Gluconeogenesis

Gluconeogenesis: The synthesis of glucose from noncarbohydrate precursors (e.g., lactate , pyruvate, glycerol, citric acid cycle intermediates, amino acids).

- Glucose is the major fuel source for the brain, nervous system, erythrocytes, and kidney medulla.

- Daily requirement: 160 grams, the brain alone 120grams.

- Approx. 190 grams is available as stored glycogen.

- Thus sufficient reserves for 1 day's requirement.

•During starvation or intense exercise, glucose must be replaced by gluconeogenesis.

•Major site of gluconeogenesis: Liver

•Secondary site: Kidney cortex.

•Thus gluconeogensis in the liver and kidney helps to maintain the glucose level in the blood so that brain and muscle can extract sufficient glucose to meet their metabolic demands.

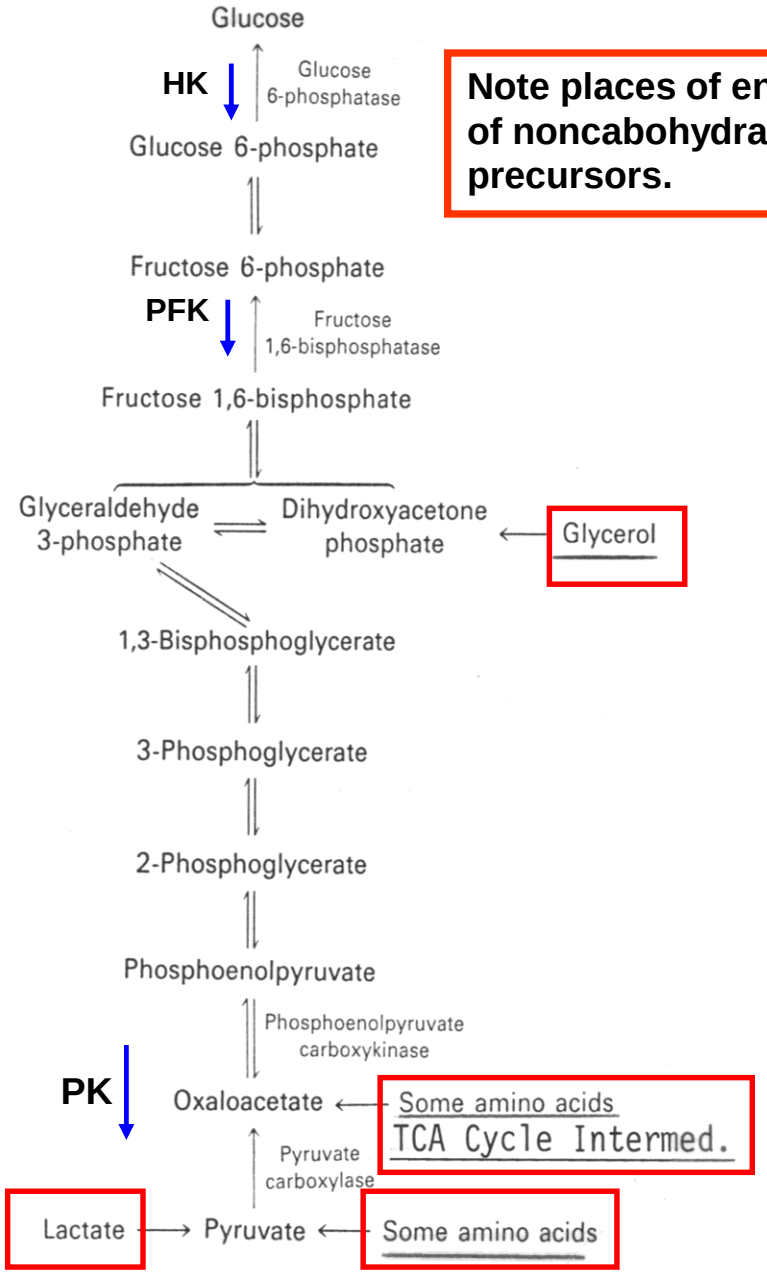
Entry of Noncarbohydrate Precursors

Pyruvate ⟶ Glucose

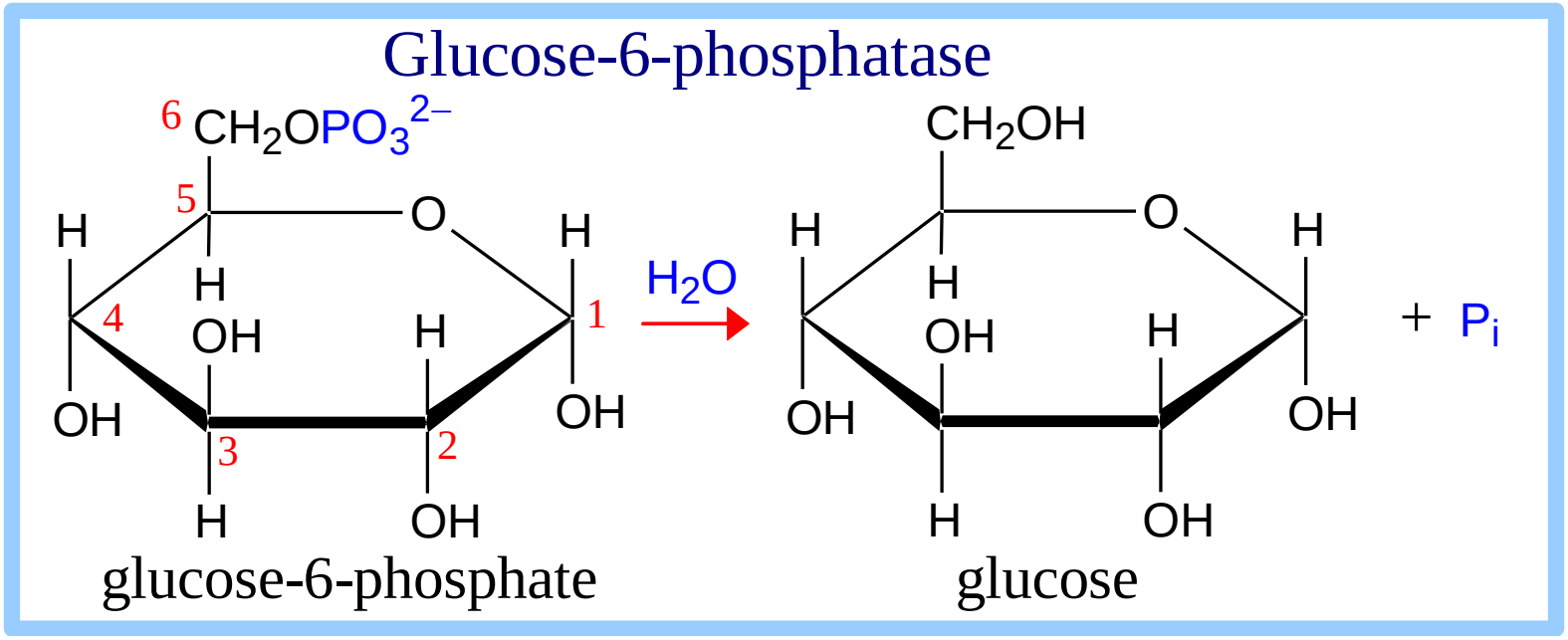
Seven out of ten reactions of gluconeogenesis are exact reversals of glycolysis.

Three steps in glycolysis are irreversible and thus cannot be used in gluco-neogenesis.

Therefore there are 3 steps for which bypass reactions are needed.



Note places of entry of noncabohydrate precursors.



