

GLYCOLYSIS

Key Notes

Overview

Glycolysis is a set of reactions that take place in the cytoplasm of prokaryotes and eukaryotes. The roles of glycolysis are to produce energy (both directly and by supplying substrate for the citric acid cycle and oxidative phosphorylation) and to produce intermediates for biosynthetic pathways.

The pathway

Glucose is phosphorylated to glucose 6-phosphate (by hexokinase) which is converted to fructose 6-phosphate (by phosphoglucosomerase) and then to fructose 1,6-bisphosphate (by phosphofructokinase, PFK). The fructose 1,6-bisphosphate is split into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate (by aldolase) and these two trioses are interconverted by triose phosphate isomerase. Glyceraldehyde 3-phosphate is converted to 1,3-bisphosphoglycerate (by glyceraldehyde 3-phosphate dehydrogenase) which reacts with ADP to give 3-phosphoglycerate and ATP (catalyzed by phosphoglycerate kinase). The 3-phosphoglycerate is converted to 2-phosphoglycerate (by phosphoglycerate mutase) and then to phosphoenolpyruvate (PEP) by enolase. Finally, PEP and ADP react to form pyruvate and ATP (catalyzed by pyruvate kinase).

Fates of pyruvate

Under aerobic conditions, pyruvate can be converted by pyruvate dehydrogenase to acetyl coenzyme A (CoA) which can then enter the citric acid cycle. Under anaerobic conditions, pyruvate is converted to lactate by lactate dehydrogenase (LDH). The NAD^+ regenerated by this reaction allows glycolysis to continue, despite the lack of oxygen. When oxygen becomes available, the lactate is converted back to pyruvate. In anaerobic conditions, yeast and other organisms carry out alcoholic fermentation that converts pyruvate to acetaldehyde and then to ethanol, regenerating NAD^+ that allows glycolysis to continue.

Energy yield

Two ATPs are used in glycolysis and four ATPs are synthesized for each molecule of glucose so that the net yield is two ATPs per glucose. Under aerobic conditions, the two NADH molecules arising from glycolysis also yield energy via oxidative phosphorylation.

Metabolism of fructose

Fructose can be metabolized by two routes. In adipose tissue and muscle, hexokinase can phosphorylate fructose to fructose 6-phosphate that then enters glycolysis. In liver, most of the enzyme present is glucokinase not hexokinase and this does not phosphorylate fructose. In this tissue, fructose is metabolized instead by the fructose 1-phosphate pathway.

Metabolism of galactose

Galactose enters glycolysis via the galactose–glucose interconversion pathway, a four-step reaction sequence. The lack of the second enzyme in this pathway, galactose 1-phosphate uridylyl transferase, leads to the disease galactosemia through the accumulation of toxic products, including galactitol formed by the reduction of galactose.

Regulation of glycolysis

The main control step is that catalyzed by PFK but hexokinase and pyruvate kinase are additional control sites. PFK is allosterically inhibited by ATP, but this inhibition is relieved by AMP. Citrate also inhibits PFK. The build up of fructose 6-phosphate stimulates the formation of fructose 2,6-bisphosphate that in turn stimulates PFK. The enzyme that synthesizes fructose 2,6-bisphosphate (phosphofructokinase 2; PFK2) and the enzyme that hydrolyzes it back to fructose 6-phosphate (fructose bisphosphatase 2; FBPase2) are also regulated hormonally by glucagon that causes glycolysis to slow down when the blood glucose level falls. PFK is also inhibited by H⁺ ions, thus preventing excessive formation of lactate under anaerobic conditions. Hexokinase is inhibited by glucose 6-phosphate which builds up after PFK is inhibited. Pyruvate kinase is activated by fructose 1,6-bisphosphate but allosterically inhibited by ATP and alanine. Like PFK, it is also regulated hormonally by glucagon.

Related topics

Monosaccharides and disaccharides (J1)	Pentose phosphate pathway (J5)
Polysaccharides and oligosaccharides (J2)	Glycogen metabolism (J6)
Gluconeogenesis (J4)	Citric acid cycle (L1)

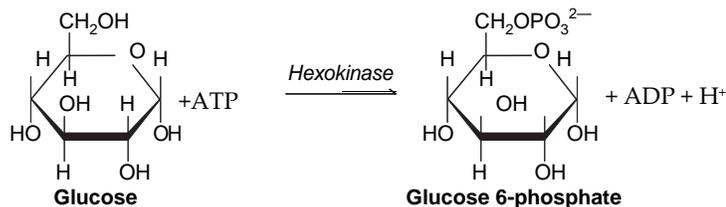
Overview

Glycolysis is a series of reactions (*Fig. 1*) that takes place in the cytoplasm of all prokaryotes and eukaryotes. Glycolysis converts one molecule of glucose into two molecules of pyruvate [which are then converted to acetyl coenzyme A (CoA) ready for entry into the citric acid cycle]. Two ATP molecules are needed for early reactions in the glycolytic pathway but four ATPs are generated later, giving a net yield of two ATPs per molecule of glucose degraded. Overall, glycolysis has a dual role. The first is to generate ATP. Although only two ATPs per glucose are made directly from the reactions of the glycolytic pathway, it also feeds substrates into the citric acid cycle and oxidative phosphorylation, where most ATP is made. The second role is to produce intermediates that act as precursors for a number of biosynthetic pathways. Thus acetyl CoA, for example, is the precursor for fatty acid synthesis (see Topic K3).

The pathway

The individual steps in glycolysis are described below.

1. Glucose is phosphorylated by ATP to form glucose 6-phosphate and ADP. The reaction is catalyzed by the enzyme **hexokinase**.



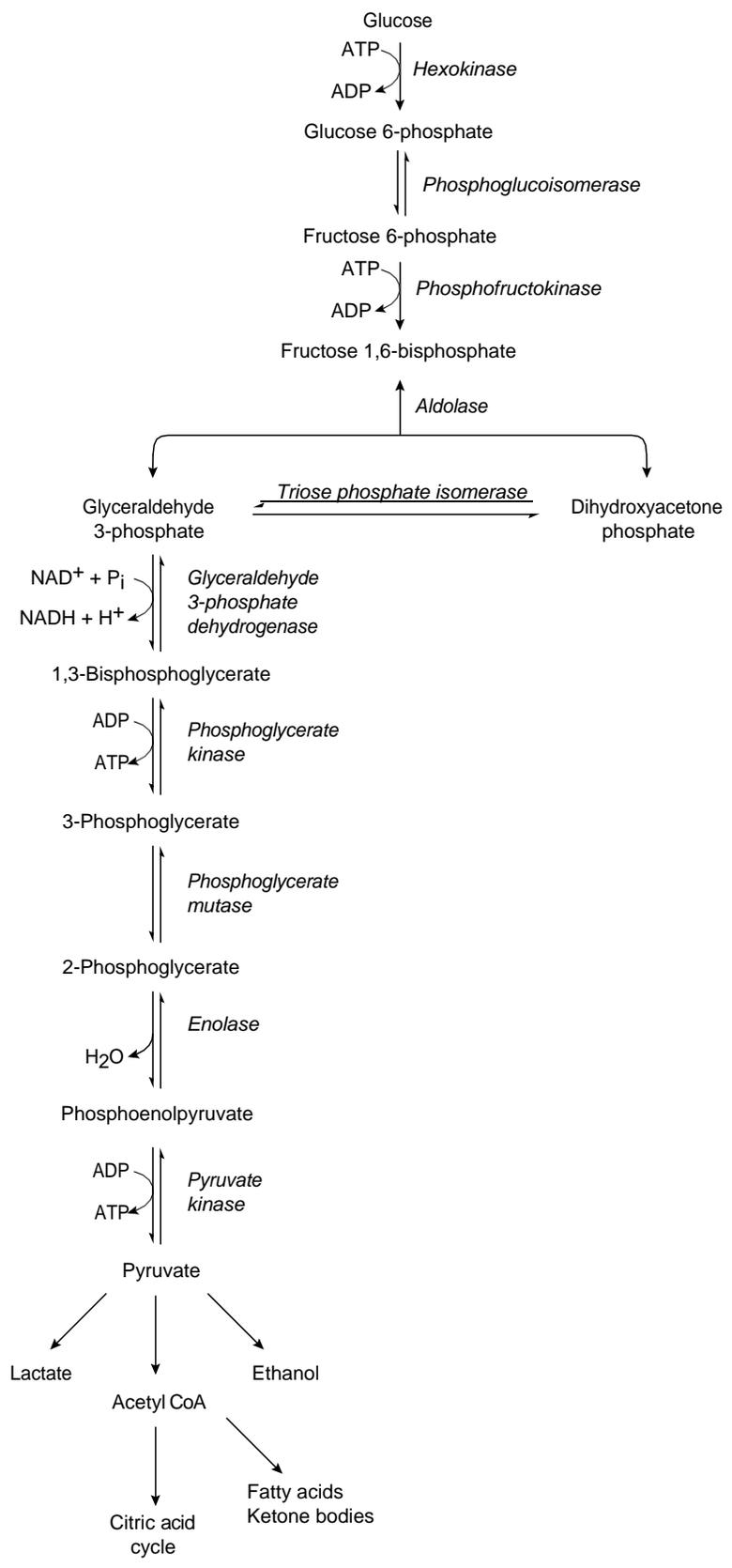
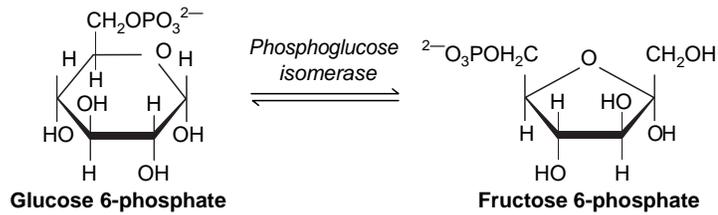
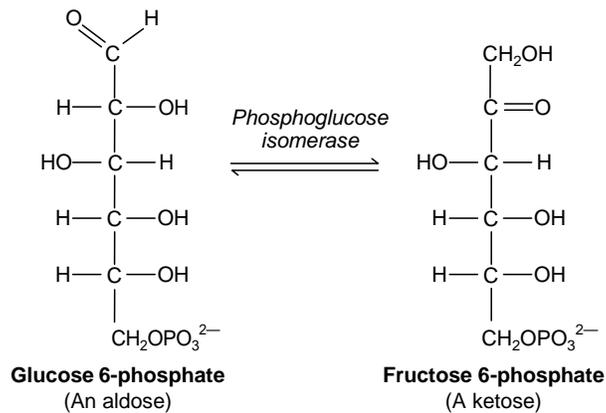


Fig. 1. The reactions of glycolysis (glucose to pyruvate) plus fates of pyruvate.

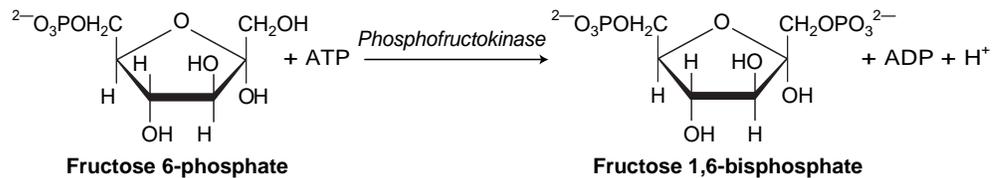
Glucose 6-phosphate is converted to fructose 6-phosphate by **phospho- glucoisomerase**.



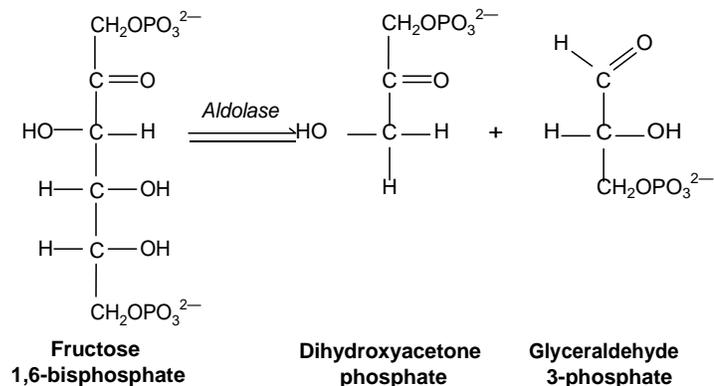
This isomerization involves the conversion of an aldose to a ketose, a conversion that is better seen by viewing the open chain representations of these molecules.



