Gluconeogenesis;

Regulation of Glycolysis & Gluconeogenesis

Gluconeogenesis occurs mainly in **liver**.

Gluconeogenesis occurs to a more limited extent in kidney & small intestine under some conditions.

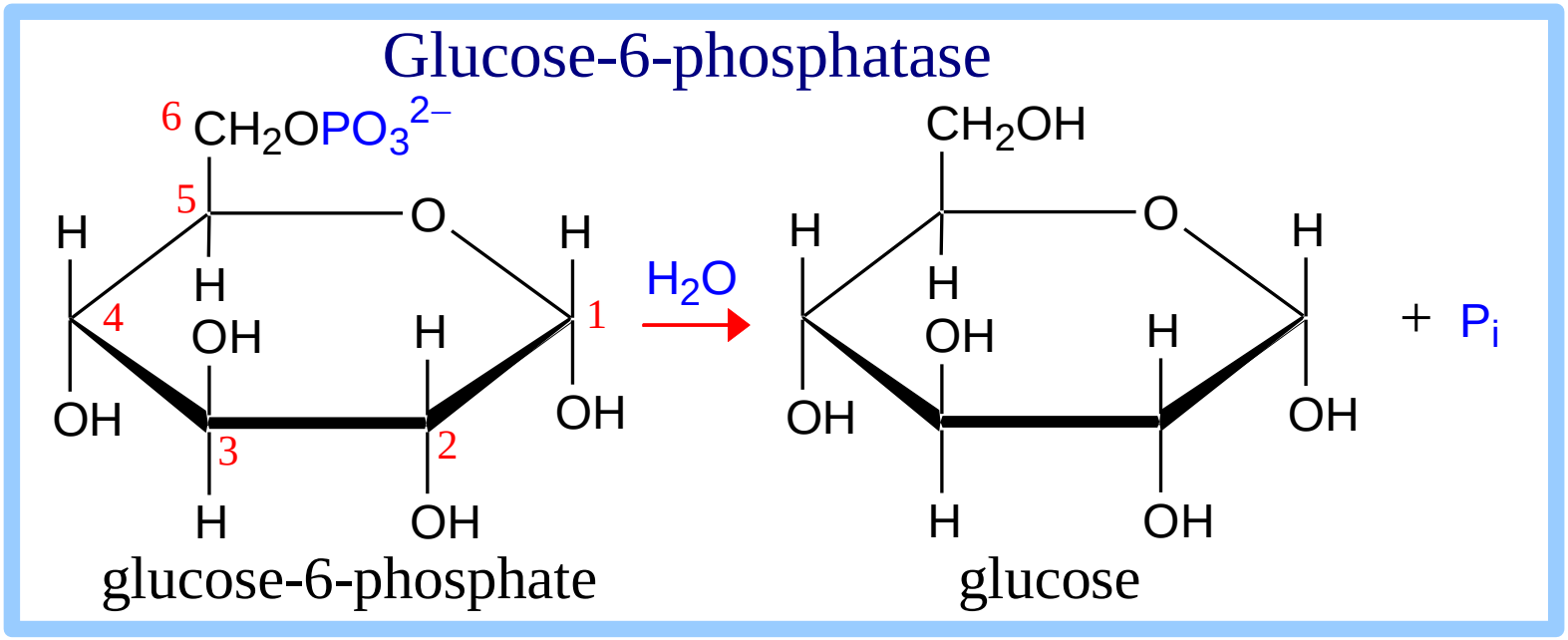
Synthesis of glucose from pyruvate utilizes many of the same enzymes as **Glycolysis**.

Three Glycolysis reactions have such a large negative ΔG that they are essentially **irreversible**.

- **Hexokinase** (or Glucokinase)
- **Phosphofructokinase**
- **Pyruvate Kinase.**

These steps must be **bypassed** in Gluconeogenesis.

Two of the bypass reactions involve simple **hydrolysis** reactions.

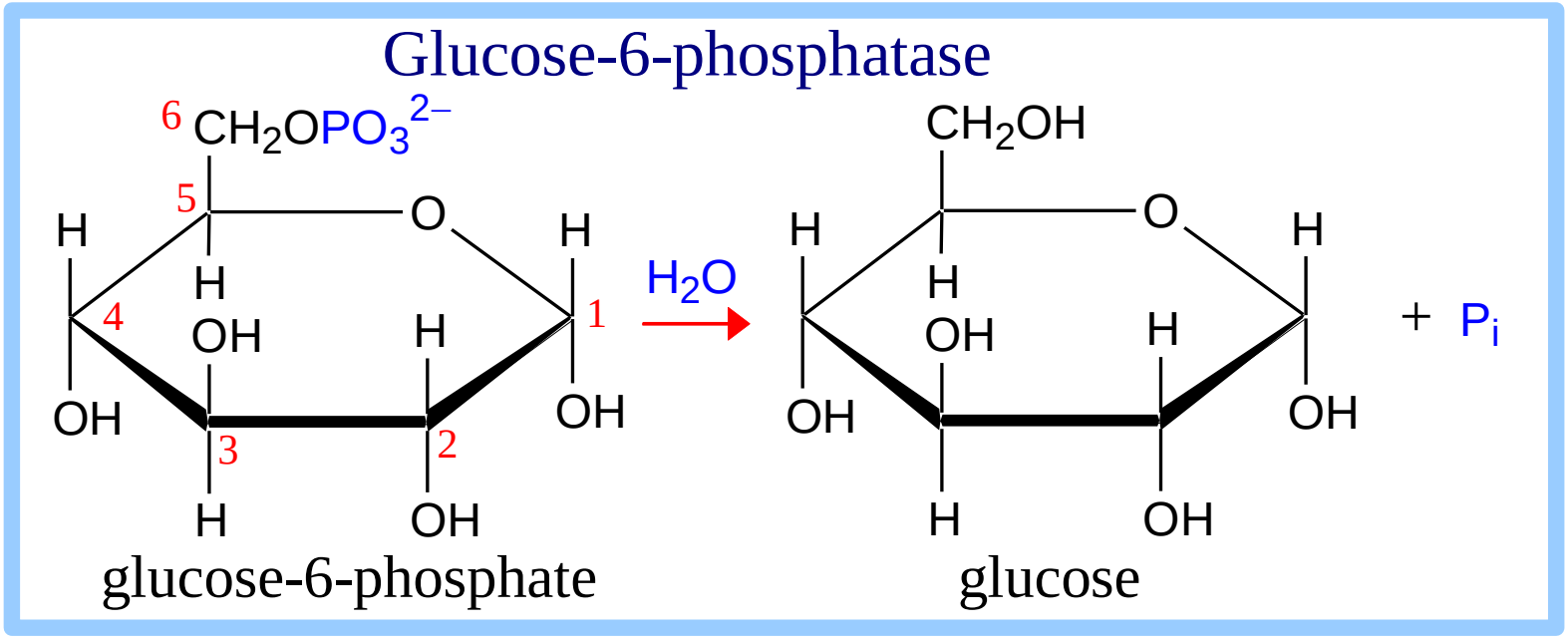


Hexokinase or Glucokinase (Glycolysis) catalyzes:

glucose + ATP → glucose-6-phosphate + ADP

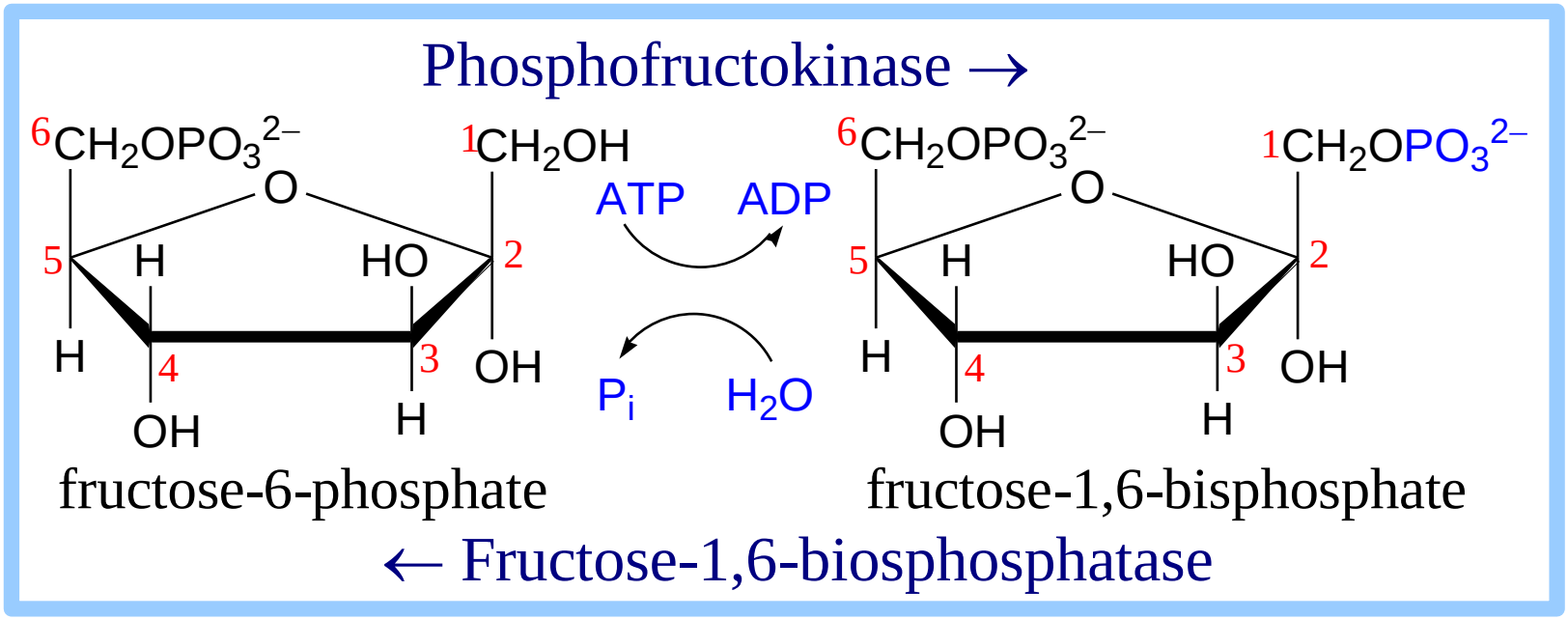
Glucose-6-Phosphatase (Gluconeogenesis) catalyzes:

glucose-6-phosphate + H_2O → glucose + P_i



Glucose-6-phosphatase enzyme is embedded in the endoplasmic reticulum (ER) membrane in liver cells.

The catalytic site is found to be exposed to the ER lumen. Another subunit may function as a translocase, providing access of substrate to the active site.



