid and reversible control* over glycogen phosphorylase activity. Such reciprocal regulation ensures that the production of energy (ATP) is commensurate with cellular needs.

To summarize, muscle glycogen phosphorylase is allosterically activated by AMP and inhibited by ATP and glucose-6-P; caffeine can also act as an allosteric inhibitor. When ATP and glucose-6-P are abundant, glycogen breakdown is inhibited. When cellular energy reserves are low (i.e., high [AMP] and low [ATP] and [G-6-P]), glycogen catabolism is stimulated.

The dimeric skeletal muscle phosphorylase exists in two interconvertible forms: the active form of the enzyme designated the **R state** and the inactive form denoted as the **T state**. Thus, AMP promotes the conversion to the active R state, whereas ATP, glucose-6-P, and caffeine favor conversion to the inactive T state.

Regulation of glycogen phosphorylase by covalent modification

As early as 1938, it was known that glycogen phosphorylase existed in two forms: the less active **phosphorylase *b*** and the more active **phosphorylase *a.*** In 1956, Edwin Krebs and Edmond Fischer reported that a "converting enzyme" could convert phosphorylase *b* to phosphorylase *a.* Three years later, Krebs and Fischer demonstrated that the conversion of phosphorylase *b* to phosphorylase *involved* covalent phosphorylation.

Enzyme cascades regulate glycogen phosphorylase

The phosphorylation reaction that activates glycogen phosphorylase is mediated by an **enzyme cascade**. The first part of the cascade leads to hormonal stimulation of **adenylyl cyclase,** a membrane-bound enzyme that converts ATP to *adenosine-3,5-cyclic monophos- phate,* denoted as *cyclic AMP* or simply *cAMP.* This regulatory molecule is found in all eukaryotic cells and acts as an intracellular messenger molecule, controlling a wide variety of processes. Cyclic AMP is known as a **second messenger** because it is the intracellular agent of a hormone (the "first messenger").

Cyclic AMP is an essential activator of *cAMP-dependent protein kinase (PKA).* This enzyme is normally inactive because its two catalytic subunits (C) are strongly associated with a pair of regulatory subunits (R), which serve to block activity. Binding of cyclic AMP to the regulatory subunits induces a conformation change that causes the dissociation of the C monomers from the R dimer. The free C subunits are active and can phosphorylate other proteins. One of the many proteins phosphorylated by PKA is *phosphorylase kinase*. Phosphorylase kinase is inactive in the unphosphorylated state and active in the phosphorylated form. As its name implies, phosphorylase kinase functions to phosphorylate (and activate) glycogen phosphorylase. Thus, stimulation of adenylyl cyclase leads to activation of glycogen breakdown.

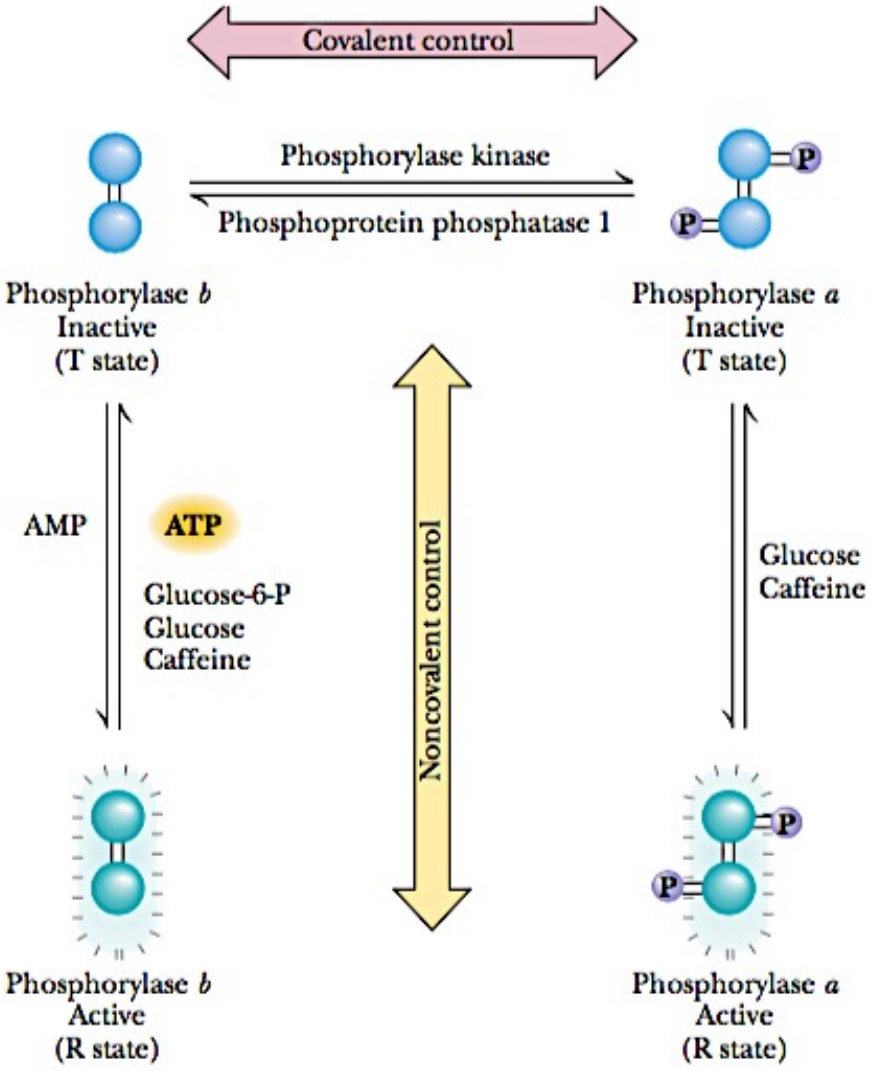


Figure 15 The mechanism of covalent modification and allosteric regulation of glycogen phosphorylase. The T states are blue and the R states blue-green.

Regulation of glycogen synthase by covalent modification

Glycogen synthase also exists in two distinct forms which can be interconverted by the action of specific enzymes: active, dephosphorylated **glycogen synthase I** (glucose-6-P-independent) and less active phosphorylated **glycogen synthase D** (glucose-6-P-dependent). The nature of phosphorylation is more complex with glycogen synthase. As many as nine serine residues on the enzyme appear to be subject to phosphorylation, each site's phosphorylation having some effect on enzyme activity.

Dephosphorylation of both glycogen phosphorylase and glycogen synthase is carried out by **phosphoprotein phosphatase 1.** The action of phosphoprotein phosphatase 1 inactivates glycogen phosphorylase and activates glycogen synthase.

Hormones regulate glycogen synthesis and degradation

Storage and utilization of tissue glycogen, maintenance of blood glucose concentration, and other aspects of carbohydrate metabolism are meticulously regulated by hormones, including *insulin, glucagon, epinephrine,* and the *glucocorticoids.*

Insulin is a response to increased blood glucose levels

The primary hormone responsible for conversion of glucose to glycogen is **insulin**. Insulin is secreted by special cells in the pancreas called the **islets of Langerhans.** Secretion of insulin is a response to increased glucose in the blood. Insulin acts to rapidly lower blood glucose concentration in several ways. Insulin stimulates glycogen synthesis and inhibits glycogen breakdown in liver and muscle.

Several other physiological effects of insulin also serve to lower blood and tissue glucose levels. Insulin stimulates the active transport of glucose (and amino acids) across the plasma membranes of muscle and adipose tissue. Insulin also increases cellular utilization of glucose by inducing the synthesis of several important glycolytic enzymes, namely, glucokinase, phosphofructokinase, and pyruvate kinase. In addition, insulin acts to inhibit several enzymes of gluconeogenesis. These various actions enable the organism to respond quickly to increases in blood glucose levels.

Glucagon and epinephrine stimulate glycogen breakdown

Catabolism of tissue glycogen is triggered by the actions of the hormones **epinephrine** and **glucagon.** In response to decreased blood glucose, glucagon is released from the cells in pancreatic islets of Langerhans. This peptide hormone travels through the blood to specific receptors on liver cell membranes. (Glucagon is active in liver and adipose tissue, but not in other tissues.) Similarly, signals from the central nervous system cause release of epinephrine —also known as adrenaline—from the adrenal glands into the bloodstream. Epinephrine acts on liver and muscles. When either hormone binds to its receptor on the outside surface of the cell membrane, a cascade is initiated that activates glycogen phosphorylase and inhibits glycogen synthase. The result of these actions is tightly coordinated stimulation of glycogen breakdown and inhibition of glycogen synthesis.

Cortisol and glucocorticoid effects on glycogen metabolism

Glucocorticoids are a class of steroid hormones that exert distinct effects on liver, skeletal muscle, and adipose tissue. The effects of cortisol, a typical glucocorticoid, are best described as *catabolic* because cortisol promotes protein breakdown and decreases protein synthesis in skeletal muscle. In the liver, however, it stimulates gluconeogenesis and increases glycogen synthesis. Cortisol- induced gluconeogenesis results primarily from increased conversion of amino acids into glucose. Specific effects of cortisol in the liver include increased gene expression of several of the enzymes of the gluconeogenic pathway, activation of enzymes involved in amino acid metabolism, and stimulation of the urea cycle, which disposes of nitrogen liberated during amino acid catabolism.

4.4.5 CITRIC ACID CYCLE

Glucose can be metabolized to pyruvate anaerobically to synthesize ATP through the glycolytic pathway. Glycolysis, however, harvests a fraction of the ATP available from glucose. Whereas, aerobic processing of glucose, is the source of most of the ATP generated in metabolism. This

aerobic phase of catabolism is called respiration or more precisely cellular respiration. The aerobic processing of glucose starts with the complete oxidation of glucose derivatives to carbon dioxide. This oxidation takes place in the *citric acid cycle,* a series of reactions also known as the *tricarboxylic acid (TCA) cycle* or the *Krebs cycle.* **The citric acid cycle is the *final common pathway for the oxidation of fuel molecules* —amino acids, fatty acids, and carbohydrates. Most fuel molecules enter the cycle as acetyl coenzyme A.**

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 group of acetyl-coenzyme A (acetyl-CoA).

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

Production of Acetyl-CoA

The formation of acetyl CoA from carbohydrates is less direct than from fat. Recall that carbohydrates, most notably glucose, are processed by glycolysis into pyruvate. Under anaerobic conditions, the pyruvate is converted into lactic acid or ethanol, depending on the organism. Note that, in the preparation of the glucose derivative pyruvate for the citric acid cycle, an oxidative decarboxylation takes place and high transfer-potential electrons in the form of NADH are captured. Thus, the pyruvate dehydrogenase reaction has many of the key features of the reactions of the citric acid cycle itself.

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 and fatty acids are degraded to the acetyl group of acetyl-CoA, the form in which the cycle accepts most of its fuel input. Many amino acid carbons also enter the cycle this way, although several amino acids are degraded to other cycle intermediates. Under aerobic conditions, the pyruvate is transported into mitochondria in exchange for OH^- by the pyruvate carrier, an antiporter. In the mitochondrial matrix, pyruvate is oxidatively decarboxylated by the *pyruvate dehydrogenase complex* to form acetyl CoA. *This irreversible reaction is the link between glycolysis and the citric acid cycle.*the pyruvate dehydrogenase (PDH) complex, is a cluster of enzymes—multiple copies of each of three enzymes—located in the mitochondria of eukaryotic cells and in the cytosol of prokaryotes.

Pyruvate Is Oxidized to Acetyl-CoA and CO_2

The overall reaction catalyzed by the pyruvate dehydrogenase complex is an oxidative decarboxylation, 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. 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 pyruvate dehydrogenase complex requires five coenzymes

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, nicotinamide adenine dinucleotide (NAD), and lipoate. *thiamine pyrophosphate (TPP), lipoic acid,* and *FAD* serve as catalytic cofactors, and CoA and NAD+ are stoichiometric cofactors.

The PDH complex contains three enzymes—pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3)—each present in multiple copies. E2 is the point of connection for the prosthetic group lipoate, attached through an amide bond to the ε-amino group of a Lys residue. E2 has three functionally distinct domains: the amino-terminal lipoyl domain, containing the lipoyl-Lys residue(s); the central E1- and E3-binding domain; and the inner-core acyltransferase domain, which contains the acyltransferase active site. The active site of E1 has bound TPP, and that of E3 has bound FAD. Also part of the complex are two regulatory proteins, a protein kinase and a phosphoprotein phosphatase. This basic E1-E2-E3 structure has been conserved during evolution.