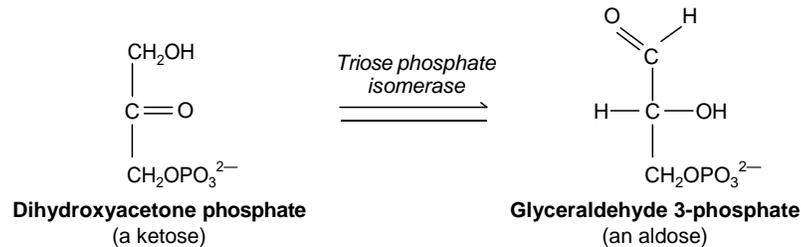
2. Fructose 6-phosphate is phosphorylated by ATP to form fructose 1,6-bisphosphate and ADP. The enzyme catalyzing this step is **phospho-fructokinase (PFK)**.



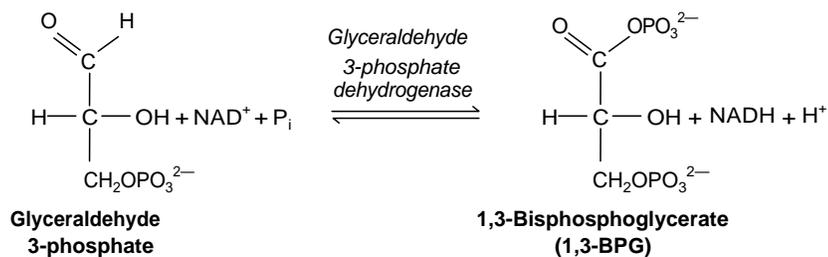
3. **Aldolase** splits fructose 1,6-bisphosphate (a six-carbon molecule) into two three-carbon molecules, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate.



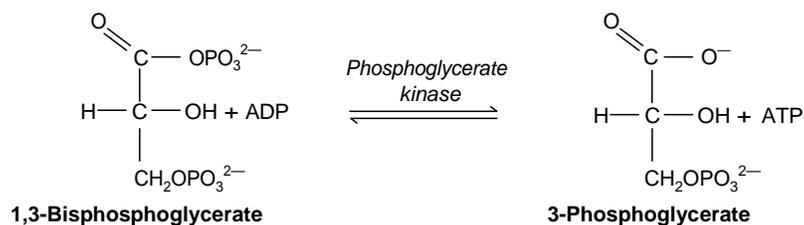
Glyceraldehyde 3-phosphate is the only molecule that can be used for the rest of glycolysis. However, the dihydroxyacetone phosphate formed in the previous step can rapidly be converted to glyceraldehyde 3-phosphate by **triose phosphate isomerase**. This is an equilibrium reaction; as the glyceraldehyde 3-phosphate is used by the rest of glycolysis, more dihydroxyacetone phosphate is converted to glyceraldehyde 3-phosphate as replacement. Thus effectively, for each molecule of fructose 1,6-bisphosphate that is cleaved in step 4, two molecules of glyceraldehyde 3-phosphate continue down the pathway.



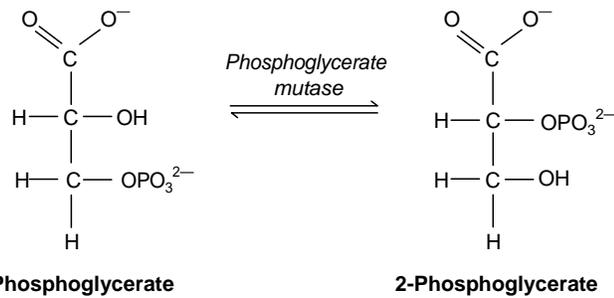
4. Glyceraldehyde 3-phosphate is converted to 1,3-bisphosphoglycerate. The reaction is catalyzed by **glyceraldehyde 3-phosphate dehydrogenase** and uses inorganic phosphate and NAD^+ . The other product is NADH . The energy for creating this new high-energy phosphate bond comes from the oxidation of the aldehyde group of the glyceraldehyde 3-phosphate.



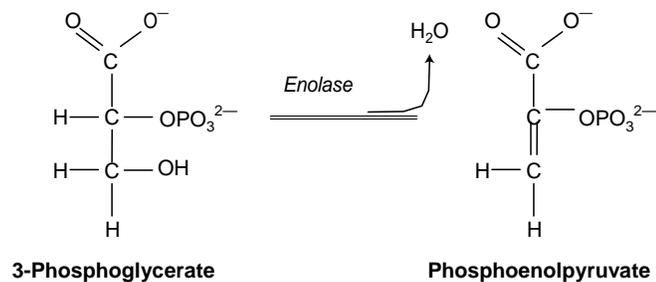
5. The newly created high-energy phosphate bond of 1,3-bisphosphoglycerate is now used to synthesize ATP. **Phosphoglycerate kinase** catalyzes the transfer of the phosphoryl group from the 1,3-bisphosphoglycerate to ADP, generating ATP and 3-phosphoglycerate.



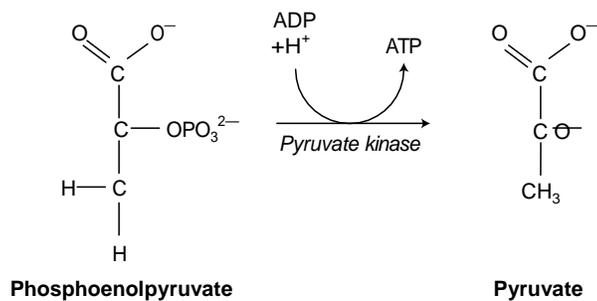
6. 3-Phosphoglycerate is converted to 2-phosphoglycerate by **phosphoglycerate mutase**. Thus the reaction is a movement of the phosphate group to a different carbon atom within the same molecule.



7. **Enolase** catalyzes the dehydration of 2-phosphoglycerate to form phosphoenolpyruvate (PEP). This reaction converts the low-energy phosphate ester bond of 2-phosphoglycerate into the high-energy phosphate bond of PEP.



8. In the last reaction, **pyruvate kinase** catalyzes the physiologically irreversible transfer of the phosphoryl group from PEP to ADP to form ATP and pyruvate.

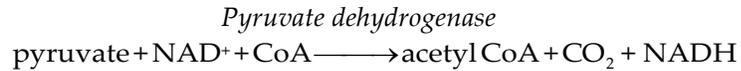


Substrate-level phosphorylation

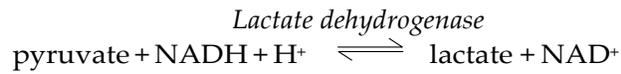
There are two distinct methods by which cells synthesize ATP. In **oxidative phosphorylation**, involving the electron transport chain, the generation of ATP is linked to the oxidation of NADH and FADH₂ to NAD⁺ and FAD respectively (see Topic L2), and occurs via the generation of a proton gradient across the inner mitochondrial membrane. In contrast, the two ATP synthetic reactions in glycolysis (catalyzed by phosphoglycerate kinase and pyruvate kinase) involve the direct transfer of a phosphate from a sugar-phosphate intermediate to ADP; these reactions are examples of **substrate-level phosphorylation**. A third example of substrate-level phosphorylation is the synthesis of GTP by succinate dehydrogenase in the citric acid cycle (see Topic L1). The GTP can be used to phosphorylate ADP to form ATP.

Fates of pyruvate ● *Entry into the citric acid cycle.* Glycolysis releases relatively little of the energy present in a glucose molecule; much more is released by the subsequent operation of the citric acid cycle and oxidative phosphorylation.

Following this route under aerobic conditions, pyruvate is converted to acetyl CoA by the enzyme **pyruvate dehydrogenase** and the acetyl CoA then enters the citric acid cycle. The pyruvate dehydrogenase reaction is an **oxidative decarboxylation** (see Topic L1 for details):

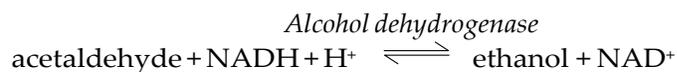
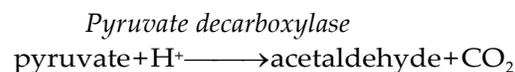


- *Conversion to fatty acid or ketone bodies.* When the cellular energy level is high (ATP in excess), the rate of the citric acid cycle (Topic L1) decreases and acetyl CoA begins to accumulate. Under these conditions, acetyl CoA can be used for fatty acid synthesis or the synthesis of ketone bodies (Topic K3).
- *Conversion to lactate.* The NAD^+ used during glycolysis (in the formation of 1,3-bisphosphoglycerate by glyceraldehyde 3-phosphate dehydrogenase; Fig. 1) must be regenerated if glycolysis is to continue. Under aerobic conditions, NAD^+ is regenerated by the reoxidation of NADH via the electron transport chain (see Topic L2). When oxygen is limiting, as in muscle during vigorous contraction, the reoxidation of NADH to NAD^+ by the electron transport chain becomes insufficient to maintain glycolysis. Under these conditions, NAD^+ is regenerated instead by conversion of the pyruvate to lactate by **lactate dehydrogenase**:



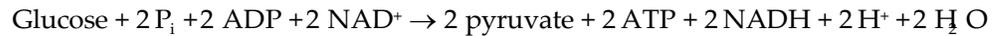
When sufficient oxygen becomes available once more, NAD^+ levels rise through operation of the electron transport chain. The lactate dehydrogenase reaction then reverses to regenerate pyruvate that is converted by pyruvate dehydrogenase to acetyl CoA which can enter the citric acid cycle (see above). Thus the operation of lactate dehydrogenase in mammals is a mechanism for the reoxidation of NADH to NAD^+ hence allowing glycolysis to continue, and ATP to be made, under anaerobic conditions. The process is even more sophisticated in the case of vigorously contracting skeletal muscle. Here the lactate produced is transported in the bloodstream to the liver where it is converted back to glucose and can return once again via the bloodstream to the skeletal muscle to be metabolized to yield energy. This is the Cori cycle and is described in Topic J4. Finally, in some microorganisms, lactate is the normal product from pyruvate.

- *Conversion to ethanol.* In yeast and some other microorganisms under anaerobic conditions, the NAD^+ required for the continuation of glycolysis is regenerated by a process called **alcoholic fermentation**. The pyruvate is converted to acetaldehyde (by **pyruvate decarboxylase**) and then to ethanol (by **alcohol dehydrogenase**), the latter reaction reoxidizing the NADH to NAD^+ :



Energy yield

Early in glycolysis, two ATPs are required for the conversion of glucose to glucose 6-phosphate by hexokinase and for the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate by PFK. However, fructose 1,6-bisphosphate then gives rise to two three-carbon units, each of which generates two ATPs in subsequent steps (catalyzed by phosphoglycerate kinase and pyruvate kinase) giving a net yield of two ATPs per original glucose molecule (*Fig. 1*). The overall reaction is:



Note that, under aerobic conditions, the two NADH molecules that are synthesized are reoxidized via the electron transport chain generating ATP. Given the cytoplasmic location of these NADH molecules, each is reoxidized via the glycerol 3-phosphate shuttle (see Topic L2) and produces approximately two ATPs during oxidative phosphorylation or via the malate–aspartate shuttle (see Topic L2) and produces approximately three ATPs during oxidative phosphorylation.

Metabolism of fructose

Fructose is an abundant sugar in the human diet; sucrose (table sugar) is a disaccharide which when hydrolyzed yields fructose and glucose (see Topic J1) and fructose is also a major sugar in fruits and honey. There are two pathways for the metabolism of fructose; one occurs in muscle and adipose tissue, the other in liver.

1. In muscle and adipose tissue, fructose can be phosphorylated by hexokinase (which is capable of phosphorylating both glucose and fructose) to form fructose 6-phosphate which then enters glycolysis.
2. In liver, the cells contain mainly glucokinase instead of hexokinase and this enzyme phosphorylates only glucose. Thus in liver, fructose is metabolized instead by the **fructose 1-phosphate pathway** (*Fig. 2*).

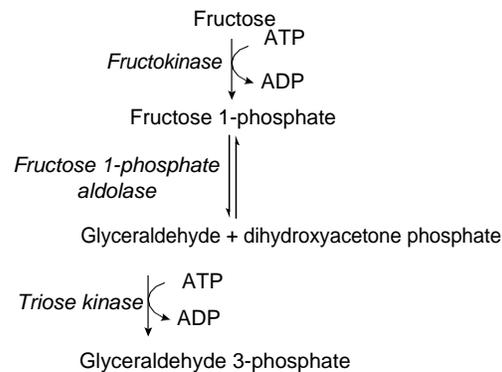


Fig. 2. The fructose 1-phosphate pathway.

- Fructose is converted to fructose 1-phosphate by **fructokinase**.
- Fructose 1-phosphate is then split into glyceraldehyde and dihydroxyacetone phosphate by **fructose 1-phosphate aldolase**. The dihydroxyacetone feeds into glycolysis at the triose phosphate isomerase step (*Fig. 1*).
- The glyceraldehyde is phosphorylated by **triose kinase** to glyceraldehyde 3-phosphate and so also enters glycolysis.

