

Potent Biology: Stem Cells, Cloning, and Regeneration (2006)
Lecture Two—Adult Stem Cells and Regeneration
Nadia Rosenthal, Ph.D.

1. Start of Lecture Two (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 second lecture is titled... And now to introduce our program the vice president for Grants and Special Programs of the Howard Hughes Medical Institute Dr. Peter Bruns.*

2. Welcome by HHMI Vice President Dr. Peter Bruns (01:09)

[DR. BRUNS:] Well thanks for rejoining us for these lectures on stem cells. Before I introduce our next speaker I'm going to make a short commercial message here for our other web site called BioInteractive.org. I recommend highly that you go to that site and take a look what's there. You'll find all sorts of interactive things animations, virtual labs, streaming video previous Holiday Lectures and all sorts of links to other places that are relevant for the topics. You also on that site can order for free DVD's of any of the Holiday Lectures and as Tom mentioned before starting next week we're going to be putting on that site the lectures as podcasts as well. So our next speaker is Nadia Rosenthal who's the lab director and senior scientist at the European Molecular Biology Lab in Italy. It's a reflection of the international nature of science that she has a lab that is full of people from all over the world working on her topic and asking how basic science can yield medical breakthroughs. Nadia has long been interested in understanding how muscle cells develop and function which has led to her interest in regeneration and aging. In her first lecture she'll focus on the role of stem cells in wound healing and regeneration. So here's a short video to introduce Nadia.

3. Dr. Nadia Rosenthal (02:39)

[music] [DR. ROSENTHAL:] Obviously living in Italy is a wonderful inspiration and in fact as a child I was obsessed with the Renaissance. So to come back and spend as much time as I possibly can when I'm not in the lab roaming the churches and looking at the art that just suffuses this city in Rome is an absolute delight. A lot of the people in the lab are also really enjoying the fact that we live in Italy. It hasn't been hard to recruit people to come and work in this lab because of the wonderful city we sit in. I've been aware of the responsibility for teaching and not just teaching, but teaching in a way that is inspiring and engaging. It's unfair to ask a young adult to sit and listen to you unless you're telling them something that's really interesting. I find science endlessly interesting so I have to figure out a way to make it as interesting to the students that I teach. The Holiday Lectures are a wonderful institution. I've already I think engaged just about my entire lab in the project to convey to as many young people as possible the excitement that we feel in stem cell research and it will be a real challenge for Doug Melton and me to meet the same sort of standards that we've seen in some of the previous lecture series. But we're lucky because we have a really exciting subject to work with. Stem cells are something that everybody's heard about and what we're hoping to be able to do with the series is to make it crystal clear exactly what stem cells are, what they can and can't do and why scientists are so excited about working on them.

4. Overview by differentiation (04:46)

Buon giorno, ragazzi! **[laughter]** Don't worry, I'm not going to go on in Italian but it's really wonderful to be here and specifically to be here with all of you. I have to say that it was just about when I was your age when

I was 15 that I first became obsessed with biology. I had a fantastic teacher in high school and I was thinking I was going to be an artist and I got this course that just made me realize that this was absolutely what I wanted to do for the rest of my life and I think that the excitement of biology is very infectious and I hope that some of you will get it and catch it at this event. I'm also very excited to be able to do this with Doug who for many years has shared with me this insatiable curiosity for developing embryos. But today I'm going to talk specifically about my most favorite topic of this particular part of my life which is regeneration. So from Doug you heard that the embryonic stem cell the ultimate embryonic stem cell which is the fertilized egg can give rise to a number of different tissues, in fact to all of the tissues in your body and it does so with a gradual process of differentiation and specification into these tissues that you see rendered here.

5. Replenishment and renewal of the body (06:02)

But what Doug touched on and what I'm going to spend the rest of my time talking to you about is the fact that we must keep the deep and very complex formation of our body intact for the entire time we're alive. We have to keep our skin intact, we have to keep our bones intact and even such very highly turning-over tissues as some of the tissues that Doug mentioned, have to stay the same shape, the size and the function has to remain as well. So we're going to be talking about replenishment and renewal which actually is happening even in you as young people. It's happening as soon as you form your body and so it's a very interesting and engaging part of science.

6. Some cells replenish constantly (06:47)

Now I'll just point out a few of the organs that we know are very good at replenishing themselves and Doug mentioned these. In the intestine we are continuously sloughing off cells inside of our intestinal wall in response to the food that passes through and that has to be continuously renewed. Or the liver, the liver clears our system our circulation of toxins and waste products and therefore has a heavy duty job. The liver cells turn over and new ones are replacing all the time. And finally our skin, which of course is our barrier to the outside world is something that is getting sloughed off all the time and has to be replaced and replaced and replaced. And for those tissues there are specialized cells that are set aside to do this very job. So

