Metabolism

Graeme K. Ambler

June 14, 2006

This is an attempt to distill the biochemistry handouts into an order that I find more logical. It is also cross-linked in an attempt to show the connections between the different parts of the subject. It is not intended to totally replace the notes, as the diagrams in the notes are really helpful and it would be a waste of time to simply copy them here.

1 Insulin

Insulin does many things in the body. In general, it promotes activities associated with the **fed state** and suppresses activities associated with the **fasting state**.

1. In myocytes:
    - Increases glucose uptake
    - Increases glycogenesis
    - Increases protein synthesis
    - Decreases proteolysis
2. In adipocytes
    - Increases glucose uptake
    - Increases lipogenesis
    - Decreases lipolysis
    - Increases protein synthesis
3. In hepatocytes:
    - Increases glycogenesis
    - Increases lipogenesis
    - Decreases gluconeogenesis
    - Decreases glycogenolysis
4. In the central nervous system (CNS):

- Suppresses appetite (evidence?)
- Promotes fertility (gonadal maturation) (evidence?)

5. In pancreatic β-cells:
   - Promotes insulin synthesis and secretion (evidence?)
   - Promotes proliferation (evidence?)

What insulin does *not* do is possibly as important as what it *does*. Namely it does *not* promote glucose uptake in hepatocytes or the CNS (or red blood cells). Nor does it promote glucose metabolism in the CNS.

Each of the topics mentioned briefly above will be covered more deeply later on in these notes.

1.1 Insulin synthesis and secretion

Insulin is synthesised in pancreatic β-cells from a precursor called **proinsulin**. Proinsulin is directed to vesicles, where it is cleaved (cut up!) to make insulin. It is secreted in response to glucose levels above 5.0mM. The insulin-containing vesicles in the pancreatic β-cells fuse with the cell membrane, releasing the insulin in a process called exocytosis. Insulin is not secreted as a direct consequence of the glucose level. Instead, the enzyme **glucokinase** acts as a glucose sensor by beginning glycolysis, which leads to generation of **ATP**. ATP in turn regulates the activity of ion channels in the β-cell membrane. It is the **ionic environment** which regulates insulin release.

1.1.1 Summary

Proinsulin $\rightarrow$ vesicles $\rightarrow$ insulin.

High glucose concentration $\Rightarrow$ high ATP concentration $\Rightarrow$ favourable ionic conditions $\Rightarrow$ insulin secretion (exocytosis).

1.2 How does insulin work?

First of all, insulin binds to receptors on the surface of cells which are highly specific, and also bind the insulin really tightly. These receptors belong to a class called **protein tyrosine kinases**, because in their active state (in this case when insulin is bound) they cause phosphorylation (hence kinase) of tyrosine residues of other proteins. This phosphorylation triggers switch mechanisms in membrane lipids and membrane-bound proteins, causing cascades of **protein serine kinases** (you can guess what they do), which bring about the actions of insulin in these cells (more than just glucose transport, see later). It is important to know this outline pathway because insulin has many effects on myocytes and adipocytes, but all come about from this sequence of events.

1.2.1 Summary

Insulin binds $\Rightarrow$ Protein tyrosine kinase activated $\Rightarrow$ Switch mechanisms $\Rightarrow$ Protein serine kinase cascade.

1.3 Glucose uptake in myocytes and adipocytes

Protein serine kinases activated by the insulin pathway discussed above stimulate the uptake of glucose in myocytes and adipocytes by increasing the number of **GLUT4 glucose transporters** in the plasma membrane. GLUT4 is present in these cells in the membranes of intracellular vesicles. Activation of the aforementioned protein serine kinases causes these vesicles to move to the cell membrane and fuse with it, exposing the GLUT4 transporters to the outside of the cell. GLUT4 is a large polypeptide which spans the membrane 12 times in such a way as to create a large pore through which glucose passes through **facilitated diffusion**.

1.3.1 Summary

1. GLUT4 in membrane of intracellular vesicles
2. Protein serine kinase activated by insulin pathway
3. GLUT4 vesicles fuse with plasma membrane
4. Glucose diffuses into cell.

2 Glycolysis

2.1 Process

Glycolysis is the backbone of cellular metabolism. Once glycolysis is understood, all of the other major parts of metabolism can be tacked on in one place or another. The first part of glycolysis (often called the *energy investment* phase) is as follows:

$$\text{Glucose} \xrightarrow[\text{hexokinase}^0]{\text{ATP}\rightarrow\text{ADP}} \text{G6P} \underset{\text{isomerase}}{\rightleftharpoons} \text{F6P} \xrightarrow[\text{PFK}]{\text{ATP}\rightarrow\text{ADP}} \text{F1,6}P_2 \underset{\text{aldolase}}{\rightleftharpoons} \text{GAP} + \text{GP}$$

The full names of the molecules at each of the steps above are glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), phosphofructokinase (PFK), fructose 1,6 bisphosphate (F1,6P_2), glyceraldehyde-3-phosphate (GAP) and glycerone phosphate (GP). At this point we have split a six carbon molecule into two three carbon molecules, with a phosphate on each. The first four steps are thus.

1. Phosphorylate.
2. Rearrange.
3. Phosphorylate.
4. Split.

[0]There are several isoenzymes of hexokinase. One important version is glucokinase, which we met when discussing insulin secretion.

The names of the enzymes which perform these steps make a lot of sense when you think about them. Steps one and three are both catalyzed by **kinases**, as they are both phosphorylation steps. Step two is a rearrangement from one isomer to another, so the enzyme is an isomerase. The purpose (from the point of view of glycolysis) of step four is to create GAP, which is an aldehyde, so it is performed by **aldolase**. The reason that GAP is the important molecule rather than GP is that it is GAP which goes on to complete glycolysis. GAP and GP are isomers of each other, and it should not surprise you to discover that there is a further isomerase which keeps these two substances in equilibrium with each other so that if GAP is broken down, thus dropping the concentration of GAP, the isomeration will favour conversion of GP to GAP, so that in the end both "halves" of glucose are used up[1].

The remainder of glycolysis goes as follows.

$$\mathrm{GAP} + P_i \xrightarrow{\mathrm{NAD}\rightarrow\mathrm{NADH}} \mathrm{1{,}3BPG} \xrightarrow{\mathrm{ADP}\rightarrow\mathrm{ATP}} \mathrm{3PGA} \rightleftharpoons \mathrm{2PGA} \xrightarrow[\text{enolase}]{} \mathrm{PEP} \xrightarrow[\text{pyruvate kinase}]{\mathrm{ADP}\rightarrow\mathrm{ATP}} \text{pyruvate}$$

The full names of each of the molecules above are glyceraldehyde-3-phosphate (GAP), 1,3-bisphosphoglycetate (1,3BPG), 3-phosphoglycerate (3PGA), 2-phosphoglycerate (2PGA) and phosphoenolpyruvate (PEP). The only named enzymes here are enolase and pyruvate kinase. Again, these names make sense, as enolase converts a ketone (2PGA) to an enol (PEP); and pyruvate kinase phosphorylates ADP to make ATP. NAD/NADH is a coenzyme.

2.2 Regulation

There are three key points of regulation for glycolysis: hexokinase, phosphofructokinase and pyruvate kinase. I will deal with these in reverse, since it is easiest to see their combined action that way.

