What I want to talk to you today about is the creation of 3-dimensional structures. And I want to emphasize their connection to the function of the organisms and the organ. Let's look, for example, at the kidney. This is a fantastic example of structure- function relationships. The kidney comprises thousand of tubules that are involved in filtering the blood and collecting urine for excretion. Now, not only do these tubules function to transport the urine as it is being processed, they also are connected very closely to various cell types that are involved in this filtration process. So the 3-dimensional structure of the kidney is integral, is required for its normal function. And this is true in essentially every organ. So it's very important to think about the relationship of different cells to one another in a particular organ. And if you're thinking about, for example, using tissue engineering to develop an artificial kidney and making some kind of pastiche of cells and polymers and putting that together in some functional way -- -- it's important to understand these structure-function relationships in the normal organ before you try to engineer something that is a surrogate. Individual cells also have particular shapes, and this is integral to their function. We'll talk a bunch about neurons, cells that have very long processes that transmit nervous impulses or transmit electrical impulses and communicate in that way. The shape of the neuron, the structure, the 3-dimensional structure is absolutely required for its function. Tom, I think we could have a little more light at the back there. It looks really dark if you guys are trying to take notes. Thank you. OK. We have, you've seen this movie previously. OK. This is the zebra fish. The zebra fish embryo. Isn't it dark at the back? Yeah. Tom? Oh, it takes a while. It takes a while. OK. I want to show you this movie again that you've seen a while. You have it on your website. And I want to show it to you now in a different way. We're actually going to first show that during the early development of the fish, these two cells on top initially give rise to a ball of cells sitting on top of this yoke cell that is just a ball of cells. And then suddenly these cells start to move during the gastrula and then the neurula phases of development. And it's this cell movement that builds structure. And I want to try to address with you how this cell movement occurs and how it builds structure. So let's look at this movie again. Here we go. Cell division, building up the raw materials to make the embryo. A group of cells sitting on top of the yolk. And now watch here. It starts to move. Those cells have made the decision to move, and they going to move towards this one dorsal side of the embryo. And they are going to move and move some more to build the eye, the brain and the various somites along the length of the axis. OK. So that process, the generation of 3-dimensional structure is a very large part of the process of building the embryo. And it's interdigitated with making the cell types. A muscle cell is not a muscle cell and it's not functional unless it is a fused myotube that has the appropriate 3-dimensional structure. So cell type and 3-dimensional structure are very closely related. OK. So let's talk about a toolkit that is involved in this process. And the first thing that we can discuss is what is in this toolkit. Well, actually, you don't really have much in the toolkit to build an organism. I can think of one thing. What's in your toolkit to build your 3-dimensional organism? Yes. Did I hear cells? Yeah. I hope I heard cells. Well, there are cells. And then if you want to look for something else to build the organism with there are cells and then there are cells. That's what you've got. In your toolkit you've got cells. And the challenge of the organism is to use that singular building material to build the various and the huge array of structures that there are. So what about these cells? And the first thing about these cells is that they come in two forms. They come as so-called epithelia or epithelial sheets. And they come as single cells which are called mesenchyme. And these two types of cells interconvert with one another. And it's from these two groups of cells, that can be different cell types, but they're either sheets or they're single cells, that one builds the organism. So the epithelia are sheets, the mesenchyme are single cells. And let me look at the next diagram that I drew for you. And you have it in front of you. If you don't have the handout, does anyone not have the handout? Could I have, Lesley, could you [UNINTELLIGIBLE] please? Thanks a lot. OK. If you look at the handout, you have this except for one thing I added this morning. Here is a sheet of cells. So I'm going to do minimal board work today because you have a lot of the information right in front of you. You have a sheet of cells here. And this sheet of cells called an epithelium is a sheet of cells because it is joined together very tightly. And the cells are joined together by various junctions that I've shown here in yellow or that I've shown in blue. And this sheet of cells, as Professor Jacks told you, has two part, has two sides, an apical side and a basal side. OK? Remember from cell biology? And it touches something called the basement membrane or the basement lamina, which is part of the extracellular matrix that I'll mention again later. Now, this epithelium, this sheet of cells can