Gluconeogenesis

In animals, glucose is required by the brain, and is important to the proper functioning of most tissues. A fall in plasma glucose can result in unconsciousness, and, if untreated, can be fatal. If dietary glucose is insufficient to maintain normal circulating levels of glucose, additional glucose must be released from the liver. The liver has some glucose stored in the form of glycogen but these stores only last for about 12 hours in the absence of dietary glucose.

Considerably before the glucose stores have been consumed, the organism must begin synthesizing additional glucose from other molecules in a process called gluconeogenesis (literally "the birth of new glucose"). The vast majority of **gluconeogenesis occurs in the liver**, with some additional glucose synthesis occurring in the kidney.

Glucose can be synthesized from pyruvate, from nearly all of the standard amino acids, or from TCA cycle intermediates. **Animals cannot convert acetyl-CoA to any of these compounds**. Because the breakdown of fat results nearly exclusively in acetyl-CoA, fat (with minor exceptions) cannot be used to synthesize glucose. Instead, most glucose is synthesized either from lactate (produced during anaerobic glycolysis) or from amino acids (derived from breakdown of proteins).

Some glucose is synthesized from glycerol (a breakdown product of triacylglycerol and phospholipids), and some can be synthesized from fatty acids containing an odd number of carbons. Cows and other ruminants use a breakdown product of chlorophyll as a gluconeogenic substrate (in humans, this pathway is present but is far less important). In general, however, gluconeogenesis uses amino acids either derived from dietary protein or from breakdown of proteins stores as the source of the substrate required for the process.
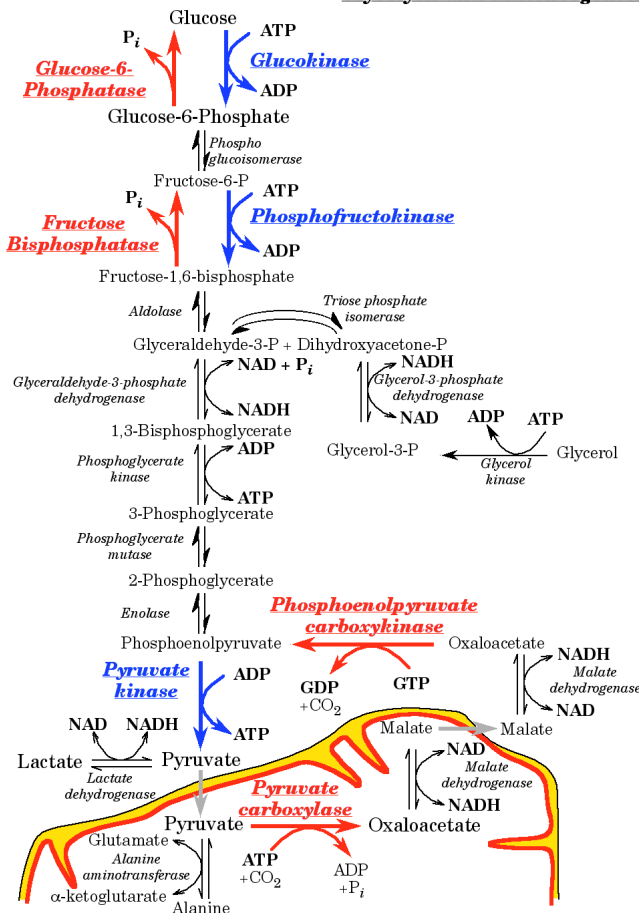
Enzymes of the gluconeogenic pathway

Most of the enzymes used to synthesize glucose are also involved in the glycolytic pathway. Some glycolytic reactions, however, are irreversible under physiological conditions, and reversing these steps requires separate enzymes. The irreversible steps tend to act as regulatory control points.

The diagram below summarizes the glycolytic and gluconeogenic pathways. The enzymes shown in blue are the regulated glycolytic steps; the enzymes in red are regulated gluconeogenic enzymes. The enzymes shown in black are common to both pathways.

The irregular shape at the bottom of the diagram is a mitochondrion. Note that the pyruvate carboxylase reaction occurs exclusively in this compartment. The phospho-enolpyruvate carboxykinase reaction is shown in the cytoplasm; this is true for some species; in humans the reaction occurs in both the cytoplasm (as shown) and in the mitochondria; in the latter case, the phosphoenolpyruvate is transported out of the mitochondria to allow the remainder of the gluconeogenic reactions to proceed.

