MITOCW | 7.013-S05-L17

We're going to continue our discussion today about cell biology, and specifically about the subject of signalling which we talked about in general terms last time. I gave you some of the general principles about signaling, signal propagation and signal processing in biological systems. And today we're going to talk about two specific examples. First a short-range response which results in the production of second messenger cyclic AMP and the results of that in terms of downstream events. And the second will be an example of a long-range response which requires new gene expression, specifically in the stimulation of cells to divide. And the example I'll give you in the context of wound healing. So just to finish up what I started at the end of last lecture. As I mentioned, the energy associated with the binding of a single ligand to a single receptor molecule at the cell's surface is insufficient to trigger any of the downstream events that are necessary either for the short-term or long-term responses. And so it's necessary that the signal be amplified. And there are various ways that this happens. The first, which I mentioned last time, is the production of molecules which we refer to as second messengers. Second because they are not the primary thing. The primary thing is the ligand binding to the receptor. They come after that messenger because they transmit the message related to the binding of the ligand to the receptor inside the cell. And the example that I mentioned and that we'll talk about in more detail today is a second messenger called cyclic AMP or cAMP. I also mentioned that there are examples of enzymatic cascades. And, again, the purpose here is to amplify the signal. One enzyme turns on another enzyme which turns on many more enzymes, et cetera. It's like a pyramid effect where something little grows to something big. And the example I gave you last time was the analogy to dominos. Pushing one over basically spreads the signal down the chain. So signal amplification with respect to second messengers, the principle is quite simple. Imagine an enzyme which is in an inactive state. The binding of ligand to receptor makes that enzyme go to an active state. And in its active state it can convert a substrate into a product. Now, if that product can stimulate a second enzyme, imagine enzyme two, which is in an inactive state now in the presence of this product, is converted to an active state, you've amplified the signal. You've turned on a lot of enzymes which make even more of this product which now stimulate yet another enzyme. So this process then results in a form of signal amplification. The same is true for enzyme cascades. If you imagine an enzyme which is in an inactive state. The binding of a ligand to a receptor converts that enzyme to an active state. If that enzyme now acts on another enzyme, E2 goes from inactive state to an active state, and that enzyme then acts on another enzyme, E3 goes from an inactive state to an active state, again, you've gone from a relatively small amount of stimulus propagated through the stimulation of this enzymatic activity, which acts on a number of these to make even more of these active, which acts on even more of these to make even more of these active, again, one develops signal amplification. OK? Now, these changes that take place, both in the generation of second messenger, as well as in the turning on of enzymes, often result from changes in protein states. I tried to give you indications last time that proteins are not static things. They can move, they can interact, and they can actually change shape. And changes in protein states are often reflected in changes in protein structure, subtle changes in protein structure, but nevertheless changes in protein structure. And these can come in noncovalent forms. The protein is not changed by any new covalent bonds but by binding to something in a noncovalent interaction. And the classic example of this are G-binding proteins. These proteins bind guanine nucleotides. That's why they're called G-binding proteins. And they're often shortened and referred to as G proteins. We'll meet two G proteins later in today's lecture. G proteins change their structure depending on whether or not they're bound to GTP or GDP. Typically, in their inactive state, they're bound to GDP. However, in response to a signal transduction pathway, there's an exchange event whereby GTP binds to the molecule and GDP is released. This reaction is usually catalyzed by another protein known as a nucleotide exchange factor. We're exchanging this nucleotide for this nucleotide, and in so doing we change the shape of the molecule. When it's bound to GTP now it has a different shape. And by virtue of this different shape, it might now be able to interact preferentially with another molecule. So imagine that we have a signaling molecule which has a shape that looks something like this. A signaling protein. Given the lack of complementarity between this shape and this shape, these proteins will not interact. However, upon nucleotide exchange such that the protein now binds GTP and now changes its shape, now we can get a productive interaction between the G protein and the signaling molecule. And this can lead to a form of signal amplification. OK? So that's an example of a noncovalent change, and