2.2.1 Regulation of pyruvate kinase

Pyruvate kinase is deactivated by phosphorylation by PKA in response to glucagon, using cyclic-AMP (cAMP) as a second messenger. This makes sense, as glucagon is secreted when blood glucose levels fall below about 5mM as it is not sensible to break down glucose when it is in short supply. In addition, it is allosterically inhibited by alanine. We will see the importance of this inhibition when we discuss gluconeogenesis.

2.2.2 Regulation of phosphofructokinase

Phosphofructokinase (PFK) is allosterically inhibited by ATP and citrate, and allosterically activated by ADP, AMP and fructose 2,6-bisphosphate[2] (F2,6P_2). We will begin by discussing the roles of the nucleotides.

[1] Alternatively, GP can be converted to glycerol if there is demand for triacylglycerol synthesis.

[2] Fructose 2,6-bisphosphate, an *allosteric regulator* of PFK, should not be confused with Fructose 1,6-bisphosphate, the *product* of PFK.

High concentrations of ATP imply that the cell's energy demands are being met, thus it makes sense that high ATP should inhibit glycolysis. Conversely, high levels of AMP and ADP imply that the cell requires energy, so again it makes sense that these should promote glycolysis. PFK is a tetramer with two distinct stable three dimensional conformations, called T and R. In the T state, PFK binds ATP at both its substrate and inhibitor sites, while in the R state only the substrate site is available. Thus ATP stabilises the T state. AMP and ADP on the other hand will only bind PFK in the R state, thus stabilising the R state. It should now be easy to see that if F6P binds to PFK in only the R state, that the inhibition/activation actions of the different nucleotides can be accounted for. This is indeed the case. In the R state, the active site of PFK contains a positively charged arginine residue, thus attracting its negatively charged substrate F6P. In the T state, however, the active site instead contains a negatively charged glutamate residue, thus repelling F6P. The three adenine nucleotides are kept in equilibrium by the enzyme adenylate kinase:

$$\mathrm{ADP} + \mathrm{ADP} \underset{\text{adenylate kinase}}{\rightleftharpoons} \mathrm{ATP} + \mathrm{AMP}.$$

The allosteric inhibition of citrate will become clear when we discuss the citric acid cycle. Essentially, it keeps the rates of glycolysis and the citric acid cycle in step, preventing a build-up of pyruvate.

Discussion of allosteric activation by F2,6P_2 is postponed until the section on gluconeogenesis control, except to say that this is another way in which glucagon exerts control over glycolysis.

2.2.3 Regulation of hexokinase

Given available glucose, decrease in the rate of PFK will naturally lead to an increase in the concentration of F6P, as it will not be converted into F1,6P_2. Since the conversion of G6P to F6P is reversible, this in turn will lead to an increase in G6P concentration. Hexokinase is regulated both competitively and allosterically by G6P, thus a build up of G6P caused by inhibition of PFK will in turn cause inhibition of hexokinase. Conversely, if PFK is working quickly, any G6P made from glucose will quickly be broken down, thus hexokinase will work at its maximal rate.

We mentioned previously that glucokinase was an isoenzyme of hexokinase. It is important to note at this point that one of the distinctive features of glucokinase as opposed to other hexokinases is that it is *not* allosterically inhibited by G6P. In addition, it has a far lower glucose affinity, reaching half $V_{\max}$ at about 5mM. Glucokinase is the isoenzyme of hexokinase found in hepatocytes and pancreatic β-cells.

3 Gluconeogenesis

3.1 Process

Gluconeogenesis is the opposite of glycolysis: it is the formation of glucose from pyruvate. Most of the steps of glycolysis are freely reversible and controlled solely by the concentration of reactants and products. However there are three steps which are not freely

reversible: pyruvate to PEP, F1,6P_2 to F6P and G6P to glucose. These are reasonably easy to remember for two reasons.

1. They are the three steps used to regulate glycolysis.

2. They are three out of the four steps of glycolysis which involve ATP.

The last two reactions are exergonic and simply catalyzed hydrolysis reactions:

$$\mathrm{G6P} + \mathrm{H_2O} \xrightarrow[\text{G6Pase}]{} \mathrm{Glucose} + P_i,$$

and

$$\mathrm{F1,6}P_2 + \mathrm{H_2O} \xrightarrow[\text{FBPase}]{} \mathrm{F6P} + P_i.$$

Getting from pyruvate to PEP is a little more complicated. Firstly, pyruvate is transported into the mitochondrion in exchange for hydroxide ions (OH^-), an example of the proton motive force in action. Pyruvate is then carboxylated in the mitochondrion to give oxaloacetate. This requires biotin as a coenzyme to carry CO_2.

$$\mathrm{pyruvate} + \mathrm{CO_2} + \mathrm{ATP} \xrightarrow[\text{pyruvate carboxylase}]{\text{biotin prosthetic group}} \mathrm{oxaloacetate} + \mathrm{ADP} + P_i.$$

The oxaloacetate is converted to malate, which is transported out of the mitochondrion. The malate is then converted back to oxaloacetate, which loses CO_2 again and acquires phosphate from GTP to form PEP.

$$\mathrm{oxaloacetate} + \mathrm{GTP} \xrightarrow[\text{PEP carboxylase}]{} \mathrm{PEP} + \mathrm{GDP} + \mathrm{CO_2}.$$

There are several sources for gluconeogenesis. Lactate and amino acids such as alanine can be converted to pyruvate. In addition, glycerol can be converted to glycerone phosphate (GP) and glycogen can be converted to glucose-6-phosphate.

3.2 Regulation

Control of gluconeogenesis and glycolysis go hand in hand. The inhibition of pyruvate kinase by glucagon and alanine could easily be seen as mechanisms for activating gluconeogenesis, and perhaps make more sense in that context.

One of the most important steps in the control of gluconeogenesis is the regulation of conversion between F1,6P_2 and F6P by phosphofructokinase (PFK) and fructose bisphosphatase (FBPase). This was alluded to in the section on PFK regulation where we said that an important activator of PFK is fructose 2,6-bisphosphate (F2,6P_2). F2,6P_2 is converted from F6P by the action of PFK2 and broken down to F6P by the action of FBPase2. Thus F2,6P_2 is a measure of F6P concentration. PFK2/FBPase2 is actually a single dual-function enzyme of which there are at least three different isoenzymes. The version present in the liver is regulated by phosphorylation of a certain serine residue. In the phosphorylated state the action of PFK2 is inhibited, while FBPase2 is activated.

Glucagon, again acting via its usual second messenger cAMP, activates protein kinase A, which phosphorylates PFK2/FBPase2. This lowers the concentration of F2,6P_2, which has two complementary actions. First of all, as mentioned above, F2,6P_2 is an allosteric activator of PFK. Thus lowering the concentration of F2,6P_2 will reduce the activity of PFK, and thus favour gluconeogenesis over glycolysis. In addition, F2,6P_2 is also an allosteric *inhibitor* of FBPase, so reducing its concentration will also increase the activity of FBPase and thus further promote gluconeogenesis. This makes sense, as glucagon is secreted when blood glucose concentration falls below about 5.0mM.

