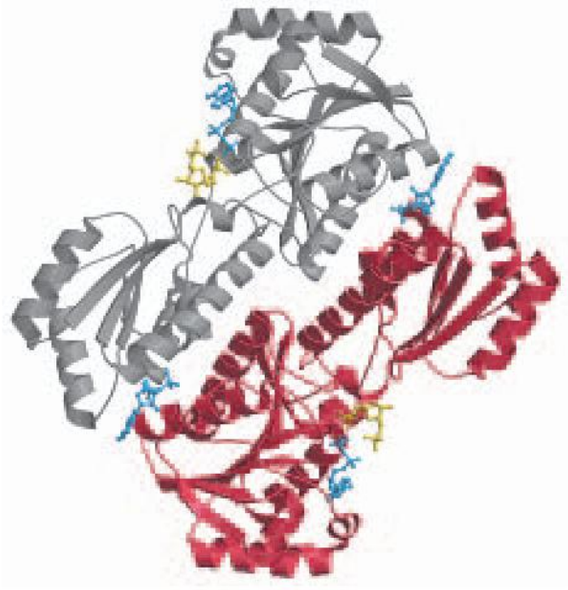
Source – Lehninger (Principle of Biochemistry)

complex enzyme has several regulatory sites at which allosteric activators or inhibitors bind.

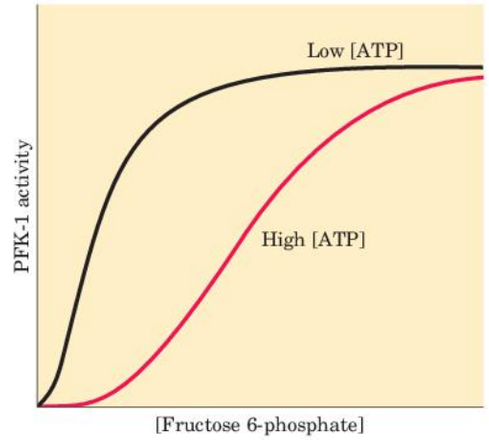
ATP is not only a substrate for PFK-1 but also an end product of the glycolytic pathway. When high cellular [ATP] signals that ATP is being produced faster than it is being consumed, ATP inhibits PFK-1 by binding to an allosteric site and lowering the affinity of the enzyme for fructose 6-phosphate (Fig. 15–18). 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.

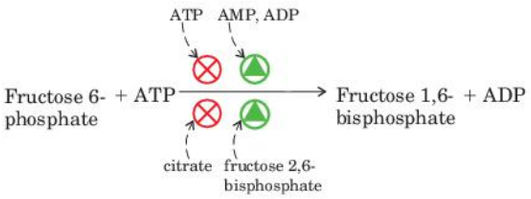
The most significant allosteric regulator of PFK-1 is fructose 2,6-bisphosphate, which strongly activates the enzyme. We return to this role of fructose 2,6-bisphosphate later.



(a)



(b)



(c)

Pyruvate Kinase Is Allosterically Inhibited by ATP

At least three isozymes of pyruvate kinase are found in vertebrates, differing in their tissue distribution and their response to modulators. High concentrations of ATP, acetyl-CoA, and long-chain fatty acids (signs of abundant energy supply) allosterically inhibit all isozymes of pyruvate kinase (Fig. 15–19). 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.

FIGURE 15–18 Phosphofructokinase-1 (PFK-1) and its regulation. **(a)** Ribbon diagram of *E. coli* phosphofructokinase-1, showing two of its four identical subunits (PDB ID 1PFK). Each subunit has its own catalytic site, where ADP (blue) and fructose 1,6-bisphosphate (yellow) are almost in contact, and its own binding sites for the allosteric regulator ADP (blue), located at the interface between subunits. **(b)** Allosteric regulation of muscle PFK-1 by ATP, shown by a substrate-activity curve. At low concentrations of ATP, the $K_{0.5}$ for fructose 6-phosphate is relatively low, enabling the enzyme to function at a high rate at relatively low concentrations of fructose 6-phosphate. (Recall from Chapter 6 that $K_{0.5}$ or K_m is equivalent to the substrate concentration at which half-maximal enzyme activity occurs.) When the concentration of ATP is high, $K_{0.5}$ for fructose 6-phosphate is greatly increased, as indicated by the sigmoid relationship between substrate concentration and enzyme activity. **(c)** Summary of the regulators affecting PFK-1 activity.

