And he took what was left, which much be very, very small, smaller than the size of a cell because the cells were removed by filtration, and he injected it into another bird. Now, a healthy bird. And strikingly that bird developed a tumor. So he was able to transfer whatever agent it was that was associated with tumor development from this bird to this bird. He concluded that it was something smaller than a cell, and he concluded that it was a virus. And indeed he was right. Now, the tumor that these birds developed is called a sarcoma. It's a tumor of the muscle in this case. And the virus that was responsible for this tumor was named after Rous, and it goes by the name of Rous sarcoma virus or RSV. Now, there was great skepticism about the connections between viruses and cancer at that time, and actually for many decades thereafter. And so Rous' work was not fully appreciated for some time. But it was eventually appreciated. And Rous actually won the Nobel Prize 50 years after he made this discovery, when it was confirmed that indeed the stuff that he was studying was highly relevant to the situation in humans. Not analogous, necessarily, but highly relevant. Over the years, since that discovery and discoveries made by others, demonstrated that the Rous sarcoma virus viral genome carried a set of genes that were familiar. These are the same sorts of genes carried by many viruses of this class. Rous sarcoma virus happens to be a retrovirus. I'll teach you more about those in a future lecture. It's the same general class that includes HIV. And there were some familiar genes that were responsible for the replication functions of the virus. But then there was a new gene present in this strain of the virus, specific to Rous sarcoma virus, which was named Src and was later considered to be the relevant oncogene, the gene that was responsible for the tumor forming capabilities of this Rous sarcoma virus. So there was then great interest in what was this Src gene? What was this special gene that could cause normal cells to become cancer cells? And research then went on for, again, a number of years studying Src, studying its biochemical functions, but the real question that was of great interest over the years was where did Src come from? Most viruses in this class don't carry Src, and it wasn't at all clear what the origins of this cancer-causing gene were. Was it a rare viral gene or perhaps did it come from the host cells themselves? And in experiments that were done, actually by my PhD advisor, Harold Varmus and his colleague Mike Bishop, it was shown that the Src gene actually does indeed reside in the cells of the host and gets picked up by a recombination process by the virus. And I'll just illustrate that for you. It turns out that there's a related virus fairly common in chickens called avian leukosis virus. And this is the predecessor of Rous sarcoma virus. It's a virus that has in its genome just the replication genes. And I'll just draw a viral capsid around this virus. So this is the avian leukosis virus with its genome and replication genes. Now, what they showed was that the avian leukosis virus, when it infects cells, here's a normal chicken cell. Most of the time it just makes more copies of itself like viruses will do. But occasionally, through a recombination mechanism, the avian leukosis virus will actually pick up a gene which is present in the normal genome of the chicken, a gene which looks very much like the Src gene present in Rous sarcoma virus. And through this recombination mechanism, the details of which I won't go through, very rarely you'll get a recombinant virus produced which has in its genome the replication genes, but now also Src gene. And this virus, which modifies the Src gene slightly in the course of the development of the virus, now, when introduced into birds, will very efficiently cause tumors. And what this showed, what this actually Nobel Prize winning experiment showed was that the oncogene, powerful cancer-causing gene resides in the DNA of the chicken. And actually there were similar genes, very analogous genes residing in the DNA of all vertebrate species, including humans. You are sitting there with two copies of the Src gene inside of you. So a question is, if that's true, if we all have these oncogenes in our cells why don't we get cancer? Why aren't we all sitting there as one large sarcoma? Anybody? Well, there are two answers. Yeah? Somebody. Claudette is pointing to somebody. Am I blind? Oh, hi. Excellent. Yes. So what's different about the situation in the chicken cell and the situation in the viral genome is how the gene is expressed. Here it might be expressed properly under what we would call physiological control, express where it's supposed to be when it's supposed to be. And here it's been removed from that regulatory network and is expressed perhaps at two high levels at the wrong time. And under those conditions it can push cells to inappropriately divide. So the virus has hijacked this gene and changes its regulation. That's one explanation. There's potentially another explanation for why we're not all sitting around with tumors, and I'll come to that in a moment. Now, as I said, Varmus and Bishop won the Nobel Prize for this work in 1989. And the day that they won the Nobel Prize Harold Varmus' sister-in-law was standing in a cafeteria line at Berkeley and she overheard two guys talking behind her. One guy said to the other, what are you going to have for lunch? And the other guy said, I don't know but it ain't going to be the chicken because two guys just won the Nobel Prize for showing that chicken causes cancer. [LAUGHTER] Which is not exactly true. But