7. Replenishment vs. regeneration (07:37)

are those daily replenishing and renewing kinds of processes regeneration? What is regeneration? We hear a lot about regeneration. In fact most of the beauty products that make your skin look better are apparently touted as great regeneration agents. So I'd like to actually get down to the real meaning of regeneration as we as biologists consider it and to do so I'm going to use a myth, the myth of Prometheus. So the ancients were very much aware of regeneration and that's clear from this myth that came out of the ancient world

8. The myth of Prometheus and liver regeneration (08:13)

in which Prometheus who is this giant Titan stole fire from the gods and fire stood for art and civilization and he gave it to humans and Jupiter was enraged by this theft and so he took Prometheus and put him up against the Mount Caucasus rock and tied him there chained him there and then sent a vulture everyday to tear out his liver and devour it and every night when the vulture departed Prometheus would lie there in agony but his liver would regenerate so that in the morning the vulture had breakfast. And this went on and on and of course we as mere mortals aren't quite as good at doing that trick of regenerating our livers every night, although some of us probably wish we could, and I should also say that if the vulture had chosen another organ to devour, such as Prometheus' heart, he wouldn't have been around to tell the tale.

9. Is wound healing regeneration (09:10)

But the idea here is that regeneration is the reactivation of developmental processes such as those that gave rise to your liver in the first place to restore the missing and damaged tissues. Now is that the same as wound healing? It's not exactly the same. So let's look at two different tissues in the adult, both of which can get injured, the liver on the right which is healthy at this point and a healthy muscle. Let's imagine that both of these are injured by a crush to your muscle, or by some sort of damage to your liver. Now in response to injury the first thing your body does of course is to close the wound as fast as possible to avoid loss of blood and to start to launch a response against any infection which usually creates a fibrotic scar, and in fact that's exactly what happens if healing is allowed to proceed and it's an absolutely essential way in which we take care of less gruesome injuries than the Promethean vultures and the devouring of the liver. Now the difference between wound healing and regeneration is beautifully illustrated by these two tissues because a muscle, although you'll see has some regenerative capacity, can't really regrow and anyone who's been in a serious car accident knows that the muscle is the hardest thing to actually get back, whereas the liver can fully regenerate. It attains its form and function just as if it was new.

10. Stem cells are rare in adults (10:37)

So what is going on here? Are stem cells involved? What's the difference between wound healing and regeneration. I'd like to take a minute to just recapitulate some of the points that Doug made in his lecture about stem cells. On the left here you see cells that are dividing. The blue cell knows to make two daughter cells that are blue, the green makes two daughter cells that are green. This is the way the majority of the cells in your body actually divide to replenish your body. Now a stem cell is slightly different. A stem cell can do exactly the same thing, it can divide to make two stem cells, but the thing that makes it truly a stem cell is what you'll see on the right. Here a cell is giving rise to two daughters, one of which will maintain a stem cell nature and the other which will go on to make either a blue, a green or a yellow cell. So this is the difference between the division of a regular cell that can only make itself, and stem cell that can make itself but can make other things as well and in the embryo of course the stem cells hold sway at the very early stages that you saw Doug talking about with those eight cells sitting as an early embryo, but soon all sorts of specification has to occur as we saw. We have to be able to make livers and pancreases and skin and all of those other colors then are represented here in the embryo and the stem cells get diluted out and become less and less until in the adult there are actually very, very few stem cells in our body. You heard about the most prevalent ones, but even in the blood only about one in ten thousand cells in your bone marrow is actually a stem cells. So stem cells in the adult are there but they're much, much less prevalent.

11. Some animals can regenerate body parts (12:19)

Now that's true for us but it's not necessarily the case for organisms that rely on being able to regenerate truly regenerate in order to survive and we're going to have a little look at some of these because I'm a biologist and I love animals and we're going to see how simple animals such as the planarian that you see on the left on the top, the hydra which is the green thing, it looks like a sprig, and the famous starfish are capable of regenerating. They regenerate in the most miraculous fashion. You can cut these things in half and literally both halves will make a new organism and I'll show you a bit about that. The complex animals you see below also are very good at regenerating but they do it with a little less drama in that they can regenerate a limb or a tail, but they can't regenerate an entire animal from just a single chunk.

12. Planaria regeneration and stem cells (13:13)

So let's look at planaria, some of you in the audience have actually done experiments with this as preparation for this talk and so this will be old hat to you, but a planaria that's cut in half can actually regenerate the part that's missing. That means that the whole head of the planaria can regenerate and the whole tail can regenerate. And this is because planaria are actually full of stem cells. They have little cells called neoblasts

in planaria-speak which are capable of regenerating large portions or whole planaria. And so that's the reason why the limit of regeneration in a planaria is so extraordinarily dramatic. Cutting a planarian to many little pieces gives you a planaria for each piece and that's because each piece contains some of those stem cells.

