

Potent Biology: Stem Cells, Cloning, and Regeneration (2006)
Lecture Three—Coaxing Embryonic Stem Cells
Douglas A. Melton, Ph.D.

1. Start of Lecture Three (00:17)

[ANNOUNCER:] *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 third lecture is titled... And now to introduce our program, the grants program director of the Howard Hughes Medical Institute Dr. Dennis Liu.*

2. Welcome by HHMI Program Director Dr. Dennis Liu (01:07)

[DR. LIU:] Good morning and welcome to the Howard Hughes Medical Institute and our topic of stem cells for the 2006 Holiday Lectures on Science. Our speaker this morning is Doug Melton. He's an HHMI Investigator at Harvard University. In his previous lecture, Doug emphasized the essential roles that stem cells play in embryonic development and understanding development was really Doug's scientific passion. As has been mentioned previously, Doug was well aware of the promise of stem cells but it was actually events in his personal life that led him to refocus his research efforts on stem cells and unleashing their therapeutic potential. Some years ago he found out that his son and daughter both were afflicted with juvenile diabetes and this sort of parental passion really changed Doug's research focus. In this lecture Doug will show how understanding stem cells and developmental biology is key to understanding how to use stem cells to cure and treat human disease. And now a brief video to introduce Doug.

3. Dr. Douglas Melton on teaching (02:23)

[DR. MELTON:] When I was young I'm not sure I knew I wanted to be a scientist, but I always liked science. I liked the idea that you could ask and try to answer questions about how things worked using an experiment so I found them fun and interesting. And I have vague recollections as a young boy being interested in how eggs made animals, like how could a frog egg make a frog and a salamander egg make a salamander and yet the eggs looked the same. I just found that puzzling. And then sort of by accident I ended up working on frogs for the first part of my career or my Ph.D. and when I came here to Harvard I worked on early frog development. I'm really lucky to have a wonderful group of colleagues to work with me here and they range from people who already have a Ph.D. And an M.D. or both, a Ph.D. or an M.D. People who are graduate students studying for a Ph.D. But also undergraduates. My lab has in the summers usually about seven or eight undergraduates who are fully engaged in the research with us and it's also a fun place to be because we have people literally from all over the world, people interested in stem cells and possible treatments for diabetes have agreed to join us here and I really like that so it makes lunch time conversation a lot of fun. I think what I would say to high school student thinking about science is if you want to do something in your life that can literally change the world, there aren't that many options. Modern biomedicine has made enormous progress treating some specific human conditions but on the whole the major degenerative diseases have not been effectively impacted. Therefore there's a possibility of working in areas like stem cell biology where there's a completely different approach to treating the disease that can make a huge difference. So science offers enormous opportunity for young people to do things that are fun and important at the same time.

4. Stem cells and cloning (04:28)

Good morning again. I'm pleased to be back to tell you about a fun part of stem cell biology today and I'm going to make two points. The first is to talk about how to use stem cells to make specialized parts of the body, particular kinds of cells like a nerve cell or a muscle cell or an insulin producing cell and obviously the object there is to not only learn how cells specialize during development but also to look forward to the possibility of new treatments for people that suffer from diseases where these cells are missing. In the second part I'm going to combine cloning with stem cell biology to talk about a sort of future in biomedicine in my view, a way where one can move the study of degenerative diseases from patients where it's obviously quite difficult to study to move it from a patient into a petri dish to try to find new drugs to treat degenerative diseases. Now you'll note that I use the word cloning in that second part and generally when I say cloning people don't think about making genetically identical copies of cells. That's not the first thing that comes to mind.

5. Cloning animals depends on reprogramming cells (05:33)

When I say cloning you think no doubt about cloning animals. So here are some animals that have been cloned in laboratories and I'm going to start off today by talking about cloning, why it was done and what we've learned from it and then how we can use that technique for stem cell biology. There you see a few pictures of animals, clones of animals and if you think about what cloning involves it is a process where we're kind of turning back the clock on specialized cells. We talked yesterday about cells becoming ever more restricted in their fates and you might remember this picture where we talked about going from a fertilized egg to a gradual process of cells turning some genes on and others off, giving rise eventually to an adult animal. So cloning then means that somehow one is taking these fully specialized cells and putting them all the way back to the beginning, not going from left to right in this slide, but instead from right to left. How can that be done?

6. Plants: The original cloned organism (06:34)

Well the first hint that that might be possible is something that you might know yourself and that is many people commonly take little clippings from plants and then grow a whole other plant from it. If you see a begonia or some type of plant you like, often you'll take a clipping and it will be re-rooted and grow a whole other plant. In fact the first part of cloning science really began from those observations and the first clone of a whole organism from a single cell was done in a plant, not a very exciting plant, you could say, it's not my favorite, the carrot, but nevertheless it was possible to take a single cell, Steward did this in the 1950s and grow an entire carrot plant from a single root cell. You see there on the right the flask has this whole carrot plant grown in it, it's sort of amusing to note that in animal biology we use serum to grow cells in culture. I'm always pleased to remember that in the case of growing carrot cells they use coconut milk as the sort of serum to grow cells in culture and here you see then this full plant grown from a single cell. I also am reminded here that the word clone, the Greek word "klon" means twig, which again relates to the plant biology of making a copy of something.