a reversible one. The GTP can be hydrolyzed -- -- back to GDP, thereby shutting the signal off. OK? Another example of a structural change in a protein but now a covalent one -- -- is in the form of phosphorylation. So imagine that we have a protein here, an enzyme which is in its inactive state. And this protein has among its amino acids, in some critical position, an amino acid with a hydroxyl residue. Does anybody remember back in the Dark Ages when you were learning your amino acid structures which amino acids have hydroxyl residues? I'm tempted to offer money to anybody who would remember all three. Anybody. Does anybody remember even one? Serine it is. Yes, that's one. Serine. Anything else? Tyrosine is another. Very good. And the last one? Threonine. So these three amino acids have hydroxyl residues. And by virtue of that they are subject to, or can be subject to protein phosphorylation at the hands of enzymes, which I've referred to in previous lectures, called protein kinases. These proteins can be modified such that they have a phosphate group on them. And this can change the structure of the protein and make it go from an inactive to an active state. There are actually examples where it goes the other way, phosphorylation takes an active protein and makes it inactive, but you see the point. Phosphorylation can change the activity. And, once again, this is reversible. There are protein phosphatases which will remove the phosphate and return the protein to its baseline state. OK? So just to give you an example of how this might be useful. Imagine that I have an enzyme here which has an active site, but this active site, by virtue of the confirmation of the protein, is closed. That is the substrates cannot get in there in order to be modified. At the hands of a specific kinase that might recognize a hydroxyl residue right here, the shape of this protein might change. So now the active site becomes open and the enzyme becomes active. OK? And likewise this can be

change. So now the active site becomes open and the enzyme becomes active. OK? And likewise this can be reversed through the action of a phosphatase. OK? So protein structures change, activities of proteins, enzymes change. And that's one way in which we can propagate signals. OK. So let's now go to a specific example. And the example that I want to give you is one that hopefully not many of you are familiar with. If you're camping and you run across this fellow here, what is your immediate reaction? You run. You run. You never try to feed the bear a marshmallow with your mouth. That's the first thing I learned about camping, you run. This is the famous fight or flight response. And when you're dealing with a bear, you want to take the flight option. It's stimulated by fear, some perception of fear. And upon perception of fear -- -- through a complex physiology, a small molecule is released from your adrenal glands. Glands that sit on top of your kidneys. This molecule is called epinephrine, otherwise known as adrenaline. So this is released from your adrenal glands and various things happen. One, that we're going to spend a little time talking about, is that your blood glucose levels go up. Why? Why do you want your blood glucose levels to go up when you see this fellow here? So you can run. Remember, that's the first thing you want to do when you see a bear, run. In addition, your heart rate goes up so you can pump more blood to your big muscles, your breathing rate goes up so you can oxygenate those muscles, and your blood pressure goes up. And there are other things that happen as well, but these are the most important with respect to the fight and flight response. This happens because this hormone, epinephrine, triggers all of these responses, actually in different cells within your body. Because it's acting at distance sites within your body, from the adrenal glands to the muscles to the liver to the heart to your brain, this is referred to as an endocrine response. An endocrine response is basically defined as hormones acting at a distance. OK? Produced somewhere. Acting somewhere else in your body. We differentiate that with the paracrine response in which there is a local release of growth factors and other ligands. So here there's a local issue. Something happens locally, factors are released right in the environment, and they act upon cells right in their neighborhood. And finally a related turn called autocrine which is cells -- -- which release the factor and bind it themselves. Autocrine. So they stimulate themselves. And very often you'll start a process one of these ways. And then the cell will take over. Basically amplifying the signal for itself. OK? Now, in the example that I want to talk about in the first portion of the lecture. We're going to deal with this one here, blood glucose, the increase levels of blood glucose. As you probably know, your body stores energy different ways. One way is in a polymer of glucose called glycogen, which is stored in your liver. Another way is through the storage of fat. For this response we want to liberate the energy that's stored in the form of glycogen and turn it into glucose which is much more consumable. It can be used directly by your muscles to make ATP which drives various reactions like contractions and so on. So the analogy here is money in your bank account