So today's lecture talks about the cell cycle, the control of the cell cycle, and also cell death. So obviously, cell division is extremely important in multi-cellular organisms. We've talked a fair bit about control of the cell cycle, in terms of mitosis and meiosis, in earlier lectures. Obviously, cell division is necessary in a variety of circumstances, probably the most obvious is development. You go from one cell to ten to the thirteenth to ten to the fourteenth cells. That's accomplished by a tremendous amount of cell division that goes on over your gestational period. I've also pointed out, in fact we talked about it last time, that cell, that wound healing is, in part, a process of cell division. Fibroblasts for example, and other epithelial cells, get recruited to divide, in order to repair damage to a tissue. And even without damage, there's a lot of cell turnover, for many tissues. You may not realize this, but your blood, for example, turns over about every month, so you have to replace all your red blood cells, and all your white blood cells, and so on, periodically. Cells in other tissues, in the skin for example, your skin cells are born, they migrate up to the superficial layers of your skin, and then they get sloughed off, and of course, they have to be replaced. There's a lot of cell division that's going on naturally, in the process of what we call homeostasis, and to give you an example of that, if you consider your intestines in a human, your intestines undergo ten to the eleventh cell divisions per day. That's a remarkable number. Ten to the eleventh cells dividing in your intestines per day. If you imagine that a cell in your intestine is about ten microns in diameter, if you lined up all the cells next to each other that were born in a given period of time, in 38 days you would have enough cells lined up end-to-end to go around the earth, and in a year you'd have enough cells to make it from the earth to the moon. So you produce a tremendous number of cells just naturally, in the process of organ function and homeostasis. Of course we can have too much cell division, and this is pathological condition, which we typically associate with cancer. And indeed, many of the factors that I'm going to talk to you about today, that relate to the normal control of cell division, and also cell death, are perturbed in the development of cancer. Cancer, as you almost certainly know, is a disease of too many cells, and a major factor in that is excessive cell division. [UNINTELLIGIBLE PHRASE]. So this happens to be a human cancer cell line growing in tissue culture, and one of the hallmark features of cancer cells is that they have, in a sense, unlimited and unregulated cell division. So if you were to watch this movie, which actually comes from your book, supplementary materials from your book, you could watch these cells dividing, and they would do so in an unnatural fashion. Without response to proper growth factors that would stimulate cell division, they would divide on top of one another, which normal cells don't do, and other places and times when other cells are kept in check, cancer cells are not. So the basic process, which again is well familiar to you, is to take a single cell and turn it into two. And we discussed the very last stages of this in earlier lectures on mitosis, how the chromosomes get divided from the mother to the two daughter cells. We also have briefly alluded to the fact that there's a second, critical event, and you talked about the mechanisms of DNA replication. The DNA needs to get duplicated so that it can be properly divided, and this, as you know, occurs during a particular phase of the cell cycle, known as S-phase, for synthesis phase. And then there's chromosome segregation, and this occurs in a distinct phase of the cell cycle called M-phase, or mitosis phase. And the development of cells over the course of, say development, or in wound healing, can be thought of as a successive iteration of DNA duplication, DNA replication, and chromosome segregation. So in a sense, you go from mitosis to S-phase to mitosis to S-phase. These segments are separated by periods of time when cells are accumulating the biomaterials that they need, basically to function, and also to carry out the next phases of the cell cycle, and these are refereed to as gap phases, G1 for the one that separates mitosis from S-phase, and G2, the one that separates the S-phase from mitosis. Now, this is a cyclic process, one cell gives rise to two, that then undergoes this process again, and so we think of these things as cycles from M-to-S, connected by these gap phases, G1 and G2. Now there's another phase that we haven't told you about, which many of your cells are actually in right now, and that's a phase called G0. This is a resting phase, and this can be either permanent or temporary. Many of your cells, when they're born, will stop dividing forever. Cells in your brain, for example, or most cells in your brain, cells of your cardiac muscle, and there are many other examples. Once they get born, they undergo mitosis, they go into this resting phase, and they'll never come back