13. Student experiments on planaria regeneration (14:05)

So some of the students did an experiment with planaria helped by Alejandro Alvarez who's an expert on this, and the question they had was in a planaria shown here, with the head on the left stained up blue and the tail on the right, if one cuts the planaria in two at either position one two, or three, leaving an almost whole planaria minus a head, a half a planaria or just a tail what happens at the regeneration point? Basically what happens is you get a new head in each case and you can see this by looking for the eyespots, those cute little eyespots are actually photosensitive. Now the question was, is a planaria's capability for making a head different if it's here or if you have to start here? And so the ability to do this was tested by looking at the time it took to put that head back into place and as you can see the best, the winner of this game was the largest piece which had a very short time before the head was formed, whereas the loser was the tail, it took a bit longer, but even so we got a tail growing a head.

14. Stem cells activated in regenerating planaria (15:21)

And here are just some beautiful shots of the students' work in which you see the stem cells those neoblasts, those sparkly cells shown up on the right as a stain for the stem cells and on the left in the context of the whole animal. Now it just so happens the one on the left had its tail cut off and the one right had its head cut off and that's why those cells seem to be so active at this point. Okay, so now that's one kind of very dramatic regeneration, but

15. Demo: The fire-bellied newt (15:55)

it's hard to relate to a planaria I mean it's got cute little eyes but for the rest of it doesn't have much else that looks like us. However I'm going to show you an organism, if I can put this glove on straight, that looks a lot more like us than that planaria, although you may not think so. For a biologist this guy is one short step away from mankind. And I'm going to bring him out here and see if I can get him on the close TV. There he is, this is a salamander or a newt. I'm going to put him here out so that you can see him I hope, on the screen. He's really quiet at the moment but these guys can scamper like crazy and they're very, very small and very easy to lose but they have, as I hope you can see, a head, you can see he's moving it now. They have two forelimbs, two hind limbs, they even have fingers, I think these guys have five or four and a tail and inside they have a beating heart lungs, a liver, a pancreas, intestines skin, and a very small brain and they're very very capable of running and out running their prey but if by any chance part of their tail or their limb gets cut off, they can regenerate the entire thing. So you see I'm just going to see if I can make him move a little bit so that you can see those limbs. You see how detailed they are? Now Doug was entranced at the rehearsal and asked me to get this little guy, now this didn't happen in the rehearsal, to get this little guy and show you what his belly looks like, because his belly is beautiful and red. Isn't that pretty? Now I'm going to do another trick which is he's so cute, I'm going to kiss him and turn him into a prince. That was for you, Doug. Okay guy, that's enough for you, back in the story. Now he's lucky, he didn't get his leg cut off he just got a kiss, but what I'm going to show you is what happens if you do cut off the leg of a newt. And for that we have a beautiful video. So let's start the video.

16. Animation: Newt limb regeneration (18:16)

Now we're going to have a look at the way in which an amputated limb grows over the course of about 90 days in a salamander. That's a time-lapse movie watching that thing grow. And now we're going to see

what's really going on. Here is the salamander and it's got a completely new limb. It looks perfect, it has inside bone, it has nerves and muscle and it can even wiggle. In fact it's perfect. Now we're going to cut it off. But here's the good news, it grows back so don't be too scared. First wound healing, did you see that wound heal? Now we're watching what the newt can do that we cannot do. Cells are streaming out of the surrounding tissues into the area of the wound and forming what we call a blastema which is a group of undifferentiated cells that are in fact really just like stem cells and they're multi-colored because they come from skin, from muscle and even from cartilage. And these have a miraculous memory of what they used to be and are able to form a perfectly functional limb and that happens all within anywhere from 30 to 90 days depending on the size. Those little guys will do it faster.

17. Muscle cells contribute to skin in regenerating limb (19:37)

So we'd like to know more about how that works. One thing that I should say is that unlike the planaria the limb that's cut off cannot make a new newt, and you might want to know why that is. Because in theory you could ask, well, maybe you could just clone the nose as Woody Allen would say but we can't and here's why. Let's have a closer look at what's going on when that regeneration blastema or group of cells at the tip starts to really do its trick. So there's the amputation plane of a really early stage in which all those cells have moved up and inside that blastema are cells that are differentiating, that come from a number of different sources within the stump, an epithelium that closes over, dedifferentiated cells that are probably stem cells, and extracellular matrix that holds the whole thing together. Those are the players. Now what we know from some beautiful experiments that were done by Jeremy Brockes and Elly Tanaka is that you can label cells from the unamputated limb that are in fact muscle stem cells and I'm going to tell you a bit more about those in a minute, and those can actually be reinjected into this amputated stump and then they will actually begin to form part of the regenerating limb and if we label those we can see what they become and what you're seeing here is that muscle stem cells became cartilage and in another picture you see here that muscle stem cells became skin. So this tells you there is some potency within the regenerating limb that allows cells to make decisions about being things that they normally weren't going to do if this traumatic event hadn't happened. And this kind of plasticity is something that is extremely interesting to biologists because it's the key to understanding how it is that stem cells in the adult are capable of recapitulating some of that marvelous potency that we found in the embryo.

