Metabolic Control Systems

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This lecture will cover the basics of metabolic control, including how enzymes and transport systems control the fate of a metabolite and how systems are integrated to maintain homeostasis. These concepts should be a review of material covered in your Biochemistry classes. For further details please refer to the books on reserve for this course[1,2]

[1] Jeremy M Berg, John L Tymockzko, and L Stryer. *Biochemistry*, volume New York. 2013. ISBN 0-7167-3051-0

[2] Denise Ferrier. *Lippincott Illustrated Reviews: Biochemistry*. LWW, 1496344499, 2017. ISBN 1-4963-4449-9

Contents

Learning Objectives

- Explain what a rate limiting enzyme is, what a committed enzyme step is and what a reversible reaction is.
- Predict the differences in speed and persistence of allosteric, post-translational and transcriptional regulation of metabolism.
- Describe the role of cellular transport in macromolecular regulation. Understand the differences between active and passive transport.

Key Terms and Concepts

- Activation Energy
- Allosteric Regulation
- Cofactor
- Compartmentalization
- Concentration Gradient
- Enzyme
- Feedback Inhibition
- Isoenzymes
- Post-Translational Modification
- Rate Limiting Enzyme
- Transcription Factor
- Transporters (Active and Passive)

Control of Metabolic Flux

Cells need to control the rates at which nutrients are taken up, stored, or used and there are several ways by which this occurs. Here we will review the biochemistry of both nutrient transport and enzyme function. Understanding these concepts will be very important to understanding how the metabolic pathways we will discuss are controlled.

Cellular Transport Systems

First we will describe the ways in which cells control nutrient permeability. Most of the nutrients we will discuss[3] are unable to pass through the plasma membrane of the cell. Allowing or denying access to a nutrient is one way by which cells can control nutrient metabolism. Without these transport mechanisms we would be unable to absorb digested food, or transport nutrients from cell to cell. While we normally think of transporters as getting nutrients into or out of a cell, they are also important *within* cells, for example getting pyruvate into the mitochondria, or storing calcium in the endoplasmic reticulum.

[3] The exceptions are sterols and some other lipids

Types of Membrane Transporters

Membrane transporters are generally fairly specific for the molecule they transport. For example GLUT4 transports glucose, but GLUT5 transports fructose. Transporters can broadly be separated into two major types, passive transporters and active transporters. These can be differentiated by considering whether they work *with* or *against* the concentration gradient, with active transporters typically working against the concentration gradient.

PASSIVE TRANSPORTERS allow for nutrients to pass down a concentration gradient into the cell. As an example, the liver expresses a glucose transporter named GLUT2. Glucose can either enter the liver (if there is more glucose in the blood than the liver) or exit the liver (if the reverse is true). Passive transporters will only allow a nutrient to enter a cell *if there is less of the nutrient in a cell than in the blood*. This is quite efficient for disposing of excess nutrients, such as after a meal, but is not effective in storing things away against a concentration gradient. It may seem like passive transporters are not regulated, but as we will see in the case of GLUT4, the amount of transporters at the cell surface can be controlled by cell signaling[4]. The rate of a passive transport is defined by three things, the gradient of the transported molecule, the number of transporters at the relevant membrane, and the efficiency of the transporter.

[4] if you want to jump ahead, here is a review on that process [Leto and Saltiel, 2012]

ACTIVE TRANSPORTERS can force nutrients into a cell *against* the concentration gradient. These transporters function like pumps and have to use energy of one sort or another to force the molecule into the cell. You may think that this is a bad idea, but there are lots of examples where this matters physiologically. One example is retaining salt. If your kidneys weren't actively retrieving sodim out of urine and back into the blood, then you would rapidly lose osmotic pressure in your blood. The key is to think about the concentration inside or outside the cell, and if you are pushing against the transport gradient, you need active transport.

Powering Active Transport

Active transport requires energy of some type. This energy can come from several sources such as ATP, other concentration gradients, or even light. Some examples are described in Figure 1. The key to controlling the rate of these transporters is not only the concentration gradient of the transported molecule, but also the levels (or gradient) of the powering force. In the cases where molecules are co-transported they can either be pulled in simultaneously (this is known as a symporter) with the molecule of interest as shown on the left of Figure 1, or can be exchanged where one molecule exits, powering the entry of the molecule of interest (this is known as an antiporter). A classic example of an antiporter is the sodium:glucose exchanger SGLT1, which extrudes sodium down its concentration gradient (into the gut lumen) to force uptake of glucose from the gut into cells. This allows for efficient carbohydrate uptake in a meal[5] even if the gastrointestinal cells have similar or higher glucose levels to the gut lumen.

[5] SGLT2 does a similar thing, retrieving glucose from urine back into the blood. Therefore inhibiting SGLT2 prevents glucose retrieval back to the blood, and is the target of several drugs which try to lower blood glucose in diabetics. The trade names for these drugs include Invokana, Farxiga and Jardiance.