Metabolism of galactose

The hydrolysis of the disaccharide lactose (in milk) yields galactose and glucose. Thus galactose is also a major dietary sugar for humans. Galactose and glucose are epimers that differ in their configuration at C-4 (Topic J1, Fig. 4). Thus the entry of galactose into glycolysis requires an epimerization reaction. This occurs via a four-step pathway called the **galactose–glucose interconversion pathway** (Fig. 3):

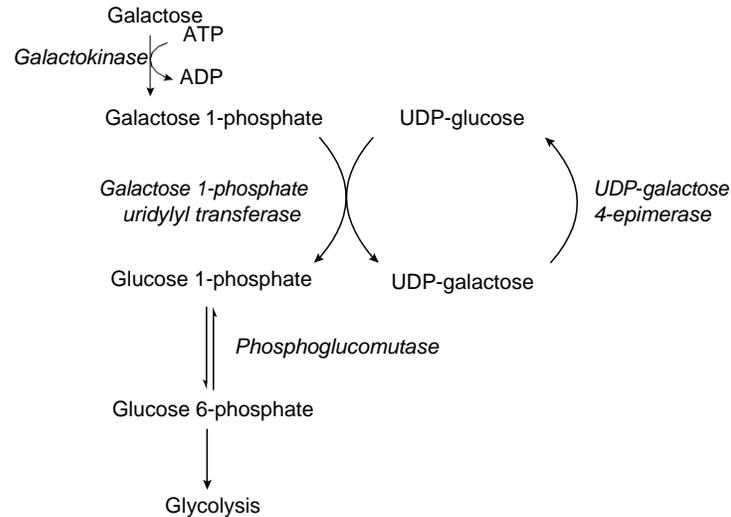
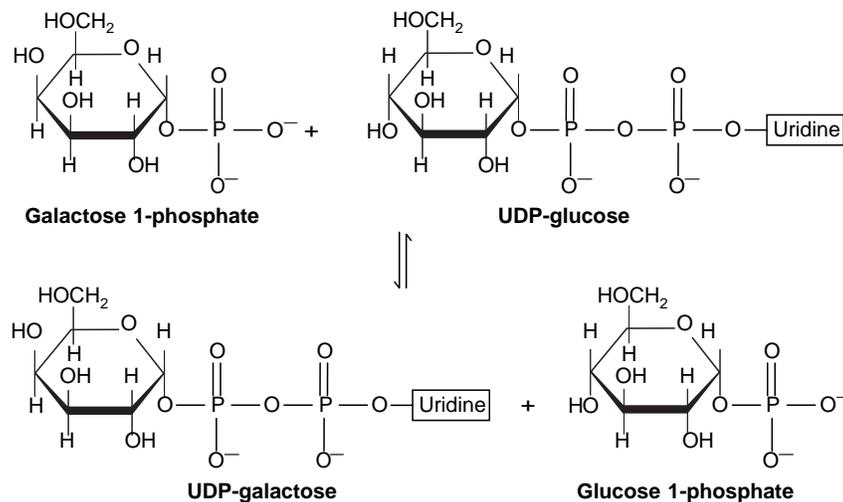


Fig. 3. The galactose–glucose interconversion pathway.

- Galactose is phosphorylated by **galactokinase** to give galactose 1-phosphate.
- Galactose 1-phosphate uridylyl transferase** catalyzes the transfer of a uridylyl group from UDP-glucose to galactose 1-phosphate to form UDP-galactose and glucose 1-phosphate.



- The UDP-galactose is converted back to UDP-glucose by **UDP-galactose 4-epimerase**. Thus, overall, UDP-glucose is not consumed in the reaction pathway.
- Finally the glucose 1-phosphate is converted to glucose 6-phosphate by **phosphoglucomutase**. The glucose 6-phosphate then enters glycolysis.

Galactosemia is a genetic disease caused by an inability to convert galactose to glucose. Toxic substances accumulate such as **galactitol**, formed by the reduction of galactose, and lead to dire consequences for the individual. Children who have the disease fail to thrive, may vomit or have diarrhea after drinking milk, and often have an enlarged liver and jaundice. The formation of cataracts in the eyes, mental retardation and an early death from liver damage are also possible. Most cases of galactosemia are due to a deficiency of the galactose 1 - phosphate uridylyl transferase enzyme and hence these individuals cannot metabolize galactose. The disease is treated by prescribing a galactose-free diet which causes all the symptoms to regress except mental retardation which may be irreversible. Since such patients have normal levels of UDP-galactose 4-epimerase, they can still synthesize UDP-galactose from UDP-glucose and so can still synthesize, for example, oligosaccharides in glycoproteins that involve Gal residues.

Regulation of glycolysis

Phosphofructokinase

The most important control step of glycolysis is the irreversible reaction catalyzed by phosphofructokinase (PFK). The enzyme is regulated in several ways:

- **ATP/AMP.** PFK is allosterically inhibited by ATP but this inhibition is reversed by AMP. This allows glycolysis to be responsive to the energy needs of the cell, speeding up when ATP is in short supply (and AMP is plentiful) so that more ATP can be made, and slowing down when sufficient ATP is already available.
- **Citrate.** PFK is also inhibited by citrate, the first product of the citric acid cycle proper (see Topic L1). A high level of citrate signals that there is a plentiful supply of citric acid cycle intermediates already and hence no additional breakdown of glucose via glycolysis is needed.
- **Fructose 2,6-bisphosphate.** Fructose 2,6-bisphosphate (F-2,6-BP) is synthesized (Fig. 4) from fructose 6-phosphate by an enzyme called **phosphofructokinase 2 (PFK2)**, a different enzyme from PFK. F-2,6-BP is hydrolyzed back to fructose 6-phosphate (Fig. 4) by **fructose biphosphatase 2 (FBPase2)**. Amazingly, both PFK2 and FBPase2 are activities catalyzed by the same polypeptide; hence this is a **bi-functional enzyme**. Fructose 6-phosphate stimulates the synthesis of F-2,6-BP and inhibits its hydrolysis (Fig. 4). F-2,6-BP in turn strongly activates PFK and hence stimulates glycolysis. The overall effect is that when fructose 6-phosphate levels are high, PFK (and hence glycolysis) is stimulated. PFK2 and

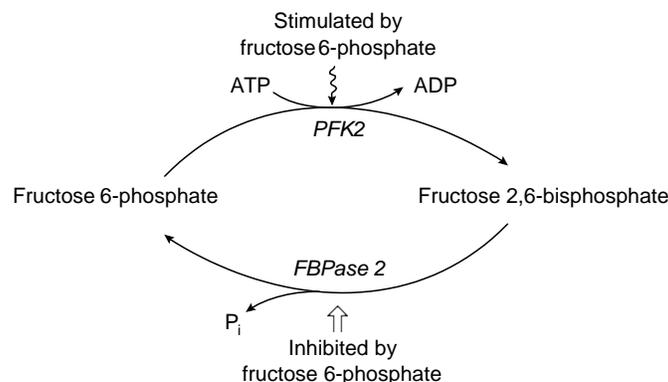


Fig. 4. Synthesis and degradation of fructose 2,6-bisphosphate.

FBPase2 are also controlled by covalent modification (see Topic C4). When blood glucose levels fall, the hormone glucagon is released into the bloodstream and triggers a cAMP cascade (see Topic J7) that leads to phosphorylation of the PFK2/FBPase2 polypeptide at a single serine residue. This activates FBPase2 and inhibits PFK2, lowering the level of F-2,6-BP and hence decreasing the rate of glycolysis. The reverse is true as glucose levels rise; the phosphate group is removed from the PFK2/FBPase2 polypeptide by a phosphatase, thus inhibiting FBPase2 and activating PFK2, raising the level of F-2,6-BP and hence increasing the rate of glycolysis.

F-2,6-BP is also important in preventing glycolysis (glucose degradation) and gluconeogenesis (glucose synthesis) operating simultaneously. This is called **reciprocal regulation** and is described in Topic J4.

- *H⁺ ions.* PFK is inhibited by H⁺ ions and hence the rate of glycolysis decreases when the pH falls significantly. This prevents the excessive formation of lactate (i.e. lactic acid) under anaerobic conditions (see above) and hence prevents the medical condition known as acidosis (a deleterious drop in blood pH).

Hexokinase

Hexokinase, which catalyzes the first irreversible step of glycolysis, is inhibited by glucose 6-phosphate. Thus when PFK is inhibited, fructose 6-phosphate builds up and so does glucose 6-phosphate since these two metabolites are in equilibrium via phosphoglucosomerase (see Fig. 1). The hexokinase inhibition then reinforces the inhibition at the PFK step. At first sight this seems unusual since it is usually the first irreversible step of a pathway (the committed step) that is the main control step. On this basis, it may appear that hexokinase should be the main control enzyme, not PFK. However, glucose 6-phosphate, the product of the hexokinase reaction, can also feed into glycogen synthesis (see Topic J6) or the pentose phosphate pathway (see Topic J5). Thus the first irreversible step that is unique to glycolysis is that catalyzed by PFK and hence this is the main control step.

Pyruvate kinase

Pyruvate kinase catalyzes the third irreversible step in glycolysis. It is activated by fructose 1,6-bisphosphate. ATP and the amino acid alanine allosterically inhibit the enzyme so that glycolysis slows when supplies of ATP and biosynthetic precursors (indicated by the levels of Ala) are already significantly high. In addition, in a control similar to that for PFK (see above), when the blood glucose concentration is low, glucagon is released and stimulates phosphorylation of the enzyme via a cAMP cascade (see Topic J7). This covalent modification inhibits the enzyme so that glycolysis slows down in times of low blood glucose levels.

GLUCONEOGENESIS

Key Notes

Overview

Gluconeogenesis synthesizes glucose from noncarbohydrate precursors and is important for the maintenance of blood glucose levels during starvation or during vigorous exercise. The brain and erythrocytes depend almost entirely on blood glucose as an energy source. Gluconeogenesis occurs mainly in the liver and to a lesser extent in the kidney. Most enzymes of gluconeogenesis are cytosolic, but pyruvate carboxylase and glucose 6-phosphatase are located in the mitochondrial matrix and bound to the smooth endoplasmic reticulum, respectively.

The pathway

Pyruvate is converted to oxaloacetate (by pyruvate carboxylase). The oxaloacetate is decarboxylated and phosphorylated to phosphoenolpyruvate (PEP) by phosphoenolpyruvate carboxykinase (PEP carboxykinase). PEP is converted to fructose 1,6-bisphosphate by a direct reversal of several reactions in glycolysis. Next, fructose 1,6-bisphosphate is dephosphorylated to fructose 6-phosphate (by fructose 1,6-bisphosphatase) and this is then converted to glucose 6-phosphate (by phosphoglucosomerase). Finally, glucose 6-phosphate is dephosphorylated (by glucose 6-phosphatase) to yield glucose.

Energy used

The synthesis of one molecule of glucose from two molecules of pyruvate requires six molecules of ATP.

Transport of oxaloacetate

Oxaloacetate, the product of the first step in gluconeogenesis, must leave the mitochondrion and enter the cytosol where the subsequent enzyme steps take place. Since the inner mitochondrial membrane is impermeable to oxaloacetate, it is converted to malate by mitochondrial malate dehydrogenase. This leaves the mitochondrion and is converted back to oxaloacetate in the cytosol by cytoplasmic malate dehydrogenase.

Pyruvate carboxylase activation

Oxaloacetate, the product of the pyruvate carboxylase reaction, functions both as an important citric acid cycle intermediate in the oxidation of acetyl CoA and as a precursor for gluconeogenesis. The activity of pyruvate carboxylase depends on the presence of acetyl CoA so that more oxaloacetate is made when acetyl CoA levels rise.

Reciprocal regulation glycolysis and gluconeogenesis

If glycolysis and gluconeogenesis operated simultaneously, the net effect would be a futile cycle resulting in the hydrolysis of two ATP and two GTP molecules. This is prevented by reciprocal regulation at the enzyme steps that are distinct in each pathway. AMP activates phosphofructokinase (PFK) (glycolysis) but inhibits fructose 1,6-bisphosphatase (gluconeogenesis). ATP and citrate inhibit PFK but citrate stimulates fructose 1,6-bisphosphatase. Glycolysis and gluconeogenesis are also responsive to starvation conditions via the concentration of fructose 2,6-bisphosphate (F-2,6-BP). During starvation, glucagon is released into the bloodstream and inhibits the synthesis of F-2,6-BP. In the fed state, insulin is released into the bloodstream and causes the accumulation of F-2,6-BP. Since F-2,6-BP activates PFK and inhibits

fructose 1,6-bisphosphatase, glycolysis is stimulated and gluconeogenesis is inhibited in the fed animal and vice versa, during starvation.

