Reciprocal regulation is intended to prevent concurrent activity in two closely parallel pathways, as represented by gluconeogenesis and glycolysis. These pathways include potential futile cycles, which would simply waste ATP if allowed to run freely.

The net direction of a reaction is governed solely by its free energy change $\Delta G$. When enzymes catalyze a reaction, both forward and reverse directions speed up by the same factor.

Similarly the action of an inhibitor or activator has the same effect on forward and reverse direction. Therefore it is not possible to govern the overall direction of the parallel pathways by regulating any of the reversible enzymes.

The sites for regulation must lie with the irreversible reactions, which are also the points where the parallel pathways diverge, and offer potential for futile cycles.

### Regulation of pyruvate kinase

Pyruvate kinase is primarily under allosteric regulation (Lehninger p. 556-557).

**Negative effectors:** ATP and acetyl CoA indicate that energy status is being satisfied by other pathways. Alanine indicates that starting substrate for gluconeogenesis is available.

**Positive effector:** Presence of fructose-1,6-bisphosphate indicates that phosphofructokinase 1 is passing substrate into the glycolysis sequence.

**Phosphorylation:** The liver form of pyruvate kinase is a substrate for protein kinase A, and phosphorylation decreases activity. The liver responds to glucagon by releasing glucose into blood, so slows its glucose catabolism. The liver uses fatty acid or amino acids as energy sources.
**Regulation of pyruvate carboxylase**

Acetyl CoA is a positive allosteric effector for pyruvate carboxylase, and the enzyme is virtually inactive in its absence (Lehninger p.731). Presence of acetyl CoA indicates either that there is a need for oxaloacetate to support the TCA cycle, or that the cell has an alternative substrate for energy production that can provide ATP for gluconeogenesis.

**Reciprocal regulation** by acetyl CoA, which inhibits pyruvate kinase, is sufficient to prevent a futile cycle looping through pyruvate kinase and pyruvate carboxylase.

**Regulation of pyruvate dehydrogenase**

Although pyruvate dehydrogenase does not lie in either the glycolysis or gluconeogenesis sequences, its activity could divert pyruvate into the TCA cycle. Pyruvate dehydrogenase is regulated by **product inhibition** as well as by **phosphorylation**, which decrease activity.

**Product inhibition** occurs when high product concentration in the cytoplasm causes product to be retained in the catalytic site, slowing progress of the enzymes' catalytic cycle. Both acetyl CoA and NADH exert product inhibition.

**Phosphorylation** is mediated by a specific **pyruvate dehydrogenase kinase**. Acetyl CoA and ATP stimulate this protein kinase activity, and thereby inhibit the dehydrogenase; ADP inhibits the kinase and therefore promotes dehydrogenase activity (Lehninger p.586-587).

**Phosphoglycerate kinase is unregulated and is not a futile cycle**

Phosphoglycerate kinase transfers phosphate from 1,3-bisphosphoglycerate to ADP in glycolysis. Due to the low concentration of 1,3-bisphosphoglycerate as reactant, $\Delta G$ is near zero even though $\Delta G^\circ = -24.2$ kJ/mol, so the reaction is near equilibrium and easily reversed for gluconeogenesis.
As a reversible reaction, the phosphoglycerate kinase can't be regulated to control the overall direction, since any regulation affects forward and reverse rates in an identical fashion. Nor is phosphoglycerate kinase a potential futile cycle, even though the reaction goes in both directions, because ATP can't be wasted by substrate shuttling back and forth. Even though the reaction 3-phosphoglycerate to 1,3-bisphosphoglycerate consumes ATP, the opposite reaction 1,3-bisphosphoglycerate to 3-phosphoglycerate replaces the ATP, which is an exact reversal.