Hexokinase or Glucokinase (Glycolysis) catalyzes:

glucose + ATP → glucose-6-phosphate + ADP

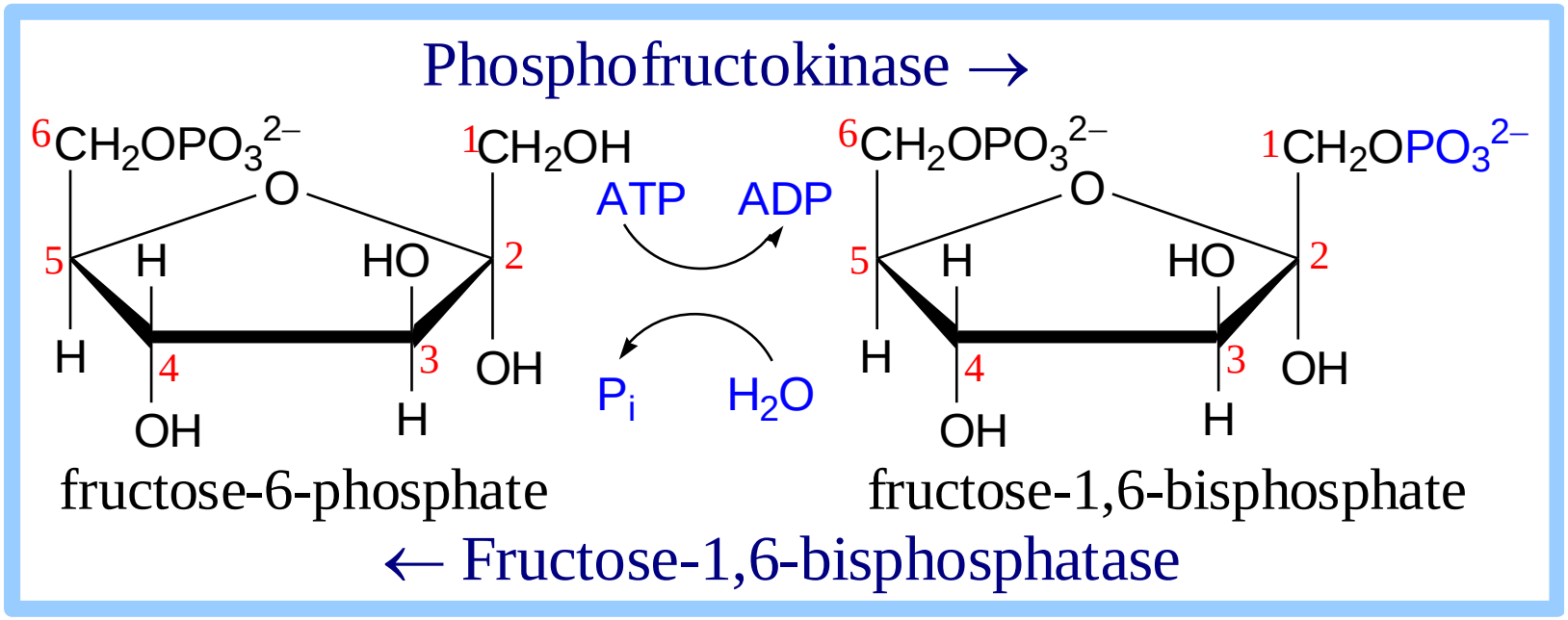
Glucose-6-Phosphatase (Gluconeogenesis) catalyzes:

glucose-6-phosphate + H_2O → glucose + P_i

•**Glucose-6-phosphatase** enzyme is embedded in the endoplasmic reticulum (ER) membrane in liver cells. but absent in brain and muscle.

•Thus, glucose produced by gluconeogenesis in the liver is delivered by the bloodstream to brain and muscle

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

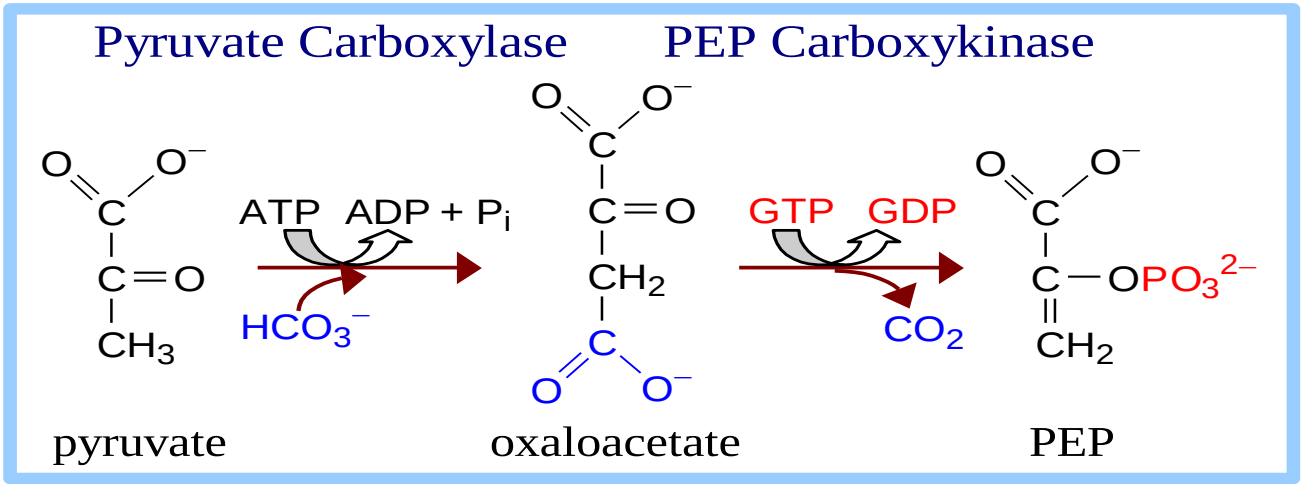
Bypass of Pyruvate Kinase:

Pyruvate Kinase (last step of Glycolysis) catalyzes:
phosphoenolpyruvate + ADP → pyruvate + ATP

Bypass of Pyruvate Kinase (requires 2 enzymes):

Pyruvate Carboxylase (Gluconeogenesis) catalyzes:
pyruvate + HCO3- + ATP → oxaloacetate + ADP + Pi

PEP Carboxykinase (Gluconeogenesis) catalyzes:
oxaloacetate + GTP → PEP + GDP + CO2



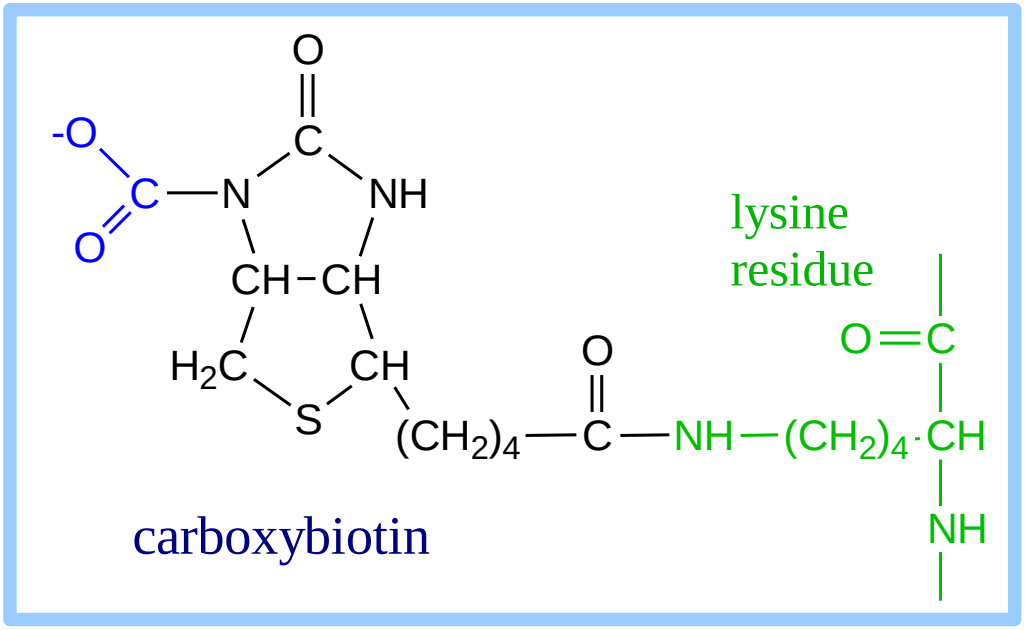
Pyruvate Carboxylase uses **biotin** as prosthetic group.