Pyruvate dehydrogenase complex carries out the five consecutive reactions in the decarboxylation and dehydrogenation of pyruvate (Figure 16).

Step 1 -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. At this stage the PDH complex exercises its substrate specificity.

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^+.

Central to the mechanism of the PDH complex are the swinging lipoyllysyl arms of E2, which accept from E1 the two electrons and the acetyl group derived from pyruvate, passing them to E3. All these enzymes and coenzymes are clustered, allowing the intermediates to react quickly without diffusing away from the surface of the enzyme complex. The five-reaction sequence is thus an example of substrate channeling. The intermediates of the multistep sequence never leave the complex, and the local concentration of the substrate of E2 is kept very high. Channeling also prevents theft of the activated acetyl group by other enzymes that use this group as substrate.

Reactions of the Citric Acid Cycle

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 these intermediates cells employ anaplerotic (replenishing) reactions

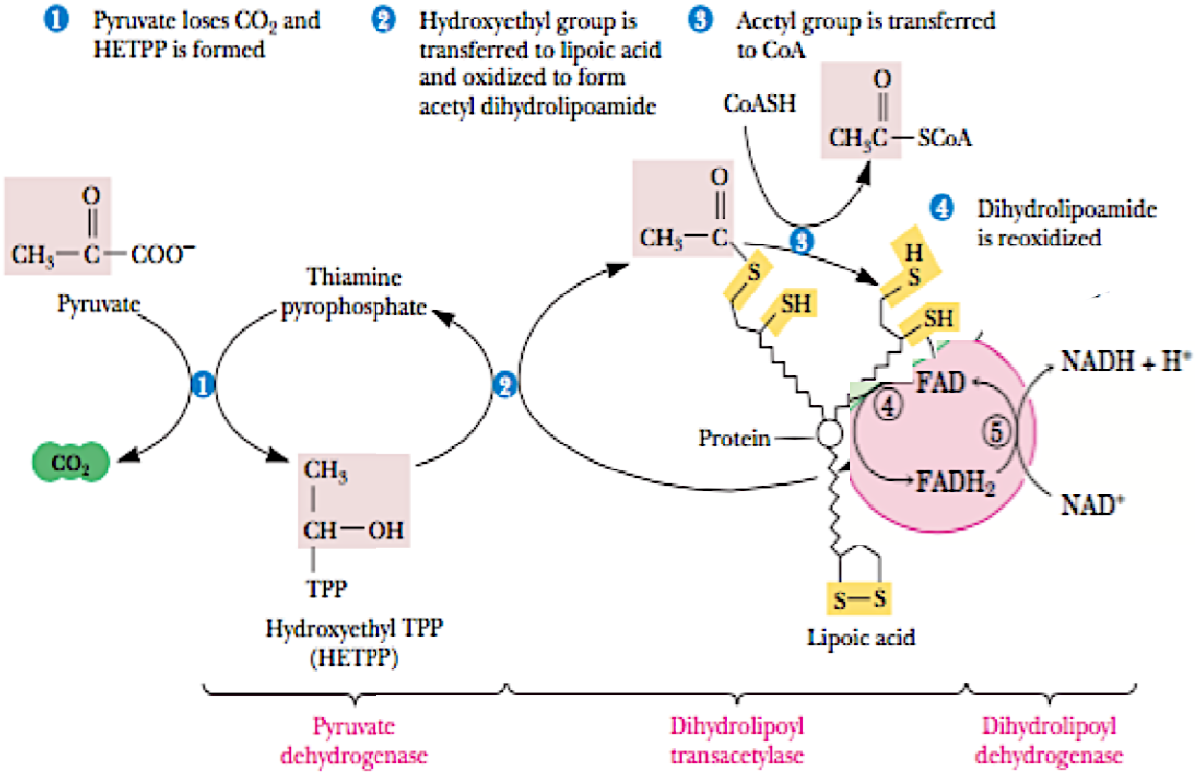


Figure 16 Oxidative decarboxylation of pyruvate to acetyl-CoA by the PDH complex.

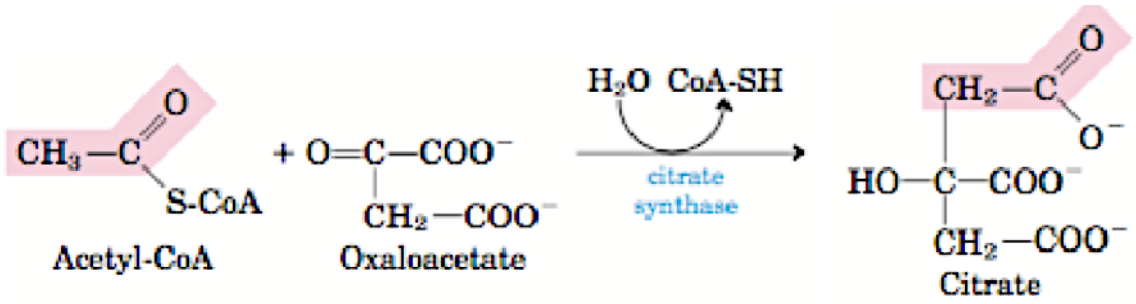
The Citrate Synthase Reaction

The first reaction within the TCA cycle, the one by which carbon atoms are introduced, is the **citrate synthase reaction**. Here acetyl-CoA reacts with oxaloacetate in a **Perkin condensation** (a carbon–carbon condensation between a ketone or aldehyde and an ester). In this reaction the methyl carbon of the acetyl group is joined to the carbonyl group (C-2) of oxaloacetate. Citroyl-CoA is a transient intermediate formed on the active site of the enzyme. It rapidly undergoes hydrolysis to free CoA and citrate, which are released from the active site. The hydrolysis of this high-energy thioester intermediate makes the forward reaction highly exergonic. The large, negative standard free-energy change of the citrate synthase reaction is essential to the operation of the cycle because, as noted earlier, the concentration of oxaloacetate is normally very low. The CoA liberated in this reaction is recycled to participate in the oxidative decarboxylation of an- other molecule of pyruvate by the PDH complex.

Citrate synthase in mammals is a dimer of 49-kD subunits. On each subunit, oxaloacetate and acetyl-CoA bind to the active site, which lies in a cleft between two domains and is surrounded

mainly by α-helical segments. Binding of oxaloacetate induces a conformational change that facilitates the binding of acetyl-CoA and closes the active site, so that the reactive carbanion of acetyl-CoA is protected from protonation by water.

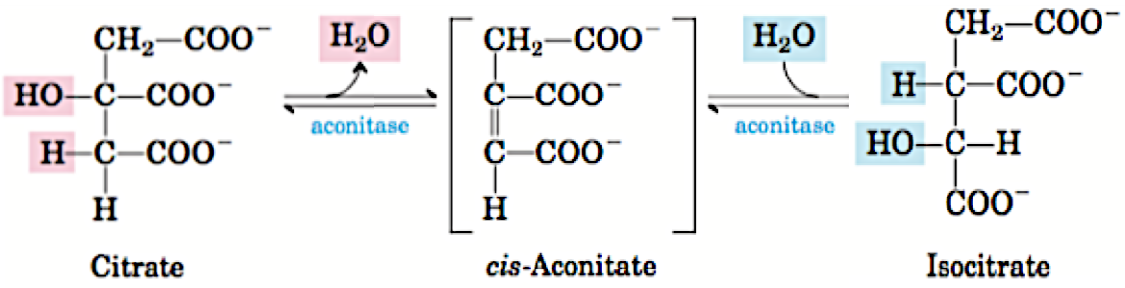
Citrate synthase is the first step in this metabolic pathway and this reaction has a large negative $\Delta G'°$. As expected, citrate synthase is a highly regulated enzyme. NADH, a product of the TCA cycle, is an allosteric inhibitor of citrate synthase, as is succinyl-CoA, the product of the fifth step in the cycle (and an acetyl-CoA analog).



Formation of isocitrate via cis-aconitate

Citrate itself poses a problem: it is a poor candidate for further oxidation because it contains a tertiary alcohol, which could be oxidized only by breaking a carbon–carbon bond. An obvious solution to this problem is to isomerize the tertiary alcohol to a secondary alcohol, which the cycle proceeds to do in the next step

The enzyme aconitase catalyzes the reversible transformation of citrate to isocitrate, through the intermediary formation of the tricarboxylic acid cis-aconitate, which normally does not dissociate from the active site. Aconitase promotes the reversible addition of H_2O to the double bond of enzyme-bound cis-aconitate in two different ways, one leading to citrate and the other to isocitrate:

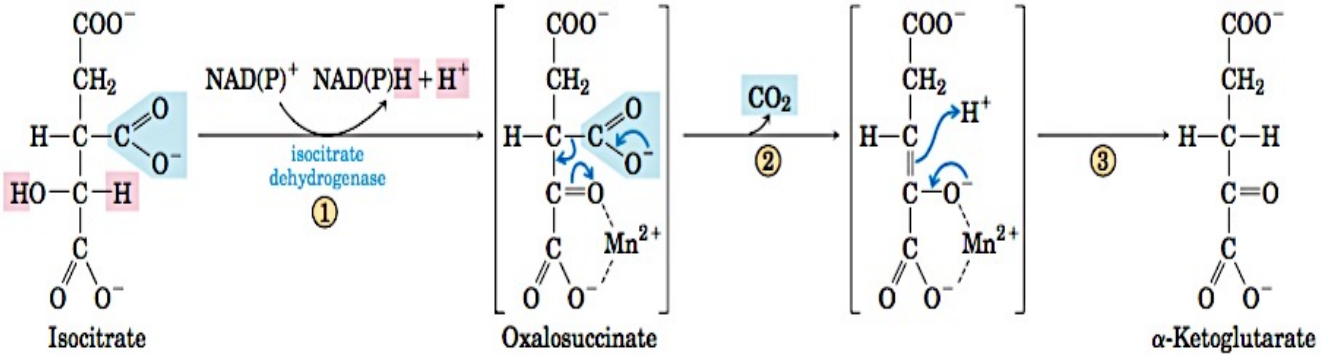


Isocitrate Dehydrogenase—The First Oxidation in the Cycle

In the next step of the TCA cycle, isocitrate is oxidatively decarboxylated to yield α-ketoglutarate, with concomitant reduction of NAD to NADH in the isocitrate dehydrogenase reaction. This two-step reaction involves (1) oxidation of the C-2 alcohol of isocitrate to form oxalosuccinate, followed by (2) a β-decarboxylation reaction that expels the central carboxyl group as **CO_2**, leaving the product α-ketoglutarate. Oxalosuccinate, the β-keto acid produced by the initial dehydrogenation reaction, is unstable and thus is readily decarboxylated.

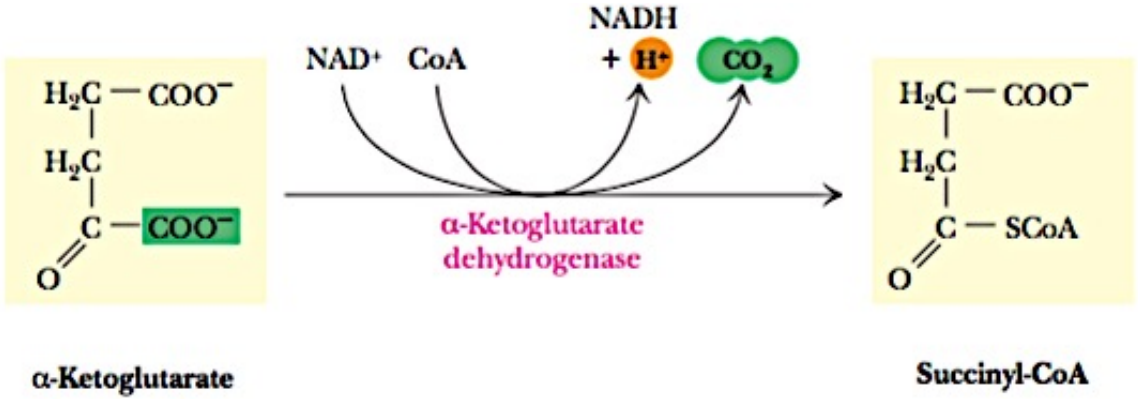
Isocitrate dehydrogenase links the TCA cycle and electron transport

Isocitrate dehydrogenase provides the first connection between the TCA cycle and the electron transport pathway and oxidative phosphorylation, via its production of NADH. As a connecting point between two metabolic pathways, dehydrogenase of isocitrate is a regulated reaction. NADH and ATP are allosteric inhibitors, whereas ADP acts as an allosteric activator. The enzyme is virtually inactive in the absence of ADP. The product, α-ketoglutarate, is a crucial α-keto acid for aminotransferase reactions, connecting the TCA cycle (that is, carbon metabolism) with nitrogen metabolism.



