

Good morning, class. Nice to see you here, you loyal holdouts, the stalwarts who haven't gone home early for Thanksgiving. You recall that last time we were talking about the Matevoidic system, and much of the rationale for studying it stems from two reasons. First of all, it recapitulates in a formal sense what happens during embryogenesis, i.e. one has relatively undifferentiated stem cells which are able to differentiate into a number of different directions by committing themselves to either the myeloid or lymphoid compartment, and then going down yet other pathways, more detailed pathways to generate a whole variety of cell types.

Secondly, we really understand the differentiation pathways of Matevoisis better than we understand any tissue in the body, in no small part because it's much easier to study the soluble cells in the blood and in the immune system than it is to study how these processes happen in normal tissues. But having said that, I want to emphasize the fact that in each of our tissues there are oligopotential stem cells. When I say oligopotential I mean they can go down several different pathways. Recall up there on that diagram we talked about pluripotential which means multiple, and today we're going to talk a bit about todipotential stem cells, which are able to disperse descendants into all the different differentiation lineages in the body.

At the end of our last lecture, we were focusing on the red blood cells. And this is sometimes called erythropoiesis, which is to say the process by which red blood cells are generated.

We mentioned the concept of homeostasis, and homeostasis just refers to the fact that all of these systems are in very delicate balance so that the body can respond to the physiologic needs of the organism at any one point in time. We talked about the fact that for example when there's a massive infection in the body, then the homeostatic mechanisms allow an increase in these kinds of immune cells in order to encounter the infection.

And at the end of our last lecture, we were talking about this specific branch, and how in fact homeostasis is maintained there.

And what we see here is a series of committed progenitors.

So when I talk about committed progenitors I'm referring to cells that have already made the commitment to go down one or another pathway. They're not yet fully differentiated.

As you can see here, we have first forming cells and colony forming cells. We don't need to remember all the different abbreviations except to say that these cells here are in a relative undifferentiated state. And the only end stage differentiation comes at the very end here when we get to red blood cells. We said in general that it's the case that most highly differentiated cells are post-mitotic, which is to say they're never going to reenter into the growth and division cycle of the cell that we talked about earlier in the semester.

And that's obviously dictated here by the fact that this erythrocyte lacks a nucleus, i.e. during the final stage of differentiation, in addition to accumulating large amounts of hemoglobin in its cytoplasm, this cell actually pops out its nucleus, and that obviously represents an irrevocable change in that cell can never again enter into growth and

division cycle. The immediate precursor of an erythrocyte is often called an erythroblast. And the term blast here refers to a cell of embryonic appearance. Blast is used often to indicate, we'll mention that again shortly, a cell which looks very primitive, and embryonic, and undifferentiated. And that ends up going into an erythrocyte, which we said is actually a synonym for a red blood cell, an RBC, a red blood cell.

And we talked about the fact that this progression is actually maintained and furthered by the stimulus of the compound called erythropoietin. So, we're using some of the same words over and over again. And erythropoietin is essentially a growth factor which stimulates the end stage differentiation of the erythroblast into the erythrocyte.

Epo, as erythropoietin's often abbreviated, is actually made in the kidneys. And it's made in the kidneys in response to the physiological stimulus of hypoxia. Hypoxia means inadequate oxygenation of the tissues. You might ask, well, why is red blood cell contractions controlled, as they are, in the kidney?

And the fact is, we don't really know why evolution has chosen the kidney as the site of monitoring the degree of oxygenation of the blood. And in response to hypoxia, it begins to crank out erythropoietin, or Epo. You can think of erythropoietin as an extracellular ligand just like a growth factor.

It has its own cognate receptor on the surface of the erythroblast, and when Epo released by the kidney hits an erythroblast in the context of the bone marrow, it actually has two effects.

It happens to be the case that roughly even 95% of the erythroblast that are made routinely are forced to go into apoptosis under routine conditions. So, this is an enormously wasteful system, i.e. as every moment we speak, 90 or 95% of the erythroblast that have come into existence in your bone marrow apoptose.

They never go into end stage differentiation.

But when Epo is around, Epo provides a strong anti-apoptotic signal to the red blood saves some and maybe even all of the erythroblasts from their normal fate of undergoing apoptosis.