18. Can humans regenerate body parts? (21:49)

So I'm going to leave you then before questions with this question of why we can't regenerate missing body parts. Is it because we're missing certain kinds of signals? Is it because there aren't enough stem cells? What are the reasons? And what we'll hopefully do after questions is then to explore some of the mechanisms that we have discovered in this fast moving field to solve some of these mysteries.

19. Q&A: Why can't the newt arm grow a new newt? (22:15)

So let's take some questions. Yes.

[STUDENT:] Why was the newt not able to regenerate completely like the arm regenerate a complete newt?

[DR. ROSENTHAL:] I'm sorry if I didn't make it clear. The question was why isn't the arm capable of regenerating the whole newt. Well, there are a couple of answers to that. Number one, as you'll see, an arm, as you can imagine is a very complicated structure. Planaria are extraordinary but they're not as complicated. The second point is that because of our complicated structure we need nerves and blood vessels and other kinds of cells that float around our body to maintain life and so basically it's impossible to keep that little newt limb alive long enough for it to grow back anything. But there's another reason. We think that the

information that you need to grow back a limb isn't actually in the limb and we'll get to that in the next session. Now let's see how good I am at this, I'm really... hand-eye coordination is not my specialty. Yes.

20. Q&A: Could you put newt stem cells into a human? (23:22)

[STUDENT:] Could we take a newt stem cells and implant them in a human's body or would the DNA not match or....

[DR. ROSENTHAL:] Well, we could do that, I wouldn't recommend it for all sorts of reasons one of which is we are trained as highly involved immuno-competent organisms to reject things like newt cells unless we're in the process of eating them, which we're not going to do either. So I think to get to your question in a little more detail, the issue of whether a stem cell from one species can function in the context of another species is something we're going to hear about a little more from Doug tomorrow, but suffice it to say that there are experiments that have been done in the culture dish where newt cells and mouse cells have been put together and the question is does the newt teach the mouse cell how to do some of the tricks it can do, or does the mouse cell tell the newt cell, forget it brother, I'm the boss here. And the answer is, the newt cell wins, so that's the exciting and encouraging thing.

21. Q&A: What happens to muscle in limb lengthening? (24:36)

Yes. Sorry, I can't even throw.

[STUDENT:] When people have surgeries to lengthen limbs, like when they separate the bones and the bones grow back into them, why can't they just like put stem cells there? When that happens is there more muscle? If there's an absence of stem cells there then how is it growing?

[DR. ROSENTHAL:] Sorry, which part is growing?

[STUDENT:] When they separate the bones and the bones grow back.

[DR. ROSENTHAL:] Ah, well, interestingly enough, bones are some of the most regenerative structures in our body and they actually grow back better than muscle. So for instance if you crush your leg in an accident and the bone is broken, your doctor will say no problem about growing your bone back that will heal and it will actually form the bone as it was before due to all sorts of signals that the bone somehow senses, much like the newt. The muscle just isn't as regenerative and we don't understand what it is about different tissues that make one more regenerative and another less, but we suspect that it may have something to do with stem cells. That's the big excitement about stem cells, that if you could understand how stem cells in one part of your body could do such a great job and your other organs don't seem to be able to do such a good job could you use stem cells from one part of your body to cure the other and I'm going to be talking about that after the break. I want to get someone in the back because I want to show that I can throw as well as Doug. [laughter] There, that's better.

22. Q&A: Can a newt regenerate its organs? (26:16)

Oh my God, I don't know, somebody back there the guy in black.

[STUDENTS:] Can newts, like, regenerate organ systems or is it just arms?

[DR. ROSENTHAL:] Newts can regenerate organ systems. Newts can regenerate an extraordinary number of different parts and in fact what we'll see tomorrow is another example of a highly regenerative organism, the fish, which can regenerate its fin, its tail, and its heart as can the newt not the entire heart but a large

chunk. The newt can regenerate its lower jaw if you cut that off. It gets gruesome after that but it's an amazing little organism.

23. Q&A: How many times will the newt limb regenerate? (26:55)

Okay let's take one from over there, the fella with the blue shirt.

[STUDENT:] In the example of the newt, would there be a specific resource limit as to how many limbs that a newt could regenerate according to its resources?

[DR. ROSENTHAL:] What's extraordinary about the newt is, sadistic as it may seem, you can cut a regenerated limb off as many times as you can stomach the process and it will grow back every time exactly the same way. So it appears that the newt has the whole process well sussed out and can do it exactly the same way. It's a very robust program if that's what you meant. So it appears that there isn't some sort of a stem cell pool or some kind of a molecule that runs out after the first regenerative event. Now I've got to do some more embarrassing throws to get one over here. Yup, that's terrible. And this one I'm going to do relay because it's too embarrassing.

24. Q&A: What triggers cells to form the blastema? (27:55)

Okay, let's go for the lady in light blue.

[STUDENT:] In the newt, like, what triggers the stem cells to go to, like, a wound to form the blastema?