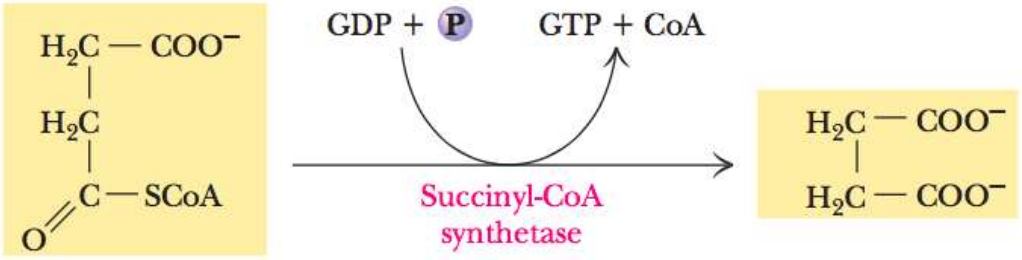
α-Ketoglutarate Dehydrogenase— A Second Decarboxylation

A second oxidative decarboxylation in the citric acid cycle is catalysed by **α-ketoglutarate dehydrogenase reaction**. Like the pyruvate dehydrogenase complex, α-ketoglutarate dehydrogenase is a multienzyme complex—consisting of *α-ketoglutarate dehydrogenase, dihydrolipoyl transsuccinylase,* and *dihydrolipoyl dehydrogenase*—that employs five different coenzyme. The dihydrolipoyl dehydrogenase in this reaction is identical to that in the pyruvate dehydrogenase reaction. The mechanism is analogous to that of pyruvate dehydrogenase. As with the pyruvate dehydrogenase reaction, this reaction produces NADH and a thioester product—in this case, succinyl-CoA. Succinyl-CoA and NADH products are energy-rich species that are important sources of metabolic energy in subsequent cellular processes.



Succinyl-CoA Synthetase—A Substrate- Level Phosphorylation

The NADH produced in the foregoing steps are routed through the electron transport pathway to make high-energy phosphates via oxidative phosphorylation. However, succinyl-CoA is itself a high-energy intermediate and is utilized in the next step of the TCA cycle to drive the phosphorylation of GDP to GTP (in mammals) or ADP to ATP (in plants and bacteria). The reaction is catalyzed by **succinyl-CoA synthetase,** sometimes called **succinate thiokinase.** The free energies of hydrolysis of succinyl-CoA and GTP or ATP are similar.



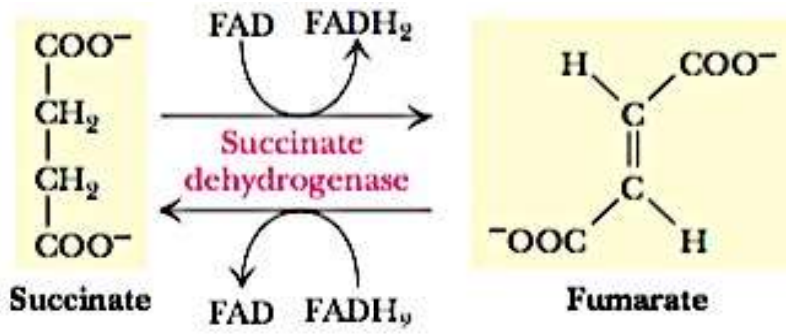
Succinyl-CoA synthetase provides another example of a **substrate-level phosphorylation**, in which a substrate, rather than an electron transport chain or proton gradient, provides the energy for phosphorylation. It is the only such reaction in the TCA cycle. The GTP produced by mammals in this reaction can exchange its terminal phosphoryl group with ADP via the **nucleoside diphosphate kinase reaction**

$$GTP + ADP \rightleftharpoons GDP + ATP$$

Succinate Dehydrogenase—Oxidation involving FAD

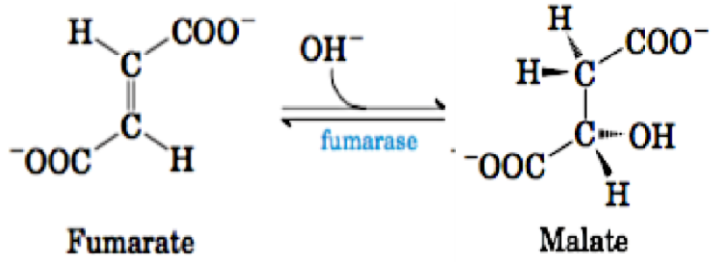
The oxidation of succinate to fumarate is carried out by **succinate dehydrogenase,** a membrane-bound enzyme that is actually part of the succinate–coenzyme Q reductase of the electron transport chain. In contrast with all of the other enzymes of the TCA cycle, which are soluble proteins found in the mitochondrial matrix, succinate dehydrogenase is an integral membrane protein tightly associated with the inner mitochondrial membrane. Succinate oxidation involves removal of H atoms across a C-C bond, rather than a C-O or C-N bond, and produces the *trans*- unsaturated fumarate. This reaction (the oxidation of an alkane to an alkene) is not sufficiently exergonic to reduce NAD^+, but it does yield enough energy to reduce [FAD].

Succinate dehydrogenase is a dimeric protein, with subunits of molecular masses 70 kD and 27 kD. The enzyme contains three different iron-sulfur clusters and one molecule of covalently bound FAD. Electrons pass from succinate through the FAD and iron-sulfur centers before entering the chain of electron carriers in the mitochondrial inner membrane (or the plasma membrane in bacteria). Electron flow from succinate through these carriers to the final electron acceptor, $\mathbf{O_2}$, is coupled to the synthesis of about 1.5 ATP molecules per pair of electrons (respiration-linked phosphorylation).



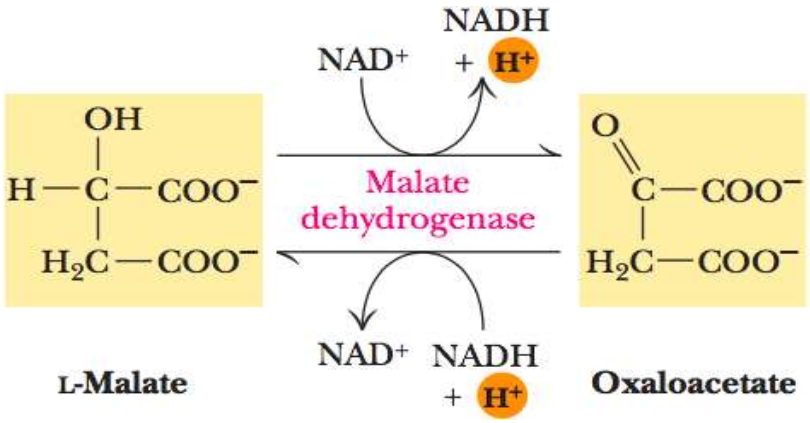
Fumarase catalyzes *trans*-hydration of fumarate

Fumarate is hydrated in a stereospecific reaction by fumarase to give L-malate. The reaction involves *trans*-addition of the elements of water across the double bond. Though the exact mechanism is uncertain, it may involve protonation of the double bond to form an intermediate carbonium ion or possibly attack by water or OH^- anion to produce a carbanion, followed by protonation. 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.



Malate Dehydrogenase—Completing the Cycle

In the last step of the TCA cycle, L-malate is oxidized to oxaloacetate by **malate dehydrogenase**. This reaction is very endergonic. Consequently, the concentration of oxaloacetate in the mitochondrial matrix is usually quite low. The reaction, however, is pulled forward by the favorable citrate synthase reaction. Oxidation of malate is coupled to reduction of yet another molecule of NAD^+, the third one of the cycle.



The energy of oxidations in the cycle is efficiently conserved

A two-carbon acetyl group enters the TCA cycle and joins 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 gets regenerated.

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.

The energy yield from the production of two molecules of pyruvate from one molecule of glucose in glycolysis 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 $6CO_2$ 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 generated per glucose.

The TCA cycle provides intermediates for biosynthetic pathways

The citric acid cycle is an amphibolic pathway 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. Four-, five-, and six-carbon species produced in the TCA cycle also act as fuel for variety of **biosynthetic processes.** α-Ketoglutarate, succinyl-CoA, fumarate and oxaloacetate are all precursors of important cellular species. A transamination reaction converts α-ketoglutarate directly to glutamate, which can then serve as a versatile precursor for proline, arginine, and glutamine. Succinyl- CoA provides most of the carbon atoms of the porphyrins. Oxaloacetate can be transaminated to produce aspartate. Aspartic acid itself is a precursor of the pyrimidine nucleotides and, in addition, is a key precursor for the synthesis of asparagine, methionine, lysine, threonine, and isoleucine. Oxaloacetate can also be decarboxylated to yield PEP, which is a key element of several pathways, namely (1) synthesis (in plants and microorganisms) of the aromatic amino acids phenylalanine, tyrosine, and tryptophan; (2) formation of 3-phospho-glycerate and conversion to the amino acids serine, glycine, and cysteine; and (3) *gluconeogenesis*

Finally, citrate can be exported from the mitochondria and then broken down by **ATP-citrate lyase** to yield oxaloacetate and acetyl-CoA, a precursor of fatty acids. Oxaloacetate produced in this reaction is rapidly reduced to malate, which can then be processed in either of two ways: it may be transported into mitochondria, where it is reoxidized to oxaloacetate, or it may be oxidatively decarboxylated to pyruvate by **malic enzyme,** with subsequent mitochondrial uptake of pyruvate. This cycle permits citrate to provide acetyl-CoA for biosynthetic processes, with return of the malate and pyruvate by-products to the mitochondria.

4.4.5.1 The Anaplerotic, or "Filling Up," Reactions

In a sort of reciprocal arrangement, the cell also feeds many intermediates back into the TCA cycle from other reactions. Since such reactions replenish the TCA cycle intermediates, Hans Kornberg proposed that they be called **anaplerotic reactions** (literally, the "filling up" reactions). Thus, **PEP carboxylase** and **pyruvate carboxylase** synthesize oxaloacetate from pyruvate.

Pyruvate carboxylase enzyme catalysed reaction is the most important of the anaplerotic reactions. It exists in the mitochondria of animal cells but not in plants, and it provides a direct link between glycolysis and the TCA cycle. The enzyme is tetrameric and contains covalently bound biotin and an Mg^{2+}site on each subunit. Pyruvate carboxylase has an absolute allosteric requirement for acetyl-CoA. Thus, when acetyl-CoA levels exceed the oxaloacetate supply, allosteric activation of pyruvate carboxylase by acetyl-CoA raises oxaloacetate levels, so that the excess acetyl-CoA can enter the TCA cycle.

PEP carboxylase occurs in yeast, bacteria, and higher plants, but not in animals. The enzyme is specifically inhibited by aspartate, which is produced by transamination of oxaloacetate. Thus, organisms utilizing this enzyme control aspartate production by regulation of PEP carboxylase. Malic enzyme is found in the cytosol or mitochondria of many animal and plant cells and is an NADPH- dependent enzyme.

CO_2 binds weakly to PEP carboxykinase, whereas oxaloacetate binds very tightly, and, as a result, the enzyme favors formation of PEP from oxaloacetate.

The catabolism of amino acids provides pyruvate, acetyl-CoA, oxaloacetate, fumarate, α-ketoglutarate, and succinate, all of which may be oxidized by the TCA cycle. In this way, proteins may serve as excellent sources of nutrient energy.

4.4.5.2 Regulation of the TCA Cycle

The link between glycolysis and the electron transport chain ensures that TCA cycle must be carefully controlled by the cell. If the cycle is permitted to run unchecked, large amounts of metabolic energy will be wasted in overproduction of reduced coenzymes and ATP; conversely, if it ran too slowly, ATP would not be produced rapidly enough to satisfy the needs of the cell. The TCA cycle is an important source of precursors for biosynthetic processes and must be able to provide them as needed.

