

Potent Biology: Stem Cells, Cloning, and Regeneration (2006)
Lecture One—Understanding Embryonic Stem Cells
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1. Start of Lecture One (00:17)

From the Howard Hughes Medical Institute... The 2006 Holiday Lectures on Science. This year's lectures, "Potent Biology: "Stem Cells, Cloning, and Regeneration" will be given by Dr. Douglas Melton, Howard Hughes Medical Institute investigator at Harvard University, and Dr. Nadia Rosenthal, senior scientist at the European Molecular Biology Laboratory. The first lecture is titled "Understanding Embryonic Stem Cells." And now to introduce our program, the president of the Howard Hughes Medical Institute, Dr. Thomas Cech.

2. Welcome by HHMI President Dr. Thomas Cech (01:09)

Welcome to the Howard Hughes Medical Institute and to our 2006 Holiday Lectures on Science. We're webcasting live here from our auditorium of our headquarters in Chevy Chase, Maryland and in the audience are almost 200 high school students from the Washington DC area including adjacent parts of northern Virginia and Maryland. To learn more about the many research and educational activities of HHMI please visit our web site, www.hhmi.org. Now at our web site you can subscribe to science news feeds or you can visit BioInteractive pages where we are making the entire series of Holiday Lectures available for video podcast starting Monday, December 4th. This year the topic is stem cells, a topic that combines exciting biology, intriguing politics and an important opportunity for ethical analysis. In this series of four lectures, Doug Melton and Nadia Rosenthal are going to tell you exactly what stem cells are and why they have such great potential for basic research and in the future for medicine. Sometimes lost in the news reports about embryonic stem cells and research is the fact that stem cells are fundamental to our normal development and health. Without stem cells none of us would be here in the first place. In his first lecture Doug Melton, who's an HHMI investigator at Harvard University, will show us the fundamental role that stem cells play in normal development from egg to adult. Doug had long been an outstanding developmental biologist. His earlier work focused on understanding how frog eggs developed into tadpoles, but he became so excited about advances in mammalian stem cell research that he has refocused his career towards harnessing the therapeutic potential of human stem cells. Doug will begin his lecture after this brief video introduction.

3. Dr. Douglas Melton in the lab (03:33)

[music] One of the things I like about teaching at Harvard is that it's a liberal arts college which means that it selects for students who may not always be certain what they want to focus on when they come and some of the students I've had in my experimental embryology class for example have been music majors and I find that really enjoyable. Many bright young people are interested in more than one thing. Science might be it, economics might be it music might be it, and a great thing about being here is we get some of the world's best undergraduates and it's a privilege to teach them, it's really energizing and a lot of fun. The most important thing to be a good scientist is to be deeply curious about how the world works. Without the curiosity or wanting to understand why things are the way they are, the sort of mechanism of how animals and plants develop, how does photosynthesis work why are we the size and shape we are, what makes us think or feel what we do, if those questions don't interest someone, then science will be quite boring. For me those are about the most interesting questions that exist is really how did life come to be as we know it and can we understand the basis of animal and plant development. I find the basic problem of early development, how does an egg divide and make different kinds of cells fascinating. I found it fascinating when I was a young boy and I still find it fascinating, it's really one of the great mysteries of life and really for me it's puzzling everyone wouldn't want to study it because it's so interesting. I'm hoping that a large number of the

students in the audience will get so excited about stem cells they'll want to come and join us and others that work in that area.

4. Introduction to stem cells (05:35)

Good morning. It's a pleasure to be here to talk to you about this very exciting and intriguing aspect of modern biology. I'm grateful to Tom Cech and HHMI for inviting me and my colleague Nadia Rosenthal to give these lectures. It's especially nice to talk to high school students in particular because this area is just now beginning to blossom or burgeon. You'll see that there are very interesting problems the answers to which we don't know and I'm hoping that some of you will decide this is an area where you'd like to make a career and help us work on and solve these problems. Now stem cells and cloning has been much in the news of course as you all know and one of the goals of my talk is going to make sure that we understand the basic scientific facts. I'm going to begin therefore by talking about the basic biology of development, about how animals develop how we come from a fertilized egg because that sets the background for understanding the special properties of stem cells. We'll talk about stem cells, about how they're involved in regeneration, possibly involved in aging and what is the role of cloning and cloning technology in all of these studies. Today then I'm going to begin in the first part of how we come from an egg. Some of this is intuitive of course but there's a lot of growth and development from a fertilized egg and I'm going to review some of the basic biology of that and then we'll move on to talk about stem cells.

5. Development is growth and differentiation (07:02)

Now there are two aspects to development. I'm going to use the example of human development and this involves both growth and differentiation. A human egg is about the size of a period in a sentence and obviously when it becomes a person like yourself there's been a lot of growth involved. Now that development then has these two aspects that we'll say something about. In the first case we can just think a bit about the growth. The growth is very rapid during embryonic development and then in the adult stage it's rather slow it's less growth and more just maintenance. A second aspect of course is that cells become different. As the embryo divides there's a period when cells have to be set aside to make different parts of the body. Now it wasn't always obvious that differentiation was a gradual process and that's one of the points I'm going to make this morning. It was in fact thought for many years, for centuries that development was just the consequence of growth that there was no change in the cells and I'm going to show you a funny picture which is how people thought about development for many centuries. This is the picture of a little animal, a human in this case, called a homunculus who was thought to be all folded up in the sperm head, as shown here and that all that was involved in development was the growth of this, that is kind of like those little sponge toys you had as a child, where you get a little sponge and you put it in water and then bingo, something comes of it. That was what was thought as being the main mechanism of animal and plant development for centuries. As you well appreciate there is this process of differentiation, cells becoming more special as development proceeds and that's what we'll talk about now.

