Chapter 23 – Metabolic Coordination, Control, and Signal Transduction

Overview of hormone action

The word “hormone” is derived from a Greek word meaning “to stir up” or “to excite”. In general, a hormone is a substance that is secreted from an organ that affects metabolic activities in tissues distant from the secretion site. Hormones can be divided into three general classes of molecules:

1. Peptides or polypeptides: examples include glucagon and insulin.
2. Steroids: examples include glucocorticoids and estrogen.
3. Amino acid derivatives: examples include dopamine and epinephrine.

Hormones generally stimulate metabolic activities by one of three mechanisms:

1. Enzyme activation or inhibition via second messengers, as we have seen with glucagon and epinephrine,
2. Stimulation of the synthesis of particular proteins through activation of specific genes, and

Your textbook has two very nice figures describing systemic hormone action and then second messengers used in signal transduction. Hormones are secreted as a result of environmental stimuli. The stimuli could be external to the body, such as a frightening experience. More often, the stimulus is internal, such as ionic strength, or a concentration of a hormone or metabolite. The stimuli are sensed by the central nervous system, which sends a message to the hypothalamus. The hypothalamus sends a message to the anterior or posterior pituitary, which sends stimulation factors to the organs that actually synthesize the hormone. For example, after a baby is born, a stimulus is recorded by the hypothalamus, which sends a message to the anterior pituitary to release prolactin, which travels to breast tissue and stimulates milk production, which the newborn can eat. In some cases, the organs that produce the hormone directly monitor the environmental agent. For example, there are receptors on the surface of pancreatic β cells that sense excess glucose in the blood; this leads to the production of insulin. Conversely, there are receptors on the surface of pancreatic α cells that sense low blood glucose, resulting in the production of glucagon.

Looking at the next figure, this describes the second messengers used by some receptor/hormone systems to send a hormonal message around a cell. The classic example are G-protein coupled receptors that lead to the activation of adenylate cyclase, generating cAMP.
Cyclic AMP will bind to and activate protein kinase A, which will phosphorylate protein substrates to activate the metabolic result intended from the hormone. Other common second messengers include cGMP, calcium ions, and diacylglycerol.

**G protein coupled receptors – Rhodopsin and vision as a model**

Let’s look in detail at one of the first second messenger systems characterized in detail. Vision occurs in organisms via specialized cells called photoreceptor cells. There are several different types of photoreceptor cells in nature. In our eyes, we have two types of photoreceptor cells, the **rod** cells and the **cone** cells. The rod cells have the ability to sense light and pass that information on to the brain. The rod cells work in dim light and are very sensitive to light. The cone cells work in brighter light, and provide our perception of color. These cells are found in an organ at the back of the eye called the retina.

Your eye is a more or less spherical organ comprised of several distinct tissues. On the outside of the eye is the cornea. The cornea provides protection against outside insults (dust, etc.), and is simply a clear covering over the center of the eye. Underneath the cornea is the lens. The lens collects light and focuses it to the retina, at the back of the eye. When light hits the retina, it is collected by light receptors in the photoreceptor cells and a message is sent to the brain via the optic nerve.

In the rod cells, the light receptor protein is rhodopsin. Rhodopsin is comprised of a protein called opsin and a visual pigment, 11-cis-retinal. The structure of 11-cis-retinal is shown on the Power Point slide. Rhodopsin is a transmembrane protein, meaning that parts of the protein extend out from both sides of the bilayer where it lives. Models have predicted that seven pieces of the protein pass through the rod outer segment membrane, the membrane in the rod cell where the rhodopsin is located. The retinal, being a fairly hydrophobic molecule, is located approximately in the middle of the bilayer. There is some carbohydrate attached at one end (the little circles sticking off of the amino terminal end) and some hydroxyl amino acids (either serine or threonine) at the carboxyl terminal end. Two fatty acids are also attached to amino acids of opsin.

When light hits the cis-retinal, it undergoes a photochemical isomerization to trans-retinal. The terminal aldehyde of the retinal is connected to the amine group of a lysine in the protein by a Schiff’s base linkage (nitrogen-carbon double bond).

When a photon of light is absorbed by 11-cis-retinal, the molecule is converted from its
ground state to an excited state. In its ground state, there is no rotation about the 11-cis double bond. Similarly, there is no rotation about the 11-trans double bond in the product all-trans retinal. However, in the excited state, there is virtually no energy barrier to rotation about the 11-double bond. Upon excitation, the excited cis-rhodopsin can convert either to cis-rhodopsin or trans-rhodopsin upon relaxation. The retinal decays to the trans form about 2/3 of the time, and to the cis form about 1/3 of the time.

The conversion of retinal from 11-cis to all-trans results in other changes in the protein as well. After the retinal is converted to the all-trans form and dissociates from the protein, the rhodopsin alters its three dimensional shape. At the transmembrane sequences, the movement is small, but this translates into much more dramatic conformational changes outside of the membrane.

What is the result of the conformational change of rhodopsin? First and foremost, the excitation of retinal and its isomerization and dissociation causes a closure of sodium ion channels in the plasma membrane of the rod cell. The rod cell is divided into two parts, called the outer segment and the inner segment. The outer segment is involved in photoreception and not much else. The nucleus, ribosomes, mitochondria, etc., the stuff normally associated with cells, is found in the inner segment. Now, the plasma membrane of the inner segment contains the sodium-potassium ATPase, which will pump sodium ions out of a cell and potassium ions in against a concentration gradient. The sodium-potassium ATPase uses the chemical energy in ATP to pump sodium out of the rod cell and potassium into the rod cell against a concentration gradient. Potassium ions can diffuse back out of the inner segment relatively easily, but the return of sodium ions back inside of the cell through the inner segment plasma membrane cannot. The result is the generation of a membrane potential; there is a difference in the ion concentration on the inside and outside of the inner segment plasma membrane, which result is differing abilities of the solutions of the two sides of the membrane to conduct current, which results in a membrane potential. The developed potential is 20 mV, negative inside (the outside is positive because there are excess sodium ions on the outside relative to the inside). This membrane potential is not particularly high, because sodium ions can return to the cytoplasm through specific sodium channels found in the plasma membrane of the rod outer segment. The sodium ions can then diffuse through the cytoplasm back to the inner segment.

