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7.012 Introduction to Biology, Fall 2004
Transcript – Lecture 19

Good morning, class. Nice to be here with you again after a long hiatus. So, we're going to talk today about the cell cycle. That is to say the life of a cell, how a cell grows and divides. And, as it turns out, this is one of our first entrees into cell biology. That is to say we're move, beginning to move away from the molecules inside cells and beginning to look at a, at the next higher level of complexity which is how cells proliferate.

It turns out this is a very critical issue in a number of different disease areas, including notably cancer which we'll start talking about next time. And this is also the transition point, the inflection point in the semester when we begin to apply some of the things we've learned earlier to learning about specific disease processes. So, you'll sense more and more introduction of discussions of human disease processes as the semester goes from here on to the end.

And here, in fact, is what we call the cell cycle. And I'm going to get into the details of it quite shortly, but the cell cycle is effectively the life cycle of a cell from the moment it is born to the moment that it, in turn, becomes the mother of two daughter cells. And this process is divided up, as you can see, into four different periods here, or as they are called phases. These are cell cycle phases. The first phase is called M or mitosis.

The second phase is called G1 phase. The third is called the S or the synthetic phase, and that refers to the fact that this is the time when cells replicate their DNA. And, as you can see, it actually takes a long time because this entire cycle in cultured mammalian cells normally takes as much as a day for a mammalian cell to divide, grow and divide. Contrast that, by the way, with many bacterial cells which can grow and divide in 30, 30 minutes.

So, our cells take a lot longer to divide, many of them, but there are exceptions to that. After cells have gone through G, through S phase they go into G2. And these two Gs are, are called Gs because of the gaps they have. Here's the gap between S phase and M phase, or

mitosis. G1 is the gap between mitosis and, and the, the subsequent S phase. And, obviously, the most important parts of the cell cycle are the time when the cell divides, which is mitosis, and the time it replicates its DNA which is in S phase.

One reason why it's so important is that one has to be sure that cells that arise from the process of growth and division, which is what this is, actually end up with a full genome's worth of DNA and chromosomes. And, therefore, during S phase there has to be a precise replication of all the 3.2 billion bases in the haploid genome, or 6.4 billion in the diploid genome.

And during M phase there has to be a precise allocation of the resulting replicated DNA in equal, exactly equal parts to the two daughter cells. And this is no mean feat. It is an extraordinarily challenging process, which cells, it turns out, with very high efficiency through a variety of complex control mechanisms. And with this outline in mind, I just want to go into some of its details to illustrate to you why we think that the scheme is organized the way it is.

The fact of the matter is that the, that the M phase, or the mitosis is actually organized into a series of sub phases. And you may remember that from high school biology but I'll just go into it in some detail, not because we're going to dwell on it but just because I want to give you a feeling for what it's composed of. It's composed of a series of, of sub, sub phases I just mentioned. And, importantly, we start out with an interphase cell.

And interphase refers to the entirety of the cell cycle outside of M. That is the entirety, entirety outside of the M phase thereby including G1S and G2. And you notice that what happens in prophase is, first of all, the chromosomes condense. And that's very important because prior to that condensation the chromatin, and chromatin represents the material out of which the chromosomes is assembled consisting largely of DNA and proteins with a big of RNA, the chromatin is dispersed through the nucleus.

It's not condensed together. And as such, as you can see here, one doesn't see any specific chromosomes because they're just scattered about the whole chromosomes in a very dilute form. But once the condensations happen, the condensation of chromosomes happen then one begins to appreciate that there are chromosomes. Indeed, if it were not for the case that this, this condensation would happen, it would have taken a very long time before we even realized at the

beginning of the 20th century that there were distinct chromosomes themselves.

Because when you look in the nucleus like this, and as you, one might do, be doing here stains for DNA, one doesn't see any distinct structures within the nucleus. Here this condensation causes a delineation of these structures in humans, as you know this being 46. And, by the way, this, this, this sequence of events is, is basically standard hardware for all eukaryote cells.

It's not as if we invented this recently. This whole sequence of events has largely been around for 1.5 billion years. One thing you begin to notice here already as we go into the Metaphase, this is the beginning of metaphase, it's sometimes called prometaphase if one wants to split it up even further, is the beginning of the disappearance of the nuclear membrane. So, here we are in a situation where the chromosomes are no longer physically separated from the cytoplasm by the nuclear membrane.

