

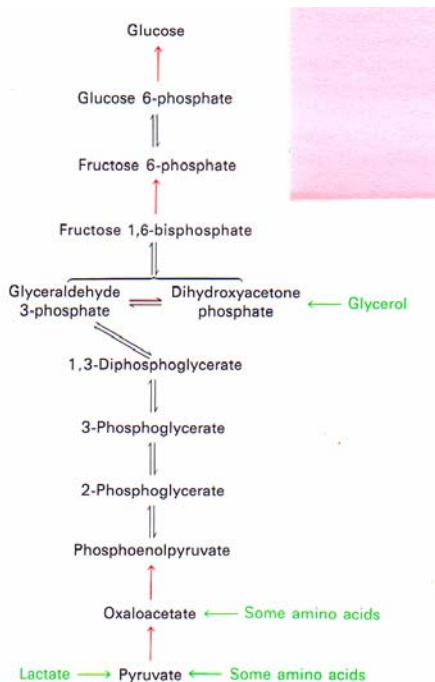
Gluconeogenesis

January 31, 2003

Bryant Miles

The synthesis of glucose from noncarbohydrate precursors is called **gluconeogenesis**.

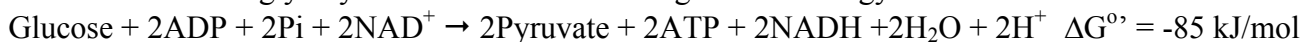
This metabolic pathway is very important because glucose is the primary energy source for the brain. Erythrocytes do not have mitochondria and derive all of their energy by glycolysis converting glucose into two molecules of lactate.



The daily requirement for glucose for a typical adult is around 160 grams. In the blood, approximately 20 grams of glucose is carried at any one time. In the liver approximately 190 grams of glucose is stored in the form of glycogen. This is a little more than a day's supply of glucose. For fasting periods longer than one day, or during periods of intense exercise, glucose must be synthesized from noncarbohydrate precursors in order to maintain the blood glucose levels. The noncarbohydrate precursors are pyruvate, lactate, oxaloacetate, amino acids and glycerol. The noncarbohydrate precursors enter the gluconeogenic pathway in the forms of pyruvate, oxaloacetate and dihydroxyacetone phosphate.

There are two major sites for gluconeogenesis, the liver and the kidneys. The liver accounts for 90% of gluconeogenesis in the body, the kidney's produce the other 10%. Very little gluconeogenesis occurs in the other tissues of the body. The liver and kidneys maintain the glucose level in the blood so that the brain, muscle and red blood cells have sufficient glucose to meet their metabolic demands.

The net reaction of glycolysis and the standard change in free energy is shown below:



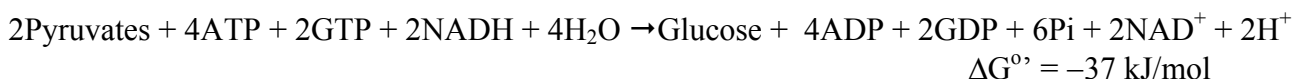
Gluconeogenesis is not merely the reversal of glycolysis because that would be highly endergonic.



Glycolysis has three irreversible steps catalyzed by hexokinase, phosphofructokinase and pyruvate kinase. In gluconeogenesis these irreversible steps have to be bypassed.

In order to make gluconeogenesis spontaneous (exergonic) six NTPs are consumed.

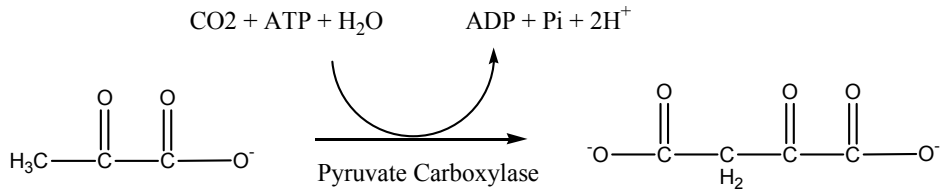
Gluconeogenesis



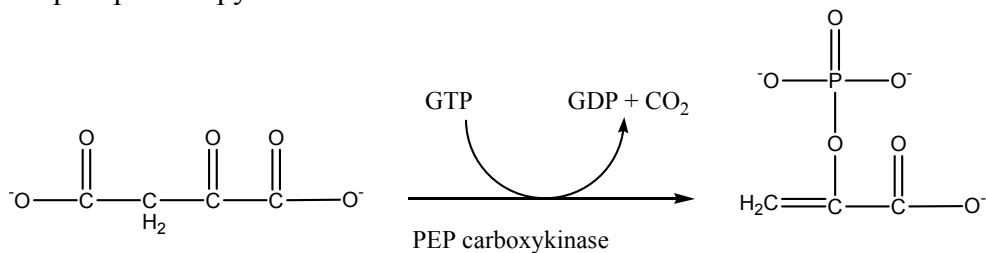
I. Overcoming the irreversible steps of glycolysis.