When rhodopsin is excited and undergoes its conformation change, one result is the
closing of some of the sodium channels in the outer segment plasma membrane. This results in a hyperpolarization of the rod cell (a sudden increase in the membrane potential). The hyperpolarization is potentiated as a nerve impulse, which is sent to the brain via the optic nerve and interpreted there. This effect is graded; the more rhodopsin molecules that are excited (up to about 100), the greater the hyperpolarization and the greater the nerve impulse.

**Rhodopsin as a G protein receptor**

How does a conformational change in rhodopsin, which is found in the outer segment disk membranes, result in closing of sodium channels in the plasma membrane? It was discovered experimentally that cyclic guanosine monophosphate (cGMP) is required to maintain the sodium permeability of the outer segment plasma membrane. If the cGMP is depleted from the cell; the permeability decreases; if replenished, the permeability increases. Here is the structure of cGMP (overhead):

The outer segment disk membranes contain another enzyme called a phosphodiesterase. A phosphodiesterase is an enzyme which hydrolyzes phosphodiester bonds, and a phosphodiester bond is a phosphate group esterified to two alcohols. cGMP contains a phosphodiester. Upon illumination, the activity of the phosphodiesterase in the outer segment disk membranes increases, causing the hydrolysis of cGMP and a lowering of its cellular concentration. The lowered cytoplasmic cGMP concentration lowers the sodium conductivity across the outer segment plasma membrane, resulting in hyperpolarization.

The next transparency describes a typical G-protein receptor and its activation cycle. The glucagon receptor is one typical example of a G-protein coupled receptor. In the resting state of the receptor, the receptor exists as a complex between the receptor, a GDP binding protein, and two other subunits labeled beta and gamma. When a hormone binds to the receptor, the receptor undergoes a conformational change, which causes the G protein to undergo a conformational change, which changes the affinity of the G protein for guanine nucleotides. The bound GDP is replaced by a molecule of GTP. The exchange between GTP and GDP causes the G protein and gamma/beta subunits to dissociate from the receptor and diffuse along the surface of the membrane. The G protein, with its bound GTP, encounters another protein called adenylate cyclase. The binding of the G protein to adenylate cyclase stimulates the cyclase, which converts ATP into cAMP. This cAMP diffuses through the cytoplasm and activates
another type of protein called a kinase. The kinase catalyzes the transfer of a phosphate group from ATP to some specific protein. Protein phosphorylation will ultimately lead to the cellular response. The G-protein has a slow GTP hydrolysis activity associated with it, so that the bound GTP will slowly hydrolyze to GDP. After GTP hydrolysis, the G protein will have GDP bound, recollect the beta and gamma subunits, and return to bind to a new receptor molecule.

With rhodopsin, a similar but not identical course of events seems to occur after stimulation with a photon of light. In the case of rhodopsin, the G protein and the beta and gamma subunits which I just described are called by a single name, transducin. Transducin has three subunits, the alpha subunit (the G protein), the beta, and gamma subunits. In the dark, transducin contains one molecule of bound GDP and exists as three associated subunits noncovalently bound to the rhodopsin protein. Upon stimulation with light, the alpha subunit undergoes a conformational change, GTP exchanges with GDP in the binding site of the alpha subunit, and the alpha subunit dissociates from the beta and gamma subunits. For the epidermal growth factor receptor, the alpha subunit remains associated with the plasma membrane, but with the alpha subunit of transducin, the conformational change increases the water solubility of the subunit, and it diffuses away from the outer segment disk membrane. The alpha subunit associates with a cytoplasmic phosphodiesterase, converting it from an inactive to an active form. The phosphodiesterase specifically hydrolyzes cGMP to GMP. As the cytoplasmic cGMP concentration decreases, cGMP will dissociate from sodium channels on the plasma membrane, where the cGMP helps them to stay open. After cGMP removal, the channels close, resulting in membrane hyperpolarization.

For your information, the reason that the accessory proteins have different names is that they were discovered at essentially the same time in different laboratories (the rhodopsin system was understood first, then the other G protein receptors). People get somewhat egotistical, and want to claim discovery of a protein or system by naming it themselves. A unified nomenclature system always occurs long after the discovery of the items needing systematic names.

There is one other response of the rod cell to light. The metarhodopsin II state of rhodopsin can be phosphorylated at the hydroxyl groups at the carboxy terminal end. After phosphorylation, the phosphorylated rhodopsin can bind to a protein called arrestin, which keeps the rhodopsin in an inactive form for an extended time. This allows the rod cell to adapt to conditions which are very bright, leading to a decrease in the sensitivity of the rod cell. This type
of adaptation is not unusual among G-protein coupled receptor pathways.

For the rhodopsin to regenerate, the cell must replenish its supply of cGMP. This reaction is carried out by an enzyme, guanylate cyclase, which converts GMP into cGMP (see Power Point slide):

Guanylate cyclase is strongly inhibited by calcium. After the light response, when the sodium channels are blocked, there is another mechanism by which sodium can return to the cytoplasm. There is a transport protein in the plasma membrane of the rod outer segment that will exchange sodium ions for calcium ions. As sodium ions get back into the rod cell, calcium ions are exported, resulting in a decrease in the cytoplasmic calcium concentration. This stimulates the guanylate cyclase, generating more cGMP and reopening the sodium channels. There are protein phosphatases in the cell which will remove the phosphate groups from the rhodopsin, regenerating opsin, which can bind to 11-cis-retinal and reassociate with the transducin, allowing the complex to respond to another photon of light.

The receptors for glucagons and epinephrine are both G-coupled protein receptors that work in the manner I just described.