7. Cloning frogs by nuclear transplantation (07:49)

But the real excitement in this area probably came from when scientists decided to test whether it would be possible to not just clone plants but to clone animals, in particular more complex animals, vertebrates. And that work was done decades ago by Briggs and King and separately by John Gurdon and their colleagues and I'm going to describe that experiment for you now. It's an experiment called nuclear transplantation. Testing whether a cell in its nucleus and all of its genes has the capacity to do something else. So here then the question is if one considers an adult cell, a fully differentiated cell, be it a muscle, a nerve or blood cell, can that cell or does that cell have within it the genetic information to make the rest of the organism or has that information been lost during normal development. In this case the cell that was used was a fully differentiated cell from the intestine and this picture here shows the nucleus from an intestinal cell being

removed or sucked up into an injection pipette. That nucleus is then being placed in a different cell, a cell that might have the capacity to reprogram or reorganize the genes in a way that they can be tested for their ability to make other kinds of cells. Obviously then the host for that transplantation is going to be an egg, an unfertilized egg and as shown here the unfertilized egg has to have its own nucleus removed, in this case it's removed by inactivating it with ultraviolet or radiation, that's the little squiggly lines at the top. Then one takes the nucleus from an intestinal cell injects it into this unfertilized or enucleated egg and the question is what does that reconstructed cell do? If the nucleus only had the capacity to make intestinal cells, one might expect that as it divided it would just make more and more cells like the intestine. The nucleus might only have the information left to make the intestine, but in fact as you know it can go on in this case and make a normal embryo first forming a blastula like the blastocyst that we spoke about, and then making a tadpole

8. Frogs were the first adult cloned animal (09:58)

and here then is the picture which you might not find as attractive as many kind of Jiminy Cricket frogs but it's a beautiful frog to many of us because it's the first adult cloned animal. So on the top is the donor from which an intestinal cell was removed and on the bottom is the first clone, 1962 by John Gurdon. This technique then which I'll describe in more detail later shows that it's possible to make a genetic copy of an adult animal through this nuclear transfer. And here's an example where we're going to look at many copies, so on the left you see the egg donor, so in this case the female frog which is I might note about 40% by weight ovary, an animal pretty much devoted to making eggs. Many eggs were removed from her and then they were transplanted, that is nuclear transfer was accomplished using the blastula from a mating of the albino frog shown in the middle and those produced then these 30 identical, that is genetically identical cloned adults on the right. You can think of this like sets of identical twins but they're genetically identical. One of the points I'd like to make here is if I could draw your attention to what is here the third row and if you look at like the third and the fifth frog over, those frogs are smaller than the other ones. That's because they probably didn't compete as well for food or didn't do as much exercise and this should just remind you of something I think you already know intuitively is that your genes are not your destiny, they're important for setting limits on what can be done, but what happens in your life can make a big difference. So here we have 30 identical frogs but they didn't all grow up to look exactly alike and if they did something more interesting than they normally do, like if they could listen to music or think, we could test whether or not they actually had different abilities but you can't do that with a frog.

9. The first cloned mammal: Dolly the sheep (11:52)

Now many of you will have heard of course, since cloning is in the news so much, that cloning frogs has not really captured the public's attention. It was cloning mammals that got everyone's attention. So here is a picture of maybe one of the most famous clones, the sheep Dolly. Dolly was cloned from a mammary epithelial cell and then the nuclear transferred embryo was put into a foster mother shown here on the right, the black-faced ewe and she then gave birth to this clone, the sheep Dolly on the left. Dolly grew up, a year or so later and then became a mother herself making her little baby Bonnie. Now there are a number of animals that have been cloned. Sheep aren't the only ones and I thought I would show you a video of how cloning occurs. So we're now going to look at the mechanism of it and before we start the video I thought I would just say that one of the things that we want to attend to here is the pipettes, one on the left and one on the right.

10. Video: Cloning by somatic cell nuclear transfer (12:52)

This video is prepared by my colleagues Dieter Egli and Kevin Eggen and you see here on the left is what's called a holding pipette which gently sucks on the egg, the egg is surrounded by a membrane called the zona and you'll watch this pipette on the right first drill a hole into the zona, then go in and suck the nucleus out and then another nucleus which has been taken from say, a somatic cell, a cell of the body is going to be put

in. So if we could start the video please. You'll see know this drilling pipette is going to drill a little hole into the membrane. You can maybe see a little bit of the hole right here at the next part. This pipette you'll note isn't really sharp like a syringe, there you can see a bit of the hole. And now the pipette's going to go in and remove the nucleus and if you look carefully in the pipette you'll see a line in the nucleus which are all the chromosomes lined up. So that nucleus is going to be squirted out now because we don't need it anymore and there we have an enucleated egg. Now the next step is to take a set of eggs like that and I'll show you two and then transfer into them a nucleus from another kind of cell, a fully differentiated somatic cell. So here the enucleated egg is set on the side and it's held by this holding pipette on the left. There's drilling the little hole in the membrane. Here we go in, here comes the nucleus from the right. Pipette goes in and these pipettes are operated with a piezoelectric device so you can't see it here, but it's like a little jack hammer going very quickly like Woody the Woodpecker getting in there to then squirt the nucleus in. Here we'll see it again, a little hole and the zona is prepared and now the nucleus is going to be squirted inside, so there's two examples of what's called somatic cell nuclear transfer.