[DR. ROSENTHAL:] That's a very good question and we believe that there are a series of acute signals that occur immediately after the wound has been created that tell the organism that there's been a traumatic event, much like a wound healing response but that second event in which instead of just closing the wound and remaining a stump like an amputee that would happen if my arm was cut off there seems to be another set of signals that we would dearly love to know about and I'm going to tell you a little bit about one of them in the second half of my talk. Now I'm afraid that's it for questions. All right. Let's go on.

25. Problems with mammalian regeneration (28:56)

So the question is why we can't grow back a limb. Obviously there are many questions in the audience that pertain to this and anticipating those questions I thought I would just put up a picture of what actually is inside your limb and this is a simplified artistic version of it but as you can see it's a very complex structure. And therefore when we consider the way in which the salamander grows back its limb we really need to think in terms of how these various complex structures know to become what they are and one possible reason why we can't do this trick is because of the risk of cancer. And I'm going to talk about each of these in turn but two others are that we've lost programs that help us to regenerate our bodies, or that we simply don't have enough stem cells such as the planaria who's full of them.

26. Dedifferentiation of cells during regeneration (29:59)

So let's look at the one about cancer. Now why do I bring this up? Any cell that can launch a proliferative response the way the explosive divisions that you see early on in embryogenesis take place, has the capacity to go wrong because cancer is cell division gone horribly wrong. And so when we think about that extraordinary growth of the little salamander's limb, we have to realize that that is putting the salamander in theory in risk of getting cancer and why is that? Differentiation as you heard from Doug involves taking a cell that is uncommitted and very potent to become many different things and assigning functions to its daughter cells. But during that regenerative event that we saw in the newt, another event occurs which I'd

like to just mention here and that is that thing one that we heard about from Doug in the pancreas: a differentiating cell actually dividing, but in this case the division is such that a backward step is taken so that now the cell is in fact potent again and can become more than one thing, and that's why we could see those muscle stem cells becoming cartilage or epidermis in the limb because dedifferentiation can occur.

27. Cancer and dedifferentiation are similar (31:34)

Now in a tumor that's exactly what happens. A normal cell divides and grows in a way that is consistent with its environment until a mutation somehow takes out some essential break on proliferation and then the cell proliferates in a way that is inconsistent with its position and it essentially grows out of control and that's a tumor. So in theory a cell that's dedifferentiated is a cell that could be a cancer cell, so what's the difference? Why don't newts get cancer?

28. Controlled cell proliferation in the newt (32:09)

Let's just look at dedifferentiation and specifically in the case of muscle, because I mentioned the fact that muscle can dedifferentiate in the case of the newt's amputated limb. Here's a close up of what muscle cells look like after you've cut the limb off at the stump. Very soon afterwards these muscle cells which are rather specialized in the sense that they have multi nuclei inside a single cytoplasm that's full of contractile proteins for their capacity to contract. These different nuclei sever themselves off compartmentalize themselves, take a few organelles put a membrane around them and run off to become new stem cells. This is a rather dramatic thing and we don't know what the signal is to do that but that would be a very interesting answer to one of our student's questions and that's perhaps why it is that the blastema can then form so rapidly because there's lots of muscle at that cut wound and all of it can start to produce these potent little progenitor cells. These cells can come from bone, they can come from muscle, and in general they regrow the limb structure very, very rapidly. So there must be something about the limb which is different from the rest of the proliferative processes that we find in our bodies that go awry. So to understand the difference between dedifferentiation in these kinds of animals and the uncontrolled dedifferentiation that we have is one of the great goals of regenerative biology. So the answer is the risk doesn't seem to be there in these animals, would it be in us?

29. Are regenerative genes lost in higher organisms? (33:56)

Now another possibility is that we have the loss or alteration of a genetic program. Well that's sort of obvious, something's lost otherwise we could do it. And in fact we know that there's an inverse relationship between evolutionary scale here shown with the bodybuilder as the epitome of evolution and the lowly planaria who probably doesn't like being at the bottom but can do a much better job of reproducing itself. So somewhere along the line we have lost some programs. We can grow muscle, that's for sure. We can look like that woman, or at least some of you probably can, but what we can't do is amputate her arm and grow it back so some kind of a program is missing. And so scientists have asked, what are the potential signals that could be present in the newt limb that are absent in our more developed and larger bodies?

30. Identifying the signals that control limb regrowth (34:56)

And a clue from this came from a beautiful experiment that Jeremy Brockes did several years ago in which he asked the question, if I cut the newt limb off below the elbow, near the wrist, what grows? And the answer is a little blastema forms, as you see and over the 70 days that it takes to regrow that limb, it grows exactly what it needs to basically just the lower part of the forearm the wrist and the hand. Then Jeremy cut off the limb above the elbow position and asked what happens and in the same period of time the blastema knew to grow an elbow, a forearm, a wrist and a hand. So something in that stump knew where it was and at the time that it was amputated, immediately launched a new program that would be different if the

amputation happened here or if it happened here. So one possible way of thinking about this is that there's something that's different in its concentration at the top of your arm and at the bottom of your arm and that gives you some kind of a zip code to know where you are along your arm even in an adult, like the newt.