In liver, ATP and alanine inhibit pyruvate kinase (glycolysis) whilst ADP inhibits pyruvate carboxylase and PEP carboxykinase (gluconeogenesis). Thus glycolysis is inhibited in times when ATP and biosynthetic intermediates are in excess whilst gluconeogenesis is inhibited in times when the ATP level is low (and ADP is high). Pyruvate kinase is also stimulated by fructose 1,6-bisphosphate so its rate increases when glycolysis is active. During starvation, glycogen secretion into the bloodstream activates a cAMP cascade that leads to the phosphorylation and inhibition of pyruvate kinase (glycolysis).

The Cori cycle

During vigorous exercise, pyruvate produced by glycolysis in muscle is converted to lactate by lactate dehydrogenase. The lactate diffuses into the bloodstream and is carried to the liver. Here it is converted to glucose by gluconeogenesis. The glucose is released into the bloodstream and becomes available for uptake by muscle (as well as other tissues, including brain). This cycle of reactions is called the Cori cycle.

Overview

Gluconeogenesis synthesizes glucose from noncarbohydrate precursors, including lactate and pyruvate, citric acid cycle intermediates, the carbon skeletons of most amino acids and glycerol. This is extremely important since the brain and erythrocytes rely almost exclusively on glucose as their energy source under normal conditions. The liver's store of glycogen is sufficient to supply the brain with glucose for only about half a day during fasting. Thus gluconeogenesis is especially important in periods of starvation or vigorous exercise. During starvation, the formation of glucose via gluconeogenesis particularly uses amino acids from protein breakdown and glycerol from fat breakdown. During exercise, the blood glucose levels required for brain and skeletal muscle function are maintained by gluconeogenesis in the liver using lactate produced by the muscle.

The main site of gluconeogenesis is the liver, although it also occurs to a far lesser extent in the kidneys. Very little gluconeogenesis occurs in brain or muscle. Within liver cells, the first enzyme of gluconeogenesis, pyruvate carboxylase, is located in the mitochondrial matrix. The last enzyme, glucose 6-phosphatase is bound to the smooth endoplasmic reticulum. The other enzymes of the pathway are located in the cytosol.

The pathway

In glycolysis (Topic J3), glucose is metabolized to pyruvate. In gluconeogenesis, pyruvate is metabolized to glucose. Thus, in principle, gluconeogenesis appears to be a reversal of glycolysis. Indeed, some of the reactions of glycolysis are reversible and so the two pathways have these steps in common. However,

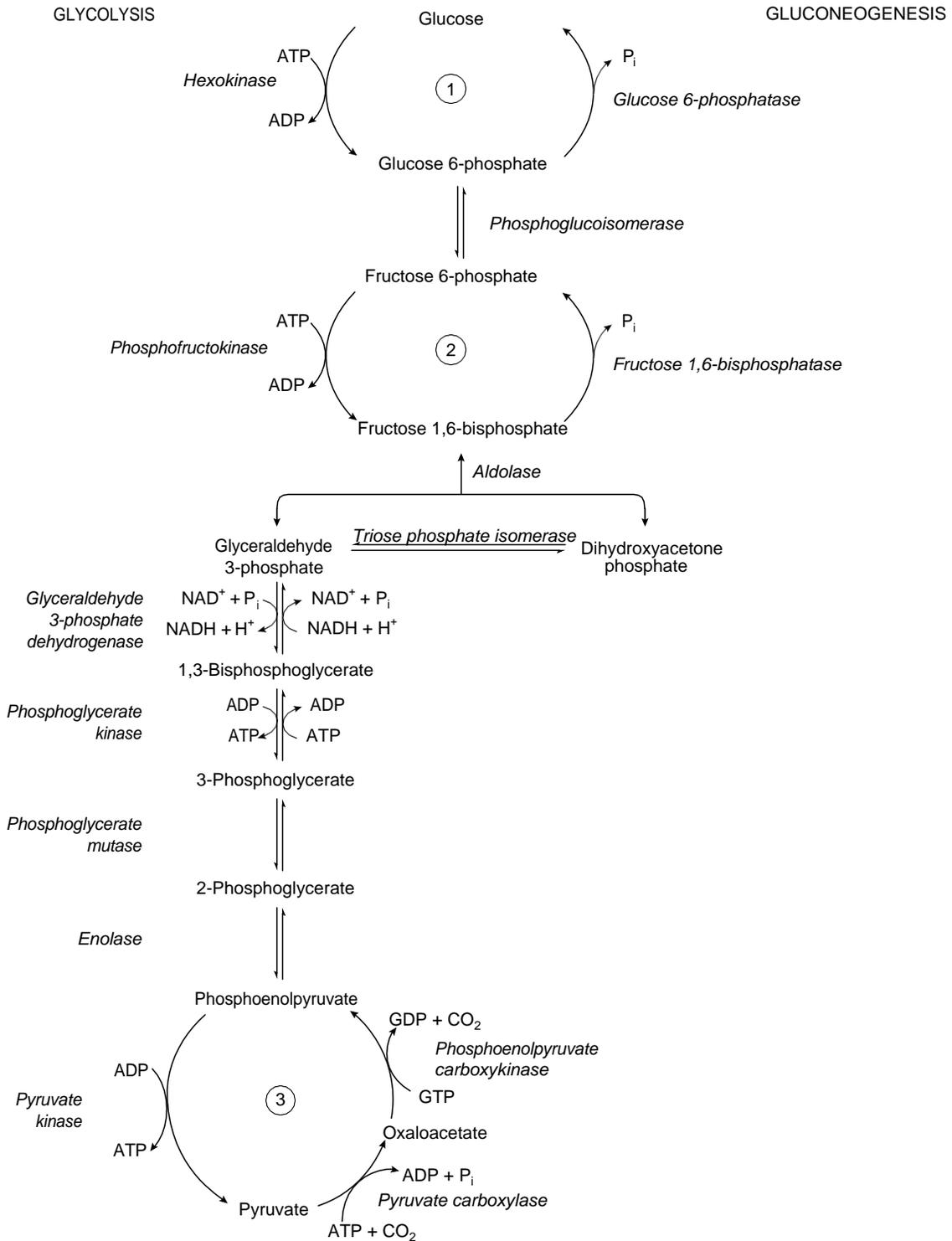


Fig. 1. Comparison of gluconeogenesis and glycolysis. The three steps of glycolysis that are irreversible are numbered. (1) Hexokinase in glycolysis is reversed by glucose 6-phosphatase in gluconeogenesis; (2) PFK in glycolysis is reversed by fructose 1,6-bisphosphatase in gluconeogenesis; (3) pyruvate kinase in glycolysis is reversed by two sequential reactions in gluconeogenesis catalyzed by pyruvate carboxylase and PEP carboxykinase.

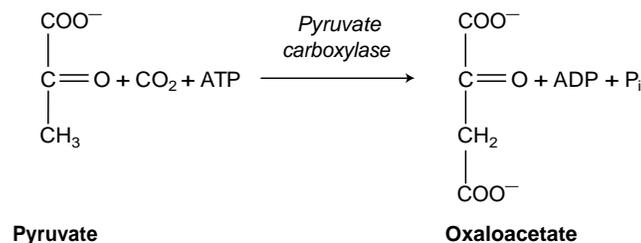
three steps in glycolysis are essentially irreversible; those catalyzed by the enzymes hexokinase, phosphofructokinase (PFK) and pyruvate kinase (see Topic J3). Indeed it is the large negative free-energy change in these reactions that normally drives glycolysis forward towards pyruvate formation. Therefore, in gluconeogenesis, these three steps have to be reversed by using other reactions as shown in *Fig. 1*; gluconeogenesis is not a simple reversal of glycolysis.

Precursors for gluconeogenesis

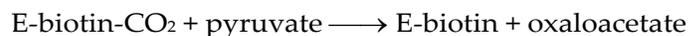
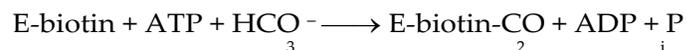
Glycerol can act as a substrate for glucose synthesis by conversion to dihydroxyacetone phosphate, an intermediate in gluconeogenesis (*Fig. 1*). In order for **lactate, pyruvate, citric acid cycle intermediates** and the carbon skeletons of most **amino acids** to act as precursors for gluconeogenesis, these compounds must first be converted to oxaloacetate. Some of the carbon skeletons of the amino acids give rise to oxaloacetate directly. Others feed into the citric acid cycle as intermediates (see Topic L1 and M2) and the cycle then converts these molecules to oxaloacetate. Lactate is converted to pyruvate by the lactate dehydrogenase reaction (see Topic J3) and some amino acids also give rise to pyruvate (see Topic M2). Therefore, for these precursors, the first step in the gluconeogenic pathway is the conversion of pyruvate to oxaloacetate.

The steps in gluconeogenesis (see *Fig. 1*) are as follows:

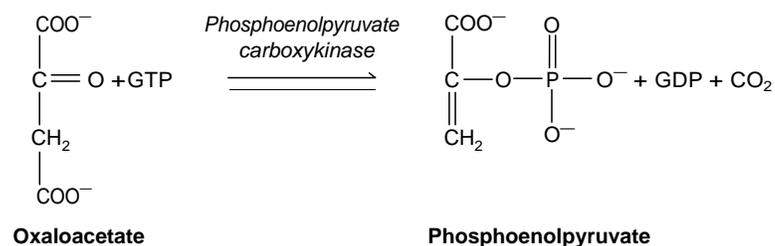
1. Pyruvate is converted to oxaloacetate by carboxylation using the enzyme **pyruvate carboxylase** that is located in the mitochondrial matrix.



This enzyme uses **biotin** as an **activated carrier of CO₂**, the reaction occurring in two stages:



2. The oxaloacetate is now acted on by **phosphoenolpyruvate carboxykinase** which simultaneously decarboxylates and phosphorylates it to form phosphoenolpyruvate (PEP), releasing CO₂ and using GTP in the process:

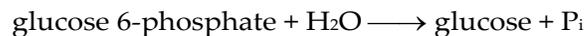


Thus, reversal of the glycolytic step from PEP to pyruvate requires two reactions in gluconeogenesis, pyruvate to oxaloacetate by pyruvate carboxylase and oxaloacetate to PEP by PEP carboxykinase. Given that the conversion of PEP to pyruvate in glycolysis synthesizes ATP, it is not surprising that the overall reversal of this step needs the input of a substantial amount of energy, one ATP for the pyruvate carboxylase step and one GTP for the PEP carboxykinase step.

3. PEP is converted to fructose 1,6-bisphosphate in a series of steps that are a direct reversal of those in glycolysis (see Topic J3), using the enzymes enolase, phosphoglycerate mutase, phosphoglycerate kinase, glyceraldehyde 3-phosphate dehydrogenase, triose phosphate isomerase and aldolase (see Fig 1). This sequence of reactions uses one ATP and one NADH for each PEP molecule metabolized.
4. Fructose 1,6-bisphosphate is dephosphorylated to form fructose 6-phosphate by the enzyme **fructose 1,6-bisphosphatase**, in the reaction:



5. Fructose 6-phosphate is converted to glucose 6-phosphate by the glycolytic enzyme phosphoglucoisomerase.
6. Glucose 6-phosphate is converted to glucose by the enzyme **glucose 6-phosphatase**. This enzyme is bound to the smooth endoplasmic reticulum and catalyzes the reaction:



Energy used

As would be expected, the synthesis of glucose by gluconeogenesis needs the input of energy. Two pyruvate molecules are required to synthesize one molecule of glucose. Energy is required at the following steps:

pyruvate carboxylase	1 ATP (× 2) = 2 ATP
PEP carboxykinase	1 GTP (× 2) = 2 ATP
phosphoglycerate kinase	1 ATP (× 2) = 2 ATP
Total = 6 ATP	

This compares with only two ATPs as the net ATP yield from glycolysis. Thus an extra four ATPs per glucose are required to reverse glycolysis.

In fact, the glyceraldehyde 3-phosphate dehydrogenase reaction also consumes NADH, equivalent to two molecules of NADH for each molecule of glucose synthesized. Since each cytosolic NADH would normally be used to generate approximately two ATP molecules via the glycerol 3-phosphate shuttle and oxidative phosphorylation (see Topic L2), this is equivalent to the input of another four ATPs per glucose synthesized.