out again. And that's why it's difficult, for example, to deal with brain injuries, or spinal cord injuries, because those cells cannot be recruited back into the cell cycle, they can't make more of them. But there are other cells in which the resting phase is temporary, and these cells can then reenter the cell cycle, going back from G0 to G1 and through the process again. And a lot of the progenitor cells that I referred to up there, in terms of the skin and the blood, and the intestines, are in that situation. They're resting, and then they be recruited back into the cell cycle, in order to make more derivative cells. It's also useful to consider what the chromosomes are doing in these different phases of the cell cycle. So in a G1 cell, if we think about a single chromosome, and we're representing it by a single-line, although this is of course, a double-helix. In the G1 phase there's a single, double-helical chromosome, in S-phase that gets duplicated in the process of DNA replication, initiation shown here, and it would be completed so that the single-chromosome would be ultimately duplicated into two. And then in M-phase, those two chromosomes get separated from one another, so these are now joined, separated from one another, eventually into two daughter cells, which can then go through this process again. OK? And much of what we think about, when we think about how the cell cycle is controlled is to deal with the initiation of DNA replication, and then the initiation of mitosis. OK, so this is a cyclical process, a highly ordered process, this is a representation of the cell cycle, as shown in your book, just so you know it's in there. Exactly as I described to you, mitosis and S-phase, where the action is, these gap phases, G1 and G2, and this little arrow represents G0, and I've also used this term before, interphase, that represents all the phases where the DNA is not evident under the light microscope. It's only evident in mitosis because of DNA condensation, so the rest of the cycle, G1, S, and G2, are called interphase. So this is a highly ordered process, each step preceded by a particular other step, leading to a particular outcome, events leading to a particular outcome, and in a topical sense, you can think about it as the NCAA pools, starting tomorrow there'll be individual

outcome, and in a typical series, you can think about it as the NCAA pools, starting tomorrow there'll be individual events, games, which will lead to next events in the next round, and next events in the next round, to an ultimate conclusion, in this case, the NCAA champion, which can only occur if the proper events have happened, prior to it. And you'll see another specific example of how the order is assessed in the development of the stages of the cell cycle. OK, so we know that the cell cycle is important. The question is, how is it controlled? How do you accomplish this orderly process of cell cycle progression? What are the genes -- -- that regulate this process? What do the genes encode, what do the proteins, and what do the proteins do? What are the pathways that they regulate to ensure the stages of the cell cycle? This was a huge question for decades, and there was rather little progress in trying to understand how it happened, especially in us. Our cells are sufficiently complex, and there's relatively imprecise, or particularly was in the past, imprecise methods for dissecting complex processes in mammalian cells. And so it took experiments performed in yeast, a single-cell, eukaryotic organism, budding yeast and also fission yeast, to allow us to understand what the details of the cell cycle are. Budding yeast, as shown here in a picture from your book, is a single-cell organism. It's also haploid, or it can be at least, haploid, which means that it has a single set of chromosomes, it doesn't have two sets, single set of genes, which makes it amenable to genetic analysis. You can make mutants more easily, if you only have one copy of each gene to worry about. So that's another reason that yeast was attractive. And another reason, which is kind of seen here, not really obvious here, but when yeast cells divide, they go through a very characteristic, morphological change. They start off as spheres, like this one, and as they go through the cell cycle, they develop a small bud. That bud then gets bigger, and eventually the two, the joint between the mother cell and the bud, get severed to form two cells. And you can actually figure out where you are in the cell cycle, by examining exactly what the morphology of the yeast cell is, and I'll show you that on the next slide. This is the yeast cell cycle, with overlay of the diagram of what the cells look like in the different phases, so a yeast cell that's in G0, let's say, looks like this, fairly round, nondescript, as it enters G1 it creates a small bud, that bud then gets bigger as the cells are now duplicating their DNA, the bud actually gets bigger. It gets bigger still, during G2 phase, and then at M-phase you can actually see a little junction between the two cells, and the mother and the daughter cell are more-or-less the same size, and