Metabolic Control and Integration of Metabolism

Introduction

There are three reasons why control is necessary in metabolic pathways:

1. To avoid substrate (“futile”) cycles.
   For example, the constant interconversion of fructose 6-phosphate to fructose 1,6-bisphosphate and vice versa, which consumes ATP.

   The “useless flow” of metabolites through such cycles is useful in that it allows rapid increases in flux through a given pathway. By slightly altering the rate of either of the two enzymes, huge variations in the net flux can be obtained, if there was originally a large futile flux through both the enzymes. [For example, the flux down the glycolytic pathway has been suggested to increase as much as 1000-fold at the initiation of intense exercise. Allosteric activation of enzymes alone seems unlikely to explain this increased flux!] These cycles can also be useful in generating heat from the hydrolysis of ATP. For example, bumblebees must keep a thoracic temperature of about 30° C to be able to fly. It turns out that their bisphosphatase is not inhibited by AMP, which seems to suggest it evolved for heat production.

2. To link energy production to energy usage.

3. To respond to physiological changes

In controlling catabolic vs. metabolic pathways, effective control can only happen at irreversible steps. At these steps, the forwards and backwards reactions are catalysed by distinct enzymes, which means that the activity of one can be increased while that of the other is decreased. In totally reversible steps, changing the activity of the enzyme changes the rate of the forwards and backwards steps equally.

Enzyme activity is controlled in two ways:
• The amount of enzyme can be changed by tweaking its rate of synthesis or rate of destruction.

In mammals, however, this is a fairly slow process, and \( t_{\frac{1}{2}} \) can range from hours to days. Thus, it tends to occur as a result of long term changes. For example:
  o An increase in the lipoprotein lipase in lactating mammary glands.
  o Changes in liver enzymes during the shift from the fed state to starvation.
  o Increases in drug-metabolising enzymes following the intake of foreign compounds.

In bacteria, however, induction of enzyme can be rapid. For example, when E. coli is exposed to lactose, the induction of \( \beta \)-galactosidase occurs rapidly (genes can quickly be activated and deactivated).

• Metabolic control of the enzyme is a much more rapid response. It is often the case that the product of a pathway inhibits the committed step (the first non-reversible step) of the pathway. This prevents intermediates accumulating, and preserves the reagent of the cycle.

Enzyme reaction rates can be controlled in two ways:
  o Allosteric regulation is particularly important in metabolic regulation. The binding of an allosteric effector changes the affinity of the enzyme of its substrate(s). The effect can be positive (increase in affinity) or negative (decrease in affinity), and is very fast.
Covalent modification most commonly involves phosphorylation by protein kinases. This causes a conformational change in the protein. [Less important in prokaryotes]. One of the main reasons this is an important form of enzyme control is because it is usually the final step in a cascade of reactions, that are triggered by a very low concentration of a signal molecule.

This provides two levels of control within the cell:

- Allosteric regulation is very rapid, and signals are usually intracellular.
- Phosphorylation is usually regulated by extracellular agents (eg: hormones). For example, glucagon, insulin and adrenaline are particularly important in controlling fat and carbohydrate metabolism. Glucagon and insulin are produced in response to low and high glucose levels, and adrenaline is released from the adrenal gland and stimulates the release of food reserves.

Other ways to control metabolic pathways (not in lecture notes) are:

- Compartmentation.
- Specialisation of organs.

### Metabolic Control in Glycolysis

The control of glycolysis will vary according to the type of tissue:

- Muscle uses glycolysis to generate ATP.
- The liver produces nearly no energy, but it is a net producer of glucose in the fasted state, and synthesises triglycerides and glycogen in the fed state.

The metabolic control can be considered in key stages.
Transport of Glucose into the Cell

Different transporters mediate the thermodynamically downhill movement of glucose across the plasma membrane of animal cells. Different members of the family have distinctive roles:

- GLUT1 and GLUT3 are present in nearly all mammalian cells, and are responsible for basal glucose uptake. Their $K_M$ value for glucose is 1 mM, significantly less than the normal serum-glucose level. Hence, these transport glucose into cells are a roughly constant rate.