G proteins are targets for some disease processes as well. A bacteria called *Vibrio cholerae* is responsible for the disease cholera. Cholera is characterized by uncontrolled diarrhea, and people die from cholera primarily due to dehydration. The cholera bacterium produces a toxin called the cholera toxin that gets into cells and catalyzes an enzymatic reaction. It catalyzes the transfer of an ADP-ribose group from NAD to the α-subunit of the G protein. This irreversibly activates the G protein. In the intestinal epithelial cells, activation of this G protein activates the secretion of water and sodium ions from the cells into the intestine. This causes the diarrhea, and is also the cause of the dehydration. The whooping cough bacterium, *Bordetella pertussis*, causes a similar activation of a different G protein. More specifically, the pertussis toxin causes the irreversible activation of an inhibitory G protein. There are two types of G proteins – activating and inhibitory – the activating ones lead to protein activation (such as adenylate cyclase) and the inhibitory ones inhibit adenylate cyclase or other target protein. This manifests itself by lowered blood glucose and a hypersensitivity to histamine, which leads to the characteristic cough associated with the disease. I just read in the newspaper this week that whooping cough is experiencing a resurgence, and many physicians are advocating the re-immunization of patients against whooping cough as adults.
In the case of glucagon and epinephrine, the activated G protein binds to adenylate cyclase, increasing the cellular concentration of cAMP. The cAMP acts on a protein kinase, stimulating its activity and generating phosphorylated proteins that are either activated or inhibited due to their phosphorylated state.

Other activated G-proteins work in different ways. A common target protein for an activated G-protein is a phospholipase, either phospholipase A2 or phospholipase C. Phospholipase A2 will hydrolyze the central fatty acid from a phospholipids, usually phosphatidylinositol. This free fatty acid can be used to synthesize other molecules. In many cases, the fatty acid is arachidonic acid; this is used to synthesize prostaglandins or other eicosanoids.

Here is a figure from Voet and Voet that describes how the binding of a hormone to a G-coupled protein receptor can lead to activation of a phospholipase. Upon hormone binding, the activated G protein binds to a membrane-associated phospholipase C. This phospholipase C will act upon phosphatidylinositol, specifically phosphatidylinositol where the inositol group is phosphorylated at positions 4 and 5. The products of the phospholipase reaction are 1,4,5-inositol tris-phosphate and diacylglycerol. The inositol tris-phosphate diffuses in the cell and opens calcium channels in the endoplasmic reticulum, increasing the cellular calcium concentrations. This activates a series of calcium-dependent enzymes. The diacylglycerol will diffuse through the plasma membrane and activate membrane associated protein kinases, leading to protein phosphorylation and activation.

**Receptor Agonists and Antagonists**

Many drugs work by binding to cellular receptors and interrupting normal hormone binding. If we are working with enzymes, small molecules that binding to enzymes and decrease their activity are called **inhibitors**, while small molecules that bind to enzymes and increase their activity are called **activators**. Molecules that bind to receptors have similar classifications. If a molecule binds to a hormone receptor and mimics the activity of the normal hormone, this molecule is called a hormone **agonist**. If the molecule binds to the receptor that does not provoke the normal hormone response, this is called a hormone **antagonist**. Your textbook gives a nice example of two drugs that bind to receptors that would normally bind epinephrine. Isoproterenol is an epinephrine analog that acts as an agonist when bound to one class of epinephrine receptors (called **adrenergic receptors**). Isoproterenol is a drug used to treat
asthma, and the affected receptor is in the bronchial muscles in the lung. The drug propranolol is an antagonist of adrenergic receptors in blood vessels; this helps to control blood pressure and heart rate.

Tyrosine Kinase-based signaling

In addition to G-protein coupled receptors, there is a second class of receptor that I would like to introduce—these are called receptor tyrosine kinases. As you recall, a kinase is an enzyme that transfers a phosphate group from ATP onto some other molecule. A tyrosine kinase is an enzyme that transfers the phosphate group onto a tyrosine –OH amino acid side chain on a protein. Examples of receptor tyrosine kinases include growth factor receptors and the insulin receptor. They are also involved in cancer, as we’ll get into in a bit.

Receptor tyrosine kinases work a little differently than the G-protein coupled receptors. The binding of the hormone to the receptor tyrosine kinase will, in general, cause a conformational change in the receptor that will induce receptor dimerization. That is, two molecules of the receptor will come together and form a dimer. Upon dimerization, the tyrosine kinase activity of the receptor becomes activated, and the receptors will phosphorylate tyrosine residues on the receptor, using ATP as the phosphate donor. The active receptor can then activate specific target enzymes, resulting in the cellular response.

To get some idea of how the target enzymes are activated, let’s take a look at the structural characteristics of two different receptor tyrosine kinases, the receptor for Platelet-Derived Growth Factor (PDGF) and Epidermal Growth Factor (EGF). For the PDGF receptor, the structure can be divided into several distinct domains. You might recall that a protein domain is a part of a protein that is structurally distinct from other parts of the protein, and which usually also performs a distinct function. Going from top to bottom, the first domain that we see is labeled the SH2 domain. Let’s take some time to look at this type of domain in detail.

The term “SH2” is short for Src Homology 2, meaning that the amino acid sequence and presumably the structure is similar to domains found in a cytoplasmic tyrosine kinase named Src. SH2 domains bind to phosphotyrosine amino acid residues on the receptor. The reason that these phosphotyrosine groups bind to the SH2 domains of associated proteins is that the tyrosine side chain fits into a pocket in the structure of those proteins. This pocket is too long for phosphoserine or phosphothreonine amino acids to totally fit into it, providing binding
specificity.

A second domain common to many proteins is the SH3 domain, analogous to another domain found in the cytoplasmic src protein. This domain binds proteins that are rich in proline amino acids, specifically those containing the motif pro-x-x-pro, where x is any amino acid.

Going back to the receptor diagram, the next domain is called the tyrosine kinase domain. This is the part of the receptor that carries out the transfer of a phosphate group from ATP to tyrosine amino acids. The tyrosine amino acids are part of the receptor itself; on this diagram, Y is the one letter abbreviation for tyrosine. Specific proteins bind to specific phosphotyrosine residues on the receptor; binding to these residues activates the proteins and increases their activity.