then they separate from one another, and they can go through the cycle again. And this is useful because you can tell if you have a mutant, as you'll see in a moment, if you have a mutant in a gene that affects one of these processes, you can actually figure out where the mutant gene acts within the cell cycle, based on what the yeast cell looks like. And these individuals here, won the Nobel prize for their efforts to understand the cell cycle, they won it about five years ago, or so. This is Lee Hartwell, he's an American, happens to run a cancer center out at the University of Washington, very suave and sophisticated guy. These two goofballs are Brits, very nice guys actually, Paul Nurse, who's now the president of Rockefeller University in New York, and Tim Hunt, actually Sir Tim Hunt, because he's knighted after this Nobel Prize. And I'm going to explain the experiments that all three of these guys did today, which won them the Nobel prize, and gave us tremendous insight into the control of the cell cycle in yeast, which turned out to tell us how the cell cycle is controlled, also, in us. OK, so the first thing we need to do is to create mutants. That's a general theme in biology. If you want to understand a process, find a mutant that can't do it, and understand the genes that get mutated in that process, and so that's what happened in the hands of Lee Hartwell, the guy in the upper-right, he took this same yeast, the budding yeast, which is also Brewer's yeast, the yeast that makes beer. He took a population of these yeast cells, and he mutagenized them. I told you about this before, you can add chemical mutagens that soak into the cells, bind to the DNA, cause mutations. Each cell might have one, or a few nucleotides changed, and at some frequency those mutations will affect genes, and at some frequency the genes affected will influence the process that you're trying to study. So he was trying to study growth control, cell division, so he asked about the ability of the cells to grow, and particularly, he did so at two different temperatures. He tested the ability of the cells to grow at their normal temperature, which is about 25 degrees centigrade, and he found that many of these yeast cells would grow, following mutagenesis, at 25 degrees. Probably some of them wouldn't grow because they would've inactivated some critical gene, and even at 25 degrees, they couldn't grow. However, he then took these plates, and he used a technique, which I referred to in a previous lecture, called replica plating. He took a stamp, basically, of these colonies, transferred them onto a fresh plate, and examined how the cells grew at 30 degrees centigrade. And what he found was that, whereas many of the cells that were able to grow at 25 degrees continued to grow at 30 degrees, so they produced colonies in exactly the pattern that he had seen previously. Some of the cells didn't. This colony here, while it was successful, the cells were successful in growing at 25 degrees, failed to grow at 30 degrees. And this type of mutant is referred to as a temperature-sensitive mutant, -- [PAUSE} -- abbreviated TS. We imagine that this mutation affects the amino acid sequence of the protein, at low temperatures the protein is still able to function, but at higher temperatures, maybe it becomes a little unstable, and now it can't function, and that's why you see no growth here. So we had a TS-mutant that affected the growth of the cells, OK? So my question to you is, is that TS-mutant interesting? Is it interesting? Well we can't actually know whether it's interesting yet, because there are two general classes of mutations that would give you this same phenotype. One of them is interesting, does that cement always show, or do we have a problem here? It's always there? Never noticed it, in all these years. You can distinguish between these two classes of boring and interesting mutants, based on the morphology of the yeast cells when you shift the temperature. If you start with a population of yeast cells growing just randomly at 25 degrees centigrade, including these mutants, if you were to look under the microscope, you would find mutants, cells that were at various stages of the cell cycle. OK? Now, let's imagine that our mutation affects a general enzyme that's required for cell viability, DNA polymerase or maybe, better still, some ribosomal protein that all cells need all the time, in order to function. If I were to take these cells which were growing at 25 degrees centigrade, and I were to shift them to 30 degrees centigrade, and look under the microscope, what would I see? Well, if it's a ribosomal protein, and now translation just stops, these cells are going nowhere. Regardless of where they were in the cell cycle, they don't go any further because you need protein synthesis to do anything, so if you look under the microscope at these cells, following a temperature shift, you would still find a distribution of cells in