Glucose

P_i ATP

Glucose-6-Phosphatase   *Glucokinase*

ADP

Glucose-6-Phosphate

Phospho glucoisomerase

Fructose-6-P   ATP

P_i

Fructose Bisphosphatase   *Phosphofructokinase*

ADP

Fructose-1,6-bisphosphate

Aldolase   *Triose phosphate isomerase*

Glyceraldehyde-3-P + Dihydroxyacetone-P

Glyceraldehyde-3-phosphate dehydrogenase   **NAD + P_i**   **NADH** *Glycerol-3-phosphate dehydrogenase*

NADH   **NAD**   ADP   ATP

1,3-Bisphosphoglycerate   **ADP**

Glycerol-3-P   Glycerol

Phosphoglycerate kinase   **ATP**   *Glycerol kinase*

3-Phosphoglycerate

Phosphoglycerate mutase

2-Phosphoglycerate

Enolase   *Phosphoenolpyruvate carboxykinase*

Phosphoenolpyruvate   Oxaloacetate   **NADH** *Malate dehydrogenase*

Pyruvate kinase   **ADP**   **GDP +CO$_2$**   **GTP**   **NAD**

NAD   **NADH**   **ATP**   Malate   Malate

Lactate   Pyruvate   **NAD** *Malate dehydrogenase*

Lactate dehydrogenase   *Pyruvate carboxylase*   **NADH**

Pyruvate   Oxaloacetate

Glutamate *Alanine aminotransferase*   **ATP +CO$_2$**   ADP +P$_i$
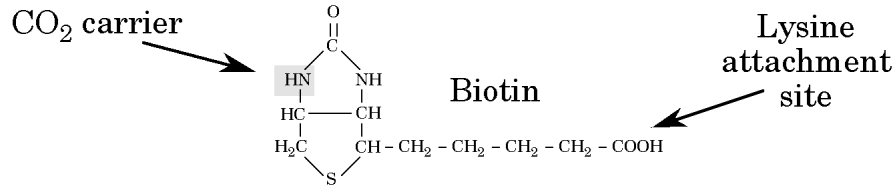
α-ketoglutarate   Alanine

Pyruvate carboxylase

The pyruvate kinase reaction is physiologically irreversible. As a result, under physiological conditions, converting pyruvate to phosphoenolpyruvate requires a short pathway containing two important enzymes: **pyruvate carboxylase** and **phosphoenolpyruvate carboxykinase**. Pyruvate carboxylase is a mitochondrial enzyme that converts pyruvate to oxaloacetate. The pyruvate carboxylase reaction acts as both a mechanism for increasing the amount of TCA cycle intermediates (this function will be discussed further in the context of TCA cycle regulation) and as the first step of gluconeogenesis.

ATP   ADP
+   +
CO_2 P_i

$$CH_3 - \overset{\overset{O}{\|}}{C} - COO^- \xrightarrow{\textit{Pyruvate Carboxylase}} O = C - COO^-$$

Pyruvate   Oxaloacetate
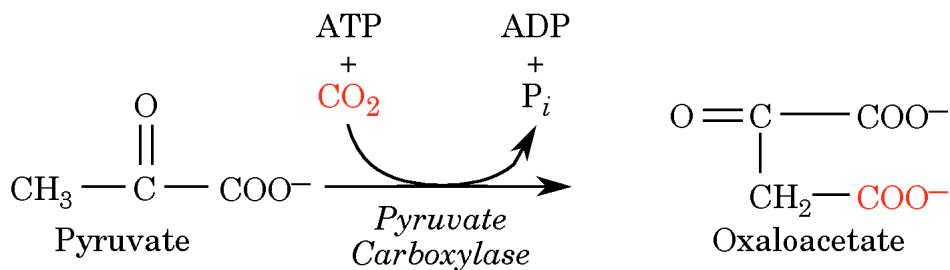
Pyruvate carboxylase is a **biotin-dependent** enzyme; biotin is covalently bound to the amino group of a pyruvate carboxylase lysine side-chain. Pyruvate carboxylase catalyzes formation of a covalent between biotin and carbon dioxide (in the form of

29

carbonate) in an ATP-dependent reaction; this carbonate is then transferred to the pyruvate substrate to produce oxaloacetate. (Because the ATP required for this reaction is derived from catabolism of carbon compounds, the pyruvate carboxylase reaction does **not** involve net carbon fixation.)