Let’s take a minute to decipher the alphabet soup of proteins bound to the PDGF receptor. Src is a cytoplasmic protein kinase that will phosphorylate other proteins – these stimulate cell growth and division. The name src is derived from sarcoma virus; the protein was first discovered in virus-infected cells. PI3 kinase will add phosphate groups to the alcohols on the headgroup of phosphatidylinositol. RasGAP accelerates the hydrolysis of GTP to GDP while the GTP is bound to Ras. Ras is a special G protein that when stimulated sends a message to the nucleus of the cell to transcriptional machinery in the nucleus, promoting the transcription of some genes and turning off the transcription of other. We’ll examine this in a little more detail later. SHP-2 is a protein tyrosine phosphatase; it will remove phosphate groups that are covalently bound to protein tyrosine residues. These bind to growth factor receptors and will remove phosphates from the phosphotyrosines on the receptors, removing their activation. Finally, the protein PLC-γ1 is a specific type of phospholipase C that is activated by growth factors.

How about the EGF receptor? There is a phospholipase C attached to this receptor, along with Grb 2 and Shc. Grb 2 is an adaptor protein. It binds to an activated (phosphorylated) receptor tyrosine kinase, while at the same time binding to another protein named Sos (for Son of Sevenless, named from a drosophila mutation), which in turn binds to Ras. Again, Ras is involved in a chain that leads to changes in transcription. Shc is another adaptor protein; it links the activated receptor tyrosine kinases to Ras.

Cancer

I want to take just a few moments to look at Figure 19-38 from another textbook (Voet
and Voet) to begin to understand some of the processes that might lead to cancer. Cancer is a disease where groups of cells grow in an uncontrolled manner. Cancers can either be metastatic or nonmetastatic, where metastatic cancers are more dangerous. In metastatic cancers, the original tumor will shed cells, where they may be transported around the body and eventually implant into an organ distant from the original target organ, leading to additional tumors. For many cancers, the spread of the cancer is often assessed by testing the lymph nodes adjacent to the cancerous tissue. If cells are metastasized from the tumor, they will likely circulate through the body in the lymph system, and the first place that they might implant are the lymph nodes. If the lymph nodes contain no cancerous tissues, it is likely that the tumor has not spread. However, if cancerous cells are found in the lymph nodes, this is a sign that the tumor was metastatic and more dramatic methods of treatment are needed in order to bring the patient back to complete health.

Almost all cancers begin with the transformation of a single cell. A cancerous cell is characterized by rapid and uncontrolled cell division. In some ways, the cancerous cell reverts back to its form before it was differentiated. That is, we begin as embryos containing cells that rapidly divide into a larger and larger cell mass. After some cycles of division, the cells start to differentiate, forming tissues and organs. A cancerous cell reverts back to the rapidly dividing form, generating a growing mass of cells. The mass may interfere with the normal function of the organ or tissue where it is found, and eventually will cause the organ to fail, resulting in nasty consequences.

Let’s now take some time to understand Figure 19-38. When a receptor tyrosine kinase is activated, the result is dimerization and the generation of phosphotyrosine residues on the cytoplasmic side of the receptor. The phosphotyrosine residues and their surrounding amino acids are binding sites for proteins that will be activated. The shape of the protein that binds to the phosphotyrosine residues is called the SH2 domain, because it is analogous to the second domain on the cytoplasmic protein kinase called src. To get some idea about protein binding, let’s look at the structure of one protein that binds to phosphotyrosine residues, namely the protein grb-2.

Grb-2 is a three domain protein comprised of two SH3 domains and 1 SH2 domain. SH3 domains, as you recall, bind to proteins with the amino acid sequence pro-x-x-pro. The two SH3 domains are identified depending upon whether it is found on the C-terminus or N-terminus of
the Grb-2 protein. In the figure, the SH2 domain of Grb-2 binds to the receptor, while the two SH3 domains bind to another protein called sos. Sos is the son of sevenless protein – it is analogous to a drosophila protein that is necessary for the proper development of the photoreceptor VII cell. In its activated form, when it is associated with Grb-2, sos will bind to another protein called Ras that is imbedded into the cytoplasmic side of the plasma membrane. When sos binds to Ras, this catalyzes the exchange of GTP for GDP that is found bound to Ras in its resting state. The GDP to GTP exchange activates Ras and allows it to carry out other reactions.

Ras is a very important protein in terms of the activation of a variety of different cellular processes associated with cell growth and differentiation. Therefore, regulation of its activation and inactivation is also extremely important. Activation of Ras is accomplished by Sos, and its inactivation is catalyzed by a protein called GAP. When GAP binds to RAS, it accelerates the hydrolysis of the GTP bound to Ras, converting it back into its inactive form.

What does Ras activate? Ras activates a number of different proteins, but the one that we will focus on is Raf. Raf is a protein kinase that phosphorylates serine or threonine hydroxyl groups on other effector proteins. One protein in particular that is phosphorylated is MEK, also called mitogen-activated protein kinase kinase (or MAP kinase kinase for short). MEK will then phosphorylate MAP kinase (MAPK on the diagram), which is a kinase that will phosphorylate several different transcription factors including Fos, Jun, and Myc. These transcription factors, upon phosphorylation, bind to DNA and promote the transcription of specific genes.

MUTATIONS LEADING TO CANCER AND VIRAL CANCERS.

With this understanding of how growth factors activate transcription, which leads to increased protein production, cell growth, and cell division, let us return to look at cancer and how certain types of viruses or mutations will result in cancer. First, let’s look at virally induced cancers, such as the Rous sarcoma virus. This is a chicken virus discovered by Peyton Rous that generates tumors when a cell-free extract is injected into a chicken. The Rous sarcoma virus is a retrovirus, meaning that it uses RNA as its genetic material. After infecting a cell, the RNA is converted into DNA by reverse transcriptase, and the viral DNA is integrated into the genomic DNA.