The isoenzyme present in the heart is also regulated by phosphorylation, but in contrast with the version found in the liver, phosphorylation *activates* PFK2 and *inhibits* FBPase2. The pathway for phosphorylation is controlled by adrenaline in this case, but the other details are very similar. Thus adrenaline promotes glycolysis and inhibits gluconeogenesis in the heart.

A third isoenzyme is present in skeletal muscle. This version is not regulated by phosphorylation, and is thus not under hormonal control. Thus F2,6P_2 acts purely as a sensor of F6P concentration in skeletal muscle.

As we will see when we discuss regulation of glycogen metabolism, glucagon and adrenaline both promote glycogenolysis as well. Thus in the liver glycogenolysis is coupled to gluconeogenesis, while in the heart it is coupled to glycolysis. This arrangement makes sense when the functions of these different organs are considered.

One final regulator of gluconeogenesis is acetyl-CoA. We will postpone discussion of this regulatory step until we discuss the citric acid cycle below and only mention that it activates pyruvate carboxylase and inhibits pyruvate dehydrogenase, an enzyme involved in syphoning off pyruvate into another metabolic pathway.

4 Glycogen metabolism

4.1 Glycogenolysis

Glycogenolysis feeds into glycolysis/gluconeogenesis at the G6P stage. Breakdown of glycogen to G6P is achieved in two steps as follows.

$$\text{Glycogen}_n + P_i \xrightarrow[\text{Glycogen phosphorylase}]{} \text{Glycogen}_{n-1} + \text{G1P},$$

and

$$\text{G1P} \underset{\text{isomerase}}{\rightleftharpoons} \text{G6P}.$$

Glycogen phosphorylase uses pyridoxal-5′-phosphate (PLP), derived from vitamin B_6 (pyridoxine), as a coenzyme. Note: Glycogenolysis requires inorganic phosphate *not* ATP.

4.2 Glycogen synthesis (glycogenesis)

Glucose-1-phosphate is not a powerful enough glucose donor to make glycogen directly. Instead, another nucleoside triphosphate is used to power the reaction, this time UTP.

$$\text{G1P} + \text{UTP} \rightarrow \text{UDPG} + PP_i.$$

The Uridine diphosphoglucose (UDPG) then adds its glucose to glycogen.

$$\text{UDPG} + \text{Glycogen}_n \xrightarrow[\text{Glycogen synthase}]{} \text{Glycogen}_{n+1} + \text{UDP}.$$

4.3 Regulation

This is a little complicated, but can be summarised as follows. Glycogen synthase and phosphorylase are regulated in a reciprocal way by several factors. Adrenaline, glucagon and calcium promote glycogenolysis and inhibit glycogenesis. Insulin promotes glycogenesis and inhibits glycogenolysis.

Adrenaline binds to a cell surface receptor which stimulates adenylate cyclase to make the second messenger cAMP. cAMP allosterically activates protein kinase A, which in turn phosphorylates and activates phosphorylase kinase. Phosphorylase kinase phosphorylates glycogen phosphorylase, converting it from the inactive 'b' form to the active 'a' form. This cascade incorporates amplification at every step, turning a small change in adrenaline concentration into a massive production of G1P from glycogen. In addition to activating glycogen phosphorylase, protein kinase A also phosphorylates glycogen synthase, which inhibits it[3]. The adrenaline receptor is a G-protein linked receptor. More details on this in the cell signalling part of the course.

Recall from homeostasis the action of Ca^{2+} in excitation-contraction coupling. In a wonderful example of the body using a single step to carry out several physiologically related processes, Ca^{2+} also activates phosphorylase kinase, whose actions are discussed in the previous paragraph. Thus whenever a muscle is stimulated to contract, glycogen is automatically mobilised to provide energy for that action through glycogenolysis and glycolysis. We will see later on that this is by no means the only place where Ca^{2+} plays a role in the regulation of metabolism (pyruvate dehydrogenase, citric acid cycle).

While adrenaline normally acts to increase glycogenolysis in the muscles within the context of glycolysis, glucagon acts in the liver in much the same way within the context of gluconeogenesis. Again, the pathway is through cAMP as a second messenger and the actions of protein kinase A on glycogen synthase and phosphorylase. The action of Ca^{2+} is important in the liver also, though it is mediated through adrenaline rather than excitation-contraction coupling. Thus adrenaline not only hydrolyses glycogen stores in the muscles via glycolysis, but also mobilises glycogen stores in the liver for transport in the blood in the form of glucose. This is another example of a G-protein linked receptor.

Insulin has the opposite action of glucagon, adrenaline and Ca^{2+}. Its actions are complex and poorly understood, but include switching off a key glycogen synthase kinase (GSK3) by phosphorylation, thus lowering the level of phosphorylation of glycogen synthase.

Glycogen phosphorylase is also allosterically inhibited by ATP (a sign of high energy levels so we want to store energy) and activated by AMP (a sign of low energy levels).

[3]Glycogen synthase can be phosphorylated at a number of different sites. The effects are cumulative.

5 The Aerobic fate of pyruvate : the citric acid cycle

The citric acid cycle is the hub of metabolism. It is linked to all of the other pathways in one way or another and so is highly regulated by a number of means. Fortunately, we need only know the general principles, not all of the details.

5.1 Acetyl-CoA formation

Though not strictly part of the citric acid cycle, we begin this section with a brief discussion of the conversion of pyruvate to acetyl-CoA. This is a key regulatory point, as there is no way to get back from acetyl-CoA to pyruvate in humans and thus once this step is performed, it is not possible to get back to glucose[4]. Firstly, as in gluconeogenesis, pyruvate is transported into the mitochondrion in exchange for hydroxide ions (OH^-). The conversion into acetyl-CoA is then catalyzed by pyruvate dehydrogenase, reducing NAD to NADH in the process. Pyruvate dehydrogenase is a large multi-subunit complex including three coenzymes (thiamine pyrophosphate[5], lipoic acid and FAD) and converts pyruvate to acetyl-CoA using a number of intermediate steps. Fortunately we do not need to know about the intermediate steps, only a little about its regulation. The reaction is as follows:

$$\text{pyruvate} + \text{CoA} \xrightarrow[\text{pyruvate dehydrogenase}]{\text{NAD}\rightarrow\text{NADH}} \text{acetyl-CoA} + CO_2.$$

5.1.1 Regulation

There are three ways in which pyruvate dehydrogenase is regulated. Firstly, feedback inhibition: acetyl-CoA itself inhibits pyruvate dehydrogenase. Build-up of acetyl-CoA implies that the citric acid cycle (which disposes of acetyl-CoA) is not keeping pace with acetyl-CoA formation, so it makes sense to slow formation down. In this context it is more likely to be a good idea to make glucose from pyruvate, providing essential fuel for the CNS, than break it down to acetyl-CoA, further exacerbating the build-up there. This argument also suggests that activation of pyruvate carboxylase by acetyl-CoA might also be a good course of action, and indeed this also occurs.

Secondly, pyruvate dehydrogenase is completely inhibited by phosphorylation of its subunits. It is not clear what agents act to phosphorylate pyruvate dehydrogenase, though there are certainly a number of good candidates (glucagon and adrenaline, for example). What is clear, though again the mode of action is not, is that insulin causes an increase in the proportion of the active (dephosphorylated) kind, resulting in greater activity.