nevertheless. Now we know since that work that there are a large number of these viruses which are called collectively acutely transforming viruses. There are a large number of acutely transforming viruses that carry in their genomes and oncogene which they have cooperated from the host cell. These viruses are not viruses of human beings. They're viruses of experimental animals and usually generated in an experimental setting. Mice, rats, chickens, turkeys, other species have been used to generate such viruses. And about 50 or so oncogenes have been discovered through this context, including one that's hopefully familiar to you already and will come again, members of the Ras gene family which I've told you about as an important signaling molecule in mitogenic signaling pathways, and another important oncogene that I'll mention briefly later called mic, and about 50 more. And it turns out that they were a very useful source to identify cancer-associated genes in humans. Many of the genes that we now know are important in human cancer were initially discovered through that process. Now, as I said, most of the time human cancer has nothing to do with viruses so don't be confused. There are a few human cancers that are virally associated. The major one is cervical cancer. About 50% of cervical cancers, particularly in the Developing World, are associated with the virus called human papillomavirus or HPV, and specifically the high-risk types. Not all papillomaviruses will cause cancer. Papillomavirus is the same virus that causes warts, for example. And there are many papillomaviruses that are not associated with true malignancies, but human papillomaviruses of the high-risk

many papillomaviruses that are not associated with cervical malignancies, but human papillomaviruses of the high risk type are. And they can give rise to cervical cancer. Fortunately, companies are now developing vaccines against HPVs which are greatly affecting the risk of cervical cancer around the world. So this is a major step forward in controlling this particular cancer type. So because most human cancers are not virally associated, there was still some skepticism about the importance of this discovery for human cancer. Maybe it was true of the viruses, maybe it was true of these experimental animals, but is it true of human beings? Do these oncogenes have anything to do with human cancer? And this debate went on for a little while longer until Bob Weinberg here at MIT in about 1980 did the following experiment. He took DNA not from a bird but from a human being who carried a tumor in his bladder. So this individual had bladder cancer. This cancer was put into cell culture and turned into a cancer cell line, and it was actually this material that Weinberg's lab worked with. And they asked the question, are there genes in the cancer cell line that are cancer-causing? Are there oncogenes that I can discover within those cancer cells? So the experiment that they did was to take DNA, they isolated DNA from the cancer cells, and they introduced it into mouse cells in the laboratory that were "normal". And what I mean by that is they looked pretty normal compared to the cancer cells which had a deranged sort of architecture, as I mentioned last time. They grew flat and didn't grow on top of each other like cancer cells will do. And, importantly, if you were to inject these cells into an immunocompromised mouse they wouldn't form a tumor. They were nontumorigenic. Whereas, these cells, if injected into a mouse, would form tumors. OK? So they isolated DNA from the cancer cells and put it on the mouse cells. The mouse cells will, at some frequency, take up the DNA, incorporate the human DNA into their own genomes and begin to express the genes from the human DNA. And they found at low frequency that within this population of otherwise normal-looking mouse cells abnormal colonies of "transformed" cells could be found. And these transformed cells looked a lot like the cancer cells. Their shape was different, they grew on top of one another, and they had other properties that also made them similar to cancer cells in the sense that if they injected these cells into an immunocompromised animal, whereas the normal cells would not form a tumor, these transformed cells would. So they were able to convert normal cells into cancer cells through the addition of DNA from a cancer cell line. So what's going on here? What did they do in this experiment? Yeah. They eventually did. To get to that point they were able to isolate from the full genome of the cancer cell an individual gene that had been transferred into these mouse cells. They isolated that human gene away from the mouse genes, and then they preformed DNA sequencing. And they discovered the first human oncogene that was isolated from a cancer as opposed to a virus. And, as I alluded to last time, the gene that they isolated in this fashion was a familiar one. It was the Ras gene, a gene that had been discovered in the context of acutely transforming retroviruses. It was already known to be cancer-associated, but now it's in the context of real human cancer. And this was a major advance. Moreover, they sequenced the Ras gene from the cancer and compared it to the sequence of the Ras gene from normal cells and they found a difference. They found that it had been mutated, just as the genetic theory of cancer would predict. Compared to the normal sequence of the Ras gene, which is shown above, the sequence of the Ras gene isolated from the human bladder cancer sample carried a single mutation which converted a glycine amino acid to a valine amino acid. And the consequence of that mutation changed the Ras