Phosphofructokinase (Glycolysis) catalyzes:

fructose-6-P + ATP → fructose-1,6-bisP + ADP

Fructose-1,6-bisphosphatase (Gluconeogenesis) catalyzes:

fructose-1,6-bisP + H_2O → fructose-6-P + P_i

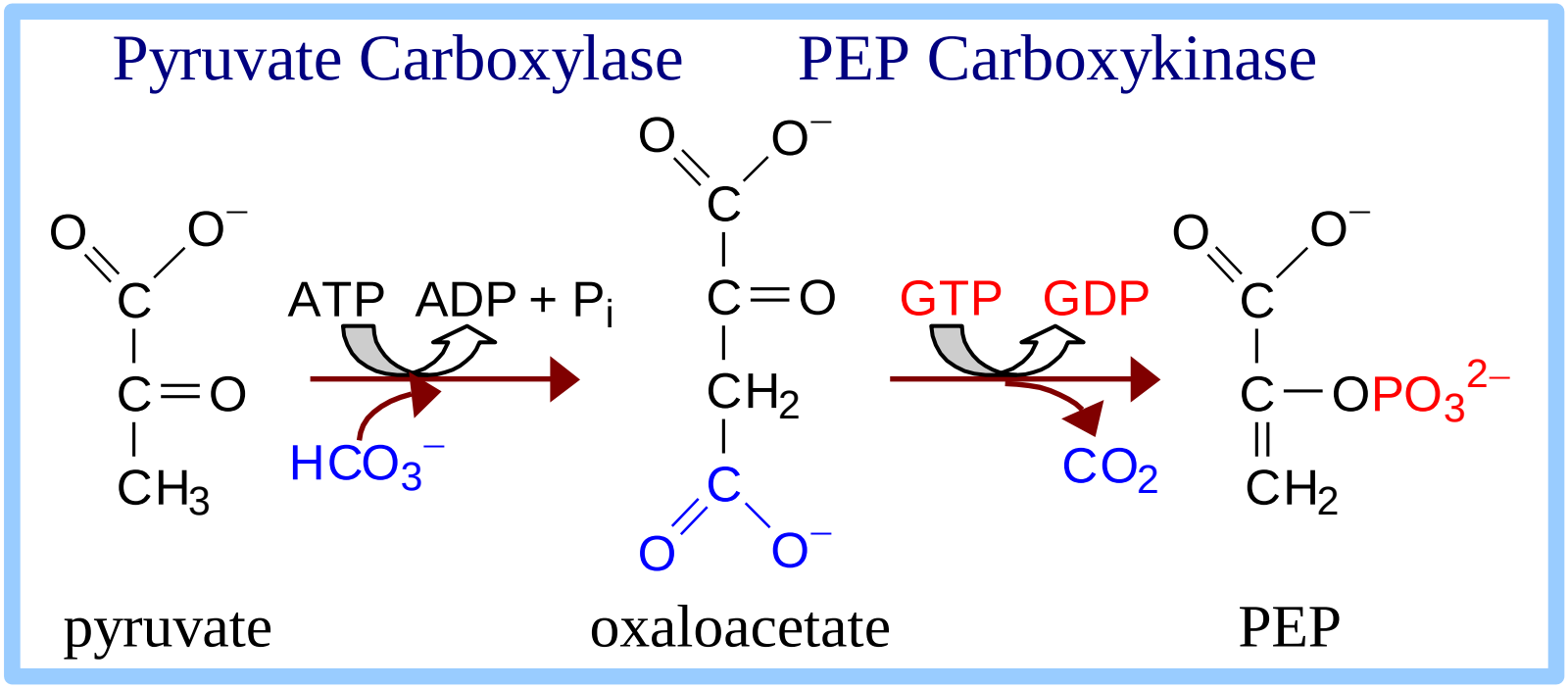
Bypass of Pyruvate Kinase:

Pyruvate Kinase (last step of Glycolysis) catalyzes:

phosphoenolpyruvate + ADP → pyruvate + ATP

For bypass of the Pyruvate Kinase reaction, cleavage of **2 ~P** bonds is required.

- ΔG for cleavage of one ~P bond of ATP is insufficient to drive synthesis of phosphoenolpyruvate (PEP).
- PEP has a higher negative ΔG of phosphate hydrolysis than ATP.



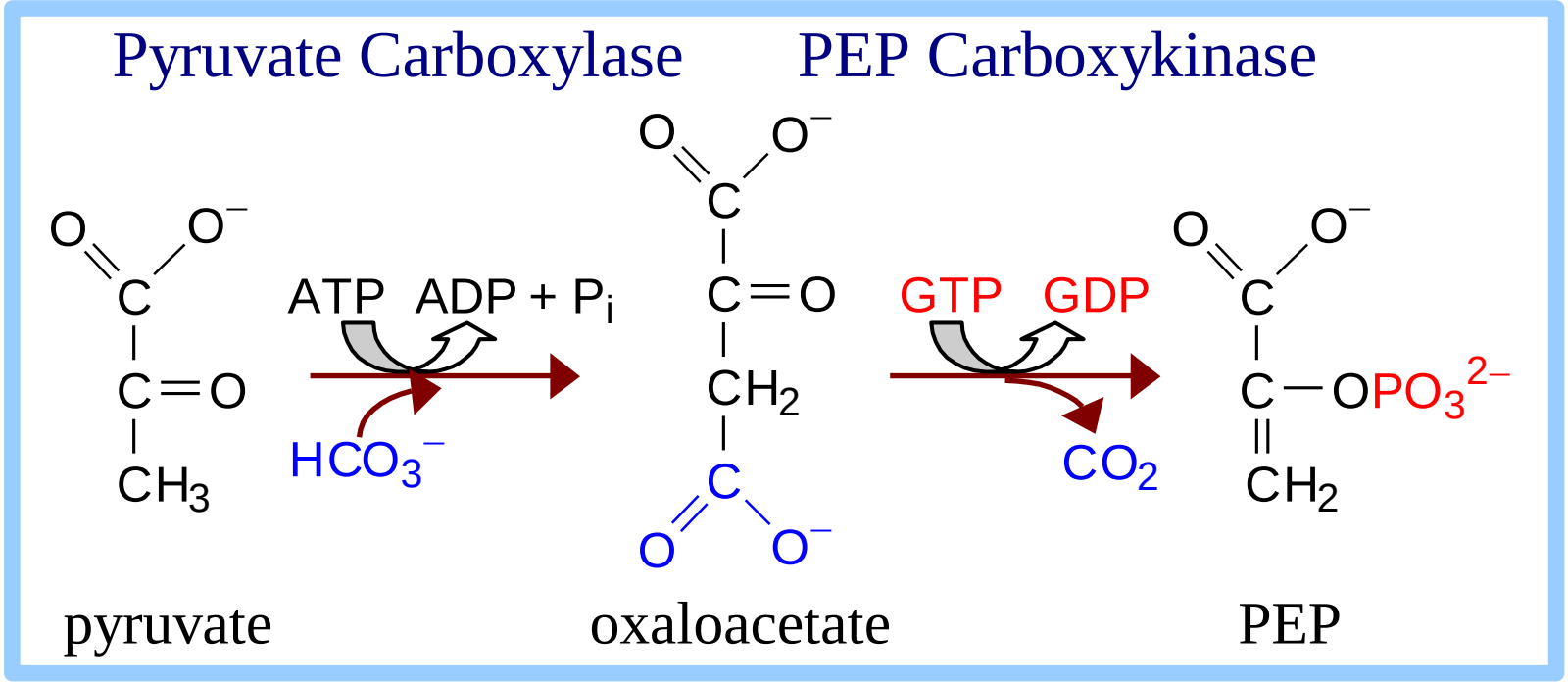
Bypass of Pyruvate Kinase (2 enzymes):

Pyruvate Carboxylase (Gluconeogenesis) catalyzes:

pyruvate + HCO_3^- + ATP → oxaloacetate + ADP + P_i

PEP Carboxykinase (Gluconeogenesis) catalyzes:

oxaloacetate + GTP → PEP + GDP + CO_2



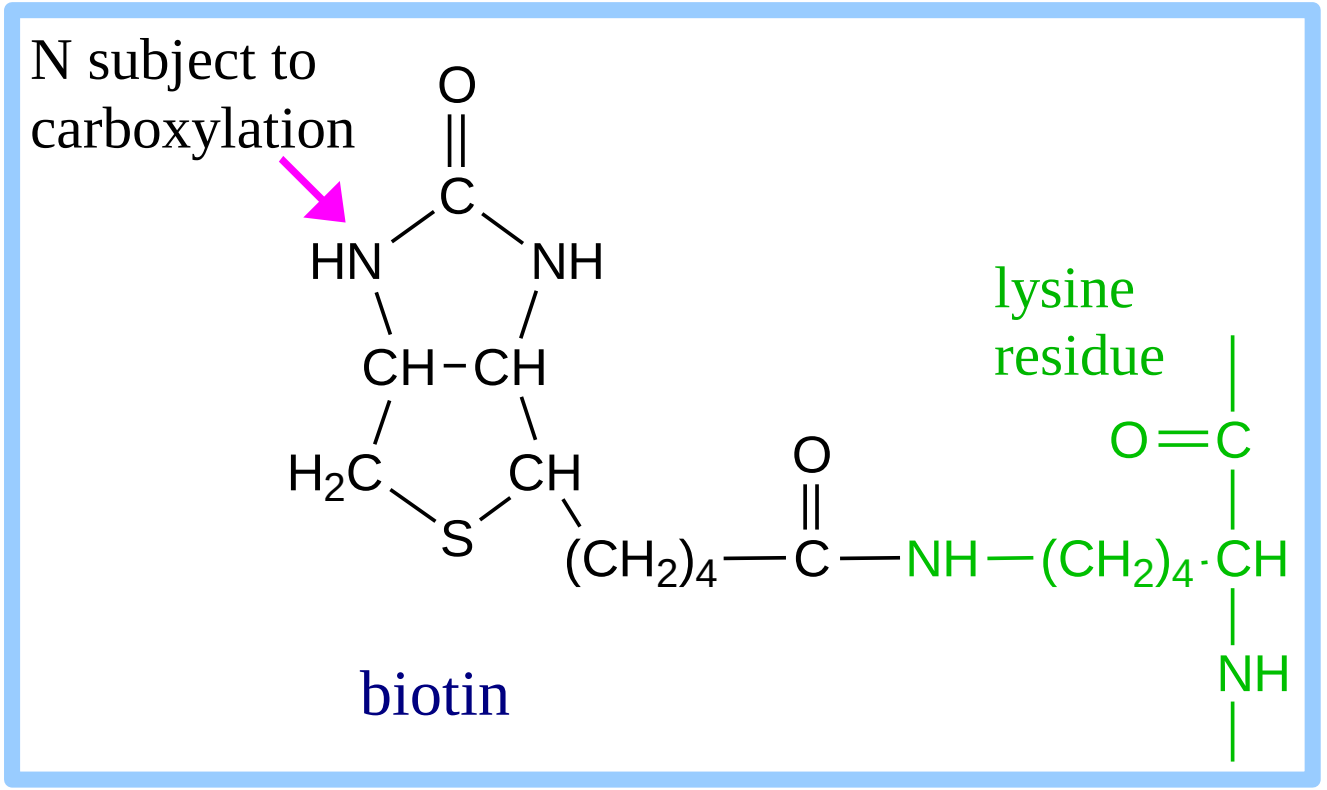
Contributing to spontaneity of the 2-step process:

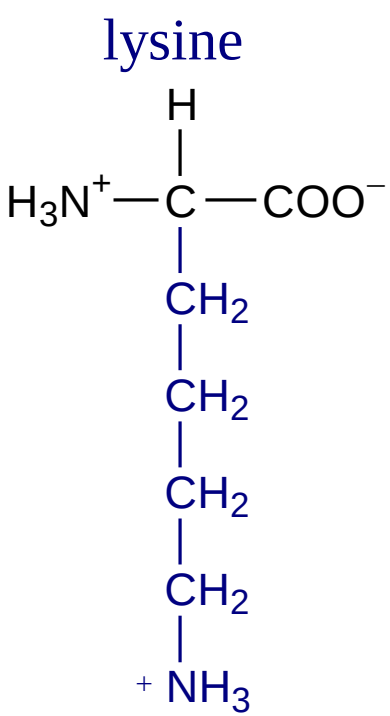
Free energy of one **~P** bond of **ATP** is conserved in the carboxylation reaction.