11. Many types of animals have been cloned (14:52)

So that's a sort of long way of describing cloning but it's technically correct because it reminds one that the somatic cells are cells of the body, the soma and they're fully differentiated cells and nuclear transfer is the process you just saw. Now a large number of animals have now been cloned by using this technique. I'm going to show you what is probably the most useful animal for this purpose which is the laboratory mouse. Here we can see an example of cloning of laboratory mice where the nuclear donor is shown here on the left and a nucleus is taken from an adult cell from that animal, and then injected into an enucleated egg, an egg from the little mouse over far on the right, then that recombined cell that is with the nucleus from a mouse on the left and the egg cytoplasm from the mouse on the right is transplanted into a foster mother, here called the surrogate mother and this is an albino mouse giving rise to the two little baby mice there, the two clones, which should look and do look like the mouse on the left. Now this is extremely valuable to be able to make genetically identical copies in mice to study how animals develop and the role of genes in development and other physiological functions. But as I've already shown you in the first slide this isn't the only animal that's been cloned. One can have a look here at, I think that's about ten or eleven cows that have been cloned, and you might say to yourself, why would you want to clone a cow? Turns out that cows that are very good at milk production are quite valuable and to breed them takes many years, multiple generations of breeding but here one can have a very good milk producer some cows produce up to 30 gallons of milk in a day and you can then make ten copies of that one to really improve your herd production. But cows, like horses can also be cloned, there's a little baby horse and this has of course, or maybe not of course but has at least moved into the area of pets cloning, so here we see the cloning of cats. The cat on the left was apparently a very favorite of some person who was willing to pay significant sums to create a clone. The foster mother there is shown on the right next to the little baby clone, it's a little baby kitten and then dogs have also been cloned. Here's a donor on the left of an Afghan hound sitting next to her clone and then you see in the picture on the right is the clone again, in this case next to the surrogate mother which was a Labrador retriever. So I've often wondered if, since we have dogs ourselves, whether that Labrador was a bit surprised when it gave rise to the Afghan hound. So what animals have been cloned? Well so far reported somatic cell nuclear transfer in mammals has been for sheep, Dolly as I said, cows, mice, goats, and pigs. I didn't show you cats and dogs and rabbits, horses and ferrets.

12. Cloning shows that differentiation can be reversed (17:54)

Now this isn't just a trick that biologists do for fun, as I said there are reasons to have done cloning and in this case it was initially begun by testing for the information present in fully differentiated cells. So one of the things I'd like you to remember from today is that cloning was done with the point of testing for whether there are irreversible changes in the genome during development and this conclusion then is one I'd like you to think a bit about. That is, from these experiments it's been possible to conclude that the nuclei of some

fully differentiated cells can be reprogrammed, the clock can be turned back so that they can become fully potent stem cells. There are no irreversible changes in all of our genes in the genomes during development. Now before I take questions I want to point out one final thing which is that cloning is a very inefficient process. The way I presented it makes it seem like we could go home tonight in our garage and clone your favorite pet. In fact, something we don't well understand is that the cloning process, the reprogramming is generally very inefficient. In the very best cases, like in hands of professionals, it can approach a 2% efficiency and we don't yet understand the reasons for that, but it's not something that occurs easily or all the time. I'm going to talk shortly about how we can use cloning and combine it with stem cells to study disease, but I think now would be a good time to stop and take some questions.

13. Q&A: Is the clone's surrogate mother different on purpose? (19:33)

Yes.

[STUDENT:] Do you purposely use different types of animals, like you had a Labrador retriever as the foster mother with the other type of dog, do you do that so you can see that the clone doesn't look like the surrogate mother?

[DR. MELTON:] Your question is asking about how can one really be certain that the clone came from the nuclear donor and wasn't say a pregnancy in the surrogate mother and one way is to use different species as you described the Labrador retriever and the Afghan. In mice one does that by using genetically marked animals so there can be an animal that has a different set of genes that's put into the foster mother and then one can be certain about the providence or the origin of the clone. It's a good question.

14. Q&A: Do clones have any deformities? (20:24)

Yes.

[STUDENT:] When doing these experiments have you come across any kind of deformities and if you have, what kind were they?

[DR. MELTON:] Yes, I'm glad you asked that. I didn't mention that studies in mice where it's been possible to look most carefully at the clones strongly suggest that the clones are not entirely normal. It's difficult to be certain as yet because one needs large numbers of animals to really help us define normal. One way to think about it is if I said who in this room is normal, it would be hard to say with any certainty who's willing to say I'm an example of a normal human being. Similarly if I showed you the mice they all look normal but they might have subtle differences and that's an active area of experimentation.

15. Q&A: Do clones have a greater risk of cancer? (21:11)

Yes, there.

[STUDENT:] Since cancer is caused through the dedifferentiation of already differentiated cells, wouldn't this process of cloning put the clones at a greater risk of cancer since you've taken a differentiated cell and given it its full potential again?

[DR. MELTON:] I don't think it would put clones at greater risk for cancer. You're right that cancer involves a change in the genetic composition of cells and so you might be thinking that by reprogramming them if you don't do it entirely accurately or with real fidelity you could open up that cell and its progeny to some susceptibility for cancer. There's no evidence for that at the moment but it is a possibility. Now you unfortunately are way in the back and I'll see if I can reach you. There we go.

16. Q&A: Why did the cloned cows have different markings (22:00)

Yes, here.

[STUDENT:] You said that the frogs that were smaller, that was due to the fact that they didn't eat enough, or whatever, so why when the cows were cloned did they not look exactly the same as each other?

