HAZEL SIVE: I want to discuss with you today a very topical and interesting question, which is the notion of stem cells. In fact, I'm going to discuss two things, the first of which is another concept that you need, following on from the concepts we had right at the beginning of lecture. I feel like this microphone-- The first is a concept that you need in addition to the ones we started lecture with, and then we'll talk about stem cells.

So today, we'll talk about potency, and then we'll talk about stem cells. Potency, along with fate, determination, and differentiation, is one of those terms that you need to know and you need to understand in order to understand stem cells. Potency refers to the number of possible fates that a cell can acquire, number of possible fates open to a cell.

And this is a very important concept of development because, in general, potency decreases with age, and decreases as different parts of the organism become specialized. So in general, potency decreases with age. But I will put in here, and we'll explore this more in a moment, except for some stem cells. And we haven't defined a stem cell yet, but we will.

What kinds of potencies are there? There's the big one, totipotent, where a cell can become all fates. And there's really only one cell that can do this in the normal animal, and is the zygote. And in most animals, even as the zygote becomes just two cells or a few cells, that full potency is lost.

And cells instead, in the embryo, are multi or pluripotent, which means that they can acquire many fates, but not all fates. Embryonic cells, especially in the early embryo, and many stem cells can also become, are also multipotent or pluripotent. And then as time progresses-- is that a hand up? Yes, sir.

AUDIENCE: How do you reconcile the fact that human cells, we can separate them. Even at the eight cell stage.

HAZEL SIVE: That's a great question. The question is how do I reconcile what I'm telling you with the fact that you can get identical sextuplets, or octuplets actually? It's a good question. That's true. In different animals, the very early embryonic cells are sometimes totipotent up to a while. OK?

And so for example, in armadillo, here's a piece of, you know, fact for your back pockets. In

the armadillo, the eight-cell embryo almost always splits into eight single cells, each of which becomes a baby armadillo. OK? So those cells are totipotent.

In mice, even at the two cell stage, the two mouse cells are probably not equivalently potent and they're not totipotent. So very, very seldom-- almost never-- get identical mouse twins. OK? So it's one of these generalities. And if you ask me, you get the specifics are a bit different.

As cell fate restriction continues, cells can become bipotent, or unipotent, whereby one or just two fates are open to them. And so if we look, taking this concept, let's now start the lecture about stem cells. You're going to need this concept. Stem cells, I'll point out, whatever they are, got almost 3,000 hits yesterday on Google News.

This is way below baseball, which got 45,000. I checked. But still, you know, as science topics go, stem cells are really up there. And they're on the covers of magazines, over and over again. And we'll talk more about why that is.

Here's a diagram-- it's not on your handouts-- that I drew for you. Let's not dwell on it. But let's now move on to Topic Number 2, which will fold in this concept of potency and the concepts of fate, determination, and differentiation, and talk about stem cells. And let's do, as is our custom, let's define what a stem cell is.

I think that a stem cell can be defined as a cell of variable potency that has the capacity to self-renew. Cells of variable potency that can self-renew. They can make more of themselves. Despite the hype, despite covers of *Time Magazine* and almost every front page of every newspaper across the world, stem cells are normally found in our bodies. And normally, as we'll explore, they're used for organ maintenance and repair, organ maintenance and repair.

But the thing, you know, that has everyone fired up is that you can somehow harness these cells for therapeutic purposes. And that you can repair what the body cannot, by being clever, and using the power of these cells as they normally have it, or as you can give it to them. And so there's this question of therapy and therapeutic stem cells, where the idea, again, is that you would repair a damaged organ by introducing somehow, injecting or otherwise introducing, extra or somehow special stem cells-- which I am going to abbreviate heretofore as SC-- by introducing extra stem cells into a damaged body.

Does this work? It does work. It works for the hematopoietic system, as in bone marrow

transplants. And it also works for skin cell transplants. Well, let's just put skin cells. OK. Skin stem cells can be grown from your own skin. And in the case of burn victims, this has really saved countless lives. The original technology began to be developed here at MIT by Professor Howard Green, who is now at Harvard.

But the idea is to take your skin and grow it on something like gauze or some kind of some solid support, and then to cover a burn patient with layers of support on which there are some stem cells. And these stem cells will help fill in the holes in the skin left by the burn. Normally when a wound heals, as I'm sure you've noticed, it heals from the sides. The only way a wound can heal is from the side.