versus money in your wallet. When you're in a situation like that, you want to transfer money from your bank account to your wallet. That's the direction we're heading in this example. In others situations, where you've just had a huge meal and you're not running from a bear, you go the other way. You take glucose and you turn it into glycogen, which I said is a polymer of glucose. Now, this balance between glycogen and glucose is controlled by enzymes which are sort of in the center of today's example. In this direction the enzyme is glycogen phosphorylase. Glycogen phosphorylase is the last enzyme that breaks down glycogen into a form of glucose that can be then further metabolized. Glycogen phosphorylase. And the other, in the other direction, is an enzyme called glycogen synthase. OK? So the goal of this reaction, or set of signals, will be to stimulate this process and actually inhibit this process. And you'll see how that's done in a second. This is epinephrine. Again, it's a small chemical produced by your adrenal glands. As I said earlier, it's released during the flight or fight response. It binds to a class of receptors which I'll take you through. They're called beta-adrenergic receptors because they bind to a factor produced in the adrenal glands, beta-adrenergic receptors. These are receptors, as you'll see, that have a complex tortuous root through the membrane. They go through the membrane seven times. They're actually sometimes referred to as serpentine receptors for that reason. And they bind to and activate GTP binding proteins, G proteins, as I mentioned down here. And this is a particular form of G protein called trimeric G proteins. And you'll see why in a second. As I mentioned, epinephrine binds to cells in different parts of the body resulting in various physiological effects. The ones I listed here and another one which I didn't list here which I find paradoxical. Actually, I know what, I know what the evolutionary advantage to all of these things is, but I don't know what the evolutionary advantage is to the relaxation of the smooth muscles around the colon during the fight or flight response. Some of you may have felt this before when you're extremely fearful. I'm not sure why it is our bodies do that. But anyway. OK. So how does this work? So we're going to consider just the mobilization of glucose. And the site of action is the liver. That's where glycogen is stored. And on the cells in the liver, the hepatocytes, on the plasma membrane of those cells, there are these receptors, these serpentine receptors. They look like that. They look like snakes, as I mentioned. Beta-adrenergic receptors. There are also alpha-adrenergic receptors, but we're going to focus on beta-adrenergic receptors. And these bind to epinephrine directly. So here's our molecule of epinephrine. It physically binds to a portion of the polypeptide chain that's sticking out from the cell's surface. Now, bound on the inside to this receptor is this trimeric G protein. It's called trimeric because there are three subunits. There's an alpha subunit, a beta subunit and a gamma subunit. And in this configuration, prior to the binding of epinephrine, the alpha subunit is bound to GDP. And, as I mentioned before, that's usually associated with inactivity. So the alpha subunit is inactive. The binding of epinephrine to the surface has two effects. The first is an exchange wherein the alpha subunit now binds GTP. And the GDP that was present there comes off. The other effect is that the G protein, the alpha subunit releases from the beta and gamma subunits and they float away. In this, now GTP-bound configuration, the alpha subunit is now active. And it can move from this position to another position within the membrane and bind to another protein. In this case an enzyme called adenylate cyclase. The GTP bound version of the alpha subunit associates with adenylate cyclase, thereby converting it to an active form. When bound to this it becomes active. And the consequence of that is that the adenylate cyclase can now convert ATP to the second messenger cyclic AMP. OK? And this process that we've gone through from binding of a single molecule to the production of a lot of a cyclic AMP is an example. OK? We've made a lot of second messenger from the binding of a single molecule because we've turned on a lot of

these, which can go on and turn on a lot of these, which can go on and make a lot of this. Now, there's another step in signal amplification here in this pathway. There's an enzyme known as protein kinase A which becomes activated in the presence of cyclic AMP. So protein kinase A in the presence of cyclic AMP goes from an inactive state to an active state. And when it becomes active it converts another enzyme, glycogen phosphorylase, which I mentioned to you a moment ago, from an inactive state to an active state. OK? So this is a phosphorylation reaction, similar to the one that I drew up here. PKA phosphorylates glycogen phosphorylase, making it go from an inactive to an active state. Another step of signal amplification. We can make some of this active which makes a lot of this active. OK? And this is the enzyme we needed to turn on in order to break down glycogen to glucose. Now, there's another kind of cool thing that happens in the control of the reverse reaction, because it turns out that the enzyme responsible for making glycogen, glycogen synthase is inactivated in the presence of cyclic AMP. So it goes from an active state in the presence of cyclic AMP to an inactive state. So at once we turn that off, turn this on, and we can generate a lot of glucose. OK. So this is the reaction. We focused exclusively on the mobilization of glucose here. Just to remind you, this same pathway controls heart rate, breathing rate, blood flow. And, actually, some of you might have heard of beta-blockers. And beta-blockers are used to control this exact reaction. Beta-blockers bind to beta-adrenergic receptors so that your body won't respond to adrenaline or epinephrine when you don't want it to. So people who get really nervous talking in front of audiences or taking exams in 7. 