The membrane, in effect, disappears. It dissolves. Obviously, at another point it's going to have to reassemble when one starts making daughter cells. And at this point one begins to see quite distinctly the, that, that each chromosome is composed of two chromatids. A chromatid is each one of these arms. And the fact that there are two of them is a direct consequence of the previous DNA replication which made from one chromatid two chromatids.

So, these two chromatids are identical, one to the other. They're the consequence of the previous S phase of, the previous DNA replication. And if all things have gone well then these two chromatids have identical DNA sequences because they're the descendants of a single chromatid that existed, actually a chromosome as it's called at that point, existed at the beginning of S phase. In fact, the fidelity of DNA replication, that is to say the accuracy with which DNA is replicated is so good that often one can demonstrate that the mistakes that are made are less than one in ten to the ninth bases.

That's stunning. One in ten to the ninth, only one in ten to the ninth bases actually is, ends up being miscopied. In fact, we now realize that the reason why there's such enormous fidelity is that the DNA polymerases that previously begin to replicate the DNA to generate these chromatids make a mistake of about one in ten to the minus five bases.

So, they're much more error prone. But there are subsequent proofreading mechanisms. Just the way you would copy edit text, there are proofreading mechanism which erase those errors and reduce the end result of miscopying, of misreplication down in one, down to one and ten to the minus ninth. And given that the genome itself is only, the haploid genome is only 3.2 billion, that is 3.2 times ten to the ninth basis of DNA, that means that there are only about, roughly speaking, three mistakes made in the entire genome each time a cell goes through a cell cycle in the haploid genome.

Now, what we begin to notice here in prometaphase is also the beginning of the assembly of the machinery that will eventually divide those two chromatids from one another. And here, in the midst of metaphase, as you see in the top left, now the chromosomes line up in what is effectively a two-dimensional plane in the middle.

It's called an equatorial plane in the middle of the cell. Where, as you can see here in this schematic, each chromosome is, here's once again the two chromatids, and the places where these two chromatids are held together are lined up in this plane with the arms of the chromosomes kind of flopping around, as you can see here. What's critical here is the points where these, these two chromatids are joined.

And those points of joining, as I'll mention in a moment, those are called centromeres. Here is a more schematized view of what's about to happen, just to give you a feeling for what this really looks like. And here, as, as we begin to move later into the mitosis, we begin to see that each of the chromatids is joined here by a centromere and that there are mitotic spindles which are reaching from these paired chromosomes to centrioles at either side of the cell.

So, these two centrioles, and the whole apparatus itself is called a centrosome, have microtubules that reach from the centromeres here in the chromosomes, so the structure in the middle of the chromosome is called a centromere.

Via microtubules that reach all the way over to one pole or the other. So, you see, one effectively has two poles here. And if we begin to develop this further, we, we've just gotten into serious metaphase here. And now what we see in anaphase is that the two chromatids are being separated apart, they're being pulled apart by these microtubule spindles.

These are called spindle fibers, which reach from each of those centrosomes over here back to the chromosomes, and literally are involved in the mechanical pulling them apart, one from the other. And to state the obvious, if we're talking about the fidelity of DNA replication, here we need also to talk about the fact that when one has a set of paired chromatids, one of the chromatids has to go to one side and one to the other. That, in itself, is in principle also an error-prone mechanism, or an error-prone process.

Mistakes could be made. And indeed in cancer cells where many of these processes break down sometimes one has both chromatids going to one or the other side. And as you can intuit from that, that leads obviously to a disruption of the normal makeup of the chromosomes that are eventually allocated to each of the two daughter cells. In fact, this allocation happens here at telophase, the last of the four major sub phases of mitosis.

Here you see that what's happened is that the, these chromatids have now been pulled apart. And the moment that they're pulled apart and no longer paired up one with the other they now are recognized, once again, to be card-carrying chromosomes. So, now here, here you see the nomenclature has changed. Now they're chromosomes. They're being pulled apart. And, obviously, one wants an identical set of chromosomes in both daughter cells. And once they're allocated to the two future daughter cells the entire mitotic spindle dissolve.