the different phases of the cell cycle. Lee Hartwell was not interested in this class of mutants. They could be anything, he didn't care. However, he reasoned that if he were dealing with a mutant that affected specifically a stage in the cell cycle, he might find that the cells, upon temperature shift from 25 degrees to 30 degrees, got to a particular point in the cell cycle and couldn't go any further, that this gene affected one of these important transitions, and so for example -- -- if he were to look under the microscope, all of the cells would have arrested with a small bud, would have arrested in G1, or maybe they would've arrested looking like this, just prior to mitosis, or like this, somewhere in G2. But importantly, they would arrest with a specific morphology that would indicate that they had a specific cell cycle block. And so he did this, and he found many, many such mutants. He called them cell division cycle mutants, or CDC mutants. And it was his methodology, which really broke this field open, and won him the Nobel prize. Now one example of a CDC mutant that he discovered, called CDC2, was particularly important. It's been renamed CDK1, or cyclin-dependent kinase one, for reasons that I'll come to. Cyclin, sorry, cyclin-dependent, kinase one. And this one acts at a particular phase in the cell cycle, early on in the transition from G1 to S. So if you imagine cells in G0, going to G1, and then progressing from G1 into S-phase, into G2, and finally, in mitosis. This particular mutant blocked right here, and so it said that this gene, CDC2 or CDK1, was required for this transition. And just to emphasize the TS-temperature shift and building up of the cells in the cell cycle concept, imagine a cell that looked like this, before the temperature shift. Ok? If you now do the temperature shift, this cell will progress to this point, but it won't go any further because CDC2 is required. Maybe I'll draw this over here, to make it even more obvious. CDC2 is required to go beyond this point, so that cell will arrest right here. If there were a cell that looked like this, in the population of 25 degrees, and now you shifted the temperature, it would complete this phase because CDC2 is not required. It would make it all the way to here, it would keep going, and then it would get stuck here again. And that's why you get cells building up with a particular morphology. OK? So this was successful, and as I said, he isolated a large number of such CDC mutants, which have taught us not about just that transition, but many other transitions in the cell. Now what Paul Nurse did, this guy here, he actually was performing very similar experiments in a related yeast species, but the critical experiment that he did was to clone the gene that is responsible for CDC2 function. So he took CDC2 mutant cells, which at 30 degrees -- -- will not grow. And he added, through a cDNA library, CDC2 cDNA, of yeast origin. So he made a cDNA library from yeast, he introduced it into these mutant cells, and then he asked whether the cells could now grow at 30 degrees -- -- and the answer was yes. That is, if the cells now carried an extra copy of CDC2, in the form of this cDNA, they now could grow at 30 degrees. He complemented the mutation through the addition of a normal copy of the CDC2 cDNA. Quite surprisingly, he did the same experiment -- -- using not a yeast gene, but a human gene. Frankly, there was great skepticism in the field about whether what these guys were doing in yeast had anything to do with cell cycle control in humans. Most people actually assumed that it probably had nothing to do with it, and so therefore, when Paul Nurse proposed to try to complement his yeast mutation with a human gene, people were skeptical. But in fact, it worked. And this told him, and the field, that the machinery that controls the cell cycle in this simple, single-celled eukaryote, is highly conserved, all the way through to humans, and that we could therefore understand cell cycle control in us, by doing experiments like this in yeast. So this really broke the field open, and that's why Nurse won the Nobel prize at that same time. However, there was a problem. They did in fact clone the gene, they were able to sequence the gene, and they found that the sequence of the gene, which I'm going to refer to now by the other name, CDK1, looked like a kinase. It had amino acid sequence, which made it resemble known kinases, so it was assumed to be a protein kinase. However, it didn't have any kinase activity. If you mixed it with various substrate molecules in the presence of ATP, those substrates were not changed, they were not phosphorylated. So the purified CDK enzyme had no kinase activity, and therefore, it wasn't entirely clear what its function really was, and the field sort of got stuck there for a while, trying to understand the biochemical function of CDK1, and other cell cycle regulators. And that then led to the experiments done by Tim Hunt, this guy here. And Tim Hunt, actually doing experiments at Woods Hole, down at the Cape, in a summer course, with summer students, did a famous experiment using sea urchins. Sea urchins, when they're fertilized, undergo very rapid, and very synchronous, cell divisions. In the first few hours after you fertilize sea urchin eggs, they will divide and divide again. And