What are the sites of regulation in the TCA cycle? We might anticipate that some of the reactions of the TCA cycle would operate near equilibrium under cellular conditions (with $\Delta G = 0$), whereas others—the sites of regulation—would be characterized by large, negative ΔG values. Three reactions of the cycle—citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase—operate with large, negative G values under mitochondrial conditions and are thus the primary sites of regulation in the cycle.

The principal regulatory "signals" are the concentrations of acetyl-CoA, ATP, NAD^+, and NADH, with additional effects provided by several other metabolites. The main sites of regulation are pyruvate dehydrogenase, citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase. NADH inhibits all of these enzymes, so that when the cell has produced all the NADH that can conveniently be turned into ATP, the cycle shuts down. For similar reasons, ATP is an inhibitor of pyruvate dehydrogenase and isocitrate dehydrogenase. The TCA cycle is turned on, however, when either the ADP/ATP or NAD^+ /NADH ratio is

high, an indication that the cell has run low on ATP or NADH. Regulation of the TCA cycle by NADH, NAD^+, ATP, and ADP thus reflects the energy status of the cell. On the other hand, succinyl-CoA is an *intracycle regulator,* inhibiting citrate synthase and α-ketoglutarate dehydrogenase. Acetyl-CoA acts as a signal to the TCA cycle that glycolysis or fatty acid breakdown produces two-carbon units. Acetyl-CoA activates pyruvate carboxylase, the anaplerotic reaction that provides oxaloacetate, the acceptor for increased flux of acetyl-CoA into the TCA cycle.

Regulation of Pyruvate Dehydrogenase

High levels of either product, acetyl-CoA or NADH, allosterically inhibit the pyruvate dehydrogenase complex. Acetyl-CoA specifically blocks dihydrolipoyl transacetylase, and NADH acts on dihydrolipoyl dehydrogenase. The mammalian pyruvate dehydrogenase is also regulated by covalent modifications. A Mg^{2+}-dependent **pyruvate dehydrogenase kinase** is allosterically activated by NADH and acetyl-CoA, and when levels of these metabolites rise in the mitochondrion, they stimulate phosphorylation of a serine residue on the pyruvate dehydrogenase subunit, blocking the first step of the pyruvate dehydrogenase reaction, the decarboxylation of pyruvate. Inhibition of the dehydrogenase in this manner eventually lowers the levels of NADH and acetyl-CoA in the matrix of the mitochondrion. Reactivation of the enzyme is carried out by **pyruvate dehydrogenase phosphatase,** a Ca^{2+}-activated enzyme that binds to the dehydrogenase complex and hydrolyzes the phosphoserine moiety on the dehydrogenase subunit. At low ratios of NADH to $NAD^+$and low acetyl-CoA levels, the phosphatase maintains the dehydrogenase in an activated state, but a high level of acetyl-CoA or NADH once again activates the kinase and leads to the inhibition of the dehydrogenase. Insulin and Ca^{2+}ions activate dephosphorylation, and pyruvate inhibits the phosphorylation reaction.

Pyruvate dehydrogenase is also sensitive to the energy status of the cell. AMP activates pyruvate dehydrogenase, whereas GTP inhibits it. High levels of AMP indicate that the cell may become energy-poor. Activation of pyruvate dehydrogenase under such conditions commits pyruvate to energy production.

Regulation of Isocitrate Dehydrogenase

The mechanism of regulation of isocitrate dehydrogenase is in some respects the reverse of pyruvate dehydrogenase. The mammalian isocitrate dehydrogenase is subject only to allosteric activation by ADP and NAD^+ and to inhibition by ATP and NADH. Thus, high NAD^+ /NADH and ADP/ATP ratios stimulate isocitrate dehydrogenase and TCA cycle activity. The *Escherichia coli* enzyme, on the other hand, is regulated by covalent modification. Serine residues on each subunit of the dimeric enzyme are phosphorylated by a protein kinase, causing inhibition of the isocitrate dehydrogenase activity. Activity is restored by the action of a specific phosphatase. When TCA cycle and glycolytic intermediates—such as isocitrate, 3-phosphoglycerate, pyruvate, PEP, and oxaloacetate—are high, the kinase is inhibited, the phosphatase is activated, and the TCA cycle operates normally. When levels of these intermediates fall, the kinase is activated, isocitrate dehydrogenase is inhibited, and isocitrate is diverted to the *glyoxylate pathway*.

4.4.6- THE GLYOXYLATE CYCLE OF PLANTS AND BACTERIA

Plants (particularly seedlings, which cannot yet accomplish efficient photosynthesis), as well as some bacteria and algae, acetate can serve both as an energy-rich fuel and as a source of phosphoenolpyruvate for carbohydrate synthesis.

Although TCA cycle can supply intermediates for some biosynthetic processes, the cycle gives off $2CO_2$ for every two-carbon acetate group that enters and cannot affect the *net synthesis* of TCA cycle intermediates. Thus, it would not be possible for the cycle to produce massive amounts of biosynthetic intermediates needed for acetate-based growth unless alternative reactions are available. In essence, the TCA cycle is geared primarily to energy production, and it "wastes" carbon units by giving off CO_2. Modification of the cycle to support acetate-based growth would require elimination of the CO_2-producing reactions and enhancement in the net production of four-carbon units (i.e., oxaloacetate). Plants and bacteria employ a modification of the TCA cycle called the **glyoxylate cycle** to produce four-carbon dicarboxylic acids (and eventually even sugars) from two-carbon acetate units. The glyoxylate cycle catalyze the net conversion of acetate to succinate or other four- carbon intermediates of the citric acid cycle:

$$2 \text{ Acetyl-CoA} + NAD^+ + 2H_2O \rightarrow \text{succinate} + 2CoA + NADH + H^+$$

The site of the Glyoxylate Cycle

The enzymes of the glyoxylate cycle in plants are present in **glyoxysomes,** organelles devoted to this cycle. Yeast and algae carry out the glyoxylate cycle in the cytoplasm.

The Cycle

In the glyoxylate cycle (Figure 17), acetyl-CoA condenses with oxaloacetate to form citrate, and citrate is converted to isocitrate, exactly as in the citric acid cycle. The next step, however, is not the breakdown of isocitrate by isocitrate dehydrogenase but the cleavage of isocitrate by isocitrate lyase, forming succinate and glyoxylate. The glyoxylate then condenses with a second molecule of acetyl-CoA to yield malate, in a reaction catalyzed by malate synthase. The malate is subsequently oxidized to oxaloacetate, which can condense with another molecule of acetyl-CoA to start another turn of the cycle. 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. Succinate is converted through fumarate and malate into oxaloacetate, which can then be converted to phosphoenolpyruvate by PEP carboxykinase, and thus to glucose by gluconeogenesis.

Note* 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 lipids.

The glyoxylate cycle helps plants grow in the dark

The existence of the glyoxylate cycle explains how certain seeds grow underground (or in the dark), where photosynthesis is impossible. Many seeds (peanuts, soybeans, and castor beans, for example) are rich in lipids, most organisms degrade the fatty acids of lipids to acetyl- CoA. Glyoxysomes form in seeds as germination begins, and the glyoxylate cycle uses the acetyl-CoA produced in fatty acid oxidation to provide large amounts of oxaloacetate and other intermediates for carbohydrate synthesis. Once the growing plant begins photosynthesis and fix CO_2 to produce carbohydrates, the glyoxysomes disappear.

Glyoxysomes must borrow three reactions from mitochondria

Glyoxysomes does not contain all the enzymes needed to run the glyoxylate cycle; succinate dehydrogenase, fumarase, and malate dehydrogenase are absent. Consequently, glyoxysomes must cooperate with mitochondria to run their cycle. Succinate travels from the glyoxysomes to the mitochondria, where it is converted to oxaloacetate. Transamination to aspartate follows because oxaloacetate cannot be transported out of the mitochondria. Aspartate formed in this way then moves from the mitochondria back to the glyoxysomes, where a reverse transamination with α-ketoglutarate forms oxaloacetate, completing the shuttle. Finally, to balance the transaminations, glutamate shuttles from glyoxysomes to mitochondria.

4.4.6.1 The Citric Acid and Glyoxylate Cycles are coordinately regulated

The sharing of common intermediates between **Citric Acid and Glyoxylate Cycles** requires that these pathways be coordinately regulated (Figure 18). 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.

The phosphoprotein phosphatase that activates isocitrate dehydrogenase is stimulated by intermediates of the citric acid cycle and glycolysis and by indicators of reduced cellular energy supply. The same metabolites inhibit the protein kinase activity of the bifunctional polypeptide. Thus, the accumulation of intermediates of the central energy-yielding pathways—indicating energy depletion—results in the activation of isocitrate dehydrogenase. When the concentration of these regulators falls, signaling a sufficient flux through the energy-yielding citric acid cycle, isocitrate dehydrogenase is inactivated by the protein kinase. The same intermediates of glycolysis and the citric acid cycle that activate isocitrate dehydrogenase are allosteric inhibitors of isocitrate lyase. When energy-yielding metabolism is sufficiently fast to keep the concentrations of glycolytic and citric acid cycle intermediates low, isocitrate dehydrogenase is inactivated, the inhibition of isocitrate lyase is relieved, and isocitrate flows into the glyoxylate pathway, to be used in the biosynthesis of carbohydrates, amino acids, and other cellular components.