1 will sometimes take beta-blockers so that they don't suffer, you know, fast heart rate or this feeling of anxiety and so on. OK. So we've turned on this signal in response to binding of epinephrine to the cell's surface. We've started all these happening. How do we turn it off? How do we deal with signal termination? How do we turn the signal off? Anybody have any ideas? Well, one might be not so obvious. And that is -- -- a process which I introduced to you last time called pathway modulation in which the activation of a second pathway can actually inhibit the activation of the primary pathway. So, for example, I've been telling you about the beta-adrenergic receptors, which through the binding of this kicks off a series of events which results in the activation of this pathway. There are many examples in biology where the binding of a second ligand to a distinct receptor results in the propagation of a signal which actually inhibits the first. So, for example, I mentioned here that there's a kinase that's activated, protein kinase A. This pathway might activate the relevant phosphatase, thereby turning this signal down. So modulation of the pathway, very important in biology. We're only really beginning to appreciate how important it is the more we learn. Another obvious example is ligand diffusion. You make a certain amount of ligand from your adrenal glands. And it just diffuses eventually or breaks down, thereby turning the signal off. There's also a phenomenon known as receptor internalization. Very often when a receptor binds to its ligand, it doesn't just stay out there on the surface but actually gets internalized inside the cell. So I'll draw a different class of receptors here. Here's the receptor. Here it's bound to its ligand. I told you last time that proteins can get to the cell's surface through a complex sorting pathway that involves actually vesicles encapsulating the proteins and then bringing them to the cell surface. Well, the reverse can happen as well where the proteins can be removed from the cell's surface into vesicles. Removing them from the cell's surface then takes them away from the possibility of being activated, and thereby turning down the signal. Another possibility would be to degrade the second messenger. OK? Cyclic AMP is an example. You need to make it in order to stimulate these various downstream events, so get rid of it. And, in fact, there's a clear pathway for that process. As I told you, ATP can be converted through the actions of adenylate cyclase to make cyclic AMP. But likewise there's an enzyme, actually, a whole class of enzymes known as phosphodiesterases which converts cyclic AMP to AMP, thereby inactivating the signal. These enzymes are actually very important in pharmacology. There are lots of inhibitors of phosphodiesterases which stimulate the production or the maintenance of high levels of cyclic AMP and other cyclic nucleotides. So caffeine for example, one of the reasons you get a buzz from caffeine is that it blocks a class of these enzymes leading to more cyclic AMP which gives you that kind of nervous energy. OK? Another related process involving another cyclic nucleotide, this time cyclic GTP. This is broken down by a different phosphodiesterase. In Viagra and like chemicals are inhibitors of this class of phosphodiesterases, thereby leading to an over stimulation of that pathway. And, finally, you can reverse the structural changes that I mentioned previously. So I said earlier that enzymes can be activated through kinases. And they can be inactivated by phosphatases. I'm going to have to move to a different board here. And G proteins which get activated in the beginning of these processes, from a GDP bound form, through the action of exchange factors to the GTP bound form, can be inactivated by GTP hydrolysis. And this is often through proteins called GAPs, which are called GTPase activating proteins. They activate the GTP hydrolysis activity of the G protein, thereby returning the G protein to its inactive state. OK. So that's the beta-adrenergic signaling pathway. It's a classic. You should know it in principle and in some detail. It's covered extensively in your book. Here's a figure from your book. I won't go through the details of this because we just did them on the board, but just so you know it's in there. Here's the production of the second messenger, cyclic AMP. And then it triggers this kinase cascade that I drew up for you before, activation of PKA, activation of glycogen phosphorylase, and the production ultimately of glucose. And