importantly, they will do so in a very synchronous manner, so all the cells will produce two cells at around the same time, and those will all produce four cells at around the same time. And this is important if one wants to do biochemical experiments, to understand what is changing within these cells as they're going through these various cell cycles. And so, what Tim and his students did was to label the proteins in these dividing sea urchin cells, with a radioactive amino acid, S35 methionine. And then they simply made extracts of these cells at different time points thereafter, and asked whether anything was changing in an interesting pattern, at different times that correspond to different stages of the cell cycle. So they ran protein gels, they separated the proteins and then exposed the gels to x-ray film, to visual what the protein concentrations were at different time points. They took cells at time zero, they took cells after 30 minutes, after 60 minutes, after 90 minutes, 120 minutes, 150 minutes, 180 minutes. And they knew already, from their earlier analysis, that this corresponded to the first cell cycle, and this corresponded to the second cell cycle. OK? Now some proteins, when they visualize them that way, didn't change. At the different time points they saw roughly equal concentrations of that protein throughout, but interestingly, other proteins changed in abundance, and did so according to a pattern. They seemed to oscillate, they seemed to cycle, in a pattern that corresponded with the cell cycle. And so he called these cyclins, and he suggested that they might have something to do with the control of the cell division cycle. Well, meanwhile, Nurse and Hartwell were doing their thing on CDKs, and so they came up with the idea that maybe these two things have something to do with one another. And particularly, maybe the failure of CDK to function as a kinase was due to the fact that it didn't have an accessory protein that it needed, mainly the cyclin. And so, whereas CDK2, sorry, CDK1, was an inactive protein kinase. Now in a test tube, in a biochemical experiment, if they mixed CDK1 with one of these cyclins, they observed kinase activity. And thus the name, cyclin-dependent kinase. It's not a kinase, it's not an active kinase in the absence of cyclin, it only becomes

name, cyclin-dependent kinase is not a kinase, it's not an active kinase in the absence of cyclin, it only becomes active in the presence of cyclin. So let me give you two quick examples of proteins that are then phosphorylated by this active kinase, to give you a sense of how this kinase regulates cell cycle transitions. There's a class of proteins that are involved in the regulation of replication initiation. We'll just call them replication initiation factors. In the absence of phosphorylation, they're inactive, and that's one of the reasons that replication is not initiated at those times in the cell cycle. However, in the presence of CDK cyclin, now the protein becomes phosphorylated, and becomes active. OK? So one of the ways you trigger the transition from G1 into S-phase, is to turn on this enzyme which phosphorylated this target protein, and stimulates S-phase entry. A second example is a protein called pRB. In its un-phosphorylated state, it is active, different from here where, in its un-phosphorylated state, it was inactive, and the function of the RB protein, in its active state, is to block again, the transition from S-phase to G1. This is actually an important cancer gene, it's mutated in a large number of cancers, and so we'll talk about its function, exactly how it blocks the transition, in later lectures, but suffice it to say, it does that. And when it is phosphorylated by CDK cyclins, it becomes inactive, thereby allowing cells to progress from G1 into S-phase. OK? So those are two examples of how CDK cyclins operate. Now, importantly, cyclin kinase activity is determined by the level of the cyclin. As I told you, cyclin levels oscillate, where this is cycle one, cycle two, cycle three. So these are cyclin concentrations inside the cell. CDK levels do not oscillate. Concentration of the CDKs inside the cells is rather constant. However, at this point in the cell cycle, when there's not enough cyclin, you don't have kinase activity. Only when you go past the threshold, do you get kinase activity. When it drops again, you lose kinase activity. But in the next cell cycle, the cyclin levels increase again, and you get kinase activity, and so on. So the oscillation of cyclins determines the oscillation of kinase activity, which determines the periodicity of the cell cycle. Now, for the truth in advertising, the situation is actually more complex, there are actually multiple CDKs and multiple cyclins. So in our cells, for example, if we imagine G1, S, M, G2, G1, S, M, G2, there's a cyclin called cyclin-D, which comes up in G1, goes down, comes up in the next G1, goes down. There's another cyclin called cyclin-E, which comes up a little bit later, in S-phase goes down, stays down, comes up in the next S-phase. And there's finally another one called cyclin-B, which comes up in G2, and comes