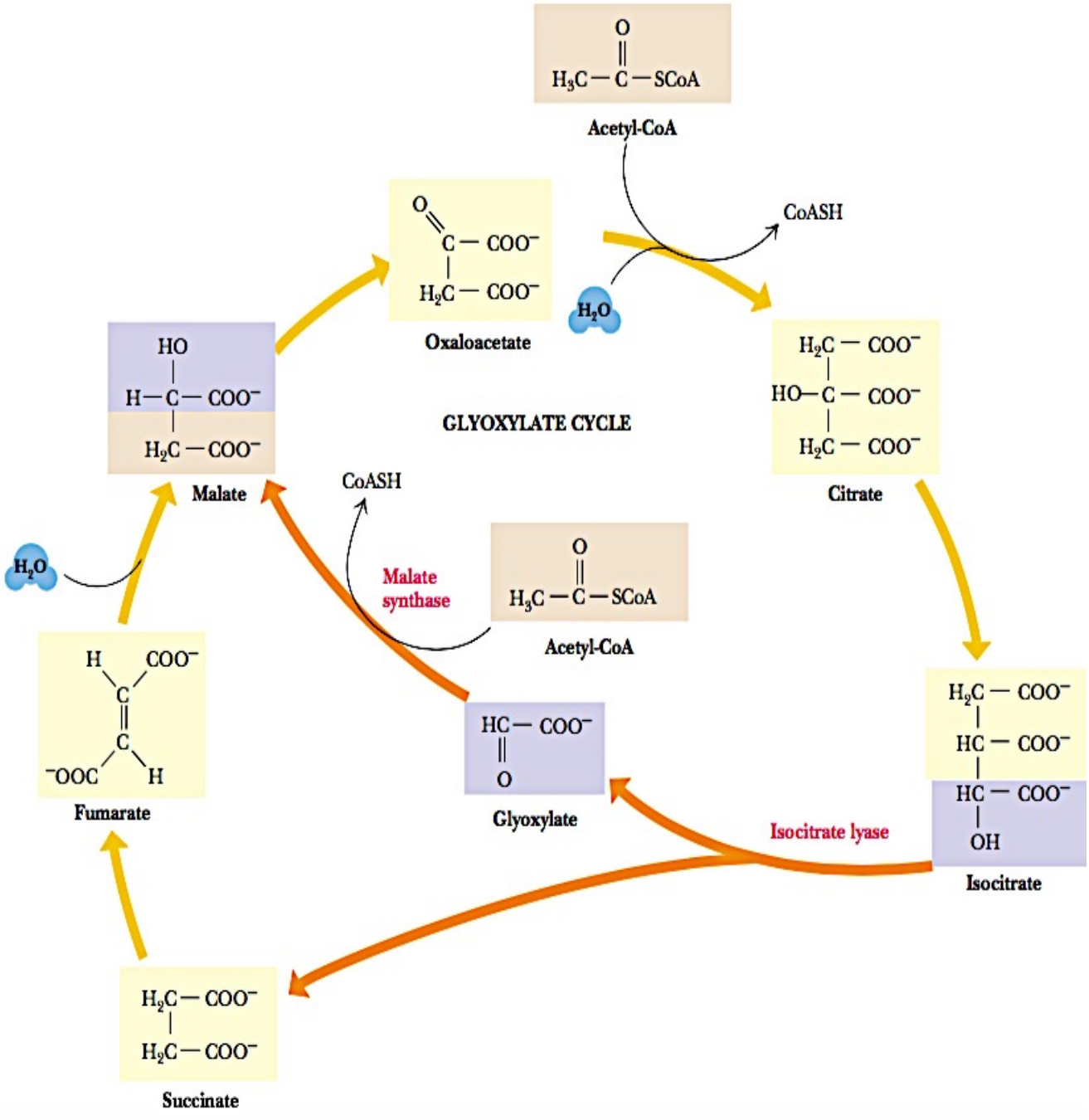


Figure 17 The glyoxylate cycle

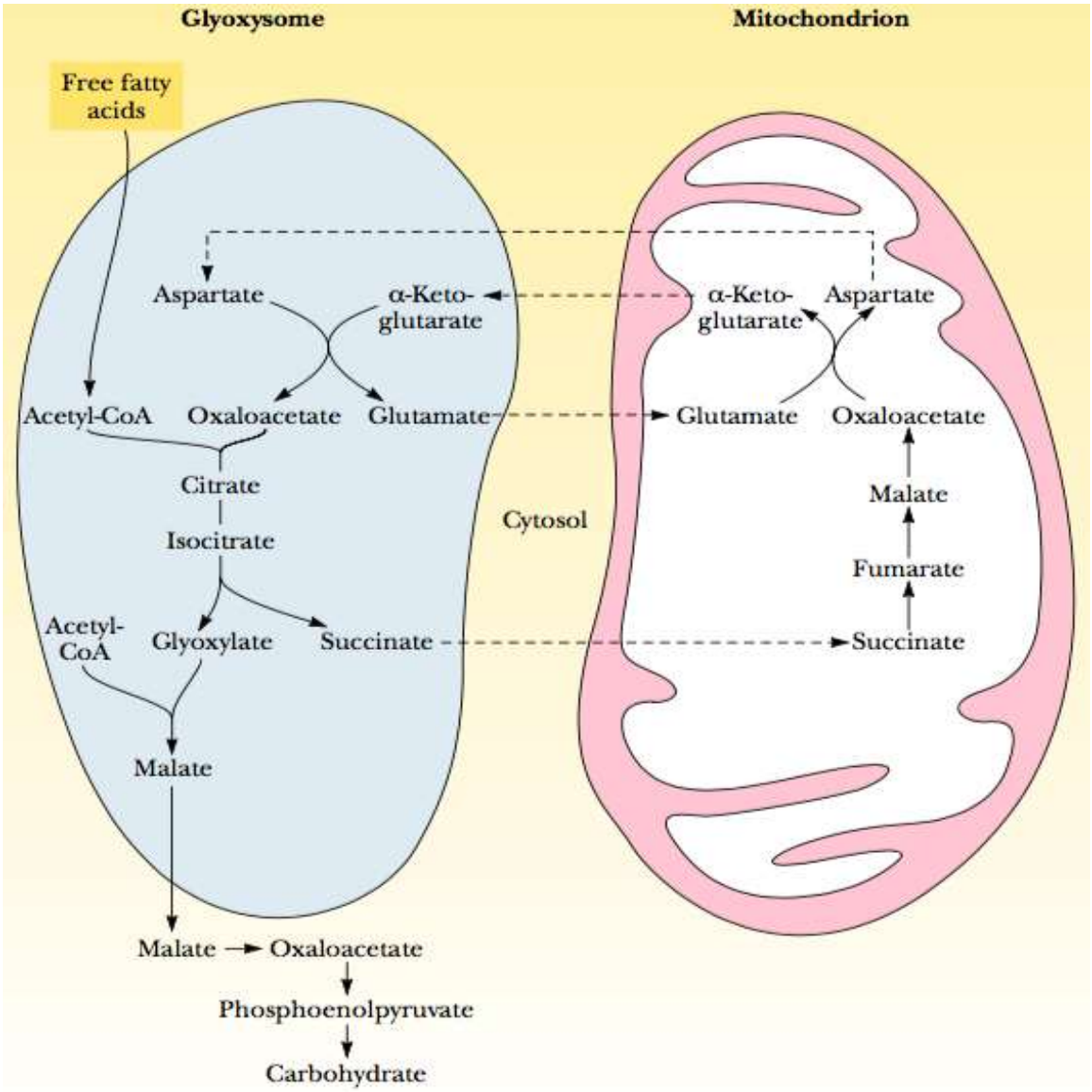


Figure 18 Relationship between the glyoxylate and citric acid cycles.

4.4.7 - ELECTRON TRANSPORT AND OXIDATIVE PHOSPHORYLATION

Most of the metabolic energy that is obtainable from substrates entering glycolysis and the TCA cycle is funneled via oxidation–reduction reactions into NADH and reduced flavoproteins [$FADH_2$]. ATP produced in glycolysis and the TCA cycle is the result of substrate-level phosphorylation. NADH-dependent ATP synthesis is the result of oxidative phosphorylation. Electrons stored in the form of the reduced coenzymes, NADH or [$FADH_2$], are passed through an elaborate and highly organized chain of proteins and coenzymes, the so-called **electron transport chain,** finally reaching O_2 (molecular oxygen), the terminal electron acceptor. Each component of the chain can exist in (at least) two oxidation states, and each component is successively reduced and reoxidized as electrons move through the chain from NADH (or [$FADH_2$]) to O_2. In the course of electron transport, a proton gradient is established across the

inner mitochondrial membrane. It is the energy of this proton gradient that drives ATP synthesis.

Electron transport and oxidative phosphorylation are membrane-associated processes

The processes of electron transport and oxidative phosphorylation are **membrane-associated.** Bacteria are the simplest life form, and bacterial cells typically consist of a single cellular compartment surrounded by a plasma membrane and a more rigid cell wall. In such a system, the conversion of energy from NADH and [$FADH_2$] to the energy of ATP via electron transport and oxidative phosphorylation is carried out at (and across) the plasma membrane. In eukaryotic cells, electron transport and oxidative phosphorylation are localized in mitochondria, which are also the sites of TCA cycle activity and fatty acid oxidation.

Mitochondrion is surrounded by a simple outer membrane and more complex inner membrane. The space between the inner and outer membranes is referred to as the intermembrane space. The outer membrane functions mainly to maintain the shape of the mitochondrion. The inner membrane lacks cholesterol and is quite impermeable to molecules and ions. Species that must cross the mitochondrial inner membrane—ions, substrates, fatty acids for oxidation, and so on— are carried by specific transport proteins in the membrane. Notably, the inner membrane has extensive folds, known as cristae, providing the inner membrane with a large surface area in a small volume.

The Electron Transport Chain—an Overview

The electron transport chain involves several different molecular species, including:

(a) Flavoproteins contain tightly bound FMN or FAD as prosthetic groups which may participate in one- or two- electron transfer events.

(b) Coenzyme Q, also called **ubiquinone** (and abbreviated **CoQ** or **UQ**), which can function in either one- or two-electron transfer reactions.

(c) Several **cytochromes** (proteins containing heme prosthetic groups, which function by carrying or transferring electrons), including cytochromes *b*, *c*, c_1, *a*, and a_3. Cytochromes are one-electron transfer agents, in which the heme iron is converted from Fe^{2+} to Fe^{3+} and back.

(d) A number of **iron–sulfur proteins,** which participate in one-electron transfers involving the Fe^{2+} and Fe^{3+}states.

(e) Protein-bound **copper,** a one-electron transfer site, which converts between Cu^{+} and Cu^{2+}.

All these intermediates except for cytochrome *c* are membrane-associated (either in the mitochondrial inner membrane of eukaryotes or in the plasma membrane of prokaryotes). All three types of proteins involved in this chain— flavoproteins, cytochromes, and iron–sulfur proteins—possess electron-transferring **prosthetic groups.**

Electron carriers function in multienzyme complexes

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. (I) **NADH–coenzyme Q reductase,** (II) **succinate–coenzyme Q reductase,** (III) **coenzyme Q–cytochrome c reductase,** and (IV) **cytochrome c oxidase**

Complex I: NADH–Coenzyme Q Reductase

As name implies, this complex transfers a pair of electrons from NADH to coenzyme Q, a small, hydrophobic, yellow compound. Another common name for this enzyme complex is NADH: ubiquinone oxidoreductase or *NADH dehydrogenase.* The complex (Figure 19) involves more than 42 polypeptide chains, one molecule of flavin mononucleotide (FMN), and as many as six Fe-S clusters. By virtue of its dependence on FMN, NADH–UQ reductase is a *flavoprotein*. Although the precise mechanism of the NADH–UQ reductase is not known, the first step involves binding of NADH to the enzyme on the *matrix* side of the inner mitochondrial membrane, and transfer of electrons from NADH to tightly bound FMN:

$$NADH + [FMN] + H^{+} \rightarrow [FMNH_2] + NAD^{+}$$

The second step involves the transfer of electrons from the reduced $[FMNH_2]$ to a series of Fe-S proteins, including both 2Fe-2S and 4Fe-4S clusters. NADH is a two-electron donor, whereas the Fe-S proteins are one-electron transfer agents. The flavin of FMN has three redox states—the oxidized, semiquinone, and reduced states. It can act as *either* a one-electron *or* a two-electron transfer agent and may serve as a critical link between NADH and the Fe-S proteins.

The final step of the reaction involves the transfer of two electrons from iron–a sulfur cluster to coenzyme Q. Coenzyme Q is a **mobile electron carrier.** Its isoprenoid tail makes it highly hydrophobic, and it diffuses freely in the hydrophobic core of the inner mitochondrial membrane. As a result, it shuttles electrons from Complexes I and II to Complex III. The oxidation of one NADH and the reduction of one UQ by NADH–UQ reductase results in the net transport of protons from the matrix side to the cytosolic side of the inner membrane. The cytosolic side, where H accumulates, is referred to as the **P** (for *positive*) face; similarly, the matrix side is the **N** (for *negative*) face. Some of the energy liberated by the flow of electrons through this complex is used in a *coupled process* to drive the transport of protons across the membrane.

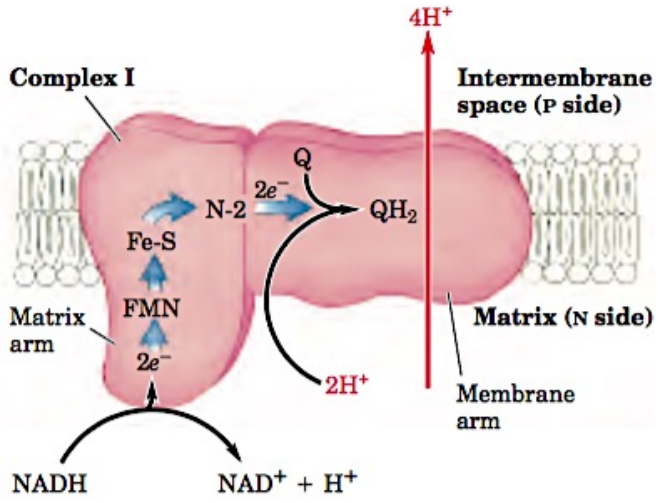


Figure 19 NADH:ubiquinoneoxidoreductase(ComplexI)

Complex II: Succinate–Coenzyme Q Reductase

Complex II is perhaps better known by its other name—**succinate dehydrogenase,** the only TCA cycle enzyme that is an integral membrane protein in the inner mitochondrial membrane (Figure 20). This enzyme is composed of four subunits: two Fe-S proteins, and two other peptides. Also known as *flavoprotein 2 (FP_2),* it contains an FAD covalently bound to a histidine residue, and three Fe-S centers: a 4Fe-4S cluster, a 3Fe-4S cluster, and a 2Fe-2S cluster. When succinate is converted to fumarate in the TCA cycle, concomitant reduction of bound FAD to $FADH_2$occurs in succinate dehydrogenase. This $FADH_2$ transfers its electrons immediately to Fe-S centers, which pass them on to UQ. Electron flow from succinate to UQ,

Succinate $\rightarrow$ Fumarate + $2H^+ + 2\ e^-$

$UQ + 2H^+ + 2\ e^- \rightarrow UQH_2$

Net reaction: Succinate +UQ$\rightarrow$fumarate + UQH_2 $\Delta G'° = -5.6$kJ/mol

The small free energy change of this reaction is not sufficient to drive the transport of protons across the inner mitochondrial membrane.