Rous sarcoma virus DNA encodes for a protein that is named v-src, which is analogous to a src protein common to all cells and which is important for cell growth and proliferation.
However, v-src is not regulated in the same manner as c-src. Proteins infected with v-src have their cell proliferation machinery constantly turned on, resulting in uncontrolled cell division and tumor formation.

Other types of viruses encode proteins that are again analogous to proteins along this proliferation cascade, but which are not under normal cellular control. Examples include v-ras, v-jun, and v-fos. These proteins again promote cell division and proliferation, but are not under normal cellular control.

In a similar way, mutations can also cause problems with this cell proliferation pathway leading to cancer. Mutations to Ras are fairly commonly observed in cancers; these mutations make Ras insensitive to GAP proteins and hence remain in an activated state more often than normally. Clearly, mutations to other proteins along the pathway may also have harmful effects.

**Protein Kinase C’s**

In the phosphoinositide cascade, an activated G protein will in turn activate phospholipase C, leading to the generation of inositol-1,4,5-triphosphate and diacylglycerol. The diacylglycerol is an activator of a different protein kinase, protein kinase A. Let’s take a brief look at protein kinase C’s before we leave chapter 19.

Protein kinase C are a family of eleven different isozymes in three families. There are three domains in the structure of protein kinase C. The C1 and C2 domains are used to bind protein kinase C to the cytoplasmic surface of the membrane, and the third domain is a catalytic domain. Again, the first step in the activation of protein kinase C is the hydrolysis of the membrane phospholipids phosphatidylinositol into inositol-1,4,5-triphosphate and diacylglycerol. In the resting state, the protein kinase C is loosely bound to the cytoplasmic surface of the plasma membrane. The inositol triphosphate will (among other things) activate a membrane enzyme, protein kinase-1, which will phosphorylate protein kinase C at thr-500. This initial phosphorylation step begins the activation of protein kinase C. After thr-500 is phosphorylated, protein kinase C undergoes an autophosphorylation step, with phosphorylation occurring at thr-641 and ser-660. The phosphorylation causes the protein kinase C to dissociate from the membrane, but the protein remains inactive because the amino terminal end of the protein becomes bound to the enzyme active site. The protein kinase C will migrate back to the plasma membrane and bind to the membrane surface, with binding caused by diacylglycerol (generated from the breakdown of phosphatidylinositol) and calcium ions. The calcium ions
come from the endoplasmic reticulum. The inositol-1,4,5-triphosphate will diffuse through the cell and open calcium channels in the endoplasmic reticulum, causing an increase in cytoplasmic calcium concentrations and binding of protein kinase C to the plasma membrane. The protein kinase C active site is now open and available to phosphorylate target proteins, which as in the case of receptor tyrosine kinases, are often involved in cell proliferation.

Phorbol esters are one of the most potent carcinogens around; the structure of a phorbol ester is shown on the next overhead. It looks a little like diacylglycerol, and the protein kinase C thinks so much more than we do. Phorbol esters bind much more tightly to protein kinase C than does diacylglycerol, and as a result, protein kinase C remains in an active state, leading to uncontrolled cellular proliferation and tumor generation.

**Insulin**

Finally, just to drive home the point that insulin is a very difficult hormone to understand and that it is responsible for many different cellular functions/reactions, this last overhead describes insulin function. Let’s take a look at this picture to see if we can interpret exactly what is going on here. Let’s begin on the left side, which should look familiar. What do you think the function of the following proteins might be?

- Shc
- Gab-1
- pY
- SHP-2
- Grb2
- Sos
- Ras
- Raf1
- MEK
- MAPK
- Myc/Jun/Fos
- P90 (heat shock protein)
- APS/Cbl (adapter protein containing plekstrin homology and src homology-2 domains/ Cbl is a SH2/SH3 docking protein that is a proto-oncogene product)
- PI3K
CHAPTER 23 – METABOLIC COORDINATION, CONTROL, AND SIGNAL TRANSDUCTION

For the final few classes, I would like to take the opportunity to review a bit the metabolic pathways we've discussed throughout the semester, and the relative importance of all of them. Now that we've seen all of the details of each pathway, we finally have sufficient knowledge to make this assessment. After this review, I would like to discuss how the action of several specific hormones activates or inactivates specific enzymes in key pathways in order to cause their desired effects.

First, let's take one more look at the more important biomolecules involves with most of the pathways we've discussed.

1. ATP. All of the pathways we've talked about this semester have involved ATP. The catabolic pathways resulted in the net synthesis of ATP; the anabolic processes utilized ATP for synthetic purposes. Therefore, ATP acts in our cells as a simple energy carrier. ATP is analogous to the furnace in our homes.

   To say that glucose provides energy for biosynthetic reactions is equivalent to saying oil keeps us warm. We don't stay warm by smearing ourselves with oil; we must burn it in an intermediary device, which is our oil furnace. Glucose is the fuel burned by our cells; the energy is transported to processes which need it by the action of our chemical heating ducts, ATP.

   ATP is primarily generated in our bodies by oxidative phosphorylation. Oxidative phosphorylation is an energetically efficient way to capture the energy stored in covalent bonds as ATP. Note that many types of cells do not use oxidative phosphorylation to any great extent. Red blood cells, for example, lack mitochondria, which means that they do not have any of the enzymes responsible for the citric acid cycle or oxidative phosphorylation. The difficulty with oxidative phosphorylation is that the process requires oxygen, which for many organisms doesn't fit into their life style.

2. NADH and NADPH. NADH is important because it efficiently carries electrons derived from carbon-containing compounds to the electron transport chain to be converted into ATP. In a sense, NADPH is more important, because its function is more diverse. NADPH provides
reducing equivalents for many biosynthetic reactions in our cell. Therefore, the production of
NADPH is crucially important to allow biosynthetic processes in our cells to occur. Most of the
NADPH production in our cells results from the pentose phosphate pathway, which requires
glucose-6-phosphate as a necessary substrate.

3. **Carbohydrate based biosynthetic precursors.** Just what molecules have we discussed this
semester act as precursors for the synthesis of larger molecules?