Two steps are required to convert pyruvate back into PEP.

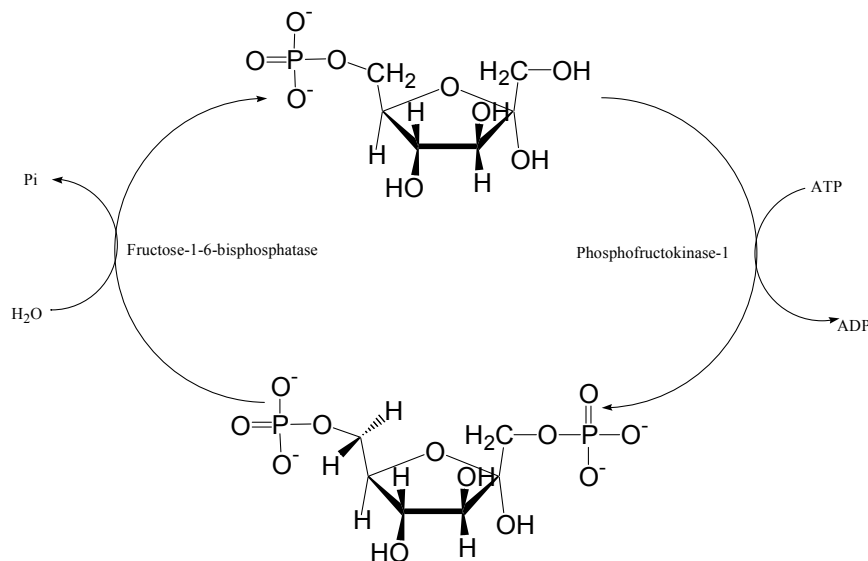
1. The first step is the carboxylation of pyruvate to form oxaloacetate. This reaction is catalyzed by pyruvate carboxylase and requires ATP.



2. In the second step oxaloacetate is decarboxylated and phosphorylated to produce phosphoenolpyruvate.



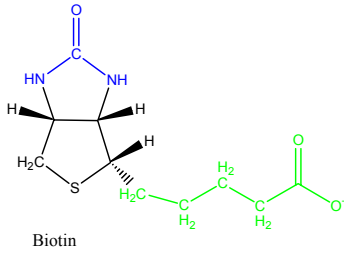
Since all of the reactions between phosphofructokinase and pyruvate kinase are reversible and near equilibrium, the same enzymes can function in both glycolysis and gluconeogenesis. The next irreversible step that must be overcome is to generate fructose-6-phosphate from fructose-1,6-bisphosphate. The enzyme that catalyzes this reaction is Fructose 1,6-bisphosphatase.



The isomerization of glucose-6-phosphate into fructose-6-phosphate is another reversible reaction, near equilibrium. The last irreversible step to overcome is the phosphorylation reaction catalyzed by hexokinase. The enzyme that reverses this step, converting glucose-6-phosphate into glucose is glucose-6-phosphatase. This enzyme is a membrane bound protein found in the lumen of the endoplasmic reticulum. A specific carrier protein carries glucose-6-phosphate from the cytosol to the lumen of the

endoplasmic reticulum. This enzyme is not present in the muscles or the brain and hence glucose can not be produced by these organs.

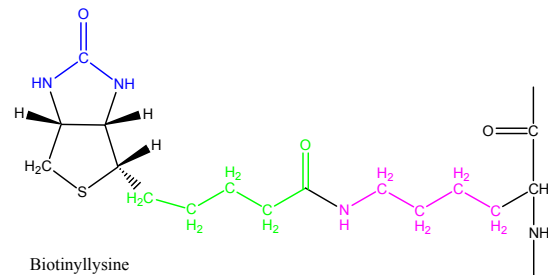
II. The reactions of Gluconeogenesis.