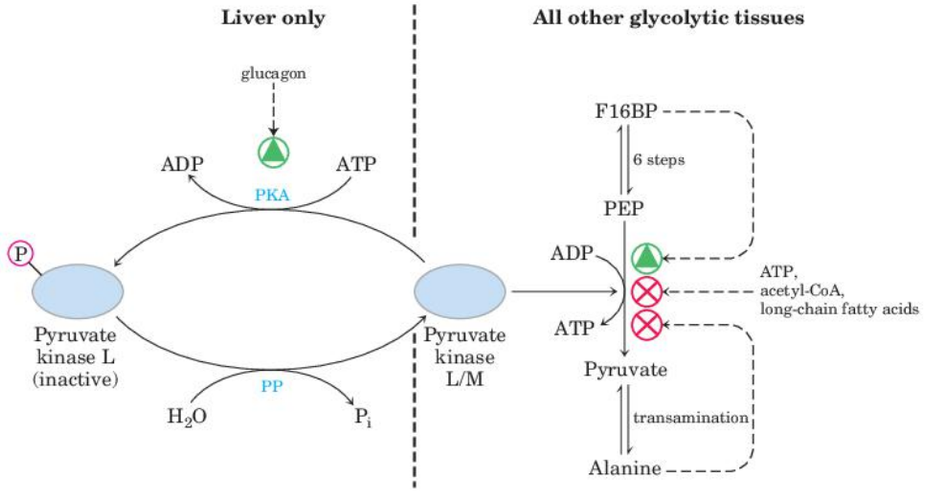


FIGURE 15–19 Regulation of pyruvate kinase. The enzyme is allosterically inhibited by ATP, acetyl-CoA, and long-chain fatty acids (all signs of an abundant energy supply), and the accumulation of fructose 1,6-bisphosphate triggers its activation. Accumulation of alanine, which can be synthesized from pyruvate in one step, allosterically inhibits pyruvate kinase, slowing the production of pyruvate by glycolysis. The liver isozyme (L form) is also regulated hormonally; glucagon activates cAMP-dependent protein kinase (PKA; see Fig. 15–25), which phosphorylates the pyruvate kinase L isozyme, inactivating it. When the glucagon level drops, a protein phosphatase (PP) dephosphorylates pyruvate kinase, activating it. This mechanism prevents the liver from consuming glucose by glycolysis when the blood glucose concentration is low; instead, liver exports glucose. The muscle isozyme (M form) is not affected by this phosphorylation mechanism.

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 (by the pyruvate dehydrogenase complex; Chapter 16) to fuel the citric acid cycle, or to oxaloacetate (by pyruvate carboxylase) to start the process of gluconeogenesis (Fig. 15–20). 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 accumulates. 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 (Fig. 15–21), 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

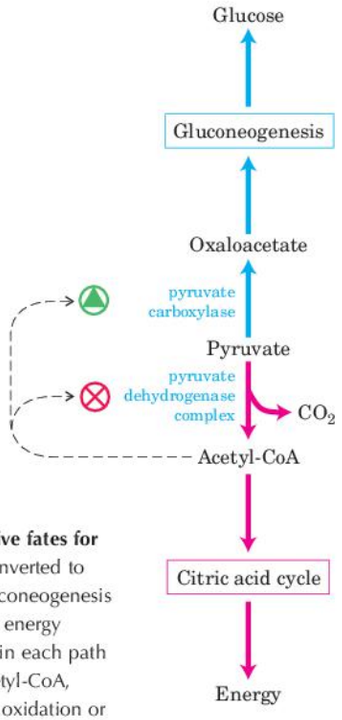


FIGURE 15–20 Two alternative fates for pyruvate. Pyruvate can be converted to glucose and glycogen via gluconeogenesis or oxidized to acetyl-CoA for energy production. The first enzyme in each path is regulated allosterically; acetyl-CoA, produced either by fatty acid oxidation or by the pyruvate dehydrogenase complex, stimulates pyruvate carboxylase and inhibits pyruvate dehydrogenase.