[DR. MELTON:] Yes, so you noticed that the cows didn't have the same spot pattern in their coat. I'm pretty certain that cows are like cats in that regard, in that the patches of the skin result from random inactivation during development of the X chromosome. And since that's a random event, they'll all have skin but the color coding will be slightly different. In fact I'm told that the person who paid to have her cat cloned was upset because the coat pattern wasn't exactly the same. Here we go, over here in the green sweater.

17. Q&A: In SCNT, why doesn't the nucleus change the cell? (22:47)

[STUDENT:] Why did the cells, when you put them in the egg cell, why would the nucleus not change that cell to be the function that it was before? Why would the cell change it to be dedifferentiated into a stem cell?

[DR. MELTON:] Right, that's a great question because it sort of asks in another way, who's in charge of the cell? Is the cytoplasm telling the nucleus what to do or is the nucleus calling all the shots? In fact there's an interaction between the two and factors that are found in the cytoplasm transcription factors, for example, proteins that turn genes on and off, go into the nucleus, turn some genes on, those will then go into the cytoplasm and change the whole composition. But it is undeniably the case that in this context when a nucleus is put into an enucleated egg the egg cytoplasm is calling the shots. If it weren't, then one would have just made more intestinal cells in that first experiment.

18. Q&A: Do cloned animals have similar personalities? (23:45)

I think we have time for one more question. Yes.

[STUDENT:] I was wondering, the cloned animals, although they look and have the same like genetic material and the same coloring as the parent, do they have the same personality? Is their personality drastically different or just sort of different?

[DR. MELTON:] All right, well I worked with frogs for a long time – for ten years and could never detect any hint of a personality. But your question is a good one. I would be willing to bet that the answer would be no, because your personality is not hardwired in your genes but is a consequence of all of your interactions with your parents, with your siblings, with your classmates, so that's sort of what I meant when I said DNA is not one's destiny, it's interaction with the environment that has a lot to do with what we all become. So thanks for those great questions, we're going to move on now and we'll have time for questions later.

19. Combining cloning and stem cells for studying disease (24:44)

I want to finish up talking less about what has been demonstrated so far and more about the future, following up on my challenge to you all to join us in this field to try to find new ways for treating disease. And in order to do that I'm going to show a slide which I'd like you to keep in mind which is this sort of circle of a research program of how to combine cloning and stem cells in studies on disease. In this slide you see a patient up in the upper left and we're going to talk about experiments where the nucleus from a skin cell of the patient is used in nuclear transfer to create embryonic stem cells like we described yesterday. You'll

remember then that following nuclear transfer a blastocyst can be grown and from the blastocyst the inner cell mass can be used to derive embryonic stem cells. The two aspects that I want to concentrate on are first using embryonic stem cells to make special types of cells. That's shown there where it says genetically matched differentiated cells. And then I'm going to finish up with talking about using these cells to study diseases in a petri dish, essentially moving the study of degenerative disease from patients to a petri dish.

20. Characteristics of degenerative diseases (25:59)

Now I've mentioned a couple of times degenerative diseases and I'm going to bunch them all together today, even though technically that's not the right thing to do, but they have some things in common and I'm going to show you some examples of degenerative diseases and tell you why we think that these can all be studied using stem cells. So by degenerative disease I mean to group together all of the afflictions that affect people as our bodies age and this would mean purposely to include say all of the neurodegenerative diseases like Alzheimer's, Parkinson's, Lou Gehrig's disease which is sometimes abbreviated as ALS for amyotrophic lateral sclerosis and I'll just refer to it as ALS today. Cardiovascular disease, the degeneration of the heart that Nadia talked about yesterday and diabetes, the case I'll focus on where particular cells in the pancreas are lost. Now in all of these cases these diseases have a few things in common. First they are not the result of a single genetic defect. Instead they're the result of many genes being combined to make the patient susceptible to getting the disease and secondly they all involve an interaction with the environment. There's some environmental signal, in almost all of these cases unknown, which results in the eventual progression of the disease.

21. Specific cells affected by degenerative diseases (27:18)

But one thing they have in common is that in every case these problems come crashing down on a single kind of cell, so that's what I'm going to talk about now. Think about the case of Alzheimer's which affects the brain. In this case it's the forebrain basal neurons as they're called, which become defective, and in this slide here I blacked those out. They become defective leading to memory loss and then other more serious problems as the disease progresses. Relatedly in the disease called Parkinson's it's not the forebrain neurons, but the midbrain neurons that make the chemical signal dopamine, which are lost. So here you see in the midbrain cells going away. A disease I'll say a bit more about, ALS is one in which motor neurons become defective. So the motor neurons innervate, or talk to the muscles of the body and so without that all muscular control is eventually lost. So here you see the motor neurons sort of being blacked out as the disease progresses. In cardiovascular disease it's quite obvious that unless Nadia figures out how to regenerate the heart soon, these cells are going to be degenerating and you see here I have the heart blackened in a bit.