Pyruvate Carboxylase uses the energy of ATP to carboxylate pyruvate to form oxaloacetate.

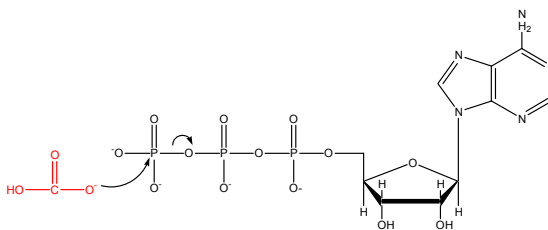
The enzyme requires biotin, a cofactor that is covalently attached to a lysine residue.

Biotin serves as a carrier of activated carbon dioxide, just as acyl-CoA carries activated acyl groups.



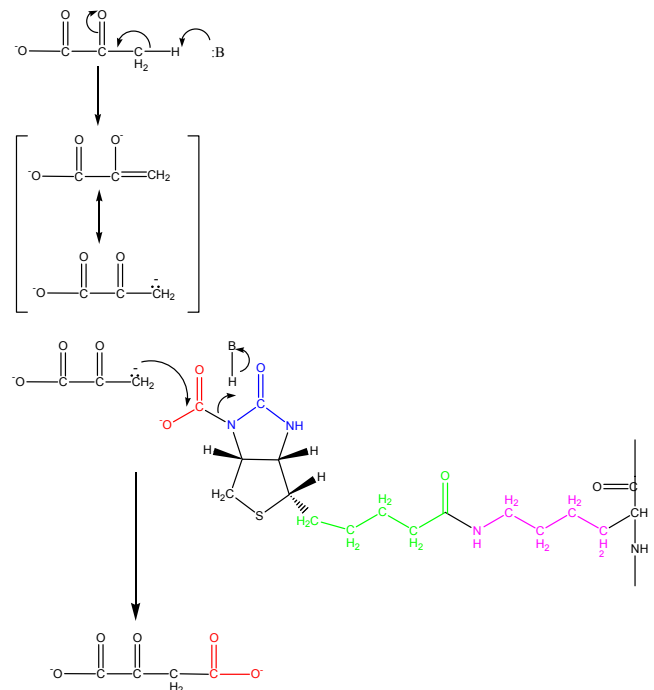
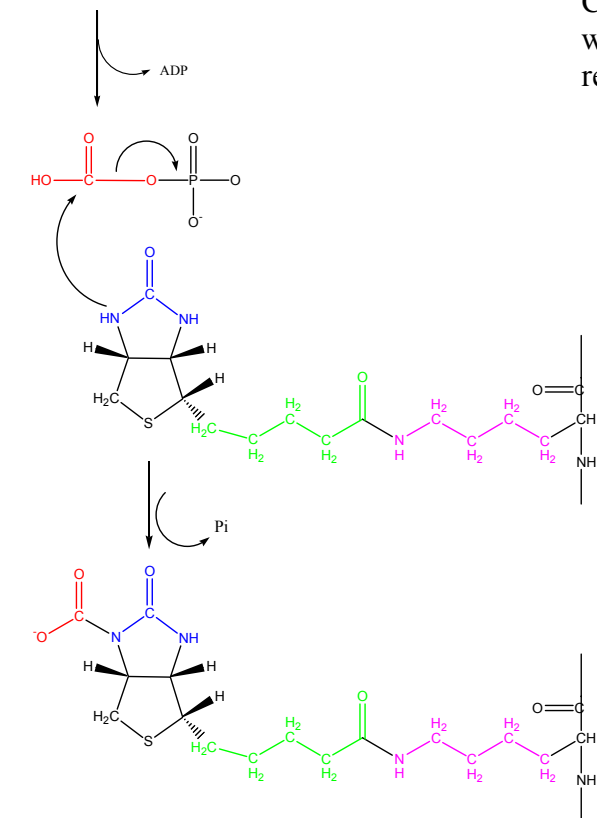
The first step of pyruvate carboxylase is to activate a molecule of bicarbonate for nucleophilic attack.

Bicarbonate is activated by phosphorylation with ATP to produce a high energy carboxyphosphate intermediate.



Carboxyphosphate then undergoes nucleophilic attack by the N1 atom of biotin to form N-carboxybiotin.