CO_2 carrier → Biotin ← Lysine attachment site

O
‖
C
HN   NH
| |
HC────CH
| |
H_2C $CH - CH_2 - CH_2 - CH_2 - CH_2 - COOH$
   \S/

Phosphoenolpyruvate carboxykinase

Phosphoenolpyruvate is an energetic molecule. Its production by phospho-enolpyruvate carboxykinase requires significant energy to form the high-energy phosphate bond in the molecule. The reaction is driven by GTP hydrolysis; in addition, the reaction involves the loss of carbon dioxide, which acts as an entropic driving force.

$$O = C - COO^-$$
$$|$$
$$CH_2 - COO^-$$

GTP → GDP + CO_2

Phosphoenolpyruvate carboxykinase

$$O^-$$
$$|$$
$$O = P - O - C - COO^-$$
$$|\qquad\qquad \|$$
$$O^-\qquad\quad CH_2$$

Oxaloacetate   Phosphoenolpyruvate
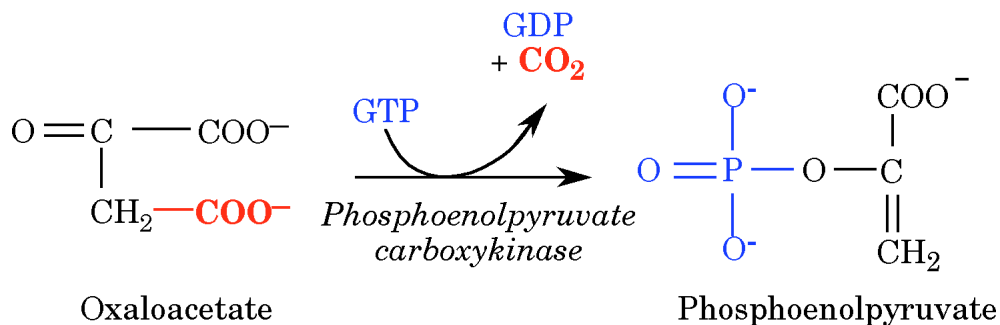
Oxaloacetate cannot leave the mitochondria. This is potentially important, because the majority of the gluconeogenic enzymes are located in the cytoplasm. In order to allow gluconeogenesis to proceed, therefore, oxaloacetate must be converted to a useful molecule that can be transported out of the mitochondria. Two molecules fit this description: **malate** and **phosphoenolpyruvate**.

In some animals (*e.g.*, chickens and rabbits) the formation of phosphoenolpyruvate by phosphoenolpyruvate carboxykinase occurs in the mitochondria. In other animals, such as rats and mice, the phosphoenolpyruvate carboxykinase is located in the cytoplasm. In humans, phosphoenolpyruvate carboxykinase is found both in the mitochondria and in the cytoplasm. Phosphoenolpyruvate produced in the mitochondria can leave via a specific transporter.

The alternative to mitochondrial phosphoenolpyruvate synthesis is to produce malate from oxaloacetate, because malate can be transported out of the mitochondria. In this case, cytoplasmic malate dehydrogenase then reforms the oxaloacetate for conversion to phosphoenolpyruvate. The use of malate in the cytoplasm has advantages, because the conversion of malate to oxaloacetate produces the NADH that will be required for the glyceraldehyde-3-phosphate dehydrogenase step. Thus, the use of malate transport effectively allows the

30

transfer of reducing equivalents from the mitochondrion to the cytoplasm. In humans, the location of phosphoenolpyruvate production is to some extent regulated by availability of NADH in the cytoplasm; if the cytoplasmic NADH level is high, mitochondrial reducing equivalents are unnecessary for gluconeogenesis, and therefore phosphoenolpyruvate is produced in the mitochondria.

(Thought question: different gluconeogenic precursors result in phospho-enolpyruvate production in the different locations. Which precursor(s) would favor the use of mitochondrial phosphoenolpyruvate carboxykinase? Which precursor(s) would favor the use of cytoplasmic phosphoenolpyruvate carboxykinase? Why?)

The same reversible enzymes that are used in glycolysis catalyze the reactions that convert phosphoenolpyruvate to fructose 1,6-bisphosphate. As with glycolysis, the enzymatic reactions at the termini of the pathway supply the ΔG required to determine the **direction** of the carbon flow through the pathway. In gluconeogenesis, these irreversible enzymes are **pyruvate carboxylase** and **phosphoenolpyruvate carboxykinase** at the beginning, and **fructose bisphosphatase** at the end.

Fructose bisphosphatase
Fructose bisphosphatase catalyzes the hydrolysis of fructose 1,6-bisphosphate. This reverses the phosphofructokinase step, except that the result is the release of free inorganic phosphate, rather than reformation of ATP. The difference between the two reactions means that both phosphofructokinase and fructose bisphosphatase catalyze physiologically irreversible processes.