22. Pancreatic β cells and type 1 diabetes (28:31)

And I want to take the example of diabetes where a particular cell in the pancreas, the insulin-producing beta cell, is lost. I'm going to use this as an example then to ask the question how might one use an embryonic stem cell to make a specialized cell type to replace it in patients that are missing or have defective cells of a certain type. So the cell we're talking about here then harkens back to the pancreatic islet, the endocrine portion of the pancreas that makes hormones and in this case, that is the case of Type I or juvenile diabetes, the insulin producing beta cells are lost because the body makes a big mistake and the immune cells attack one kind of cell in the body, these beta cells and kill it off. So if you watch carefully here the blue cells are going to go away, no insulin can be produced in these patients, and so diabetics, as you may well know, have to take blood tests five to ten times a day and insulin injections in order to survive because their body doesn't have the capacity to make this necessary hormone, insulin. So if we take that as a puzzle, on the one hand you have diseases where there are cell types missing, in this case the pancreatic beta cell,

23. Can cultured ES cells differentiate to pancreatic β cells (29:46)

and you'll have cells which can make any part of the body, you don't have to think too hard to say how could we connect those dots? How do we use that information to possibly find a way to make new cells? Here then is the plan for that. In normal development you'll remember that the inner cell mass cells progressively become specialized or have their fates instructed to become pancreatic beta cells. Now of course, not every inner cell mass cell does that some will become muscle and nerve, but this just shows the progression to one kind of differentiated cell. Because embryonic cells, embryonic stem cells which I abbreviate as ES cells, can do that I replace as you see here, the ICM cell with an ES cell because it can become any part of the body. So our challenge then is how to instruct this cell in a tissue culture dish to become a pancreatic beta cell. During this progression genes have to be turned on and off, they control the differentiation and mark it for us so we know whether or not it's happening. And here are little pictures of the DNA chips from my last slide, from my last lecture showing the progression. So our challenge is to figure out how to tell that cell at each stage what it should become and

24. Interactions between different cells affect differentiation (31:01)

I'm going to give you one example of how we discover the signals that are responsible for instructing these cells. We're going to talk about the signals at this step here, what causes this step to occur. The hint from this came not from any complicated experiment but from the simplest kind of thing a scientist does which is to just watch, to observe what happens in normal development. And by watching the normal development of pancreatic beta cells in mice, my colleagues and I observed an interesting fact which is that at every stage of their development, pancreatic islets seem to be right next to blood vessels. You see here on the left an embryonic islet where the islet is just beginning to grow, it's stained in green and it's next to a red blood vessel there and the other nuclei are just stained blue. As development proceeded to a mature adult, again the islet is in green and you see it's invested with vessels, all like a spider web all through it they're always right next to it. Well, given that they're right next to each other we wanted to look at that very carefully at the earliest stages of development and I'll show you a picture of sections through a developing mouse here. The cartoon at the bottom shows blood vessels. On the top are the two dorsal aorta and then the yellow tube is the gut tube which is going to become a pancreas and the blue dots are the buds that will eventually make the pancreas. If you look at the left on section A you see that the two dorsal aorta do not directly touch the gut tube and there's no sign of pancreatic development whereas in the middle the blood vessel touches the gut tube and we use the genetic trick to show that when cells are making their first commitment to become pancreas, turning on a gene called PDX, they turn those cells blue and there you see, aha, those cells have now been told, we think, to become pancreas, and going a little bit farther towards the back the aorta no longer touches the gut tube and this gene doesn't come on. But that's the sort of circumstantial evidence. That doesn't demonstrate cause and effect,

25. Blood vessel proximity and pancreatic β cells (33:06)

so a nice simple experiment is to try and demonstrate that the blood vessel sends a signal by pulling the pieces apart and recombining them in a petri dish. That's shown here. Here looking at a picture of a mouse embryo that's been dissected. Those little balls, those little sausage-shaped things are called somites and they're running along both sides of the neural tube and we're looking at the embryo up from the bottom and in the left picture you can see peeling away a piece of the endoderm the endoderm that doesn't yet know what to do, and on the right those two little ribbons are the dorsal aorta which have been dissected away. So we're now going to do a simple experiment of mixing and matching. If we take endoderm alone and put it in the petri dish we ask what happens, or we combine endoderm with the aorta and ask what happens. This slide here shows that only when the endoderm is combined with the aorta is it capable of turning on insulin, that is can it produce cells that are like the pancreatic beta cell. So there's a really simple experiment with a straight forward conclusion. The aorta is sending some signal to the endoderm telling those cells "your job is to become "pancreatic beta cells." Now while we don't know the chemical nature of that signal, we

obviously know a way forward to find it because we know where its source is, the aorta has this signal and we'd like to now know what are the gene products that it's secreting to tell endoderm to become pancreatic. So the answer to the question of what causes this step is some signal from adjacent blood vessels and it will be possible to identify that signal.

26. Molecular signals for each differentiation step are unknown (34:42)

I want to now step back a bit and say well this is an example of how one moves from an undifferentiated ES cell eventually to a fully differentiated pancreatic beta cell but we don't know all of the steps yet. We think we know how to go about finding them, but if you said to me "Can you do it tomorrow?" the answer is no, we don't know the signals for every step. This doesn't mean of course that one shouldn't try to find out how to turn embryonic stem cells into these different kinds of cells shown here on this slide, the neurons for Alzheimer's or the midbrain neurons for Parkinson's

27. Deriving motor neurons from ES cells (35:17)

and I thought I'd give an example, I talked already about the pancreatic beta cells, but I want to give an example of where I think we know the most, which is in the case of motor neurons, how would one make a motor neuron? And this comes from a nice set of experiments by my colleagues Hynek Wichterle and Tom Jessell where they've been able to take a mouse embryonic stem cell and turn it into a motor neuron in a dish. So this is quite amazing because it doesn't depend on letting the cell develop in an animal but rather in a petri dish, they can make a motor neuron. You can see that here where a mouse embryonic stem cell is treated with two different factors and then it turns into this bright green neuron you see over at the right. The two factors they used which they had been studied for some years have to be added at the right time and the right concentration. One is retinoic acid, related to a vitamin, and the other is this protein that we talked about before, the growth factor sonic hedgehog.