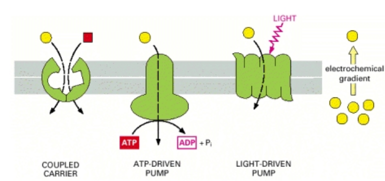

Figure 1: Examples of active transport. Reproduced from Alberts et al. [2002]

TRANSPORTERS THEMSELVES CAN BE REGULATED. This is often done by changing the number of transporters at the cell surface, or by changing the activity of the transporter. For example, in the case of GLUT4, insulin stimulates the translocation of GLUT4 from intracellular vesicles to the cell surface, increasing the amount of glucose that can be taken up by the cell. This is a common mechanism by which cells can rapidly respond to changes in nutrient availability. Other transporters can be regulated by changing their activity, for example by phosphorylation. See the section below Integrated Control of Metabolism by Regulation of Enzymes for more details on how this happens.

Enzymes

Thermodynamics

EVERY CHEMICAL IN THE BODY HAS A CERTAIN AMOUNT OF ENERGY. When we eat, some of this chemical energy is converted into ATP to allow for function. This is known as catabolism. When we are storing nutrients, we use ATP to generate higher energy molecules such as fats or glycogen. This is known as anabolism. Every molecule in our body has a set amount of energy and a chemical reaction can be considered endothermic (requiring energy) or exothermic (releasing energy), depending on whether the reactants or products have higher energy. The levels of these metabolites at equilibrium can be calculated with the following equation where K_{eq} is the *Eqiulibrium constant*[6]:

[6] The square brackets mean the concentration of A or B

$$K_{eq} = \frac{[B]}{[A]} \tag{1}$$

The equilibrium constant can be calculated from the *free energy* of the reactants and products.

$$\Delta G_o = G_o' - RT \ln K_{eq} \tag{2}$$

$$\Delta G_o' = G_o'(reactants) - G_o'(products) \tag{3}$$

Some reactions have products with very similar energy levels and the balance between the reactants and the products is based primarily on their concentrations. This is known as an *equilibrium* reaction which would have a K_{eq} of near to 1. If a reaction requires a lot of energy to occur, this is often an *irreversible* or *committed* step[7]. This means that once this reaction happens, there is no going back. If you think about the metabolic pathway in Figure 2, this would mean that once you proceed through step 2 to make C you cannot go back to B. Given the free energy (G_o') and concentration of the reactants and products in a reaction you can calculate the ΔG and equillibrium constant for a reaction and estimate whether it is reversible[8] under normal conditions.

[7] This would have a large, negative G_o

[8] There is a good blog post explaining how the steady state ΔG is determined on this basis at `http://sandwalk.blogspot.com/2007/10/aldolase-reaction-and-steady-state.html`

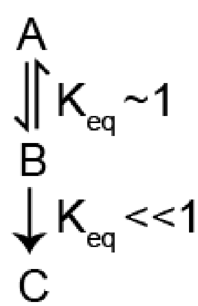

Figure 2: Example schematic of a metabolic pathway.

Enzyme Kinetics

Without enzymes, many reactions occur very slowly due to the *activation energy* needed for the reaction to occur. Enzymes increase the rate of a chemical reaction by reducing the activation energy required for a reaction to occur. This does not change the equilibrium constant, it just allows the reaction to reach equilibrium faster. This is sketched out in Figure 3, note that ΔG is not changed, but the dashed line has a higher activation energy, and therefore slower reaction rate than the solid line.

MOST METABOLIC PATHWAYS ARE CONTROLLED BY ALTERING THE RATES at which metabolites are converted to final products. The overall rate of a metabolic pathway is controlled by the *rate-limiting step*[9]. In a linear pathway, the speed of this step's enzymatic reaction controls the overall rate. Quite often the rate-limiting enzyme is an

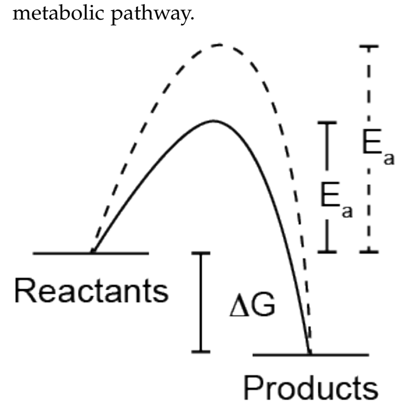

Figure 3: Example schematic of the activation energy (E_a) of an enzymatic

important point of regulation, as adjusting the speed of this reaction can speed up or slow down and entire pathway.

