HAZEL SIVE: All right, moving along, I want to talk about three things today.

The first is breaking symmetry in the embryo, leading to the first differences that then go on to amplify into many differences in a multicellular organism. And then I'm going to talk about organic genesis and then about morphogenesis. And we'll define these terms as we go along.

Let's start with this question of breaking symmetry. And I'm going to start by going back to the last handout of last lecture that you should have looked at-- hm, I wonder if our AV expert could do something to the screen. I don't think it's the computer. I think it's the screens. Let's see if we can do this.

The last handout I gave you was a consideration of how different parts of the embryo become different. And I started off-- and you might have this in front of you still-- I started off with an embryo, a fertilized egg, that's got some kind of asymmetry to it. And I've called it a determinant.

And at the first cell division, look, I've put that determinant into one cell and not the other cell. One cell and not the other cell.

Now, let's say that that determinant has some effect on changing the secreted factors-- oh, fantastic, thank you very much. It's like magic-- that determinant has an effect. And it actually elicits signals to be secreted from the cell.

Then look what happens at the next stage, at the four cell stage, here is a cell that's secreting a signal. And it's doing something to the cell that receives the signal. It's making it brown. And that signal in turn then elicits other signals. So the brown cells start signaling to the cell next door. And as cell division precedes, you now get gray cell.

And this is a theoretical, but a real, demonstration of how the embryo becomes different over time, starting with an initial asymmetry. That is then built upon due to segregation of localized factors and also due to cell-cell signaling. And all of this comes together to make these different regions, which I've cool territories, which eventually will go on to give rise to different kinds of cells or different groups of kinds of cells.

If you look in a real embryo-- this is a fruit fly embryo, Drosophila embryo. You don't have a

handout. Just look on the screen.

You can see the sequential division. And you can see this by looking at changes in gene expression patterns. And it's really spectacular.

This is a drawing. But you can actually look at this in terms of in situ hybridization, that technique I mentioned last time, where one can look at where RNAs or proteins, and in the case of in situ hybridization, it's RNA, where RNAs are localized. And in the very early embryo, there is an asymmetry of a protein called bicoid, where there's more on one side than the other.

A little later, after fertilization, you start to see literally these stripes of RNA or protein across the embryo. And then the stripes of different proteins and RNA get narrower as the embryo is divided up into its segments and so on. And so what you see out of this is the embryo being divided up from one large territory into smaller and smaller territories.

But the question is, where does it all start? And so the first asymmetry has got something to do with fertilization. It can either occur before, at, or after fertilization, where the haploid egg and sperm fuse to become a zygote. And somewhere in this process, this asymmetry develops.

In the case of frogs, the first asymmetries are formed before fertilization, and also in Drosophila, fruit flies. In the case of Caenorhabditis, also known as the worm, asymmetry forms at fertilization. And in the case of mammals, like ourselves, the asymmetries appear to form of to fertilization.

And these asymmetries eventually build up to give rise to a system of positional coordinates, kind of like the lines of latitude and longitude on the globe. And these are called the axes, which are positional values. And there are three of them. I'm just going to put the initials on the board. And then I'll show you a slide, and you can get these later.

They're called a A/P, D/V, and L/R. And if you look on the screen, you'll see my diagram of a mouse, the same in ourselves, where there is this A/P coordinate, which stands for anteroposterior, where antero is the head and posterior is the tail, or as far back as your body goes. Then there's dorsal which refers to back and ventral, belly. And then there's left and right.

And these values are kind of like north, south, east, west, and then degrees on either side of

the meridian. They help the embryo figure out where to put different organs in different cell types. And I'm not going to dwell on them in the interest of time, but there are a whole sets of genes that are involved in setting up these coordinates.

The one thing that I will dwell on more is a specific example of setting up the first asymmetry in Caenorhabditis, where this first asymmetry is set up at fertilization. And the bottom line-- we'll look on it on your hand out in a moment-- is that the sperm entry and particularly the centriole brought in by the sperm causes a massive rearrangement of the cytoskeleton in the egg or in the zygote.

The cytoskeleton we mentioned way back in Lecture 2. It is the system of actin, filaments, and microtubule tubules that give the cell shape and allow it, as we'll discuss later on, to move and to help build the animal or the plant.