6. The variety of cell types in the human body (08:49)

Now the end point of the differentiation is that there are many cell types in your body. The exact number isn't known but there are hundreds of different kinds of cells in your body. In this slide here we see an adult human in the middle and around her is examples of a few kinds of cells. You can see a skin cell up at the top left, there's muscle then blood cells, bone, I'll be talking quite a lot about the pancreas and a cell there called the beta cell. There's the liver, the intestine, and two examples of nerves. So how do these different cells arise? To introduce us to that I'm going to show you a short video and that video will show you that it's a gradual process and attend to a very important early stage when cells begin to become different.

7. Animation: Human embryonic development (09:38)

In this video then we'll look at the beginning of development, looking into the ovary at an unfertilized egg. It then gets fertilized by sperm which travel down the fallopian tube, so here are millions of sperm coming along, several of them will hit the egg and try to penetrate it but one will win as it were, go into the nucleus and then there's a reprogramming process where the male and female nuclei have their genes set aside to be turned on and off for early development. Here you see early cleavage stages occurring and this is one of the early growth phases as the embryo moves down the fallopian tube. It's going to form an important stage called the blastocyst here in a few seconds. Of course in real life that takes days, about five days. At this stage then I'd like to draw your attention to the inside of the blastocyst where there are cells called the inner cell mass which I'll be abbreviating as ICM. Those are the cells that make the entire animal and the outer cells give rise to the placenta and other supporting tissues. At this stage the embryo implants into the wall of the uterus this is when the pregnancy is really initiated and now we'll see those blue inner cell mass cells form a disc and then as the cells continue to grow they change their physical positions, their kind of geographical relationship to one another and you'll see that represented here as this disc gets transformed into an embryo. Those lines represent sites where cells are migrating in and out and here's an important stage when the three beginning layers of the embryo, the so called germ layers are formed and I'll come back to that in a few minutes. As development proceeds there's more growth and movement of cells, it will begin to form a neural tube here it turns and appendages start to bud out, you see the head forming and the eye, and then eventually we get a small embryo and some months later of course this would be born as a young baby.

8. Germ layer make specific tissues (11:56)

Now what you saw there is both the complicated movements and the growth of the embryo and I want to focus in a bit on these early stages of the formation of the germ layers as they're called, the first signs when cells are being told what they will become. As you saw in the picture, there were three germ layers. I'm not exactly sure how to explain or describe why they're called germ layers but they're germ in the sense that these are like buds or something that will develop into something new as the animal proceeds. We can think of them as sort of the primary colors. They're not drawn here as primary colors as red, blue and yellow, but rather as blue, green and yellow and you can think of them as the subset or the sort of sub stratum which is used to make the animal. And one point I'd like to emphasize then is that the outer layer, the blue layer, the ectoderm is going to give rise to the whole nervous system and the skin. The inner middle layer called the mesoderm will make the bone, blood, and muscle, like the heart muscle and the skeletal muscle, etcetera and I'll spend a fair amount of time talking about the third layer, the endoderm which makes the gut tube, running from the mouth to the anus and what is formed from the gut tube are the lung the liver, the pancreas, the stomach, the intestine etcetera, and in the next movie I'll show you to emphasize this point, you'll see that at the end we begin to focus in on the development of the pancreas as one example. If I could have the next animation please.

9. Animation: Germ layers and cell fate (13:31)

So here we're going to go through quickly the early stages of development again and then we're going to get to this stage when the germ layers are set aside the three germ layers, the ectoderm, mesoderm, and endoderm. So here you'll see then that the inner cell mass gives rise to the entire adult animal, in this case a human. This same process would occur in a mouse as we'll see a bit later. If we now look at the embryo after the three germ layers have formed, this video will highlight what comes from the blue germ layer, the ectoderm. You'll see that it makes the nervous system including the brain, and the skin. The middle part, the mesoderm, green here, gives rise to the muscle, including the kidneys, the heart, and the endoderm gives rise to the whole gut tube. Here you see the lung, the liver, the intestine and to give one more detailed example of this let's think about the development of the endoderm and in this case the formation of the pancreas, so there's the pancreatic bud which comes out of the endodermal derivative. Now I'm going to talk to you a bit

today about the pancreas as one example of how an organ, and then the cell types within that organ, get made.