Biotin serves as a carrier of activated CO2

Enzyme -biotin + ATP + HCO3 ⟶ Enzyme-biotin -CO2 + ADP + Pi

Enzyme-biotin -CO2 + Pyruvate ⟶ Oxaloacetate + Enzyme-biotin

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



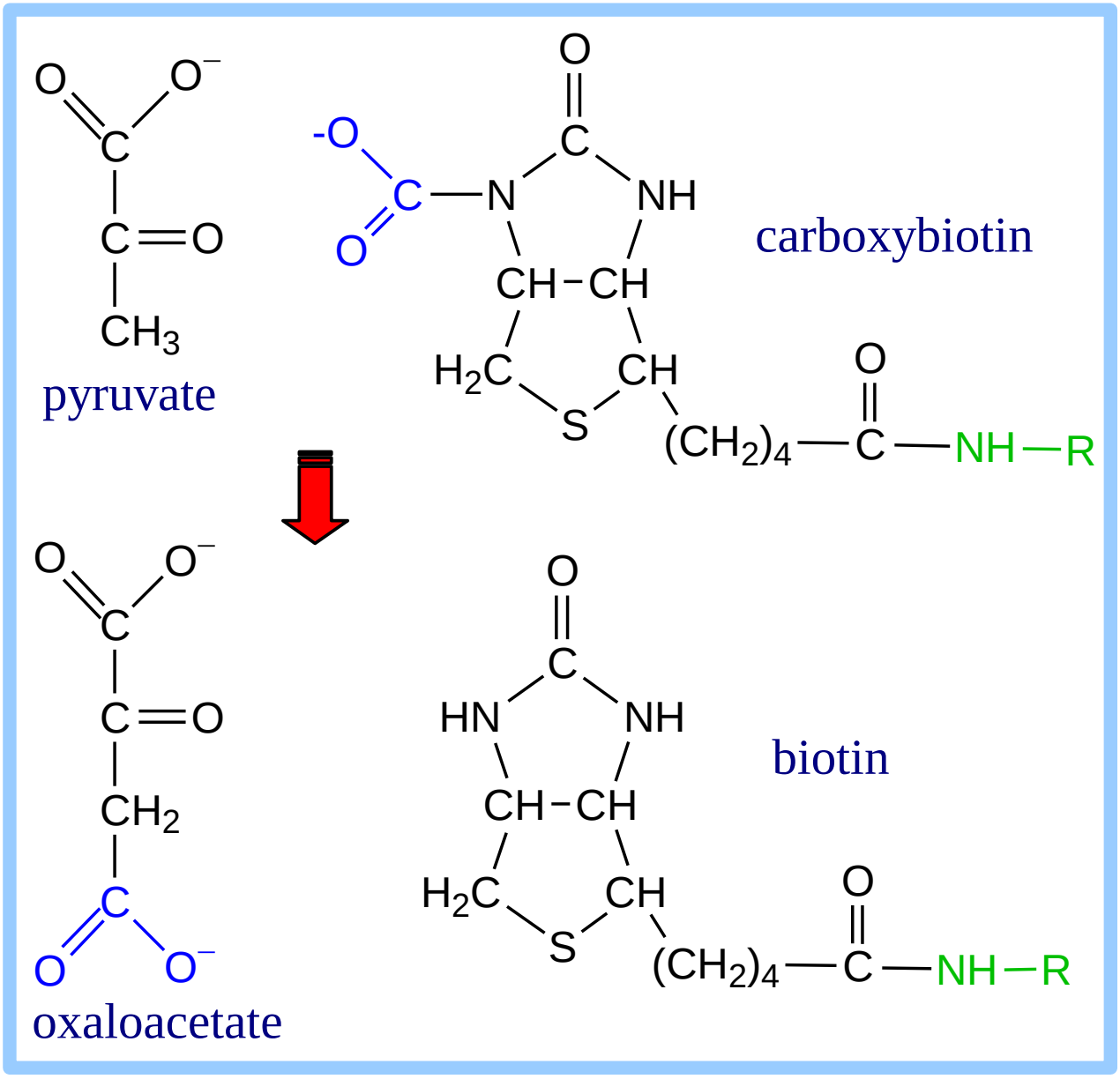
biotin + ATP + HCO_3^- → carboxybiotin + ADP + P_i

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

carboxybiotin + pyruvate

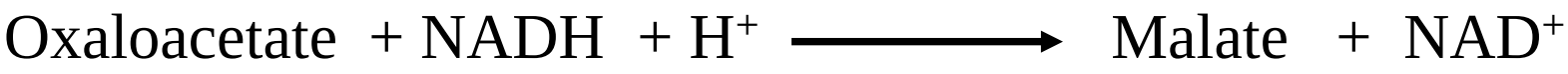
↓

biotin + oxaloacetate



Since pyruvate carboxylase is a mitochondrial enzyme and gluconeogenesis occurs in cytosol:

Oxaloacetate reduced to malate by mitochondrial *malate dehydrogenase* at the expense of mitochondrial NADH.



Malate exits the mitochondrion via the malate/α-ketoglutarate carrier.

In the cytosol, malate is reoxidized to oxaloacetate via cytosolic *malate dehydrogenase* with the production of cytosolic NADH.

Physiological control point

Biotin is not carboxylated unless acetyl-CoA is bound to the enzyme.