**Dihydroxyacetone phosphate** - glycerol synthesis for glycerolipids.

**Acetyl-CoA** - The most important biosynthetic precursor. From this humble beginning fatty
acids, cholesterol, ketone bodies, and some amino acids are formed.

**Citric acid cycle intermediates** - oxaloacetate leads to amino acids and glucose, citrate provides
a source of cytoplasmic acetyl-CoA, α-ketoglutarate is converted to glutamate, important in
many biosynthetic processes, succinyl-CoA is the starting molecule for porphyrin synthesis.

Another point to emphasize in review is that the degradative and biosynthetic pathways
leading to and from a biomolecule are always enzymatically distinct. Different enzymes perform
key steps in the opposing pathways. This is important, because it allows for regulation of both
synthetic and degradative pathways apart from mass action.

What do I mean? Let's look at the reaction carried out by **phosphoglucone isomerase.**
This enzyme converts glucose 6-phosphate to fructose 6-phosphate. The enzyme is fully
reversible; the standard free energy change associated with the reaction is +0.4 kcal/mol, which
results in an equilibrium constant of 2 to 1 in favor of glucose 6-phosphate. If you put 1 mole of
glucose 6-phosphate in contact with this enzyme and allow the reaction to go to equilibrium, you
will get 0.67 moles of glucose 6-phosphate and 0.33 moles of fructose 6-phosphate. If you add 1
mole of fructose 6-phosphate instead, you'll get exactly the same result.

The forward and reverse reactions with this enzyme are controlled by mass balance. If
all of the enzymes in the glycolysis/gluconeogenesis pseudocycle behaved like this, the concen-
trations of all of the intermediates would solely result from the concentrations of initial
substrates and the equilibrium constants of the enzymes in the pathway.

Using two enzymes to catalyze the forward and reverse reactions at important steps in a pathway overcomes this obstacle. For example, in glycolysis, phosphofructokinase catalyzes the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate, while fructose 1,6-bisphosphatase catalyzes the dephosphorylation of fructose 1,6-bisphosphate to fructose 6-phosphate. The two enzymes are reciprocally controlled; when one is activated, the other is inactivated. This allowance for independent control allows our cell more flexibility in determining how its foodstuffs are used to the well being of the cell.

RECURRING MOTIFS IN REGULATION

How does our cell accomplish regulation? Let's review a few common methods:

1. **Allosteric control of enzyme activity.** Simple enzyme inhibition usually requires a substrate-like molecule. Allosteric inhibition involves interaction of an effector at a site different from the enzyme active site. This site could be specific for molecules with any different type of structure. Also, multiple sites might exist, with affinities for different molecules - this is how both ATP and citrate can inhibit phosphofructokinase. Finally, activation of an enzyme is difficult to obtain using a simple active site interactions, but allosteric activation is easily explained and a commonly used regulatory mechanism in cells.

2. **Covalent modification of enzymes.** The method of covalent modification most familiar to us is phosphorylation, such as the phosphorylation of glycogen phosphorylase. Covalent modification provides a faster method of regulation than allosteric effects; the enzyme is turned on or off as fast as a kinase or phosphatase can act upon it.

3. **Enzyme levels in cells.**

4. **Compartmentalization of enzymes.** What compartmentalization does is that it adds intercompartmental transport as a factor in regulation. For example, fatty acid synthesis occurs in the cytoplasm, while fatty acid degradation occurs in the mitochondrial matrix. Newly synthesized fatty acids are not degraded immediately after synthesis not because the degredative enzymes are inhibited, but because the transport mechanism which brings fatty acids from the cytosol to the mitochondrial matrix is inhibited.

5. **Metabolic specialization of organs.** Some metabolic pathways are allowed in some cells; others are not allowed. We'll discuss this concept in a bit.
MAJOR CONTROL SITES IN METABOLIC PATHWAYS - A REVIEW

1. **Glycolysis** - The pathway converts glucose to two moles of pyruvate, with production of two ATP and two NADH. The pathway required NAD+ to carry out the glyceraldehyde 3-phosphate dehydrogenase reaction.

   Phosphofructokinase is the primary site of regulatory control. ATP inhibits phosphofructokinase. Citrate enhances ATP inhibition. ATP inhibition is reversed by AMP.

   In liver, the most important regulator of the enzyme is fructose 2,6-bisphosphate. This is formed as the result of hormonal stimulation, which we will discuss shortly.

2. **Citric acid cycle** - The oxidation of one molecule of acetyl-CoA through the citric acid cycle results in the generation of three molecules of NADH and one molecule of FADH₂. These two reduced cofactors then reoxidize, donating their electrons to molecular oxygen to form water, with concurrent synthesis of ATP. NADH and FADH₂ are oxidized only when ADP is present to allow for ATP synthesis. The point - when no ADP is around, the mitochondrial concentrations of NADH and FADH₂ build up, inhibiting the citric acid cycle. This tight coupling between cofactor oxidation and ATP synthesis is called respiratory control.

   High concentrations of NADH inhibit the enzymes isocitrate dehydrogenase, alpha ketoglutarate dehydrogenase, and malate dehydrogenase. In addition, high concentrations of ATP also inhibit citric acid cycle enzymes, specifically citrate syntase, and the first two dehydrogenases above.

   A final level of regulation in the citric acid cycle is the concentration of cycle intermediates. Gluconeogenesis will draw malate from the cycle, decreasing the net concentration of intermediates and thus slowing the rate of flux of acetyl-CoA through the cycle.

3. **Pentose Phosphate Pathway** - This pathway is primary used for the generation of NADPH for biosynthetic purposes. Therefore, the major regulatory mechanism used by the pathway is the ratio of NADPH/NADP+. You might note that the ratio of NADPH/NADP+ is separate from the ratio of NADH/NAD+, meaning that both glycolysis and reductive biosyntheses can occur at high rates at the same time, when lots of glucose is present.
4. **Gluconeogenesis** - Gluconeogenesis and glycolysis are reciprocally regulated. When one pathway is turned on, the other is shut down. This reciprocal regulation is best observed at the phosphofructokinase/fructose 1,6-bisphosphatase step shown before, where the regulator fructose 2,6-bisphosphate activates phosphofructokinase and inhibits the phosphatase.