Transport of oxaloacetate

Pyruvate carboxylase is a mitochondrial matrix enzyme whereas the other enzymes of gluconeogenesis are located outside the mitochondrion. Thus oxaloacetate, produced by pyruvate carboxylase, needs to exit the mitochondrion. However the inner mitochondrial membrane is not permeable to this compound. Thus oxaloacetate is converted to malate inside the mitochondrion

by **mitochondrial malate dehydrogenase**, the malate is transported through the mitochondrial membrane by a special transport protein and then the malate is converted back to oxaloacetate in the cytoplasm by a **cytoplasmic malate dehydrogenase** (Fig. 2).

Pyruvate carboxylase activation

Oxaloacetate has two main roles. It is an intermediate that is consumed in gluconeogenesis and it is also a key intermediate in the citric acid cycle where it fuses with acetyl CoA to form citrate, eventually being regenerated by the cycle. Thus pyruvate carboxylase generates oxaloacetate for gluconeogenesis but also must maintain oxaloacetate levels for citric acid cycle function. For the latter reason, the activity of pyruvate carboxylase depends absolutely on the presence of acetyl CoA; the biotin prosthetic group of the enzyme cannot be carboxylated unless acetyl CoA is bound to the enzyme. This allosteric activation by acetyl CoA ensures that more oxaloacetate is made when excess acetyl CoA is present. In this role of maintaining the level of citric acid cycle intermediates, the pyruvate carboxylase reaction is said to be **anaplerotic**, that is 'filling up'.

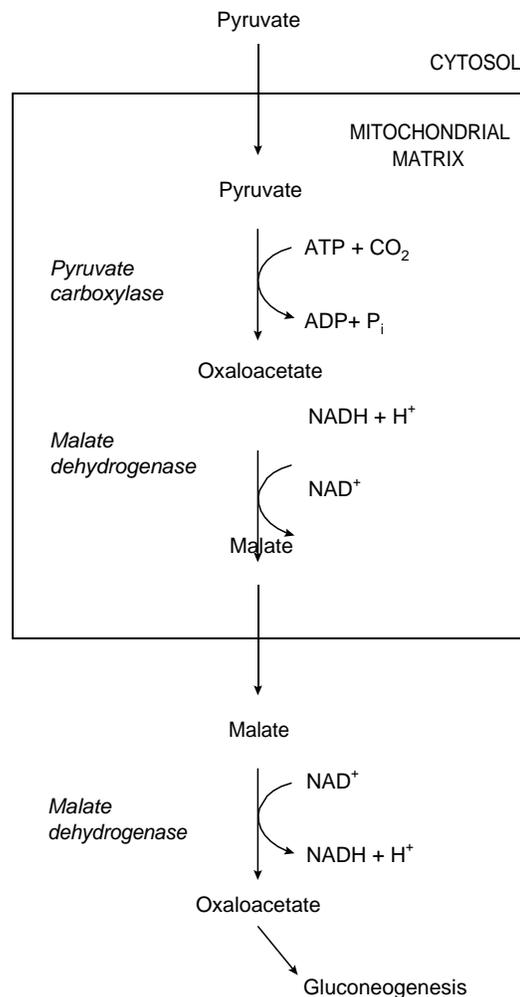


Fig. 2. Transport of oxaloacetate out of the mitochondrion.

Reciprocal regulation of glycolysis and gluconeogenesis

Glycolysis generates two ATPs net per glucose whereas gluconeogenesis uses four ATPs and two GTPs per glucose. Thus, if both glycolysis and gluconeogenesis were allowed to operate simultaneously, converting glucose to pyruvate and back again, the only net result would be the utilization of two ATPs and two GTPs, a so-called **futile cycle**. This is prevented by tight coordinate regulation of glycolysis and gluconeogenesis. Since many of the steps of the two pathways are common, the steps that are distinct in each pathway are the sites of this regulation, in particular the interconversions between fructose 6-phosphate and fructose 1,6-bisphosphate and between PEP and pyruvate. The situation is summarized in *Fig. 3* and described in detail below.

Regulation of PFK and fructose 1,6-bisphosphatase

When the level of AMP is high, this indicates the need for more ATP synthesis. AMP stimulates PFK, increasing the rate of glycolysis, and inhibits fructose 1,6-bisphosphatase, turning off gluconeogenesis. Conversely, when ATP and citrate levels are high, this signals that no more ATP need be made. ATP and citrate inhibit PFK, decreasing the rate of glycolysis, and citrate stimulates fructose 1,6-bisphosphatase, increasing the rate of gluconeogenesis.

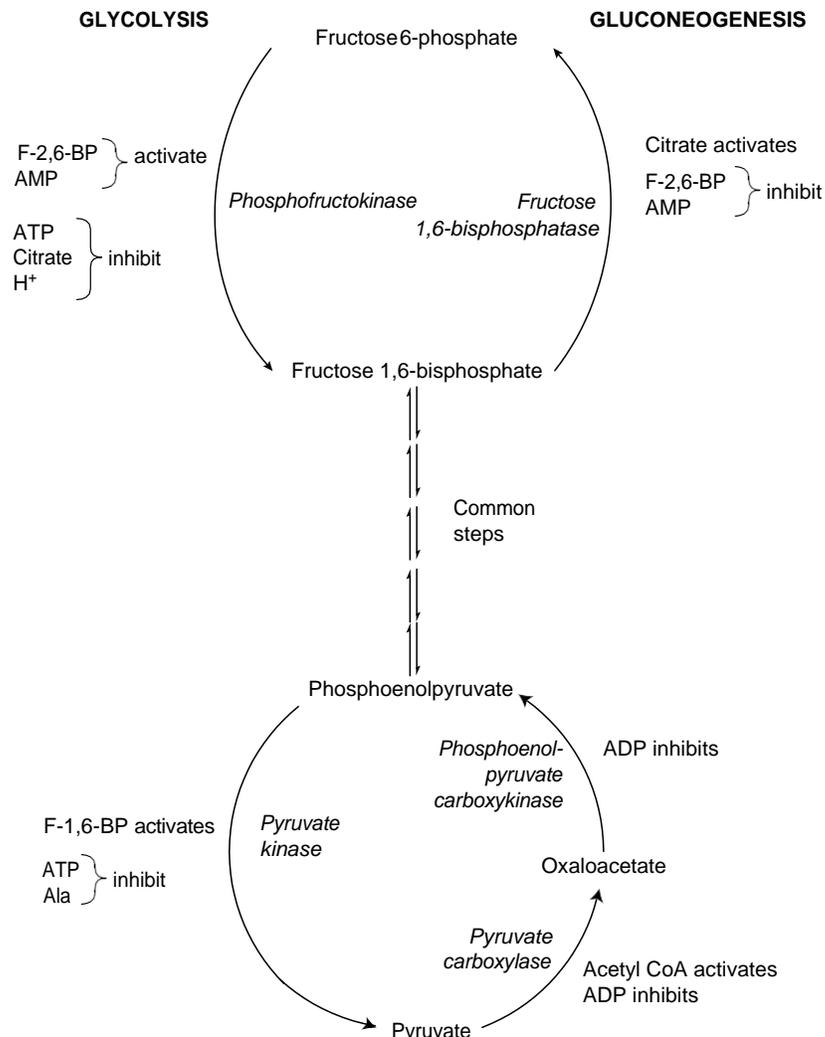


Fig. 3. Reciprocal regulation of glycolysis and gluconeogenesis.

Glycolysis and gluconeogenesis are made responsive to starvation by the level of the regulatory molecule **fructose 2,6-bisphosphate (F-2,6-BP)**. F-2,6-BP is synthesized from fructose 6-phosphate and hydrolyzed back to fructose 6-phosphate by a single polypeptide with two enzymatic activities (PFK2 and FBPase2; see Topic J3). The level of F-2,6-BP is under hormonal control. During starvation, when the level of blood glucose is low, the hormone glucagon is released into the bloodstream and triggers a cAMP cascade (Topic J7), eventually causing phosphorylation of the PFK2/FBPase2 polypeptide. This activates FBPase2 and inhibits PFK2, lowering the level of F-2,6-BP (see Topic J3). In the fed state, when blood glucose is at a high level, the hormone insulin is released and has the opposite effect, causing an elevation in the level of F-2,6-BP. Since F-2,6-BP strongly stimulates PFK and inhibits fructose 1,6-bisphosphatase (Fig. 3), glycolysis is stimulated and gluconeogenesis is inhibited in the fed animal. Conversely, during starvation, the low level of F-2,6-BP allows gluconeogenesis to predominate.

Regulation of pyruvate kinase, pyruvate carboxylase and PEP carboxykinase

- In liver, pyruvate kinase is inhibited by high levels of ATP and alanine so that glycolysis is inhibited when ATP and biosynthetic intermediates are already plentiful (see Topic J3). Acetyl CoA is also abundant under these conditions and activates pyruvate carboxylase, favoring gluconeogenesis. Conversely, when the energy status of the cell is low, the ADP concentration is high and this inhibits both pyruvate carboxylase and PEP carboxykinase, switching off gluconeogenesis. At this time, the ATP level will be low so pyruvate kinase is not inhibited and glycolysis will operate.
- Pyruvate kinase is also stimulated by fructose 1,6-bisphosphate (see Topic J3; feedforward activation) so that its activity rises when needed, as glycolysis speeds up.
- During starvation, the priority is to conserve blood glucose for the brain and muscle. Thus, under these conditions, pyruvate kinase in the liver is switched off. This occurs because the hormone glucagon is secreted into the bloodstream and activates a cAMP cascade (see Topic J7) that leads to the phosphorylation and inhibition of this enzyme.

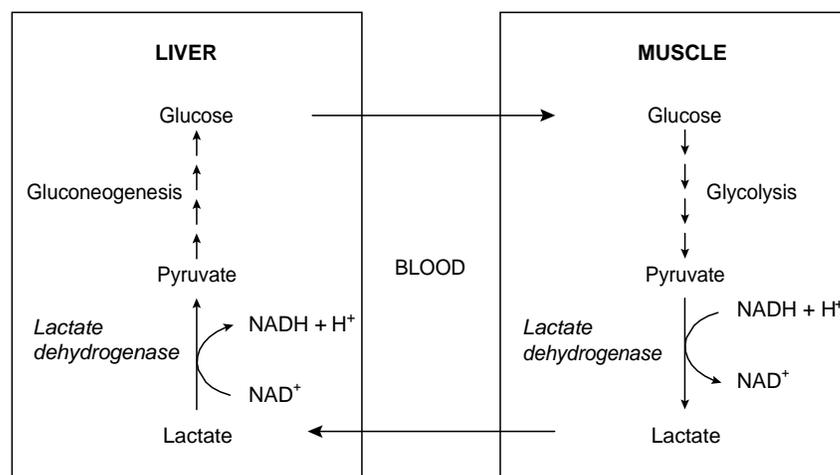


Fig. 4. The Cori cycle.

The Cori cycle Under the limiting oxygen conditions experienced during vigorous exercise, the formation of NADH by glycolysis exceeds the ability of the respiratory chain to oxidize it back to NAD⁺. The pyruvate produced by glycolysis in muscle is then converted to lactate by lactate dehydrogenase, a reaction that regenerates NAD⁺ and so allows glycolysis to continue to produce ATP (see Topic J3). However, lactate is a metabolic dead-end in that it cannot be metabolized further until it is converted back to pyruvate. Lactate diffuses out of the muscle and is carried in the bloodstream to the liver. Here it diffuses into liver cells and is converted back to pyruvate by lactate dehydrogenase. The pyruvate is then converted to glucose by gluconeogenesis and the glucose is released back into the bloodstream ready to be taken up by skeletal muscle (and brain). This cycle of reactions (*Fig. 4*) is called **the Cori cycle**.

PENTOSE PHOSPHATE WAY

Key Notes

Overview

The two major products of the pathway are nicotinamide adenine dinucleotide (reduced form; NADPH) and ribose 5-phosphate. Ribose 5-phosphate and its derivatives are components of important cellular molecules such as RNA, DNA, NAD⁺, flavine adenine dinucleotide (FAD), ATP and coenzyme A (CoA). NADPH is required for many biosynthetic pathways and particularly for synthesis of fatty acids and steroids. Hence the pathway is very active in tissues such as adipose tissue, mammary gland and the adrenal cortex.

Main reactions of the pathway

The reactions of the pathway can be grouped into three stages. In the first stage, oxidative reactions convert glucose 6-phosphate into ribulose 5-phosphate, generating two NADPH molecules. In the second stage, ribulose 5-phosphate is converted to ribose 5-phosphate by isomerization. The third stage of reactions, catalyzed by transketolase and transaldolase, converts ribose 5-phosphate into fructose 6-phosphate and glyceraldehyde 3-phosphate and hence links the pentose phosphate pathway with glycolysis.