Enzyme -biotin + ATP + HCO_3 ⟶ Enzyme-biotin -CO_2 + ADP + Pi

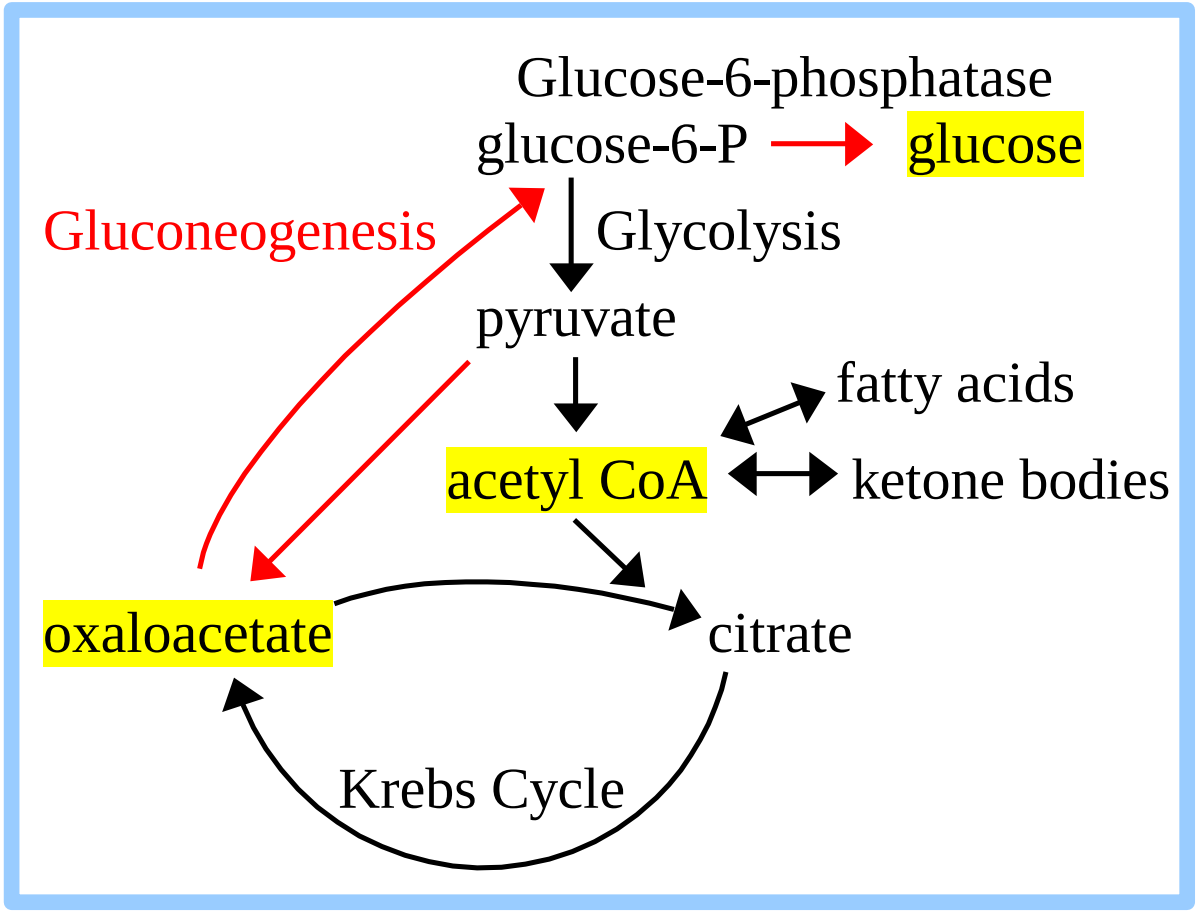
Enzyme-biotin -CO_2 + Pyruvate ⟶ Oxaloacetate + Enzyme-biotin

high levels of Acetyl CoA signals the need for more Oxaloacetate ,
if [ATP]⇧ oxaloacetate enters gluconeogenesis
if [ATP]⇩ oxaloacetate enters krebs cycle

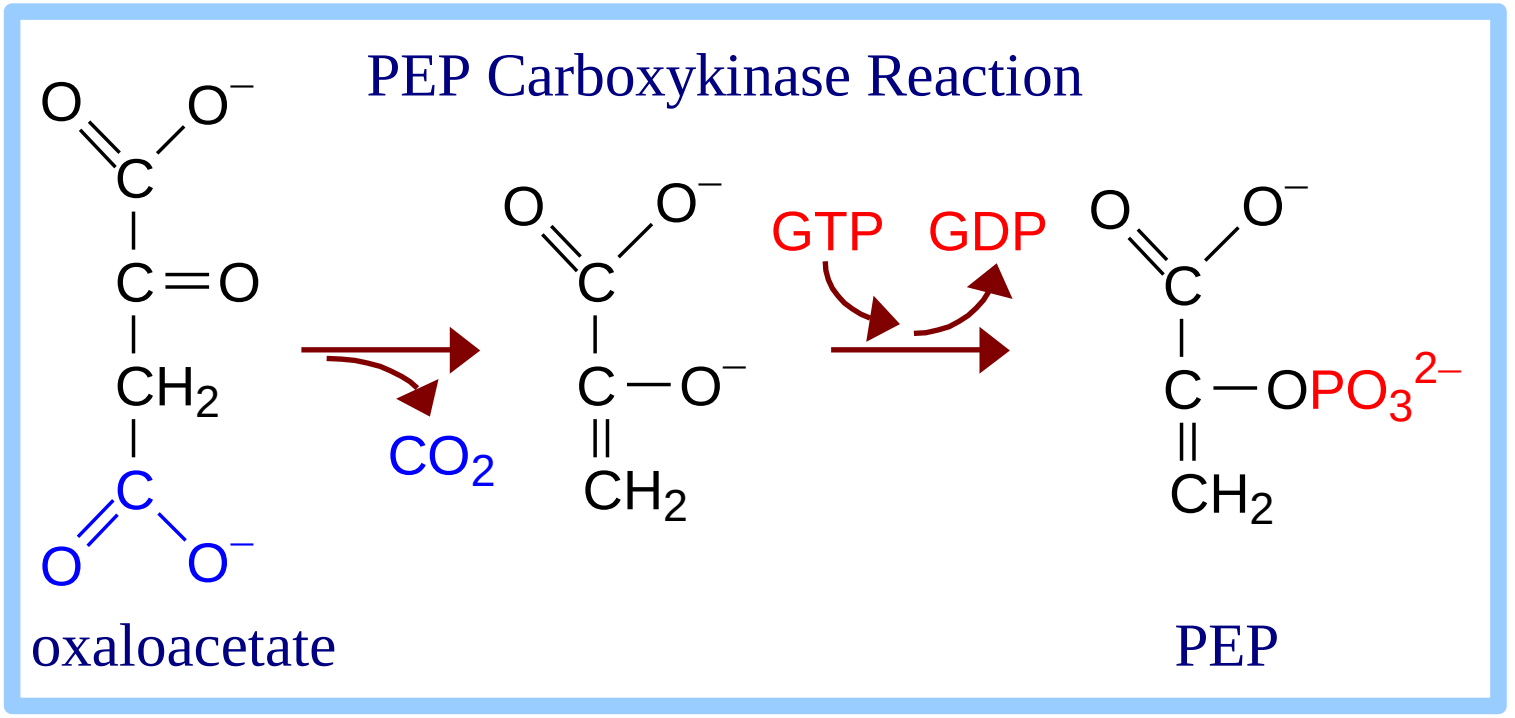
Oxaloacetate plays a role in gluconeogenesis and a critical role in maintaining the level of krebs intermediates

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

[Oxaloacetate] tends to be limiting for Krebs cycle.