Spontaneous decarboxylation contributes to spontaneity of the 2nd reaction.

Cleavage of a second **~P** bond of **GTP** also contributes to driving synthesis of PEP.

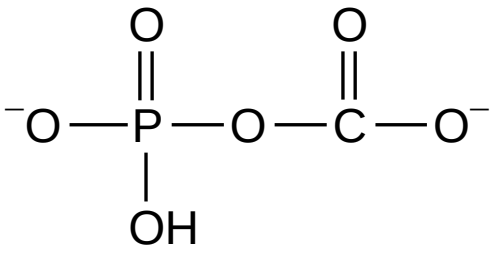
Pyruvate Carboxylase uses **biotin** as prosthetic group.

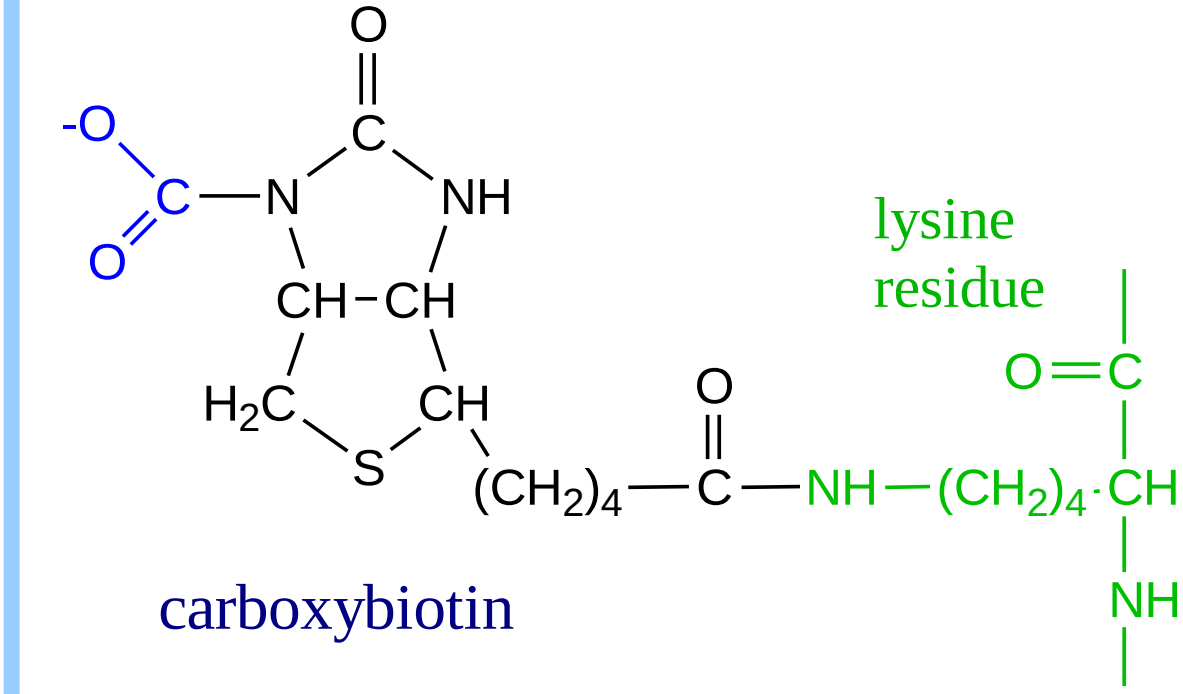




Biotin has a 5-C side chain whose terminal carboxyl is in amide linkage to the ε-amino group of an enzyme **lysine**.

The biotin & lysine side chains form a **long swinging arm** that allows the biotin ring to swing back & forth between 2 active sites.





Biotin carboxylation is catalyzed at **one active site** of Pyruvate Carboxylase.

ATP reacts with HCO_3^- to yield **carboxyphosphate**.

The carboxyl is transferred from this ~P intermediate to **N** of a ureido group of the biotin ring. Overall:

biotin + ATP + HCO_3^- → carboxybiotin + ADP + P_i

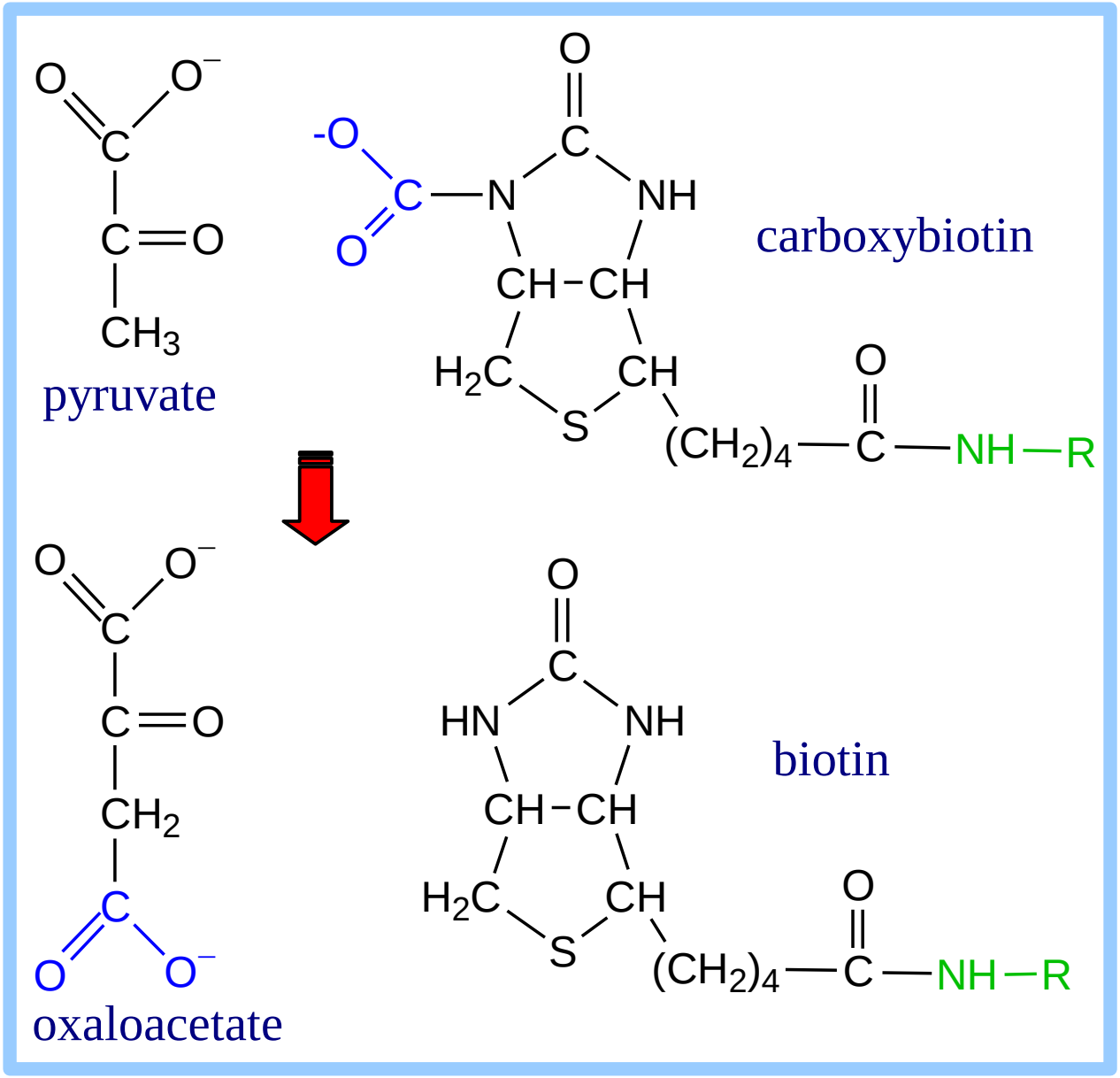
At the **other active site** of Pyruvate Carboxylase the activated CO_2 is transferred from biotin to pyruvate:

carboxybiotin + pyruvate

↓

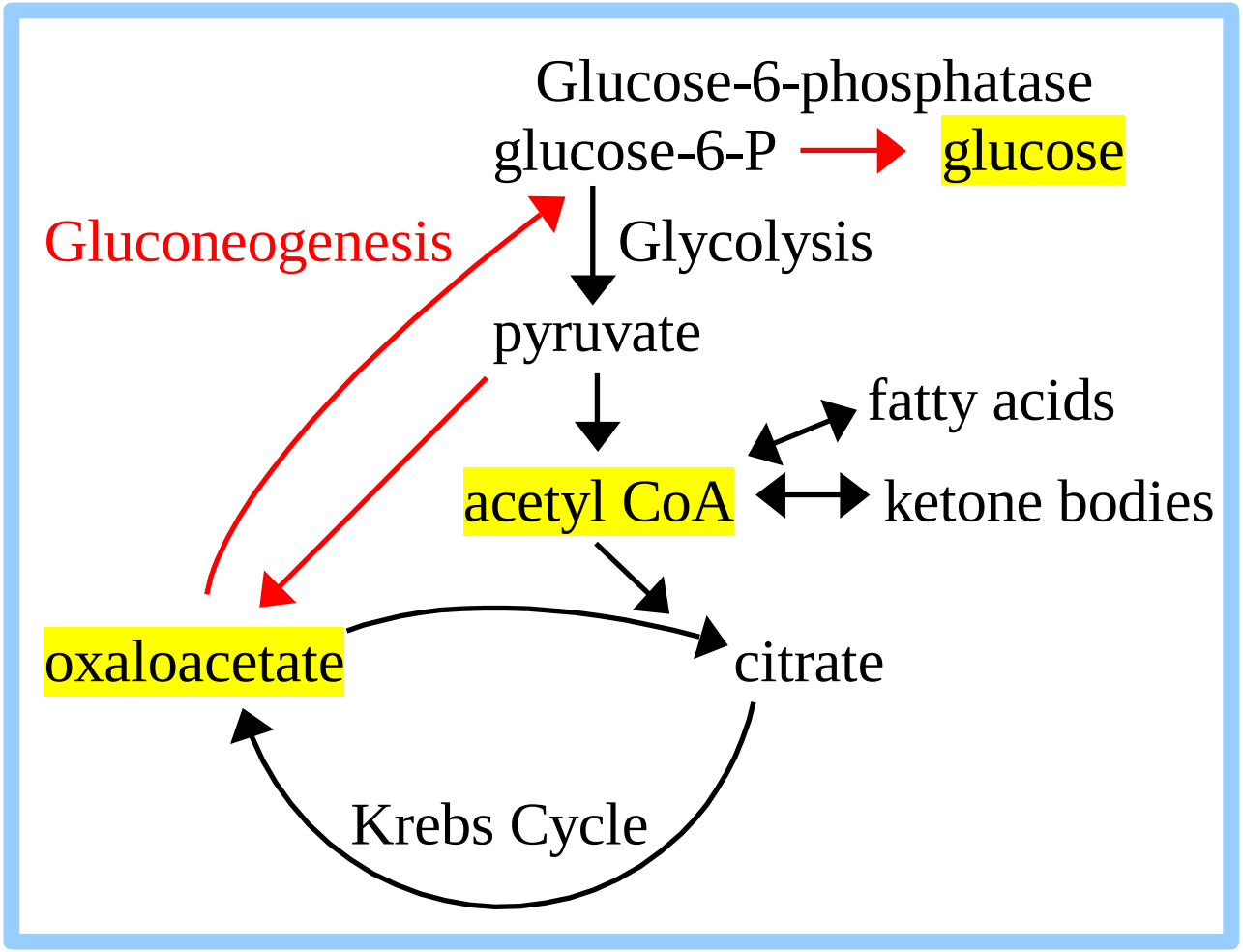
biotin + oxaloacetate

View an animation.