5. **Glycogen synthesis and degradation** - The enzymes glycogen synthase and phosphorylase are both sensitive to hormonal stimulation. The hormonal stimulus which activates one of these enzymes will inhibit the other, ensuring that maximal response to the stimuli is observed.

6. **Fatty acid synthesis and degradation** - The citric acid intermediate citrate activates acetyl-CoA carboxylase, the enzyme which catalyzes the committed step in fatty acid synthesis. High levels of citrate are found in the cytoplasm when both the cellular concentration of ATP is high, and when acetyl-CoA is abundant. If you think about it, under these conditions, we have energy to spare, and can store this energy by synthesizing fat.

   Fatty acids are degraded to acetyl-CoA. The acetyl-CoA will then be oxidized by the citric acid cycle if there is enough oxaloacetate to condense with the acetyl-CoA. The level of citric acid cycle intermediates are high when ATP is needed. Also, beta-oxidation will process only when significant concentrations of NAD+ and FAD+ are around to accept the electrons generated from the process. If lots of ATP is around, there won't be much NAD+ or FAD+ around; the reduced cofactors will build up because the flux through oxidative phosphorylation is slow.

   The bottom line here: **When ATP is high, fatty acid oxidation is slow!**
METABOLIC PROFILES OF MAJOR ORGANS

BRAIN: The brain is the organ around which all other organs revolve. The brain uses glucose almost primarily as an energy source. In the event of starvation, the brain will lower itself to use ketone bodies for energy, but only as a last resort. The brain is an energy hog – it uses about 15% of the total energy consumed by an individual in one day. The brain also requires a large amount of oxygen to complete the glucose oxidation; about 20% of our oxygen is consumed by the brain. The brain cannot use fatty acids for energy because fatty acids cannot pass through the blood/brain barrier. Also, the brain does not have any glycogen, due to a lack of glycogen synthase. The brain also does not have the enzyme glucose 6-phosphatase, and therefore cannot export glucose.

MUSCLE: The major fuel sources for muscle are glucose, fatty acids, and ketone bodies. Muscle has large stores of glycogen available to them; in times when blood glucose is low, glycogen can be used to generate glucose. Muscle, like the brain, lacks glucose 6-phosphatase, and therefore cannot supply glucose to the blood. However, since muscle does prefer to use glucose for energy during periods of activity, the glycogen store in muscle allows for muscle to use glucose for energy without depleting serum glucose.

When muscle is working hard, it cannot absorb enough oxygen to fuel oxidative phosphorylation. During these times, muscle metabolizes glucose anaerobically to lactate, which is excreted from muscle and brought to the liver to be resynthesized into glucose. In addition, during times of work alanine is generated from muscle; this too is transported to the liver for glucose synthesis and nitrogen elimination.

Resting muscle does not need glucose for energy, but in fact prefers ketone bodies or fatty acids under these conditions.

Also, muscle has a unique form of energy storage, creatine phosphate, that it can use to replenish ATP in times of heavy exercise or stress.

3. ADIPOSE TISSUE: Adipose tissue stores fatty acids as triacylglycerol. Adipose tissue does not contain glycerol kinase, and hence the triacylglycerol synthesized by adipose tissue must begin with dihydroxyacetone phosphate or glycerol 3-phosphate derived from glucose.

Triacylglycerols in adipose tissue are constantly being synthesized and hydrolyzed. If a lot of glucose is present, the cellular concentration of glycerol 3-phosphate will then be high, and
fatty acids produced by saponification of triacylglycerol will be reesterified. However, if serum glucose is low, then cellular glycerol 3-phosphate will be depleted, and the fatty acids will be transported out of the adipocyte.

4. **LIVER**: The liver's role in metabolism is primarily to keep the rest of the body happy. Most of the material absorbed by the intestine enters circulation and immediately passes through the liver. The liver then picks out the choicest morcels for its own consumption (as well as eliminating all of the trash). The liver, immediately after a meal, will absorb a great deal of glucose and convert it into glycogen. The liver has this unique ability because it contains two different enzymes which phosphorylate glucose, hexokinase and glucokinase. Remember that glucokinase has a high $K_M$ for glucose; because of this, the enzyme is only active when glucose concentrations are high. The presence of the second enzyme allows the liver to concentrate glucose when serum glucose is high, allowing the liver to store the glucose as glycogen for times of need.

The liver also regulates lipid metabolism. When energy sources are abundant, the liver will synthesize fatty acids. These are esterified and secreted into the blood as VLDL. The VLDL will then go to adipose tissue and muscle and pass the fat onto them. In times of fasting, however, the liver will make ketone bodies instead of fatty acids. What regulates this choice? Fatty acids are transported from the cytoplasm into the mitochondria as acyl carnitine, which is synthesized by the enzyme carnitine acyltransferase I. This enzyme is inhibited by malonyl-CoA, which is the product of the committed step in fatty acid synthesis. Therefore, when the cell is synthesizing fatty acids (when energy is high), these will not be metabolized in the mitochondrial matrix. However, when fuels are scarce, the cellular concentration of malonyl-CoA is low, and fatty acids are transported into the mitochondrial matrix for oxidation. Under the same conditions, the citric acid cycle intermediates are also low in concentration, so the acetyl-CoA formed is converted into ketone bodies for use by the entire organism, instead of into energy for the liver to enjoy by itself.

What does the liver like to eat? It cannot eat ketone bodies, because it has little of the transferase necessary to convert acetoacetate into acetoacetyl-CoA. The liver meets most of its energy needs by oxidizing $\alpha$-keto acids derived from amino acids.

