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**.

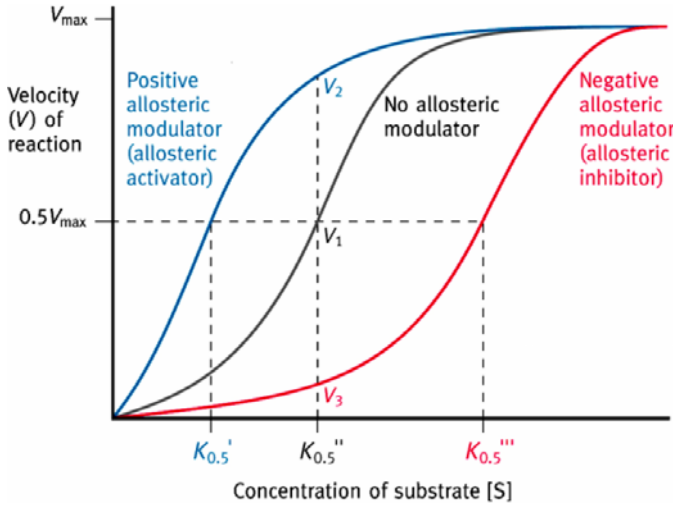
  In **mammals**, however, this is a fairly **slow** process, and $t_{½}$ can range from **hours** to **days**. Thus, it tends to occur as a result of **long term changes**. For example:

  - An **increase** in the **lipoprotein lipase** in **lactating mammary glands**.
  - Changes **in liver enzymes** during the shift from the **fed state** to **starvation**.
  - **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 **β-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**:

  - **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 **adrenalin**e 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**:

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

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**:

$$\text{Fructose } 6-\text{phosphate} \underset{\text{fructose bisphosphatase 2 (FBPase2)}}{\overset{\text{phosphofructokinase 2 (PFK2)}}{\rightleftharpoons}} \text{Fructose } 2{,}6-\text{bisphosphate}$$

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 **phsophorylated**, **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 CO_2** and **incorporation into lipid**.

This **critical enzyme** is **stringently controlled**:

- **Acetyl CoA** ***inhibits*** the **transacetylase (E_2)** compoenent of the enzyme by **direction binding**.
- **NADH** ***inhibits*** the **dihydropropyl dehydrogenase (E_3)** 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 **pyruvaste dehydrogenase** (E_1) compoenent 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 **Ca^{2+}** (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**.]

α-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):

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

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**.

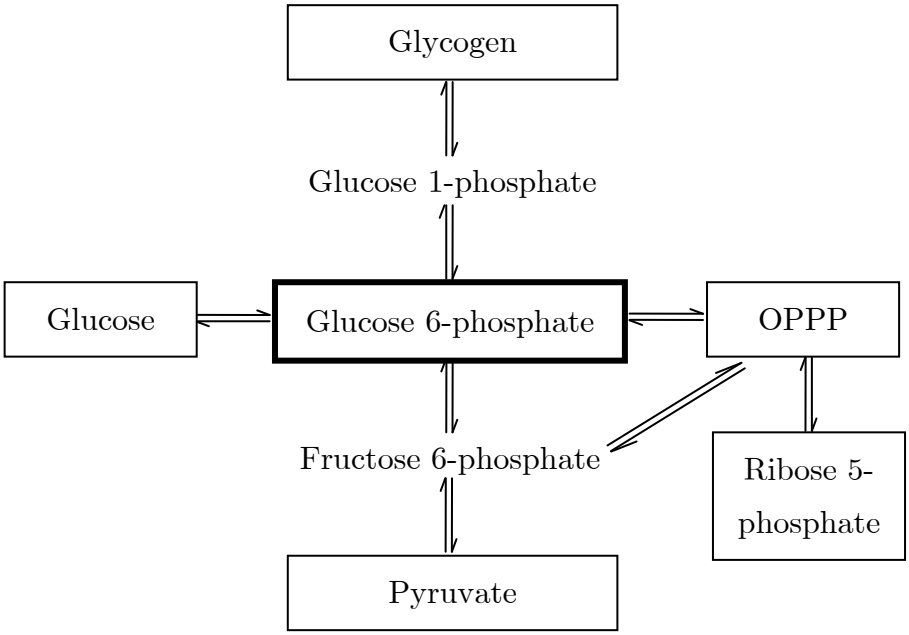
For **glycolysis**, it turns out that even though **PFK-1** is a **"key control enzyme"**, the **rate of lactate production** is ***not*** simply controlled by this enzyme because of the influence of others. **Feedback inhibition** may operate, and **metabolites** may **flow into other pathways**. **MCA** 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**.

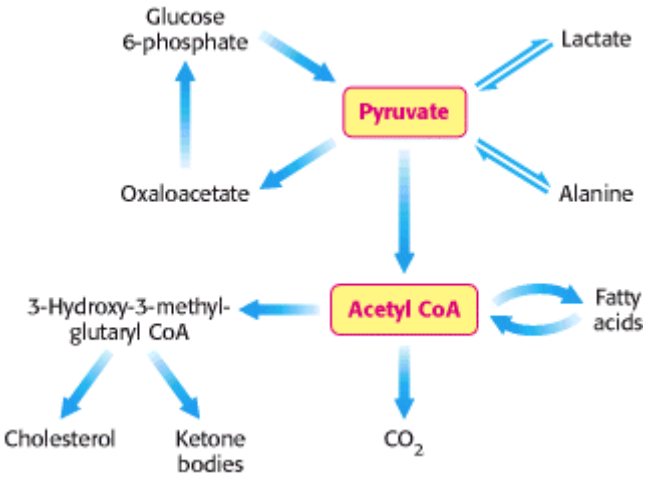
Three major intermediates

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 **lacatate** 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**.