- GLUT2 is present in liver and pancreatic β cells is insulin independent but has a very high $K_M$ value (about 15-20 mM). Thus, glucose enters these tissues only when there is much glucose in the blood. Thus, the pancreas can sense the glucose level, and liver cells only utilise glucose to produce triglycerides and glycogen when it is abundant.

- GLUT4, which has a $K_M$ of 5 mM transports glucose into muscle and fat (adipocyte) cells. However, in the absence of insulin, these transporters are trapped inside intracellular vesicles. Insulin recruits these vesicles to the cell membrane, allowing the transport of glucose.

- [GLUT5, in the small intestine, functions primarily as a fructose transporter].

Control at Phosphofructokinase

This is the most important control site in the mammalian glycolytic pathway. The reason for this is that it is the committed step in glycolysis, despite the fact that it is not the first reaction. The reaction catalysed by hexokinase does not have this status simply because glucose 6-phosphate is not only a glycolytic intermediate – it can also be converted to glycogen and used in the OPPP.

- This enzyme is inhibited as the energy charge ($ATP/AMP$ ratio) rises:
- ATP is both a substrate and an allosteric inhibitor of phosphofructokinase-1. ATP binds to a specific regulatory site that is distinct from the catalytic site.
- AMP, however, reverses the inhibitory action. This is perfect, because in muscle cells, ATP is primarily needed to power contraction, a process which requires a high energy charge.

[Note that it is AMP and not ADP which is the positive regulator of the enzyme. This is first of all because adenylate kinase catalyses the following reaction in muscles, to salvage some ATP back:

$$\text{ADP} + \text{ADP} \rightleftharpoons \text{ATP} + \text{AMP}$$

Furthermore, the use of AMP provides very sensitive control. This is because the concentration of the adenylate pool stays roughly constant over time. The concentration of ADP, however, is much higher than the concentration of AMP. Thus, a small percentage change in [ATP] results in a much larger percentage change in [AMP] than it does in [ADP].]

- Low pH also enhances the inhibitory effect of ATP in muscular phosphofructokinase. Such acidic environments could be produced by high concentrations of lactate. In such conditions, muscle must halt glycolysis to prevent damage to muscle tissue. In the liver, lactate is rarely formed.
- Citrate enhances the inhibitory effect of ATP in liver phosphofructokinase. This is, again, ideal, because high [citrate] signifies that the citric acid cycle is “filling up”, and that no further glycolytic products are required for biosynthesis.

**Control at Hexokinase**

Hexokinase has a very low $K_M$ indeed (about 0.1 mM) and so can operate well under low glucose conditions. However, it is inhibited by its product, glucose-6-phosphate. It signals that the cell no longer needs
G6P for biosynthesis or energy. G6P is the way phosphofructokinase communicates with hexokinase.

The liver, however, also possesses an isozyme of hexokinase, called glucokinase, which has two key differences:

- It is not inhibited by glucose-6-phosphate. Thus, it can provide glucose-6-phosphate for the synthesis of glycogen and fatty acids, two of the major roles of the liver, even if no energy is needed.
- The $K_m$ value of glucokinase is very high (10 mM). This ensures that the brain and muscle have first call on glucose when it is sparse. [The liver, for example, will not re-absorb the low concentrations of glucose that it releases during fasting!]

**Control at Pyruvate Kinase**

This enzyme catalyses the last irreversible reaction of glycolysis.

- ATP allosterically inhibits the reaction to slow it when energy charge is high.
- Alanine (synthesised in one step from pyruvate) also inhibits pyruvate kinase. In this case, to signal that building blocks are abundant.
- Fructose 1,6-bisphosphate (the product of the previous irreversibly reaction) activates this enzyme, to make sure it is able to keep pace with the oncoming high flux of intermediates. This is an example of “feedforward stimulation”.
- In the liver, the L (as opposed to the M) isoenzyme is present, which can be phosphorylated and therefore made less active. This occurs as a result of the cyclic AMP cascade triggered by glucagon (which is released when blood glucose levels are low).