transform into single cells. And these single cells have the property of being non-adherent. And they don't attach very tightly to the extracellular matrix, although they are in contact with it. And in order to go from an epithelium to a mesenchymal state there are changes in gene expression that take place, we know, at the transcriptional level. And these result in changes in cell adhesion and changes in the organization of the cytoskeleton. This is a reversible process, and mesenchyme can go and turn back into epithelium. Now, the epithelial sheet is a very important part of the body, both as a building block for various structures, but also as a barrier. So your skin is a barrier because the cells in it are very tightly joined together and they form an impermeable barrier. But that's true of essentially every organ you have. Every organ you have is surrounded by an epithelium, or one or more epithelia, and these surface barriers so that stuff in the organ doesn't get out, stuff outside the organ doesn't get in. And if there is a lesion, a break in this epithelium it is a big deal. And that is wound. That's what a wound is. It's a break in the epithelium. And there is a very rapid and profound system of wound healing to repair these epithelial sheets. Now, during development there are many transitions from epithelium to mesenchyme. So the term that you really need to know is abbreviated EMT. Not to be confused with other EMTs. This refers to the epithelial mesenchymal transition, which I did not write on your handout so I will write it here. The epithelial mesenchymal transition. And it's always said that way, even if it's actually a mesenchymal epithelial transition. One of the most profound examples of epithelial mesenchymal transitions is during the formation of the nervous system. Your central nervous system, as we'll discuss in a few lectures, forms from a tube that rolls up during development. And this tube is an epithelial sheet. As the tube is rolling up, a group of cells, shown here in yellow,

development. And this tube is an epithelial sheet. As the tube is rolling up, a group of cells, shown here in yellow, moves away, breaks away from the tube and undergoes an epithelial to mesenchymal transition. This group of cells is called the neural crest cell population, and these neural crest cells then migrate away from the neural tube and go and set up the entire peripheral nervous system. Not eripheral nerves, peripheral nerves. They also make all the pigment cells in the body and the adrenal medulla. This epithelial mesenchymal transition is not only crucial during development. It's also believed now to be crucial for metastasis of tumors during progression of cancer. And Professor Jacks will address that later in the course. This is a movie demonstrating the migration of the neural crest cells out from the neural tube which is in the middle here, this white tube. And these little dots migrating out are shown over a period of about 12 hours. The single cells migrating out from the neural tube as they have undergone that epithelial mesenchymal transition. All right. So I wasn't wrong in facetiously saying what's in your toolkit is cells. That's what's in your toolkit. But clearly the cells are slightly different from one another or profoundly different from one another in their disposition. And we'll talk about what mesenchyme and what epithelial can do in a moment. The other thing that's different or the other thing that's important that makes cells actually very good for building is that they are plastic. So let's go through a few things that they have going for them. I want to talk about adhesion. I'm going to mention junctions, I'm going to mention cell sorting, and I'm going to mention the extracellular matrix. And some of this stuff you've had before so I'm going to go through it quickly. This is a diagram that's on your handout, it's from your book, to indicate that there are many ways epithelial sheets are stuck together that involve very tight apposition of the cell membranes, or in the case of tight junctions or slightly less tight apposition in the case of these things called desmosomes. You can go back and remind yourselves. We mentioned these previously. The most important thing is you understand that cells are joined together. In this micrograph, to demonstrate how tightly cells are joined together, this is a sheet of cells where the nuclei are stained green and the red is staining for a particular specific protein that is found in a kind of junction called a tight junction. And these tight junctions outline the cells. In other words, the cells are glued together very tightly. Here's another one. Cell sorting. There is another kind of cell adhesion interaction that's very important for cell adhesion. I've indicated here something called cadherins. Cadherins are interesting. They're calcium-dependent adhesion molecules. And if you have sharp eyes you'll see up here something that's got a beta and then there's a word here catenin. Remember beta-catenin from dorsal-ventral axis formation? This is the same beta-catenin. Not only is it a transcription factor, it is also involved in cell adhesion. There's an interesting complication of biology for you, but I don't want to dwell on that. Cadherins are essential for sticking cells together. These are pictures of frog embryos. This is a normal embryo that's been cut open and the cells remain a tight mass. However, if you take an embryo and you inject it with inhibitors