10. The pancreas: Structure and function (15:01)

And in order to do that I have to remind you a bit about what the pancreas does. I doubt any of you would say that the pancreas is one of your favorite organs or that you even think much about it but I'll remind you that when you had breakfast this morning you did that to gain nourishment and you couldn't do that without the important role of the pancreas. So where is the pancreas and what does it do? Let's have a look here at our little human model and the pancreas is a banana size organ found right next to your stomach and your intestines. I'm going to remove the stomach here from this little fellow and you can see this white organ here which is the pancreas. As I said it's about the size of a banana and in the next slide I show a picture of what it looks like, that's here. So you can see it's nestled in next to the top part of the intestine and the reason is that it serves its physiological function related to the digestion and the use of food. That purple line there is a duct into which are secreted all the enzymes that the pancreas makes that allow your intestine to digest the food that you've eaten

11. Function of specific pancreatic cells (16:11)

and we can see that in a bit more detail as to what at the cellular level of this tissue or organ is involved in digestion. You can see here a cell called an exocrine cell which is the cell that makes these enzymes. They're secreted then into that inner tube that flow into the intestine to digest food as I said. There's a blood vessel right next to the endocrine function of this organ, the endocrine component which is called an islet, standing for island. Now I want to look a little more detail at this pancreatic islet because within it, it has just four kinds of cells and an important one that I'm going to use as an example of cell differentiation is called the beta cell shown in yellow at the top and the beta cell makes the hormone insulin which many of you will have heard of. The other three cell types make different hormones like glucagon and somatostatin. I want to show you a real life picture of this which is actually a stained picture of an islet which is done with a microscope that measures the fluorescence of this stain called a confocal microscope. So while that cartoon there gives you an idea of what it looks like in real life an islet looks like this. In this case the colors are a little different, the insulin producing cells are in blue.

12. Role of the pancreas in diabetes (17:28)

Now why am I so obsessed with this pancreatic islet why have I chosen this as an example? I could have of course chosen any example: a cell in your eye a cell in your heart, a muscle cell or a blood cell as an example of differentiation but one of the important reasons that I've chosen this one is that the production of insulin is very important in order for your body to make use of the food you eat and once the sugar enters your blood and once your food is digested into sugar and is flowing through the blood stream the islet measures the amount of sugar in the blood and then secretes this hormone insulin in order for the rest of your cells to take up sugar and use it. Without insulin one doesn't survive and there are two diseases related to insulin and sugar utilization they're both called diabetes, type I and type II diabetes and I'll say a bit more about those today and tomorrow but for now I want to make the point that the pancreas is very important in terms of thinking about medical problems like diabetes in addition to understanding normal development.

13. Progressive development creates specialized cells (18:32)

So let's talk a bit about normal development here that is how does the body make a cell type like this? As I've already said, this doesn't just happen like magic going from a completely unspecialized cell in order to a fully specialized pancreatic beta cell but it's rather a stepwise process and in this slide here we can see some of the steps involved in that process. You'll remember in the early development I showed a stage called the

blastocyst, inside of which there was the blue cells of the inner cell mass. So one of those is depicted here up at the top, ICM and that cell then makes decisions about becoming or some of its daughter cells make decisions to first become endoderm, then a pancreatic bud cell then an endocrine cell which is the islet component and then finally within the islet it's going to be a beta cell or some other type of cell as shown here. Now in order to really understand this process we need to know what's going on at each of these steps and as you will appreciate, this involves turning genes on and off which make your cells different. The reason your lens cells of your eye, for example aren't like a pancreatic beta cell is because the cells in the eye express lens crystallin and the pancreatic beta cell expresses insulin. So they're expressing different genes.

14. Demo: Using a DNA chip to study gene expression (19:54)

Now as scientists we can tell what genes are being turned on or off by using a technique that involves something commonly called a DNA chip. This is a small microscope slide onto which has been spotted all of the genes, a copy of all of the genes in the human genome, which is about 30,000 genes. And so I'll show you with an example using a nerve cell here how we would go about doing that. So if we wanted to know which genes were expressed in this nerve, so here we have the head of the nerve where the nucleus is found, and here's the axon and here's the business end that sends the signal, if we wanted to know what's going on in terms of which genes were on and off in this, we would extract the nucleic acid which I'm going to do here with this turkey baster since we just finished Thanksgiving. So you suck out the nucleic acid, extract it from the cell and then using part of the nucleic acid called RNA which represents the genes which have been expressed or turned on, one can make a probe from that and label the probe with a fluorescent marker. That's then applied to a DNA chip and I have my little DNA chip here which some of you may remember from playing games as a child. We then take our labeled probe and put it into this chip like this and this will, because there are spots representing every gene in the genome, tell us exactly which genes are on, shown here, or off, in the other color. Now I've done this with a nerve and it would be a different result, a different pattern if we used a different cell type and I'll show you in the next slide an example of a chip, a cartoon of one and what it would look like.

15. Genes are turned on and off at each step of differentiation (21:35)

So here in principle then are the 30,000 genes found on the chip and some of them will be what are called cell type specific like the nerve I just did and others would be genes found in all cells, sometimes called housekeeping genes, things like histones, the gene for the histone protein would be expressed because all cells need histones to wrap up their chromosomes and there would be other proteins needed for this general cellular machinery. Here's then an example to make or emphasize this point that different cells express different sets of genes and if I did my little trick right you'll see that our connect toy there looks just like a nerve cell. Blood cells would have a different pattern as would the pancreatic beta cell. So the point here then is that we can use the expression of genes to monitor what's happening during development as cells are making decisions about their fate. Now how do we put this all together? Well if we want to know what happens during the life of the cell, how do you get from an unspecialized undifferentiated embryonic cell like an inner cell mass cell to a pancreatic beta cell. At each step genes are turned on and off and we can see that here in this slide. The inner cell mass cell then has the decision to make initially. Should it be part of the ectoderm in blue, mesoderm in green, or endoderm in yellow? That sort of lineage tree might remind you of like your own ancestry, trying to think of where things come from and similarly those kind of lineage studies can be done on a cell like this. The first decision to make in this picture is to become an endoderm cell, turning on some genes. The next decision is within the endoderm not to be lung not to be liver, but instead to be pancreas. Within the pancreas it's making a decision to become part of the endocrine component, the component to make hormones, and finally it makes the decision to become a pancreatic beta cell. So here if you like is this sort of molecular history of what a beta cell looks like