here's the second messenger. I'm pleased to see that I put this in the book, in my slide set last year because the book had an incorrect structure for cyclic AMP in the previous edition. I'm pleased to see they know have corrected the structure. This is the actual proper structure of cyclic AMP. OK. So now want to change signaling pathways and consider not what you do when you first encounter the bear, but what do you do, or what does your body do if you fail to successfully run from the bear? So it's funny what you can find on the Web, boy. You can find anything you want on the Web. In fact, when I was looking up, I was pretty sure that Viagra blocked phosphodiesterases. I was pretty sure, but I wasn't certain so I did a Web search. This is true. I did a Web search. And, God, I cannot believe what I came across. It's unbelievable. But you can find some pretty nasty bear wounds on the Web as well, and here's one of them. So this guy didn't run fast enough. So what's going to happen? What is his body going to do following an injury such as that? Well, obviously he needs to heal the wound. Your body is remarkable in its ability to detect damage and fix it. So we're going to heal the wound. And this is an example of

remarkable in its ability to detect damage and fix it. So we're going to heal the wound. And this is an example of one of these long-term effects of a signaling pathway. Whereas, this was short-term, everything was happening in the cytoplasm, generation of glucose to be secreted. This is a long-term effect. The effect has to take place ultimately in the nucleus because we need to turn on gene expression to convince cells to divide and heal the wound. This may be a troubling picture. Maybe I should go back to the bear. There. So if we imagine the skin, which is a tight complex of cells forming a protective layer against the elements and so on keeping stuff out that should be out, in that should be in. In a wound you breach that structure, you get a scratch. And one of the things that happens, as you bleed into that wound, is that small packets, former bits of cells called platelets bind to the edges of that wound. And the platelets then release a factor imaginatively called platelet-derived growth factor. And platelet-derived growth factor, otherwise known as PDGF stimulates other cells to divide. And specifically it stimulates cells called fibroblasts which sit underneath these cells normally as part of the connective tissue underneath the epithelial cells. In the presence of PDGF, the fibroblasts proliferate. And this results in the formation of a scar. OK? And what we want to understand now is how is it that PDGF stimulates fibroblasts to divide? So we can model this process in cell culture. We can extract fibroblasts from your skin and put them in a tissue culture dish, let's say one times ten to the fifth cells. So this is cell number on this axis and this is days in culture on this axis, one, two, three, four days. If we plate 100,000 fibroblasts in a tissue culture dish, in the absence of growth factors they won't divide. They'll just sit there. But if we add PDGF or other growth factors, the cells will divide, and therefore we'll see an increase in cell number. So, again, the question is how does that happen? What is the mechanism that allows that to happen? Oops. What did I do? Well, here we're talking about another class of receptors. These are called growth factor receptors. And these are different from seven transmembrane receptors in that they have, on their cytoplasmic side, enzymatic activity, specifically kinase domains. These receptors are also enzymes. And their enzymatic activity is to add phosphates to other proteins. In this configuration, however, they're inactive. What the growth factor does is causes those receptors to dimerize with one another. To become in close proximity to one another. So the growth factor serves to link them. Here's our growth factor. And in this now increased proximity, these kinase domains become active. And the consequence of that is we get phosphorylation of the neighboring protein. We call this transphosphorylation. So ligand induced activation, transphosphorylation. And now what we've done, on the inside of the cell, is to create a structure. And it's the formation of that structure that then allows the rest of the process to work. These phosphorylated residues act as the binding sites for other proteins. The first one is known as an adaptor protein. An adaptor protein which binds to a class of proteins to that I referred to previously, a guanine nucleotide exchange factor. And that then allows the exchange factor to stimulate the exchange of a small GTP binding protein, which is generally bound to GDP and inactive, to stimulate the exchange of GTP for GDP. This small GTP binding protein is a protein called RAS, a very important signaling molecule. And, actually, a very important cancer molecule as well. Mutationally activated in a high percentage of cancers. When you now have RAS in its GTP bound state, it can activate a kinase cascade. Which I'll go through with you on slides in a moment. It first activates a protein called RAF which then activates a protein called MEK which then activates a protein called ERK. ERK is a map kinase. It's a kinase itself. Its enzymatic activity is to phosphorylate other proteins. MEK phosphorylates