down, goes up in the next G2. Anyway, the point is that there are different cyclins that get induced in different phases of the cell cycle, and actually control, through binding to different CDKs, different transitions in the different phases of the cell cycle. OK. Let's see. So, another concept: the transitions from the cell cycle don't occur, from one cell cycle position to the next don't occur, unless the previous cell cycle event has been completed. And that makes sense because you don't want to, for example, try to divide your DNA in mitosis if you haven't fully replicated your DNA in S-phase. So there are processes that are overlaid on top of cell cycle control, which ensure the completion of one phase before the next phase is initiated. And I draw, as an analogy, your washing machine, which likewise, has checkpoints which will determine whether or not the previous phase of the wash cycle has been completed. For example, you don't spin your wash until all the water has been rinsed out. There's a sensor that will determine whether that's not true, and if that sensor is tripped, it blocks the wash cycle at that point. Your cells have very similar checkpoints that will monitor and regulate cell cycle transitions, and they're called checkpoints. There are checkpoints, actually, that operate at different phases of the cell cycle. I'll only give you one example, it's actually the one that's best known. It occurs at the transition in mitosis, from metaphase -- -- where, if you'll recall, the duplicated chromosomes line up on the metaphase plate. In the process of anaphase, where the chromosomes separate from one another, the chromatids I should say, separate from one another. There's a cell cycle checkpoint that makes sure that this happens before that can happen. And in particular, if you have a chromosome which is only attached to one side of the mitotic spindle, so if you recall, these are centered in the middle through attachment to microtubules that are emanating from the two poles. If you have a chromosome that is only attached to one of the two spindles, it's called monopolar attachment, whereas perhaps other chromosomes, maybe all of the other chromosomes, are properly attached at the metaphase plate. This one, unattached chromosome will literally send a signal, a biochemical signal, which is the equivalent of "wait for me". And that signal will inhibit cell cycle progression. It'll specifically inhibit the transition from metaphase to anaphase. While that signal is being sent, the cells will just sit there, waiting for another microtubule to bind to this end of the chromosome, thereby extinguishing the signal, relieving this inhibition, and allowing anaphase to progress. OK, so these checkpoints are critical in insuring that these things happen in a timely and ordered fashion. OK. That's it for the cell cycle, so for the final ten minutes, and I always give short shrift to the next topic, which is cell death, which is another fascinating topic, fortunately there's not a lot of board work to show you. Cell death. So cells are born, divide, as we've just been talking about, but remarkable numbers of cells in your body die. They will die, too, because of cellular injury, but they'll also die because they're programmed to die, or they'll decide to commit suicide, or they'll be murdered by other cells. This happens in development, for example, your brains, as developing fetuses, have ten times the number of cells than you end up with as a young infant, because 90%, for some of you more than 90% of the cells will actually be killed through this process of programmed cell death. Other cells, in your immune system, for example, are eliminated by this process, so as to avoid them attacking your own body. And there's many other examples of relevant and important programmed cell death. So we want to understand this process, partly because it's critical in normal development, and also because deregulation of this process is critical for many diseases. Too little cell death can give you proliferate diseases like cancer or autoimmune disease, too much cell death can give you diseases like neurodegenerative diseases, too many cells in your brain dying. So the regulation of cell death is quite important. Here are two other famous examples of programmed cell death. This is the loss of the tadpole's tail that occurs through an orderly, cellular suicide program, in which all of these cells die, giving rise to a frog with no tail. And likewise, in development, your hands are formed in such a way that the digits are actually connected by other cells, but through this process of programmed cell death, the cells in the middle, the interdigital cells, are eliminated, thereby sculpting the formation of your fingers. And this can be regulated, and has been regulated, in evolution, in some species, like ducks, the loss of the interdigital cells doesn't take place, so they have webbing. In other species of birds, like in us, the interdigital cells are removed, so they have sculpted