protein from being a signaling molecule that could be regulated from an on state to an off state to a signaling molecule that could not be regulated. Once it go turned on it couldn't' get turned off. So it was a constitutively active signaling molecule, which makes sense for a cancer cell that is continually proliferating. Now, there's an issue here that I would like you to ponder. And that is that this is a single point mutation, a single base change. In all of your cells, during cell division, there's a particular mutation rate. It's not known exactly, at least for all cells in your body what that rate is. But let's estimate that it's about ten to the minus ninth per base pair per cell division. Now, if you calculate how many cell divisions are going on in your body at any given time, you can estimate that you have about ten to the third to ten to the fifth Ras mutant cells inside you as you sit there today. Maybe as many 100, 00. Maybe more than a million cells that carry that very mutation in your bodies. So I ask you again, why is it that you don't all have tumors? This time it's not the explanation that we heard before about regulation because this is the native gene which is being changed. It's being regulated the same way. So why is it that we don't all have bladder cancer, for example, or some other type of tumor? Well, it relates to what I told you last time, that cancer is rarely a single-step event. Cancers rarely are associated with a single mutation. A single mutation may be involved, may be involved in initiation, but it's not sufficient to give you all the steps you need for a full cancer. As I mentioned last time, you might need five, ten or twenty distinct mutations to get a true cancer. So you might have mutant cells in you, they might be totally normal, but if layered on top of those mutations are other mutations they could progress. OK? Now, that's particularly important because the mutations that we're talking about are, at least by some assays, dominant. And the old genetics terminology of dominant and recessive, these mutations are dominant. Note, in this assay we're transferring a single gene into an otherwise normal cell and seeing a phenotype. That's an example of dominance. And these genes can, in the right context, function in a dominant fashion on top of a normal copy or normal copies of the gene. And you'll see why a distinction between dominant and recessive is important in a moment. Now, as I hope you remember from earlier lectures about signal transduction, the Ras proteins fall within a signal transduction pathway that's very well worked out. They link transmembrane growth factor receptors through intermediary proteins, adaptors and exchange factors, to downstream components involved in, for example, phosphorylation kinases and transcription factors that turn on the expression of target genes. This is the pathway that I've illustrated to you before. And we now know that many of the components of this pathway, this so-called mitogenic signaling pathway that drives cells to divide are mutated in cancers. Not just Ras, which is actually mutated at pretty high frequency in cancers, but many of these components are mutated as well. And they're mutated through different mechanisms. Subtle mutations, like I've just told you about for Ras, where single nucleotides can be changed and a single amino acid can be changed to convert a normally regulated protein into an abnormally regulated protein like Ras. Some of the growth factor receptors are likewise activated by that mechanism. But there are other mechanisms, other activation mechanisms for cancer-associated genes, including gene amplification and translocation which I'll illustrate on the

mechanisms for cancer associated genes, including gene amplification and translocation which I indicate on the board. I hope you noticed on the right here, Review Sessions for Monday night for the exam on Wednesday, as well as Tutoring Sessions and indications of where you're supposed to go. So gene amplification is important in cancer. There are quite a few genes that are amplified in tumors compared to normal cells. And I'll give you one example which is relevant to breast cancer and ovarian cancer. In normal cells, in the DNA of normal cells there's a gene called HER2 which is one of these growth factor receptors like number two up there. And of course the normal cells are diploid so they have two copies of the HER2 gene. And through normal transcription and translation that's going to give rise to a particular concentration of the growth factor receptor on the surface of the cells. And that's going to give rise to a certain signal emanating from that growth factor receptor which will cause the cells to divide at the appropriate time and place. In about 30% of breast cancers and a similar percentage of ovarian cancers, the number of copies of the HER2 gene has been increased dramatically, sometimes as much as a hundred fold. And these are due to errors in DNA replication. Instead of having DNA copied once in this region, it gets copied again and again and again and again. And now, in these cancer cells, you don't have that same concentration of the growth factor receptor. You have a much higher concentration of the receptor. And because there's a much higher concentration of the receptor, you might get as much as ten or a hundred times the level of signaling. And the consequences of this are that a cell that shouldn't divide doesn't have actually enough concentration of growth factors where it should normally divide will now divide anyway. Inappropriate proliferation, the hallmark of cancer. Now, the good news here is that there are antibodies against