Pyruvate Carboxylase (pyruvate → oxaloactate) is allosterically **activated by acetyl CoA**.

[Oxaloacetate] tends to be limiting for Krebs cycle.

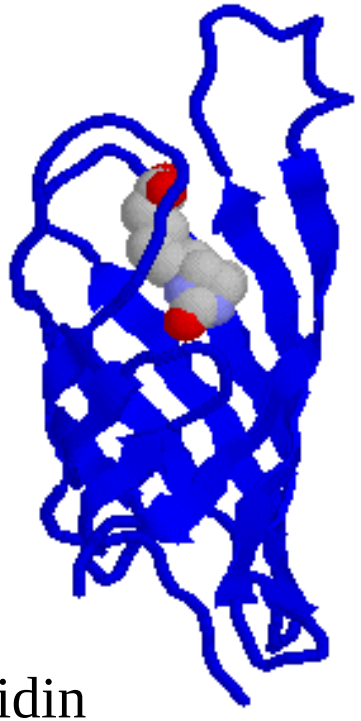


When gluconeogenesis is active in liver, oxaloacetate is diverted to form glucose. Oxaloacetate depletion hinders acetyl CoA entry into Krebs Cycle. The increase in [acetyl CoA] activates Pyruvate Carboxylase to make oxaloacetate.

Avidin, a protein in egg whites with a **β barrel** structure, tightly binds biotin.

Excess consumption of raw eggs can cause nutritional deficiency of biotin.

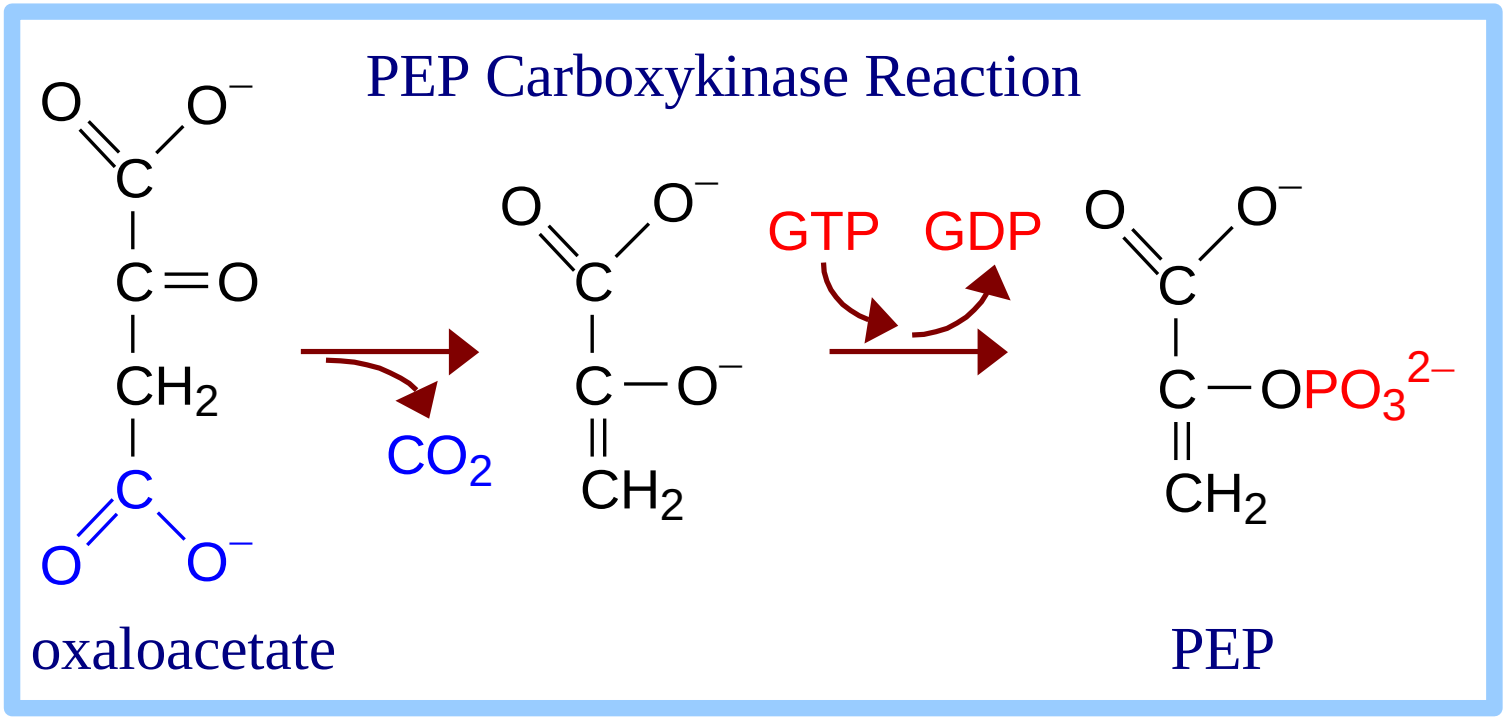
The strong **avidin-to-biotin affinity** is used by biochemists as a specific "glue."



avidin
with bound biotin

If it is desired to bind 2 proteins together for an experiment, biotin may be covalently linked to one protein and avidin to the other.

Explore with Chime the biotinyl domain of a carboxylase and the avidin-biotin complex.

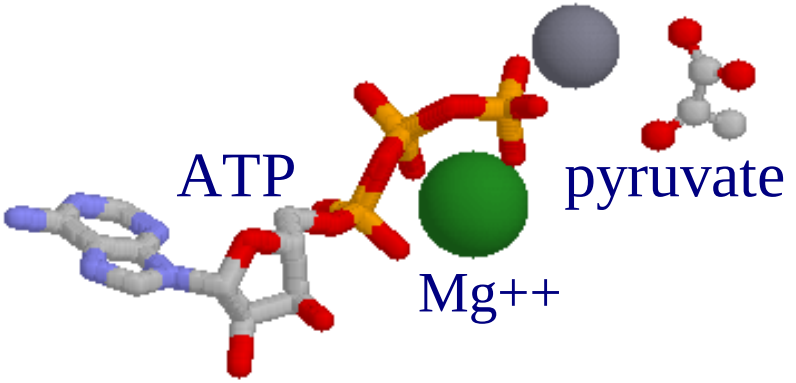


PEP Carboxykinase catalyzes GTP-dependent oxaloacetate → PEP. It is thought to proceed in 2 steps:

- Oxaloacetate is first **decarboxylated** to yield a pyruvate enolate anion intermediate.
- **Phosphate transfer** from **GTP** then yields phosphoenolpyruvate (PEP).

In the **bacterial** enzyme, ATP is P_i donor instead of GTP.

In this crystal structure of an *E. Coli* PEP Carboxykinase, pyruvate is at the active site as an analog of PEP/ oxaloacetate.



PEP Carboxykinase
active site ligands PDB 1AQ2

A metal ion such as **Mn^{++}** is required for the PEP Carboxykinase reaction, in addition to a **Mg^{++}** ion that binds with the nucleotide substrate at the active site.

Mn^{++} is thought to promote P_i transfer by interacting simultaneously with the enolate oxygen atom and an oxygen atom of the terminal phosphate of GTP or ATP.

The **source of pyruvate and oxaloacetate** for gluconeogenesis during fasting or carbohydrate starvation is mainly **amino acid catabolism**.

Some amino acids are catabolized to pyruvate, oxaloacetate, or precursors of these.

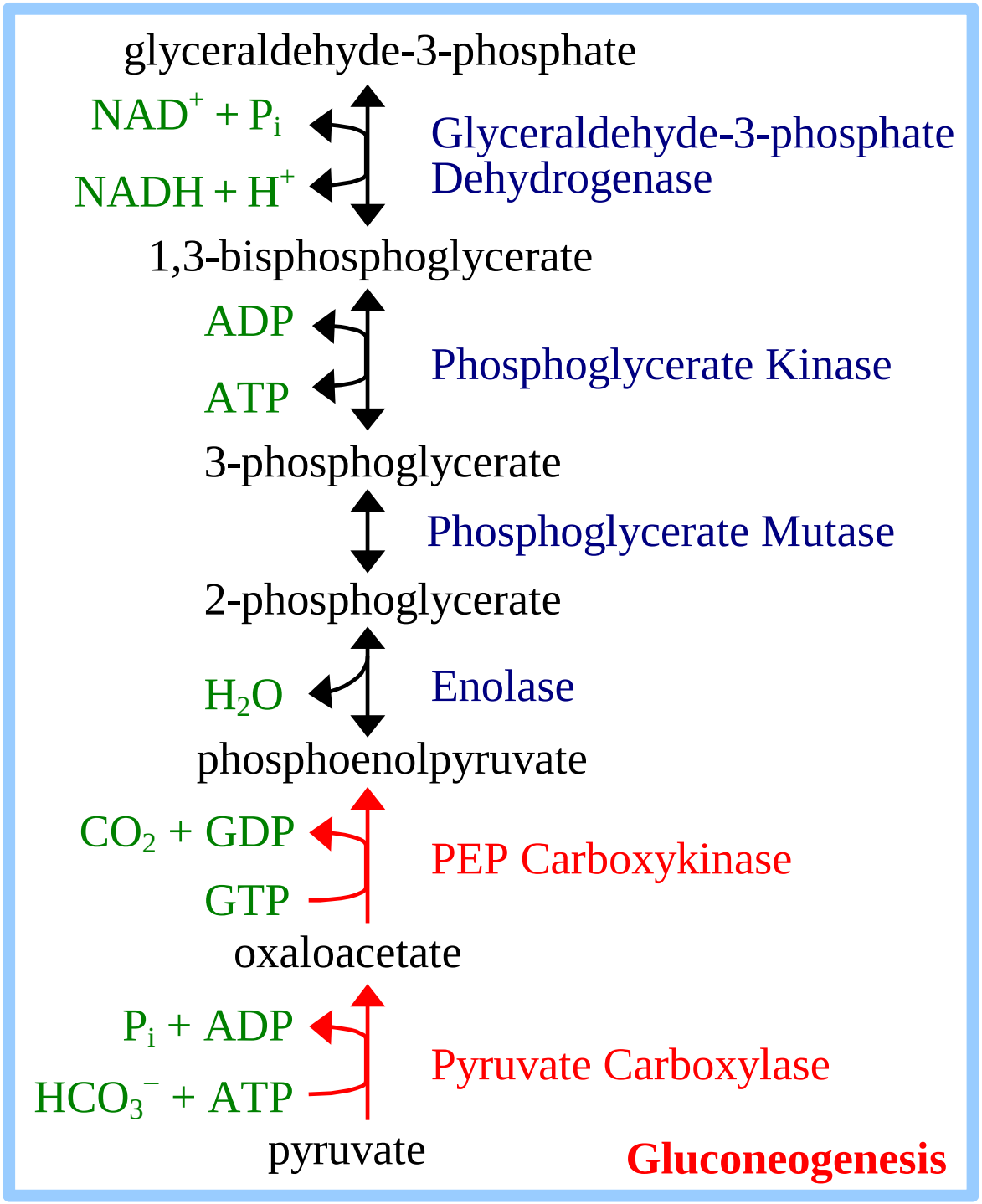
Muscle proteins may break down to supply amino acids. These are transported to liver where they are deaminated and converted to gluconeogenesis inputs.