28. Motor neurons derived from ES cells are functional (36:14)

Now the amazing thing here then is that one make a progenitor for the motor neuron and ask could we put it in an animal and show that it has not just the morphology, the shape of a neuron but can it have the function of a neuron? And that slide here shows that they took these mouse progenitors made from embryonic stem cells and injected them into a chicken embryo. The chicken embryo is shown there on the left in a cartoon. That embryo then developed into a little baby chick on the right and you can see the cells were marked with a green fluorescent protein and they made motor neurons that went out and innervated muscle. And you can see this at the bottom where the red and the green show motor neurons that have gone and made contacts with the muscle. So here we have a chick embryo that has mouse neurons talking to its muscle, demonstrating that the mouse ES cell can be directed to differentiate into a functional neuron. Now those two examples of course are not complete in that we're not at the point where we can take human embryonic stem cells and turn them into any cell type of choice, but the examples were intended to convince you that this is possible and it's now a matter of working out the details of which signals at what concentration and at what time have to be added to cells to tell them what to do. Should they be a motor neuron, a pancreatic beta cell or a cardiomyocyte?

29. Challenges in studying degenerative diseases (37:39)

I want to finish up before questions by talking about connecting cloning now with this presumed ability to make stem cells to study degenerative diseases. So let me go back to degenerative diseases and remind you about why these have been so confoundingly difficult to treat. Degenerative diseases, the diseases I mentioned before where cell types go missing, are difficult to study because they're complex genetic disorders it's not a single gene but a set of genes that lead to the defect. Moreover as I've already said, there's

an interaction with the environment and the environmental signal or signals in general aren't known. Now the reason we know that is there are cases of identical twins, so they have the same genes, where one gets a disease and the other doesn't, so that shows that it was the environment, their growth and development which allowed for some signal, added to the genetic background to give rise to the disease state. Finally what makes it even more difficult is on the whole these diseases really are only well known and studied in people and if the cause of the disease occurs many years before the patient appears in the doctor's office, then the doctor doesn't really have any way of trying to figure out what caused it. Was it that they ate cucumbers when they were 15 or they didn't eat enough cereal? What was the cause of a particular disease? You might even say it's all those confounding factors which can explain why biomedicine has not been very effective to date in treating these diseases. So I'm going to describe a proposal that many of us think is an important way forward and it combines cloning with embryonic stem cells. So let's begin by looking at an animation about how we might make an embryonic stem cell which contains all of the genes that we know are going to cause a person to get a disease. So this animation here please,

30. Animation: Combining SCNT and ES cells (39:37)

will show us the nuclear transfer we'd seen before, but now we're going to see it as an example where a skin cell nucleus is going to be used to make an embryonic stem cell. So the egg cell has the nucleus removed. The nucleus from a skin cell is then injected into that egg. That egg, you'll remember, can then initiate development by dividing, making a blastocyst. Here's this important reprogramming phenomena going on, the details of which are still unknown. The cells now begin to divide, continue to divide to make a blastocyst with an inner cell mass, and then it's from the inner cell mass and that blastocyst that an embryonic stem cell can be derived.

31. Creating ALS-diseased cells by SCNT for research (40:28)

There's our inner cell mass, the source of new cells. So how might we do that with one of these diseases? I'm going to only give one example, the example of ALS but I'd like you to keep in mind that this could pertain to any of the degenerative diseases that I've described this morning. Let's look at what would be involved. Well the first step, an important one is to get the host cytoplasm, in this case it's from a human female, it's an unfertilized egg that would be taken in the same methods used to do in vitro fertilization procedures. In fact one could note that our society encourages people and allows people to donate this tissue for the treatment of infertility and what I'm proposing here is the similar donation for the treatment of other diseases. In this case the patient, the ALS patient which we then know has the genes that give rise to the disease, donates a skin cell, the skin cell fibroblast shown there. The nucleus from the fibroblast is removed and injected into the unfertilized and enucleated oocyte. That can be used then to give rise to these embryonic stem cells, all in a dish.

32. Using ES cells to study ALS development (41:42)

Now what would we do with these cells? An important point that I think has been missed in the popular press is that these cells are not useful in terms of transplanting them back into patients. If a person has a disease where they're missing a particular kind of neuron, it doesn't do us any good to make more of those neurons. What we want to do is to use the genetic makeup of these ES cells to understand why a person gets that disease in the first place. To get at the mechanism or the root cause. And I'm sure you'll agree that if you understand the mechanism you're in a much better place to try to figure out how to do something about it. So let's have a look at what that would involve. In this case then we have two kinds of embryonic stem cells. On the left, the disease-specific cells, here ALS and the hES stands for human embryonic stem cell. On the right, control human embryonic stem cells. In both cases scientists will cause them to become different, that is to differentiate in a petri dish by the kinds of methods I've described already. So this is directed differentiation protocols telling the cells what to do, telling them to become motor neurons. I think it's

obvious to you now that what you want to know is what happens to the cells on the left? Where do they screw up? At what stage do things go wrong? Did some gene come on which shouldn't? Did some gene stay on too long? Did some gene go off which was important? At the moment we don't know any of those facts about these diseases and we can test these cells because they're in a dish, not in a person's body or in a person's brain. And we can test them with a number of assays. Here's the assay beginning on the right that we've already covered. We can look at their gene expression. In theory one could look at their gene expression every few minutes and say which genes came on and off at any time. We can also do electrophysiology to test for the function of these neurons at any point and one very useful assay is to look at precipitates which form in these cells, particularly in this disease, they're so called amyloid inclusion bodies and these are precipitates of cellular proteins which are closely correlated with the dysfunction or the failure of these cells both to survive and to function.