And if it's a big wound, it can take a very, very long time to heal. And you can get infections and so on while the healing process is going on. So seeding the inside of a wound with stem cells that can start the skin regeneration process and seal up the body against infection, that's been incredibly useful. And we'll talk more about bone marrow transplants in a moment.

OK. We previously talked about this process by which cells decide, are undecided initially, they decide what they're going to become. And then they differentiate into their final function. Stem cells fit into this litany somewhere between the commitment stage and the differentiation stage. And in this diagram, these multiple arrows are there for a reason.

There are multiple steps between commitment and differentiation. And somewhere along the way, a group of cells with capacities we'll talk about, leaves this lineage and sits around and waits, partially determined, so that it can go on and make more differentiated cells when they're needed. And I've added on there the potency timeline, decreasing with age, of the developing animal.

But let's diagram the notion of stem cells on the board. Stem cells generally divide slowly. Here's one. It's a variable potency. It may be multipotent. It may be bipotential. And it is somewhat committed, which is a slightly difficult concept.

Because last time we talked about committed versus uncommitted. But now I'm telling you something can be somewhat committed. And that gets to this multiple arrows there were as cells progress in their fate decisions, they change their molecular signature and they really do become closer and closer to a cell that's made a decision.

But it's kind of like, you know, if you're weighing up going to med school or going to graduate

school in bioengineering, you know, you have decided that it will be one or the other, but you haven't decided which. You're somewhat committed. And then when you make the decision to go to graduate school, you have now become committed. OK? So the cell is doing the same kind of notion there.

There's your stem cell, variable potency. Under the correct stimulus, that stem cell will divide to give rise to two different cells. One is another stem cell. And the other is something we'll call a progenitor. The progenitor is more committed than the stem cell. The progenitor cell is going to go on and divide, usually a lot.

Progenitors divide rapidly. And their progeny will eventually go on and differentiate into one or more different kinds of cells, maybe a stripey cell, and a spotted cell type, and a cell type with squiggles. And so here are the differentiated cell types. And the number of different differentiated cells that comes out of this process is a reflection of the potency of the stem cell. OK?

So here you've got these progenitors. The idea is that these progenitors will have similar potency. But as I'll show you, there's a whole variation on this. But here the number of differentiated cell types, the number of cell types reflects potency of the stem cell. OK?

This kind of diagram is called a lineage diagram. It tells you what-- not only what the final fate of the cell is, it tells you something about the progress towards that final fate. So a lineage we can define as the set of cell types arising from a stem cell or a progenitor.

Let's talk about the discovery of stem cells, because this is something that really was pivotal in helping understand whether or not there was some way that the body normally repaired itself. It was clear that during early development, there was lots of cell division and lots of changes. Cell types were formed and organs were formed. But it really wasn't clear in the adult how much repair there was, how much turnover of tissues there were, and really what the whole dynamic process of maintaining the adult was.

And the discovery of stem cells came about because people looked to see how long cells lived. And what they found was found using a turnover assay that measures the half life of cells. And they found that in almost all organs, in fact, probably in all organs, cells did not live forever. They turned over. They died. And they were replaced by new cells.

And this turnover assay implied that there was some kind of replacement. And the cells doing

the replacement were called stem cells. You find this by a pulse/chase assay, which we'll go over on your handout in a moment. And what was found was really variable for different organs.

Firstly, all organs, about, show cell turn over. Red blood cells have a half life of about 120 days. There are a lot of red blood cells in your body. And in fact, that implies that there are about 10 to the seventh new red blood cells made a day. In your intestine, the half life of cells is three to five days, in the small intestine. And the hair on your head has a half life of about four years. So it's variable for different kinds of cells.

If you look at your first handout, it diagrams a pulse/chase assay where a cell population is labeled with a nucleotide analog. It's a normal nucleotide, but it's got a bromine added to it. And it acts like deoxythymidine, gets incorporated into DNA, and you give just a short pulse of this nucleotide analog. So only some of the cells get labeled. And you only give it for a short time.

So you get a labeled cell population. And then you stop the labeling by adding lots of unlabeled thymidine, and that's called a chase. And you follow the cells over this long chase period. And then you can watch and see what happens to those cells that you initially labeled over a very short time.

And so in this example, I've got four cells initially labeled. Over time, they're only two cells left. And if you measure the time from going from four cells to two cells, you can get to the half life of that cell population. OK?

You can also-- if you don't have a handout for this, just look on the screen-- you can also follow the labeled cell population and see what those cells become. And you can see that they go on to differentiate as particular cell types. So this is a kind of way of labeling the lineage of these cells. And that is useful, too.