Control of the pathway

The transketolase and transaldolase reactions are reversible and so allow either the conversion of ribose 5-phosphate into glycolytic intermediates when it is not needed for other cellular reactions, or the generation of ribose 5-phosphate from glycolytic intermediates when more is required. The rate of the pentose phosphate pathway is controlled by NADP⁺ regulation of the first step, catalyzed by glucose 6-phosphate dehydrogenase.

Overview

Reducing power is available in a cell both as NADH and NADPH but these have quite distinct roles. NADH is oxidized by the respiratory chain to generate ATP via oxidative phosphorylation (see Topic L2). NADPH is used for biosynthetic reactions that require reducing power. Despite their similar structures (see Topic C1), NADH and NADPH are not metabolically interchangeable and so the cell must carry out a set of reactions that specifically create NADPH. This set of reactions is the pentose phosphate pathway (also known as the **hexose monophosphate shunt** or the **phosphogluconate pathway**). It takes place in the cytosol and is particularly important in tissues such as adipose tissue, mammary gland and the adrenal cortex that synthesizes fatty acids and steroids from acetyl CoA (see Topic K3). The activity of the pathway is very low in skeletal muscle, for example, which does not synthesize fatty acids or steroids.

The core set of reactions of the pathway oxidize glucose 6-phosphate to ribose 5-phosphate and generate NADPH. Thus, as well as generating NADPH, the pathway has a second important role in converting hexoses into pentoses, in particular ribose 5-phosphate. Ribose 5-phosphate or derivatives of it are required for the synthesis of RNA, DNA, NAD⁺, flavine adenine dinucleotide (FAD), ATP, coenzyme A (CoA) and other important molecules. Thus the two main products of the pathway are NADPH and ribose 5-phosphate.

**Main reactions
of the pathway**

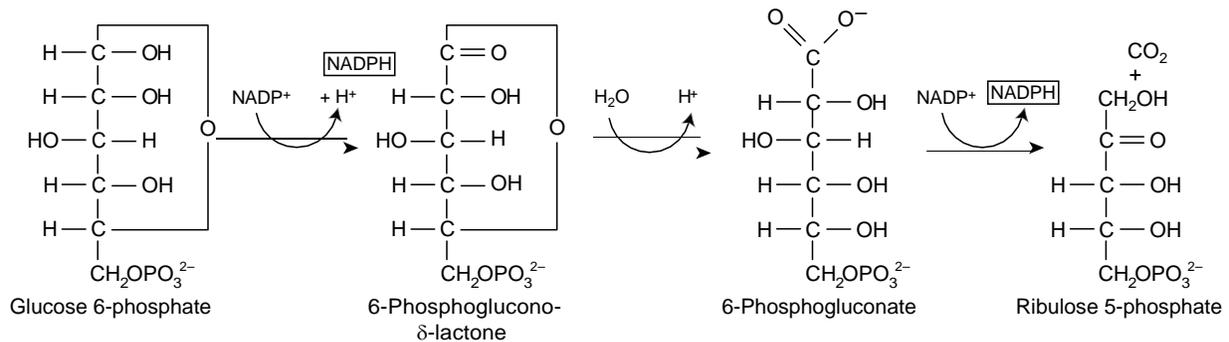
The core reactions of the pathway can be summarized as:



The pathway has three stages:

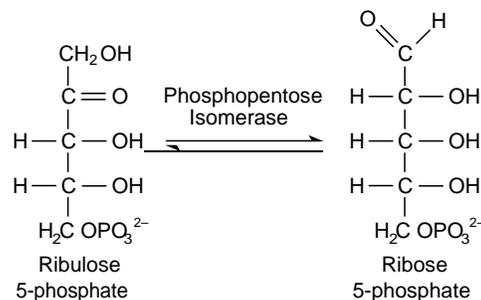
Stage 1 – Oxidative reactions that convert glucose 6-phosphate into ribulose 5-phosphate, generating two NADPH molecules

Glucose 6-phosphate is oxidized by **glucose 6-phosphate dehydrogenase** to 6-phosphoglucono-6-lactone (producing NADPH) and this is then hydrolyzed by **lactonase** to 6-phosphogluconate. The 6-phosphogluconate is subsequently converted by **6-phosphogluconate dehydrogenase** to ribulose 5-phosphate. This is an **oxidative decarboxylation** (i.e. the 6-phosphogluconate is oxidized and a carbon is removed as CO₂). These reactions are shown below:



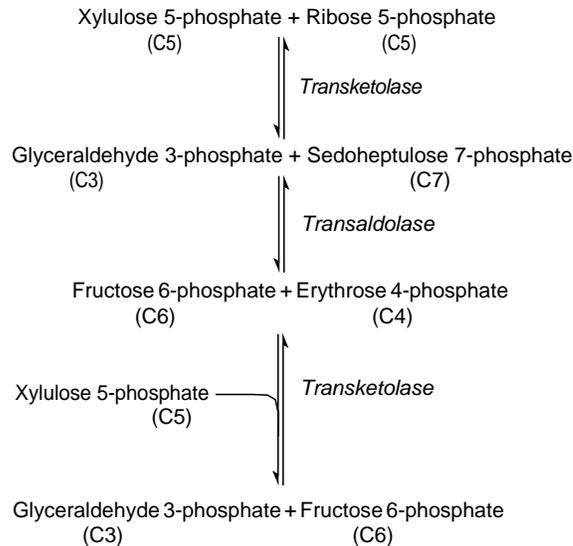
Stage 2 – Isomerization of ribulose 5-phosphate to ribose 5-phosphate

The ribulose 5-phosphate is now converted to ribose 5-phosphate by isomerization, a reaction catalyzed by **phosphopentose isomerase**:

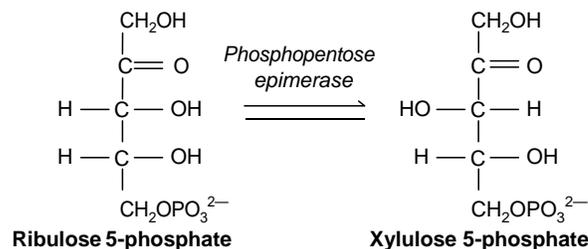


Stage 3 – Linkage of the pentose phosphate pathway to glycolysis via **transketolase** and **transaldolase**

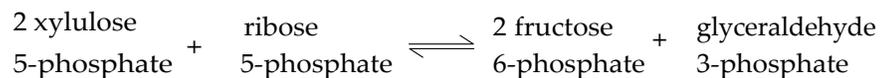
If at any time only a little ribose 5-phosphate is required for nucleic acid synthesis and other synthetic reactions, it will tend to accumulate and is then converted to fructose 6-phosphate and glyceraldehyde 3-phosphate by the enzymes **transketolase** and **transaldolase**. These two products are intermediates of glycolysis. Therefore, these reactions provide a link between the pentose phosphate pathway and glycolysis. The outline reactions are shown below.



Details of these reactions, showing the structures of the molecules involved, are given in *Fig. 1*. These reactions require xylulose 5-phosphate as well as ribose-5-phosphate. Xylulose 5-phosphate is an epimer (see Topic J1) of ribulose 5-phosphate and is made by **phosphopentose epimerase**:



Overall the reactions in this stage can be summarized as:



Control of the pathway

The transketolase and transaldolase reactions are reversible, so the final products of the pentose phosphate pathway can change depending on the metabolic needs of the cell. Thus when the cell needs NADPH but not ribose 5-phosphate, the latter is converted to glycolytic intermediates and enters glycolysis. At the other extreme, when the need for ribose 5-phosphate exceeds that for

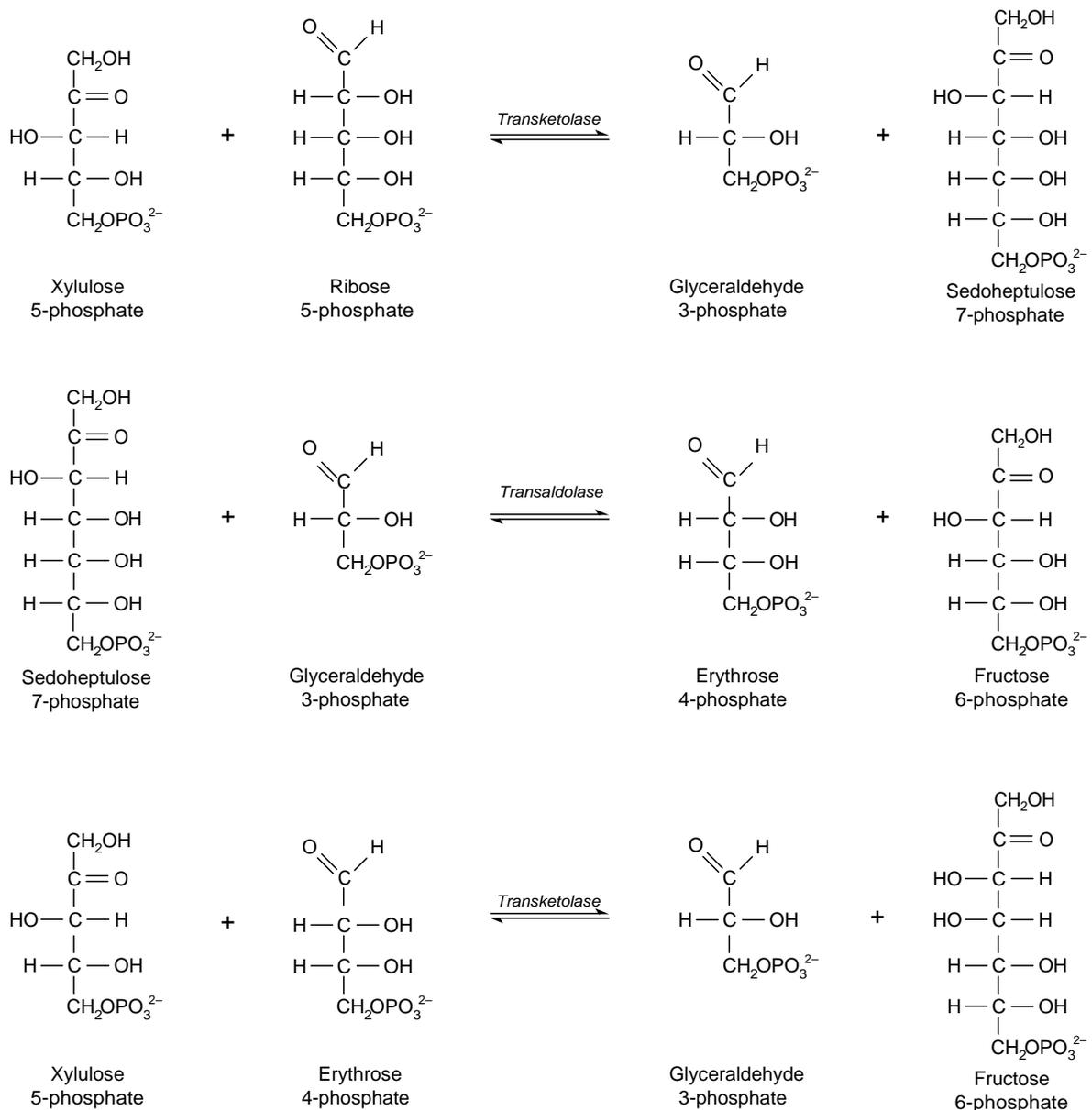


Fig. 1. Details of the transaldolase and transketolase reactions.

NADPH, fructose 6-phosphate and glyceraldehyde 3-phosphate can be taken from glycolysis and converted into ribose 5-phosphate by reversal of the transketolase and transaldolase reactions.

The first reaction of the pathway, the oxidation of glucose 6-phosphate by glucose 6-phosphate dehydrogenase, is rate limiting and irreversible. The enzyme is regulated by NADP⁺. As the cell uses NADPH, the concentration of NADP⁺ rises, stimulating glucose 6-phosphate dehydrogenase and so increasing the rate of the pathway and NADPH regeneration.

GLYCOGEN METABOLISM

Roles of glycogen Glycogen is a large polymer of glucose residues linked by α 1–4 glycosidic bonds

Key Notes

Roles of glycogen metabolism

Glycogen is stored mainly by the liver and skeletal muscle as an energy reserve. The role of stored glycogen in muscle is to provide a source of energy upon prolonged muscle contraction. In contrast, glycogen stored in the liver is used to maintain blood glucose levels.