this HER2 protein. And they are actually effective in the treatment of breast cancer. They can prolong the life of women with breast cancer, specifically those who have amplifications of this gene. This drug actually does nothing for women who don't have amplifications, but it prolongs the life of women who do. So that's an important link between basic biology and treatment. We'll talk more about those next time. Another mechanism of activation of oncogenes is translocation. I showed you last time karyotypes of cancer cells in which one piece of one chromosome gets linked to a piece of a different chromosome. The reason that happens and is selected for is that new regulatory networks or new genes can be produced by those kinds of break and joining reactions. And one famous one involves this gene called myc which is a transcription factor like the bottom of this pathway, like number eight there, transcription factor which is important in driving cells into the cell cycle. myc is normally expressed from a "weak promoter". Which means that not a whole lot of myc mRNA is produced, which means that not that much myc protein is produced at any given time in a cell. And this gives rise to regulated cell division. However, occasionally in cancers, particularly in certain types of leukemia and lymphoma, translocation events take place where a new segment of DNA is produced which joins the normal myc gene to a very strong promoter. And now, just like this gentlemen mentioned before about the RSV, now a gene is not regulated properly. It's now regulated, in this case, too strongly. So a lot of myc mRNA is produced which gives rise to a lot of myc protein which gives rise to unregulated cell growth. Again, the hallmark of cancer. So the DNA of tumor cells changes by point mutations, by gene amplifications, by translocations, as well as by gene deletions, which I haven't shown you but also occur. And together these mutations are found in virtually all human cancers. Sometimes RAS, sometimes in growth factor receptor, sometimes in transcription factor, but one place or another you would typically find a mutation in this pathway. So signaling proliferation is key to tumor development. And that's not too surprising given how we think about how cancers arise. But these oncogenes, these positive regulators of tumorogenesis are not the only genes that are important. Normal cells do, in fact, use these signals. And I've equated them with like the gas peddle on your car, go signals, which cause normal cells to divide when it's appropriate to divide during development or injury repair or cell replenishment. But there's another class of genes that turns on when it's appropriate for cells to stop dividing. So these genes are the equivalent of the stop signals which would impose themselves when cell division should cease. Now, oncogenes promote cell division. Too much oncogene product, a mutant oncogene product will cause normal cells to divide too often. But in addition to those we find mutations in the stop signals, the signals that would normally halt proliferation. And this allows an even greater accumulation of cells. The go signals are the equivalent of oncogene mutations and the stop mutations are the equivalent of another class of genes known as tumor suppressor genes. Tumor suppressor genes have been known for about 20 years now. And the first one comes from this tumor here. This is a child with a tumor called retinoblastoma. It's a tumor of the retina. It's actually really rare. It occurs in about one in 40, 00 births in the general population, but there are individuals who are familialy predisposed to retinoblastoma. And in these kids 90% of the time they will develop retinoblastoma actually affecting both eyes. So can be actually a fairly common and severe disease in that sense. This gene, the gene responsible for retinoblastoma was cloned, again in Bob Weinberg's lab several years ago. And we now know that it's an important cell cycle regulator, as is indicated here. So the retinoblastoma gene encodes a retinoblastoma protein. The retinoblastoma protein is called pRB. And, as is illustrated on this slide, the RB protein is a negative regulator of the cell cycle. As you recall, cells normally cycle from M phase to G1/S phase through again. And the RB protein acts normally to restrain cell division. It blocks cells in the G1 phase of the cell cycle when it's active. When cells receive signals from growth factor pathways they inactivate the RB gene through phosphorylation, the RB protein through phosphorylation. And this occurs by cyclin-CDK complexes, which I told you about in the cell cycle lectures. There are actually two different cyclin-CDK proteins which phosphorylate RB and inactivate it. So when you need to divide you inactive this brake on the cell cycle and the cells can divide. When you need to stop dividing you activate certain growth inhibitory pathways that inhibit the kinases that block the phosphorylation of RB so RB stays in its active state. And now the cells cannot divide anymore. So RB is a molecular brake on the cell cycle. RB mutations, and mutations in other components of this pathway occur in about 90% of human tumors. It's a very, very commonly affected pathway in tumor development. What kind of mutations would you expect to see in the RB gene in human cancer? Would you expect to see gene amplifications, translocations that cause too much of the RB protein to be produced? Does that sound right? Remember, it's a brake. So what kinds of mutations do you expect to find? Nonsense mutations. Or more generally inactivating mutations. Nonsense mutations. Maybe deletions which would remove the gene all together.