**Glycolysis and Gluconeogenesis**

Glycolysis and gluconeogenesis are reciprocally regulated. Energy charge will determine which is most active at two points (these, of course, are the
irreversible points in the pathways, which therefore have substrate cycles associated with them.

**Reciprocal control at Pyruvate Kinase**

We mentioned above that pyruvate kinase is activated by F-1,6-BP and alanine. It turns out that pyruvate carboxylase, one of the enzymes catalysing the opposite reaction, is inhibited by ADP and activated by Acetyl CoA.

The presence of cAMP (as a result of glucagon or adrenaline) also causes this enzyme to be phosphorylated, which inhibits its activity.

**Reciprocal control at Phosphofructokinase**

The enzyme that catalyses the opposite reaction is fructose-1,6-bisphosphatase. As expected, it turns out that this enzyme is inhibited by AMP and activated by citrate.

There is another factor, however, which we have not yet taken into account; that is the regulation through fructose-2,6-bisphosphate. This molecule is synthesised from fructose 6-phosphate:

\[
\text{Fructose 6 – phosphate} \xrightarrow{\text{phosphofructokinase 2 (PFK2)}} \text{fructose bisphosphatase 2 (FBPase2)} \text{Fructose 2,6 – bisphosphatase}
\]

This molecule activates phosphofructokinase (and therefore glycolysis) and inhibits fructose 1,6-bisphosphate (and therefore gluconeogenesis).

Several factors control the synthesis and breakdown of this molecule:

- Large quantities of fructose 6-phosphate *increase* the rate of production of fructose 2,6-bisphosphate (since fructose 6-phosphate is the substrate of the synthesis, AND since it stimulates phosphoprotein phosphatase which de-phosphorylates the double-headed enzyme – see below) This is another example of feedforward stimulation.
• The **blood glucose level** has an effect on the production of **fructose 2,6-bisphosphate** in the liver. **Low blood glucose level** causes the release of the **hormone glucagon** into the blood, which results in a **cAMP cascade**. This **activates FBPase2** and **inhibits PFK2**, **decreases** the concentration of **fructose 2,6-bisphosphate** in the liver and **increases the rate of gluconeogenesis**. The same occurs **vice-versa**. [Note that **adrenaline** has a **similar effect** on the liver].

Interestingly, **both these enzymes** are on a **single polypeptide chain**! Which part of the chain is active at one point depends on the **phosphorylation** of a **single serine residue**. When the residue is **phosphorylated**, **FBPase2 is activated** and **PFK2 is inhibited**, and vice versa. The addition and removal of the phosphate group is catalysed by **protein kinase A** (addition) and **phosphoprotein phosphatase** (removal).

Note also that a **substrate cycle** exists at this point, and so **small changes** in both the enzymes caused by one of the factors described above can cause **large changes** in the **flux**. In **muscle**, this cycle is controlled by **AMP**.

**Glycogen Synthesis**

A few points worthy of attention:

- **Glycogen synthase** is **activated** by **insulin** and **inhibited** by **cAMP** (as a result of **glucagon** or **adrenaline**).
- **Glycogen phosphorylase** is **activated** by **cAMP** and **AMP**, but **inhibited** by **ATP**.

**The Citric Acid Cycle**

The **citric acid cycle** is central in many ways, and is controlled at several points:
Respiratory Control

First, the **tight coupling** of the citric acid cycle and the electron transport chain is an **important control mechanism** (see TCA cycle notes).

Pyruvate Dehydrogenase

Pyruvate dehydrogenase is the **point of no return** for glucose derived carbon, because Acetyl CoA cannot be turned back to glucose. This conversion essential commits the carbon to two principle fates – oxidation to CO₂ and incorporation into lipid.

This critical enzyme is stringently controlled:

- **Acetyl CoA inhibits** the transacetylase (E₂) component of the enzyme by direction binding.
- **NADH inhibits** the dihydropropyl dehydrogenase (E₃) component.