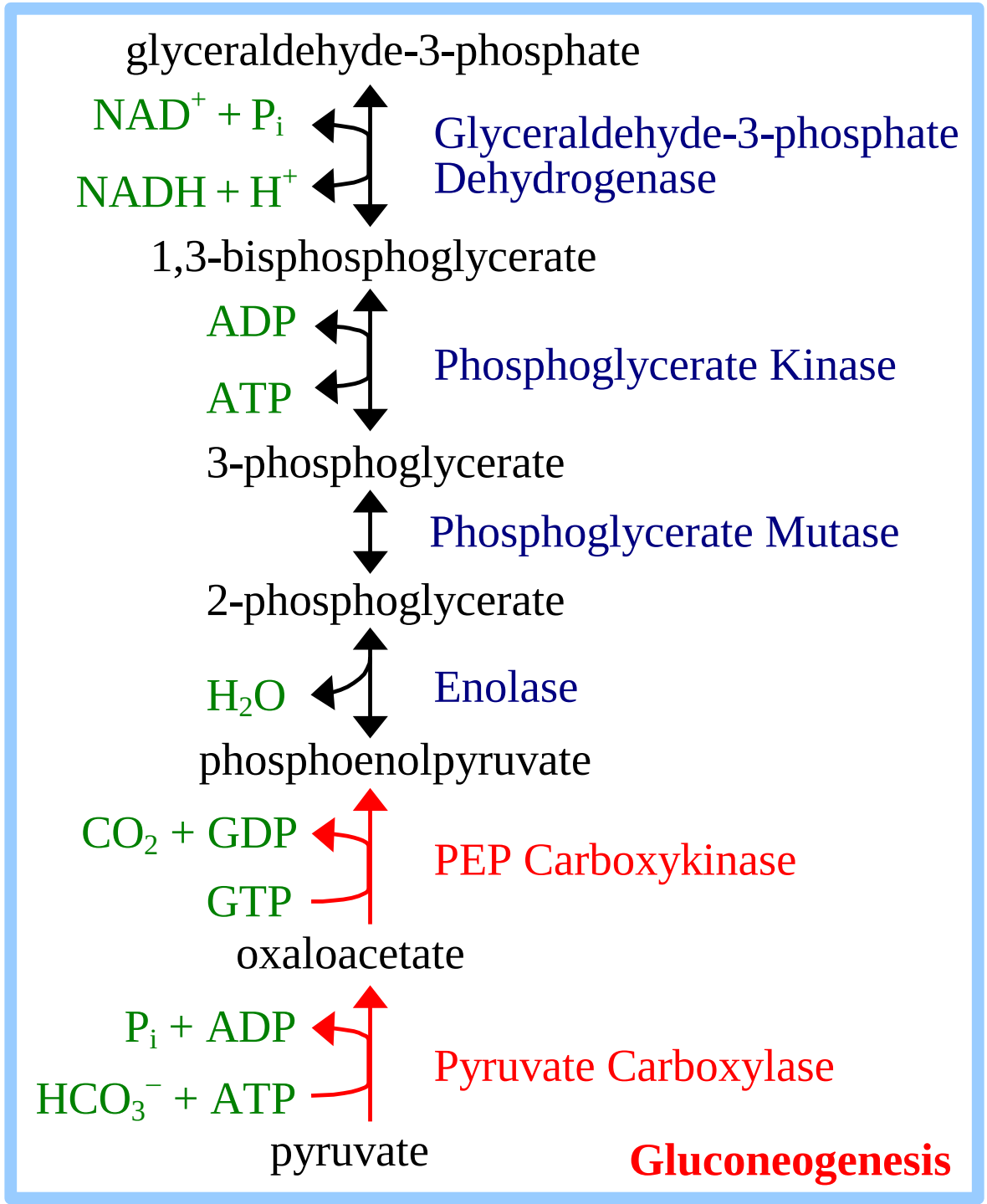
When gluconeogenesis is active in liver, oxaloacetate is diverted to form glucose. Oxaloacetate depletion hinders acetyl CoA entry into Krebs Cycle. The increase in [acetyl CoA] activates Pyruvate Carboxylase to make oxaloacetate.

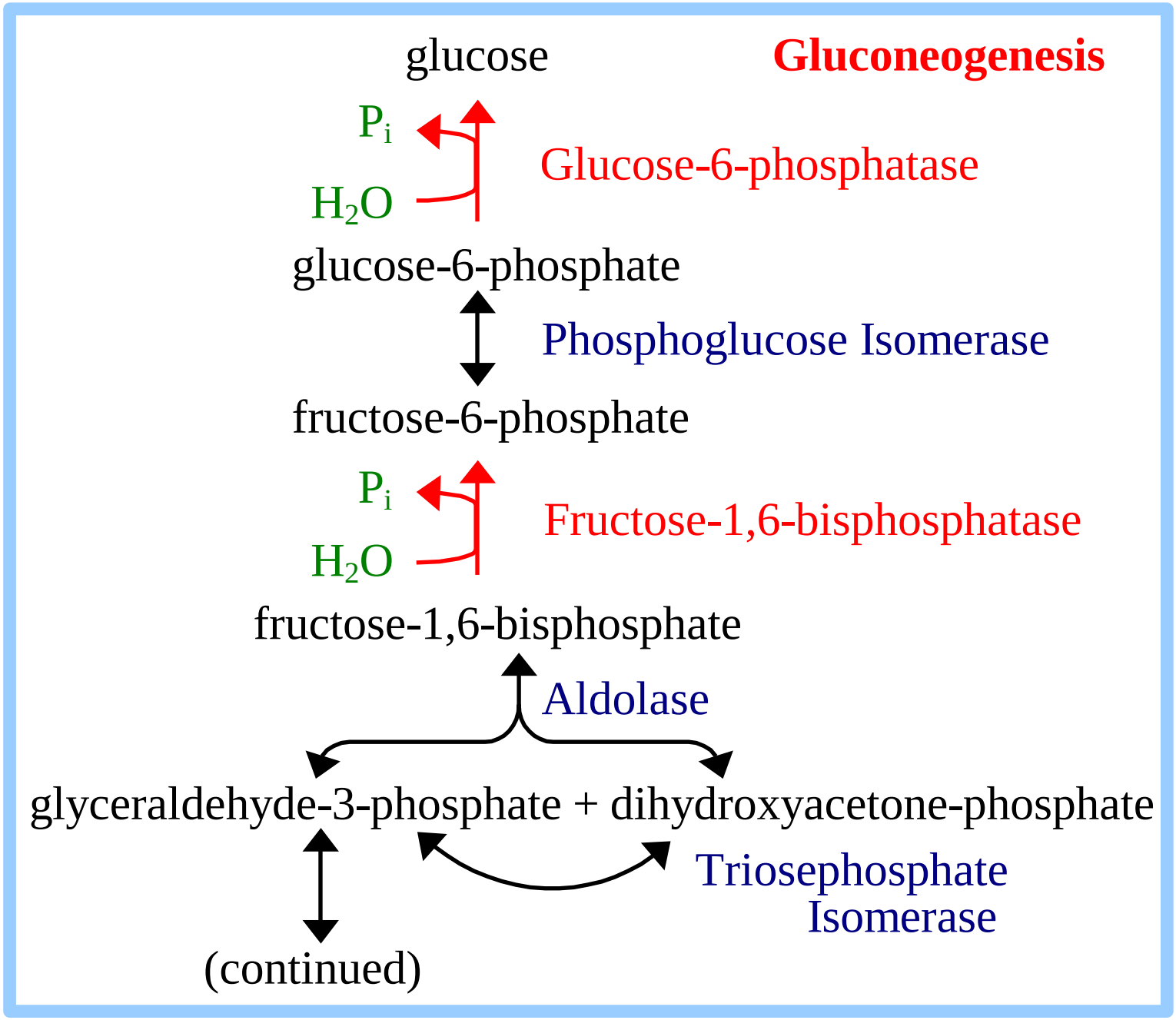


PEP Carboxykinase catalyzes GTP-dependent oxaloacetate → PEP. It is thought to proceed in 2 steps:

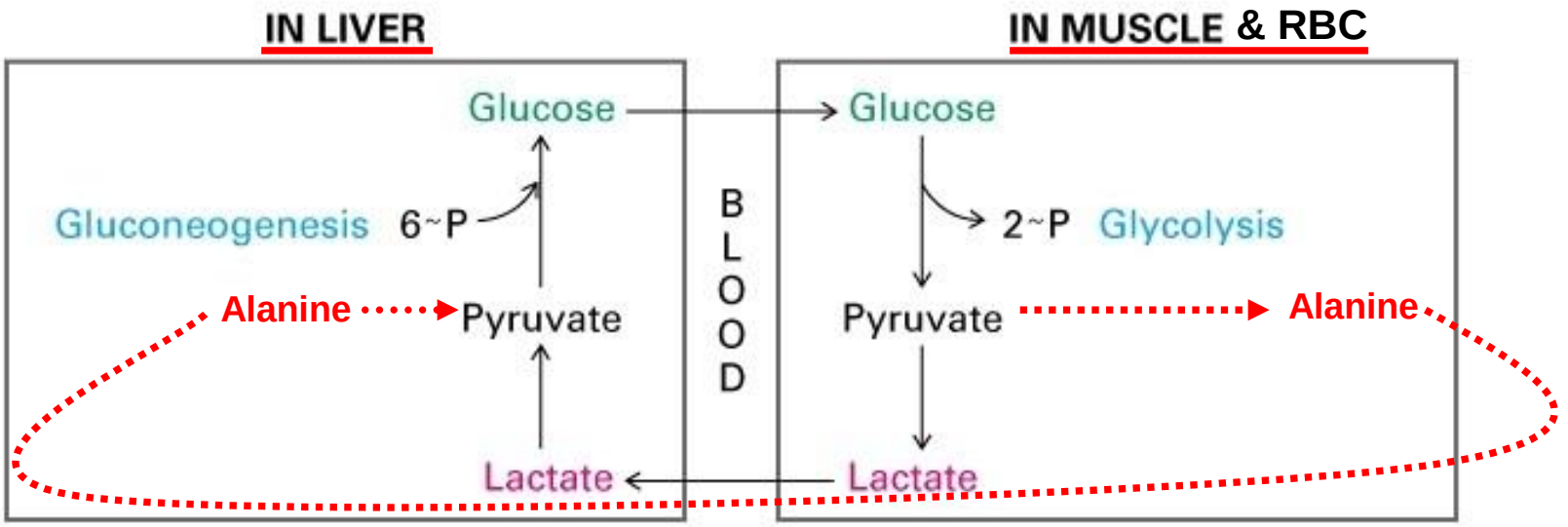
- Oxaloacetate is first **decarboxylated** to yield a pyruvate enolate anion intermediate.
- **Phosphate transfer** from **GTP** then yields phosphoenolpyruvate (PEP).