So this the sperm entry causes cytoskeletal re-arrangement. And this particularly pertains to actin and tubulin, where the tubulin forms microtubules and the actin forms microfilaments.

And with the cytoskeletal re-arrangement, a protein called Par 6, that is firmly attached to the cytoskeleton, is pulled to one side of the zygote, leaving one side of the zygote with lots of par-6 on the other side with none. And at the first cell division, one cell gets lots of Par 6, and the other gets none. And that gives you your first asymmetry upon which the embryo builds to give rise to all its different cell types and organs.

So the cytoskeletal re-arrangement pulls the attached Par 6 protein-- and this is a regulatory protein. It doesn't matter exactly what it does-- to the future anterior side of the embryo. And in doing so, it actually sets up the anterior side of the embryo.

Let's look at your first slide or your first handout. This is a diagram of the early worm egg, actually the very early worm zygote, because here is the egg nucleus, called a pronucleus. It's haploid. The sperm pronucleus has just entered. And the cytoskeleton I've represented by this grid. And Par 6 protein is represented by the red that you can see on the screen surrounding the outside of the zygote.

Here's the sperm entry, causing cytoskeletal contraction. And with it, because par-6 is attached to the cytoskeleton, Par 6 is moved to one side of the cell. That's the most important thing.

Later on, the first cell division along this dotted line here. And you can see that the first cell, in

the first cell division, one of the cells would get most of the Par 6 and the other cell would get very little. This is a very beautiful mechanism that's been worked out really beautifully in worms.

Here's a movie that I guess isn't going to work. You know what, it was working great before I walked in this room. But it's not going to work. So I'm going to post it as a movie. And you can go to the URL and you can get it.

And you'll see what this movie does will show you fluorescently labeled Par protein. It's actually a different protein than Par 6. And you'll be able to see it moving beautifully to one side of the embryo and not the other.

Number 2, organogenesis.

An organ, as I've written on the screen, is a functional unit of many cell types that are arranged obligatorily in a three-dimensional structure that allows them to function together. So the problem we have here is that an organ is many cell types, arranged in 3-D, such that there is a specific functional outcome, like the heart pumps blood, the kidney filters blood, the eyes see. All of these things require both of these correct cell types and 3-D organization.

And just this sentence or just this phrase raises two questions. How do you get all the cell types to the correct place? And how are they put into the correct structure?

Let's phrase the problem by looking at the kidney. The kidney is one of the most complex organs. It does two things. It filters the blood and gets waste products out of the blood, particularly products of amino acid metabolism. And it also maintains water balance. Both of those things are crucial functions of that kidney, which consists of about 15 different cell types, which are connected into several types of tubes that lead on fluidly from one to the next.

You have one type of tube that does one thing. It's connected to the next tube, which does the next thing, connected to the next tube and so on. The tubes are very long. They form a 3-D organization. And they form filtration units, called nephrons.

Here are part of the nephrons. And you just have to look at this without any knowledge to see that this is a complex structure. This is a distant view, so you can't see the cells here. Low magnification structure. This is a really complex organ. And it takes a moment's thought to wonder how something like this gets put together.

And, of course, this is not only important for developments. It's important for thinking about how you would replace someone's kidney with something that was artificial, and not just using donated organs. Could you build something like a kidney and use it in place of donated organs?

So the question of organogenesis is of huge importance both to basic researches, to physicians trying to understand what goes wrong when organs fail, and to bioengineers who are trying to build organs that would replace human versions.

Let's start by tackling the first question. How do you put different cell types in the correct place? And there are three possibilities, all of which are used by the body.

OK, so one, they can move there. There can be all these cell types all over the body. And they can say, oh, OK, we need to go move over here to build a kidney. OK, it seems unlikely, but in fact, it's true. And that process would be migration, where the cells come from all over the place to build the organ.

The cells need to know where to go. And that just pushes the problem back one. But the great example of cell migration is the limb. Most of the cells in your limbs and mine did not come right from where the limb grew. They migrated there from the spinal cord. They migrated there from the muscles on the sides of the body. And the blood vessels also grew in from the cells that were distant.

So the limb puts it all together by moving cells around. How else could you get cells to the right place?