These two conditions inform the cell that the energy needs of the cell have been met, or that the cell is degrading fatty acids to produce NADH and Acetyl CoA. In this case, glucose can be spared. [Note that it is the energy charge that is important here].

The **key means of regulation**, however, is covalent modification. PDH kinase and PDH phosphatase catalysed the phosphorylation and dephosphorylation respectively of the pyruvate dehydrogenase (E₁) component of the complex. These are associated to the complex.

- The kinase is **inhibited** by pyruvate, CoA, NAD⁺, but activated by acetyl CoA, NADH and ATP (the immediate and eventual products of this enzyme’s action).
- The phosphatase is **activated** by Ca²⁺ (the signal that gives rise to muscle contraction). Similarly, insulin (= fed state) stimulates this enzyme in tissues capable of fat synthesis (eg: adipocytes), since they synthesise lipids from Acetyl CoA.
Citrate Synthase

This enzyme is allosterically inhibited by ATP (ATP increases $K_M$ of the enzyme for Acetyl CoA). This is important for gluconeogenesis during starvation; if the energy charge is high, then oxaloacetate can be used for gluconeogenesis, and Acetyl CoA can be used to generate ketone bodies. Both of these pathways can be used to fuel the brain (the latter only in an emergency).

Isocitrate Dehydrogenase

This is inhibited by ATP and NADH (which displaces NAD$^+$), and activated by ADP and NAD$^+$. [A great illustration of control – if this is inhibited, citrate then builds up and travels to the cytoplasm where it inhibits glycolysis and can act as a source of Acetyl CoA for fatty acid synthesis.]

$\alpha$-ketoglutarate dehydrogenase

This is inhibited by high energy charge and by its products – succinyl-CoA and NADH, but activated by Ca$^{2+}$.

Metabolic Control Analysis

Often, the analysis of metabolic enzymes separately cannot generate a complete picture of how the overall system is controlled. For example, increasing the amount of enzyme for ethanol production in yeast do not increase the amount of ethanol produced, but increasing the need for ATP does.

Metabolic control analysis attempts to predict the effect of varying one or more of the enzymes of a pathway. The concept of demand control is often used.
For example, if flow is through three enzymes, the first and that last of which catalyse reactions near equilibrium whereas the middle one catalyses the forwards reaction very efficiently, then it is the concentration of the middle enzyme that will have the potential to control flux in the pathway, even if flux through that enzyme is much less. In other words, it has the largest flux control coefficient, which is defined as the rate of fractional changes in flux with respect to the fractional changes in enzyme concentration (ranges from 0 – no effect on the flux, to 1 – all the effect on the flux): 

\[
\frac{\partial J}{\partial [E]} \frac{[J]}{[E]}
\]

The sum of all the flux control coefficients involved in controlling a pathway must be equal to 1. This is the additivity theorem of metabolic control.

For glycolysis, it turns out that even though PFK-1 is a “key control enzyme”, the rate of lactate production is not simply controlled by this enzyme because of the influence of others. Feedback inhibition may operate, and metabolites may flow into other pathways. MCA demonstrates that a large number of parameters affect the rate of lactate production and there is no rate determining step!

When acetate is the only carbons source of the cell, citrate synthase has a flux control coefficient close to 1. It seems to have less of an impact when glucose is the energy source.

**Three major intermediates**

**Glucose 6-phosphate**

As soon as glucose enters into the cell, it is converted to glucose 6-phosphate. This prevents its exit across the cell membrane. Note that that entry of glucose 6-phosphate into the glycolytic pathway can be both catabolic (when this is done for energy) or anabolic (when this is done to provide carbon skeletons).
Pyruvate and Acetyl CoA

A few notes:

- The conversion to lactate regenerates redox balance and basically buys time, and shifts the metabolic burden to other organs.
- The conversion of pyruvate to alanine is a transamination, and several other amino acids can be transaminated to pyruvate. Thus, transamination is a major link between amino acid and carbohydrate metabolism.