**HORMONAL REGULATION OF METABOLIC PATHWAYS**
Now that we have reviewed the essentials of metabolism and regulation, we can now begin to discuss hormone action on metabolism, and the interrelationships between different hormones in different organs in our bodies. Let's talk about several polypeptide hormones, in order of complexity of response. (TABLE 23.2 P. 835)

1. **Glucagon**

   Glucagon is synthesized in the pancreas in response to the organism being in a fasted state - that is, when blood glucose is low. The main target organs of glucagon are muscle and the liver. Glucagon works by binding to its receptor and triggering the adenylate kinase cascade. Increased concentrations of cyclic AMP activates a protein kinase, which phosphorylates both **phosphorylase kinase** and **glycogen synthase**. Phosphorylation of phosphorylase kinase turns on this enzyme, which catalyzes the phosphorylation of **phosphorylase** to convert the inactive \( b \) form to the active \( a \) form. Phosphorylation of glycogen synthase immediately switches off this enzyme. The result of these operations is the increased production of glucose from glycogen in the liver. This newly produced glucose is released to the blood to increase the serum glucose level.

   Other actions of glucagon are
   
   1. The elevated cAMP concentrations in the liver resulting from glucagon binding also phosphorylates **phosphofructokinase 2**. This enzyme is the enzyme responsible for both the synthesis of fructose 2,6-bisphosphate from fructose 6-phosphate, and also for the dephosphorylation of fructose 2,6-bisphosphate to fructose 6-phosphate. The activity of this enzyme depends upon the phosphorylation state of the enzyme. When phosphorylated, the enzyme works to hydrolyze fructose 2,6-bisphosphate to fructose 6-phosphate. This activity inhibits glycolysis and promotes gluconeogenesis. This process prevents the liver from metabolizing all of the glucose generated from glycogen.
   2. Glucagon causes phosphorylation of the pyruvate dehydrogenase complex in the liver mitochondria. This inactivates the complex, preventing pyruvate from accumulating in the mitochondria as acetyl-CoA and its metabolic products.
   3. Glucagon also works on adipose tissue, increasing cAMP in these cells. The cAMP activates a lipase which hydrolyzes triacylglycerols. This process results in the net increase in serum fatty acids, which can be used by cells other than the brain for energy, leaving all of the glucose generated by the liver to be eaten by the brain.

2. **Insulin**

   Insulin is another pancreatic hormone. Its release is stimulated by glucose, and also
by signals from the parasympathetic nervous system. The presence of insulin in serum indicates that our body is well fed, and promotes metabolic responses to this state.

Binding of insulin to its receptor promotes phosphorylation of cellular enzymes. The insulin receptor has its own tyrosine kinase activity, which we spoke about last class. However, the phosphorylation caused by the insulin receptor is somewhat cryptic; we don't know for certain the function behind insulin-induced phosphorylation.

It does appear that this phosphorylation event sends a message to some sealed vesicles within adipose and muscle cells (most mammalian cells lack insulin receptors and hence do not respond to insulin). Muscle and adipose tissue have sealed vesicles which act as storage areas for glucose transport proteins. After insulin stimulation, the vesicles with the glucose transported fuse with the plasma membrane, increasing the concentration of the glucose transport enzyme in the plasma membrane, resulting in an increase in net glucose absorption by these tissues. Glucose is used in muscle for glycogen synthesis and for energy; in adipose tissue, it is used to generate more glycerol 3-phosphate for triacylglycerol synthesis.

Insulin apparently also stimulates the uptake of some amino acids into muscle cells, and in general promotes protein synthesis in muscle. These processes are not yet well understood.

3. Epinephrin and norepinephrin - These two hormones are released by the adrenal medulla and sympathetic nerve endings in response to a low blood sugar level. These two hormones are what are generally called catecholamines. These are also called "fight or flight" hormones; these are excreted in response to fear or anger. The action of the catecholamines is more complicated than the action of glucagon, even though some of the responses of the two types of hormones overlap.

The catecholamines have receptors both on liver and muscle cells. In both tissues, binding of catecholamine to receptor results in a increase in the cellular concentration of cAMP and an activation of phosphorylase. Glycogen is broken down into glucose by both tissues; the glucose made in the liver is excreted to the blood, while muscle, which has no glucose 6-phosphatase, uses the glucose it generates for energy.

This is the perfect response for fighting or fleeing. Lots of glucose is generated, but not for use by the brain primarily, but by muscle. Since the brain is used to avoid a confrontation like this, the body has bypassed the thinking organ and increased the energy at the working organs.

FED VS. FASTING VS. STARVATION
Let’s now refer to Figure 23.4, which describes the metabolic relationships between organs at different fed states in people. Let’s begin by examining the well fed state immediately following a meal. Blood glucose will be high, so insulin will be released by the pancreas, promoting glucose uptake into many different tissues. In the liver, the glucose will be converted into glycogen (until the liver is full of glycogen) and fatty acids. The fatty acids are packaged into VLDL, which is released into the bloodstream. The fats are broken down by lipoprotein lipase outside of adipose tissue and are taken up into the adipose, where they are stored as triacylglycerides. Brain is using glucose as usual. Muscle is using glucose; this helps to reduce the blood glucose levels after the meal. Muscle glycogen stores are regenerated if needed after the meal.

Move to a time of low blood glucose, say several hours after a meal but not in a fasting state. In this state, the body will want to keep blood glucose constant by recruiting glucose from glycogen storage in the liver. The liver will use fatty acids for its own energy. Adipose tissue will be breaking down fat and releasing fatty acids into the bloodstream to provide energy for the liver, for skeletal muscle, and heart muscle. The brain, of course, continues to eat glucose, because the brain is a pig at heart.

Finally, the fasted state. You haven’t eaten for three days, and you refuse to eat the maggoty bread that is in your pack. Adipose keeps breaking down triacylglycerides to fatty acids, which are distributed throughout the body for energy. Glycogen stores are depleted, so we need to generate glucose in other ways. Muscle proteins are broken down into amino acids; these are sent to the liver and when possible made back into glucose. The liver is also taking fatty acids and converting them into ketone bodies, which are used by many tissues for energy. The brain is using glucose still, but if there isn’t enough glucose around, it will lower itself to eat ketone bodies for energy.