See, it's not so easy. OK, so here's another one. Well, you could in one place tell a bunch of different cells to form at the same time. You could have your organ and say, all right, we need cell types 1 to 10. Form. Here's some signals. You will form in this one place.

And in fact that happens. This is called co-induction, where many cell types or several cell types form in one place, often because one signal can act at different concentrations to give rise to different cell types. So many cell types form in one place, often due to a concentration gradient of an inducer, of a signal. And the great example of this is the spinal cord, and the nerves in the spinal cord, which arise by co-induction of actually about two different inducers.

And the third one is called sequential induction. And this is how the kidneys forms, where the

idea with sequential induction is that a cell type forms. And it says, OK, I need this other cell type to work with. And it instructs the cells around to become another cell type. And those cells in turn might instruct some cells around them to become another cell type. And there's this conversation going on sequentially in the place where the organ will form.

So cell type 1 induces or signals to type 2, which induces type 3, etc. And a great example of this is the kidney, where I will say it is still not known how you build a kidney. Some of the steps are understood. But there's no organ where we can say these are all the steps that build the organ. It's incredibly complex.

Good. That's all I'm going to say about organogenesis, to throw out at your this complex problem. And you can explore it more in later courses. I am going to talk more, though, about this question of 3D structure. And that is the question of morphogenesis.

Oranogenesis, morphogenesis, you might have heard of histogenesis, which means building tissues. The genesis part refers to building. That's what it means, building of something.

Morphogenesis, building a three-dimensional structure, or the generation of form is what you'll see. And what that really means is building 3D structure. And this relates to organogenesis because in organogenesis one of the things that's hidden on the board above was the question of getting cells of an organ into the correct 3D structure.

And, of course, this question of 3D structure is intuitively quite clear. You can imagine a human heart or having some kind of animal's heart in front of you. It's an extraordinary structure. It's actually a folded tube, where the walls of the tube have become very muscular. And the heart will pump with great regularity. It's a controlled pump that can speed up or slow down depending on the how the animal is doing. And that heart contains about 10 different kinds of cells.

And you can imagine having one in front of you. And you can imagine my coming along with some kind of protease or calcium free medium that will cause the cells of the heart to leave one another, deconstruct the structure of the heart. Now you've got a heart that's not this pump looking tube structure. It's a pile of cells. The same cells that were in the organ of the heart, but, of course, they're not. They're just a pile of cells.

They're all there. They might all be alive. But they're not acting. And that's really an engineering problem, which is what morphogenesis is all about. And I want to just discuss

morphogenesis with you because I think as a science and engineering problem, it's really an extraordinary one to think about.

And it's extraordinary to think about because really there's only one building material by which all the structures of all organisms are built. And that's cells. You can't find different plastics or different metals or different alloys to build things with, it's just one building material. And it's really amazing what life has managed to do with cells. And that's what we'll explore for the rest of the lecture. So getting cells in organs into the correct 3D structure.

This is a great example of 3D structure, another one, the lungs, which are about a 20-fold iteration of branching. You start off with one tube, the trachea, which branches into the two bronchi. And those branch and they branch and they branch. It's about a 20-fold branching. And you end up with many, many little tubes.

In this rendition, the ends of the tubes, which are sacs of cells, have been taken away. But it's through these tubes that oxygen moves or air moves. The oxygen is extracted from the air, moves into the bloodstream, and then the waste air is exhaled. And the same thing starts again. How do you build these lungs with this branching arrangement of cells? And indeed, how do you build the kidney?

You know, the problem really phrases like this-- and if we had more time today, I would sit and challenge you for five or 10 minutes to go and think about how you take a pile of cells from the early embryo and build that structure, which could be what it is or could be something that represents an organ. And while I'm moving the boards, you can think about how you would take those cells and turn them into a 3D structure. And we'll see if we have concordance in our thinking here.

All right, so let's turn this pile of cells into a 3D structure. And let's pull out some processes that we can talk about in lay terms, no scientific terms. And then I'll put some molecular labels on that will help you understand what we're talking about. And process indeed has two s's. There we go.

Well, one thing you know if you look at my pile of cells in the 3D structure is that those cells have been sorted out. I've put them in different colors, in groups of different colors. So I'm going to write that the cells sort out. And there is a molecular basis for that. It's called homotypic adhesion, where like cells tend to bind to one another, so that they can function as a unit.