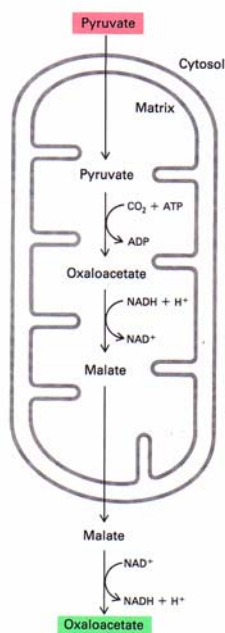
The third step to the reaction is to remove a proton from the C3 of pyruvate to generate a resonance stabilized carbanion which is a good nucleophile, attacking the N-carboxybiotin to regenerate biotin and form oxaloacetate.



Regulation of pyruvate carboxylase

Pyruvate carboxylase is allosterically activated by acyl-CoA. In order to activate bicarbonate, an acyl-CoA must be bound to an allosteric binding site of the enzyme. The second half of the enzyme catalyzed reaction, the nucleophilic attack of the pyruvate enolate on N-carboxybiotin, is not affected by this allosteric regulator. The activation by acyl-CoA provides important physiological regulation. If the concentration of ATP is low (remember that ATP is a substrate for this enzyme) and/or the concentration of acyl-CoA's is low, then pyruvate is directed into the citric acid cycle, to promote the synthesis of ATP. If the concentrations of ATP and acyl-CoAs are high, then pyruvate is converted in oxaloacetate and consumed in gluconeogenesis. High concentrations of ATP and acyl-CoA's are signals that the cells energy level is high and metabolites are converted into glucose. If the energy status of the cell is low, then the concentrations of ATP and acyl-CoAs are low and pyruvate is directed towards the TCA cycle.

Compartmentalization of pyruvate carboxylase and PEP carboxykinase

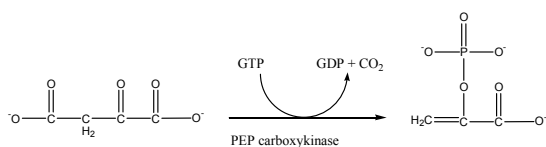


Pyruvate carboxylase is found only in the matrix of the mitochondria. The next enzyme involved in gluconeogenesis is PEP carboxykinase is found in both the mitochondria or in the cytosol or both depending on the organism and the tissue. In human livers, PEP carboxykinase is found in both the mitochondria and the cytosol. Pyruvate is transported into the mitochondria where it is either converted into acetyl-CoA by pyruvate dehydrogenase and enters the citric acid cycle, or it is converted into oxaloacetate by pyruvate carboxylase and used for gluconeogenesis.

In tissues where PEP carboxykinase is found only in the mitochondria, oxaloacetate is converted into PEP in the mitochondria. PEP can be transported to the cytosol.

In tissues where PEP carboxykinase is found only in the cytosol, oxaloacetate must be reduced to malate so that it can be transported across the mitochondrial membrane to the cytosol where the malate is reoxidized into oxaloacetate and goes on through gluconeogenesis.

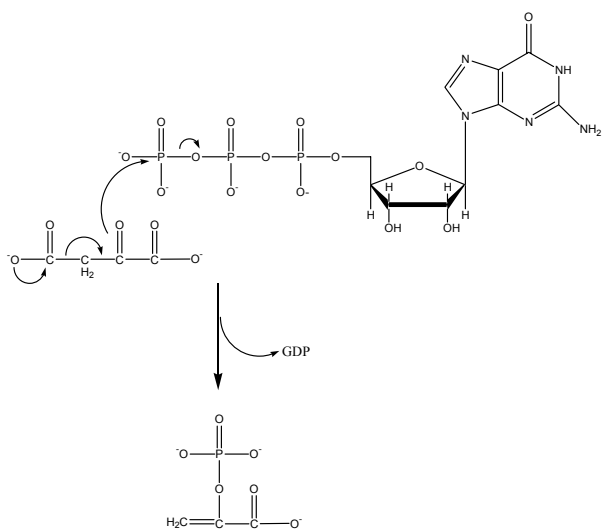
PEP Carboxykinase



PEP carboxykinase simultaneously decarboxylates and phosphorylates oxaloacetate.