So here, if we imagine there are actually two fates, one is to become an erythrocyte, and the other is to apoptose, where the apoptosis is paradoxically enough the dominant fate of the cell, the moment that an Epo comes on the scene, it blocks this alternative fate, allowing these cells to mature. Epo at the same time stimulates the erythroblast to differentiate. Now, you might ask yourself the question, why is there this enormously inefficient process?

An enormous effort is made to crank out large, astronomical numbers of erythroblasts, and yet most of them are wasted even before they've had a chance to undergo end stage differentiation.

And the rationale here is as follows. This is a terrific system for rapidly ramping up the level of red blood cells in your circulation because here, within a matter of a day or two, one can crank up, actually in a matter of hours, you can crank up the rate of production of red blood cells by maybe even a factor of ten.

Instead of having 90% of the erythroblast apoptose, let's say 0% of them do so, and therefore, instead of having 10% of the erythroblasts becoming red blood cells, 100% of them will do so.

And therefore, you have the virtually miraculous response that if you go from here high up in the rocky mountains at ten or 12,000 feet, within a matter of two or three days, your red blood cell concentration actually has compensated, has risen up to create the oxygen carrying capacity that enables you to deal with the thin oxygen, with the low oxygen tension that's present at high altitudes. Now, having said that, the fact is that there is an Epo receptor on the surface of the erythroblast, and what we see there is the following.

Let's talk about the erythroblast and just blow it up a little bit.

So, here's the erythroblast. That's the undifferentiated precursor. And by the way, the erythroblast is actually still a white blood cell. Often we call a white blood cell a leukocyte. You may know that gluco means white. So, a leukocyte, it's still white. And after the erythropoietin impinges on it, one of the things it starts doing is to make the hemoglobin, which turns it into a red blood cell.

At this stage, it's still white. On the surface of the erythroblast are these Epo receptors. I'll just abbreviate them like this, Epo receptor, and once it binds the ligand Epo just like the growth factor receptors, we talked early in the semester, the receptors signals are sent into the erythroblast to stimulate both differentiation and to prevent the initiation of the cell suicide program that we call apoptosis. Interestingly, one of the things that happens normally is the following, that when these signals come in, there is an enzyme called a phosphatase which is attracted to the receptor. The Epo receptor works like a tyrosine kinase growth factor receptor that we talked about earlier in the semester. And here, we have an enzyme, a phosphatase, which actually counteracts the function of the tyrosine kinases. So, after the Epo receptor has bound its ligand, here's the plasma membrane, it has a whole series of I'll draw Y here for tyrosine.

It has a whole series of phosphates attached to it because of the actions of tyrosine kinase enzymes that are associated with its cytoplasmic domain indirect analogy to what we talked about in the case of growth factor receptors. But, one of the things that happens is that this phosphatase, which removes phosphates, then gloms onto the receptor like this. It grabs hold of some of these tyrosine kinases. And what this phosphatase does is reach around. It reaches around and it begins to prune off all of these phosphates because that's what a phosphatase does.

It cuts away all the phosphates, thereby directly reversing the previous actions of the tyrosine kinase that led to the formation of these phosphates, and that in turn allows downstream signaling to occur. This is obviously a functional negative feedback loop, i.e. whenever there is an agonist you want an antagonist. Whenever there's a stimulus which is induced in the body, there has to be an inhibitory signal, and this is part of the whole issue of homeostasis, the balance between forward and backward. Interestingly enough, there's a family in Finland, I believe, which has a mutant receptor.

And their mutant receptor lacks this tyrosine.

And what happens as a consequence is that that particular tyrosine doesn't get phosphorylated. Because that tyrosine doesn't get phosphorylated, the phosphatase cannot be attracted to the receptor because there isn't a tyrosine there.

There's some other amino acid residue. I don't know what it is.

It's not important, but it's not a tyrosine. And this cannot happen because they don't have this tyrosine. This phosphatase could not be attracted to the receptor to shut it down as it normally would be. So normally homeostasis is imbalanced, and several members of this family have become Olympic cross-country ski winners. They've become Olympic champions.

Why? Because their Epo receptor's hyperactive. Because the Epo receptor's hyperactive, they have higher than normal levels of red blood cells in the circulation, and this clearly allows them to function better in cross country skiing, which as you know is a really physically demanding task.

Again, I'm not saying this is a good thing for them necessarily.