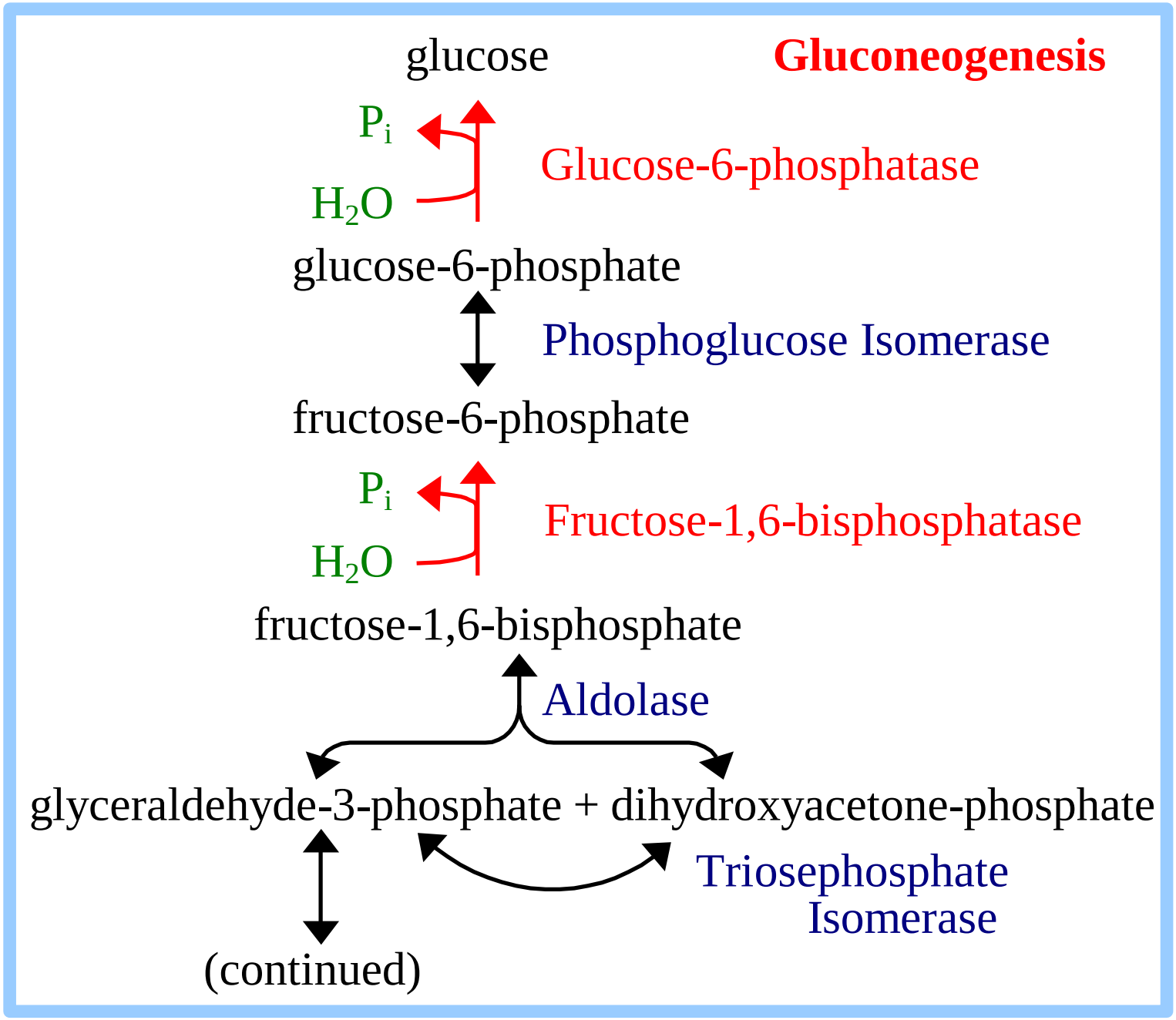
Glycerol, derived from hydrolysis of triacylglycerols in fat cells, is also a significant input to gluconeogenesis.

Summary of Gluconeogenesis Pathway:

Gluconeogenesis enzyme names in red.

Glycolysis enzyme names in blue.




Gluconeogenesis
glucose
P_i
H_2O
Glucose-6-phosphatase
glucose-6-phosphate
Phosphoglucose Isomerase
fructose-6-phosphate
P_i
H_2O
Fructose-1,6-bisphosphatase
fructose-1,6-bisphosphate
Aldolase
glyceraldehyde-3-phosphate + dihydroxyacetone-phosphate
Triosephosphate Isomerase
(continued)

Glycolysis & **Gluconeogenesis** are **both spontaneous**. If both pathways were simultaneously active in a cell, it would constitute a "**futile cycle**" that would waste energy.

Glycolysis:

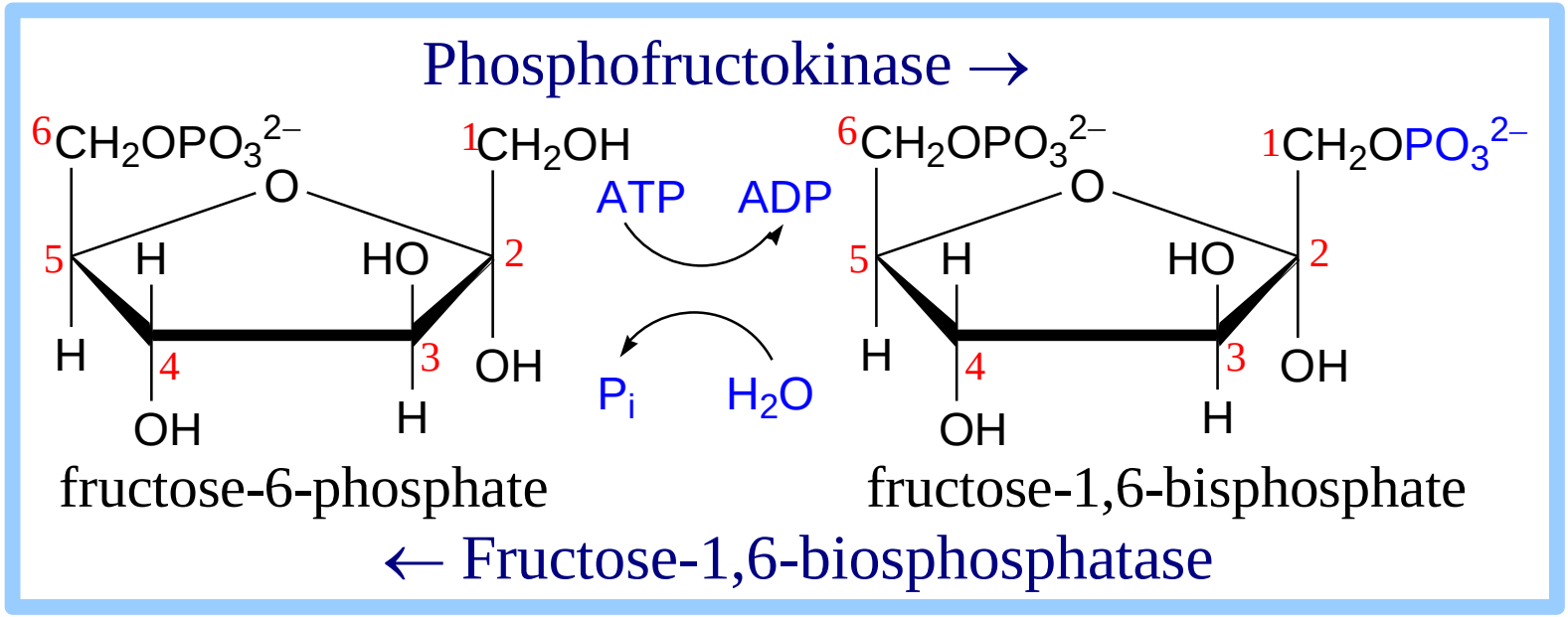
glucose + 2 NAD^+ + 2 ADP + 2 P_i → 2 pyruvate + 2 NADH + 2 ATP

Gluconeogenesis:

2 pyruvate + 2 NADH + 4 ATP + 2 GTP → glucose + 2 NAD^+ + 4 ADP + 2 GDP + 6 P_i

Questions:

1. **Glycolysis** yields **how many ~P** ? **2**
2. **Gluconeogenesis** expends **how many ~P** ? **6**
3. A **futile cycle** of both pathways would waste **how many ~P per cycle** ? **4**



To prevent the waste of a futile cycle, Glycolysis & Gluconeogenesis are **reciprocally regulated**.

Local Control includes reciprocal allosteric regulation by **adenine nucleotides**.

- **Phosphofructokinase** (Glycolysis) is inhibited by ATP and stimulated by AMP.
- **Fructose-1,6-bisphosphatase** (Gluconeogenesis) is inhibited by AMP.

The **opposite effects of adenine nucleotides** on

- **Phosphofructokinase** (Glycolysis)
- **Fructose-1,6-bisphosphatase** (Gluconeogenesis)

insures that when cellular ATP is high (AMP would then be low), glucose is not degraded to make ATP.

When ATP is high it is more useful to the cell to store glucose as glycogen.

When ATP is low (AMP would then be high), the cell does not expend energy in synthesizing glucose.

Global Control in **liver** cells includes reciprocal effects of a **cyclic AMP cascade**, triggered by the hormone glucagon when blood glucose is low.

Phosphorylation of enzymes & regulatory proteins in liver by Protein Kinase A (cAMP Dependent Protein Kinase) results in

- **inhibition of glycolysis**
- **stimulation of gluconeogenesis**,

making glucose available for release to the blood.

Enzymes relevant to these pathways that are **phosphorylated** by Protein Kinase A include:

- **Pyruvate Kinase**, a glycolysis enzyme that is **inhibited** when phosphorylated.
- **CREB** (cAMP response element binding protein) which activates, through other factors, transcription of the gene for **PEP Carboxykinase**, leading to **increased gluconeogenesis**.
- A **bi-functional enzyme** that makes and degrades an allosteric regulator, **fructose-2,6-bisphosphate**.

Reciprocal regulation by fructose-2,6-bisphosphate:

- **Fructose-2,6-bisphosphate stimulates Glycolysis**.

  Fructose-2,6-bisphosphate allosterically **activates** the Glycolysis enzyme **Phosphofructokinase**.