16. Differentiation is like making life decisions (23:42)

and I'd like you to think about it as an analogy that this relates to how your own life might unfold. What are the decisions involved in your life that tell you to become say a soccer player, or a lawyer, or a scientist? There are all kinds of influences that impinge on those decisions and it's not a process where when you arrive from your mother's womb, you decided right then to be a scientist. This is a stepwise process that involves your education you're affected by your neighbors in school, by your parents, and by others and similarly cells have to make these decisions by influences both internal and external.

17. Cytoplasmic factors affect cell fate (24:21)

And so I'm going to now move to this point of how do cells know what genes to turn on and off? What are the signals that tell a cell what to do? And in general there are two kinds of signals. One internal and the other external. The internal signals which happen earliest in development come from cytoplasmic factors. These are factors which are in the cytoplasm of the egg and begin by sending signals into the nucleus usually by a transcription factor to tell it which gene to turn on and off and we have a little video if I could have that next, to show you what that would look like. So here you see these factors, they're color-coded to correspond to the germ layers and they're going in and out of the nucleus to tell that nucleus which genes it should turn on and off for example, to initiate development. We'll now move to the other kind of signal so as initially cells are being told which part of the germ layer, which germ layer they should pick,

18. Cell-cell interaction also affects cell fate (25:26)

and then they're going to move to the issue of signals by adjacent cells. Here are adjacent cells that are involved in determining cell fates. In this case I'm showing the example from the endoderm where adjacent cells, either other endodermal cells or cells from the mesenchyme which is a mesodermal derivative, send the signal and as you see here these signals go back and forth telling the cell what it should do and then once it makes a decision it will send signals back to the signaling cell. That can then result in the end of the cell making the decision to become a pancreatic bud cell. I've talked about these signals in a general sense what are they in particular? They are almost always a kind of gene product called a growth factor. Now that's a little bit confusing because I'm telling you that they're not involved in telling the cells to grow but they're called growth factors because they were initially discovered by their ability to make cells grow in a tissue culture dish. There are families of growth factor molecules and nearly 100 or 200 important such signals known in development. What this looks like in this picture here is that a neighboring cell will synthesize a growth factor here shown as a little circle or a polygon, and then it gets secreted by that cell to its neighboring cell and when the neighboring cell receives it through a protein receptor or trans-membrane receptor on its surface it changes the fate of the cell and tells it to become a pancreatic bud cell. I will show you in a few minutes examples of these signals telling stem cells what to do. But for now I want to summarize at this point and say what have we covered so far? We've covered the point that from a fertilized egg to a full adult differentiated animal involves a multi-step process, a gradual stepwise signaling to cells to tell them what their fate should be. After I take some questions we're going to talk about how those different cell types are maintained, but for now I want to see what questions you might have about this brief summary of early development.

19. Q&A: How many genes are involved in differentiation? (27:33)

Yes. When you were showing the pictures of the nine cells with the green or red showing on or off, those nine were different for each different kind of cell, do you basically use the nine or were those just graphic representations of those kinds of cells? Yeah, that was a highly simplified version. In practice what we use is all 30,000 genes on a chip to say exactly which genes are on and off out of that 30,000. And you can imagine this requires some serious statistical and computational work because it's too big of a number. It would be easy if it were like our little toy but given there are so many kinds of cells in the body and they

change their gene expression pattern during development, it's not surprising that so many genes are involved. But I forgot to tell you that there's a reward for asking a good question like that, and here's a Harvard t-shirt.

20. Q&A: Does the ectoderm determine pigmentation? (28:28)

Any other questions? Yes, up there in the back. I was wondering, you said that the endoderm is responsible for the skin. Is it also responsible for pigmentation later on? Yes, the question was about the germ layer that makes the skin and the nerve, the ectoderm, and yes it does give rise to not just the outer layer of the skin but also the hair, the so called sebaceous glands which make the oil to lubricate the skin and many of the pigment cells. Some of the pigment cells come from the neural crest which is also part of the ectoderm. Now you're pretty far back but I'm going to see if I can get this all the way up to you. Yes, another question, yes here.

21. Q&A: Does a cell respond to multiple growth factors? (29:18)

You said that a cell can choose what it's going to be by growth factors. Can it have multiple growth factors like from, say a cell might be a blood cell or a skin cell because they're so close can it have multiple growth factors and how would that affect the cell? Yeah, that's a great question. The way I showed it implied that there would be a single growth factor to tell each cell what it should do but that wouldn't really make sense because you couldn't have one factor for each stage of development. In fact what cells do is kind of take the temperature or make multiple readings. I guess we kind of think about it like multitasking when you're using your computer, it might be reading signals from three or four growth factors and its next decision might also depend on its history what it is at that time. And again here I think the analogy is quite apt thinking about your own education. The signals you receive now and from the classes and from your friends and your parents are read by you or interpreted by you in part based on your history but also in part on what you're willing to listen to not what receptors you have. Good question.