There are other things in life besides, believe it or not, winning cross country Olympic competitions because as I mentioned last time, having too many red blood cells in your circulation, there's a downside to it which is that you have a much greater tendency to have occlusions, to have blood clots in your circulation which obviously is not a very good thing to have.

Oh, so is there a threshold of Epo receptor activation before phosphatase shuts it down?

These things are not really well understood, are not well studied.

The fact is, you might be able to say we should make a mathematical model of all of these different circuitry. But the fact is if you want to make a mathematical model, you have to know some of the constants. You have to know some of the parameters, the binding constants. And in fact, for most of the signaling interactions, no one's ever

really studied them in such great detail. So, one really doesn't know how much phosphate you need here before the phosphatase becomes really active. And so, there's not a really good mathematical model of this feedback loop, even though we know without any doubt that it exists. So, I want to get into other issues that are related to the whole issue of accumulated differentiation traits as one moves down this pathway.

Again, we've used this as a model for how differentiation takes place in the entire body. The faith that's been implicit in this kind of scheme for the last 20 or 30 years is that this acquisition of different kinds of phenotypes is not accompanied by genetic changes, that is, in the genomes of these cells. I.e. one can accomplish these different kinds of differentiation not by rearranging genes but just by rearranging transcriptional programs, and that the DNA sequence of these cells as they proliferate and differentiate is fully unchanged. And that's a matter of faith because you could say to me, how do you know that it's really true. The fact is that people have looked at genes in many kinds of cell types, but it's essentially impossible, or it has been at least until recently, to preclude the possibility that as cells move down these differentiation pathways, they begin to change the nucleotide sequences of different ones of their genes. In fact, I've already told you about one instance where that's clearly the case. And that is in the differentiation of the B cells of the immune system, which happen to be right up here on this chart, because as you recall from our discussion vis-à-vis immunology, the B cells actually do rearrange their genes in order to cobble together DNA sequences that together are able to enable them to make antibodies that are able to react to specific antigens. So there, there's no doubt at all that there's a somatic rearrangement of the genes, somatic meaning it's not a germ line change. It's happening in the soma outside of the germ line. There's a somatic mutation.

It's not a mutation that's deleterious, but rather is directed towards a physiologically normal and desirable end point.

But for example, how do you know that when you remember things in the brain, part of the memory does not derive from changing the DNA sequence and different neurons in the brain?

What's the molecular basis of memory? Could it be that each time we learn some things that there are different nucleotide sequences, critical nucleotide sequences, that are changed in neurons in the brain, and that those nucleotide sequence changes represent an important basis for ensuring that memory is retained over decades of time. Or, rather than having genetic changes in the brain, might it all be epigenetic, i.e. all the other changes that happen to the cell besides changing DNA sequences in the chromosomal DNA.

So, here we're dealing with the dialectic between epigenetic and genetic. And, have we talked about DNA methylation here? Yes, so we talked about DNA methylation, and do you recall or having discussed the fact that when DNA gets methylated, that suppresses the transcription of a gene.

But that doesn't change the nucleotide sequence, and that methylation configuration of a gene can be passed to one cell generation to the next. It's heritable, but it's not genetic in the strictest sense of the term, i.e.

it doesn't involve a change in nucleotide sequence, which is what we want to limit this term to referring.

So, epigenetic can represent all the changes in the cell including DNA methylation, alterations in transcription, and all other downstream events that result in changes in the cell.

And how can one address this? Well, there are different ways of addressing this question or addressing the possibility that in fact there are changes in the nucleotide sequence of the gene.

One way to do this is the following. And that is to take cells from an early embryo, and here we see an early vertebrate embryo.

This looks really more like a frog embryo or a slightly different shape, and here we see an early embryo. It's after a blastula. It's called a blastocyst. Here again we have the word blast.

How about one question per lecture? We have to have some equity here.

Other people can ask questions. It's good to ask questions, but how about one per lecture; that's fair, equitable.

All right, so here's an early vertebrate embryo.

Here we see the blastocyst. This comes after the earlier stages in the embryo, and here we see the inner cell mass.

And as it turns out, the inner cell mass is going to be the precursor of many of the tissues of the ultimately arising embryo.

And here, one can do an interesting experiment. One can take cells out of the inner cell mass. And one can begin to propagate them in culture. And what one ends up with is embryonic stem cells.

And the intrinsic interest of embryonic stem cells is manifold.