[4]This is not strictly speaking true. As we saw in our discussion of gluconeogenesis, it is possible to convert oxaloacetate (the end product of the citric acid cycle) to glucose. However, oxaloacetate is also a necessary substrate for the citric acid cycle, which can only be made by either the cycle itself or from pyruvate. Thus in order to make glucose from acetyl-CoA we must use oxaloacetate, which is derived from glucose. What this all means is that for every molecule of glucose which is made from acetyl-CoA, we need a molecule of glucose to provide the intermediaries. Thus although the carbon atoms from the acetyl-CoA may end up in a glucose molecule, there is no net gain of glucose.

[5]Thiamine pyrophosphate is made from thiamine (vitamin B_1).

Finally, our old friend Ca^{2+} activates pyruvate dehydrogenase. I (personally, without any research supporting my view) suspect that this may be a second messenger action of adrenaline, though it could simply be its presence in excitation-contraction coupling... I should look into this.

5.2 The citric acid cycle

The citric acid cycle is an eight step cycle, starting with oxaloacetate and acetyl-CoA and ending with oxaloacetate. The cycle has four oxidative steps where coenzymes are reduced (3NAD and 1FAD), makes one GTP from GDP, produces two molecules of CO_2 and uses one molecule of water. I have to admit that I don't understand the chemical arithmetic in this, as there are several steps where the number of hydrogens in the reactants and products seems to be a little a-rye, and one step where an oxygen mysteriously appears out of thin air. The eight steps are as follows.

$$\text{acetyl-CoA} + \text{oxaloacetate} \xrightarrow[\text{citrate synthase}]{} \text{citrate} \tag{1}$$

$$\text{citrate} \xrightarrow[\text{aconitase}]{} \text{isocitrate} \tag{2}$$

$$\text{isocitrate} \xrightarrow[\text{isocitrate dehydrogenase}]{\text{NAD}\rightarrow\text{NADH}} \text{2-oxoglutarate} + CO_2 \tag{3}$$

$$\text{2-oxoglutarate} + \text{CoA} \xrightarrow[\text{oxoglutarate dehydrogenase}]{\text{NAD}\rightarrow\text{NADH}} \text{succinyl-CoA} + CO_2 \tag{4}$$

$$\text{succinyl-CoA} \xrightarrow[\text{succinyl-CoA synthetase}]{\text{GDP}\rightarrow\text{GTP}} \text{succinate} + \text{CoA} \tag{5}$$

$$\text{succinate} \xrightarrow[\text{succinate dehydrogenase}]{\text{FAD}\rightarrow\text{FADH}_2} \text{fumarate} \tag{6}$$

$$\text{fumarate} + H_2O \xrightarrow[\text{fumarase}]{} \text{malate} \tag{7}$$

$$\text{malate} \xrightarrow[\text{malate dehydrogenase}]{\text{NAD}\rightarrow\text{NADH}} \text{oxaloacetate} \tag{8}$$

Many of the molecules involved in the citric acid cycle are removed from the cycle to make other biological molecules. For example 2-oxoglutarate is converted to glutamate, succinyl-CoA is a precursor of the heme ring structure and as we mentioned above, oxaloacetate can be made into glucose in gluconeogenesis. In order to make up for this loss and keep the cycle running we need pyruvate to be converted to oxaloacetate by pyruvate carboxylase.

5.2.1 Regulation

The first thing to note about the regulation of the citric acid cycle is that its speed is mainly determined by the availability of its substrates. Availability of oxaloacetate and acetyl-CoA, for example, regulate the rate of citrate synthase activity, as citrate synthase

can generally make citrate faster than the rate at which its substrates become available. It is also possible to see the dual action of acetyl-CoA (activation of pyruvate carboxylase and inhibition of pyruvate dehydrogenase) as an attempt to balance the supply of substrate to the rate of the citric acid cycle. Availability of NAD is also an issue, which is determined in part by ADP availability for oxidative phosphorylation.

In addition to activating pyruvate dehydrogenase (as mentioned above) and thus controlling the availability of acetyl-CoA, Ca^{2+} also activates isocitrate dehydrogenase and oxoglutarate dehydrogenase (steps 3 and 4 above). This counters the effect of citrate, which inhibits its own formation, by decreasing the citrate concentration.

Finally, ADP is an allosteric activator of isocitrate dehydrogenase, thus stimulating respiration when cellular energy levels are low.

6 Oxidative phosphorylation

The high energy product of each of the catabolic processes described in this note (glycolysis, citric acid cycle, β-oxidation) has mainly been NADH[6], rather than the cell's preferred fuel source, ATP. The story of oxidative phosphorylation is the story of how the energy stored in NADH is converted to ATP. In order for this to be done efficiently, there has to be some high-energy intermediate, as there is enough energy stored in NADH to make at least three ATP from ADP and P_i. It is difficult to see how this could be done directly without making only a single ATP from NADH, which would be incredibly wasteful (not to mention dangerous, as it would lead to a great deal of heat production). In fact, since active transport of P_i is also required only about 2.5 ATP are made from each NADH, but this is still significantly more efficient than the direct route.

Peter Mitchell came up with the solution in the middle of the 20th century in the form of his **chemiosmotic theory**. The high energy intermediate turns out to be an electrochemical potential difference of H^+ ions across the inner mitochondrial membrane known as the **proton motive force** (PMF). The energy of NADH (and $FADH_2$) is used to pump H^+ ions out of the inner mitochondrial matrix. Because H^+ ions are not just carriers of electrical charge but are also 'acidic', the proton motive force is made up of two components, the voltage difference and the pH difference. It is expressed in millivolts per mole of protons:

$$\text{PMF} = \Delta\psi - \left(\frac{RT}{\log_{10}(e)F}\right)\Delta\text{pH}.$$

At a temperature of 310K (37°C), PMF$\approx \Delta\psi - 60\Delta$pH. Volts are related to joules via the Faraday constant. For the transfer of n electrons

$$\Delta G = -nFE,$$

where ΔG is change in free energy, F is the Faraday constant (96494 coulombs per mole) and E is voltage.

[6] And $FADH_2$.

6.1 The electron transport chain

Having said that the proton motive force couples oxidation of NADH to phosphorylation of ADP it is natural to ask how the proton motive force is created and maintained. This is perhaps the most complicated part of metabolism that we are required to know, and requires four large multi-enzyme complexes in the inner mitochondrial membrane called (conveniently) complex I, complex II, complex III and (no surprises here) complex IV. Electrons are shuttled between them on their way from NADH (or $FADH_2$) to oxygen by smaller, more mobile carriers called ubiquinone and cytochrome *c*. In total about 70 different polypeptides making up 20 redox centres are required for the electron transport chain.

Complex I is the largest, with around 40 polypeptides contributing to its formation. This is the point where the electrons from NADH enter the electron transport chain. They are passed first to FMN, and then to Q via several iron-sulphur proteins. See the appendix for a discussion of these and other coenzymes involved in metabolism. During this process, four H^+ ions are pumped out of the mitochondrial matrix though the detailed mechanism for this is unknown.

Complex II is actually involved in the citric acid cycle as well as the electron transport chain, as it is the site of succinate dehydrogenase. It should be easy to guess that this is the place that electrons from $FADH_2$ enter the electron transport chain, as succinate dehydrogenase is involved with converting FAD into $FADH_2$. The electrons are passed to Q via several iron-sulphur proteins, and no H^+ ions are pumped across the membrane at this complex.