generally inactivating mutations. Nonsense mutations, maybe deletions which would remove the gene altogether. Now, are these mutations dominant or recessive? Inactivating mutations are recessive. So what's going on here? We all have two copies of the RB gene, and yet we're saying that the mutations that take place in this gene are loss of function mutations, inactivating mutations. So how is it that you ever find RB mutations in cancer? Well, the answer is that one mutation is not enough. One mutation of one copy of the RB gene is not enough. For tumor suppressor genes were the mutations are recessive, you need two hits. Two mutational events are required to now fully deprive the cell of the brake. One mutation is not enough. And this is now known in the field as the two-hit model of tumor development, at least for tumor suppressor genes. And it's illustrated here. Because we carry two copies of all of our genes, two mutational events are required to eliminate the function of a tumor suppressor gene. And this is what it looks like. If this is a normal cell which has two normal copies of the RB gene, the first thing to happen is that one copy of the RB gene incurs a mutation. It might be a nonsense mutation or a deletion, but whatever it is it eliminates the function of the RB gene. But this cell itself is normal because it still has one normal copy of the RB gene and this is a recessive mutation. So this cell here is actually normal, but it's one step away from lacking the RB gene all together. And there are a number of ways that that second copy of the gene can be lost. And they're illustrated by these different arrows. The simplest thing to think about is that that second copy of the RB gene picks up its own mutation. We call that a de novo mutation. And now you have a cell which has two independently mutated copies of RB, and that's a cell that now has lost control of proliferation. But there are other mechanisms, which are illustrated here, where the second copy of the RB gene is actually lost all together because the chromosome that carries that gene is itself lost giving rise to a cell that only has one copy of the chromosome. And it's the one that has the mutant RB gene. Or there's a recombination event which replaces the good copy of the RB gene with the bad copy of the RB gene giving rise to a cell that is lacking functional RB once again. And this term here is called loss of heterozygosity or LOH. And it's a hallmark of tumor suppressor gene mutations. It's an indication that there's a tumor suppressor gene mutation in the region. It's usually detected not by a change in the tumor suppressor gene itself but rather by some linked polymorphic marker like a SNP which is close by to the RB gene or whatever tumor suppressor gene it is. This individual has a big A, little A, and therefore is heterozygous. He or she is heterozygous, big A, little A. But if you notice in the tumors, if the tumors arise by chromosome loss or by mitotic recombination, the individual has lost the little A allele. Either there's only big A or there are two copies of big A, in which case we've seen loss of heterozygosity, LOH. And that's a common feature of tumor suppressor gene mutations. And I suspect you'll hear about it in problem sets and beyond. So LOH is an important mechanism which allows us to go from one hit to two hits. OK. Now, RB is not the only tumor suppressor gene that's mutated in cancer. And proliferation is not the only process that affected in tumorigenesis. As I told you, at the end of the day in cancer we care about how many cells we have. And you can have too many cells by increasing proliferation. And the RAS mutations and RB loss are examples of how you can have too much proliferation. But you can also have too little cell death. And we actually have examples of both oncogenes, which are activated to prevent cell death, and tumor suppressor genes that are lost to prevent cell death. So let me tell you a little bit about those. The first gene that was associated with apoptosis and cancer was this gene called Bcl2. It's related to one of the first apoptosis genes discovered in the Horvitz lab here at MIT, and it's involved in regulating caspases. You've probably forgotten by now but caspases are proteases that are kept normally inside your cells in an inactive state. And they get activated by cell death signals. Signals that would trigger the cells to commit apoptosis which causes the caspases to become active. And this then leads to the program of cell death. The Bcl2 gene functions to block, downstream of the death signals, the activation of caspases. Is Bcl2 an oncogene or a tumor suppressor gene? Does it get activated during cancer or inactivated during cancer? Activated. You want to suppress cell death in tumorigenesis. And Bcl2 does that, so Bcl2 gets activated. It's an oncogene. And it's typically activated by translocation, in the cases where it is involved. Translocation, like with myc, too much Bcl2, inappropriate cell survival. Another very important gene in tumorigenesis that regulates apoptosis is p53. p53 is actually the most commonly affected gene in all human cancer. 