For one thing, you can take embryonic stem cells and you can genetically alter them. You can put a new gene in, in the case of a mouse, or you can take another gene out.

And then what you can do is you can inject the genetically altered embryonic stem cell into the blastocyst of another embryo.

So let's say we take the cells out of the inner cell mass.

We develop embryonic stem cells. We can call them ES cells. That's what they're called in the trade, ES cells. We take them out. We can propagate them in culture. And then, what we can find is we'll put a genetic marker in those ES cells. Let's say we put in those embryonic stem cells the marker for the gene beta-galactosidase.

And beta-galactosidase in the presence of a proper indicator, if you put a proper indicator and make a cell turn blue.

So now we have an ES cell line that produces the beta-galactosidase enzyme. The beta-galactosidase enzyme beta-gal itself has no effect on the biology of the cells. It's only a marker. And now, we take those ES cells, and we inject them into another embryo, a wild type embryo that lacks this beta-gal marker.

And what we can see is that we inject the ES cells into this blastocyst. The injected ES cells will now insinuate themselves, will now intrude into the massive cells in this embryo into which we injected the ES cells, and they will become part of the entire embryo genesis that follows. I.e. soon these foreign ES cells will weasel their way into this inner cell mass.

And they will become established and become functionally equivalent to the inner cell mass cells that were resident there prior to this injection. And what you can do then is follow the subsequent fate of, in this case, a mouse. And what will happen often is that you can find blue cells all over the mouse sometimes in the paws, sometimes in the coat. Let's imagine that the hair would turn blue, which in fact is not the case. But let's imagine the hair would turn blue. So here's the mouse, happy because it's part of an important experiment.

And what you'll sometimes see is that, well, remember that art was not my forte. Anyhow, here you might see stripes of blue cells on the skin. The hair won't turn blue actually, but the skin may if you give it the proper indicator.

And what this indicates is that in this case, the cells that were injected into the blastocyst could become part of lineages which committed themselves to becoming skin cells.

Or, the cells in the brain might be blue. Or, the cells in the gut might be blue. Or under certain conditions, the cells in the intestine might be blue. In telling you that, I mean to indicate that the cells that we injected into this blastocyst, which carry beta-gal were totipotent.

They could create all the tissues of the mouse under the proper conditions. The proper conditions are obviously being put into this very special environment in which all kinds of differentiation inducing signals, which we don't really understand, can induce this cell to commit itself to enter into one or another differentiation lineage. And in

principal, you can make a whole organism out of an ES cell. ES cell has as much plasticity, as much flexibility, as a fertilized egg.

It has not yet lost the ability to make all the parts of the body.

On some occasions, the ES cell will even get into the gonads of the mouse, which are down here somewhere. And if that's so, if the ES cell which you injected has been able to seed the formation of these cells down here, then what will happen is that either the sperm or the egg coming from this mouse will now transmit the blue gene. And now, in the next generation, all of the mice will inherit the blue beta-galactosidase gene in all of their cells because now this will have entered into the germ line.

If these blue cells happen to colonize the testes, the ovary, or the testes, then these blue cells will become ancestors to the sperm or the egg. And now, in the next generation, mice will inherit a blue gene in all of their cells.

And now this mouse is really happy because it's now part of an extremely important experiment because now all of its cells will become blue, having inherited them as part of the oocyte which led to its formation. In this kind of an animal, we call this animal a kind of a chimera. Chimera is a mythical beast which is, let's say, half human and half horse or something like that. Or a chimera means it has genetically different parts in it. That is not to say that these parts carrying the blue gene are necessarily defective, they're just genetically different, one from the other. But they can participate in embryogenesis in a fashion that's indistinguishable from the non-blue cells. They just do everything they're supposed to do, and they pretend as if they were in this embryo from the get go, from the very beginning, from the moment of fertilization. So they are totipotent.

There's an alternative experiment you can do, and you can take the ES cells, and you can inject them under the skin of a mouse, let's say. So now, you're putting them in a very unfamiliar environment. And what you see then on many occasions is you can actually get a tumor. You can get what's called an embryonal carcinoma.

Now you'll say, well, so what? That's not so interesting. But it's very interesting. Why?

Because if you look at the genome of those embryonal carcinoma cells which we can call EC cells if you want, those cells are genetically full wild type. And yet, we're getting a tumor here.