Complex III accepts electrons from QH_2 (which may have come from either complex I or complex II). The reactions which take place at this complex are quite complicated and form the Q-cycle, which is thankfully beyond the scope of the course. What we do need to know is that electrons are passed to another iron-sulphur protein and then on to cytochrome c_1, which passes them to cytochrome *c*. Along the way, four H^+ ions are pumped out of the mitochondrial matrix using two *b* cytochromes as a kind of channel. The two *b* cytochromes are positioned at opposite sides of the membrane, giving them slightly different redox potentials owing to their different environments. Consequently they are called b_H and b_L (high and low) and have absorption peaks at 562 nm and 566 nm respectively.

Complex IV is the last port of call in the electron transport chain and delivers electrons from cytochrome *c* and H^+ ions to oxygen to make water. It first transfers two electrons to reduce O_2 directly to peroxide (O_2^{2-}), avoiding the highly reactive O_2^- form. Then two more electrons are used to break the O–O bond and $4H^+$ are consumed to make $2H_2O$. These four electron transfers happen at four redox centres: cytochrome *a*, cytochrome a_3, the Cu_A centre (2Cu ions bridged by 2S from cysteine residues) and the Cu_B centre. The phenol group of a tyrosine residue is involved with O–O bond cleavage. In addition to the four H^+ ions mentioned above, four more are pumped out of the mitochondrial matrix (thus $2H^+$ per NADH or $FADH_2$)[7]. The pumped protons are kept strictly separate from

[7]I'm a little confused by that, as surely that should make the net effect of complex IV a transfer of either $6H^+$ or $2H^+$ out of the inner mitochondrial matrix (thus either $3H^+$ or $1H^+$ per NADH or $FADH_2$)? Or do two of the chemical protons come from each side of the membrane? I'm sure that it does all add

the chemical protons by the configuration of complex IV.

6.2 ATP synthesis

Although not the only use of the proton motive force, ATP synthesis by F_0F_1ATPase is arguably the most important[8]. F_0F_1ATPase catalyses the reaction

$$\text{ADP} + P_i \underset{F_0F_1\text{ATPase}}{\rightleftharpoons} \text{ATP} + H_2O.$$

It is organised into two building blocks, F_0 and F_1. It is only active in the forward direction when

1. The two are bound with F_0 straddling the inner mitochondrial matrix, and
2. The proton motive force is present to drive the conformational change of F_1 in the right direction.

Interestingly it is the *release of ATP* from the enzyme which requires energy in the form of the proton motive force to drive it, not formation of ATP from ADP and P_i, which happens spontaneously. For a change I am going to have to confess that the way that F_0F_1ATPase works is actually described beautifully in the notes. I could reproduce them here, but what would be the point? Instead, I simply refer to pages 15–17 of the handout.

7 Fat metabolism

When discussing fatty acid synthesis, it is important to remember that while superficially similar, the process of synthesising fatty acids is actually very different from the process of breaking them down. (Synthesis adds two-carbon chunks from acetyl-CoA to a growing chain, oxidation removes two carbon chunks to acetyl-CoA). This is the source of many nasty MCQs. One example of this difference is that fatty acid synthesis takes place in the cytoplasm whereas the process of breaking down fatty acids, called β-oxidation, takes place in the mitochondrial matrix. Other differences will become clear as we discuss the two processes below.

7.1 Fatty acid synthesis

Fortunately, we do not need to know all of the scary details behind fatty acid synthesis and can reduce the process to a few key steps. First, acetyl-CoA is converted to malonyl-CoA using acetyl-CoA carboxylase[9]. Then malonyl-CoA donates two carbons to a growing

up, but it seems darned confusing to me!

[8]There are three other important uses of the proton motive force which we need to know about. One is pyruvate transport into the mitochondria in exchange for OH^-, driven by the ΔpH component of the PMF. The second is ADP/ATP exchange across the inner mitochondrial membrane, which is driven by the $\Delta\psi$ component of the PMF. The third is phosphate transport into the inner mitochondrial matrix in exchange for OH^-, driven by the ΔpH component.

[9]Acetyl-CoA carboxylase uses a biotin prosthetic group to carry CO_2.

fatty acid chain using fatty acid synthase. Finally, when the chain has grown to about 16 carbons long it is released from fatty acid synthase by hydrolysis and attached to CoA. The fatty acyl-CoA, as it is then called, then undergoes some final processing before donating its acyl group in the formation of triacylglycerols or phospholipids.

The donation of a two carbon chunk to a growing fatty acyl-CoA requires some reducing power, which it gets in the form of NADPH, which is oxidised to NADP (two lots of NADPH are required to add each two-carbon chunk). The re-reduction of NADP back to NADPH is actually part of the pentose phosphate pathway[10] and is another place where glucose-6-phosphate (G6P) enters the picture:

$$\text{G6P} + 2\text{NADP} \longrightarrow \text{Ribulose-5-P} + 2\text{NADPH} + CO_2.$$

The ratio of NADPH to NADP is kept high in order to favour reductions (such as those in fatty acid synthesis). This is in complete contrast to the NADH to NAD ratio, which is kept low to favour oxidations such as those in glycolysis. Thus it makes sense that a key anabolic process like fatty acid synthesis would use NADPH for reducing power, whereas key energy-producing catabolic processes are more likely to use NAD than NADP as an oxidising agent.

This pathway can be linked into the citric acid cycle, though not in the most immediately obvious way of simply using acetyl-CoA directly. As mentioned above, synthesis of fatty acids occurs in the cytoplasm, but acetyl-CoA is formed in the mitochondion and as we discuss below, cannot cross the mitochondrial membrane. Citrate has no such problem and thus it is at this stage that the two pathways are linked. Once out of the mitochondrion ATP citrate lyase catalyses the formation of acetyl-CoA so that fatty acid synthesis can begin:

$$\text{Citrate} + \text{ATP} + \text{CoA} \xrightarrow[\text{ATP citrate lyase}]{} \text{Acetyl-CoA} + \text{oxaloacetate} + \text{ATP} + P_i.$$

7.2 β-oxidation of fatty acids

As mentioned above, β-oxidation of fatty acids takes place in the mitochondrial matrix. Fatty acyl CoA's are moved into the mitochondrial matrix by a process of facilitated diffusion through the **carnitine shuttle**. Carnitine palmitoyl transferase I and carnitine palmitoyl transferase II are present on the outside and inside of the mitochondrial matrix respectively. The first swaps the CoA group of the fatty acyl CoA for a carnitine molecule. The fatty acyl carnitine is then transported into the mitochondrial matrix. Inside the mitochondrial matrix the second catalyses the reverse reaction and the free carnitine is transported back out into the cytoplasm. This process is necessary for two reasons.

1. Coenzyme A cannot cross the mitochondrial membrane.

2. Free fatty acids are far too reactive to be allowed to simply separate from coenzyme A, so something needs to attach to the carboxylic acid group at all times to prevent

[10]The pentose phosphate pathway is important in the synthesis of nucleotides, as it makes their ribose/deoxyribose components.

cell damage[11].