33. Using ES cells to screen for effective treatments (43:57)

This then tells us about what the causes of the disease might be but I think even more exciting is trying to combine this with looking for drugs that would prevent the disease progression. Again this is something one can't really do in patients. One doesn't take 100 people that you imagine are going to get ALS and then start randomly treating them with drugs to see what effect they might have. Here however we can do the following experiment. We can take the embryonic stem cells, have them go through the differentiation protocol in a petri dish and then do chemical screening. I think this holds enormous process for the next few years to try to find drugs that slow or prevent this neural degeneration. Now I should emphasize this does not cure the disease, it doesn't reverse the process but it does slow it down. So if a patient is suffering from such a disease it would be an enormous benefit to slow the process say just by a factor of two. If it took twice as long for you to lose your memory or lose motor function that would be a major advance. Now before I end I want to point out something else with this slide. To achieve this kind of goal requires the interaction between scientists with lots of different talents or expertise. We need developmental biologists to make the stem cells, cell biologists to cause them to differentiate, and chemical biologists to help with this chemical screening. So I'd like you to think about as your career goes forward about working with teams of people that can bring different talents to bear on complicated problems like this. In my own case I benefit enormously from my colleagues Lee Rubin and Stu Schreiber in thinking about the chemical screening and so I just point out that that could be very important and helpful in trying to take on complex problems.

34. Two approaches to using ES cells to study diseases (45:43)

Let me finish before questions with this last slide, the one I'd like you to sort of dream about, which is how does one combine stem cell biology with treatments of new diseases? I propose two ways today. One is to take human embryonic stem cells and to understand how we can tell them what to do, how we can determine their fates, turn them into a pancreatic beta cell or some other cell type. The other is to derive specific kinds of stem cells, what we would call disease specific stem cells and figure out why these cells screw up in human development. Study them in a dish and try to find ways to prevent them from causing so much trouble. So before I take questions I just wanted to say that of course the work I described today isn't all my own, it's from many colleagues not all of whom I've been able to note and I'll be happy to show on the DVD all of the people who have been involved in this research

35. Q&A: Can lack of diversity in cloned cows cause problems? (46:41)

and I thank you for your attention and let's take some questions now. Yes, let's go here.

[STUDENT:] I had a question about, actually from the first part that you were talking about. I was really interested in the cows. You said that the farmers or the people, the scientists who clone them they use then for economic purposes, like the certain traits that they have are you know really profitable. But wouldn't that

have also a very negative effect because since you're cloning like all of them, the same genetic, like almost similar wouldn't that have a real effect later on, like a real, since they're not like, how can I explain let's say a disease comes, and they have...

[DR. MELTON:] No, I think you have a very good intuition there, I think I understand what you mean. Wouldn't it be a bad idea if every cow was genetically the same. That's right, this problem is true for all of agriculture, both plants and animals. I doubt that any farmer, let alone whole states or the whole nation is thinking about having all genetically identical cows but you raise a very good point. Another use of animals like that has been to produce human proteins in milk. So the way human proteins are now made is expensive and complicated in large incubators, but it turns out one can very efficiently produce human proteins in a sheep or a goat or cow milk, and so that's another use of that kind of technology but I'm glad you brought up that point because it's true for not just farm animals but also for plants. One doesn't want to have to lose our important variation in nature. There you go.

36. Q&A: Are there cells that can't be used for cloning? (48:30)

Up here in the hat.

[STUDENT:] I was wondering you said that some differentiated cells can be used to make clones. Does that imply that some can't?

[DR. MELTON:] Yes. I was careful in my saying some and I'm glad you caught that because it would be wrong to conclude that all cells contain all of the information. We know from years of study in immunology for example, that cells of your immune system have had genetic rearrangements and they would be unlikely to be capable of giving rise to a full animal. There could also be other mutations that have arisen during the course of the animal's development which would make it incapable, that is make the nucleus incapable of giving rise to a full animal. I would be careful though to focus on the issue of trying to make adult animals because as you'll remember from the second part of my talk, it's not making cloned adult animals that one is so concerned with, it's making genetic copies of cells and the cells don't require the full array of all reprogramming, probably in order to enable us to say direct the synthesis of a muscle cell or a nerve cell. I'm running out of t-shirts here, so I'll have to give a rain check on those that I don't get to. Yes, here.

37. Q&A: How do you treat prion-based degenerative diseases? (49:51)

[STUDENT:] I was wondering if there is like any way that stem cells could be used to treat degenerative diseases caused by prions, like, I read in an article that Parkinson's might actually be caused by a prion, not anything else.