Reaction rates increase in rate as the concentration of substrates increase until the enzymes are saturated (see the solid line in Figure 4). This is known as Michaelis-Menten kinetics. The reaction rate constant (k) and rate can be calculated from the activation energy with these equations[10]:

$$k = Ae^{-\frac{E_a}{RT}} \tag{4}$$

$$rate = k\frac{[Reactant][Enzyme]}{[Reactant]+K_m} \tag{5}$$

If products build up the reaction becomes more complex and now looks like this where K_p is the binding constant for the product:

$$rate = k\frac{[Reactant][Enzyme]}{[Reactant]+K_m\left\{1+\frac{[Product]}{Kp}\right\}} \tag{6}$$

[10] A and R are constants, T is temperature and e is Euler's number. K_m is the Michaelis constant for an enzyme.

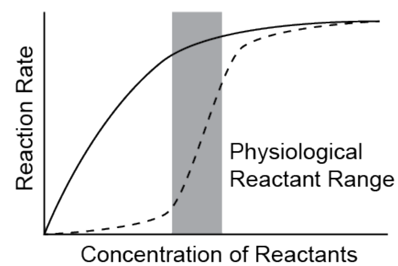

Figure 4: Example of Michaelis-Menten (solid line) and allosteric (dashed line) kinetics.

Allosteric regulation is another way by which enzymes can control reaction rates. Allosteric enzymes are generally multi-subunit enzymes that change their K*m* as more products bind. An example of this is the dashed line in Figure 4. This has several advantages in terms of regulation. One advantage is that the reaction rate can be effectively zero or at maximum in a much narrower range, bracketing the actual range of substrates present physiologically. Another advantage is that allosteric activators or inhibitors can shift the curve to the left or right, to effectively increase or decrease the reaction rate. This is a common mechanism by which the activity of rate-limiting enzymes are regulated.

On the basis of these equations, reaction rates (and the rate of a particular metabolic pathway) can be increased by several things[11]. Try to convince yourselves how this happens based on the equations listed above. Can you think of any other things that would affect pathway flux?

[11] Some examples include:

1. Decreasing the activation energy
2. Increase the amount of the reactants
3. Decrease the amount of the products
4. Increase the number of enzymes
5. Decreasing the K_m of the enzyme
6. Shifting the substrate sensitivity of the allosteric enzyme

While linear flow through a pathway is important, another aspect of pathway control is how the fate of a particular nutrient is decided. This is illustrated in Figure 5. In the example on top the nutrient would be equally distributed between three products, but in the bottom example, by adjusting the rates of the specific pathways, a nutrient can be directed to a particular product. At several points during this class, we will describe how the *fates* of particular metabolites are controlled by the relative rates of metabolic pathways.

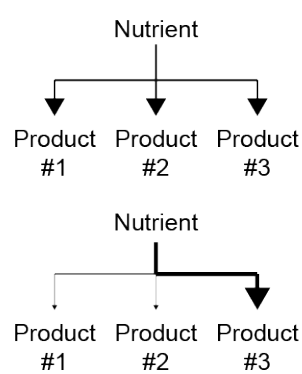

Figure 5: Example of how regulated pathways control nutrient fate.

Cofactors

Many enzymes require non-protein helper molecules to catalyze their reactions. These are known as *cofactors*. Table 1 lists some important cofactors, and the dietary vitamins from which they are derived. Other important roles of vitamins, for example in activating molecules like Co-enzyme A and electron carrier molecules like NADH will be described later in the semester. A lack of a dietary source of a cofactor can often impair the activity of an enzyme.[12]

[12] For example, a lack of vitamin B_1 (thiamine) can impair the activity of pyruvate dehydrogenase, which is important for converting pyruvate to acetyl-CoA. This can lead to a condition known as Wernicke-Korsakoff syndrome, which is characterized by confusion, ataxia and ophthalmoplegia. This is all because pyruvate dehydrogenase cannot function properly.

Table 1: Some examples of cofactors that are important for enzymatic catalysis.

Cofactor	**Source**
TPP	Vitamin B_1
Pyrdioxal Phosphate	Vitamin B_6
Biotin	Vitamin B_7
THF	Folic Acid
Iron	Dietary Fe
Selenium	Dietary Se

Isoenzymes, Compartmentalization and Tissue Specificity

ENZYMES CAN BE TISSUE SPECIFIC. This means that the same reaction can be catalyzed by different enzymes in different tissues. These are known as *isoenzymes* or *isozymes*. An example of this is lactate dehydrogenase, which has several isoforms that are expressed in different tissues. The liver isoform is more active at lower pH than the muscle isoform, which is more active at higher pH. This means that the liver can continue to produce glucose from lactate even when the blood pH is low, such as during exercise. This is an important concept to understand, as it allows for different tissues to have different metabolic priorities, and will be discussed in more detail later in the course.