Once inside the mitochondrial matrix the actual process of β oxidation can begin. It is actually remarkably similar to steps 6–8 in the citric acid cycle. One FAD is reduced to $FADH_2$, one molecule of water is added and one NAD is reduced to NADH, just as in those three reactions. Details of the reactions can be found on page 37 of Dr Rubery's handout. The process is called β oxidation because it is the β carbon (i.e. the second after the COCoA group) of the fatty acyl CoA which is oxidised (from CH_2 to $C{=}O$).

7.3 Fat storage and transport

Any section on fat metabolism would be incomplete without a discussion of triacylglycerols, the long-term energy store of the body. Triacylglycerols are exactly what their name suggests — three acyl groups attached to a glycerol molecule ($CH_2OH{\cdot}CHOH{\cdot}CH_2OH$). The source of glycerol is glycerone-P (GP), the "unwanted" three-carbon molecule formed when aldolase splits F1,6P_2 in glycolysis[12]. The reducing power for this reaction is provided by NADH:

$$\text{glycerone-}P \xrightarrow[\text{glycerol-3-phosphate dehydrogenase}]{\text{NADH}\rightarrow\text{NAD}} \text{glycerol-}P.$$

This reaction is actually reversible, thus although fatty acids cannot be constructively made into glucose, it is possible to make the glycerol component of fat into glucose through gluconeogenesis[13]. Triacylglycerol synthesis takes place mainly in adipocytes and the liver, though it also occurs in the mammary glands of lactating females.

Triacylglycerol is transported around the body in two different ways. Firstly, dietary triacylglycerol is emulsified in the small intestine by bile acids from the gall bladder and then hydrolysed by a lipase[14]. The emulsification increases the surface area upon which lipases in the gut can act and thus speeds up the process. The products are then absorbed by the epithelial cells lining the gut and converted back into triacylglycerol. Amazingly inefficient! The resulting triacylglycerol is then packaged into large lipoproteins called chylomicrons and transported through the lymphatic system until it eventually enters the blood at the thoracic duct. Secondly, fat made in the liver is packaged together with some proteins ('apoproteins') in large complexes called very low density lipoproteins (vLDL) and secreted into the blood.

[11] One can get an appreciation of the problems which free fatty acids would cause in living cells if one considers the fact that fatty acids dissolve phospholipid membranes.

[12] Glycerol is also made from glyceraldehyde in fructose metabolism. The reaction is catalysed by alcohol dehydrogenase and the reducing power required is again provided by NADH.

[13] In insect flight muscle this reaction is part of the **glycerophosphate shuttle**, which is a method whereby high energy electrons from NADH are transported into the mitochondria to be used in the electron transport chain. This reduces the need for oxygen and allows the tissue to generate the largest known sustained power-to-weight ratio of any living tissue. The second component is provided by flavoprotein dehydrogenase, which straddles the inner mitochondrial membrane and couples the oxidation of glycerol-P (back to glycerone-P) to the reduction of FAD to $FADH_2$, which then passes its electrons to ubiquinone and enters the electron transport chain at complex III.

[14] The emulsified globules of fat with bile acid surfactants on the outside are called micelles.

Adipocytes and myocytes have receptors on their surface (lipoprotein lipase, or LPL) which recognise a protein component of chylomicrons and vLDL and hydrolyse the triacylglycerol, releasing fatty acids which enter the cells.

7.4 Regulation

Fatty acid synthesis starts with and β-oxidation ends up with acetyl-CoA, so it is clear that anything which regulates pyruvate dehydrogenase also has an impact on fat metabolism. However there is also some regulation of the intermediate steps, the most important of which is the regulation of acetyl-CoA carboxylase (ACC).

The first way that ACC is regulated is through phosphorylation. Both cAMP-dependent kinase (protein kinase A) and AMP-dependent kinases inhibit ACC by phosphorylating it. AMP-dependent kinases are activated by AMP, whose concentration increases when supplies of ATP run low (consider what happens to the adenylate kinase-mediated reaction when supplies of ATP are short). Thus fatty acid synthesis is turned off when cellular energy levels are low — exactly what we would want to happen. cAMP concentrations on the other hand are regulated by the activity of adenylate cyclase which, as we saw when we discussed glycogen regulation is part of the adrenaline pathway. Thus fatty acid synthesis is inhibited by adrenaline, which is secreted in the fear, fight or flight response, when we would like energy reserves to be directed towards keeping us alive rather than building up fat reserves!

Glucagon also stimulates adenylate cyclase and thus has a similar effect to adrenaline. Insulin on the other hand causes a decrease in the degree of phosphorylation of ACC and thus activates fatty acid synthesis. It is not clear exactly how this effect is mediated at present.

In addition to covalent modification by addition of phosphate groups, ACC is also allosterically activated by citrate, activating fatty acid synthesis when the citric acid cycle stalls and citrate builds up. In yet another example of feedback inhibition, it is also inhibited by fatty acyl-CoA, helping fatty acid synthesis to keep pace with triacylglycerol synthesis.

Fatty acid oxidation is controlled simply by the availability of substrate. This is controlled by the rate of fatty acid release from adipocytes, which is controlled by hormone-sensitive triacylglycerol lipase. Glucagon and adrenaline both cause activation of this enzyme by phosphorylation, again using cAMP as a second messenger. The pathway is very similar to the one which inactivates ACC (described above) except that phosphorylation of the enzyme causes activation in this case rather than inhibition. Thus adrenaline and glucagon both inhibit fatty acid synthesis and activate fatty acid oxidation.

A slightly more direct method of regulation of fatty acid oxidation is provided by the inhibition of carnitine palmitoyl transferase I by malonyl-CoA, again emphasising the importance of the acetyl-CoA $\rightarrow$ malonyl-CoA step in regulating this pathway. Inhibition of this enzyme keeps long-chain fatty acids out of the mitochondrial matrix and thus effects the availability of substrate once more.

The methods of regulation discussed above all have short-term effects. In contrast, there are long term effects which also affect fat metabolism. These take place at the gene expression level and are (I believe — check this!) part of the insulin-stimulated MAPK

pathway. These are caused by a high carbohydrate diet. Apparently we cover this pathway in more detail next term.

In the notes it mentions that insulin-stimulated glucose uptake could be seen as regulating fat metabolism by providing substrate for fatty acid synthesis through glycolysis and pyruvate dehydrogenase, but this really is stretching it a little I feel!

8 The Anaerobic fate of pyruvate : fermentation

One of the really important things to remember about respiration is that it is almost totally reliant on oxygen. At the end of the citric acid cycle, each glucose molecule has given rise to:

1. 2ATP from glycolysis,
2. 2NADH from glycolysis,
3. 2NADH from conversion of pyruvate to acetyl-CoA,
4. 6NADH from the citric acid cycle,
5. 2$FADH_2$ from the citric acid cycle,
6. 2GTP from the citric acid cycle.

In the presence of oxygen this can be turned into a total of 27ATP in the electron transport chain[15]. However in the absence of oxygen the citric acid cycle will stall from lack of NAD and FAD. This shows how important it is to be able to recycle some of that NADH in the absence of oxygen, so that glycolysis does not also stall, leaving the cell without any source of energy whatsoever.