31. The CD59 protein may guide newt limb regrowth (36:09)

And in fact recently the same group has come upon a cell surface molecule which has this really sexy name, CD59, and CD59 turns out to be at very high levels on the surface of cells of the limb proximal to the body and at much lower levels on cells in the limb down at the wrist. And so the question they asked is, could CD59 give cells an address, or is it just a correlation? And to answer that question they did some very elegant experiments. They tried to change a cell's address by giving a cell more CD59 than it should see. So in this case what they did on the top is a control in which they took a cell out of the limb before it was amputated and labeled it with a red dye and then replaced it into the growing blastema of an amputated limb and asked, where do the progeny of that cell end up? So all the cells have the mark and the progeny have the mark and as you see, the cell ends up at the right place in the wrist because that's where it came from. Now we do the same experiment except this time we engineer that cell to express a lot of this cell surface protein CD59 and then we put the cell back into the same position on the blastema and as you can see on the lower panel, that cell thinks that it should be down here somewhere because of the higher levels of CD59 that it's expressed. So it's probably cordoned off and the guy says, hey you should be down at that end.

32. Overexpressing CD59 causes malformation (37:48)

The problem is that it's not a benign arrangement because in fact the whole experiment results in a deformation of the limb so that not only does this tell the cell where to go and it's a different place, but once it's there it produces some sort of deformity and in fact what we see is that on the control on the left there are a number of different concentrations of CD59 shown on the bottom in a schematic way with different colors and those color orders are in disarray and are disrupted by the presence of cells expressing too much CD59. So there's no more green cells because the cells that are there are expressing CD59 at a level that should be red. So in theory what you could say then is that we obviously have lost the CD59 zip code and therefore we simply don't have the right programs to do the job and will never be able to regenerate because we wouldn't be able to know how to grow that new limb.

33. Demo: Regeneration of deer antlers (38:53)

So a question then is, are humans and mammals just incapable of regeneration because we can't afford it because of cancer or because we don't have the right programs? Well the answer is no. And improbable as it may seem, this is the only case of true regeneration in the mammalian kingdom. Now for those of you who have never seen one of these before, it's a deer with antlers. And for those of you who know nothing about deer with antlers--this is very seasonal--it turns out that every year these antlers fall off, every single year and every year they're replaced, new antlers grow and every time a new antler grows it acquires a new point. And so eventually we talk about six-point bucks these are deer that have had six years to develop these different points. Think what that means. At the base of the skull of the deer, right where my finger is, right there, is an area on the skull of the deer which is different from our skull called a pedicle and on that pedicle are a series of little tissues that are in a little ring that are full of stem cells and at an appropriate moment, when the deer antler has fallen off, those stem cells then get into action and grow an entire new antler that's perfect every time, and not only that, it has a different pattern according to how old the deer is and finally it does it at an enormous speed. So when this guy is going full tilt for growing an antler because this has to happen every season because he uses these for display to get girls this area right here has to be pumping out new growth at two centimeters a day and the growth is all from the tip so it looks just like a blastema. Okay. Now with that being said it doesn't obviously lend itself particularly to any kind of medical applications and

34. Adult stem cells in bone marrow and muscle (41:09)

I'd like to finish off today by talking about a slightly more serious subject and that is the possibility that we have smaller numbers of stem cells and that's why we can't keep up with trauma and serious injury. Now you heard from Doug that in fact the bone marrow is a very rich source of stem cells that turn over very, very rapidly although they are proportionally in very small numbers. And so the possibility might be that these bone marrow cells could zoom around on a highway in our bodies and fix things as they found problems and this was a very popular idea and has actually given rise to a number of very exciting clinical trials in which bone marrow cells are being used to try to cure various diseases. But I'd like to talk about another source of stem cells, namely cells that are resident within a particular tissue and don't circulate on the stem cell highway and in this case I'll talk about muscle because I know a little bit about it and in muscle as I said fibers contain many nuclei but they also contain within them rare little cells stem cells called satellite cells which I've mentioned before that are capable of responding to injury by proliferating. So they have in some ways a sort of a stem-cell-like nature. The problem is that there aren't that many of them

35. Muscular dystrophy overwhelms stem cell capacity (42:38)

and in fact in a devastating disease like muscular dystrophy where the muscle is weakened by a genetic lesion, robbing it of an essential protein and causing it to be fragile--every time it's used it actually breaks and has to be replaced--the stem cells can keep up with this for awhile but eventually there just aren't enough and so at that point what happens is that the muscle loses its capacity to contract. The injuries instead of being replenished actually act like a wound heal and turn into scar and fibrotic tissue and the muscle becomes actually quite paralyzed, so an obvious way to think about this would be to replenish their waning cells in their muscles with some kind of a stem cell that could at least help with the consequences of this disease if not the cause.