**Phosphofructokinase 1 and fructose-1,6-bisphosphatase represent the key point of reciprocal regulation**

\[
\text{Fructose-1,6-bisphosphate} \xrightleftharpoons{\text{Fructose-1,6-bisphosphatase}} H_2O \xrightleftharpoons{-} \text{AMP} \xrightleftharpoons{+} \text{Fru-2,6-BP} \xrightleftharpoons{+} \text{citrate} \xrightarrow{-} \text{ADP} \xrightarrow{\text{phosphofructokinase 1}} \text{ATP} \xrightarrow{-} \text{ATP, PEP}
\]

Fructose-6-phosphate

Allosteric regulation of phosphofructokinase 1 (PFK1) was discussed in detail in lecture 7. Negative effectors for PFK1 include ATP, PEP and citrate; positive effectors are AMP (ADP in bacteria) and fructose-2,6-bisphosphate. Of these, **AMP, citrate and fructose-2,6-bisphosphate have the opposite effect on fructose-1,6-bisphosphatase** (FBPase1), and limit the potential futile cycle (Lehninger p.731-732).

**Citrate** indicates that the TCA cycle is provided with substrate, hence energy and oxaloacetate can be spared for gluconeogenesis. **AMP** is an indicator of cellular energy status, while **fructose-2,6-bisphosphate** relays hormonal signals to the PFK1 / FBPase1 pair.

**Control of fructose-2,6-bisphosphate concentration**

\[
\text{Glo-6-P} \xrightleftharpoons{\text{fructose-2,6-bisphosphatase}} \text{Fructose-6-P} \xrightleftharpoons{\text{phosphofructokinase 2}} \text{Fructose-1,6-bisP} \xrightleftharpoons{\text{etc}} \text{etc}
\]

Fructose-2,6-bisphosphate promotes glycolysis and inhibits gluconeogenesis.

Fructose-2,6-bisphosphate does not lie directly on any metabolic pathway, and is not the precursor for any other compound. It is made from fructose-6-phosphate as needed, and returns to fructose-6-phosphate when its need is over.
Fructose-2,6-bisphosphate is made by a special **bifunctional enzyme found in liver and in a slightly different form in muscle**. The N-terminal domain has phosphofructokinase 2 (PFK2) activity, while the C-terminal domain has fructose-2,6-bisphosphatase (FBPase 2) activity. The bifunctional enzyme is a substrate of protein kinase A, and therefore responds to the phosphorylation cascade (Lehninger p.453).

The **liver** enzyme (470 amino acids) is phosphorylated on Ser 32, **which activates FBPase 2 and deactivates PFK2** (Lehninger p.732-733). Glucagon is the hormone that signals low blood glucose, and stimulates the protein kinase A cascade in liver. This causes fructose-2,6-bisphosphate levels to **fall**, and thus promote gluconeogenesis. This drives the liver to export glucose and restore the blood glucose level.

The **muscle** enzyme (530 amino acids) is phosphorylated on Ser 406 and Thr 475, which **inhibits FBPase 2 and activates PFK2**. Muscles respond to epinephrine by activating the protein kinase A phosphorylation cascade. This causes fructose-2,6-bisphosphate levels to **rise**, promoting glycolysis in the muscle.

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**Glucose secretion prevents a futile cycle at the hexokinase/glucose-6-phosphatase step**

Glucose produced by the liver is quickly secreted, and this breaks the circuit that might give rise to a futile cycle.

**Hexokinase is regulated to control glucose distribution**

Hexokinase exists in two major isozymes in animals. Isozymes are enzymes of similar catalytic function but differing in other properties (Lehninger p.555).

The major hexokinase is strongly inhibited by its product, glucose-6-phosphate. Thus if cell metabolism is not consuming the glucose-6-phosphate, the reaction is halted. Glucokinase or hexokinase IV is found in liver. This isozyme is not subject to product inhibition, but has an unusually high $K_m$ for glucose (> 10 mM). This is higher than normal blood glucose levels, so the rate of conversion to glucose-6-phosphate is normally low in liver. After a glucose rich meal however, glucokinase allows excess glucose to be consumed in the liver to bring the blood glucose level down to normal.