Glycogen degradation

Glycogen degradation is carried out by glycogen phosphorylase and glycogen-debranching enzyme. Phosphorylase removes glucose units sequentially from the nonreducing ends of a glycogen molecule, producing glucose 1-phosphate as the product. It breaks only α 1–4 glycosidic bonds and cannot break the α 1–6 branchpoints. The glucose 1-phosphate is converted to glucose 6-phosphate by phosphoglucomutase. In liver this is further converted to glucose by glucose 6-phosphatase and the glucose enters the bloodstream. Muscle lacks glucose 6-phosphatase. Rather, here the glucose 6-phosphate enters glycolysis and is oxidized to yield energy for muscle contraction.

Glycogen synthesis

UDP-glucose is synthesized by UDP-glucose pyrophosphorylase from UTP and glucose 1-phosphate. Glycogen synthase then uses the UDP-glucose as a substrate to synthesize glycogen, adding one residue at a time to the nonreducing end of the glycogen molecule, forming α 1–4 bonds between neighboring glucosyl residues. The enzyme can only extend chains and therefore requires a primer, called glycogenin, in order to begin synthesis. Glycogenin is a protein with eight glucose units joined by α 1–4 bonds. The branches in glycogen are created by branching enzyme that breaks an α 1–4 bond in the glycogen chain and moves about seven residues to an internal location, joining them to the main chain by an α 1–6 bond.

metabolism

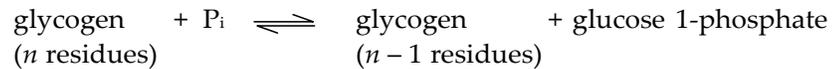
with branches every 10 residues or so via α 1–6 glycosidic bonds (see Topic J2 for structure). Glycogen provides an important energy reserve for the body. The two main storage sites are the liver and skeletal muscle where the glycogen is stored as granules in the cytosol. The granules contain not only glycogen but also the enzymes and regulatory proteins that are required for glycogen degradation and synthesis. Glycogen metabolism is important because it enables the blood glucose level to be maintained between meals (via glycogen stores in the liver) and also provides an energy reserve for muscular activity. The maintenance of blood glucose is essential in order to supply tissues with an easily metabolizable energy source, particularly the brain which uses only glucose

except after a long starvation period.

Glycogen degradation

Glycogen degradation requires two enzymes; **glycogen phosphorylase** and **glycogen-debranching enzyme**.

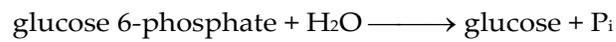
Glycogen phosphorylase (often called simply **phosphorylase**) degrades glycogen by breaking α 1–4 glycosidic bonds to release glucose units one at a time from the nonreducing end of the glycogen molecule (the end with a free 4'OH group; see Topic J2) as glucose 1-phosphate. The other substrate required is inorganic phosphate (P_i). The reaction is an example of **phosphorolysis**, that is breakage of a covalent bond by the addition of a phosphate group. The (reversible) reaction is as follows:



However, glycogen phosphorylase can remove only those glucose residues that are more than five residues from a branchpoint. Glycogen-debranching enzyme removes the α 1–6 branches and so allows phosphorylase to continue degrading the glycogen molecule. The glucose 1-phosphate produced is converted to glucose 6-phosphate by the enzyme **phosphoglucomutase**:



The fate of the glucose 6-phosphate depends on the tissue. Liver contains the enzyme glucose 6-phosphatase which converts the glucose 6-phosphate to glucose, which then diffuses out into the bloodstream and so maintains the blood glucose concentration:



During glycogen degradation in muscle, the main aim is to produce energy quickly and so the glucose 6-phosphate is metabolized immediately via glycolysis. This tissue does not contain glucose 6-phosphatase.

Glycogen synthesis

Three enzymes are needed to synthesize glycogen:

1. **UDP-glucose pyrophosphorylase** catalyzes the synthesis of UDP-glucose (see Fig. 1) from UTP and glucose 1-phosphate:



The pyrophosphate (PP_i) is immediately hydrolyzed by inorganic pyrophosphatase, releasing energy. Thus the overall reaction is very exergonic and essentially irreversible.

2. **Glycogen synthase** now transfers the glucosyl residue from UDP-glucose to the C4 OH group at the nonreducing end of a glycogen molecule, forming an α 1–4 glycosidic bond (see Fig. 2). Interestingly, glycogen synthase can only extend an existing chain. Thus it needs a primer; this is a protein called **glycogenin**. Glycogenin contains eight glucosyl units linked via α 1–4 linkages, which are added to the protein by itself (i.e. autocatalysis). It is this molecule that glycogen synthase then extends. Each glycogen granule contains only a single glycogenin molecule at its core. The fact that glycogen synthase is fully active only when in contact with glycogenin limits the size of the glycogen granule.
3. **Branching enzyme [amylo-(1–4→1–6) transglycosylase]** is a different

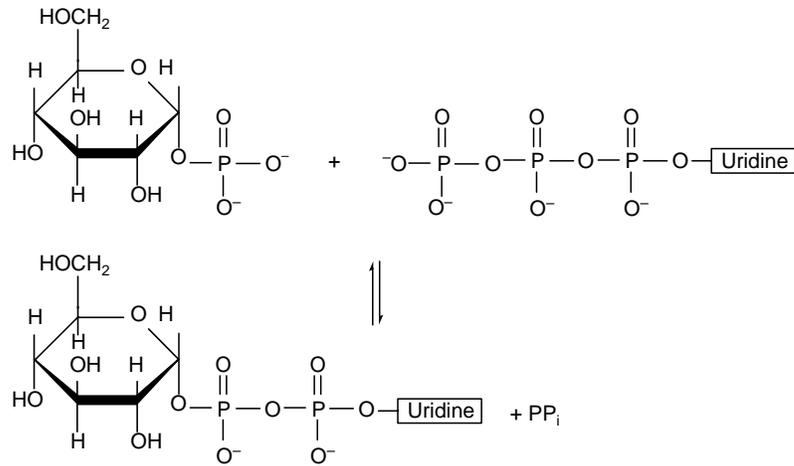


Fig. 1. The UDP-glucose pyrophosphorylase reaction.

enzyme from glycogen-debranching enzyme. After a number of glucose units have been joined as a straight chain with α 1–4 linkages, branching enzyme breaks one of the α 1–4 bonds and transfers a block of residues (usually about seven) to a more interior site in the glycogen molecule, reattaching these by creating an α 1–6 bond. The branches are important because the enzymes that degrade and synthesize glycogen (glycogen synthase and glycogen phosphorylase, respectively) work only at the ends of the glycogen molecule. Thus the existence of many termini allows a far more rapid rate of synthesis and degradation than would be possible with a nonbranched polymer.

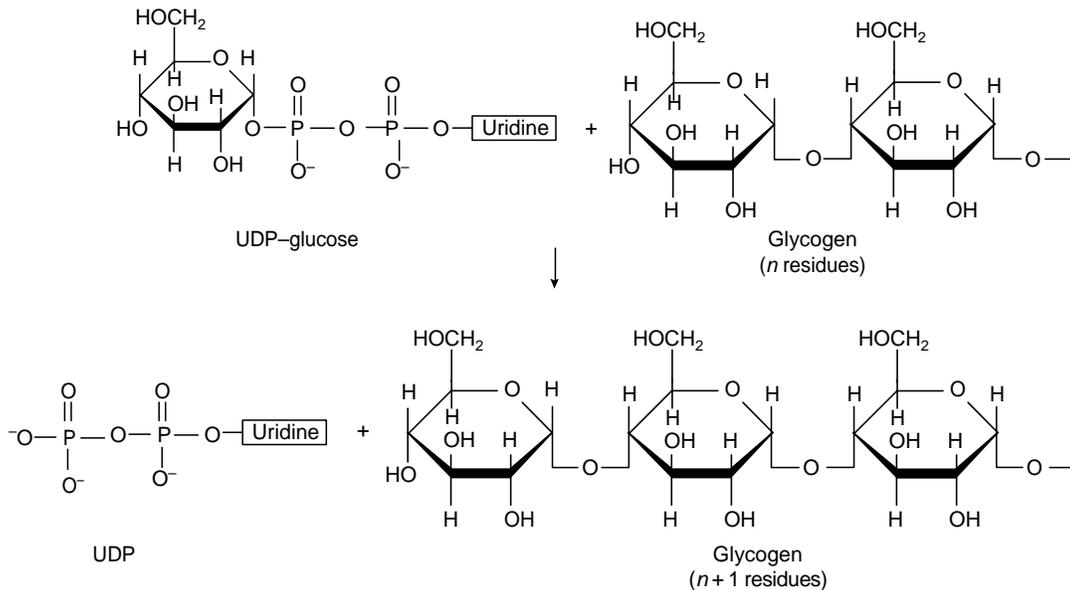


Fig. 2. Synthesis of glycogen by glycogen synthase.

CONTROL OF GLYCOGEN METABOLISM

Key Notes

Overview

Glycogen degradation and glycogen synthesis are controlled both by allosteric regulation and by hormonal control.

Allosteric control and covalent modification

Phosphorylase exists in a phosphorylated active *a* form and a dephosphorylated normally inactive *b* form. The two forms are interconverted by phosphorylase kinase and protein phosphatase I. In muscle, phosphorylase *b* is activated by the high concentrations of AMP generated by strenuous exercise and thus degrades glycogen, but the AMP stimulation is opposed by high concentrations of ATP and glucose 6-phosphate and so the enzyme is inactive in resting muscle. In liver, phosphorylase *b* is not responsive to AMP but phosphorylase *a* is deactivated by glucose so that glycogen degradation to produce glucose occurs only when glucose levels are low. Glycogen synthase exists as a phosphorylated normally inactive *b* form and a dephosphorylated active *a* form. A high concentration of glucose 6-phosphate can activate synthase *b* in resting muscle, stimulating glycogen synthesis, but the enzyme is inactive in contracting muscle where the glucose 6-phosphate concentration is low.

Hormonal control by epinephrine and glucagon

Epinephrine (adrenaline) stimulates glycogen degradation in skeletal muscle. Epinephrine and glucagon stimulate glycogen degradation in liver. The hormone binds to a plasma membrane receptor and activates adenylate cyclase via a G protein. Adenylate cyclase synthesizes cAMP from ATP which in turn activates protein kinase A. Protein kinase A phosphorylates phosphorylase kinase which activates it. The phosphorylase kinase then converts inactive phosphorylase *b* to active phosphorylase *a* by phosphorylation. The same active protein kinase A inactivates glycogen synthase by phosphorylation, converting glycogen synthase *a* to glycogen synthase *b*. When hormone levels fall, stimulation of glycogen degradation is turned off by degradation of cAMP to 5' AMP by phosphodiesterase and by dephosphorylation of the phosphorylated forms of phosphorylase and synthase by protein phosphatase I.

Hormonal control by insulin

Insulin is released into the bloodstream when the blood glucose concentration is high and it stimulates glycogen synthesis. It binds to and activates a receptor protein kinase in the plasma membrane of target cells. This leads to activation of an insulin-responsive protein kinase then activates protein phosphatase I by phosphorylation. Activated protein phosphatase I ensures that phosphorylase and glycogen synthase are dephosphorylated, thus inhibiting glycogen degradation and activating glycogen synthesis.

Calcium control of glycogen metabolism

During muscle contraction, Ca^{2+} ions released from the sarcoplasmic reticulum partially activate dephosphorylated phosphorylase kinase and this in turn activates phosphorylase, stimulating glycogen degradation.

Overview

If glycogen synthesis and glycogen degradation were allowed to occur simultaneously, the net effect would be hydrolysis of UTP, a so-called **futile cycle** (Fig. 1). To avoid this, both pathways need to be tightly controlled. This control is carried out via allosteric regulation and covalent modification of both the glycogen synthase and phosphorylase. In addition, the covalent modification is under close hormonal control.

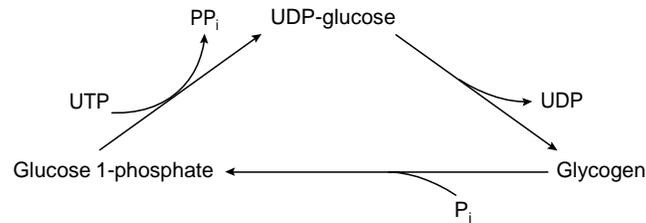


Fig. 1. Simultaneous operation of glycogen synthesis and degradation resulting in net hydrolysis of UTP.

Allosteric control and covalent modification

Phosphorylase exists in two interchangeable forms; active **phosphorylase a** and a normally inactive **phosphorylase b**. Phosphorylase *b* is a dimer and is converted into phosphorylase *a* by phosphorylation of a single serine residue on each subunit by the enzyme **phosphorylase kinase**. The process can be reversed and phosphorylase inactivated by removal of the phosphate group by **protein phosphatase I** (Fig. 2a) (see Topic C5).