36. Using bone marrow cells to repair dystrophic muscle (43:33)

So in my lab I've been working on just such a question with my colleague Antonio Musaro at the University of Rome. So together we wanted to develop a question of just such a nature. Could we take a bone marrow cell and make it contribute in some way to muscle that was injured? So to do this experiment we had to set it up and because we work with mice we can engineer mice to express all sorts of wonderful proteins and in this case we used a mouse that we had in the lab in which the same alkaline phosphatase gene you heard about from Doug as a marker was driven by a regulatory element that activates it only in those skeletal muscle fibers. So if you look inside this mouse as you see on the right the muscle fibers are for the most part a sort of a dark purpley blue when we stain them and that means they're expressing this gene which we call hAP. So if we see hAP it means the cell is a muscle cell. Now what we do is we remove bone marrow from this mouse. Now because the mouse is transgenic for that marker that means it's genetically engineered so that all of its cells have that marker the bone marrow cells actually contain this marker but it's silent. Why? Because it's not a muscle cell at the moment, it's a bone marrow cell and so it doesn't recognize the signals to turn on the hAP gene. So the question is will these bone marrow cells ever turn purple if we put them into the right context? Now what's the right context? In this case we're going to use a mouse model with muscular dystrophy, called the MDX mouse. This mouse is actually a very well studied mouse it undergoes some of the same problems as the boys, mice escape the real devastating effects of muscular dystrophy, but their muscle looks awful it looks just like that picture of that little boy's muscle constantly regenerating, constantly degenerating. And so we ask the question, could we improve the muscle of the mouse functionally and could we stave off the devastating effects of the disease? So we did the experiment, we introduced into a vein in the mouse's leg some of these bone marrow cells from our hAP mouse and asked, do we ever see any cells that engraft into the muscle and turn purple because if we see them turning purple we know they come from the hAP mouse, they come from bone marrow and they're becoming muscle. And

every once in a while we saw one of these, about one to two percent of the skeletal muscles in the mouse actually turned purple. Unfortunately one to two percent isn't going to cure a muscular dystrophic boy,

37. Growth factor IGF-1: Function and mechanism (46:18)

so we had to think of other ways in which we could augment this rare but very exciting result. And we did so using a growth factor. Now in this case we used a growth factor called insulin-like growth factor 1 and like the growth factors you heard about from Doug it's a growth factor that actually promotes growth but it promotes a number of other things as well. It's important for growth in the fetus, it's not so important for growth in our bodies, it promotes all sorts of different kinds of cell functions and then eventually it is very interestingly induced in response to injury locally. So if you injure muscle or you injure any part of your body, IGF-1 is transiently expressed. Now what does it do? It's a growth factor, it's a molecule and it circulates around the cell milieu looking for a receptor to bind to. Once it binds to the surface of the cell it sends a cell signal, an intracellular signal that eventually ends up in a transcriptional event which turns on a whole set of genes and we can study those genes in the same way that Doug explained to you with chips. But for today's lecture we'll just talk about the way in which IGF-1 works at the level of the body. Locally it's expressed as I said in response to injury and in muscle and in heart it tends to induce growth and it's also expressed by the liver and in this case it circulates throughout the body. But we can get away without the stuff that's circulating in the body and in fact we find that the circulating form of IGF-1 has none of the same properties as the form that's made in the muscle itself.

38. IGF-1 improves bone marrow's ability to regrow muscle cells (48:01)

So we had the chance then to make a mouse that was expressing more than its normal share of IGF-1 in the skeletal muscle and we did that with transgenesis and it's the same way we made the hAP mouse and we do it by taking a fertilized egg out of a mouse and injecting it physically with DNA encoding our IGF-1 protein, the gene of IGF-1 goes in there, in this case driven with a muscle specific regulatory element. That embryo is transferred into a foster mother and that foster mother gives rise to pups, some of which hopefully got the gene and if so those pups then can be used to study the effects of IGF-1 in the muscle. And what we found was when we mated those IGF-1 animals to the MDX mouse and did the same experiment we got a very exciting result which you can see on the right. The one on the left is the same story I told you before. And the one on the right is what happens when we inject bone marrow cells marked with hAP into an MDX mouse that's also expressing this growth factor in the muscle at which point 15 to 20% of the cells are blue or purple instead of 1 to 2%. So we're seeing a remarkable increase in the capacity for these cells to actually get taken up and become muscle.

39. Summary of regeneration schemes (49:26)

So the moral of the story then is that salamanders if we go back to our initial survey of regenerative processes in the animal kingdom can actually use local cells to replace damaged tissue. So if you cut off the end of a salamander's limb you can regenerate it. With mammals we're basically looking at a slightly less efficient arrangement whereby circulating cells can have some effect along with local cells but to really make that a clinically relevant phenomenon we're definitely going to have to resort to some tricks and in this case we used a genetic trick in which we used growth factors. So then in conclusion, tomorrow I'll be able to tell you a bit more about the mechanisms that we envision to identify the factors that we'll need in order to augment the extraordinary capacity of stem cells to do a better job for us in our own bodies

40. Q&A: Could stem cells reverse the effects of liver cancer? (50:29)

and I'll stop there and take questions.