And, as you can imagine, there is a reformation, as is indicated here schematically and not very clearly, in the nuclear membrane. So now the whole cell is reconstructed and now each of the two daughter cells is able, in principle, to split off one from the other and to go off on its merry way. And this looks like a really neat process. And in some eukaryotic cells it happens much more rapidly than the 24 hours that I just indicated to you.

There are indications that in some lymphocyte populations it only takes three or four hours. And in some early embryonic cell populations, where there's an absolutely frenetic pace of cell growth and division, it may happen every 30 minutes rather than the 24 hours I just talked about. And you'll say, so what, that, who cares about that. But 30 minutes for replicating an entire 6.4 billion bases of DNA and then allocating them to two daughter cells is actually quite an achievement.

And we don't really understand how that happens. I've talked implicitly about the process of cell growth and division. And what I mean to say in more detail about that is the following. After one gets these two initial daughter cells they're obviously only half the size of the previously existing mother cell. And, therefore, what must happen in the subsequent cell cycle is that each of these daughter cells must actually physically grow in size so that it once again becomes a big mama over here after the next cell cycle.

And so that growth begins immediately. And much of it occurs just through the generation of new sets of ribosomes, new proteins, new membranes in the cell. All of the complex constituents of a cell need to be duplicated for the next cell cycle including, obviously, all of the organelles, the small structures in the cytoplasm and including, indeed, the mitochondria.

So, imagine now the complexity of this because not only is the eukaryotic cell so complex but its entire contents must be faithfully replicated during the subsequent cell cycle. And that obviously implies the intervention, the involvement of very complex control mechanisms about which we understand almost nothing. Now, how, let's get back to this for a moment, because what I've been talking about over the last six to eight minutes is simply mitosis, the M phase here.

In fact, in many of these early embryonic cell cycles, what happens is that cells go from M phase directly into S phase and from S phase directly into M. In other words, they don't indulge themselves in a period over here, a long period, and in some cultured mammalian cells this can be 12 or 14 hours of getting set, getting prepared for DNA replication.

Similarly, after cells have replicated their DNA, and as we just said, for example, converted one chromosome into two paired chromatids, after they get through S phase they'll also wait another four or five hours, most cultured mammalian cells before they go back into M phase, these two gap periods. Well, how do we actually know how long each of these gaps takes? Well, as is usual, I'm glad I asked that question. And one way we can do so is the following.

What we can do is we can take cells and we can block them in mitosis. How can we block them in mitosis? We can use a microtubule antagonist. So, I told you before that the spindle fibers that pull the chromosomes apart are made of microtubules. Microtubules happen to be one of the constituents of the cytoskeleton that gives structure

to the cell, and these microtubules are used as the fibers for pulling apart the two chromatids during anaphase, as I just mentioned.

And we can use a microtubule antagonist. And in biochemistry, when you want to antagonize something often you use a symbol like this. And, for instance, here's a drug called colcemide, and colcemide is a microtubule antagonist. And even though it has a bit of an effect on the overall cytoskeleton, if you put colcemide on cells then they'll go into mitosis like this and they'll go into metaphase, as we indicated before, but the subsequent sub phases of mitosis will, will grind to a halt.

Why? Because the microtubule spindle fibers will be unable to assemble properly because of this, now, I forget whether there's an E here or not. Probably not. OK. Typo. The colcemid prevents the spindle fibers from forming, so therefore cells get hung up, they get blocked in M phase.

In fact, if you're interested in looking at the, at the array of chromosomes in a cell, the simplest way of doing it with a mammalian, with a human cell is to put colcemid in it, and the colcemid will block them in M phase, they'll block them in metaphase, they can't get out of metaphase simply because these pink fibers here are unable to assemble properly, and therefore they get hung up right here while the chromosomes are condensed.

And this allows you to visualize the chromosomes under the microscope. Why do need to do that? Well, in the absence of blocking cells and trapping them in metaphase, cells will just spin right around the cell cycle and they'll only be in metaphase for maybe only 10 or 15 minutes out of the 24 hours. And, therefore, in a population of cells which is moving around the cell cycle willy-nilly very rapidly, only a minute fraction of the cells at any one point in time will, by chance, be in metaphase when you want to study their chromosomes.