ERK so it's called a map kinase kinase. And RAF is a kinase itself which phosphorylates this kinase so it's referred to as a map kinase kinase kinase. I'm not making it up. OK? So it stimulates a kinase cascade, similar to what I introduced you to before. And then, as you'll see, ERK, which is a cytoplasmic protein, moves from the cytoplasm into the nucleus. I'll show this to you on slides, and it's in your book as well. And in the nucleus this kinase phosphorylates transcription factors which then bind to target genes turning them on stimulating the cell to divide. I would have drawn that for you but we're running out of time. So I will show it to you instead on the overheads. There's our wound. So here's the pathway. It's just straight out of your book. Here are the growth factor receptors, again, not dimerized, not active. The growth factor causes the two proteins to come together in close apposition. This causes the kinase activities to get stimulated resulting in the phosphorylation of the cytoplasmic tails. The adaptor protein then can bind to the phosphorylated tail. This then stimulates through an exchange factor not shown here, the exchange of GDP for GTP, making an active RAS molecule. This then can activate RAF making it now an active kinase which activates MEK making it an active kinase which activates ERK called map K here for map kinase. This now goes into the nucleus of the cell from the cytoplasm, and in the cytoplasm it can encounter transcription factors. It then phosphorylates those transcription factors which bind to their target genes turning on gene expression and leading to a change in the cellular phenotype. And the change in the cellular phenotype that we're talking about here is cell division, the details of which we'll cover next time. So this is sort of a static picture. But to give you a more dynamic picture of what's happening here, I borrowed from the Biocreations Website an animation, which I find very helpful and, actually, is kind of funny as well. So we're going to start this process much the same way that we've just talked about using the terms that I used on the board. Oop. We need sound. Let's do that again so you can hear it in all its glory. OK. So the first thing is the receptors get dimerized by the growth factor leading to the phosphorylation of these cytoplasmic tails. And then this protein, which is the adaptor, it's called grab-2 grabs onto one of those phosphorylation sites. It has attached to it an exchange factor called SAS here. OK? So that's the first thing that happens. You recruit this signaling complex to the membrane. Once you have it there it's now in close proximity to RAS which is, if you remember, a membrane associated protein. It's now in its inactive stage, its GDP bound state. Oops. Sorry. We'll just have to watch that again. Oh, no. Wait. Wait. Wait. Go back. Forget you saw that. That's the end. You never want to see the ending. Oh, wait a minute. OK. Here we go. OK. So next we're going to activate RAS. It gets a little kick in the butt. GDP comes off. GTP comes on. Now RAS is active. It's now capable of interacting productively with RAF, the first of these kinases, the map kinase kinase kinase. And it's going to cause it to become active. Once it's active it's now capable of activating the next guy in line, which is MEK. Now MEK is active, and it's going to phosphorylate ERK, and now ERK is phosphorylated and active. Now, there have been a couple of other steps, which you can look at, at home, but the RAS went from a GTP bound stage to a GDP bound state. I told you that you can turn these signals off up here.

That happens in this pathway. Another GAP comes along and turns RAS off. And here's a protein phosphatase, which is going to clip off the phosphate, thereby turning MEK off. So each of these steps is reversible. But, importantly, we're down here at this level where ERK has been phosphorylated. And now it's capable, dammit. All right. We'll have to watch it again. Wait a minute. Come on. OK. Here we go. I know. It's like a video game. OK. So ERK is active. And now it's going to go in the nucleus. Phosphorylate Jun, one of these transcription factors. And upon doing so the transcription factors dimerize, bind to the promoter of a target gene. They recruit RNA polymerase. And this leads to the production of new gene expression. mRNAs that go into the cytoplasm get translated into proteins that convince the cell now it's time to divide. OK? So I encourage you to look at this to get the details. Now, if you just wait one more second. In the last 30 seconds I want to point out the fact that these pathways, while we're teaching to you as simple linear pathways, are actually highly complex. Pay attention to this clock here. It's 11:54. Highly complex, highly interactive, not linear, and therefore we frequently analogize what's happening inside a living cell to an integrated circuit. And increasingly these days in biology, we're thinking about these things in analogy with computers, and actually relying extensively on computers to help us understand how that complexity actually works using methods from chemistry and physics and mathematics and other disciplines in a new and emerging area called systems biology, which you might be interested in. And the new Biological Engineering major will have a strong focus in this area. OK.