This was the theory behind stem cell definition. But what is a stem cell look like, and how do you isolate one? It turns out that that's really difficult. So isolation and assay in the adult stem cells are very, very rare. And that is one of the issues with using stem cells for therapy. There are very few of them and they're hard to isolate.

Hematopoietic stem cells comprise about 0.01% of the bone marrow, which is where the stem cells reside, and where the precursors of your whole blood and immune system reside. The

way that this was dealt with was through a really clever technique that has the acronym of FACS. I'll give you a slide in a moment. It stands for Fluorescence Activated Cell Sorting. We'll go to a slide in a moment so we don't have to spell it out.

And the idea behind FACS is that you label stem cells. And you might be guessing what to label them with. But you label them usually via their cell surface proteins with some kind of tag, often an antibody tag. And then you can use that tag to make them different colors. And you can then sort them, cell by cell, through the Special Fluorescence Activated Cell Sorter.

Sort individual cells, and then you can assay individual cells or small groups of them for stem cell properties. Let's look at a slide of how the FACS, the Fluorescence Activated Cell Sorter works. No, let's not. I've really gotten ahead of myself here. And I'm going to go back because I want to show you this. Hold on to that thought and let's go back to this notion of a pulse/chase assay. I forgot that I had this here. This is really important.

This is a pulse/chase assay of intestinal cells. And so your small intestine lies inside your belly. And if you look at its anatomy, it contains many tubes whose surface is thrown into folds to increase the surface area for food absorption. And if you look at these folds, they are very closely packed. So you get a huge surface area increase. And the cells of these folds that are doing the food absorption turn over every three to five days. Those are the ones I was talking about.

So if you blow up one of these folds, which is called a villus, there's the part that's sticking up into the cavity, into the lumen of the small intestine. And then there's this part that kind of dips down into the lining of the intestine, which is called a crypt. The stem cells lie somewhere at the base of the crypt. It's not exactly clear where.

But the idea is that somewhere at the bottom of this crypt there are these stem cells that under specific conditions will start to proliferate. And they will move up into the villus and replace the villus cells that are dying. You can monitor this by doing a pulse/chase experiment. So here's the crypt. And they've given, in this experiment, a pulse of thymidine has been given. And you're looking kind of at time 0, right after the pulse of thymidine has been given.

Here the cells, the black cells are in the crypt. They're labeled. And then if you look over time, you can see that these black cells move away from the crypt. They're moving up into the villus. And here they are actually right on top of the villus, replacing the cells that were turning over. So that's a really beautiful demonstration of a pulse/chase assay.

OK. Now we can go to our Fluorescence Activated Cell Sorter. The idea is that you take a mix of cells-- you don't have this on your handout, just look on the screen-- the cells are labeled with fluorescent antibodies. And you put them into a reservoir and droplet generator. And the cells drop out of this reservoir, one at a time.

And as they drop out, they go past a laser. And you can tune the laser to whatever wavelengths you want. It excites the cells. And if the cells emit in the particular wavelength you're interested in, the detector will detect that. And then it actually gives a charge to the cell that it is the correct fluorescence. And as the cells are dropping down, the cells of the correct color are deflected by an electric charge. And different color cells can be collected into different flasks.

OK? This really works. It's a fantastic machine. You can collect cells about, you know, you can collect millions of cells an hour. It's pretty quick. But you do it one cell at a time. And in that way, you can isolate cells, which have got stem cell properties. OK.

So you've used your FACS machine. You've got cells that look as though they've got stem cell properties. And now, let's look at the assays that may be used. And there are three assays that you should know. One is a repopulation assay to test stem cellness.

And this is a transplant assay where you're transplanting test cells, test stem cells, into the adult. And you have removed from the adult, endogenous cells that might be competing with those stem cells. That would include the stem cells. We'll go through a slide in a moment. I'm going to list them here.

Another one is an in-vitro induction assay, where you are going to take isolated cells and you're going to treat them with various inducers, various signaling molecules, and you're going to test and see what fates those cells can acquire. And a third assay is called an embryo incorporation assay, where you are going to take cells that may be stem cells, and you're going to test them in a chimeric embryo.

Let's go through your next slides to discuss each of these points. Bone marrow transplants resulted in a Nobel Prize in 1990 for E. Donnall Thomas and Joseph Murray. It's a technique that saved millions of lives, and here how it works in a mouse. You take the mouse and you irradiate the mouse to destroy the bone marrow and the stem cells associated with the bone marrow. If it's a person, to destroy the diseased burned bone marrow.