of cadherin function the cells become completely loose from one another and you can actually see the outlines of the cells because they are no longer stuck together. Now, this is fascinating and very important for the animal because it turns out there are lots of different adhesion molecules. And different cells types sort out according to the adhesion molecules they are expressing on their cell surfaces. So this is a rendition of a fantastic old experiment that was done in the 1950s to demonstrate how cells sort out. What was done, these are two frog embryos, and a piece of the future skin or epidermis was removed from one, and then a piece of the future neural plate, the nervous system was removed from another. And these had different colors so you could tell which cells were which. Now, if you take those cells and you put them in a medium that does not contain calcium all the cadherins and other adhesion molecules cannot work. And these cells fall into a pile of single cells. And you can take your pipette and mix them up in your little dish, and you get this salt and pepper arrangement of the two kinds of cells. And then you can add a little bit of calcium back to the medium, and the cells will form this big ball of cells. And it's salt and pepper again. The two neural plate cells and the epidermal cells are mixed up. But if you go away and have dinner or have a good night's sleep and come back the next day and look at your ball of cells, you see that amazingly the cells have sorted themselves out. The epidermal cells have gone back together and the neural cells have gone back together with one another. And you can do this with cells from almost any organ. You can mix cells from two organs particularly when they're embryonic organs. You can mix them together, but also in the adults to some extent. And these cells from different organs will sort out. And they sort out because of specific adhesive molecules that they have. And the term that one uses for this is homotypic binding where cells will interact with one another through membrane-bound receptors. Again, I'm assuming you remember this is a lipid bilayer with a protein sticking through. Two receptors, proteins sticking through the lipid bilayer interacting with one another in calcium-dependent or independent ways. And there may be more than one receptor that mediates cell-specific interactions. But these interactions keep different cells separate from one another and facilitate development and building structure. OK. Here's another one. The extracellular matrix. What is the extracellular matrix? We mentioned this at the beginning of the course. Professor Jacks threw it at you in cell biology. The extracellular matrix refers to the stuff on which the cells sit, so cells in your body are not sitting on nothing. An epithelium is not just floating free or a tube is not floating free in liquid. It is actually surrounded by a bunch of proteins and carbohydrates that are secreted by other cells and form the extracellular matrix. Or, in the case of epithelia, the basement membrane. OK? That's what the extracellular matrix is called. It's highly organized in the case of the epithelium. And it consists of proteins and various things called proteoglycans which are sugars bound to proteins. Now, one of the proteins in the extracellular matrix is collagen. And collagen is the most abundant protein in the Animal Kingdom. OK? It comprises a very high percentage of your body mass. And if collagen, if there are mutations in collagen many things can go wrong. For example, there is a disorder called osteogenesis imperfecta where your bones don't form properly. That's a mutation in one of the collagen genes. There are a lot of collagen genes, and the protein mass of collagen is enormous. Now, the cells are sitting on the extracellular matrix. But there is also a fantastic, and then that's good, that's helpful for the cells in a support sense. It gives them some support. But the extracellular matrix does a lot more than that. It actually communicates to the cells. And it does so by means of adapter proteins. You have this as a handout, if you didn't realize. This is one of your handout diagrams. OK? These adaptor proteins have the property of being transmembrane or at least integral membrane proteins that are attached to the cells. But they also attach to proteins in the extracellular

integral membrane proteins that are attached to the cells. But they also attach to proteins in the extracellular matrix. And this allows them to sense what the extracellular matrix contains and to transmit that information to the cells. Because the thing, of course, about living organisms and the 3-dimensional structures in them is that the process of both building the structure and maintaining the structure is a dynamic one. It's not equivalent to building Building 10 or Stata. It's not equivalent to putting components together and getting a structure. You get the structure, and the structure is maintained because the structure senses how it's doing, whether it's intact or not. If one of the walls in this building fell down, it would fall down until someone repaired it. If one of the tubes in your body gets a hole in it, your body will sense it and try to repair it. That same thing is true when the epithelia, and I'll tell you in a moment, when mesenchymal cells are actually doing their building process, they are sensing what is around them. And they do it through these adapter proteins. The adaptor proteins are