Fructose bisphosphatase is regulated by most of the same regulators as phosphofructokinase. This enzyme is present in many tissues; it allows the formation of glucose-6-phosphate for glycogen synthesis and other purposes.

Glucose-6-phosphatase
Glucose-6-phosphatase is present only in the liver and kidney. It hydrolyzes glucose-6-phosphate to release free glucose. Glucose-6-phosphatase is required for a tissue to be able to release glucose into circulation; therefore, only the liver and (to a much lesser extent) the kidney can contribute to the plasma glucose concentration.

Net reaction of gluconeogenesis
2 Pyruvate + 2 NADH + 4 ATP + 2 GTP $\rightarrow$ glucose + 2 NAD + 4 ADP + 2 GDP + 6 P$_i$

The ATP molecules are used for the pyruvate carboxylase step and the phosphoglycerate kinase step (each must be performed in duplicate). The GTP is used for the phosphoenolpyruvate carboxykinase step. Note that if lactate is used as the starting material, the NADH molecules required are derived from the conversion of lactate to pyruvate.

It is clear from the net reaction that considerably more energy must be put into the process of gluconeogenesis than can be obtained from glycolysis. This raises the question: **why would an organism want to perform gluconeogenesis**?

Put simply, the answer to this question is: all cells can use glucose for metabolism, and some tissues require it (especially the red blood cells and the brain, while many other energy containing molecules are much less readily used. In addition, animals can convert glucose into any carbon-containing molecule they are capable of synthesizing, while other molecules are significantly less flexible.

Substrates for gluconeogenesis

In animals, **acetyl-CoA cannot be used as a substrate for gluconeogenesis**. Therefore, some other molecules must be used to generate glucose. The other molecules used vary depending on conditions.

Cori Cycle

Under conditions in which exertion exceeds the muscle capacity for TCA cycle and electron transport, the muscle produces and exports lactate. The liver does not participate in muscle contraction directly; it can therefore use its gluconeogenic capacity to convert the lactate back to glucose to support further work by the muscle. The exchange of muscle lactate for freshly synthesized glucose is called the Cori cycle after Carl and Gerty Cori (the husband and wife team that discovered the cycle and many features of glucose metabolism).

The muscle has the ability to take up vast quantities of glucose. Because it lacks glucose-6-phosphatase, it cannot release glucose directly. Instead it releases either lactate or alanine, which can be converted to glucose by the liver.
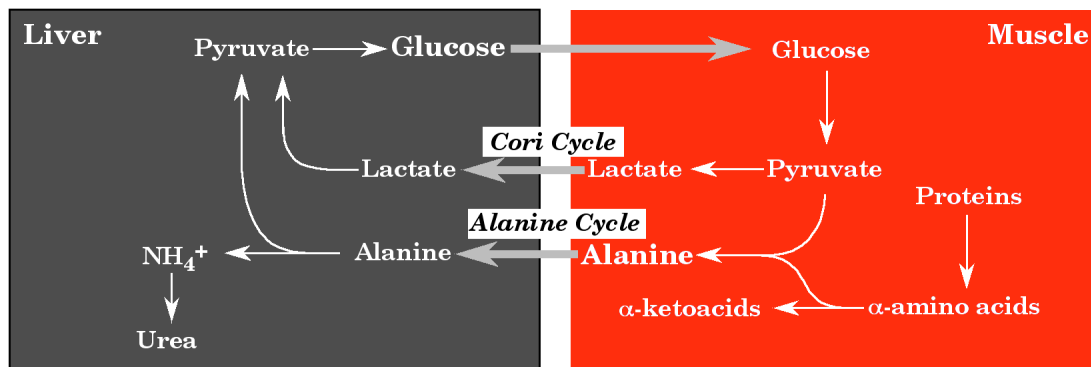
Alanine cycle

During fasting the muscle breaks down proteins. It releases a number of amino acids into circulation, but the primary amino acid released is alanine. Alanine can be converted directly to pyruvate, and used as a substrate for gluconeogenesis. Alanine also acts as a way of transferring nitrogen to the liver for disposal in the form of urea.

Glycerol

Adipose tissue contains triacylglycerol. One component of this fat storage molecule is glycerol. Glycerol can enter the gluconeogenic pathway. Note, however, that only the liver contains glycerol kinase; circulating glycerol tends to be converted to glucose by the liver.

32