So, the best thing to do is to try to block them in metaphase. Now, what you can do, in fact, is put cells in metaphase, and I'm going to draw the cell cycle around here again. Here's M, here's S phase, slightly different than what we just saw before, here's G1, and here is G2. So, now we're going to block cells with colcemid right here in metaphase.

And now what happens is that we put metaphase in. Ideally, if we put it in for a day we'd start out with a population of cells which we will

call asynchronous. Asynchronous means that at any one point in time these cells will be distributed randomly around the cell cycle. They'll be all over the place, obviously, because there's nothing to cause them to move synchronously through the cell cycle.

A random population of cells will be scattered all over the cells cycle like this. We put in colcemid, and what's gradually going to happen is that cells are moving around the cell cycle and when they get to here they'll start piling up. In principle, if all the cells are scattered around the cell cycle at the moment you put in the colcemid, and it takes them 24 hours to get all the way around the cell cycle, then after a day's worth of colcemid treatment all the cells should have piled up right here. In fact, you can begin to look.

What's the kinetics with which cells pile up into the M phase? And it looks like this. They go around, they go on piling up, and then at the end of 24 hours they plateau. Why do they plateau at 24 hours? Because by 24 hours the stragglers, which started out right here, will have had time to go all the way around the cell cycle and get trapped in M phase. And that gives you a feeling for how long the entire cell cycle time is, now, or this cycle which I called growth and division.

And, again, to emphasize, growth is literally the physical growth of the cell. Division is the production of two daughter cells at mitosis. Now, in principle, we can relieve this metaphase block by getting rid of the colcemid. And now, interestingly enough, we have a population of cells that is emerging from metaphase synchronously. What do I mean by that? I mean to indicate that they're all moving in lockstep.

They're all moving at the same time out of the cell cycle, so now there's a whole cohort of cells which starts out right here. They've all left metaphase at the same time. This is not an asynchronous culture. And they start moving around, advancing around the cell cycle together. In principle you'd say, well, they should remain synchronous forever. But the fact is all of these phases of the cell cycle, the times are quasi-stochastic, sometimes go, some cells go through G1 in ten hours, some cells go through cells in eleven hours, some through in eight hours.

And, as a consequence, as they move further and further around the cell cycle, the population of cells becomes progressively more asynchronous and they won't really enter into the next M phase totally synchronously. But now we can ask the following question, how long is G1? And the way we can do that is to do another kind of

experiment where what we do is we release cells, a population of cells from M phase by removing the colcemid, for example.

And now we treat cells, we, we treat aliquots of this synchronously advancing population. Each hour we pluck out some of these cells and we expose them for, let's say, the next hour thereafter to some tritiated thymidine. Tritiated thymidine, as you may recall, H3 thymidine as it's sometimes denoted, is obviously a radiolabeled precursor of DNA.

And it's going to get incorporated into the DNA and, and to no other macromolecules in the cell. And so the question is if we expose cells for an hour here to tritiated thymidine, how much tritiated thymidine is going to be incorporated into their DNA? How do we know what's incorporated and how much tritiated thymidine remains unincorporated? We extract the DNA from the cells and we precipitate it in an acid.

And when that happens the macromolecules of DNA go to the bottom, they precipitate, and the unincorporated tritiated thymidine, which is still soluble, it has not yet been polymerized into DNA molecules, remains in the acid solution. So, we asked how much acid-precipitable thymidine counts are there here? How many in the next, are made in the next hour with another aliquot of cells? How many in the next hour and so forth? And when we do such an experiment we find that we get a, a curve which looks like this.

All of a sudden, here we've got no cells labeling, in the second hour none, third, fourth, fifth, sixth, in the seventh hour there are some cells which make DNA, in the eighth hour there are some cells, even more cells making DNA, and by the time they're in the ninth or the tenth hour then we find a high rate of DNA synthesis. Now, in principle we could continue this experiment if we wanted to, if we had enough money for tritiated thymidine, which one does because it's very cheap.

And what we would find is if we started labeling over here with, for another hour pulse, when I say an hour pulse I mean we just put the tritiated thymidine in at the beginning of this one-hour period way over here, and an hour later we take out the cells and extract their DNA and measure how much DNA has been incorporated in that interval. And if we did a tritiated thymidine pulse over here, we'd begin to see that the rate of DNA synthesis was actually lower, and

eventually it would go down, back down to this as the cells were moving asynchronously through the culture.