And then you replace that bone marrow with normal bone marrow to either try to make the person better, or in this case, to test something about stem cells. The irradiated mouse or person would die, but the normal bone marrow will cause the mouse to live. And if you've put stem cells into that mouse, you can start getting them out of that mouse whose life you have saved, and isolate more stem cells.

Those kinds of assays led to the definition of the hematopoietic stem cell. Here it is. It's a pluripotent stem cell that gives rise to all of these different kinds of cells, the immune cells, and all of the blood cells. It's a very, very powerful stem cell. And the ability to actually make this diagram and say that there was a single cell that gave rise to all these different lineages was because of a titration assay where you could take these putative hematopoietic stem cells that were difficult to isolate-- and still haven't been isolated in their purity-- but you can titrate them down.

And you can introduce what you think is one stem cell, 10 stem cells, 100 stem cells, and so on, into an irradiated mouse, and ask how many stem cells does it take to repopulate the entire blood system and immune system. And it turns out, you have to mix these cells with carrier cells, otherwise it doesn't work. But it turns out that one cell can repopulate the entire hematopoietic system, which is really extraordinary, and led to the diagram that I showed you in the slide before.

OK. Here's another assay. This is an in-vitro induction assay. And the idea here is that you start off with something, which you think might be stem cells, by various criteria. And then to test what these cells can do, you put them into plastic tissue culture dishes, and you add some nutrients and so on to allow the cells to divide. And then you add some inducers.

And you remember a couple of lectures back, inducers are just ligands for various signaling systems. You might add fibroblast growth factor to this one, and retinoic acid to that one, and then you ask what happens to the cells. They will go on, in general, to differentiate into different cell types. And depending on what they differentiate into, you can say something about the potency of these putative stem cells. You can't test if they're really stem cells, but you can say something about their potency.

You can do a similar experiment, but in a whole mouse. The mouse is made from, the embryo is made from a part of the very early embryo called the inner cell mass. And you can inject labeled, putative stem cells into an early mouse embryo, into this inner cell mass part of the

embryo, put it into a mother, a recipient mother, and then ask what comes out, what kind of embryo comes out of that process.

And if you see that the baby that comes out of this chimeric embryo has got a green liver and green ears and green whiskers, you'll know that these cells that you put into the chimeric embryo, that you made the chimeric embryo with, had the capacity to give liver, ears, and whiskers. OK? So this is a powerful assay to, again, look at the potency of cells, not, in this case, the stem cellness of cells.

One of the things about stem cells is that you only want them to work when you want them to work. If you cut yourself, in the normal process of keeping your liver the right size, in the normal process of keeping your heart muscle correct, you want your stem cells to be working and keeping everything homeostatic. You don't want them to be dividing out of control, because then you'll get cancer.

And so something has to control what stem cells do and when they do it. And this is a question of regulation and the notion of a stem cell niche. Stem cells are kept quiescent, usually in G0 that we talked about in the cell cycle lecture. And they're kept quiescent by signals from the surrounding cells.

So their cell-cell interaction, and by signals from the surrounding cells. And those are given a special name by stem cell biologists. They're not that special, but they're given a special name. They're called niche cells, or the niche. OK? They're just surrounding cells that are secreting signals.

On some kind of activation-- you cut yourself, your organ normally needs repair-- things change. So on activation, by some kind of environmental input-- local or less local-- niche cells induce the stem cells to divide. And they do this-- and they induce the stem cells to divide into a stem cell plus a progenitor, which then goes on to do all the things that I diagrammed that progenitors do, on the first board. And the niche cells do this because they have changed their signaling.

This is yet another use, or a very related use, of the notion of cell-cell signaling in controlling life. Here's a diagram. Here are niche cells. You don't have this. Just look on the screen. Surrounding cells, maintaining stem cells quiet. When there's an environmental input, the niche cells change. They activate the stem cells to divide and form more stem cells and progenitor cells.

One really fantastic example of the niche, and the interaction between stem cells and the niche, is in the hair. This is from my colleague Elaine Fuchs at Rockefeller, who over many years has figured out that in the hair follicle-- this is the hair sticking out of the skin-- in the hair, there's a small group of cells on one side of the hair shaft called the bulge cells, and these bulge cells are the stem cells.

Her investigators isolated these bulge cells and did the following experiment to show that they were hair stem cells. There's a kind of a mouse called a nude mouse that has a very bad immune system. So it's useful for immune system experiments. But it also has no hair. And you can do a transplant into this mouse of these bulge cells. And you get little tufts of hair growing where the rest of the mouse is nude.