Summary of Gluconeogenesis Pathway:

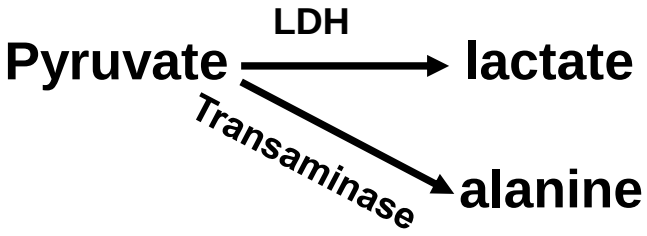



Gluconeogenesis
glucose
P_i
H_2O
Glucose-6-phosphatase
glucose-6-phosphate
Phosphoglucose Isomerase
fructose-6-phosphate
P_i
H_2O
Fructose-1,6-bisphosphatase
fructose-1,6-bisphosphate
Aldolase
glyceraldehyde-3-phosphate + dihydroxyacetone-phosphate
Triosephosphate Isomerase
(continued)

The Cori Cycle



Lactate and alanine, produced by skeletal muscle and RBCs are the major fuels for gluconeogenesis.



The cycle in which part of the metabolic burden is shifted from the muscle to the liver is known as the Cori Cycle.

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**.

Gluconeogenesis from Various Metabolites

Citric Acid Cycle Intermediates: form oxaloacetate during one turn of the cycle. Can get net synthesis of glucose from citric acid cycle intermediates.

Amino Acids: all can be metabolized to either pyruvate or certain intermediates of the citric acid cycle. Hence they are glucogenic (i.e., they can undergo net conversion to glucose). Exceptions are leucine and lysine.

Alanine and glutamine are of special importance as they are used to transport amino groups from a variety of tissues to liver → deaminated to pyruvate and α-KG → gluconeogenesis.

Glucogenic Amino Acids, Grouped by Site of Entry*

Pyruvate	**Succinyl-CoA**
Alanine	Isoleucine†
Cysteine	Methionine
Glycine	Threonine
Serine	Valine
Tryptophan†	
α-Ketoglutarate	**Fumarate**
Arginine	Phenylalanine†
Glutamate	Tyrosine†
Glutamine	**Oxaloacetate**
Histidine	Asparagine
Proline	Aspartate

Fatty Acids: ***even numbered carbon FA*** **are not converted into glucose since during catabolism they yield only acetyl CoA which can't be used as a glucose precursor.**

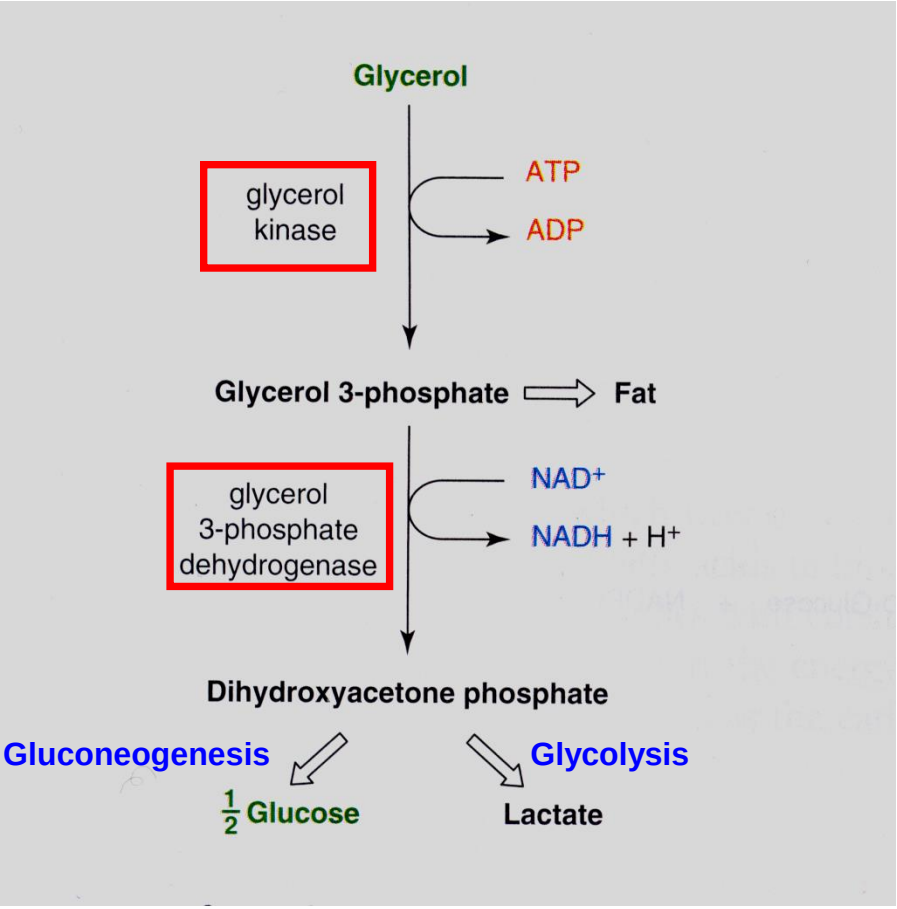
Since: for every 2 carbons that enter the cycle as acetyl CoA, 2 carbons are lost as CO_2, thus there is no net production of OAA to support glucose biosynthesis.

FA oxidation does contribute in that it provides ATP and NADH needed to fuel gluconeogenesis.

In contrast odd numbered carbon FAs ⟶ propionyl CoA succinyl CoA which enters the cycle past the decarboxylation steps.

Thus one can synthesize glucose from odd chain fatty acids.

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



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

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.