[DR. MELTON:] That's a great question, it's not in my field and I don't know a lot about it, but I would say that if there are cells which are defective for whatever reason, prion changes in conformation and then the dysfunctional cell, I think it's perfectly reasonable to imagine that one could make that kind of missing cell and use it for replacement. Now in some cases like in Parkinson's it may well be possible to do it because it seems one of the principle or most important functions of the missing neurons are to make dopamine, but in other cases like in Alzheimer's where it's the neural connections over the history of the animal that give rise to memory and cognition it's much less likely. There I think it's the second program I talked about, looking for drugs that would slow degeneration which is more likely to give benefit in the shorter term. Okay.

38. Q&A: Only two factors for motor neuron differentiation? (50:55)

And let's go back to this side here.

[STUDENT:] I was wondering when you were talking about the signals that make a cell differentiate, you just said two, is it really like that few or are there a lot more?

[DR. MELTON:] The signals that tell a cell what to become--determine its fate--are really one of the sort of hottest areas of modern biology is to try to figure out what factors are in the cytoplasm these transcription factors which go and rearrange the chromatin and set the sort of whole orchestra in play for all of early development. You're asking me how many of those signals are there. What we can say now is that in terms of the signals that come from outside the cell, what I called growth factors, those are members of families of proteins, there are only about 10 families, maybe about 100 signals in total and now the challenge for scientists is to figure out how are they mixed and matched to give rise to all these different kinds of cells. One example I sometimes use is that when you go to a Chinese restaurant you have a menu of hundreds of items, but if you were going in the kitchen you might only find six or seven pots. It's the way they're mixed and matched that make all these different dishes. Similarly the mixing and matching of the time and the concentration of those signals can give rise to all these different kinds of cells. Now that's my last t-shirt but I'll promise to get one for other questions.

39. Q&A: Why didn't the chick embryo reject mouse neurons? (52:23)

Do we have a question here?

[STUDENT:] Earlier you discussed how you injected one of the motor neurons from a mouse into a chicken and yesterday you had said that it was like possibly harmful, or it could cause rejection to move the cells from different animals. So how is it that in this case nothing was...

[DR. MELTON:] I'm glad you caught that little discrepancy. The trick here, the difference is that the mouse cells were put into a chick embryo before the chick's own immune system had developed. So one suspects that as the chick was growing and its immune system was surveying what's around saying what is self and what is not self, it didn't recognize the mouse cells as being not self. It recognized it as self. So yesterday's experiment that Nadia was talking about pertained to transplantation in an animal with a fully functioning immune system. I'm also glad you asked that question though because in all of the procedures I've talked about today I've pretty much ignored the problem of immune rejection. Even if what I've said today were successful for transplanting cells, it would require, given our present state of knowledge, some kind of immunosuppressant to be moving cells from one kind of patient to another unless we made patient specific ES cells for everybody.

40. Q&A: How would you deliver ES cells to an adult patient? (53:43)

Yes, in the green.

[STUDENT:] I understand how you would develop patient-specific cells and then possibly inject them into an embryo, but if you develop patient-specific cells how would you inject it into an adult person who had the disease? Would you inject it right to the site and expect the cells to start dividing and regenerate those missing cells?

[DR. MELTON:] Right, that's a good question and of course the answer is going to be complicated because it depends on which disease we're thinking about. In the case of diabetes it's already known that one can transplant islets from a cadaver into the liver of a patient and that they will function for a year or more. That's obviously not what one would do with a motor neuron to treat ALS--you don't want your motor neurons in your liver, right? So for each disease there would be different kinds of transplantation and I would say the cases where this field is farthest along is transplanting the dopamine-producing cells, the neurons into the midbrain for Parkinson's and for pancreatic beta cells for diabetes.

41. Q&A: Degenerative diseases that affect two cell types? (54:51)

Good question. Here.

[STUDENT:] Are there any type of degenerative diseases that attack two types of cells instead of just one?

[DR. MELTON:] I think the answer is certainly going to be yes but we don't know so much about the progression of all of these diseases. The reason I guess it's going to be yes is that once one of the cells in your body starts to screw up all of the cells are constantly interacting so the adjacent blood vessels or other tissues are likely to degenerate at the same time. This has been a special problem in the nervous system to try to figure out when neurons degenerate. Is it because the supporting cells like the glial cells are really the ones that have the defect and aren't providing enough support or nourishment, or has the neuron gone wrong and then when it's not healthy its adjacent cells fall apart.

42. Q&A: Could cells be reprogrammed to prevent cancer? (55:40)

We have time for one more question, let's go over here for our last question.

[STUDENT:] I was just wondering if this process of reprogramming nuclei could maybe one day be used to treat or reverse some of like the oncogenic mutations that ultimately lead to cancer or if the two were just completely unrelated?

[DR. MELTON:] I think they are related in the following way. If a cancerous cell appears, one wants to figure out how one can kill it as the sort of first thing to think about but it may in the end be easier in some cases to reprogram it to not to be causing so much trouble. The reprogramming in the first instance could be to tell it not to divide or it could tell it to stop dividing and differentiate. And so this problem of how programming and reprogramming occurs is connected both in the subject we talked about and in cancer biology. Thank you all very much and I'm now glad to hand it off back to Dennis.

43. Closing remarks by HHMI Program Director Dr. Dennis Liu (56:34)

[applause] [DR. LIU:] Thanks for that beautiful talk Doug and thanks for all the good questions. We're going to have a 30 minute break now and when we return Nadia Rosenthal is going to close our series and she's going to talk about exploring the relationship between stem cell potency and the natural process of aging and whether it's possible to turn back our biological clocks.