But, of course, in order to get the cells to sort out, they actually had to move. Yes? Say, yes. OK.

And they moved because of their cytoskeleton, which was rearranging and changing and allowing them to do so. So the molecular basis for movement is cytoskeletal change or rearrangement.

What else is on my list? You know, the pile of cells that I've got there, it's meant to look loose, like it would be a pile of cells that would just spread out all over the table if I dumped it out. But the organ that I drew is meant to look tight. Those cells are stuck together. And that is one of the things that happens. The cells stick together. And they do so because of cellular junctions, which are particular collections of proteins that cause cells to stick together, often in a really waterproof kind of a way.

The cells change shape. They've gone from balls to triangles to columns. And that is also driven by cytoskeletal changes.

And then the two lost processes on my list I'm not going to explore more because we had a whole lecture on them. Cells can divide, and they can die. You can get rid of the excess cells. And you can generate more cells to build your organ by the processes of cell division and cell death. And we've had those before, so we aren't going to talk about those again. But I will just put up cell cycle control and apoptosis. Good. I-- yes?

STUDENT: What about the [INAUDIBLE] the organ, [INAUDIBLE]

HAZEL SIVE: Ah, great question. The question is, how do the cells know where to go? How do they know that the dark blue ones are on the left and the columna brown ones are on the right? It's a great question. And you know, if we had more time, I think that the thing would come up with was the notion of some kind of plan, that there are instructions somewhere that are telling the cells where to go.

What are those instructions? Well, they're somehow in the genes. But it's more than that. I don't think that there is actually a set of instructions in the genes that says build this organ or the kidney or anything else. There's a set of instructions that unfolds as the organ is being built.

And I think what's in the genes are the first steps of those instructions. And then they kind of

unfold bit by bit. So the outcome is the organ. And there is some kind of a plan. But we don't have any evidence that there's any kind of plan actually written in the genes. OK, it's a fascinating question. Good.

Back to the toolkit. This toolkit to build all organs of cells. But cells, you know, are not just equivalent to bricks one shape. They can actually change. So it's not quite fair that I said to you this is different than engineering where you've only got one material. It's true you've only got one material. But it can change. It can change its shape, and it can move around.

And the two kinds of things you really need to know are involved are single cells and cell sheets. And single cells and cell sheets are interchangeable from one another. Single cells are also called mesenchyme. [INAUDIBLE] [? they ?] are. Single cell.

And groups of mesenchymal cells can associate to become sheets of cells. And these sheets are called epithelia, or singular is epithelium.

Single cells migrate. They move. And there are no junctions between the cells that allows them to be single cells.

Epithelia, cell sheets, can change shape. But they don't move around that much. So they change shape as sheets. And what that allows them to do is to form coverings and tubes. I should say one very important thing. The process is reversible.

If you look at the next handout, there is more information that you'll get. Here's your epithelial sheet. It's joined together by junctions.

Let me make a note that there are junctions between the cells. That's what sticks the cells together is a sheet. Here are the junctions. There are two kind. There's top ones called tight junctions, which are waterproof junctions. And then they these are the ones, called adhesion junctions, which aren't so strong.

Both epithelia and mesenchyme sit on the extracellular matrix, or ECM, which we mentioned way back, glycoproteins that are involved in giving support and also carrying signaling molecules to cells. And here is the conversion of the sheet to the single cells, and the single cells back to the sheet.

This process of epithelial mesenchymal transition or mesenchymal epithelial transition is very important in cancer. Tumors usually start off as epithelia. The And they become metastatic.

They disperse throughout the body when they become mesenchymal, or when they give off single cells, which can migrate and establish the tumor somewhere else. So this process is crucial both in building organs and in cancer biology.

The other thing that you should note-- we're not going to dwell on it very much-- is that the cells in a sheet have got orientation. They've got an axis of asymmetry along one side, call the apical basal axis and an axis of asymmetry along the top, called the planar axis. But we're not going to dwell on that very much.

All right, let's look what epithelial sheets do in a little more detail. So epithelial sheets or epithelial form waterproof coverings, as I've noted. And through cell shape changes, they'll also bend a cell sheet or turn it into a tube.