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:

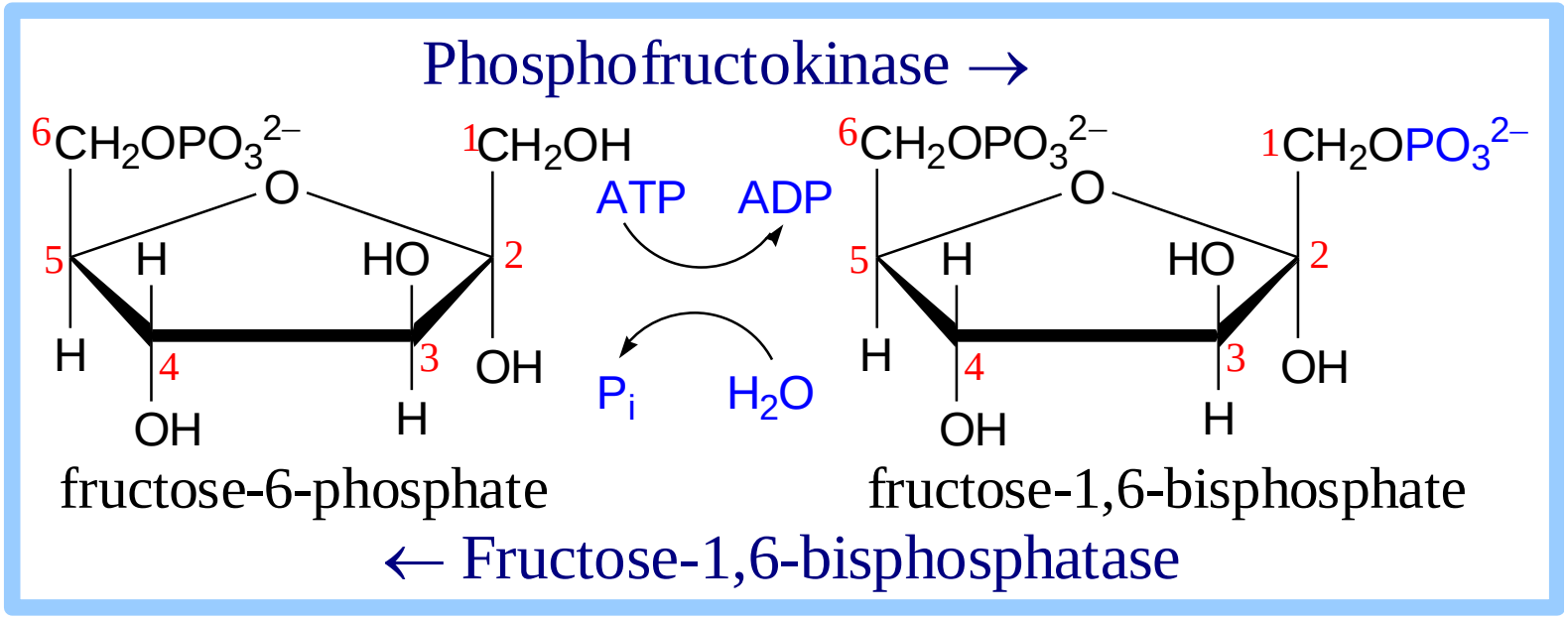
glucose + 2 NAD^+ + 2 ADP + 2 P_i →

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



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

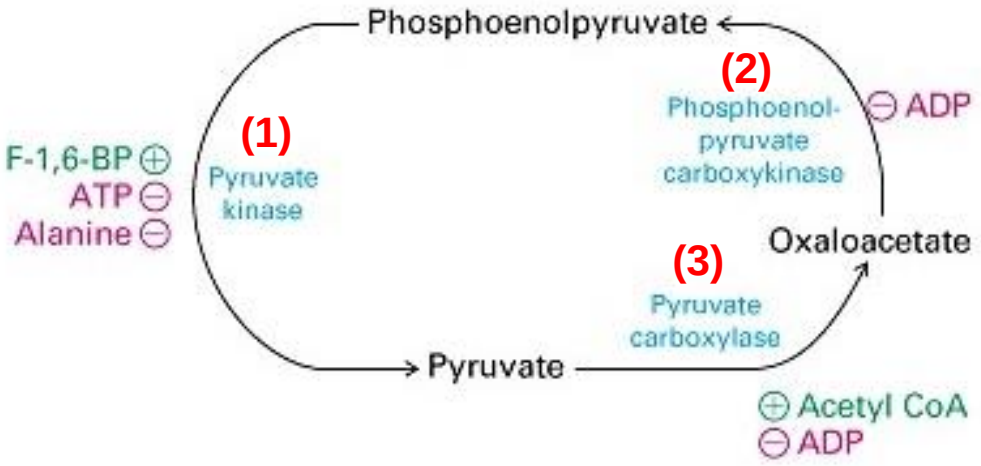
Local Control includes reciprocal allosteric regulation by **adenine nucleotides**.

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

First Control Point: Pyruvate ⟶ PEP

(1) Pyruvate Kinase:
inhibited by ATP and alanine.
Activated by F-1,6-BP.

(2) PEP Carboxykinase: ADP turns it off.
Thus when energy charge of the cell is low, the biosynthetic pathway is turned off.



(3) Pyruvate Carboxylase: stimulated by acetyl CoA. Inhibited by ADP.
Thus when excess acetyl CoA builds up glucose formation is stimulated.
When the energy charge in the cell is low, biosynthesis is turned off.

Finally, recall that *PDH* is inhibited by acetyl CoA. Thus excess acetyl CoA slows its formation from pyruvate and stimulates gluconeogenesis by activating *pyruvate carboxylase*.

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.

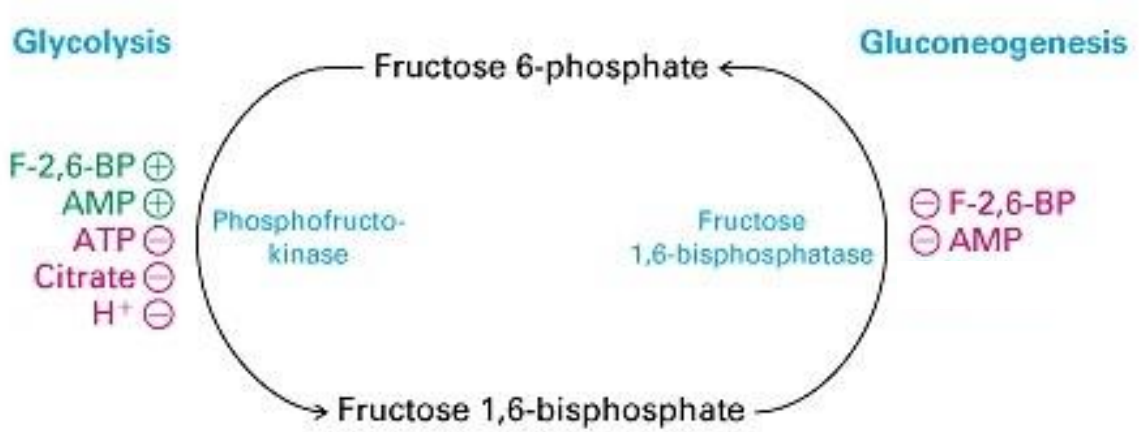
When ATP is low (AMP would then be high), the cell does not expend energy in synthesizing glucose.

Second Control Point:

Fructose 1,6-Bisphosphate ⟶ Fructose 6-phosphate

Thus F-1,6-BPase is inhibited by F-2,6-BP and AMP.

These modulators have the opposite effect on PFK1.



Further, recall that F-2,6-BP is a signal molecule that is present at low concentration during starvation and high concentration in the fed state due to the antagonistic effects of glucagon and insulin on its production.