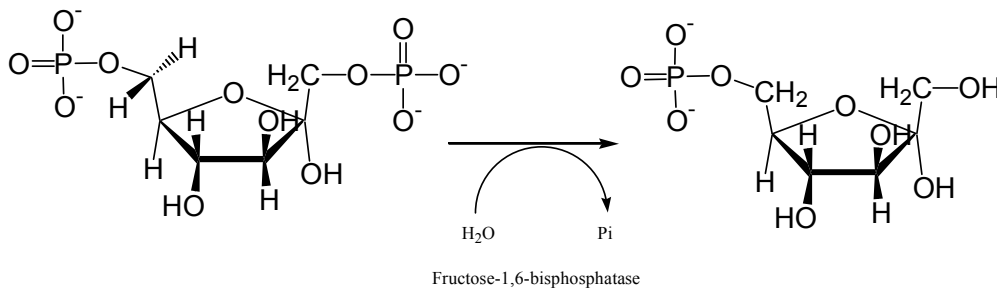
The CO₂ added in the pyruvate carboxylase step comes off in the PEP carboxykinase step.

The decarboxylation reaction is very exergonic and helps to drive the otherwise highly endergonic reaction of PEP synthesis.



After PEP is formed by PEP carboxykinase, PEP is hydrated by enolase to form 2-phosphoglycerate which in turn is isomerized into 3-phosphoglycerate by phosphoglycerate mutase, phosphoglycerokinase uses ATP to produce 1,3-bisphosphoglycerate which is reduced by GAPDH into glyceraldehydes-3-phosphate. Triose phosphate isomerase produces dihydroxyacetone phosphate. Aldolase catalyzes an aldol condensation of DHAP and glyceraldehyde-3P to form fructose-1,6-bisphosphate. All of these enzymes function in both glycolysis and gluconeogenesis because they are all near equilibrium and reversible.

The phosphorylation of fructose-6-phosphate by phosphofructokinase is the first committed step of glycolysis and is irreversible. For gluconeogenesis, fructose-1,6-bisphosphate is hydrolyzed by the enzyme fructose-1,6-bisphosphatase, an allosterically regulated enzyme. The standard change in free energy for this reaction is $\Delta G^{\circ} = -16.7 \text{ kJ/mol}$.



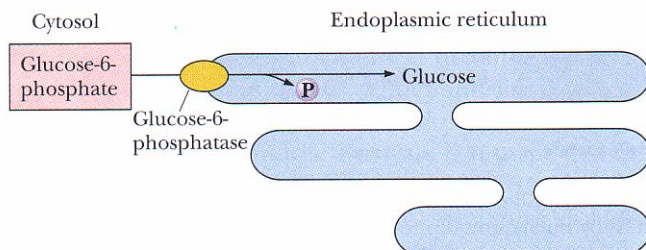
This reaction is exergonic, irreversible and regulated.

Citrate↑
Fructose-2,6-bisphosphate↓
AMP↓

AMP enhances the inhibition of Fructose-2,6-BP.

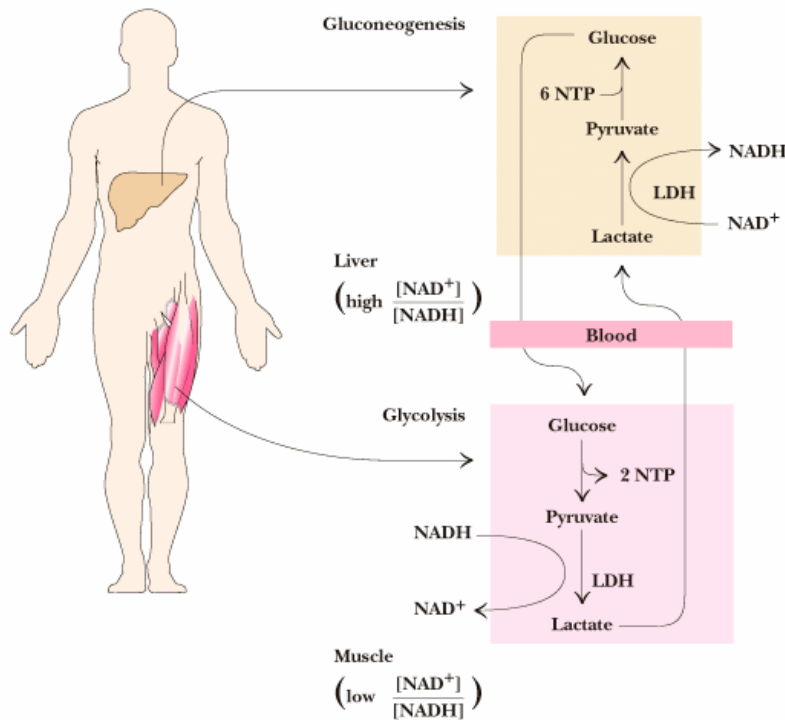
After fructose-1,6-bisphosphate is converted into fructose-1-phosphate, phosphoglucose isomerase isomerizes it to glucose-6-phosphate. The final step of gluconeogenesis is the conversion of glucose-6-phosphate to glucose. The enzyme that catalyzes this reaction is glucose-6-phosphatase. This is a membrane bound protein found in the membranes of the endoplasmic reticulum of the liver and the kidneys. This enzyme is absent in other tissues such as the brain and muscle cells.