What does that mean? Well, this is the time, obviously these cells are in S phase, these cells were in G1, and these cells have now emerged from S phase into G2 and are no longer making DNA. And on that basis we can actually calculate, we can determine how many hours it takes for cells to move through G1. We can do a similar kind of experiment to figure out how long G2 is, the gap 2, G2 phase is.

G2, recall, is the time between the ending of DNA replication and the beginning of mitosis. And how do we do that? Well, we can do an inhibitor of DNA synthesis. So, let's say, here is, I'm going to redraw the cell cycle. Here's S phase, G2, I'm drawing it again, here's M, and here's G1.

And now what we can do is we'll add a DNA synthesis inhibitor. An inhibitor of DNA synthesis has no effect, as you could imagine, on M phase. This is one of those things, it's called hydroxyurea. It actually blocks the biosynthesis of the precursors, the, the oxyribonucleoside triphosphate precursors of DNA. And, therefore, you add hydroxyurea to cells and they, their DNA replication grinds to a halt.

OK. So, let's do that. And we'll, we'll, we'll add hydroxyurea to the cells for 24 hours. What's going to happen? What will the distribution of cells be afterwards? Well, in fact, some of the cells, when we first added the hydroxyurea the cells were scattered all around, they were asynchronous. We'll add the hydroxyurea and what we'll find is the following. The cells that were in the middle of S phase when we added the hydroxyurea will be stuck dead in the water. They won't be able to move anymore. So, these cells that were in S phase, the moment we add the hydroxyurea will be trapped right here.

They cannot make anymore DNA. And, therefore, they'll be frozen at many points, times, points in time in S phase. The cells that are outside of S phase, they can continue to advance all the way around the cell cycle. And, accordingly, there'll be lots of cells over the next 24 hours that are going to just pile up at the G1-S transition. Why?

Well, the hydroxyurea has no effect at all on these other points of the cell cycle. And when these cells try to go, go from G1 into S, they cannot get into S phase because they can't make any DNA so they're trapped right over there. And now we'll take away the hydroxyurea,

which is, obviously is, to use the notation I used before, an inhibitor of DNA synthesis. And what we can do now is the following.

We're going to ask, after we take out the hydroxyurea we're going to put in colcemid. Why do we want to do that? Well, what we really want to do is to ask, after the cells have reached, have gone through S phase, how soon does a labeled cell move from S phase all the way into M phase? How long does it take? How long is G2? And so what we'll do is the following. We take away the hydroxyurea. This allows all these cells to begin to move out.

Is this a fully synchronous culture? Well, actually, no, because some of these cells are over here. There's a whole bunch over here. These are synchronous, but the rest of the ones they're already scattered out, so there's going to be some pioneers over here moving ahead of the phalanx, and they'll be some stragglers and then there's going to be a big slug of these cells that are moving as the rear guard ahead. Now, what we're going to do, after we take away the hydroxyurea we're going to add our old friend colcemid.

And colcemid, I tried to spell it right this time, colcemid is going to block cells, as we obviously said before, right over here. And then what we're going to do is the following. We're going to look, every hour we're going to take some cells and put them in colcemid, or we'll leave them in colcemid the whole time and every hour we'll take cells out of the Petri dish, which have been in colcemid since they were released from here, and we're going to look at the metaphase cells. And how do we look at the metaphase cells?

Well, here's a metaphase cell. Here will be its chromosomes. Obviously, they're chromatids at this point. They're under the microscope. And they start accumulating up here. And each and every hour we're going to take some of these metaphase cells, we'll take them out of the Petri dish, and after we've put them in, or we'll leave them in the Petri dish if you want, and after they're there and they've, they've come over here we'll fix them onto the plate.

We'll add some alcohol or something which causes them to stick to the plate, they can't swim away, and then after that we'll add some radioactive emulsion. So, here's, let's imagine here is a cell at the bottom of the plate in metaphase, we're going to add some photographic emulsion on top of that like that. And then what we're going to see is how soon we can detect radioactive metaphase chromosomes. How can we do that? Because each time an electron

leaves the tritiated thymidine, each time a beta particle leaves it's going to cause a grain of emulsion, of silver to form in the, in the photographic emulsion that we've layered above the cell.