22. Q&A: Can you change gene expression to change cell types? (30:26)

Yes. Is it possible to manipulate the gene expression in the nerve cell to make it like a blood cell or something? That's a great question because it anticipates what I'll be talking about tomorrow. How could you, once these fates are determined or specified, how permanent is it? Can you manipulate a cell with say growth factors or some other kind of signal to make it change its gene expression pattern and become another kind of cell, and as we'll here tomorrow that really is the main result from nuclear transfer or cloning. Thanks.

23. Q&A: Can cells be changed in vitro with chemical factors? (31:02)

Yes. We were talking about cytoplasmic factors and what a cell changes into. And I was wondering how much we know about like their chemical factors and like if in vitro, like how much do we know about the chemical factors so we can like, stimulate the cell, the original cell mass to grow into a certain cell? That's a very good point. If we knew the cytoplasmic factors that were going into the nucleus to program or reprogram genes that would be wonderful because then as you say we could try to find chemicals that mimic that and then for example just take a skin cell and now turn it into another kind of cell. So it's a very hot area of research, it's called reprogramming so when one puts a nucleus into the cytoplasm of an egg, it's getting these reprogramming signals and we'll say a bit about that tomorrow, but the right answer to your question is that's precisely the kind of thing we want you to be working on. We don't know what all those signals are. We have good ideas that they involve changing the conformation of the chromosome, sometimes called the chromatin and opening up regions of the genome so that it can be expressed or not, but we don't actually know the molecular mechanisms of that.

24. Q&A: Do internal/external factor's influence cells simultaneously? (32:21)

Right here in front. The two factors that make the cell differentiate, do they occur simultaneously while the cytoplasmic factors are going on, they also interact with other cells, or does the cytoplasmic factors occur first? The distinction I made between cytoplasmic factors and external factors is a little bit artificial because the external factors work by binding to a receptor on the cell membrane which then almost always involves some changes in phosphorylation of internal cytoplasmic proteins and then those go into the nucleus to turn genes on and off. So your question is a good one because it shows that these two things are not a sharp distinction, that is a cell uses one or the other. The external factors eventually become internal signals. Here you go. There's our last one for now. I'm going to move on to part 2 of this morning's talk and then we'll take some more questions at the end of that.

25. Body maintenance and cell renewal (33:24)

So this process of differentiation that we've just concluded ends up having these different kinds of cells, you might note here the boxes are color-coded for the different germ layers. But I want to point out in a sense a trick of what I've said to you so far. I've implied that the problem of developmental biology is to figure out how do you make the animal, but that's actually not true and not fair because development doesn't just involve the production or the construction of the animal, but its maintenance, and your bodies are not stable, they're constantly being turned over with new cells, so I think it's probably obvious to you that you know that your skin cells turn over for example. And I now want to talk about this. One way to think about it is sort of like looking at a river. If you go to a river or a stream that you're accustomed to looking at, most of the time it looks about the same but at any one instant there's different water of course moving through that river or stream. Similarly you didn't have any trouble recognizing yourself when you looked at your face in the mirror this morning but the cells that are present there weren't there a few months ago. So let's talk a bit about the rates of this turnover. This turnover is an important issue because it relates to the larger problem, or the related problem you could say of regeneration and Nadia Rosenthal will be talking about that. How do cells maintain their bodies... I'm sorry, how are bodies maintained by cells, and that's what I want to focus on in this part.

26. Different cell types have different renewal rates (34:59)

So let's look a bit at the rates of turnover. Differentiated cells are not static is my point, cells are continually being renewed. Among the cells which are renewed most quickly are the cells in your intestine. They last just a few days, three to five days in general, so at the tips of the inside of your intestine cells are constantly being sloughed off and new cells are differentiating to replace that. Similarly blood cells last up to a few weeks. The red blood cells shown here in the picture last up to about 10 weeks, white blood cells, the cells that fight infection last for a much shorter time less than a week. In other tissues and organs, the one I've talked about in particular the pancreas, cells live for many, many months, sometimes for years and still other cells particularly many types of neurons are essentially permanent. That doesn't mean that your whole brain is permanent as that picture shows but some of the cells in your brain are and I mentioned for example cells in the eye before the photoreceptor cells are essentially permanent. The ones you had at birth are the same ones you have now.