The association of this enzyme with the membrane is important to its function because the substrate glucose-6-phosphate is hydrolyzed as it passes through the membrane into the endoplasmic reticulum. Vesicles form from the endoplasmic reticulum membrane enclosing the molecules of glucose. The vesicles then diffuse to the plasma membrane and fuse with it. When the vesicle fuses with the plasma membrane, all of the glucose contained within the vesicle is released into the blood stream.



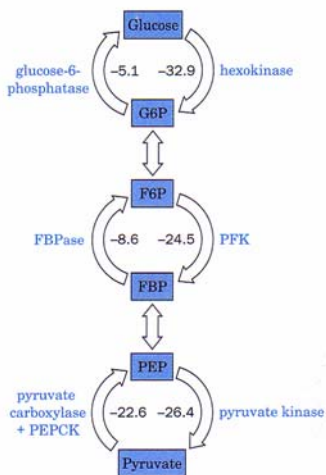
III. The Cori Cycle

Vigorous exercise leads to an oxygen shortage producing anaerobic conditions in the muscle cells. In order to regenerate NAD^+ to keep glycolysis going pyruvate is reduced to lactate. The lactate produced by the muscles is released into the blood where it is carried to the liver. In the liver lactate is reoxidized into pyruvate which is then converted to glucose via gluconeogenesis. In this way the liver shares in the metabolic stress produced by vigorous exercise. During exercise the liver releases glucose into the blood stream to fuel the muscles. The muscles produce lactate which is carried back to the liver where by gluconeogenesis it is converted back into glucose. This cycle is called the Cori cycle.



IV. Regulation of glycolysis.

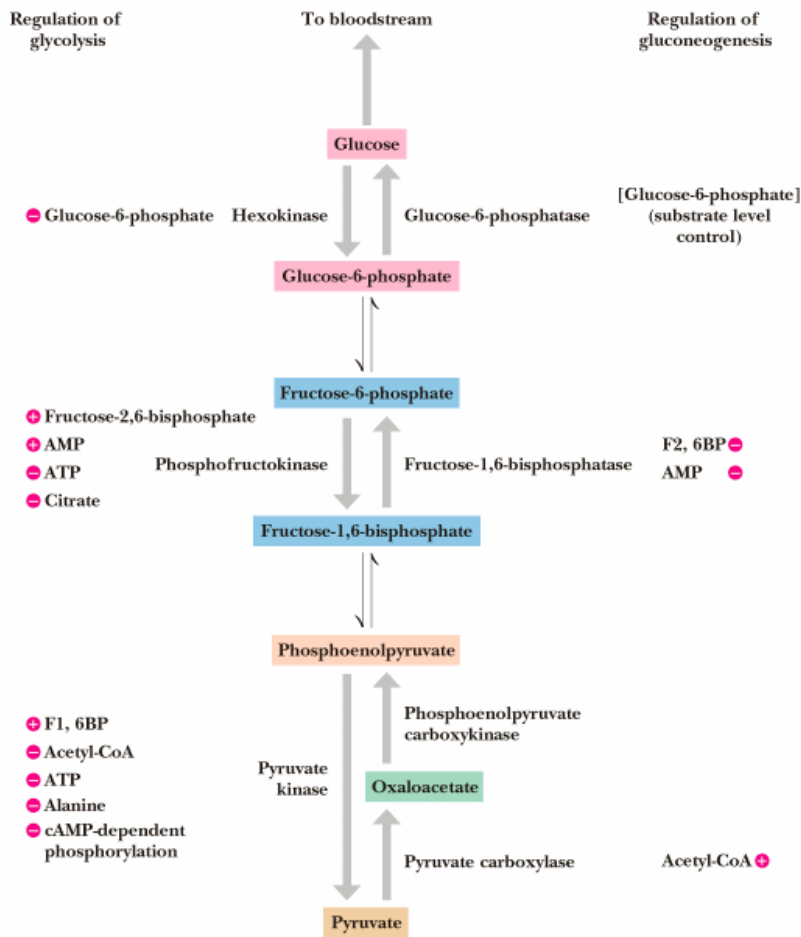
All of the reactions of glycolysis and most of the reactions of gluconeogenesis occur in the cytosol. Nearly all of the reactions of glycolysis and gluconeogenesis are reciprocally regulated. The result of this reciprocal regulation allows to the cell to rapidly catabolize glucose when the cellular energy supply is low and to synthesize and store glucose when the energy level of the cell is high.