toes. This is what programmed cell death looks like. It is a very rapid, and as I said, a very orderly process. Cells get signals to die, they can get signals because they get damaged, or they can get signals from their neighbors. When they get those signals, they undergo a series of biochemical changes. Their nucleus gets condensed, the DNA within the nucleus breaks up, and then the cells themselves break up into small fragments called apoptotic bodies, and then interestingly, the cells that surround those cells, eat the damage. It's like disposing of the body after a crime, the cells are eliminated through a process of phagocytosis, this is a very clean process then, there's very little junk, there's very little cellular debris that remains when this process of programmed cell death is completed. This is a normal looking lymphocyte, and this is a lymphocyte undergoing cell death. It's like, you know, this is your brain, this is your brain on drugs, this is a cell, this is a cell undergoing apoptosis. It's a fairly violent death, at least visually. This movie shows you an example of that. Here are cells undergoing apoptosis, I hope. Maybe not. Oh well. It comes from your book, so you can see it yourselves, in fear that that would happen, I'll show you another set of stills. Here are normal cells growing in the tissue culture dish, you add some agent which induces apoptosis, the cells begin to round up as you can see here, and their cell surfaces actually become this horrible mess of blebbing membrane, and they will eventually break up, as you can see starting to happen here. So apoptosis is critical and actually very, very interesting. As I said, too much cell death can give rise to various diseases, neurodegeneration stroke is complicated because of the effects of apoptosis, too much apoptosis, even in AIDS, there's a lot of cell death that takes place in apoptosis, and too little cell death, as I said, is important in cancer, autoimmune disease, and certain viral infections. Importantly, we know about the genes that are regulated in cell death, that regulate cell death, through genetic experiments. And here, the genetic experiments were performed, in large part, at MIT, by a professor in the biology department named Bob Horvitz. He took comfort in the fact that the process in C. elegans, a simple worm, has very many similarities to the process that I've outlined to you, and therefore, he hoped that the genes that regulate programmed cell death in C. elegans, were similar to the ones that do so in mammals. And so he then undertook a study to ask what genes regulate the cell death process in this simple organism called C. elegans, which as an adult has only 1,090 cells. And the beauty of this organism is that you can actually see through it during development, and you can therefore follow the fate of individual cells over the course of development. And he and his students and fellows did that, he observed that in the development of certain cell lineages of the developing worm, individual cells died. In fact, about 130 cells underwent this process of apoptosis. And then he carried out a genetic experiment. He asked, if I mutagenize worms, can I find ones in which this process doesn't happen, or perhaps, in which this process happens too much. What are the genes that regulate apoptosis? And he found several such genes that turn viable cells into cells that have undergone programmed cell death, or apoptosis. In particular, there were two genes that positively regulated this process. They were called Ced-3 and Ced-4. They were required for apoptosis to occur. Mutants in Ced-3 and Ced-4 didn't have this cell death process in those developing worms. And another gene called Ced-9 was required to prevent apoptosis. In a Ced-9 mutant, there was too much apoptosis going on, OK? They then cloned these genes, and it turned out they all have homologs, versions, in our cells, and those genes in our cells likewise regulate apoptosis in us. And we now know that at least members of this family of genes are important in diseases, including cancer. I'll just mention the function of one gene, right now. Ced-3, it is a protease, which cleaves target proteins -- -- into fragments. So, one of the key things that happens in apoptosis is, you turn on this enzyme, this protease, and it goes around cutting up various target proteins, which eventually lead to the death of the cell. There are many target proteins that get cleaved when these caspases, these proteases are called caspases, when these proteases get activated, and I just gave you one example of one such target. There's an enzyme that is normally involved in cleaving your DNA, this is a DNA gel, of viable cells and cells that have undergone apoptosis after a certain number of hours. The DNA of the cells is normally intact, and large, because this enzyme is kept in check. However, when its inhibitor is cleaved by the caspsase, it now goes about cleaving the DNA, and this is one of the reasons why apoptotic cells die, because their DNA gets cut up into very small fragments. There are a number of other targets, you can read a little bit more about this in your book, I apologize that we had to rush through apoptosis, it is important, and we will in fact come back to it in a discussion of cancer.