receptors, by definition, they're binding to something in the ECM. And a large number of them comprise of class of proteins called integrins, as an example of a name. All right. So let's move on here and let's mention -- -- the cytoskeleton as being required for shape and movement. We've mentioned the cytoskeleton before. I'll talk about it more in a moment. This is a diagram from your book that you had before. The cell is not a floppy bag of liquid. It contains many filaments that keep it rigid, that allow it to have particular shapes and that allow it to change its shape. We're going to be talking most about microfilaments which comprise polymers of actin, the protein actin. There are also intermediate filaments and microtubules which we mentioned last lecture when we talked about the sperm flagellum. This is a micrograph of tube stained, of cells stained for the microtubule network. The nucleus is yellow. Stained for one of the major proteins in the microtubules, which is tubulin. And you can see this very extensive meshwork throughout these cells. And the same is true for actin. The same is true for intermediate filaments. So cells are very well supported by these filaments. OK. And, finally, let me mention cell division and cell death as being part of the toolkit that allows one to build structure. And I'll show you one experiment which involves the inhibition of cell death in the embryo. This is a mouse, normal mouse embryo, and this is a mouse embryo in which a protein was removed. This protein is called caspase-9. And caspases are involved in killing cells during development, as Professor Jacks mentioned to you. In this mutant animal, one of the things you should notice is that the brain is hugely overgrown. And this is an indication of the amount of cell death that has to happen during normal brain development. OK? And the flip side I'm not going to dwell on. You need to get not only the normal amount of cell proliferation, but it needs to be in the right place. All right. So let's talk about the behavior of cells and how this works together to give 3-dimensional structures. And I want to talk very briefly about, actually, not so briefly, about the behavior of single cells -- -- and the property that is most important to them, which is the ability to move. So the point about an epithelial mesenchymal transition is that what comes out of it are single cells. And this is important in metastasis and in normal development. The difference between this epithelium and these single cells is that the single cells are free to move because they are not attached to a sheet. And that is how cells get from one place to another in the body. OK? So let's dwell on this in more detail. So how do cells know where they're going? Well, this is a very interesting question. They know where they're going because they're told to go somewhere. So I told you the neural crest migrating out of the neural tube. This is a group of cells from an organism called dictyostelium. And these are single cells. They've been fluorescently labeled. And up here in the corner someone has put invisibly a drop of the second messenger cyclic AMP. And you can see these cells that had been milling around randomly start to realize this and move as a consorted group towards the source of cyclic AMP. OK? And this is one of the ways a dictyostelium organizes itself. The cells chemotax, they follow a particular chemical stimulus. How do they move? How do cells move? Well, they move because the reorganize their cytoskeleton, particularly their actin cytoskeleton in a concerted way. So I've drawn for you a kind of animated cartoon. You have the whole thing in front of you. And I'll animate it and see how it goes. We start off here with our green cell. This is a mesenchymal cell. And it is sitting on some kind of extracellular matrix, which I've shown as kind of organized, but actually it may not be. But that doesn't matter. It is loosely attached to this extracellular matrix. It's got these receptors sticking out of its membrane that can sense extracellular matrix, but only at the rear of the cell is it actually attached by these red triangles to the ECM. OK? That's what my red triangles indicate. Now, the cell suddenly as it's sitting there or moving randomly along comes across these black circles -- -- which are some kind of molecule that can interact with these receptors and tell it that it ought to go in a particular direction. So how does it do this? Well, the first thing it does it to elicit this interaction with a substrate or with these black molecules in the substrate. This is a receptor ligand interaction. It's exactly the same kind of interaction that you talked about in cell biology, that we talked about in the formation, earlier formation lectures, cell receptor ligand interaction. And it does the same thing that other receptor ligand interactions do. It activates some kind of signal transduction process, but this signal transduction process has the property of telling the actin filaments in this local region of the cell that they should polymerize. OK? So this is cell signaling in a very local region of the cell. And I'll show you the movie in a moment. You'll see it's extraordinary. So here are the actin filaments forming in this region of the cell. And I've called this the front of the cell. OK? It's an arbitrary term. But the front of the cell is arbitrary but it's going to refer to the direction that the cell is moving. So there's actin polymerization of the