27. Stem cells are responsible for maintenance and repair (36:08)

So how is this maintenance of the body achieved when there's the constant turnover? I'm going to show you two ways our bodies do that and this allows me to introduce these very exciting cells of stem cells. So as you've already guessed stem cells are responsible in many, but not all cases, for this maintenance this replenishment, this repair of your body. So what is a stem cell? This might be the most important slide I'm going to show you today because

28. Two essential properties of stem cells (36:39)

in this cartoon we see properties of stem cells, many of which we really don't understand. We know that stem cells have these properties or this capacity, but we don't yet understand how they do it. So these are really magical cells that can do two things. The first thing a stem cell can do as shown in the top is that it's capable of self renewal and that should make you think about this process of renewing your body regeneration, replenishment and repair. If we could understand the genetic program that allows a cell to divide and make more of itself, that would give us some deep insight I suggest into how our bodies are repaired. The second thing a stem cell can do shown going to the lower right is that they can give rise to a daughter cell one of the progeny, which has the capacity to make specialized cells. In this case there are three different colored cells forming and I'll show you now that for adult stem cells those specialized cells make up the different parts of an organ. Let's think for a moment about skin or the intestine. An intestinal stem cell would be able to give rise to all the different kinds of cells in your gut that are involved in secreting the mucus of the gut, absorptive cells and other types, or similarly in skin, as the question asked before, a skin stem cell can give rise to the skin you see on the palm of your hand, as well as to the hair and the glands, the sweat glands and the sebaceous glands in your skin.

29. Blood stem cells can replenish and differentiate (38:09)

One of the best understood stem cells and one which you will intuitively know about is a blood stem cell. So many of you would have donated blood at a blood drive and you'll remember of course that when you go and give some blood, a half of pint or a pint of blood, your body isn't in permanent deficit for that blood, it tops it back up to the right number of blood cells and that's done through a blood stem cell which has the difficult name of hematopoietic stem cell and that's shown here in this slide. So blood is replenished by stem cells and this is as I said one of the very best understood cases and in fact it's used clinically. So what does a hematopoietic stem cell do or what is its capacity? Well it can self-renew, which isn't actually shown in this slide, but it can make more of itself and it can also give rise to these kinds of different daughter cells. If we look down at the bottom we see that it can give rise to a red blood cell or a B cell or a T cell which are cells of the immune system and the other ones that are unlabeled refer to things like granulocytes and megakaryocytes, different components of the blood system.

30. A single blood stem cell can replenish an entire animal (39:16)

Now what's especially interesting is that a single blood cell can make all of the blood in an animal. This was shown in a nice experiment where one takes a mouse and removes its blood or destroys its blood by irradiation. We see here then if we take the little mouse and give it x-ray irradiation that mouse's blood stops being produced and the mouse will die without any further treatment. If one then injects into that a fully differentiated red blood cell or a group of them, the mouse could survive for maybe a very short period, but not for very long because remember red blood cells don't live very long. If however one puts in just a single hematopoietic stem cell, just one of those cells, that's sufficient to have the mouse survive and make all the different kinds of blood cells we saw in the previous slide. So that experiment shows really the power of a hematopoietic stem cell and that power has been brought to clinical treatments, to medical treatments. Many of you will know of what's sometimes called bone marrow transplants, so these are given to cancer patients. So when the bone marrow is transplanted into a cancer patient after the patient has gone through chemotherapy treatment, it is the hematopoietic stem cell in the bone marrow, that's where it's found, which allows the patient to then survive. Now

31. Some cell types replenish by division, not by stem cells (40:42)

how does the animal or does one use the stem cells to replenish the different parts of the body. I've given an example and talked a bit about stem cells, shown there at the top and I now want to talk about other kinds of tissues, in particular the pancreas which don't seem to use stem cells but instead have in a sense a simpler but clinically less useful mechanism which is just to take the differentiated cells and have them divide. So let's think about the pancreas. Remember that it has a slower turnover rate, the cells live for many months to years, but nevertheless there is some turnover, the organ is not static and that is emphasized here in this picture. These little bubbles are showing the cells dying and then being replaced. Now here in this slide it would seem like it's happening every few seconds, in fact it takes months for this to happen but during your lifetime this will go on your pancreas is being renewed.

32. A pulse-chase experiment on pancreatic cell replacement (41:41)

Now in an interesting experiment called a pulse-chase experiment we tested for whether there were stem cells involved in that replacement or whether there was some other mechanism and I'm going to describe that experiment to you in some detail, it was done by my colleague, Yuval Dor and it's a little bit complicated but I'm sure you'll be able to understand it. It involves genetically labeling cells and then watching what happens to them if you wait for some period of time. So it's called a genetic pulse-chase experiment and this is how it's done. The circle there is intended to represent one of the pancreatic islets that I showed you earlier. So here the islet, we're pretending that it only has one kind of cell, the pancreatic beta cell, the insulin producing cell and it's shown there on the left and the key part of this experiment is to label cells at the beginning of the experiment, at time zero, or at the pulse, and label them in such a way that they will express an enzyme that we can then use to stain the cells blue. The enzyme is called placental alkaline phosphatase and it's arranged in this case so that the only cells that will express this enzyme are those that are already fully differentiated, and that's an important point. They are cells that are expressing the insulin gene and are fully functional beta cells. So we label them at this period and then we're going to wait to see what happens. So let's consider the two possibilities. Since the cells in the islet are turning over, if the blue and the yellow cells die and they're replaced by a stem cell, that stem cell would not have been marked because it wasn't expressing the insulin gene, so all of the new beta cells that would form from a stem cell would not be blue, that is would be yellow in this picture. That's what would happen if the pancreas were like the blood or the skin, there would be constant replacement. However the way we do the marking experiment which is a genetic and a permanent change through the genome at time zero, all of the blue cells, if they were to divide, give rise to more blue cells. So if we look at the upper right and imagine that those blue and yellow cells are slowly going to be dying off and then consider that the ones that remain can divide to give rise to more cells that are either blue or yellow, whichever one they started off as, in model two you would get maintenance by beta cell duplication. So the two extremes then are maintenance by stem cells or maintenance by the differentiated cell itself dividing.