Global Control in **liver** cells includes 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:

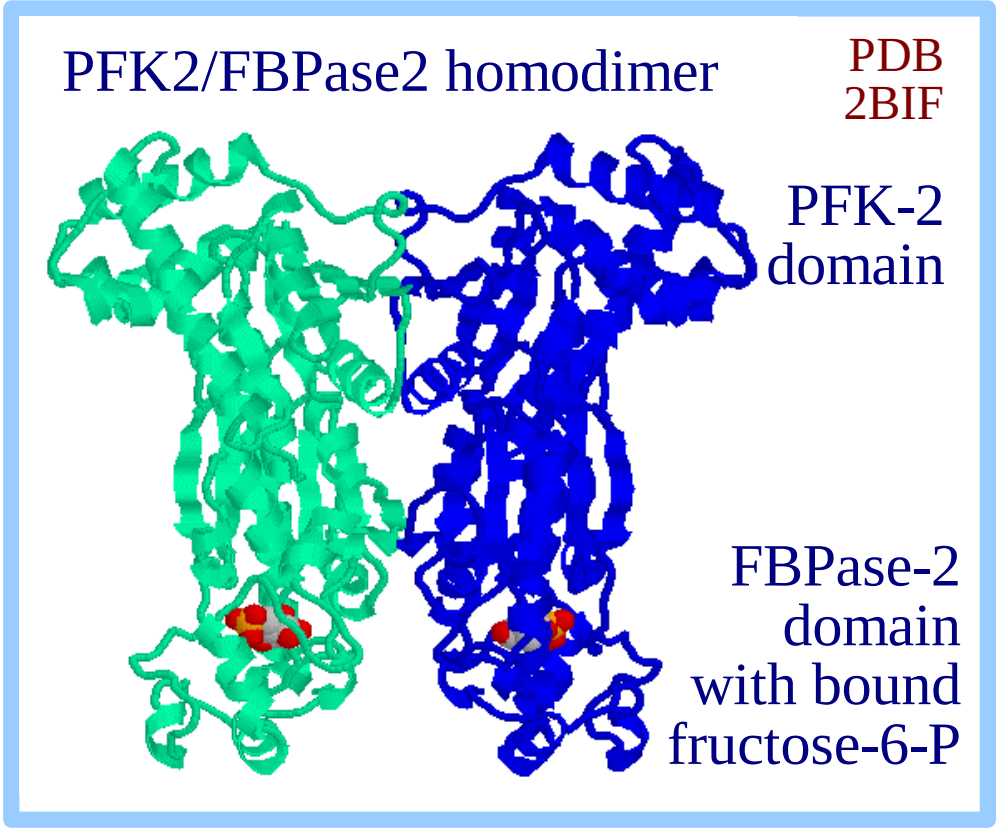
- **Fructose-2,6-bisphosphate stimulates Glycolysis**.

  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**.

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



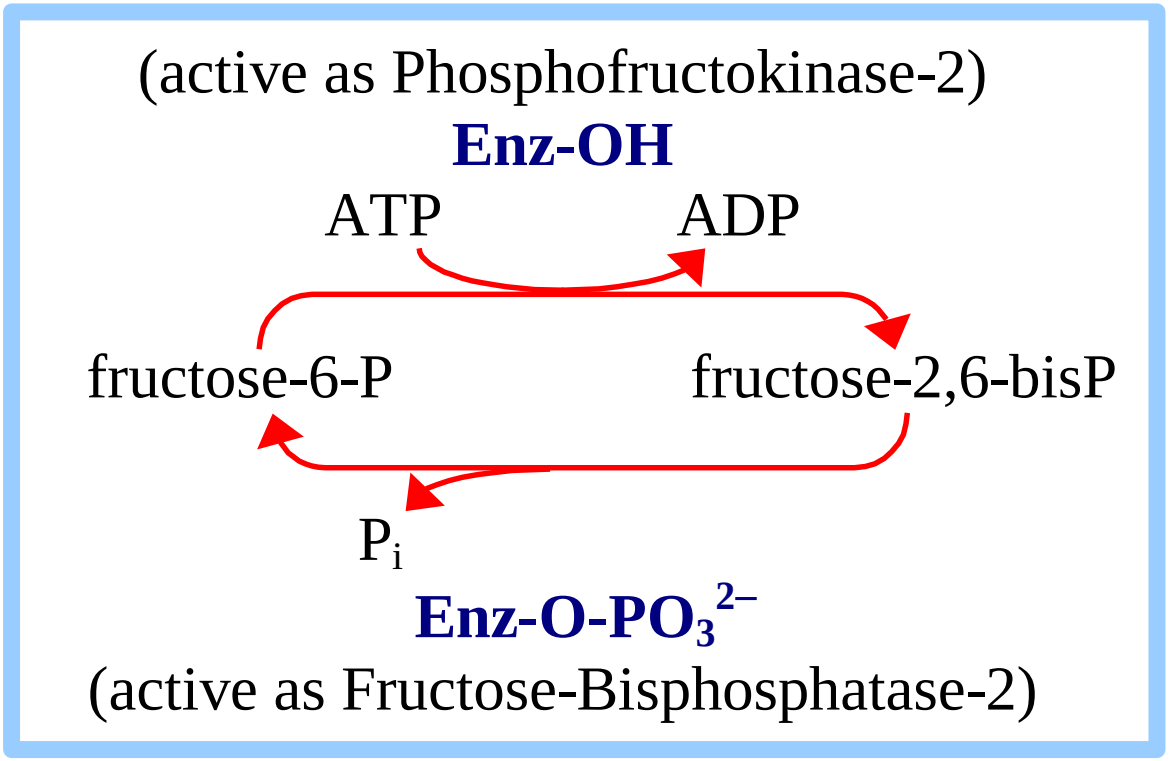
Phosphofructokinase-2 (**PFK2**) domain catalyzes:

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

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

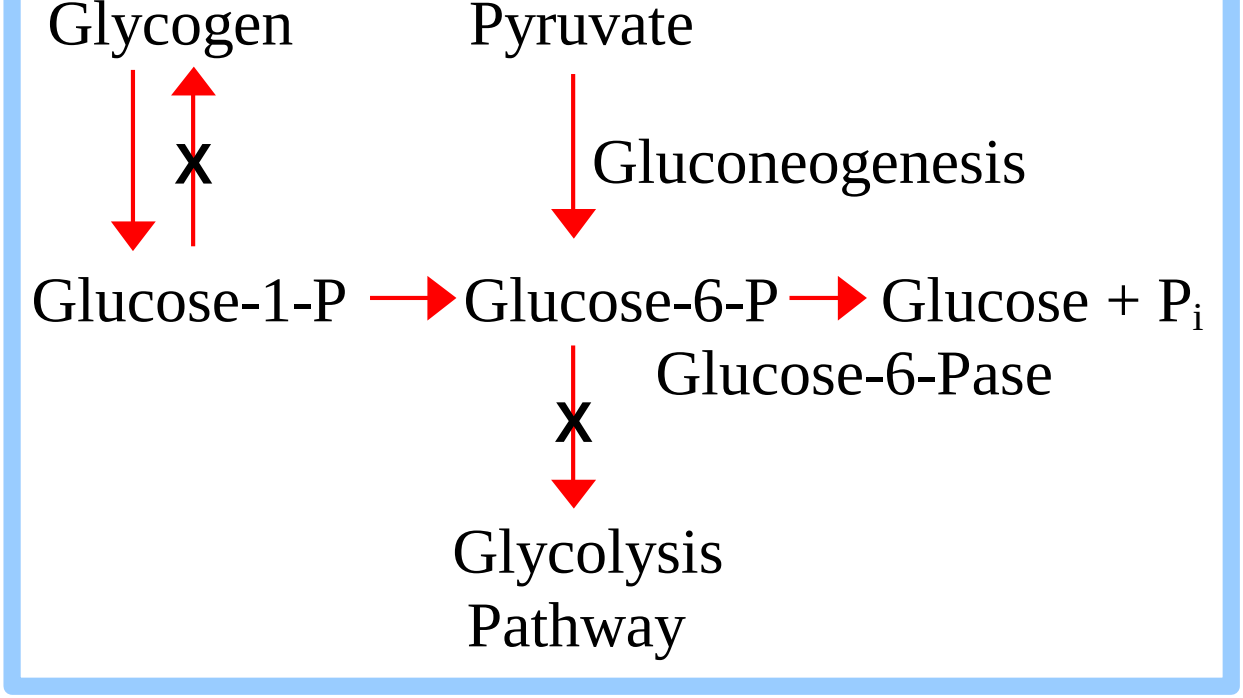
Fructose-2,6-bisphosphate + H_2O → fructose-6-phosphate + P_i

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



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.



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.