The three irreversible steps of glycolysis are catalyzed by hexokinase, phosphofructokinase and pyruvate kinase. All three of these enzymes are allosterically regulated. Gluconeogenesis replaces these irreversible reactions with corresponding irreversible reactions that are exergonic in the direction of glucose synthesis. The enzymes that catalyze these reactions are glucose-6-phosphatase, fructose-1,6-bisphosphatase and the sequential reactions of pyruvate carboxylase and PEP carboxykinase.

Glucose-6-phosphate is not under allosteric control. It is regulated in a different way. The K_m of glucose-6-phosphatase for glucose-6-phosphate is considerably higher than the cellular concentrations of

glucose-6-phosphate. As a result glucose-6-phosphate displays a linear dependence of activity as a function of glucose-6-phosphate concentration. This enzyme is said to be under **substrate-level control**.



Acetyl CoA is an allosteric effector of both glycolysis and gluconeogenesis. Acetyl-CoA inhibits pyruvate kinase and reciprocally activates pyruvate carboxylase. Acetyl CoA also inactivates pyruvate dehydrogenase providing a regulatory link between glycolysis and the citric acid cycle. High concentrations of acetyl-CoA are indicative of high energy supplies. When the energy supply is high, metabolites are directed towards storage in the form of glycogen.

Fructose-1,6-bisphosphatase is another important site of gluconeogenesis regulation. Fructose-1,6-bisphosphatase is activated by high concentrations of citrate and inhibited by high concentrations of AMP. Fructose-2,6-bisphosphate is another important allosteric regulator of fructose-1,6-bisphosphatase. Fructose-2,6-bisphosphate inactivates fructose-1,6-bisphosphatase.

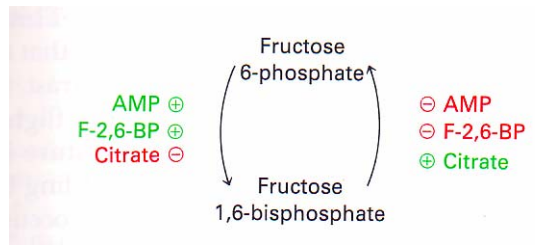
Citrate ↑

Fructose-2,6-bisphosphate ↓

AMP ↓

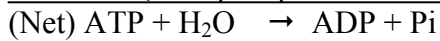
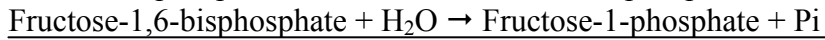
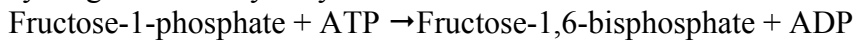
AMP enhances the inhibition of Fructose-2,6-BP.

Note that these allosteric effectors of fructose-1,6-bisphosphatase all are allosteric effectors of phosphofructokinase. These effectors reciprocally regulate both enzymes.



This provides a way for gluconeogenesis and glycolysis to be coordinated such that when one pathway is active the other pathway is inactive. If both pathways were active at the same time, the net result would be the net hydrolysis of 2ATPs and 2GTPs per reaction cycle. Both pathways are highly exergonic so there is no thermodynamic barrier to such cycling.

The cycle shown above, cycling fructose-6-phosphate and fructose-1,6-bisphosphate is called a substrate cycle. If the flux of metabolites through each pathway is the same then the net result of the substrate cycling is the net hydrolysis of ATP.



Regardless of which pathway is activated (the other pathway will be reciprocally inactivated) there is always flux of metabolites through both pathways.