So, it means that these cells, which have been placed in a fully unfamiliar environment under the skin or in the belly of a mouse will begin to form a tumor. And in fact, they represent the only type of cell that we know about where a cell having a wild type genome can actually give you a tumor.

As you sensed from our previous discussions, all other kinds of human cancer cells we know about have to have

mutant genes in order for them to grow as a malignancy. These cells are fully wild type and can grow as an embryonal carcinoma. They are very primitive. These cells have quite a bit of autonomy. They're not so responsive to all the growth factors that normally are required by many cells throughout the soma of an animal throughout the tissues.

So this allows us to begin to move on and ask other kinds of questions.

For example, you can take these embryonal carcinoma cells.

You put them in a Petri dish, and you can actually induce them to differentiate into different cell types in vitro.

How can you do that? Well, we're just beginning to learn how to do that. We don't really know how to do that.

But, if you give them the right cocktail of growth factors, they might begin to form muscle cells. If you give them another cocktail of growth factors, they might begin to give pancreatic eyelid cells that form insulin, or in this case cartilage cells.

And presumably, the cocktail of growth factors you're providing each one of these cells with in vitro, i.e. in the Petri dish, is mimicking the growth factor environment that each of these cell types is experiencing within the embryo. In other words, cells in different parts of the embryo experience different combinations of growth factors that persuade them to commit themselves to becoming these kind of cells, these kind of cells, and these kind of cells. And therefore, one of the promises of embryonic stem cell research is the possibility of being able to regenerate different kinds of tissues in a fashion that I just showed you here. But this whole experiment in the case of human beings is ethically extremely controversial.

Why? Because the experiment starts out making these ES cells here, and if we want to start out with an early embryo like this, start out with a blastocyst, in the case of a human blastocyst, this human blastocyst has the potential under the proper conditions of becoming a newborn human being. And therefore, we have this enormous ethical conflict in this country.

Is this blastocyst already a human being? Can you already afford to truncate the life of this blastocyst at this stage of development, and in so doing, are you actually extinguishing human life, or is this organism, if you want to call it that, already still much too primitive to consider it to be equal to human life?

And here, I would not, unlike my political views, be forward enough to venture an opinion because it's really something that no one really can argue about in any objective way.

It's all a matter of opinion. Is this a human being already, or is it simply an inanimate cluster, a clump of cells?

Now, in principal, how could we do this?

How could we actually create this kind of tissue therapy?

Because the fact is, as you get older, your tissues start falling apart. You haven't experienced that.

But I have. And the fact is that even if you try to stay in shape, things just start falling apart. And the older you get, the more they fall apart. Even people who eat well, which I do, and exercise well, which I don't, even they fall apart.

And so the question is, are there way of replacing and repairing tissue? And this would, in principal, represent one such strategy because it means that you could possibly inject replacement cells into an agent tissue and generate cells which could then restore and regeneration function which has somehow inevitably deteriorated over the decades. Well, that raises the question of how you can actually get a blastocyst, how you can make a blastocyst like this. To state an obvious thing which you might already have intuited, let's say you had such cells differentiated from various cell types that you want to inject into somebody's muscle or into their liver if they had diabetes and had lost their beta cells, or into their cartilage if they banged up their knee during basketball practice or something like that, or jogging, which is allegedly good for you.

Who knows? How could you deal with that? Well, the fact is, let's imagine there were such a blastocyst which we'd produce in this fashion that we differentiated like this.

OK, this is now the sequence of events. There's an important consideration we have to take into account, and that is if this blastocyst came from a different person than you, and we induced these cells to differentiate, and we injected those differentiation cells into your muscle, things wouldn't work. Why? Because these cells, if the blastocyst originated in a different person than yourself would be genetically different from you, and would be recognized as foreign tissue by your immune system. So even though you were getting an injection of cells which could regenerate your muscle perfectly well, those cells would never be given a chance to establish themselves and to thrive, and to reconstruct the tissue simple because the immune system would regard those cells as being foreigners and would go after them hammer and tongs trying to get rid of them in the same way it tries to get rid of all kinds of foreign invaders. I.e. the only way you could avoid it is if this blastocyst was genetically identical to you.

But how can you make a blastocyst which is genetically identical to you? Well, I'm glad I asked that question.

