BC 351 Exam 4 Study Guide

Lecture 12

1. What is homeostasis? What is the steady state? What is metabolic flux? How do these relate to one another? How do they relate to the control aspects of pathways? What enzymes will be regulated? How can this lead to regulation of an entire pathway?
Homeostasis is a cellular process in which a steady state is maintained, allowing the celling to respond to external stimuli. The steady state is one of non-equilibrium in which cellular components (i.e. metabolites, etc.) remain at more or less constant concentrations. Metabolic flux is the rate at which metabolites enter and pass through a pathway. These three things relate to each other in that the cell maintains homeostasis and the steady state so that it can adjust metabolic flux and metabolic pathways as necessary in response to stimuli. The enzymes that are regulated are the ones that catalyze irreversible reactions, which in turn controls the reversible ones that follow them.

2. What is an allosteric enzyme? How do these enzymes kinetics compare to the kinetics of the enzymes covered in lecture 7? What is the $K_{0.5}$? How can their activity decrease and increase?
An allosteric enzyme is one that undergoes a conformational change, resulting in an increase or decrease in activity. These enzymes exhibit a sigmoidal kinetics curve, rather than a parabolic one, which is an indicator of cooperative binding behavior and therefore means they can be regulated. The curve can be changed by adjusting $K_{0.5}$ or v_{max}. $K_{0.5}$ is a measure of the specificity of the enzyme for its substrate; in other words, it's the K_m value for allosteric enzymes; more specifically, it indicates the [S] required to reach ½ v_{max}. The activity of allosteric enzymes can be either negatively or positively modulated, resulting in either decreases or increases, respectively. Negative modulators decrease either the v_{max} for the reaction, by adjusting k_{cat}, or the specificity of the enzyme for its substrate, by adjusting $K_{0.5}$. Positive modulators will do the opposite.

3. What is a covalent modification? Which is the most common? Does it activate or inhibit enzymes? Besides covalent and allosteric regulation by what other means can enzymes be regulated?
A covalent modification is the covalent binding of some additional group to an enzyme to affect its activity. The most common form of this is phosphorylation, or the addition of a phosphate group, which is carried out by kinases within the cell. It can either activate or inhibit enzymes, though it usually inhibits them. One example, however, of activation due to covalent modification is the expression or muting of genes in DNA expression. Other means by which enzymes can be regulated include protein turnover (the degradation of existing enzyme or the production of more); protein sequestration (the removal/gathering of an enzyme in a compartment other than where it usually operates, thus separating it from its substrate); and regulatory protein binding (the attachment of some kind of modulator to either increase or decrease activity).

4. What is the energy charge? What is it a measure of? What should be happening to metabolic flux of catabolic/anabolic pathways as energy charge increases/decreases? How might the cell accomplish this increase/decrease in flux in response to energy charge? In other words what is the molecular process in which metabolic output can be integrated based upon energy charge?
Energy charge is a measure of the energy status, or ratio of ATP to ADP and AMP, within the cell. As energy charge increases, catabolic processes should decrease and anabolic pathways should increase; vice versa, as energy charge decreases, catabolic processes should be up-regulated while anabolic pathways should decrease in activity. The cell can achieve these changes because adenylyl compounds (ATP, ADP, AMP, etc.) are key modulators for most allosteric pathways, which means

that their respective concentrations can tell pathways which way to go in response to changing conditions of the cell, which is permitted by the maintenance of the steady state. In other words, the molecular process by which this occurs is allosteric regulation.

5. How do PFK-1 and FBPase-1 illustrate the principles of metabolic control specifically in regards to energy charge?
High concentrations of ADP and AMP tell the cell to increase the activity of PFK-1, a catabolic enzyme, while AMP indicates that there should be a decrease in FBPase-1 activity. On the other hand, high levels of ATP will tell the cell to decrease PFK-1.

6. What is glucagon/insulin a sign of? How do these hormones affect the PFK-1/FBPase-1 bypass reaction? What is the overall result, in terms of glycolysis flux and GNG flux in the liver cell in response to these hormones?
Glucagon is released by the pancreas in response to low blood glucose to tell the liver to release glucose, while insulin is secreted in response to high blood glucose to tell the liver to absorb it. The secretion of glucagon tells the liver cell, via binding to the membrane protein (NOT transporter) called a glucagon receptor to induce a conformational change, to release glucose. The glucagon receptor gives adenylate cyclase the "green light" to convert ATP to cAMP, which then activates the pathway in which protein kinase A (PKA) uses a phosphate group from ATP to phosphorylate the bi-functional enzyme PFK-2-FBPase-2 (two domains, NOT subunits – they have the same primary structure), which then activates the FBPase-2 domain. The active FBPase-2 then breaks F26BP down in F6P to remove the activator of glycolysis/the inhibitor of GNG, thus increasing GNG flux and decreasing glycolytic flux. With insulin signaling, the opposite occurs: the membrane protein insulin receptor, causing a conformational change, induces a series of complex enzyme-catalyzed reactions that activate the enzyme protein phosphatase (PP) to remove the phosphate group from PFK-2-FBPase-2, deactivating the FBPase-2 domain and activating the PFK-2 domain, resulting in the conversion of F6P to F26BP. The presence of F26BP results in activation of glycolysis and the inhibition of GNG. [Overall: **insulin → inc. glycolysis, dec. GNG;** *glucagon → inc. GNG, dec. glycolysis*]

Lecture 13

1. What is cellular respiration? What are its 3 phases? At what metabolite do all catabolic paths feed into?
Cellular respiration is the process by which cells consume O_2 and produce CO_2 and H_2O. The three phases are: 1) the production of acetyl-CoA, usually from pyruvate as a product of glycolysis; 2) the citric acid/TCA/Kreb's cycle; 3) the electron transport chain/oxidative phosphorylation. All catabolic paths feed into cellular respiration at the first phase, the production of acetyl-CoA, which is the "central point" for almost all metabolic pathways.