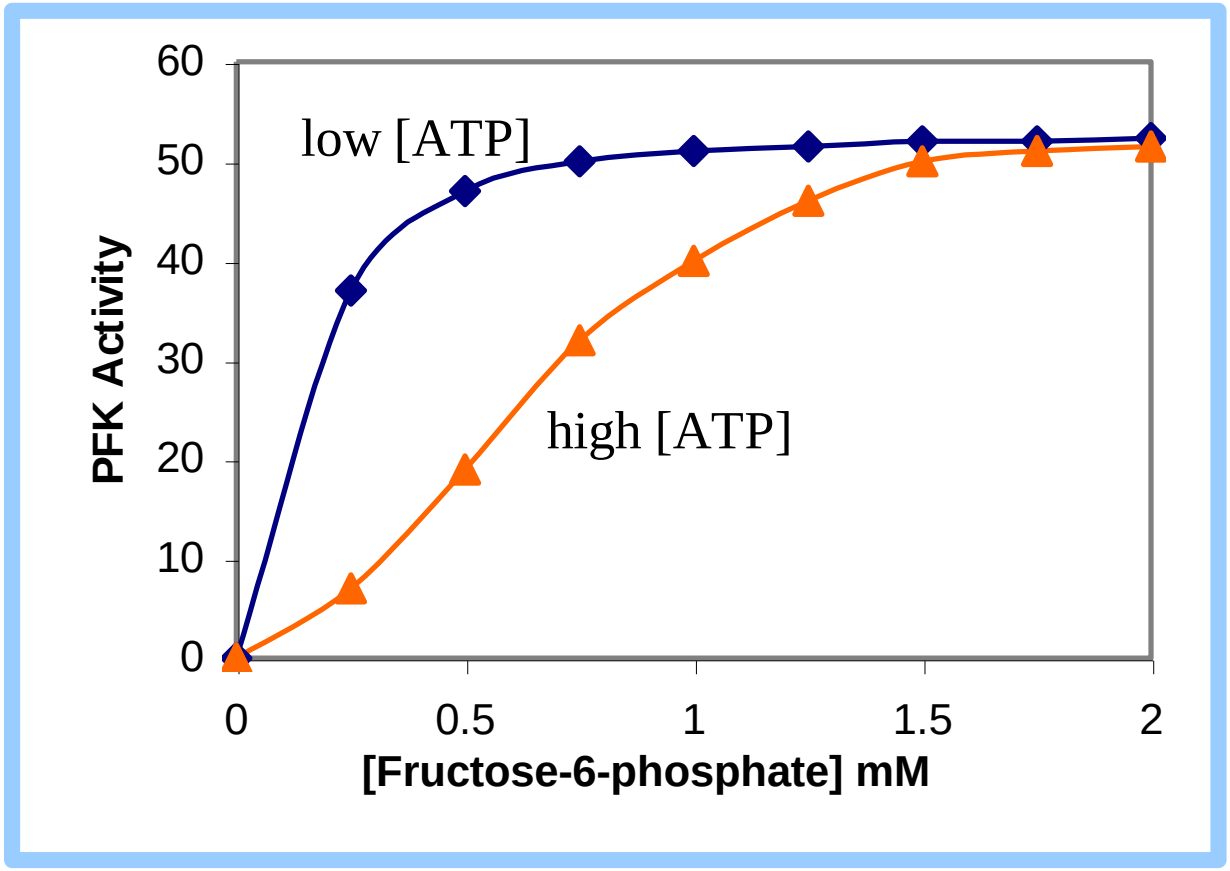
  Fructose-2,6-bisphosphate also **activates transcription** of the gene for **Glucokinase**, the liver variant of Hexokinase that phosphorylates glucose to glucose-6-phosphate, the input to Glycolysis.

- **Fructose-2,6-bisphosphate** allosterically **inhibits** the **gluconeogenesis** enzyme **Fructose-1,6-bisphosphatase**.

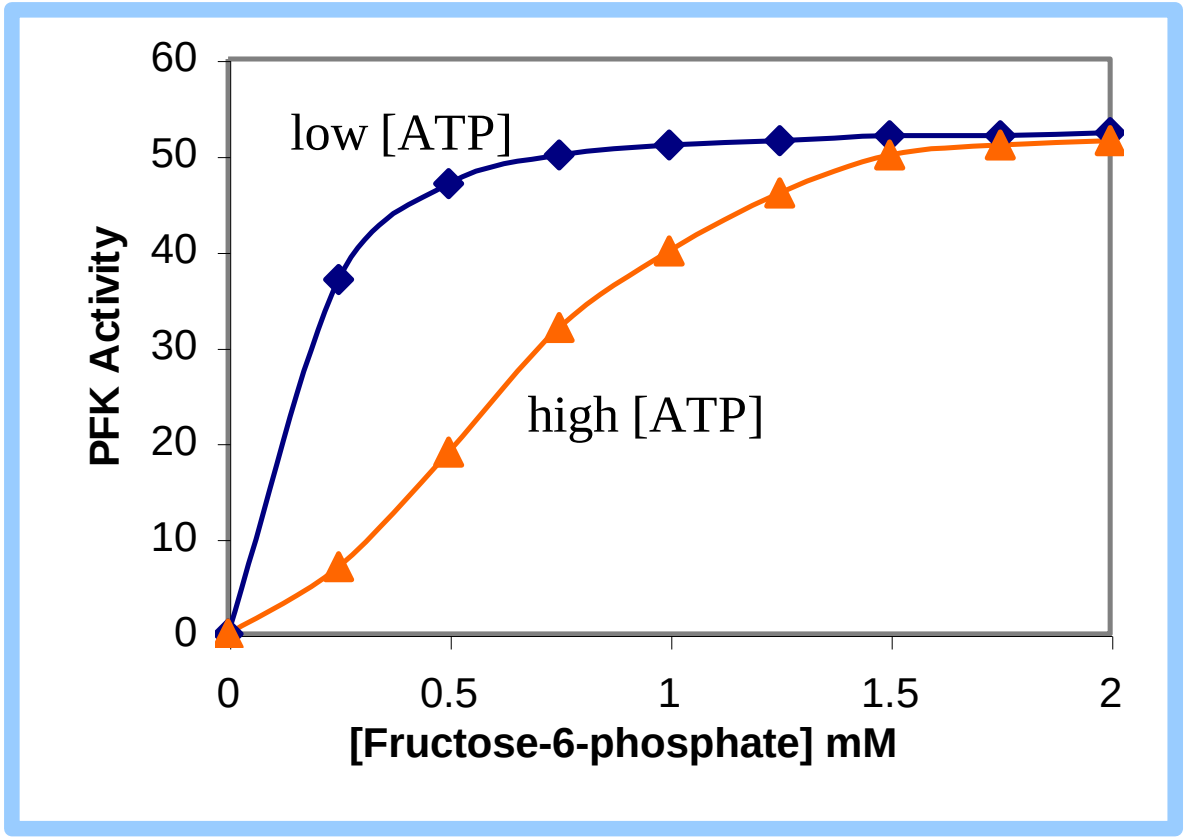
Recall that **Phosphofructokinase**, the rate-limiting step of Glycolysis, is **allosterically inhibited by ATP**.

At high concentration, ATP binds at a low-affinity **regulatory site**, promoting the tense conformation.

Sigmoidal dependence of reaction rate on [fructose-6-phosphate] is observed at high [ATP].



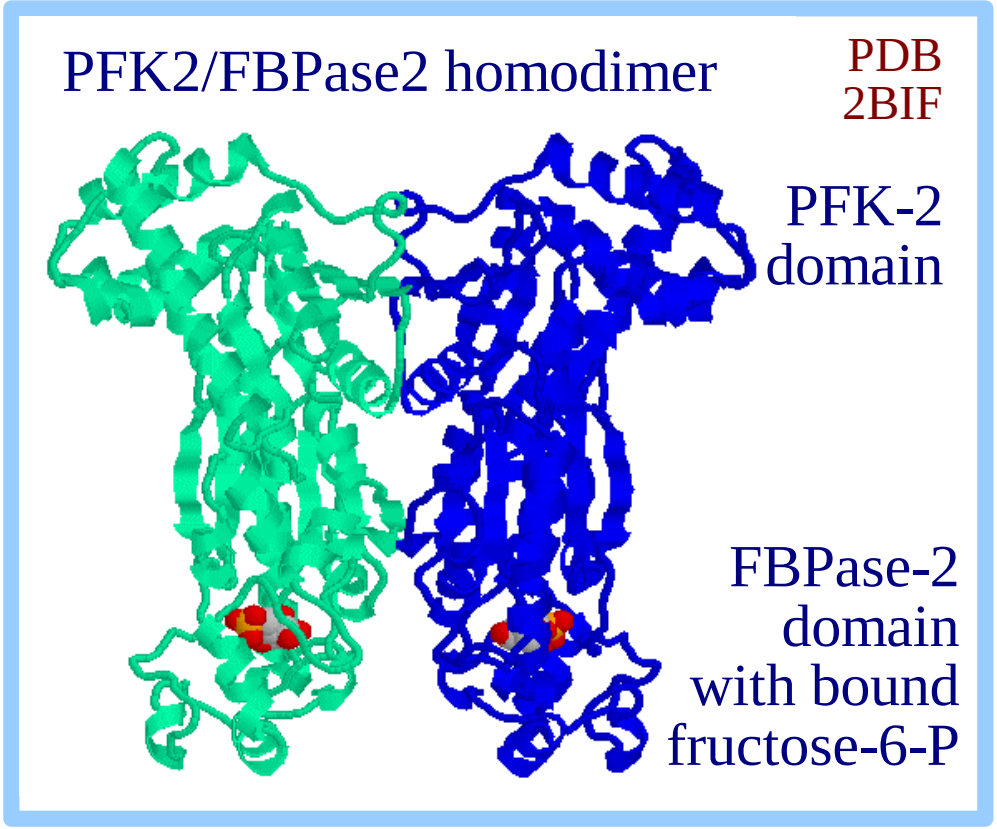
PFK activity in the presence of the **globally controlled** allosteric regulator **fructose-2,6-bisphosphate** is similar to that at low ATP.



Fructose-2,6-bisphosphate promotes the **relaxed** state, activating Phosphofructokinase even at high [ATP].

Thus **activation by fructose-2,6-bisphosphate**, whose concentration fluctuates in response to external hormonal signals, **supersedes local control** by [ATP].

The allosteric regulator **fructose-2,6-bisphosphate** is synthesized & degraded by a **bi-functional enzyme** that includes 2 catalytic domains:



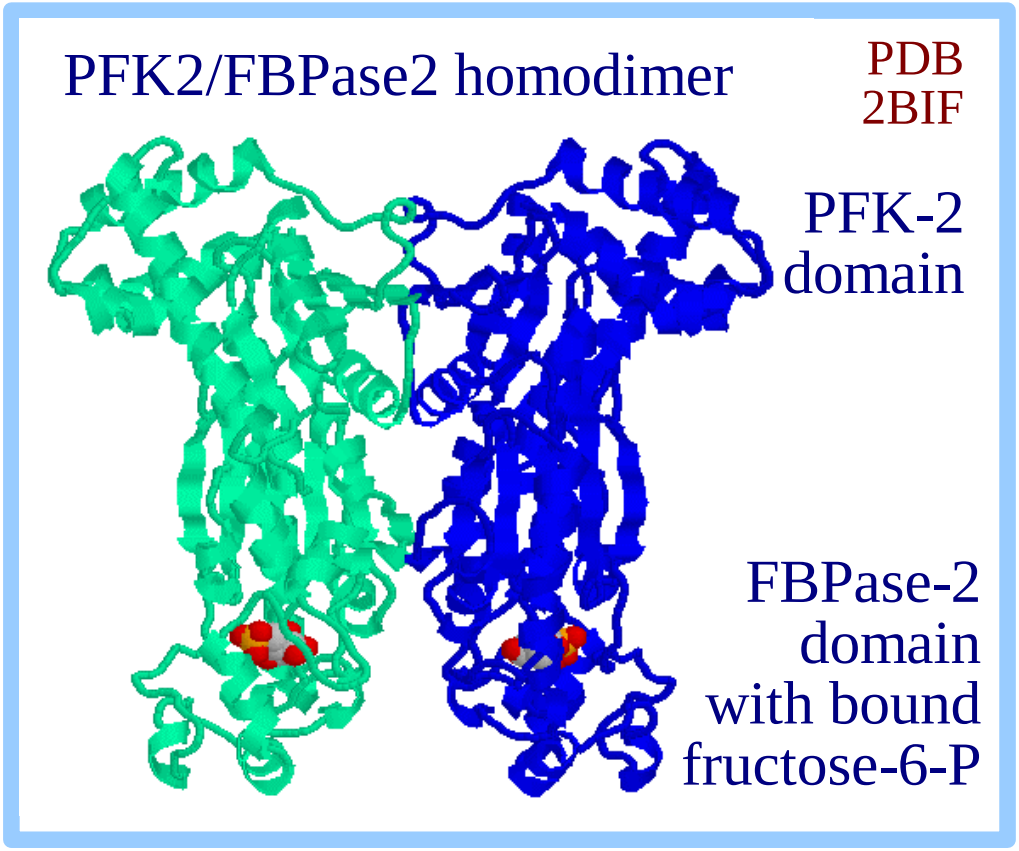
Phosphofructokinase-2 (**PFK2**) domain catalyzes:

Fructose-6-phosphate + ATP → fructose-2,6-bisphosphate + ADP

Fructose-Biophosphatase-2 (**FBPase2**) domain catalyzes:

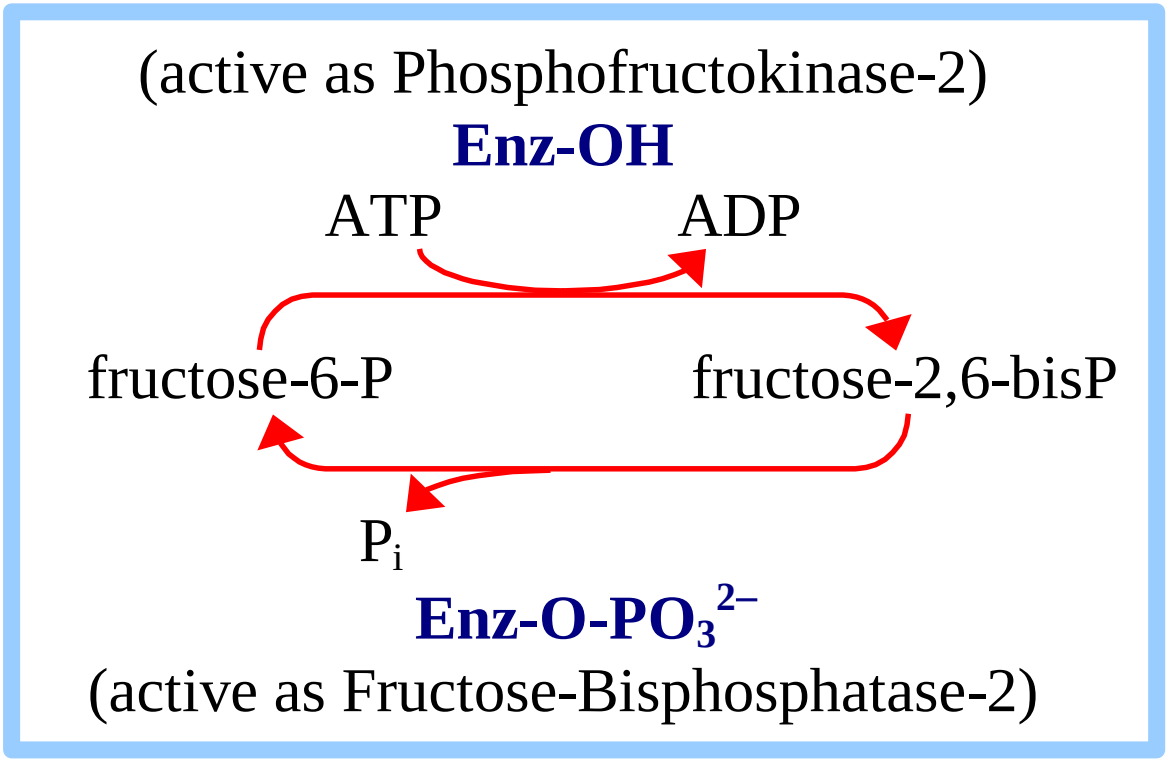
Fructose-2,6-bisphosphate + H_2O → fructose-6-phosphate + P_i

Bifunctional PFK2/FBPase2 assembles into a **homodimer**.



Adjacent to the PFK-2 domain in each copy of the liver enzyme is a **regulatory domain** subject to **phosphorylation** by cAMP-dependent Protein Kinase.

Which catalytic domains of the enzyme are active depends on whether the regulatory domains are phosphorylated.

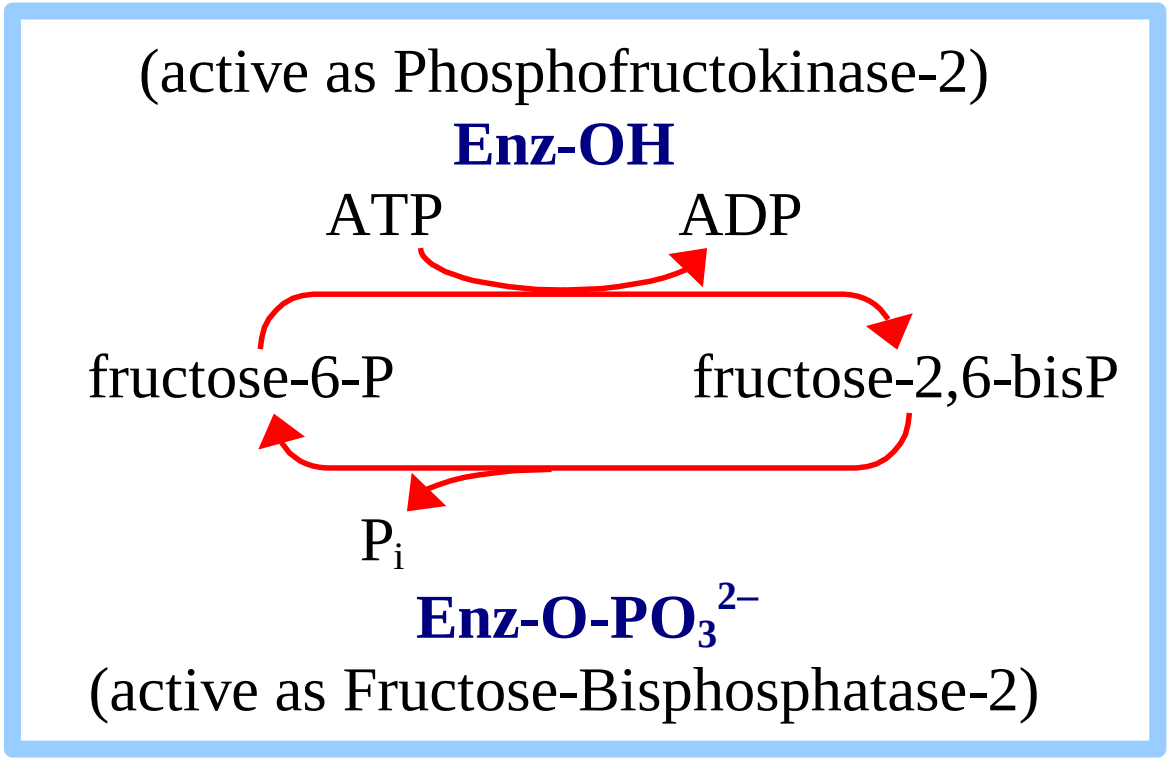


View an animation.

cAMP-dependent phosphorylation of the bi-functional enzyme **activates FBPase2** and **inhibits PFK2**.

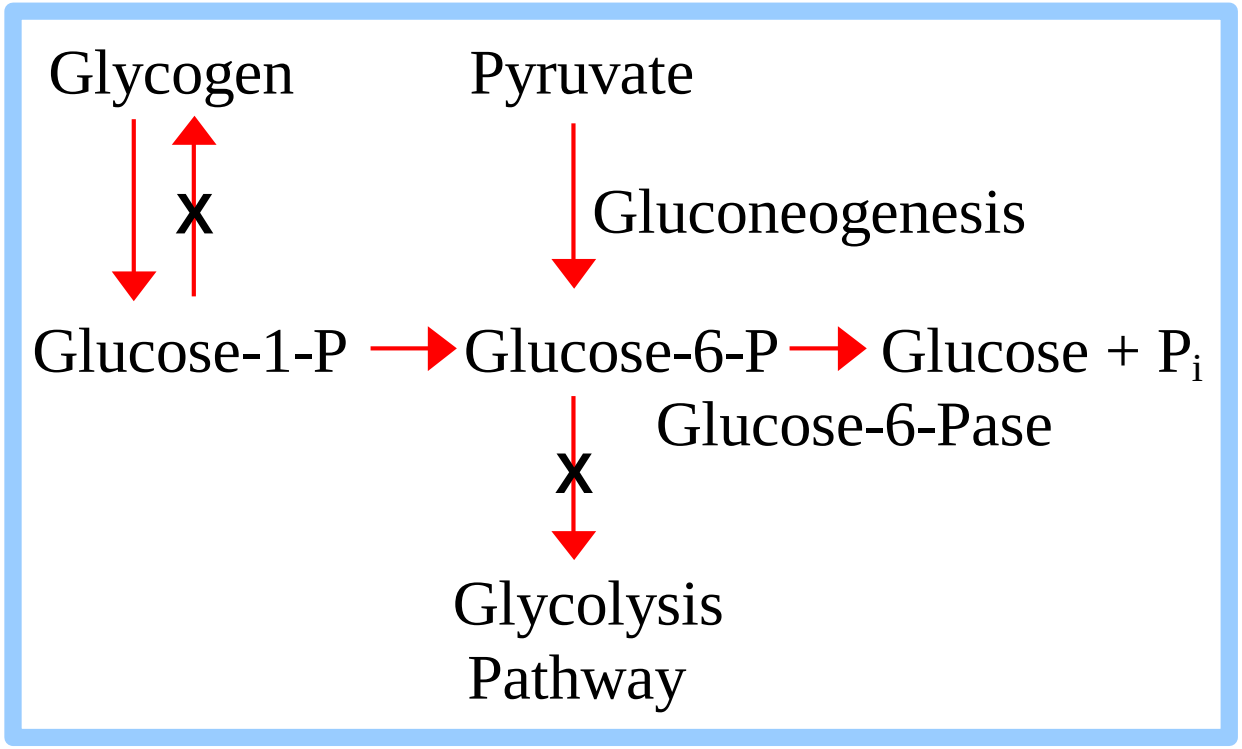
[**Fructose-2,6-bisphosphate**] thus **decreases** in liver cells in response to a cAMP signal cascade, activated by **glucagon** when blood glucose is low.

Downstream effects of the cAMP cascade:



Glycolysis slows because fructose-2,6-bisphosphate is not available to activate Phosphofructokinase.

Gluconeogenesis increases because of the decreased concentration of fructose-2,6-bisphosphate, which would otherwise inhibit the gluconeogenesis enzyme Fructose-1,6-bisphosphatase.



Summary of effects of glucagon-cAMP cascade in liver:

- Gluconeogenesis is stimulated.
- Glycolysis is inhibited.
- Glycogen breakdown is stimulated.
- Glycogen synthesis is inhibited.
- Free glucose is formed for release to the blood.