That's really the big challenge we have here because we don't want to create a situation where we have to restore somebody's tissues, but the only way we can restore them is to leave them immunosuppressed for the rest of their lives. When I say immunosuppressed I mean we have to prevent their immune system from attacking all of these cells that we've injected in them, these foreign cells, in the same way that we have to suppress the immune

system of any person who has received a graft from another individual including often bone marrow transplants. In all cases, we have at least for a while to prevent their immune system from attacking and eliminating these engrafted cells. And this is where the whole strategy comes for the whole process of cloning. You may recall the case of Dolly about five years ago, and let's remember what happened here because this would a momentous experiment in mammalian biology.

It asked the question, really, if you take cells from a somatic tissue, from here, or here, or here, are those cells, in principal, still totipotent, i.e. is the nucleus, is the genome of those cells totipotent, or has the genome, the chromosomal complement of cells in their cells undergone some kind of irrevocable, irreversible change, which precludes those cells from ever becoming totipotent? Well, in fact, if you take mammary epithelial cells from the breast of a human being or from the breast of a ewe and you put them into the blastocyst, nothing's going to happen. Those introduced mammary epithelial cells will not be able to establish themselves in the blastocyst.

And, we will not be able to insinuate themselves amidst the inner cell mass, and they will not be able to participate in embryogenesis. So therefore, the epigenetic program in these somatic cells seems to be irrevocably set to preclude the participation of the already differentiated mammary epithelial cells in subsequent embryogenesis. Therefore, you could not do this experiment all over again of introducing cells into the inner cell mass as I just described over here, injecting them into this.

But still, that doesn't answer the question. The issue is not whether the mammary epithelial cell is irrevocably committed to being a mammary epithelial cell. The issue: is its genome capable under the proper circumstances of becoming an early embryonic cell.

And therefore, what was done is the following. One took mammary epithelial cells, in this case from Dolly's quote unquote "mother, one prepared nuclei from these cells, taking them out of the cytoplasm, and then one got fertilized eggs or eggs that have been induced to become.

So here's an oocyte. An oocyte is an unfertilized egg.

In principle, you can activate an oocyte by putting a sperm in, or in fact it's actually better if you take the oocyte and you fool it into thinking it's become fertilized by treating it with different salts, high potassium concentration, and so forth.

And that will induce the egg to say I've been fertilized.

I better start embryogenesis. But what you do in this case is the following. The egg has its own haploid nucleus here, and you can take a little needle. And, you suck that nucleus right out of the egg. So, you've enucleated it.

That's what you've done, and now the egg is enucleate.

It doesn't have a nucleus in it. But keep in mind, much of what happens during early embryogenesis is programmed not only by the genes but by all array of cytoplasmic proteins that are present throughout the egg, and which play critical roles in determining the subsequent course of embryogenesis.

So now what you can do is you inject into this enucleate oocyte the nucleus of a mammary epithelial cell.

The mammary epithelial cell is obviously highly differentiated.

It's there to make milk. We'll call it an MEC if you want, and you put that in there, and under certain circumstances, and then you can treat this with a little bit of salt to mimic the physiological stimulus that comes after the sperm hits the egg.

And now this egg will think it's been fertilized.

And now it will begin to divide. But keep in mind, the genome of this quote unquote "unfertilized egg" has come not from the sperm and the preexisting nucleus of the egg. It's come because the nucleus has been injected from a mammary epithelial cell.

An experience over the last 30 years had indicated that this will never work. But finally somebody in Scotland, a man named Ian Wilmut tinkered enough with the conditions of these cells that he could actually get it to work not so often, maybe one, or two, or three times out of 100 tries. But on those conditions, this thing would begin to divide. The nucleus would begin to divide its diploid. Keep in mind that when a sperm comes into an egg, the egg is haploid. The sperm is haploid. Together they make a diploid genome. This introduced genome diploid, and the question is, the critical question is, can the genes in this introduced nucleus totally rearrange their transcriptional program so that even though these genes might all be intact in terms of nucleotide sequence, can the entire infinitely complex array of DNA associated proteins, i.e. the proteins that constitute the chromatin which is not only the histones but also the transcription factors, the TF's, can they all jump on and jump off as they should to mimic and replicate the spectrum of transcription factors that is normally present shortly after an egg is fertilized?

If they can do that, then this embryo can begin to replicate, and can ultimately develop into a complete embryo.