CELLS ARE NOT JUST A BAG OF ENZYMES. They are highly organized structures with different compartments that have different functions. This is known as *compartmentalization*. For example, the mitochondria are the site of oxidative phosphorylation, while the endoplasmic reticulum is the site of protein synthesis and lipid metabolism. This allows for different metabolic pathways to occur in different parts of the cell, which can help to control the rates of reactions and prevent unwanted side reactions. Compartmentalization also allows for the separation of metabolic pathways that may otherwise interfere with each other, such as glycolysis and gluconeogenesis.[13]

[13] Try to think of another example of a metabolic reaction and how it might be compartmentalized to a specific tissue or a subcellular location.

Integrated Control of Metabolism by Regulation of Enzymes

What we do (or do not do) with nutrients is largely governed by the activity of transporters and enzymes. There are several ways in which enzymes are regulated, both based on intracellular and extracellular signals. An example might be that a lack of intracellular ATP causes an increase in ATP producing pathways such as glycolysis. On the other hand, low circulating blood glucose levels may work to stop a glucose consuming process such as glycolysis. We will discuss this in detail throughout the class, but some of the hormones we will discuss in this course that are particularly important are listed in Table 2:

Hormone	Main Function
Insulin	Reduces blood glucose and lipid levels
Adrenaline	Increases blood flow, nutrients to muscle
Glucagon	Increases blood glucose levels acutely
Cortisol	Increases blood glucose levels chronically
GH/IGF1	Promotes protein synthesis and bone growth
Testosterone	Promotes protein synthesis
Leptin,GLP1	Suppresses appetite
CCK, Gastrin, Secretin	Regulation of digestion

Table 2: Some important metabolic hormones we will discuss in this class.

Hopefully these hormones, how they work and how they are regulated is material you are familiar with from previous classes. If not, or you want a refresher, check out the **Endocrine Regulation of Macronutrient Metabolism** handout also available on Canvas.

Allosteric Regulation

As noted above, one way by which enzymes are regulated is by allosteric regulation. This is a common mechanism by which the activity of rate-limiting enzymes are controlled. Allosteric enzymes have multiple subunits and can change their conformation when a metabolite binds to them. This can either increase or decrease the activity of the enzyme, depending on the metabolite that binds. Allosteric regulation is often rapid, and can be reversed quickly, so it is a good way to control metabolic pathways in response to changes in substrate or product levels.[14] This means that when ATP levels are high, glycolysis is slowed down, but when ATP levels are low, glycolysis is sped up.

[14] An example of this is phosphofructokinase-1, which is allosterically activated by AMP and inhibited by ATP.

Post-Translational Modification

One common way by which enzymes are regulated is by the modification of existing proteins. One common example is protein phosphorylation. In this example a phosphate molecule is attached to an existing protein, which could increase or decrease its activity. This is often reversible, so a good analogy is that post-translational regulation is like flipping a switch for an enzyme on or off. This can occur fairly rapidly, and is not a permanent change.[15] This is a good way to rapidly change the activity of a pathway in response to a stimulus, such as low blood glucose levels or exercise.

[15] An example of this is that in response to glucagon or adrenaline, glycogen phosphorylase is phosphorylated, which increases its activity and allows for glycogen breakdown to glucose.

Transcriptional Regulation

Another way to change the activity of a pathway is to selectively change the number of enzymes. If this is done at the messenger RNA level, it is known as transcriptional regulation. This is because transcription is the process by which new mRNA is made. By increasing or decreasing the rate of mRNA (and eventually protein) production, the cell can respond to a stimulus to make more or less of a particular protein.[16] The regulation of transcription is often controlled by *transcription factors*, a class of proteins that can bind to selective sites of DNA and recruit the machinery to make (or prevent the making) of mRNA. For a really great review with more details on transcription factors, take a look at Lambert et al. [2018]. These kinds of changes are slow, energetically costly and difficult to reverse. They represent a chronic response, and are not appropriate for short term modifications. The relationship between allosteric, post-translational and transcriptional regulation is demonstrated in Figure 6. Reflect on an example of metabolic regulation that you can think of. Then consider the timescale by which the changes happen, and try to think what would be the most appropriate mechanism to alter metabolism.

[16] An example we will discuss in this course is that when stress levels are high, the brain responds by increasing appetite.

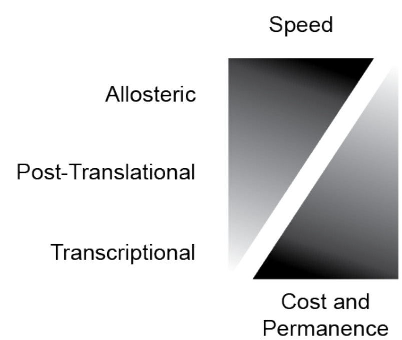

Figure 6: Shematic of the timing and permanence of some forms of enzymatic regulation.

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