[STUDENT:] You talked earlier about how the liver is able to regenerate and like heal itself and then there's also the case of liver cancer. Is it possible to inject stem cells from like a healthy donor into livers and then kind of reverse the effects of liver cancer?

[DR. ROSENTHAL:] Well it's a great idea and in fact liver transplants have been very successful precisely because of the fact that a diseased liver can be literally completely replaced with a small portion of a healthy one and it grows right back to the full size liver in the recipient. The problem with liver cancer is that cancer itself is a very nasty disease because it grows even from a single cell and so it's unlikely that you'd want to leave any cancer in the body you'd want to try to remove it before you tried to regrow the liver, but the concept of regeneration of the liver is one that's been well used and is an extremely successful operation for many people who have damaged liver.

41. Q&A: Why do we age if we can regenerate cells? (51:48)

Yes. This one I can do. Ha. Look at that...

[STUDENT:] ...she talked about regenerating the liver and if we have some stem cells in our bone marrow and those bone marrow cells then how come as we age we get shorter? Why do we age if we're able to regenerate ourselves?

[DR. ROSENTHAL:] Why do we age if we're able to regenerate ourselves? It's a great question and if you promise to come tomorrow I'll answer it for you. I have a whole talk on aging and it will take me too long to do it now, but you get a t-shirt because you're close and I feel like I can probably pull it off. Okay, notice I can't just take you in the front because it's not fair. Okay,

42. Q&A: How far up a newt's limb can you cut? (52:28)

I'll go for the fella in black who thought I was going to ask him a question at the very end.

[STUDENT:] I was just curious how far can you cut how far up the limb of the newt can you cut and it will still regenerate? Can you cut all the way, I mean I'm a bad example but if you cut off above the shoulder will it still regenerate?

[DR. ROSENTHAL:] That's a very good question, I have never cut the arm off a newt, but I presume you can get pretty much as close to the trunk as you can get a scissors and it still will regenerate. If you would then go in with a hammer and chisel and take out its whole shoulder bone I suspect we might get something a little less nice than a full limb. But I think your question is very good, how much information do you need to start with to regenerate an entire limb and I think that the issue is very very pertinent because obviously there must be something that tells the organism that I am a shoulder and then I am a limb. But I don't think that there's any boundary line there it's probably just more of a practical issue of how you could actually cut that limb off. So you can get pretty close to the bone as it were and still regenerate.

43. Q&A: Are skin markings identical on the regrown newt limb? (53:52)

The lady in white.

[STUDENT:] If you cut off the arm of a newt or a salamander whatever, will it grow back the same exact arm? Like if it had a marking on it will it grow back with the same marking and everything?

[DR. ROSENTHAL:] That's a wonderful question. The answer is that you do lose some of the actual marcation on the limb and we believe that's because some of the cells that end up in the limb to make this

demarcation originate from some place other than the limb, and so in fact you may not get the exact pattern back. You know what, I'm forgetting about the t-shirts but this is really tough. Can I do relays because otherwise it's not going to work. I'll throw it halfway there. And now for you...wheeee! At her feet.

44. Q&A: Can a newt regrow two limbs at once? (54:44)

Okay, the lady in pink.

[STUDENT:] I was wondering if you were to take a newt and cut off two limbs at the exact same time one at the wrist and then one maybe at the elbow would it focus on the more serious injury or would it regrow the same two at the time or would the CD 59 would it get it mixed up which one was cut at the wrist and which one at the elbow?

[DR. ROSENTHAL:] It's a great question. The answer is that these events are local and so the limb that needed to grow back just a hand would grow back the hand, the limb that needed to grow back the whole thing would grow back the whole thing. It appears that these are local signals and therefore they don't actually get mixed up across the body. The CD 59 molecule is actually sitting on the surface of the cell within the limb. So it's as if the surface of the cells were just in more intense pink up here and less intense down here. So it's an intrinsic capacity to read your zip code from your local information rather than some guy coming around and telling you, and I'm sorry got to get a t-shirt. I'm getting better at this but I'm afraid we've got to stop, time is up and thank you all for your attention and we'll see you in the morning.

45. Closing remarks by HHMI Vice President Dr. Peter Bruns (56:08)

[applause] [DR. BRUNS:] Thank you, Nadia and there were plenty of questions left and obviously the people out there are watching this on a DVD in the future, or on the web right now, can't ask questions right here but we have a mechanism to answer questions without fear of flying t-shirts and that's found on that web site BioInteractive.org a feature called Ask A Scientist and that's worth looking at. If you have a question on this or other topics you can send it into that site, our volunteer scientists will answer you directly and if it's a question that we feel is worthwhile we'll even post the question and answer and those are archived so it's a very interesting resource for other questions in any case. So thank you.