33. New pancreatic β cells can be made (44:15)

Here's what it looks like in the real experimental result you can see in brown the staining for the protein insulin, all of the cells in these two little islets, one is bigger than the other, would be expressing insulin but the dark blue ones have also been genetically marked so they're really blue plus brown although you can't see the brown because the blue is so strong. So this marks the cells, the important point here is half the cells are marked at time zero, at the pulse and you can see it's very specific. Looking at a section through the pancreas, the ducts and the exocrine tissue aren't marked, it's only these little raisins or islets within the pancreas which get marked. So in our experiment then we mark the cells and wait a year. Now a year is a pretty long time because we're anxious to know the result and this is in a mouse, but in you it would be as if I labeled your beta cells now and then came back when you were 45 years old, that would be the relative equivalent for how long we're waiting in the mouse, and ask what happened in your pancreas during that 30 to 40, 30 year period. Well the answer here is visually quite obvious. At the pulse and the chase there's no change in the proportion of beta cells that are labeled, 100% of the islets contain labeled cells at both times

and the proportion remains constant. This supports the conclusion then that the pancreas is not maintained by a stem cell, but by, you could say, a simple mechanism where fully differentiated cells divide to give rise to more of the same.

34. In type 1 diabetes, no new β cells can be made (45:47)

Now you might think well, then what's the big deal why is that a problem, why does it matter if a tissue uses a stem cell or not? Well in this case it matters at a minimum from the medical point of view because in diseases where the differentiated cells are lost, or in the case of injury, if there's no stem cell then the body has no capacity to replace or replenish itself. So here you see in the case where in type I or juvenile diabetes when the pancreatic beta cells are destroyed by the immune system, that patient then has no source of cells to replace the missing ones. Where are we at this point? There are two mechanisms for replacing tissues in an animal, stem cells and the division of differentiated cells. But these stem cells are the ones that have attracted our attention because they both teach us something about normal development and differentiation but also because of their potential for clinical applications and I'm going to finish up by talking about stem cells and in particular, a special stem cell called an embryonic stem cell.

35. Embryonic stem (ES) cells and their traits (46:53)

There are two places then that stem cells can be derived or be found. In the adult on the far right I've already given you an example of the blood and I talked a bit about the skin. Those cells are called multi-potent because they can give rise to a few cell types, but not all the cells of the body, so for example a blood stem cell gives rise to blood. On the left is the cell I want to draw your attention to now, that is an embryonic stem cell which comes from that early stage of development we saw in our first movie, the blastocyst stage. These cells are described as totipotent, toti as in total meaning that they can form any cell type in the body. So just to emphasize that point, there are two kinds the embryonic which have the greatest potential, can form all cell types, grow forever in culture and are very plentiful, and adult stem cells which are harder to find and harder to isolate but can still be very useful and Nadia will be describing their use for example in replenishing muscle.

36. How are ES cells derived? (47:52)

Well where do these embryonic stem cells come from? In this slide I describe for you how they are derived. They come from the early blastocyst stage using the inner cell mass cells which are those cells which haven't yet made decisions about whether they'll be ectoderm mesoderm, or endoderm. The inner cell mass is dissected out of the blastocyst and put into a petri dish which has a feeder layer, a nourishing layer of cells on the bottom. Some of those cells are capable of becoming embryonic stem cells as shown here and then they grow into colonies or cultures. We call them lines because like a line or a breed of dogs they're all derived from an original progenitor and we keep them growing in culture. I'm going to show you a movie now about the derivation of these cells and at the end of the movie it's going to show one of their properties which is that by adding factors growth factors or other signals, we can begin to tell the cells what to do. In general however, if one removes the culture conditions which allow the cells to just self renew, to make more of themselves because remember these are stem cells, the cells without those signals for self renewal will just spontaneously differentiate will start to become different kinds of tissues and I'll show you an example of that as well. Can we have the movie please? Here you see then

37. Animation: ES cell creation (49:13)

the inner cell mass cells which are going to be removed and grown in a petri dish. We first remove the outer layer that would normally form the placenta. Here the cells are put into a petri dish. Usually there are mouse embryonic fibroblasts on the bottom of it to help the cells grow. Most of them don't survive but a few do and

can grow into colonies of cells as you'll see here. So this then is that process of self renewal. The cells can continually self renew and in fact they are immortal in culture, they can be grown like this forever. Some mouse embryonic stem cells growing now in the lab were derived more than 30 years ago, so they long outlived the life of the animal from which they came. Now here we're going to see them begin to differentiate begin to start to specialize. So some of them are becoming mesoderm and some of them ectoderm. Here you see a neuron forming, here you see a different kind of tissue forming and I'll show you an example of that in a second. One of the big puzzles in biology is to try to control this process now. So these cells with all of this potential that can become any part, how do we tell it what to do? Well a growth factor, which has the funny name sonic hedgehog named after the cartoon character, is actually quite important for telling cells what to do, as is another kind of a growth factor called activin. So these are examples of experiments where we're adding growth factors to cells to tell them what to do and this is an area where we need lots of help from people like you to begin to figure out what is the combination of signals that will tell these cells what they should become.