This is a crucial point because proton transport is coupled with ATP synthesis. Oxidation of one $FADH_2$ in the electron transport chain results in synthesis of approximately two molecules of ATP, compared with the approximately three ATPs produced by the oxidation of one NADH. Other enzymes can also supply electrons to UQ, including mitochondrial glycerophosphate dehydrogenase, an inner membrane–bound shuttle enzyme, and the fatty acyl–CoA dehydrogenases, three soluble matrix enzymes involved in fatty acid oxidation. The effect of each of these electron-transferring enzymes is to contribute to the pool of reduced ubiquinone. QH_2 from all these reactions is reoxidized by Complex III.

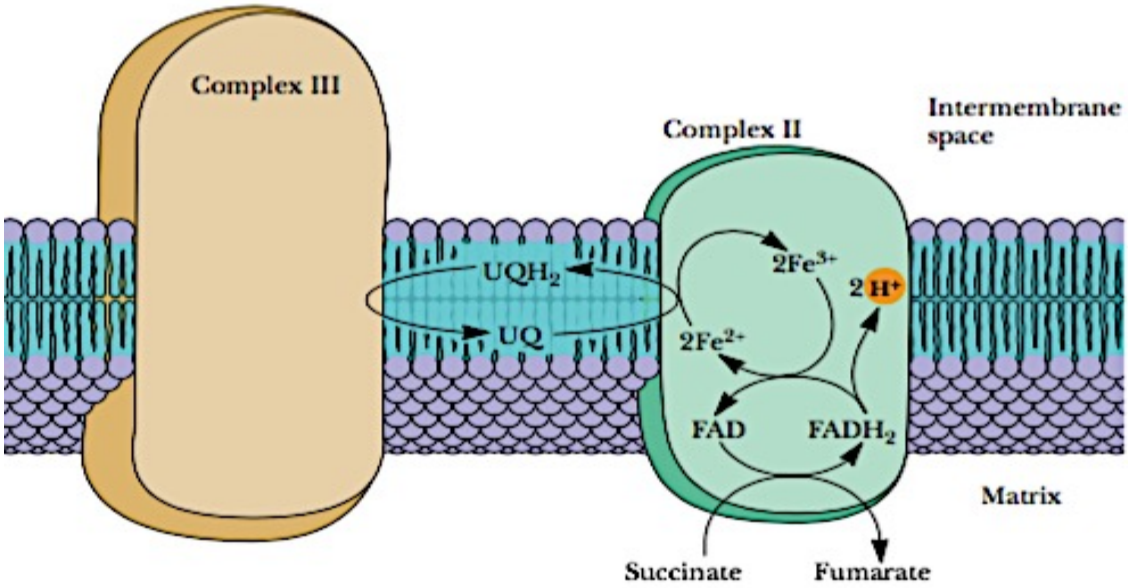


Figure 20 Scheme for electron flow in complex II

Complex III: Coenzyme Q–Cytochrome *c* Reductase

In the third complex of the electron transport chain, reduced coenzyme Q (UQH_2) passes its electrons to cytochrome *c* via a unique redox pathway known as the **Q cycle (Figure 21).** *UQ–cytochrome c reductase* (UQ–cyt *c* reductase) involves three different cytochromes and an Fe-S protein. In the cytochromes of these and similar complexes, the iron atom at the center of the porphyrin ring cycles between the reduced Fe^{2+} ferrous) and oxidized Fe^{3+} (ferric) states.

Cytochromes were first named and classified on the basis of their absorption spectra, which depend upon the structure and environment of their heme groups. The ***b* cytochromes** contain *iron–protoporphyrin IX*, the same heme found in hemoglobin and myoglobin. The ***c* cytochromes** contain *heme c,* derived from iron–protoporphyrin IX by the covalent attachment of cysteine residues from the associated protein. UQ–cyt *c reductase contains a b-type cytochrome, of 30 to 40 kD, with two different heme sites and one c-type cytochrome.*

The net equation for the redox reactions of this Q cycle is

QH_2+ 2 cyt c_1 (oxidized) + $2H^+_N \rightarrow$Q + 2 cyt c_1 (reduced) + $4H^+_P$

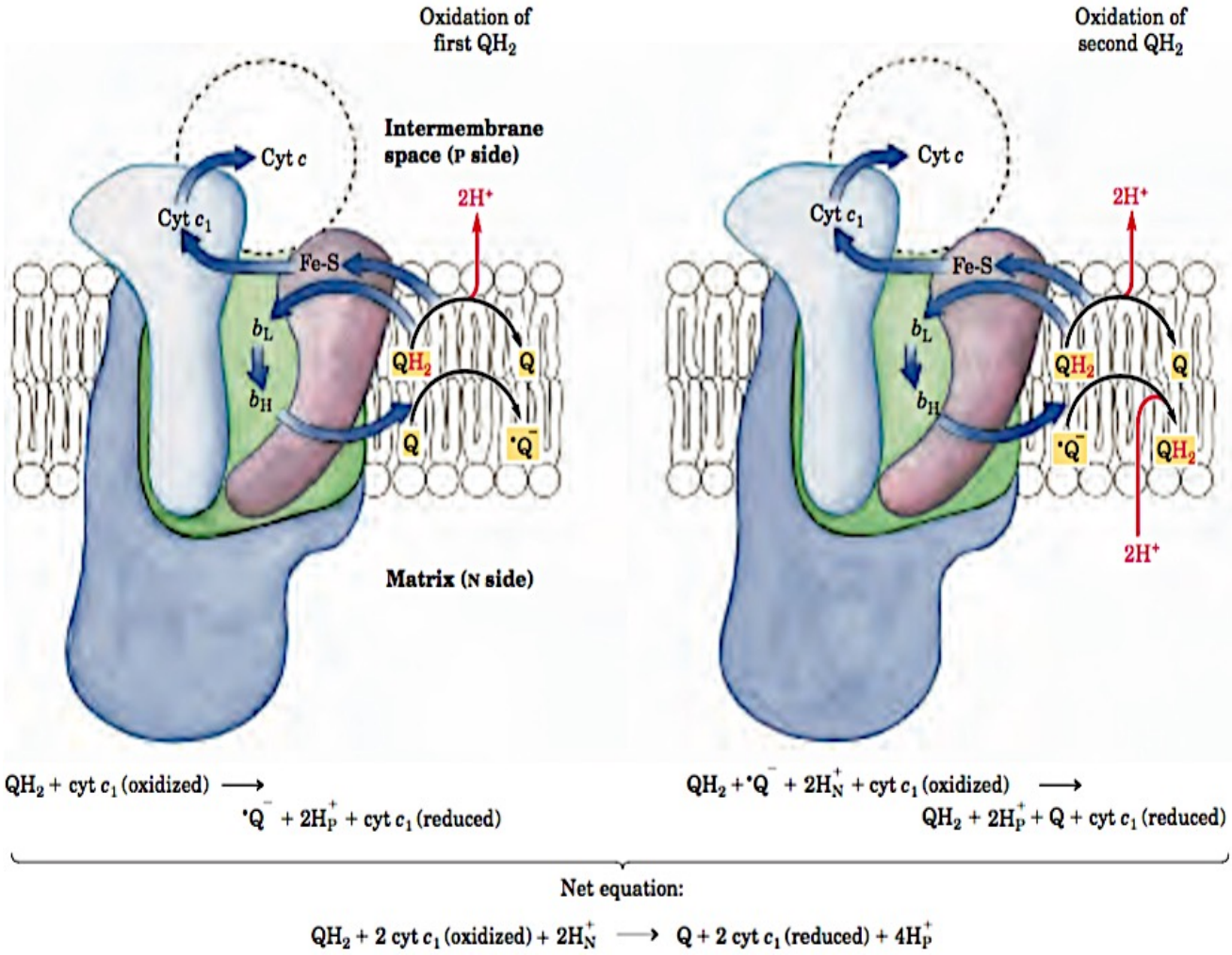


Figure 21 The Q Cycle

The Q cycle accommodates the switch between the two-electron carrier ubiquinone and the one-electron carriers—cytochromes b_{562}, b_{566}, c_1, and c—and explains the measured stoichiometry of four protons translocated per pair of electrons passing through the Complex III to cytochrome c. Although the path of electrons through this segment of the respiratory chain is complicated, the net effect of the transfer is simple: QH_2 is oxidized to Q and two molecules of cytochrome c gets reduced.

Cytochrome c is a soluble protein of the intermembrane space. After its single heme accepts an electron from Complex III, cytochrome c moves to Complex IV to donate the electron to a binuclear copper center.

Complex IV: Cytochrome c to O_2. In the final step of the respiratory chain, Complex IV (Figure 22) also called cytochrome oxidase, carries electrons from cytochrome c to molecular oxygen, reducing it to H_2O. Complex IV is a large enzyme of the inner mitochondrial membrane. Bacteria contain a form that is much simpler, with only three or four subunits, but still capable of catalyzing both electron transfer and proton pumping. Mitochondrial subunit II contains two Cu ions complexed with the -SH groups of two Cys residues in a binuclear center (Cu_A) that resembles the 2Fe-2S centers of iron-sulfur proteins. Subunit I contains two heme groups, designated a and a_3, and another copper ion (Cu_B). Heme a_3 and Cu_B form a second binuclear center that accepts electrons from heme a and transfers them to O_2 bound to heme a_3.

Electron transfer through Complex IV is from cytochrome c to the Cu_A center, to heme a, to the heme a_3–Cu_B center, and finally to O_2. For every four electrons passing through this complex, the enzyme consumes four "substrate" H^+ from the matrix (N side) in converting O_2 to $2H_2O$. It also uses the energy of this redox reaction to pump one proton outward into the intermembrane space (P side) for each electron that passes through, adding to the electrochemical potential produced by redox-driven proton transport through Complexes I and III. The overall reaction catalyzed by Complex IV is

$$4 \text{ Cyt } c \text{ (reduced)} + 8\ H^+_N + O_2 \rightarrow 4 \text{ cyt } c \text{ (oxidized)} + 4H^+_P + 2H_2O$$

This four-electron reduction of O_2involves redox centers that carry only one electron at a time, and it must occur without the release of incompletely reduced intermediates such as hydrogen peroxide or hydroxyl free radicals—very reactive species that would damage cellular components. The intermediates remain tightly bound to the complex until completely converted to water.

For each pair of electrons transferred to O_2, four protons are pumped out by Complex I, four by Complex III, and two by Complex IV (Fig. 23). The vectorial equation for the process is therefore

$$NADH + 11\ H^+_N + \tfrac{1}{2}\ O_2 \rightarrow NAD^+ + 10H^+_P + H_2O$$

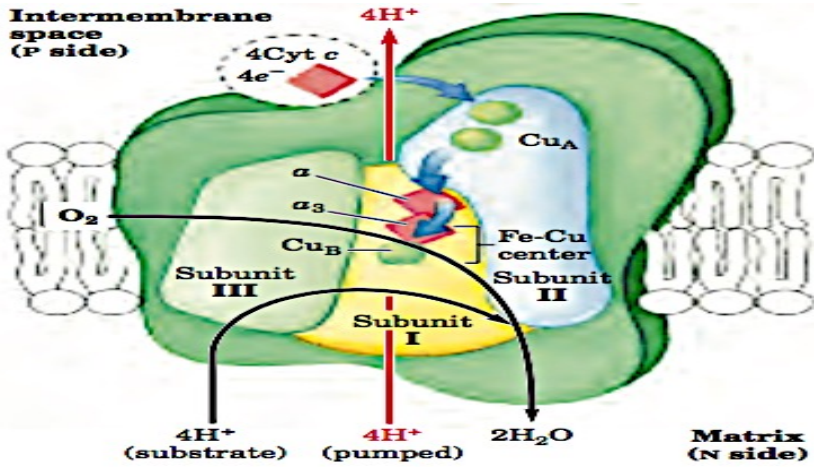


Figure 22 Path of electrons through Complex IV

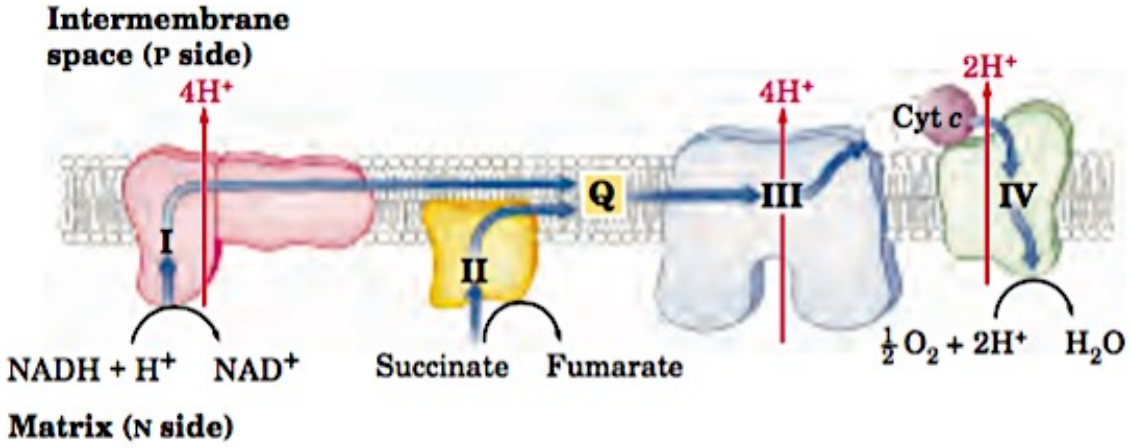


Figure 23 Summary of the flow of electrons and protons through the four complexes of the respiratory chain.