So, this photo emulsion is only not a way of detecting light but a way of detecting when there, whenever there's radioactivity in, that's being emitted. And what we're going to look for are grains, silver grains that are located above the chromosomes in the microscope.

We could look down through this plate, through the emulsion, on the chromosomes we can see them clearly. I've shown you that before, and we're going to ask ourselves the question, when can we begin to associate silver grains with the chromosomes? Because those silver grains must have been incorporated into the chromosomes during the previous S phase, that is when cells were over here.

If a cell was over here and we added colcemid and there won't be any, it won't be radioactively labeled. So, what we're really going to ask now is the following. These cells are all advancing through like this, they're advancing into M phase, when does the first radiolabeled cell get, after we release them with hydroxyurea, when does it get, when do we first see cells like this? And the fact is after five or six hours after we've released them from S phase then we begin to see cells like this which have chromosomes on which there is radio, with which there's radioactivity associated.

Well, keep in mind that when cells move through the S phase here, this is when they incorporated tritiated thymidine to their DNA. Cells can't incorporate tritiated thymidine here. They can't incorporate it here. They can't incorporate it here.

OK? Right. So, we can allow cells to incorporate a little bit of tritiated thymidine, we can freeze them in here, we'll allow, give them a little bit, bit of tritiated thymidine, let it get into the DNA here, and then we'll add hydroxyurea very shortly thereafter and freeze the cells right like this. So, they'll be, they'll be radioactive by virtue of having dwelled here at different times in the S phase. And when we add hydroxyurea then, the cells that were already in S phase and incorporated a bit of tritiated thymidine will remain in S phase.

All of the other cells which were, when we added the hydroxyurea over here, they will go all the way around here. They will move all the way around the cell cycle. They might have happened to incorporate a little bit of tritiated thymidine before we added the hydroxyurea, but

they'll get trapped over here. So the only, so the cells that have tritiated thymidine, they will be scattered at various points in the S phase. We'll remove the hydroxyurea that allows the cells to escape from S phase and move all the way around to here.

And then we begin to look for seeing when cells get radiolabeled chromosomes. Is that clearer now? A little clear. OK. Now, let's begin to ask how this is all coordinated. What determines how cells, when cells will grow and when cells with not grow? So, let's go back to our depiction of the cell cycle.

How do, what controls all this? What determines when a cell is going to move around the cell cycle? In our bodies we have roughly three times ten to the thirteenth cells. I don't know if that's a number I mentioned to you before. It's a pretty interesting number. It's probably not the most interesting number.

By now far more people are interested in the number of how many games the Red Sox have won or lost, but it's a number anyhow. How many cell divisions do we go through in a lifetime? Did we ever talk about that? How many times do we have a cell growth and division cycle in a human lifetime, lifetime? And the answer is in human lifetime there are about ten to the sixth cell divisions. It's a staggering number.

Ten to the sixteenth in one human body in a human lifetime. And if you figure out what that amounts to, I think it's something like, and you can figure it out yourself, I think it amounts to something like ten to the seventh cell divisions in each second. So, each time I'm talking with you I'm going, I've already gone through any one of my sentences. And by the time my longwinded sentence is finished, I've probably already had 100 million mitoses happening inside of me. That's --

Maybe even more because some of my sentences are really long. So, imagine that, ten to the seventh for a second. You can figure out the number. This is a lifetime of let's say you live 70 years, you can do the math of how many it is per second. A lot of that's going on, by the way, in the bone marrow and in the gut, because in the bone marrow you're constantly replacing a lot of your red blood cells. You have lots of them, but they only last for 120 days.

So, after 120 days a red blood cell is broken down and you get a new red blood cell in its place. But obviously you have more than one

blood cell in your body. The same thing happens in the gut. The cells lining the gut, those are called epithelial cells, they're lining the surface of the gut, and they are constantly being sloughed off the wall of the gut. Why? Because it's not so nice to live on the wall of the gut. It's not a very pleasant environment.