38. Confirming that ES cells are totipotent (50:53)

How do we know that cells can do everything? I told you that the cells can become any part of the body they're totipotent. Well in the case of the mouse there's a very good series of experiments which show that. One is in culture they can be seen to differentiate spontaneously as that little video described. But in addition we can take stem cells, embryonic stem cells which are genetically marked, shown here in brown. Inject them into a host blastocyst, then re-implant that blastocyst into a female mouse. She'll then give birth to a mouse where one can see here that the brown cells have given rise to different parts of the coat color, kind of like a calico cat in this case. What's not shown in this picture is that these cells if one looked inside the animal, would also give rise to part of the heart, part of the pancreas, in fact any part of the animal can come from embryonic stem cells.

39. Deriving human ES cells and their potential usefulness (51:46)

Now these stem cells are greatly important for manipulating genes to study the normal development of animals and one of the biggest excitements in this field in the last five years has been the derivation or the isolation of embryonic stem cells not just from mice but from humans. And I'm going to finish up by showing you that. Human embryonic stem cells come from blastocysts just like mice, in this case the blastocysts are previously frozen, they're left over material from in vitro fertilization clinics, so they're blastocysts which would otherwise be discarded. And with informed consent from the donors, these can be used for research purposes and as shown here the inner cell mass is removed, it grows out in a feeder layer and then there's a colony of human embryonic stem cells shown there on the left. We've been doing this with my colleagues for a few years now and when we get good healthy blastocysts from the freezer with about a 50% efficiency we can make human embryonic stem cell lines and so far in collaboration with Doug Powers at Boston IVF we've derived about 32 such human embryonic stem cell lines. These stem cells, just like their mouse counterparts, remember, can make any part of the body and that should really tease you to make you think about what you could do with them. Imagine then a cell that can differentiate into any part of your body, and you can think about injury or other cases of diseases where you might want to repair body parts. I'm going to show you just one of many possible examples of this. This is my favorite example, one that undergraduates at Harvard do because it shows both the power of these cells and the puzzle.

40. Video: Human ES cells differentiating into heart cells (53:28)

It's an example where human embryonic stem cells are grown in a culture dish and we remove the factors that allow them to self renew and they now spontaneously differentiate. Could I have the next video please? And what you'll see here is that these cells, in some cases spontaneously make beating muscle, cardiocytes, that is the muscle just like in your heart. Now of course here we're seeing four examples of that you see that

they beat at slightly different rates, and obviously they're not organized into anything like your heart. Your heart, which Nadia will be talking about is about the size of your fist, this is a tiny little group of cells thousands of cells in a petri dish. But it does raise the interesting question of how did these cells know what to do? How did they make this decision to become cardiomyocytes? What steps were involved, what were the signals that they received perhaps from their neighbors? What I'd like to leave you with then today is this fact that embryonic stem cells can make all cell types. How do they do that? We don't know how they do that but it's an exciting problem because it will teach us something about normal development and it also has the potential to treat diseases where cells are missing and I'll be talking more about that tomorrow.

41. Q&A: Would transplanted ES cells differentiate properly? (54:45)

But for today let me stop and take questions. Yes. You say you're really focused on the external factors that would cause an embryonic stem cell to differentiate. Could you inject a stem cell or implant it into a person and say they have diabetes, then the external factors would take care of itself and become a beta cell. That's a great question. So let's suppose a person didn't have a beta cell and you wanted to make more. And so you might think, let's just take that embryonic stem cell which we know can make the beta cell and inject it right into the pancreas. There are two problems though--but it's a good idea. The first is that the pancreas probably no longer has the signals that instructed the cells of what to become. That was part of the whole history of them getting to that point. The second thing is without the signals which tell every cell you inject to become a beta cell. Some of them will kind of willy-nilly go on and do other things, like maybe make those beating heart cells. And in fact there's a kind of a tumor which mimics this called a teratocarcinoma which can grow quite large in people and it has all kinds of differentiated cell types in it, but not in an organized way in a disorganized way. But your idea is the right one, is how could we take this cell which has the potential to make a beta cell and tell it to do that and then put it into a patient?

42. Q&A: What stimulates production of external/internal factors? (56:11)

I have time for one more question. What stimulates the natural production of growth factors and cytoplasmic factors? That orchestration of how does a cell know which signal it should be sending, which receptors or receivers it should have on is set up early in development in that period during gastrulation when the three germ layers were forming. We don't know exactly how those decisions are made but we have lots of examples of genes or molecules that can incline cells towards either a different germ layer or a particular cell type, but that's one of the great mysteries. If we have 30,000 genes, how do you mix and match them to get all the different cell types. Thank you all for your attention.

43. Closing remarks by HHMI President Dr. Thomas Cech (56:58)

[applause] Thanks for a terrific lecture, Doug. Where would we be without stem cells? And thank you, students, for your excellent questions. Now a 30 minute break. When we resume, Nadia Rosenthal will continue our stem cell exploration focusing on regeneration.