The electrochemical energy inherent in this difference in proton concentration and separation of charge represents a temporary conservation of much of the energy of electron transfer. The energy stored in such a gradient, termed the proton-motive force, has two components: (1) the chemical potential energy due to the difference in concentration of a chemical species (H^+) in the two regions separated by the membrane, and (2) the electrical potential energy that results from the separation of charge when a proton moves across the membrane without a counterion

When protons flow spontaneously down their electrochemical gradient, energy is made available to do work. In mitochondria, chloroplasts, and aerobic bacteria, the electrochemical energy in the proton gradient drives the synthesis of ATP from ADP and Pi.

4.4.7.1 ATP Synthesis

How concentration gradient of protons gets transformed into ATP? Electron transfer releases, and the proton-motive force conserves, more than enough free energy per "mole" of electron pairs to drive the formation of a mole of ATP. Mitochondrial oxidative phosphorylation therefore poses no thermodynamic problem. But what is the chemical mechanism that couples proton flux with phosphorylation?

In 1961, Peter Mitchell proposed a novel coupling mechanism involving a proton gradient across the inner mitochondrial membrane. In Mitchell's **chemiosmotic hypothesis (Figure 24)** protons are driven across the membrane from the matrix to the intermembrane space and cytosol by the events of electron transport. This mechanism stores the energy of electron transport in an **electrochemical potential.** As protons are driven out of the matrix, the pH rises and the matrix becomes negatively charged with respect to the cytosol. Proton pumping thus creates a pH gradient and an electrical gradient across the inner membrane, both of which tend to attract protons back into the matrix from the cytoplasm. Flow of protons down this electrochemical gradient, an energetically favorable process, then drives the synthesis of ATP. To emphasize this crucial role of the proton-motive force, the equation for ATP synthesis is sometimes written

$$ADP + Pi + nH^+_P \rightarrow ATP + H_2O + nH^+_N$$

ATP Synthase

The mitochondrial complex that carries out ATP synthesis is called **ATP synthase** or sometimes **F_1F_O–ATPase** (for the reverse reaction it catalyzes). **It Consists of Two Complexes—F_1 and F_O.** The spheres observed in electron micrographs make up the **F_1 unit,** which catalyzes ATP synthesis. These F1 spheres are attached to an integral membrane protein aggregate called the **F_O unit (Figure 25).** F1 consists of five polypeptide chains named α, β, γ, δ and ε with a subunit stoichiometry $\alpha_3\beta_3 3\ \gamma\delta\varepsilon$. F_O consists of three hydrophobic subunits denoted by *a*, *b*, and *c*, with an apparent stoichiometry of $a_1b_2\ c_{9\text{-}12}$. F_O forms the transmembrane pore or channel through which protons move to drive ATP synthesis. The α andβ subunits are homologous, and each of these subunits bind a single ATP. The catalytic sites are in the βsubunits; the function of the ATP sites in the αsubunits is unknown.

Proton Flow through ATP synthase leads to the release of tightly bound ATP: The binding-change mechanism

ATP synthase catalyzes the formation of ATP from ADP and orthophosphate.

$$ADP^{3-} + HPO_4^{2-} + H^+ \Longleftrightarrow ATP^{4-} + H_2O$$

The actual substrates are Mg^{2+} complexes of ADP and ATP, as in all known phosphoryl transfer reactions with these nucleotides. A terminal oxygen atom of ADP attacks the phosphorus atom of Pi to form a pentacovalent intermediate, which then dissociates into ATP and H_2O. The attacking oxygen atom of ADP and the departing oxygen atom of Pi occupy the apices of a trigonal bipyramid.

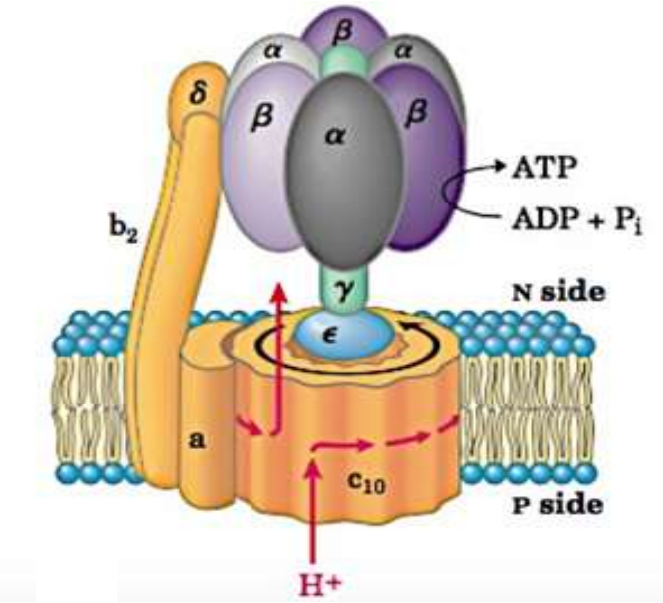


Figure 24 Diagram of the F_1F_O complex

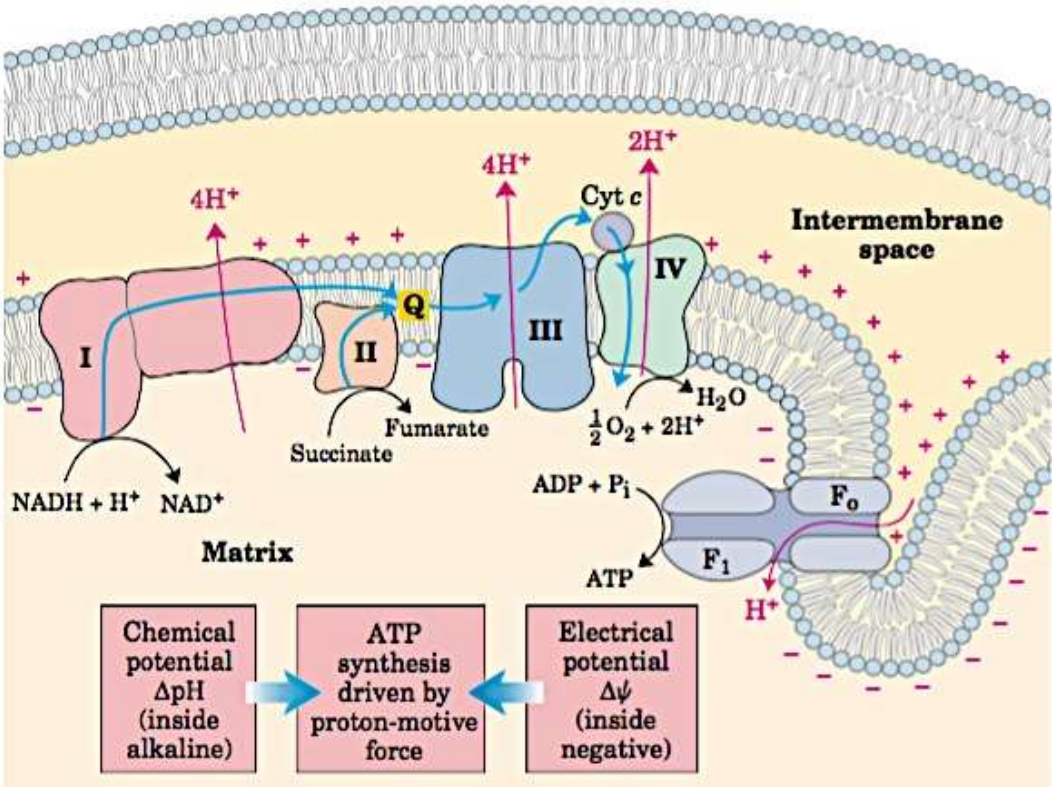


Figure 24 Chemiosmotic model.

How does the flow of protons drive the synthesis of ATP?

Paul Boyer proposed a *binding-change mechanism* (Figure 26) for proton-driven ATP synthesis. This proposal states that changes in the properties of the three βsubunits allow sequential ADP and Pi binding, ATP synthesis, and ATP release. As already noted, interactions with the γsubunit make the three β subunits inequivalent. One βsubunit can be in the T, or tight, conformation. This conformation binds ATP with great avidity. Indeed, its affinity for ATP is so high that it will convert bound ADP and Pi into ATP with equilibrium constant near 1. However, the conformation of this subunit is sufficiently constrained that it cannot release ATP. A second subunit will then be in the L, or loose, conformation. This conformation binds ADP and Pi. It, too, is sufficiently constrained that it cannot release bound nucleotides. The final subunit will be in the O, or open, form. This form can exist with a bound nucleotide in a structure that is similar to those of the T and L forms, but it can also convert to form a more open conformation and release a bound nucleotide.

The interconversion of these three forms can be driven by rotation of the γ subunit. Suppose the γ subunit is rotated 120 degrees in a counterclockwise direction (as viewed from the top). This rotation will change the subunit in the T conformation into the O conformation, allowing the subunit to release the ATP that has been formed within it. The subunit in the L conformation will be converted into the T conformation, allowing the transition of bound ADP + Pi into ATP. Finally, the subunit in the O conformation will be converted into the L conformation, trapping the bound ADP and Pi so that they cannot escape. The binding of ADP and Pi to the subunit now in the O conformation completes the cycle.

This mechanism suggests that ATP can be synthesized by driving the rotation of the γ subunit in the appropriate direction. Likewise, this mechanism suggests that the hydrolysis of ATP by the enzyme drives the rotation of the γ subunit in the opposite direction.

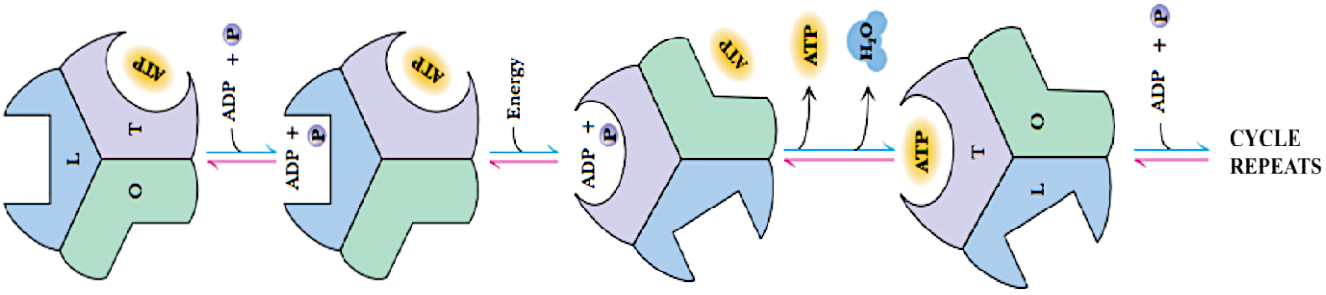


Figure 26 Binding-Change Mechanisms for ATP Synthase

4.4.7.2 Inhibitors of Oxidative Phosphorylation

Rotenone is a common insecticide that strongly inhibits the NADH–UQ reductase. Ptericidin, amytal, and other barbiturates, mercurial agents, and the widely prescribed painkiller Demerol also exert inhibitory actions on this enzyme complex. All these substances appear to inhibit reduction of coenzyme Q and the oxidation of the Fe-S clusters of NADH–UQ reductase.