If they can't, then embryogenesis is going to be truncated shortly thereafter maybe at the two cell stage, at the four cell stage, at the 16 cell stage, but shortly thereafter, not because of the DNA sequences being defective, but because the spectrum of transcription factors is up and down regulates certain genes is in fact not been able to re-assort themselves in response to what?

Initially, in response to the signals coming from the cytoplasm because one might imagine, correctly so, that the nucleus in here is getting signals from the cytoplasm telling it, in effect, telling this nucleus, you should behave functionally as if you were the nucleus of a fertilized egg. In other words, the environment of proteins here is influencing the behavior of this nucleus. That goes backwards to our normal way of thinking because keep in mind our normal vectoral way of thinking is that the nucleus is influencing the cytoplasm.

That's the direction of information flow. But here, we're having a different situation. Here, the cytoplasm is telling this injected nucleus, well, you used to be a mammary epithelial cell nucleus, but now you've got to take on a different job. And we're going to force you to do so. And to the extent that happens, then in principle, one can end up having a normal embryo.

And, it happened actually on rare occasion that this worked.

Here they used actual electrical stimulus rather than salt to get the nucleus to divide. This electrical stimulus, again, was to mimic the stimulus that the sperm entering the egg normally provides, thereby activating the egg and forcing the entire fertilized egg to proliferate.

And so, once this starts developing, let's say, the blastocyst stage, here we have a blastocyst. You can see the inner cell mass once again here. This can be transferred into a pseudo-pregnant ewe. Pseudo-pregnant means you take a female ewe and you inject it with a series of hormones that persuade her reproductive system including prolactin, and progesterone, or estrogen, persuade her reproductive system, her uterus, that she's pregnant. You inject this early embryo into her, and this early embryo will then implant into the wall of her uterus and begin to develop. And if it all works well, you get a Dolly is born. You get a new sheep coming out of this.

It doesn't work so often, one, two, three, four times after out of a hundred, and very often in the great majority of cases, there are mis-births, mis-carriages, which happen in the middle of embryogenesis. So, almost in the great majority of cases, this fails. Somehow, the reprogramming of this nucleus, which is what we're talking about, reprogramming it in terms of its transcriptional program, goes awry. And therefore, bad things happen. The fact that on a rare occasion gets and succeeds here already is extremely interesting because it proves irrevocably that the genome of a mammary epithelial cell is in principle competent to program entire embryonic development.

And that means that during the development of Dolly's mother, we'll put her up here, as she developed from one cell into 1, 00 or 10,000 billion cells, as that development occurred the DNA sequences that went from the fertilized egg to her didn't really change. I.e. the DNA sequences that were in one of her mammary epithelial cells were intact, and as capable in principle of launching the full-fledged development as would be a fertilized egg. And that is one of the proofs, by the way, that in fact differentiation does not involve, with some rare exceptions, alterations in DNA sequence.

This, in turn, ends up being connected with the whole issue of embryonic stem cells. Let's say that I wanted to have my muscles regenerated, although they're still pretty good.

So, I take a skin cell of mine, and I inject the skin cell.

I take the nucleus out, and I inject it into an oocyte.

And then I let the oocyte develop up to this stage.

And I don't put the oocyte back into a sheep or another woman, although I could in principle. I actually take the cells out of the inner cell mass. Those are ES cells, and I begin to use them to regenerate my muscles to do this strategy. So, the cells are, in this case, not used for reproductive cloning, which is what this is here.

They're used for therapeutic cloning, where instead of taking these cells and the ES cells and allowing them to form a whole embryo, they're used to form a cell line of ES cells from the blastocyst from the inner cell mass. What we talked about before, here you see the blastocyst with the inner cell mass here.

You see it again. But now, rather than allowing this blastocyst to continue development, we simply extract cells from it and again create ES cells. I could create therefore in principle, ES cells, which are genetically identical to all the cells in my body, and any one of you could as well.

And here, there's not only one, but there's two ethical complications.

First of all, here we're starting human life with the intent of truncating it very early, and secondly, where are the oocytes going to come from? Well, you could say you can get them from some women, but producing oocytes from a human female isn't so easy. You have to inject her with all kinds of stimulatory hormones, choriogonadotropin hormones. It's an unpleasant procedure. Usually women are paid \$5,000 or \$10,000 to produce some oocytes. Well, you say, that's OK, but is that OK? I don't know.