And they do this by a series of stereotypical changes, where cells can change shape, so that kind of a square cell can become an elongated cell in one direction or a cell that's elongated in a different direction. Or it can become a wedge shaped cell. And the names of these different kinds of cells don't really matter.

And they're not sequential. So this cell can become this cell. It can also become this wedge shaped cell in one fell swoop.

But if you think about it, these changes in cell shape can do lots of things. For example, if cells get long and thin, then the cell sheet will lengthen. If cells get wedge shaped, and you've got a whole bunch of them getting wedge shaped-- you can draw this out-- you'll actually bend the cell sheet.

Draw it out. Put a whole bunch of wedge shaped cells next to one another. And you'll see there's no way you can get a flat sheet. The cell sheet bends. And that is one of the things which drives building the structure of organs.

There are a lot of different ways to make tubes. I've diagrammed them on the next few slides. You don't have these.

Cell sheets can roll up to form a tube. Mesenchymal cells can condense, can come together, to form a tube. And here's a really extraordinary one. Single cells can form tubes. Tiny, single cells with a 10 micron diameter can roll up on their cell themselves or actually hollow out their middles to become tiny, tiny tubes.

What about single cells? Single cells move. And this allows things to sort out and cells to get to where they need to go.

But we're also going to use this property to understand the molecular principles that underlies cell shape change. And that you should know. Let's make a note that single cells move and that this allows cells to sort and tissues and organs to form.

And then what I'm going to tell you over the next few slides and on your handouts is that all of the movement and all of these shape changes that I drew on the board have got to do with changing the cytoskeleton of the cell. But what I'll tell you is that it's about locally changing the cytoskeleton, where you have to stop viewing the cell as something huge, where independent things can happen in different parts of the cell. And those independent changes in the structure, in the skeleton of the cell will get the cell to become long or squat or wedge shaped or moved.

So shape and movement of both sheets and single cells is controlled by changing the cytoskeleton. But it's done so locally, which means in one part of the cell.

And a most important change that occurs as the cytoskeleton changes or with cytoskeletal changes or is cytoskeletal change is a polymerization of actin from G-actin, which is unpolymerized, to F-actin-- and this is reversible-- which is polymerized, non-covalently. And this F-actin will stretch the cell and help the cell to move.

And let's explore that in this movie and in the rest of your handouts. This is a movie of a cell, where the actin has been labeled fluorescently. And what you can see as the cell is moving are these little filaments of bright green. Those are the polymerized F-actin.

And you can see if you just focus on one, over the course of minutes, it goes away. And new ones form. And the new ones are forming in the direction that the cell is moving. And this movie really illustrates the principles underlying how the cytoskeleton is controlled.

Let's draw something on the board or let's write a few things on the board and then look at your last two handouts. And what you'll understand is that the principles governing actin polymerization are really the same for both single cells and for cell sheets.

The idea here is that the extracellular matrix, the ECM, connects to the plasma membrane via receptors on the plasma membrane. When ligands bind these receptors, you know what happens now. Signal transduction is activated.

So the ligands plus the receptors-- and there are many different signaling pathways that can be involved-- go and activate a cascade of things, which involve kinases, a signal cascade via kinases and via special GTPase. Remember Ras was a GTPase, but this one is called Rho. And Rho GPTase it's critical in telling G-actin to polymerize and form F-actin. And so out of this the Rho GPTase tells G-actin to form F actin. And with that, various things happen.

So let's look at your last handouts. Here's the cell migrating. And the thing that I want you to see is that in the direction of migration, there are receptors that are bound to ligands. Signal transduction has occurred. I've diagrammed it down here. And F-actin, these little filaments, have formed in the direction in which the cell is moving. And where the cell is detaching so that the cell can move forwards-- you have to lift up your foot in order to move forward. It's the same thing with the cell-- where the cell is moving forward, they're no ligands. And there's no F-actin.

That principle is crucial also in changing cell shape and sheets. It's a little more complicated. But, again, there are receptors that interact with Rho GPTases, change the cytoskeleton and cell sheets and change the shape of the cell sheets. And this is really the molecular underpinning that will get those single cells to form tubes and bench sheets and all of the materials that are needed to build organs. And we'll stop there.