2. What is the end result of the PDH reaction? What does the production of NADH and CO2 tell us about this reaction? In other words what type of reaction is this? Where is some of the energy for this reaction conserved?
The PDH reaction is one of oxidative decarboxylation – it's a redox reaction (hence oxidative) in which a carbon is removed from a substrate as CO_2 (hence decarboxylation). So, the end result is that pyruvate is converted to acetyl-CoA and CO_2, a total of 2 moles per mole of glucose. The production of NADH indicates that it's a redox reaction, and the production of CO_2 indicates that it's a decarboxylation. The energy that's stored is conserved in the thioester bond that's present in acetyl-CoA.

3. Study carefully the irreversible steps in this pathway (citric acid cycle) and any step that results in the production of an electron carrier or some form of energy. Be able to recognize reduced or oxidized carbons.

Only three reactions are reversible: reaction 2 (aconitase), reaction 6 (succinyl DH), reaction 8 (malate DH)

Irreversible reactions (five total): reaction 1 (citrate synthase), reaction 3 (isocitrate DH), reaction 4 (alpha-KGA DH), reaction 5 (succinyl-CoA synthetase), reaction 7 (fumerase)

GTP from reaction 5 (succinyl-CoA synthetase)

FADH2 from reaction 6 (succinyl DH)

NADH from PDH, reaction 3 (isocitrate DH), reaction 4 (alpha-KGA DH), reaction 8 (malate DH)

- Reaction 1: citrate synthase – acetyl-CoA + OAA → citrate; the energy from the thioester bond in acetyl-CoA forces the reaction almost completely towards products, which greatly depletes [OAA] in mitochondrial matrix; induced fit enzyme, fits when acetyl-CoA and OAA are present
- Reaction 3: isocitrate DH – isocitrate + NAD+ → alpha-KGA + NADH + CO2
- Reaction 4: alpha-KGA DH – alpha-KGA + coenzymes + NAD+ → succinyl-CoA + NADH + CO2; thioester stores ~ 30kJ of energy derived from redox reaction
- **Reaction 5: succinyl-CoA synthetase – succinyl-CoA + GDP → succinate + GTP; energy supplied by thioester
- Reaction 6: succinyl DH – succinate + FAD → fumarate + FADH2; going from alkane in succinate to alkene in fumarate requires passing e- to something with higher affinity than NAD+ so it uses FAD
- **Reaction 7: fumerase – fumarate → L-malate
- Reaction 8: malate DH – L-malate + NAD+ → OAA + NADH; will only proceed if [OAA] = very low, so citrate synthase reaction is very necessary!

4. How many ATP equivalents will be produced in this pathway? How many ATP equivalents will be produced in this pathway if you assume that electrons will flow through the electron transport chain leading to ATP synthesis? How many carbon dioxide molecules were produced from 1 glucose if you start at glycolysis and end at the citric acid cycle?

From TCA cycle alone, starting with pyruvate and ending with OAA and assuming the oxidative phosphorylation via the ETC follows, 25 ATP equivalents are made (8 NADH = 20 ATP, 2 FADH2 = 3 ATP, 2 GTP = 2 ATP). Combined with glycolysis, a total of 30-32 are made, depending on which shuttle is used in the GAPDH reaction. Starting with glycolysis means that the PDH reaction occurs to convert pyruvate to acetyl-CoA before entering the TCA cycle, so a total of 6 CO2 molecules are produced per glucose molecule (2 from PDH, 2 from isocitrate DH, 2 from alpha-KGA DH; 3 per pyruvate, 2 pyruvates per glucose).

NADH per glucose: 2 from PDH, 2 from IDH, 2 from alpha-KGA DH, 2 from *malate DH (reversible)*

FADH2 per glucose: 2 from *succinate DH (reversible)*

GTP per glucose: 2 from succinyl-CoA synthetase

5. What citric acid cycle enzymes are regulated? How are they regulated? What about the PDH complex regulation?

The irreversible, and therefore regulated, enzymes in the citric acid cycle are: reaction one, citrate synthase (inhibited by ATP and NADH, activated by ADP/AMP); reaction three, isocitrate dehydrogenase (inhibited by ATP, activated by ADP/AMP and Ca2+); reaction four, alpha-KGA dehydrogenase complex (inhibited by NADH, activated by Ca2+); reaction five, succinyl-CoA synthetase; and reaction seven, fumerase. There are a total of five irreversible reactions, and three reversible (2, 6, 8) ones. The PDH complex reaction is also irreversible (inhibited by ATP and NADH, activated by ADP/AMP).