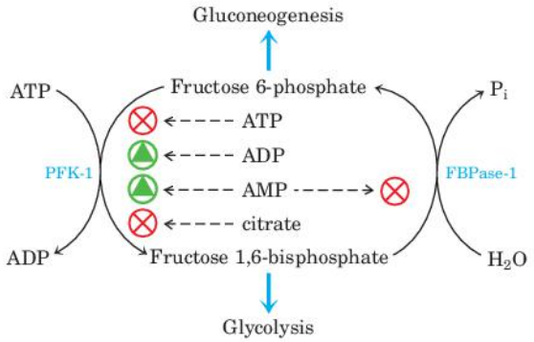


FIGURE 15–21 Regulation of fructose 1,6-bisphosphatase-1 (FBPase-1) and phosphofructokinase-1 (PFK-1). The important role of fructose 2,6-bisphosphate in the regulation of this substrate cycle is detailed in subsequent figures.

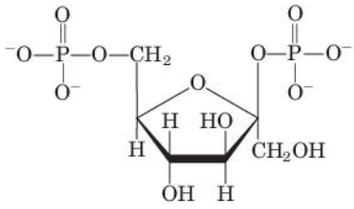
in the two pathways are regulated in a coordinated and reciprocal manner. In general, when sufficient concentrations of acetyl-CoA or citrate (the product of acetyl-CoA condensation with oxaloacetate) are present, or when a high proportion of the cell's adenylate is in the form of ATP, gluconeogenesis is favored. AMP promotes glycogen degradation and glycolysis by activating glycogen phosphorylase (via activation of phosphorylase kinase) and stimulating the activity of PFK-1.

All the regulatory actions discussed here are triggered by changes inside the cell and are mediated by very rapid, instantly reversible, allosteric mechanisms. 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 special role of liver in maintaining a constant blood glucose level requires additional regulatory mechanisms to coordinate glucose production and consumption. When the blood glucose level decreases, the hormone **glucagon** signals the liver to produce and release more glucose and to stop consuming it for its own needs. One source of glucose is glycogen stored in the liver; another source is gluconeogenesis.

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 (Fig. 15–22):



Fructose 2,6-bisphosphate

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 of its other positive and negative effectors (ATP, AMP, citrate), PFK-1 is virtually inactive in the absence of fructose 2,6-bisphosphate. Fructose 2,6-bisphosphate *activates* PFK-1 and stimulates glycolysis in liver and, at the same time, *inhibits* FBPase-1, thereby slowing gluconeogenesis.

Although structurally related to fructose 1,6-bisphosphate, fructose 2,6-bisphosphate is not an intermediate in gluconeogenesis or glycolysis; it is a *regulator* whose cellular level reflects the level of glucagon in the blood, which rises when blood glucose falls. The cellular concentration of fructose 2,6-bisphosphate is set by the relative rates of its formation and breakdown (Fig. 15–23a). 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).** (Note that these enzymes are distinct from PFK-1 and FBPase-1, which catalyze the formation and breakdown, respectively, of fructose 1,6-bisphosphate.) 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 glucagon and insulin (Fig. 15–23b). As we saw in Chapter 12 (p. 441), glucagon stimulates the adenylyl cyclase of liver to synthesize 3′,5′-cyclic AMP (cAMP) from ATP. Then cAMP activates cAMP-dependent protein kinase, which 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 glycolysis 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, stimulating 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, stimulating glycolysis, and inhibiting gluconeogenesis.