And you've done a careful experiment where you've labeled the cells you have transplanted in so that you can show that they actually came from the transplanted tissue and not from the mouse, the nude mouse tissue. And you can do that because you labeled them with GFP and they're green. So this the green hair. And it shows you that these bulge cells are stem cells.

During the life of a hair-- your hair has a life, we discussed four years on your head-- during the life of a hair, the bulge cells and the niche cells are in different places. And depending on whether they're touching or far apart from one another, there's induction of hair growth or not. So at a particular stage of the hair cycle-- this is called the hair cycle, look on the bottom of the screen here-- the bulge cells and a group of cells called the dermal papilla that lies right at the bottom of the hair shaft are touching one another.

And at that point, a particular signaling pathway called the Wnt pathway is activated. And these dermal papilla cells tell the bulge cells to start dividing and start making a new hair shaft. After that's happened, the bulge cells move away from the dermal papilla cells. Here they are during growth, the growth period. You see the bulge and the dermal papilla cells are no longer touching.

At this point, the stem cells become quiescent. And there are enough progenitor cells in the hair shaft to give you formation of the hair. And then the stem cells remain quiescent until the next hair cycle starts and they get in contact with the dermal papilla again and start making new hair. This is a really beautiful story that's shown us quite clearly how the niche cells can control these stem cells.

All right. So let's spend the last minutes talking about therapeutics. Here's the dream. You know, the dream is that you have a stem cell population for every organ in the body, including things like limbs, such that if your limb gets severed or your heart becomes really diseased or your spinal cord is injured and you can't walk anymore, that you can just inject into a patient the correct stem cells and everything gets repaired.

That's the dream. And that's really the holy grail of what thousands and thousands of investigators are going after. And it's given precedence by bone marrow transplants, which really are very successful. Turns out that it's kind of tough. It's tough because these adult stem cells are really rare. The hematopoietic stem cells are special because they're kind of liquid. They're single cells. They're not attached to anything. And they are relatively easy to identify.

But other stem cells are very difficult. So the idea is that you inject stem cells to repair a damaged organ. You need stem cells of the correct potency, otherwise you're not going to repair the specific organ. But adult stem cells are very rare.

And so the quest has been to find some kind of substitute for adult stem cells. And those substitutes come in two flavors. One are embryonic stem cells, abbreviated, ESCs. Embryonic stem cells are cells that grow from the inner cell mass of an early mammalian embryo. And you can grow from them groups of cells that will keep growing in the laboratory for a long time and have variable potency.

So the embryonic stem cells are derived from the inner cell mass of an early mammalian embryo-- human in the case of human. And you grow out so-called ESC lines, which means that these cells grow continuously in culture. And each embryonic stem cell line has a unique potency and can be used to do different things, in terms of theoretical repair.

If you look on your next handout, the idea is that you take this inner cell mass, you plate the cells as single cells, they grow and grow and grow. And if you treat them with various inducers, you can get them to become heart or neuron progenitors, inject those into animals and make them better. There are some issues with embryonic stem cells. And the problems are twofold.

One is ethical in that you have to harvest human embryos. You have to obtain and harvest human embryos to get these human stem cell lines. And currently, you're really not allowed to do much human embryo work to obtain human stem cells.

But the second, even if you were, is that these cells are non-autologous. They do not match

the person into which you're putting them. They do not match the immune system of the recipient. And so they'll be rejected. And it's the same as an organ transplant. You have lots of problems of rejection.

So the latest thing that is very exciting and wonderful and potentially might be very useful, is the use of things called IPS cells, in which you convert adult cells into stem cells. And you do so, as I'll tell you, by adding some transcription factors to them. The advantage of these is that they are self cells. You could do them from yourself.

The disadvantage is that they're really not proven. And there is still, I will just say, lots of problems. But there are a lot of people, including some of the top investigators in the world, working on these IPS cells.

So look at your last handout. We will remove our interesting calendar reminders there. Adult differentiated cells, which are unipotent, can be treated with three or four transcription factors. And finding these transcription factors is the key. And once you express these transcription factors in these adult cells, like magic, they become stem cells. It really was like magic.

You can test the potency of these stem cells in the same way we discussed. And Professor Yamanaka in Japan and Professor Jaenisch here have shown that these are really very powerful cells. And those are the promise of the future. And we'll stop there and meet on Friday.