And the cells are therefore constantly gotten, gotten rid of because they have a hard time surviving for extended periods of time. Those cells live only three or four days on the wall of the gut. So, when you go to the bathroom, I don't mean to be too graphic, but number two, a significant proportion of what comes out the bottom is actually cells that have been sloughed off from the epithelial lining of the gut. I don't know what the proportion is. It's something like 20% or 30%.

About half of what comes out is actually bacterial in the feces. We're not having lunch now so you can, you can absorb all this, right? About half of it. Almost none of the bulk of the feces actually ends up being what you ate because most of what you ate ended up, ends up getting compressed to a very small amount of solid matter. So, most of what comes out is, is not what, what came in, is not the processing of food but other things, as I've just mentioned.

And, by the way, here's another unsettling statistic. It's not so graphic but it's unsettling if you think about it. There are more bacterial cells in your gut than in the rest, than there are mammalian eukaryotic cells in the rest of your body. They're taking over. There are more of them than there are us in each one of our bodies. Go figure. Anyhow.

Why am I going on this long excursion? Well, only he knows and he's not telling. But there was a reason behind it. The reason why I'm going into this is to impress on you the fact that it's really important that the advance of cells through their growth and division cycle that we've just talked about here is very carefully controlled, because each one of these growth, cell growth and division cycles is the opportunity for disaster. What kind of disaster?

Well, if the cell makes a mistake in its chromosomes it might die. The death of a cell in our body is not a disaster. I just indicated to you that we're, we're making ten to the seventh new cells every second, but hopefully we're making exactly the same number of cells that die. So, ten to the seventh cells are made for every ten to the seventh cells that die. If you have an excess of new cells compared with dying cells you're in bad shape because that's really the disease of cancer.

And that implies, even with our knowing any, without our knowing anything else about cancer, that there have to be very careful controls over the decision on the part of a cell to whether, as to whether it can grow or divide, as to whether or not it should grow and divide. Now, what does that mean? Well, part of the decisions come from the following situation. Let's imagine here we're growing, we're talking about a human tissue. Each one of these things is a cell. And, as usual, my art is not that splendid.

But one of the things I want to impress on you is that the body cannot give license to this cell or to this cell or to this cell to make the decision about growing and dividing on its own. A cell cannot go and say I think I feel like dividing, I'm going to go do it. That's a no-no. Why is it a no-no? Because the moment one grants autonomy to such a cell one's in a very dangerous situation. Instead, what has to happen, what must happen is that cells talk to one another and cells read a consensus.

For instance, maybe there's a cell that's missing right over here in this tissue. And keep in mind an architecturally complex tissue has a precise number of cells in it organized in a very precise pattern. And the way that pattern is maintained is that the cells are constantly looking around and seeing is there a gap here? Do we need to make a new cell or not? It's not as if this cell says, well, I'm happy here and all my neighbors are happy but, still, I'm going to grow and divide because it seems like a nice thing to do.

Again, that's an invitation for disaster. And I'm saying that if only to impress upon you the fact that cells only will make the decision to grow and divide and to go into this cell cycle on the basis of consultations with their neighbors. They're constantly talking to one another. The signals, the intercellular signals within a living tissue represent constant ceaseless chatter.

It's like everybody is on a cell phone talking with six or eight or ten of his or her neighbors all the time. And if you could hear it, it would be a real din. So, that raised the question how do cells talk with one another? What messages do they exchange, one with the other, so that they can establish some kind of consensus as to whether or not it's inappropriate for one of their number to grow and divide? All right. This is an unauthorized ad. You can interpret it the way you want.

Anyhow. So how do they do that? Well, what they do, one of the most important ways they communicate is they exchange proteins

with one another called growth factors. And a growth factor goes from one cell, here's a cell, it releases a growth factor and it goes to a second cell and induces the second cell to begin to divide. That's a mechanism of transferring the signal or conveying the signal. And a growth factor itself is a relatively low molecular weight protein which travels through interstellular, interstellar, intercellular space.

It moves from one cell to the other. So, it's secreted by one cell, it moves here, and then it goes over here to a second cell. In fact, if we want to blow up the second cell, the second cell has on its surface a specific protein which is known as a receptor or, in this case, we can say a growth factor receptor, which represents a means by which that cell senses the presence of a growth factor in the extracellular space.