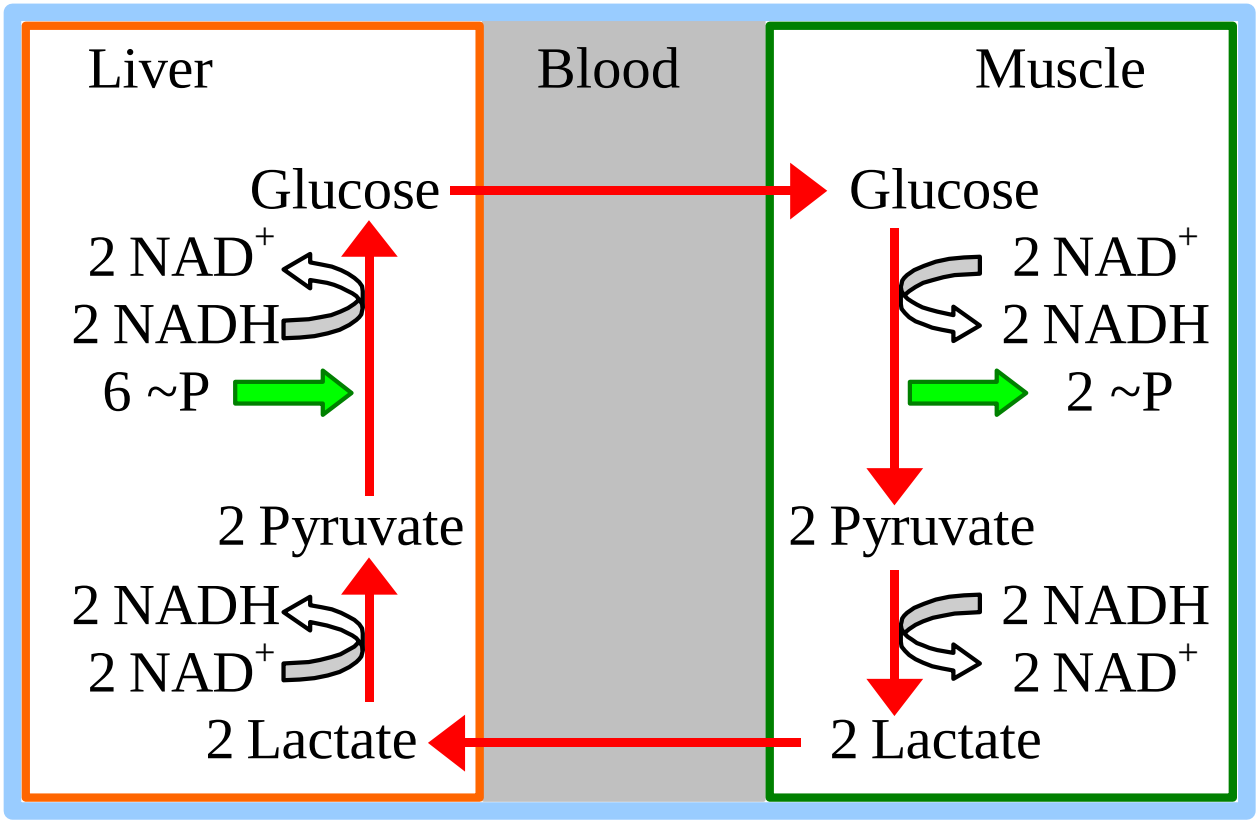
The **Cori Cycle** operates during **exercise**.

For a brief burst of **ATP** utilization, muscle cells utilize **~P** stored as **phosphocreatine**.

Once phosphocreatine is exhausted, ATP is provided mainly by **Glycolysis**, with the input coming from **glycogen** breakdown and from **glucose uptake** from the blood.

(Aerobic fat metabolism, discussed elsewhere, is more significant during a lengthy period of exertion such as a marathon run.)

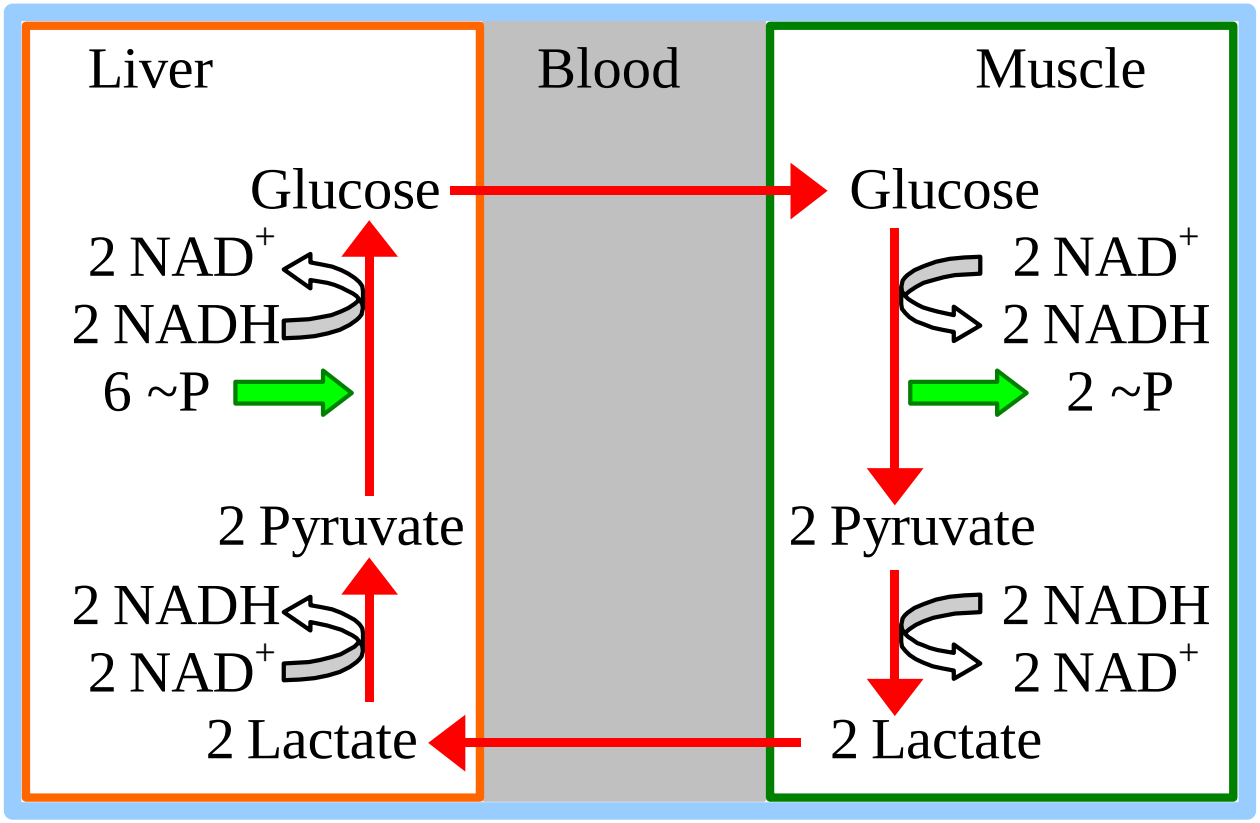
Cori Cycle



Lactate produced from pyruvate passes via the blood to the **liver**, where it may be converted to **glucose**.

The glucose may travel back to the **muscle** to fuel **Glycolysis**.

Cori Cycle

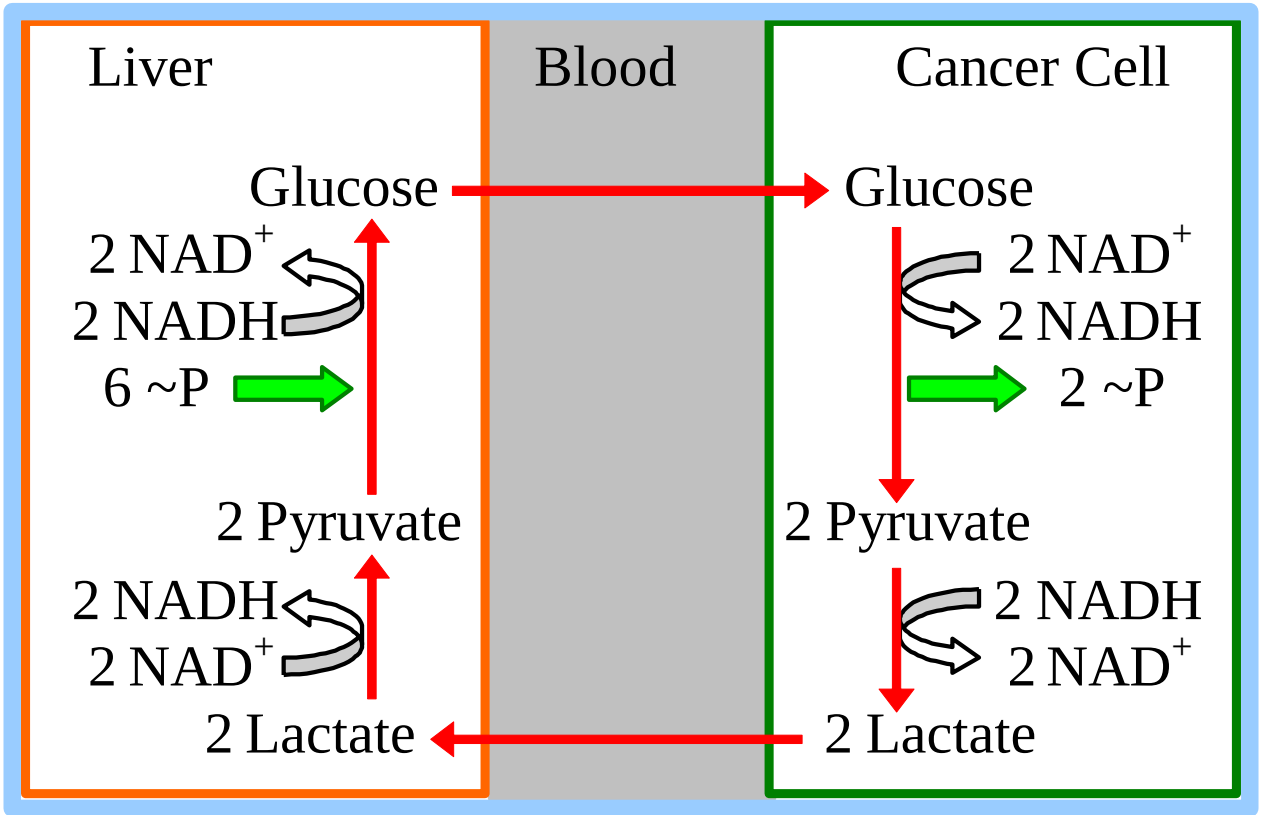


The Cori cycle **costs 6 ~P** in liver for every **2 ~P** made available in muscle. The **net cost is 4 ~P**.

Although costly in ~P bonds, the Cori Cycle allows the organism to accommodate to large fluctuations in energy needs of skeletal muscle between rest and exercise.

The equivalent of the **Cori Cycle** also operates during **cancer**.

If blood vessel development does not keep pace with growth of a solid tumor, **decreased O_2 concentration** within the tumor leads to activation of signal processes that result in a shift to **anaerobic metabolism**.



Energy dissipation by the **Cori Cycle**, which expends **6 ~P** in liver for every **2 ~P** produced via Glycolysis for utilization within the tumor, is thought to contribute to the **weight loss** that typically occurs in late-stage cancer even when food intake remains normal.