Is it OK to pay a woman to donate her oocytes to make herself into an oocyte factory? I don't know. You have to judge.

I think there's arguments both for and against it.

Clearly, any one of us would be extraordinarily naïve if we thought that this was a procedure which had no ethical encumbrances in it.

And, you have to think about them for yourself. Still, the potentials are enormous, and therefore the question exists.

Will there be ways in the future of taking differentiated cells from one's tissue, and in fact using them in these ways to make ES cells without having to go through an oocyte, and without having the potential of creating human life. The alternative to this has been to do the following, to go into our normal tissues and pull out adult stem cells. What do I mean by adult stem cells?

These are not stem cells that are totipotent. These are stem cells which are in my muscles and regenerating muscle mass, which happens believe it or not. These are stem cells which might be in my skin and are continually regenerating skin cells.

Keep in mind that in the maintenance of all our normal tissues there are stem cells whose configuration can formally be depicted like this with the transit amplifying cells we talked about before.

And maybe, if one took the stem cells out of an adult tissue right here, if we had a way of extracting them, those could be propagated in vitro, and then injected back in. Those are so-called adult stem cells. And the individuals who are against this kind of manipulation of human embryos and so forth say that adult stem cells are really the solution. You take stem cells out of a person's tissue, you expand them. Ex vivo means out of the body, in vitro, and then you use them. You inject them into somebody's tissue to regenerate their tissue.

There's only one problem with that. It's ethically far less encumbered obviously, but it doesn't work that well. In fact, some people think it hardly works at all, that the exceptions are really rather far and few between. And so, this issue will long be or continue to be debated. But it obviously represents a very new and exciting area of biomedical research. And interestingly enough, it impinges as well in a fully unexpected way on cancer because this whole paradigm of stem cells, it turns out, also applies to cancer cells. If you were to have asked me two or three years ago, what did the cells in the tumor look like? I would draw a picture like this, that these are a series of exponentially growing cells so that all the cancer cells, all the neoplastic cells in the tumor mass are biologically equivalent to one another. They all have the same mutant genome, and they all are capable of multiplying exponentially.

But it turns out that work in the Matavoidic system on Matevoidic tumors like leukemias, and now on breast cancers, yields a very different results, because it turns out that the way that the tumors are organized looks like this. The tumors also are organized in this hierarchical array just like normal tissue.

How do we know that? Again, I'm glad I asked that question.

Because if you take these cells out of the tumor and put them in another mouse, let's say, you get a new tumor.

These cells are tumorigenic, I.e. they concede a new tumor.

If you take these cells out of the tumor, they have the same mutant genome. They constitute the bulk, the vast mass of the cancer cells in a tumor. You put these into a mouse, and they're non-tumorigenic.

And, in some kinds of tumors, the tumorigenic cells can represent only 1 or 2% of the total mass of cancer cells in the tumor.

And from this, we begin to realize that you look inside tumors: the tumors deviate minimally from the organization of normal tissue. They also depend on self-renewing stem cells which can make transit amplifying cells and can give end stage cells, which although they're neoplastic, have many of the differentiated characteristics of the normal tissue from which they arose. And this has enormous implications for, for example, therapies against tumors.

If you ask somebody, how do you develop and how you judge the success of an anticancer treatment? You talk to somebody like from the pharmaceutical industry. And let's say that's easy.

If you have a new drug, and that drug reduces the mass of a tumor by 50%, that means that you've done something really good.

But let's look what's going on here. If these cells are 99% of the tumor in terms of the mass and these cells are 1% of the tumor, let's say you've invented a new drug which wipes out all of these cells but doesn't touch these cells. The bulk of the tumor has shrunk and everybody will say, eureka, we've succeeded in curing cancer. But keep in mind that the self-renewing capacity of the tumor rests in these cells. And if these cells are allowed to survive, then they'll start proliferating again and regenerate the entire tumor mass. And you won't really know that you had any success because these cells look like all the other tumor cells under the microscope. But biologically, they're very different. And therefore, the future of cancer therapy, and it will take five or ten years to do this, has to begin to focus on getting rid of these self-renewing stem cells which create this enormous regenerative capacity on the part of tumors.

See you next Monday. Have a great vacation.

Eat much turkey, and get some exercise, and don't smoke.