In skeletal muscle, high concentrations of AMP can activate phosphorylase *b* (by acting at an **allosteric site**) but this is opposed by the concentrations of

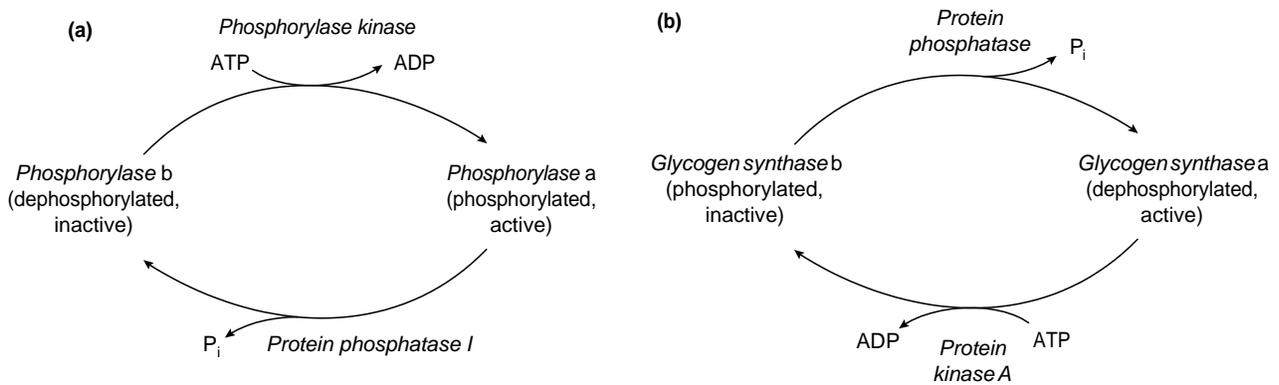


Fig. 2. Regulation of (a) glycogen phosphorylase activity and (b) glycogen synthase activity by phosphorylation (covalent modification).

ATP and glucose 6-phosphate found in resting muscle so that in this condition phosphorylase *b* is indeed inactive. Since most of the phosphorylase in resting muscle is phosphorylase *b*, significant glycogen degradation does not occur under these conditions. However, during exercise, the concentrations of ATP and glucose 6-phosphate fall and the concentration of AMP rises. Thus phosphorylase *b* becomes activated and this leads to the rapid degradation of glycogen to yield energy as required. Phosphorylase *a* is unaffected by ATP, AMP or glucose 6-phosphate and so remains active under all conditions.

In liver, phosphorylase *b* is not activated by AMP and is therefore always inactive. Unlike muscle, therefore, glycogen degradation in liver is not responsive to the energy status of the cell. Rather phosphorylase *a* is deactivated by glucose. This fits with the different role of glycogen storage in liver, namely to maintain blood levels of glucose. Thus as glucose levels rise, glycogen degradation by liver phosphorylase *a* is shut off and degradation starts again only as the glucose level falls.

Glycogen synthase is also regulated by covalent modification and allosteric interactions. The enzyme exists as an active glycogen synthase *a* and a normally inactive glycogen synthase *b*. However, in contrast to phosphorylase, it is the active form of glycogen synthase (synthase *a*) that is dephosphorylated whereas the inactive synthase *b* form is the phosphorylated form (*Fig. 2b*).

A high concentration of glucose 6-phosphate can activate glycogen synthase *b*. During muscle contraction, glucose 6-phosphate levels are low and therefore glycogen synthase *b* is inhibited. This is at the time when phosphorylase *b* is most active (see above). Thus glycogen degradation occurs and glycogen synthesis is inhibited, preventing the operation of a futile cycle. When the muscle returns to the resting state and ATP and glucose 6-phosphate levels rise, phosphorylase *b* is inhibited (see above), turning off glycogen degradation, whereas glycogen synthase is activated to rebuild glycogen reserves. The synthase *a* form is active irrespective of the concentration of glucose 6-phosphate.

Hormonal control by epinephrine and glucagon

Glycogen metabolism is tightly controlled by hormones. When blood glucose levels fall, glucagon is secreted by the α cells of the pancreas and acts on the liver to stimulate glycogen breakdown to glucose which is then released into the bloodstream to boost blood glucose levels again. Muscular contraction or nervous stimulation (the 'fight or flight' response) causes the release of epinephrine (adrenaline) from the adrenal medulla and this acts on muscle to increase glycogen breakdown ready to supply the energy needs of the cells.

Consider first the activation of glycogen degradation by epinephrine in the liver. The hormone binds to a receptor, called the **β -adrenergic receptor**, in the plasma membrane of the target cell (*Fig. 3*). Binding of the hormone to the receptor causes a conformational change in the protein which in turn activates an enzyme called **adenylate cyclase**. The receptor does not activate adenylate cyclase directly but rather by activating a **G-protein** as an intermediary in the signaling process (see Topic E5 for details). Activated adenylate cyclase converts ATP to 3'5' cyclic AMP (cAMP). The cAMP binds to **protein kinase A (PKA)**, also known as **cAMP-dependent protein kinase**. This enzyme consists of two regulatory subunits (R) and two catalytic subunits (C), making a complex, R_2C_2 , that is normally inactive (*Fig. 3*). The binding of two molecules of cAMP to each of the regulatory subunits leads to dissociation of the complex into an R_2 complex and two free C subunits that are now catalytically active. The active protein kinase A

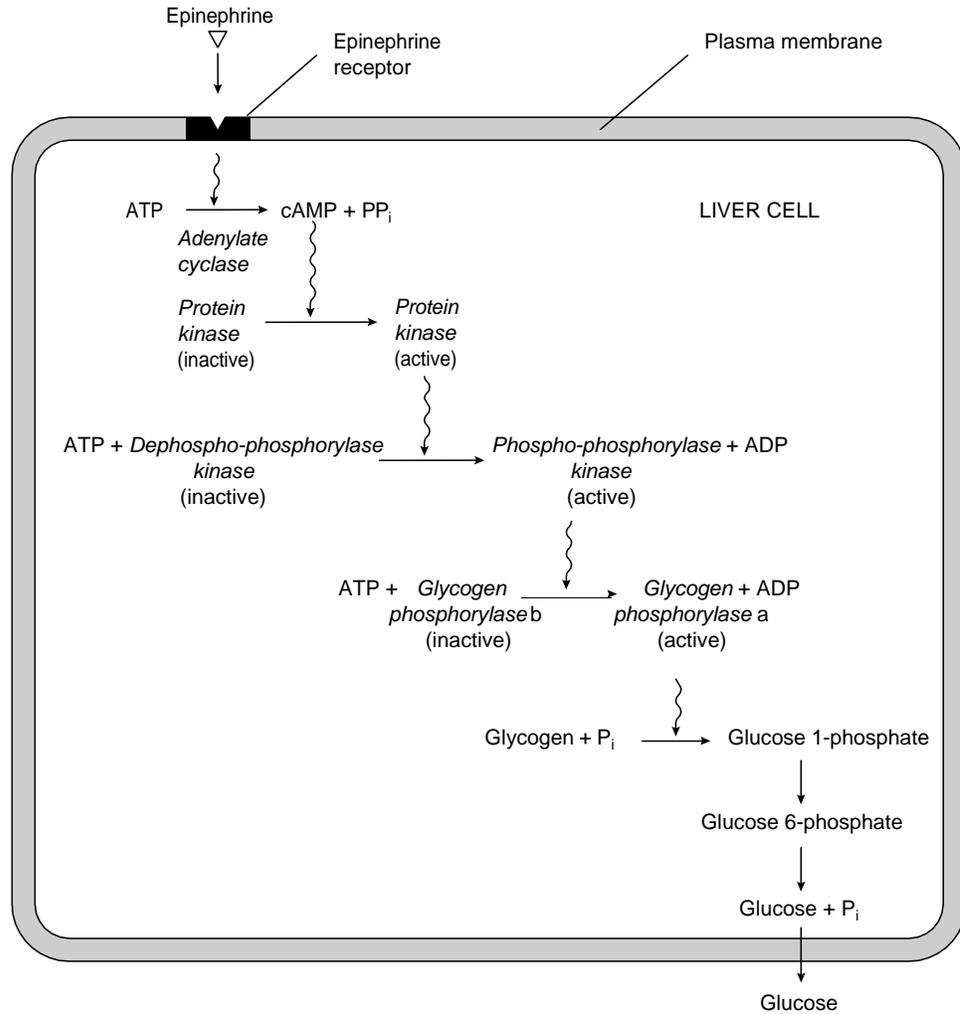


Fig. 3. Mechanism of action of epinephrine.

phosphorylates phosphorylase kinase which can exist as an inactive dephosphorylated form and an active phosphorylated form. Thus phosphorylase kinase is now also activated and in turn phosphorylates phosphorylase *b*, converting it to the active phosphorylase *a* that now carries out a rapid degradation of glycogen. This set of reactions is called a **cascade** and is organized so as to greatly amplify the original signal of a small number of hormone molecules. For example, each bound hormone causes the production of many cAMP molecules inside the cell; the activated protein kinase A in turn activates many molecules of phosphorylase kinase; each active phosphorylase kinase activates many molecules of phosphorylase. Thus a small hormonal signal can cause a major shift in cell metabolism.

To prevent the operation of a futile cycle, it is essential that glycogen synthesis is switched off during epinephrine or glucagon stimulation of glycogen breakdown. This is achieved by the activated protein kinase A that, as well as phosphorylating phosphorylase kinase, also phosphorylates glycogen synthase *a*, converting it to the inactive synthase *b* form (Fig. 4). Thus protein kinase A activates glycogen degradation and inhibits glycogen synthesis.

When epinephrine and glucagon levels in the bloodstream fall again, the hormone dissociates from the receptor, no more cAMP is made and existing

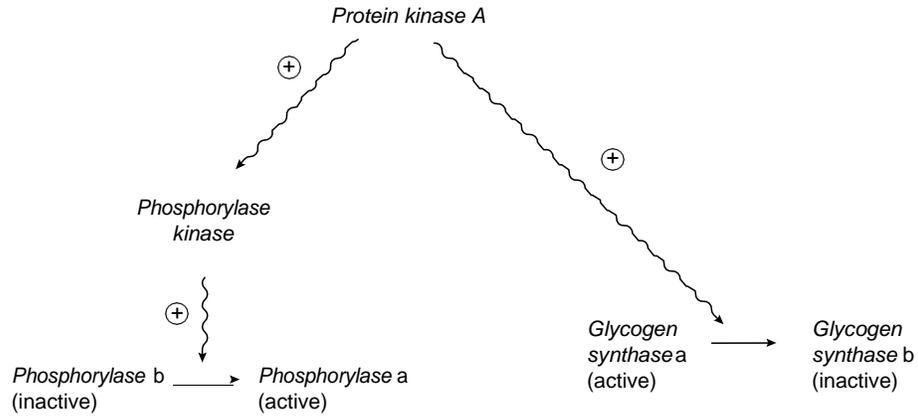


Fig. 4. Dual control of glycogen degradation and glycogen synthesis by protein kinase A.

cAMP is converted to 5' AMP (i.e. 'normal' AMP not the cyclic form) by cAMP phosphodiesterase, an enzyme that is constantly active in the cell. This decline in the cAMP level shuts off the activation cascade. The enzymes that had been phosphorylated are now dephosphorylated by protein phosphatase I, restoring them to their original condition.

Hormonal control by insulin

Insulin is released into the bloodstream by the β cells of the pancreas when blood glucose levels are high after feeding, and stimulates glycogen synthesis to store excess glucose as glycogen. This control is also achieved via phosphorylation events. Insulin binds to its receptor in the plasma membrane and activates it. This receptor has tyrosine kinase activity (i.e. it will phosphorylate selected tyrosine residues on target proteins; see Topic E5). Its activation leads to the activation of an insulin-responsive protein kinase that then phosphorylates protein phosphatase I, thus activating it. This enzyme now ensures that glycogen synthase is dephosphorylated (and hence active) and that phosphorylase kinase is also dephosphorylated (and hence inactive). The net effect is to stimulate glycogen synthesis.

Calcium control of glycogen metabolism

As we have seen, during epinephrine or glucagon hormonal control, dephosphorylated phosphorylase kinase is activated by being phosphorylated by protein kinase. This then activates phosphorylase and stimulates glycogen degradation. However, there is also another way to activate dephosphorylated phosphorylase kinase, at least partially, and that is by a high concentration of Ca^{2+} ions. This is important in muscle contraction which is triggered when Ca^{2+} is released from the internal store in the sarcoplasmic reticulum (see Topic N1). Thus, as well as allosteric control and hormonal control during muscle contraction, both of which stimulate glycogen degradation, there is also calcium control.