And, as is suggested in this very crude and poorly drawn cartoon, this receptor is a transmembrane protein. It has an extra cellular domain, it has an intracellular domain, and it moves through, it protrudes through the lipid bilayer of the plasma membrane. We talked very briefly about these at the beginning of the semester.

And what happens is these receptors are so configured that in the event that a growth factor comes over and is sent off by a neighboring cell, the growth factor, I'll draw it here as a, as a square, this growth factor binds to the receptor in a very specific fashion. And, as a consequence, the receptor responds in its intracellular or cytoplasmic domain by emitting signals into the cell informing the cell that an encounter has been had in the extracellular space with this growth factor which has been released ostensibly by another cell.

Now, the fact of the matter is there are many different kinds of growth factors. Here I've drawn a square one. Here's a triangular one. Here's a circular one. And each one of these growth factors has its own cognate receptor. So, here's a receptor that binds circular growth factors.

Here's a receptor that binds triangular growth factors. Well, obviously I'm very schematic. The point I wish to make is that there are multiple distinct kinds of receptors, and each growth factor we can, I'll abbreviate it as a, as a GF, each growth factor is what's called a ligand, or a ligand depending on where you live, a ligand for the receptor. So, in this case the square thing is the ligand for the receptor.

It binds to the receptor and, in so doing, it provokes the receptor to emit a signal which his transferred in through the plasma membrane into the cell cytoplasm. Clearly, what I also wish to indicate by this is that growth factors don't bind to inappropriate receptors. They only bind to their appropriate receptors or their cognate receptors. So, for example, there's a growth factor that we'll talk about shortly.

It's called epidermal growth factor.

And I'll just abbreviate EGF for growth factor. There's another growth factor that called platelet-derived growth factor.

So, we have EGF, we have PDGF, platelet-derived growth factor. And, in fact, there's actually 30 or 40 other kinds of growth factors. And each one binds specifically to its own receptors.

And so now we begin to understand that the signaling channels are actually very multiplexed. It's not just at one, one kind of growth factor can travel through intercellular space. And, again, this cell will only receive, will only make a decision to grow and divide if its growth factor receptors have send an adequate number of stimulatory signals into the cell which then pursued the signaling circuitry which processes these signals that it is indeed time to make the binary decision, growth versus non-growth on the basis of these signals received from the extracellular space.

From that we can already intuit something interesting about cancer cells. Cancer cells make a decision to grow even without having received the signal from their neighbors. In other words, cancer cells become quasi or totally, they become growth factor independent.

That is to say somehow they have usurped and perturbed this signaling mechanism and they have no longer become dependent on these extracellular signals in order to make the decision to grow and divide. They will just grow in essentially an autonomous or independent fashion. And we know a little bit about this from the following kind of experiment.

If you put a human connective tissue cell, a connective tissue cell is called a fibroblast in culture or even a mouse, fibroblast in culture, you

put it in a Petri dish like this, it'll sit on the bottom. You can provide this fibroblast or fibroblasts with all the nutrients it needs, with glucose and with vitamins and vital amino, essential amino acids, whatever you want, give it all those low molecular weight compounds and the, and the fibroblasts will sit down on the dish.

And it will look up at you and smile. And it will do so for days and weeks. It won't do anything. It won't grow at all. Why? Because you haven't given it the requisite signals to induce it, to persuade it, to proliferate. And what are those requisite signals? They are growth factors. So, you need to add growth factors in addition to the nutrients in order to persuade a cell to emerge from its quiescent state into the active growth and division cycle.

And so in my last minutes or my last minute, hopefully not on this earth but in this lecture, let me just say that when you, I mean nothing is sure, but if you take a cell and you deprive it, you starve it of growth factors it exits from the active growth and division cycle and it will go out into another phase called G zero.

And it will sit there like a bump on a log for an extended period of time. It will be metabolically active, viable, but it won't proliferate. It will be like the cells you put in the Petri dish without growth factors. And if you give it growth factors then the cell will go back into the G1 phase of the cell cycle and begin active growth cycling. It will pass through a successive cycles of growth and division as long as you give it growth factors.

And each time the cell emerges from M it's going to ask the question, are there any growth factors around me? If so, I might commit myself to doing this over again. And if there aren't, I'm getting out of this racetrack and going over to G zero where I may stay for days, weeks or years. On that dramatic and tense note, see you on Wednesday.