This problem is solved by the coupling of NADH oxidation and pyruvate reduction to lactate[16].

$$\text{pyruvate} + \text{NADH} \underset{\text{lactate dehydrogenase}}{\rightleftharpoons} \text{lactate} + \text{NAD}.$$

This totally cuts out steps 3–6 above, making the entire process generate only 2ATP. Given that this is only $\frac{2}{27}$ of the energy available in the presence of oxygen it is easy to see why complex organisms like humans die pretty quickly without it!

Lactate is released into the blood and taken up by the liver for use in gluconeogenesis or cardiac muscle for the citric acid cycle when oxygen levels are more favourable.

[15]If we were simply to add the NADH from glycolysis to those generated in conversion of pyruvate to acetyl-CoA and the citric acid cycle and then multiply by 2.5 for each NADH, 1.5 for each $FADH_2$ and 1 for each GTP we would get 32ATP. However, for the NADH from glycolysis to be converted into ATP it would need to enter the mitochondria to find its way to the electron transport chain. This would require active transport, and does not occur in humans. A solution to this problem has been found in the flight muscles of insects. In addition, GTP has many uses in the cell apart from generating ATP, so this conversion may well never take place.

[16]Lactate buildup during exercise can lower the cytosolic pH in myocytes during strenuous exercise, causing cramp, so this is strictly a short-term solution.

9 Ketogenesis

The brain, unlike most other cells in the body, cannot use fat as a source of fuel. This is because lipoproteins such as vLDL cannot cross the blood-brain barrier. Thus when times are hard and there is little glucose intake the body starts producing glucose through gluconeogenesis. Unfortunately, since the body also cannot make glucose from fatty acids this puts a strain on the body's ability to cope, as glucose can only be produced from certain amino acids, glycerol and glycogen. The amount of energy stored in the body in the form of glycogen and glycerol is not large, and breaking down amino acids means muscle wasting and other processes which would put the body at a serious disadvantage since proteins, which are made from amino acids, are the main functional and structural molecules of the body. Thus there is obviously a need for another solution, and this is found in the form of ketone bodies, which are made from acetyl-CoA and thus can be made from fatty acids. The brain can also utilise these molecules as an energy store and thus the fuel crisis is averted.

There are two major disadvantage of ketone bodies. One is that acetone is produced as a waste product of their metabolism, and the body can only get rid of this by exhaling it. The second problem is that both acetoacetate and β-hydroxybutyrate are acids. In excessive fasting, or metabolic disorders that mimic the endocrine features of this state such as diabetes, excessive production of ketone bodies in the liver can lead to a disruption in the acid/base balance and thus to **ketoacidosis**. This is what happens to diabetics when they get hyperglycaemic by injecting too little insulin or ingesting too much carbohydrate. About 3% of cases of type I diabetes present as ketoacidosis. Ketoacidosis is actually a really good clinical example to think about the regulation of different metabolic pathways, see how they relate, and see how they can go wrong in disease. Ketone bodies are also used by myocytes (especially cardiomyocytes).

Ketone bodies are made in the liver by a lengthy process which we don't need to know the details of, except that two molecules of acetyl-CoA are converted into one molecule of acetoacetate and the coenzyme A is released. The acetoacetate can then either be metabolised to acetone and CO_2 or reduced to β-hydroxybutyrate using NADH. Ketone bodies are used in myocytes by reversing the above steps (from either β-hydroxybutyrate or acetoacetate) to produce acetyl-CoA, which is fed into the citric acid cycle. Acetoacetate accepts CoA from succinyl-CoA in this process.

10 Nitrogen metabolism

Amino acid metabolism is a large and important topic in biochemistry, but for some reason we do not cover it in a lot of depth. Many of the amino acids commonly used in proteins cannot be made by the body and must be acquired through the diet. These are His, Ile, Leu, Lys, Met, Phe, Thr, Trp and Val. While they cannot be made by the body, they can be broken down. Most amino acids can be converted into pyruvate and thus are glucogenic, though leucine and lysine can only be converted to acetyl-CoA and are thus ketogenic. In either one of these processes nitrogen from the amino acid must be disposed of. In animals this is done through the **urea cycle**, which takes place in the

liver. Urea is highly water soluble and not easily protonated or deprotonated, making it a good candidate for excretion in the urine.

The process of transferring the amine group (and thus the nitrogen) from an amino acid is called transamination and the target is always glutamate or aspartate, though it might temporarily be passed to alanine for transport in the blood from muscle to liver. The process works by reversibly donating the amine group to a keto acid (called an acceptor). This may take several steps, for example:

$$\text{Alanine} + \text{Pyridoxal} \longrightarrow \text{Pyruvate} + \text{Pyridoxamine}$$

followed by

$$\text{Pyridoxamine} + \text{2-Oxoglutarate} \longrightarrow \text{Pyridoxal} + \text{Glutamate},$$

thus recycling the pyridoxal ready to accept another amine group. The enzymes which catalyze these transamination reactions require the coenzyme pyridoxal-5′-phosphate (PLP), which is a derivative of pyridoxine (vitamin B_6).

The deamination of glutamate to make ammonia then recycles the 2-Oxoglutarate as well:

$$\text{Glutamate} + \text{NAD} \xrightarrow[\text{Glutamate dehydrogenase}]{} \text{Oxoglutarate} + \text{NADH} + NH_3.$$

This takes place in the mitochondria, though I wasn't able to find out how the glutamate gets there.

Ammonia from glutamate and aspartate then feed into the urea cycle, which is similar to the citric acid cycle and was discovered by the same man (Hans Krebs). Still in the mitochondrial matrix, ammonia is combined with bicarbonate to make carbamoyl phosphate:

$$NH_3 + HCO_3^- + 2\text{ATP} \xrightarrow[\text{carbamoylphosphate synthase}]{} NH_2 \cdot CO \cdot OPO_3^{2-} + 2\text{ADP} + P_i.$$

The carbamoyl phosphate is then donated to ornithine to make citrulline, which is transported out of the mitochondria (in exchange for ornithine) into the cytoplasm. It then condenses with aspartate to form argininosuccinate. Argininosuccinate is then split into fumarate (which contains no nitrogen) and arginine[17], which is hydrolysed to make urea and ornithine, which is transported back into the mitochondrial matrix so that the cycle can begin again.

In the absence of bicarbonate ammonia is often donated to glutamate to form glutamine. This prevents buildup of this otherwise toxic substance. Glutamine is another important transport amino acid. This can cause problems if there is a congenital defect in one of the enzymes involved in the urea cycle, as this leads to depletion of glutamate, an important neurotransmitter.

[17]This is the synthetic pathway for arginine.

11 One-carbon metabolism

There are two key pathways involved in one-carbon metabolism, the folic acid pathway[18] and the S-adenosyl methionine (SAM) pathway. Of these, the folic acid pathway is much more flexible, as it is able to accept/donate CH_3, CH_2, CH, CHO, and occasionally $COOH$ groups, while the SAM pathway can only donate CH_3 groups.

The reason that one-carbon metabolism is important is that it is required for the formation of the bases used in RNA and DNA. One example of this is the synthesis of thymine monophosphate (TMP) from deoxyuracil monophosphate (dUMP[19]). Firstly, folic acid (more precisely tetrahydrofolic acid—THFA) accepts CH_2 from serine, forming glycine and methylene THFA:

$$\text{serine} + \text{THFA} \xrightarrow[\text{serine hydroxymethyltransferase}]{} \text{glycine} + \text{methylene THFA}.$$

Methylene THFA then donates the CH_2 group to dUMP, forming TMP and DHFA. THFA is regenerated from DHFA using dihydrofolate reductase. This enzyme is an important anti-cancer target, as disabling dihydrofolate reductase will effectively stop cell devision, as it will be impossible to duplicate DNA. Methotrexate and aminopterin work in this way.