Complex IV, the cytochrome *c* oxidase, is specifically inhibited by cyanide (CN^-), azide (N_3^-), and carbon monoxide (CO). Cyanide and azide bind tightly to the ferric form of cytochrome a_3,

whereas carbon monoxide binds only to the ferrous form. Inhibitors of ATP synthase include dicyclohexylcarbodiimide (DCCD) and oligomycin.

4.4.7.3 Uncouplers

Another important class of reagents affects ATP synthesis, but in a manner that does not involve direct binding to any of the proteins of the electron transport chain or the $\mathbf{F_1F_O}$– ATPase. These agents are known as uncouplers because they disrupt the tight coupling between electron transport and the ATP synthase. Uncouplers act by dissipating the proton gradient across the inner mitochondrial membrane created by the electron transport system. Typical examples include 2,4-dinitrophenol, dicumarol, and carbonyl cyanide-*p*-trifluoro-methoxyphenyl hydrazone (perhaps better known as fluorocarbonyl-cyanide phenylhydrazone or FCCP)

4.4.7.4 ATP exits the mitochondria via an ATP–ADP Translocase

ATP, the cellular energy currency, must exit the mitochondria to carry energy throughout the cell, and ADP must be brought into the mitochondria for reprocessing. Neither of these processes occurs spontaneously because the highly charged ATP and ADP molecules do not readily cross biological membranes. Instead, these processes are mediated by a single transport system, the **ATP– ADP translocase.** This protein tightly couples the exit of ATP with the entry of ADP so that the mitochondrial nucleotide levels remain approximately constant. For each ATP transported out, one ADP is transported into the matrix. Transport occurs via a single nucleotide-binding site, which alternately faces the matrix and the cytosol. It binds ATP on the matrix side, reorients to face the cytosol, and exchanges ATP for ADP, with subsequent movement back to the matrix face of the inner membrane.

4.4.7.5 Regulation of Oxidative Phosphorylation

Oxidative phosphorylation is regulated by cellular energy demands. The intracellular [ADP] and the mass-action ratio [ATP]/([ADP][Pi]) are measures of a cell's energy status. Normally 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. The process 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.

Note* In brown fat, which is specialized for the production of metabolic heat, electron membrane. Thermogenin (uncoupling protein) provides a path for protons to return to the matrix without passing through the Fo F1 complex and the energy of fatty acid oxidation is dissipated as heat.

ATP-Producing pathways are coordinately regulated

The major catabolic pathways arecoordinated regulated to function together in an economical manner to produce ATP and biosynthetic precursors. The relative concentrations of ATP and ADP control not only the rates of electron transfer and oxidative phosphorylation but also the rates of the citric acid cycle, pyruvate oxidation, and glycolysis. Whenever ATP consumption

increases, the rate of electron transfer and oxidative phosphorylation increases. Simultaneously, the rate of pyruvate oxidation via the citric acid cycle increases, increasing the flow of electrons into the respiratory chain. These events can in turn evoke an increase in the rate of glycolysis, increasing the rate of pyruvate formation. When conversion of ADP to ATP lowers the ADP concentration, acceptor control slows electron transfer and thus oxidative phosphorylation. Glycolysis and the citric acid cycle are also slowed, because ATP is an allosteric inhibitor of the glycolytic enzyme phosphofructokinase-1 and of pyruvate dehydrogenase.

Phosphofructokinase-1 is also inhibited by citrate, the first intermediate of the citric acid cycle. When the cycle is "idling," citrate accumulates within mitochondriaand then spills into the cytosol. When the concentrations of both ATP and citrate rise, they produce a concerted allosteric inhibition of phosphofructokinase-1 that is greater than the sum of their individual effects, slowing glycolysis.

4.5- SUMMARY

- Sugars (also called saccharides) are compounds containing an aldehyde or ketone group and two or more hydroxyl groups.
- Monosaccharides generally contain several chiral carbons and therefore exist in a variety of stereochemical forms, represented on paper as Fischer projections. Epimers are sugars that differ in configuration at only one carbon atom.
- Monosaccharides commonly form internal hemiacetals or hemiketals, in which the aldehyde or ketone group joins with a hydroxyl group of the same molecule, creating a cyclic structure; this can be represented as a Haworth perspective formula.
- Oligosaccharides are short polymers of several monosaccharides joined by glycosidic bonds. At one end of the chain, the reducing end is a monosaccharide unit whose anomeric carbon is not involved in a glycosidic bond.
- Polysaccharides (glycans) serve as stored fuel and as structural components of cell walls and extracellular matrix. The homopolysaccharides starch and glycogen are stored fuels in plant, animal, and bacterial cells. They consist of D-glucose with linkages, and all three contain some branches.
- Glycosaminoglycans are extracellular heteropolysaccharides in which one of the two monosaccharide units is a uronic acid and the other an N-acetylated amino sugar.
- Proteoglycans are glycoconjugates in which a core protein is attached covalently to one or more large glycans. Glycoproteins contain covalently linked oligosaccharides that are smaller but more structurally complex, and therefore more information-rich, than glycosaminoglycans.
- Glycolysis is a near-universal pathway by which a glucose molecule is oxidized to two molecules of pyruvate, with energy conserved as ATP and NADH.It is tightly regulated in coordination with other energy-yielding pathways to assure a steady supply of ATP. Hexokinase, PFK-1, and pyruvate kinase are all subject to allosteric regulation.
- Gluconeogenesis is a ubiquitous multistep process in which pyruvate or a related three-carbon compound (lactate, alanine) is converted to glucose.Glycolysis and gluconeogenesis are reciprocally regulated to prevent wasteful operation of both pathways at the same time.

- The oxidative pentose phosphate pathway (phosphogluconate pathway or hexose monophosphate pathway) brings about oxidation and decarboxylation at C-1 of glucose 6-phosphate, reducing $NADP^+$ to NADPH and producing pentose phosphates.
- Glycogen phosphorylase catalyzes phosphorolytic cleavage at the nonreducing ends of glycogen chains, producing glucose 1-phosphate. The debranching enzyme transfers branches onto main chains and releases the residue at the ($\alpha 1 \rightarrow 6$) branch as free glucose.Glycogen synthesis depended upon sugar nucleotides.Glycolysis and Gluconeogenesis are coordinatly Regulated.
- The citric acid cycle (Krebs cycle, TCA cycle) is a nearly universal central catabolic pathway in which compounds derived from the breakdown of carbohydrates, fats, and proteins are oxidized to CO_2, with most of the energy of oxidation temporarily held in the electron carriers $FADH_2$ and NADH. During aerobic metabolism, these electrons are transferred to O_2 and the energy of electron flow is trapped as ATP.
- In the glyoxylate cycle, the bypassing of the two decarboxylation steps of the citric acid cycle makes possible the net formation of succinate, oxaloacetate, and other cycle intermediates from acetyl-CoA.
- Oxidative phosphorylation is the culmination of energy yielding metabolism in aerobic organisms.

4.6 GLOSSARY

Aldose-a sugar containing the aldehyde group or its hemiacetal equivalent.

Allosteric regulation-is the **regulation** of an enzyme by binding an effector molecule at a site other than the enzyme's active site. The site to which the effector binds is termed the **allosteric** site.

Anomers-are diastereoisomers of cyclic forms of sugars or similar molecules differing in the configuration at the anomeric carbon (C-1 atom of an aldose or the C-2 atom of a 2-ketose).

ATP Synthase-an enzyme complex of the inner mitochondrial membrane catalyzes the formation of ATP from ADP and Pi, accompanied by the flow of protons.

Carbohydrates-are polyhydroxy aldehydes or ketones, or substances that yield such compounds on hydrolysis having the empirical formula $(CH_2O)n$.

Chemiosmotic theory -the electrochemical energy inherent in the difference in proton concentration and separation of charge across the inner mitochondrial membrane—the proton-motive force—drives the synthesis of ATP as protons flow passively back into the matrix through a proton pore associated with ATP synthase.

Citric acid cycle- a central catabolic pathway in which compounds derived from the break-down of carbohydrates, fats, and proteins are oxidized to CO_2, with most of the energy of oxidation temporarily held in the electron carriers $FADH_2$ and NADH. During aerobic metabolism, these electrons are transferred to O_2 and the energy of electron flow is trapped as ATP.

Cytochromes-are iron containing hemeproteins central to which are heme groups that are primarily responsible for the generation of ATP via electron transport.

Epimers- sugars that differ only in the configuration around one carbon atom are called epimers.

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

Gluconoegenesis- a metabolic pathway that results in the generation of glucose from certain non-carbohydrate carbon substrates.

Glycan- compounds consisting of a large number of monosaccharides linked glycosidically

Glycolysis- is the metabolic pathway that converts glucose into pyruvate.

Glycogenolysis-is the biochemical breakdown of glycogen to glucose.

Glycogenesis- is the formation of glycogen from glucose.

Glycoprotein- any class of proteins that have carbohydrate groups attached to the polypeptide chain.

Glycosidic Bond- or glycosidic linkage is a type of covalent bond that joins a carbohydrate (sugar) molecule to another group, which may or may not be another carbohydrate.

Ketose- is a monosaccharide containing one ketone group per molecule

Mutarotaion- is the change in the optical rotation because of the change in the equilibrium between two anomers, when the corresponding stereocenters interconvert.

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

Proteoglycan- Proteoglycans are proteins that are heavily glycosylated. The basic proteoglycan unit consists of a "core protein" with one or more covalently attached glycosaminoglycan chain.

Proton-motive force-The proton motive force is the energy generated from transferring protons or electrons across an energy-transducing cell membrane.

Reducing sugar- Any carbohydrate, which is capable of being oxidized and causes the reduction of other substances is known as reducing sugar.

Substrate-level phosphorylation- is a metabolic reaction that results in the formation of ATP or GTP by the direct transfer of a phosphoryl group to ADP or GDP from another phosphorylated compound.

Vice versa-in reverse order from the way something has been stated.

4.7 SELF ASSESSMENT QUESTIONS

4.7.1 Long answer type questions:-

Q 1. What are carbohydrates? Describe their classification in detail with suitable examples.
Q 2. Give a detail account of the glycolysis and its regulation.
Q 3. Give comparative account of the process of glycogenolysis and glycogenesis.
Q 4. Describe pentose Phosphate pathway and its significance.
Q 5. Describe in detail the process of gluconeogenesis and its role in metabolism.
Q 6. Give a detail account of the Citric acid cycle and its regulation.

4.7.2 Short answer type questions:-

Q 1.Write short note on the types of fermentation.
Q 2. Write notes on electron transport chain.
Q 3. Write notes on glyoxylate cycle.
Q 4. Write short note on- Monosaccharide, Oligosaccharide and polysaccharide
Q 5. Write notes on starch and glycogen.

4.7.3Fill in the blanks

Q 1. Carbohydrates have the empirical formula _____.

Q 2. Isomeric forms of monosaccharides that differ only in their configuration about the hemiacetal or hemiketal carbon atom are called _____.

Q 3. _____and_____ are reciprocally regulated to prevent wasteful operation of both pathways at the same time.

Q 4. _____is a ubiquitous multistep process in which pyruvate or a related three-carbon compound (lactate, alanine) is converted to glucose.

Q 5. Pyruvate dehydrogenase complex requires five coenzymes or prosthetic groups namely_____.

Q 6. _____replenish Citric Acid Cycle intermediates.

Q 7. The proton-motive force drives the synthesis of ATP as protons flow passively back into the matrix through a proton pore associated with_____.

Q 8. _____is also inhibited by citrate, the first intermediate of the citric acid cycle.

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ANSWERS

Fill in the blanks

1. $(CH_2O)_n$, 2. Anomers , 3. Glycolysis, Gluconeogenesis, 4.Gluconeogenesis, 5.Thiamine pyrophosphate (TPP), flavin adenine dinucleotide (FAD), coenzyme A, nicotinamide adenine dinucleotide (NAD), and lipoate.6. Anaplerotic Reactions, 7. ATP synthase, 8.Phosphofructokinase-1