front. And the cell also sends out some protrusions called filopodia or lamellipodia. And it sends out these protrusions to try to sense where it should move next. Now, once it's done that, once it's made these polymerized actin filaments it contracts. However, it doesn't go anywhere because it's still attached at the rear. So it's kind of like pushing down on your accelerator when your hand break is still engaged. OK? You don't really go very far. So the next thing it has to do is to lose the adhesion at the rear. I couldn't get this to work but it will work in a moment. OK. And it also depolymerizes the actin at the rear. So there. There we go. There's loss of the adhesion and there's loss of the actin at the rear. And now it's in a position, it's disengaged the hand break and it can move forward, so it goes forward. OK. This is my cartoon. This is the real thing. This is a cell where actin has been labeled fluorescently. And I want you to watch two things. It's on a repeat loop so we'll watch it a number of times. At the part of the cell that is sticking out, you should be able to see these bright cables or these bright lines. These are cables of polymerized actin. OK? And the cell is sticking out these protrusions and polymerizing actin. And it's moving down

polymerized actin. OK? And the cell is sticking out these protrusions and polymerizing actin. And it's moving down in this plane of the board, or the screen as it's making these polymers. OK? So this is the front of the cell. And then if you look at the back of the cell, let's wait for the loop again to start, and you will see, here's the back of the cell with initially polymerized actin. And if you watch individual lines you will see them go away as the actin is depolymerized and the cell loses its adhesiveness at the back. OK? So this process involves selective adhesion, loss of adhesion mediated by actin polymerization through the cytoskeleton. There's a signal transduction pathway involved. It involves things called GTPases that you've talked about previously. And it's really an extraordinary process. All right. But let's move on and let's talk about cell sheets. So cell migration is crucial to get from one place to another, but you cannot really build much with single cells because they're single cells. They don't really give you any kind of integral structure, any kind of cohesive structure. And so you have to turn these single cells back into sheets if you're going to use them then for building. So what do cell sheets do? Ah, OK. So let me go through a couple of things that cell sheets do. Cell sheets can get longer. They can change the number of layers that they have. They can change the number of length, the number of layers that they have, and they can bend. Here are some cartoons that I drew for you. Here's a sheet of cells. And you can lengthen it in two ways. You can stretch it out so that the surface area to volume ratio changes. And you get a longer, thinner sheet of cells. You can also take this initially bilayered sheet of cells and fit the cells in between one another by a process called intercalation, or the term that's usually used is convergent extension. And that lengthens a sheet of cells. And the reason that a small embryo becomes longer is because of this process of intercalation where the embryonic cells interdigitate with one another and make the whole embryo stretch out. You can also change the number of cell layers to get a more complex tissue, a more complex structure. You can break two cell layers into two individual layers. That's called delamination. Or you can turn two cell layers into one cell layer by another process of intercalation so that you get a single sheet from what was initially a two cell thick sheet. Again, this is in front of you and I don't want you to dwell on it. Let's talk about bending cell sheets. This is really interesting because one of the things that comes out of bending cell sheets are tubes. Now, tubes are pervasive. There is not an organ in your body that does not have some kind of tubular structure. And if you think about it, if I were to give you a pile of cells and say, here, build me a tube. You could probably come up with several ways that you build a tube. And, in fact, the organism has come up with several ways. One of the ways is to roll an epithelial sheet into a tube. So here is the sheet. And you roll it up to form a tube. This is how your neural tube is made. And also part of this is that the cells change shape as they are rolling up. So if you have a sheet of cells that is either cuboidal or columnar, to get that sheet of cells to bend you have to make wedge-shaped cells out of them. You have to give them a constriction, a so-called apical constriction. So you go from a sheet of columnar cells to a bench sheet of cells because you have apically constricted those particular cells. And you can do that through an actin process or through our old friend beta-catenin here acting as a cell adhesion molecule. All right. So here's the process of rolling up the neural tube in frogs. It's rolling up by apical constriction and by the epithelial sheets bending. OK? You've seen this movie previously, and it is on your website so you can look at it again. Here's another way. You can balloon out an epithelium. You can imagine an epithelium, and you make a little, you blow it and you get a little balloon. And then you blow it some more, you pull it some more and you can turn an indentation or a very small vesicle into