Both THFA and SAM are involved in the **activated methyl pathway**, which is responsible for almost all methylations, such as those required in the synthesis of adrenaline or choline, or methylations of the bases in DNA or RNA at the polymer level. The cycle begins with homocysteine, which is methylated by CH_3THFA (rather than CH_2THFA, used in TMP synthesis) to form methionine:

$$\text{Homocysteine} + CH_3\text{THFA} \xrightarrow[\text{methionine synthase}]{} \text{Methionine} + \text{THFA}.$$

Methionine synthase uses cobalamin (vitamin B_{12}) as a coenzyme[20]. SAM is then formed by addition of the nucleoside part of ATP to the sulphur of methionine.

$$\text{Methionine} + \text{ATP} \xrightarrow[\text{methionine adenosyl transferase}]{} \text{SAM} + PP_i + P_i.$$

This activates methionine's methyl group, as the positively charged sulphur is now quite unstable and will readily give it up.

12 Ending hormone-driven activation/inhibition

It may have occurred to the reader that there is a need for the processes described in the above sections on control to have a finite duration of action. In some cases, this happens

[18] Folic acid is also used to make choline and is thus important in many ways, for example formation of the neurotransmitter acetylcholine, but here we are interested in its role in one-carbon metabolism. I'm not all that sure that's true, though I'm sure I heard it somewhere...

[19] This should not be confused with deoxyribose uracil monophosphate, which would traditionally also be known as dUMP. Since uracil is not a normal base of DNA this is rarely going to cause confusion.

[20] Other than methylmalonyl-CoA mutase, which is involved with odd-chain fatty acid oxidation, this is the only coenzyme B_{12}-associated enzyme in mammals.

without any intervention, for example if the concentration of G6P decreases due to either glycolysis or glycogenesis then hexokinase will automatically start working at a higher rate. However in the case of hormone regulated pathways, for example, it is not immediately clear why these do not have an infinite duration of action. This is achieved in the case of cAMP second-messenger based regulation by the continuous action of phosphodiesterase and protein phosphatase. Phosphodiesterase hydrolyses cAMP to 5'AMP, while protein phosphatases reduce the phosphorylation caused by protein kinases. This is an ongoing process, ending the action of hormones when their concentration drops.

13 Metabolic inhibitors

There are many chemicals which interfere with metabolism. Those we discuss here are those which interfere with the working of oxidative phosphorylation, though I will try to add others as I come across them. There are five parts to oxidative phosphorylation, and an inhibitor for each.

1. Complex I is inhibited by rotenone, which inhibits the transfer of electrons from an iron-sulphur centre to ubiquinone.

2. Complex II is inhibited by malonate, which acts competitively with succinate to inhibit succinate dehydrogenase, thus inhibiting the electron transfer chain before it starts by preventing formation of $FADH_2$ in the citric acid cycle. This does not, of course, stall the electron transport chain completely, as there are other sources of $FADH_2$.

3. Complex III is inhibited by antimycin.

4. Complex IV is inhibited by cyanide, which is a ligand for cytochrome a_3.

5. F_0F_1ATPase is inhibited by oligomycin, which blocks the F_0 channel of the inner mitochondrial membrane, preventing the proton motive force from driving ATP synthesis.

A Important coenzymes

A.1 NAD^+ and NADH

Nicotinamide adenine dinucleotide (NAD^+) is one of the most important molecules in metabolism. It is present in glycolysis, the citric acid cycle, β-oxidation, oxidative phosphorylation and transamination. The + refers to the charge on the nitrogen atom at the end of the molecule which is primarily involved in reductions/oxidations, though the net charge on NAD^+ is actually negative as it contains two phosphate groups. NAD^+ is reduced to NADH by addition of a hydride ion, H^-, removing the positive charge. Nicotinamide comes from niacin, a vitamin B complex.

A.2 Coenzyme-A (CoA or CoASH)

Coenzyme-A appears most frequently in the form of acetyl-CoA, although it is also present in the form of succinyl-CoA, malonyl-CoA and fatty acyl CoA among others. It consists of pantothenate (a vitamin) bonded to β-Mercaptoethylamine ($NH_2 \cdot CH_2 \cdot CH_2 \cdot SH$). The pantothenate component is also bonded to a 3′-phosphoadenosine via a pyrophosphate (PP_i) bridge. It binds to other groups via a thioester linkage from the S of the β-Mercaptoethylamine part. This is a high energy bond, and it has been suggested that this is evidence that before phosphate became abundant enough in the primordial environment to be used as an energy carrier, thioesters were a major energy carrier. There is a picture and further discussion of this topic on page 413 of VVP.

A.3 Flavins

The flavin nucleotides used in metabolism are FMN and FAD (flavin mononucleotide and flavin adenine dinucleotide). Both are important coenzymes in oxidative phosphorylation, where their reduced forms $FMNH_2$ and $FADH_2$ transfer electrons to ubiquinone via iron-sulphur proteins. The flavin component of these coenzymes comes from riboflavin (vitamin B_2).

A.4 Iron-sulphur proteins

These are found in complexes I, II and III. In the notes it says that they contain either two iron bonded to two sulphur atoms ([2Fe-2S] proteins) or four iron bonded to four sulphur atoms ([4Fe-4S] proteins), but VVP seems to disagree, saying that one of the iron-sulphur proteins in complex II has a [3Fe-4S] configuration, which is just a [4Fe-4S] with a missing Fe atom. In each case the Fe atoms are bonded to the protein via the sulphur of a cysteine residue.

A.5 Ubiquinone

Ubiquinone, also called coenzyme-Q or simply Q has a long lipophilic side-chain (rather like phospholipids) which makes it membrane-soluble. It is present in the inner mitochondrial membrane and carries electrons from complexes I and II to complex III. The process of donating electrons at complex III is called the Q cycle and is involved in the proton pumping which complex III performs. Q is reduced to QH_2 (ubiquinol) in two single-electron steps at complexes I and II and donates them again in single electron steps in the Q cycle.

A.6 Cytochromes

Cytochromes are so-called because of their characteristic absorption spectra within the visible portion of the electromagnetic spectrum (i.e. they are coloured). They are again involved in electron transfer, this time within complexes III and IV. Cytochromes contain

a heme group which is Fe^{2+} in its reduced form or Fe^{3+} in its oxidised form[21]. Their reduced forms have additional absorption peaks between 500 nm and 600 nm which are not present in the oxidised form and thus a colour change is visible which can be used for diagnostic tests. Cytochromes come in several different forms, of which cytochromes b and c_1 are present in complex III; cytochrome c transfers electrons from complex III to complex IV; and cytochromes a and a_3 are present in complex IV.

A.7 Copper centres

Copper centres are important oxidants in complex IV of the electron transport chain, where they cycle between states Cu^{I} and Cu^{II}.

[21]The heme group of hemoglobin is present in the reduced (Fe^{2+}) form and is the same structure as cytochrome b.