a long tube. OK? And I'll show you. This is not the best diagram, I've got to work on this one, but the idea is that you pull this. Dr. Gardel is agreeing with me, I've got to work on this diagram. OK. But you pull out this sheet of cells into a long tube. I'll talk more about this when we talk about lung formation in a moment. Here's another way. You can take your single mesenchymal cells that have migrated to wherever they're going and you can get them to condense into an epithelial sheet. And this epithelial sheet can then form a tube. This is exactly what happens in formation of the blood vessels. Your blood vessels have got lots of layers of epithelia. The first one to form is the endothelium. It's the inner most layer of the blood vessel. And it forms by condensation of mesenchymal cells into an epithelial sheet. OK. Excellent. So I want to end by giving you a specific example and talking about the genes involved in a particular aspect of 3-dimensional structure generation. And the example I've chosen is formation of the lung and the tubules in the lung. So your lungs are made up of multiple tubules that have got little sacks on the end of them, alveoli that are the places where oxygen is exchanged between the air you breath in and between the blood vessels that feed into or that surround all the cells of the lung. Formation of the lung tubules is very interesting because mathematically, if you look at it, you can really define it by a Mandelbrot set where you have some kind of reiterative branching process. And if you look at the lung tubules and count them and look at the number of branching, you can figure out that you have to go through 20 branching events to get to the set of lung tubules that is in an adult human lung. OK? If you look at the structure, the structures during the process start with the trachea and this thing called the bronchial bud -- -- which divides into two to give two primary bronchi. Then each of those divide more, branch more and so on. So these are multi-cellular, these are epithelial sheets that are branching reiteratively during lung formation. Here's a movie of the process. You can get this to take place in a Petri dish with the appropriate signals added. And so here is the lung tubule branching reiteratively over a period of about a day or two in tissue culture. OK? So this is a 3-dimensional tissue engineering, or at least you can get this aspect of 3-dimensional tissue structure to form in tissue culture. All right. So what genes control this? As you know, as I've told you over and over and over, everything is controlled by genes and the interaction of genes. And what do we know about lung formation? Well, we know most about lung formation from insects. In insects the evolutionary relic or the evolutionary precursor or the evolutionary equivalent is the system of trachea. These are a system of tubes that branch out from the spiracles, which are holes that lead to the outside. And then tubes come off these spiracles and branch in the insect and actually carry air directly to the tissues. There's no circulatory system equivalent as there is in our cells. But the way these tubules branch and form is very equivalent to ourselves. And we know that this involves a receptor signaling system that you are familiar with, which is a receptor tyrosine kinase signaling system. And the particular one I'll tell you about is the fibroblast growth factor signaling system. You remember that growth factors bind to receptors, and these growth factors can be in the extracellular matrix. They usually are. That's one of the places where growth factors are,

factors can be in the extracellular matrix. They usually are. That's one of the places where growth factors are. They bind to a receptor. And then there is a cascade of signal transduction that, in this case, involves kinases. And eventually the final kinase moves to the nucleus where it does stuff to transcription factors and you change the transcriptional activity of a cell and you get stuff happening. OK. So this is a diagram of what happens in the fruit fly where we know most about the process. The epithelium that's going to give rise to the trachea undergoes cell division, and at the same time it balloons out to form this thing called the primary tubule. You have this diagram in front of you. It forms the primary tubule. After a bit, this primary tubules branches to give secondary tubules. And later on it branches again to give these tertiary tubules. There are a number of mutants in drosophila that affect each of these processes, the primary tubule formation secondary or tertiary tubule formation. And these are members of the fibroblast growth factor signaling system. In the primary tubule the FGF receptor is expressed, and the cells receive FGF which tells them to divide and to divide in a particular orientation to give you this primary tubule as it grows out. OK? And there's a ligand sitting around in the extracellular matrix that tells the cells to do this. Now, what's really cool is that at the point where the secondary tubule, I'm going to take 20 seconds, so I'd appreciate it if you'd listen. At the tip of the tubule an FGF inhibitor called Sprouty is made. This inhibits cell division of these cells right at the tip, but the cells on either side continue to divide. And so you get the branching, the secondary branching taking place. And I'm going to stop there. And those of you, don't forget, come along to Stata this afternoon if you need a review.