50% of all tumors -- -- carry p53 mutations. That's a remarkable number. And p53, we now know, is an important regulator of apoptosis. It doesn't *only* regulate apoptosis but it is an important regulator of apoptosis. p53 is like a molecular policeman inside your cells. It's present at very low levels normally, and it's responsive to various stress signals. When cells get stressed, for example when their DNA gets damaged or they recognize that they're proliferating abnormally or they're starved of oxygen, the levels of p53 protein go way up. And when that happens the cells either arrest to try to repair the damage or they will commit suicide killing themselves so they cannot go on to become life-threatening tumors. So is p53 an oncogene or a tumor suppressor gene? Is it activated in cancer or inactivated in cancer? Inactivated. It's a tumor suppressor gene. Cancer cells don't want to die so they get rid of p53 to prevent the death. So p53 is a tumor suppressor gene and a very frequently mutated one in human cancers. You can study p53 in a variety of ways. My lab has been studying it for a dozen years using mice that carry mutations in p53 that were generated by gene targeting technology. You don't need to know the details of this, but suffice to say it's possible to create mice which are either heterozygous for a p53 mutation or homozygous for a p53 mutation. And the question we asked in those experiments is would these heterozygous mutant mice or homozygous mutant mice be cancer prone? Should they be? Would you expect an animal that's lacking p53, one copy or both, to be cancer prone? It's a tumor suppressor gene so, yeah, absolutely. And sure enough it is. If you look at a population of mice and plot their percent survival as a function of time, one year, two years, three years, mice live about three years, normal mice with two copies of p53 will live a normal lifespan. Mice that have one functional copy of p53 die sooner. They live about a year and a half. And if you look in the tumors of these mice, the normal copy of p53 is missing, which is the prediction of the two-hit model of tumorigenesis. You need to get rid of both copies. And p53 minus-minus mice only make it about four to six months and die from cancer. So p53 is an incredibly important tumor suppressor gene. If you're mutated in it, your risk of getting cancer goes way, way up. OK. Now, in the last five minutes I just want to mention the fact that cancer, while typically a sporadic disease -- -- by which I mean there's no family history -- -- about 5% of the time there's familial predisposition. Cancer gets

passed through the family, through the generations. And you can see that in this pedigree here. This is actually a pedigree of familial retinoblastoma. The individuals who inherit the mutant copy of the gene most of the time will develop the tumor, and they'll pass along that predisposition to their children. If you were to look at this pedigree for a while, you would see that it looks like an autosomal dominant pedigree, if you remember back to that portion of the course. If you inherit the mutation, you have a high likelihood of developing the tumor. And it doesn't matter whether the mutation is coming from your mother or father or whether you're a boy or a girl. What's surprising, despite that fact, is that most familial cancer syndromes like that one are caused by tumor suppressor gene mutations. So what's being passed through the generations here is a mutant copy of a tumor suppressor gene, the RB gene, or the breast cancer susceptibility gene, or colon cancer susceptibility gene. A loss of function copy of that gene such that these individuals who are developing cancer and passing the mutation onto their kids are heterozygotes. And, as you think about it, you have to wonder why are they cancer prone? Most of their cells should be normal because most of their cells are heterozygous for the mutation. But, importantly, those cells are one step away from mutating the other copy of the gene. In contrast to the general population, it carries two normal copies of the tumor suppressor gene. And in whose cells two mutational events have to happen in succession to mutate the first copy and then the second copy. In these people all of their cells carry the first mutation. They're only one mutational event away. And the likelihood that that will happen in the retinas of their eyes or in the developing breast or colon is sufficiently high that there is a near certainty that they will develop cancer. OK? So what event must happen during tumor development? A mutation in the second copy of the gene, one of these LOH events or a second de novo mutation. I also wonder what might explain the fact that this gentleman here, who must have the mutation because he passed it onto his sons, did not himself develop the tumor. Why is that? What's going on in that guy? Excellent. That's exactly the way I phrase it, he was lucky. In his developing retina by chance no cell underwent the second hit, and so his cells were just like normal and he developed normally, but he still passed the mutation onto his children and they weren't so lucky. And, finally, what would happen if two individuals who were heterozygous produced a homozygous mutant offspring? What do you think would happen in those individuals? Well, if they survive they should be mighty cancer prone because no mutational event is required to eliminate the function in them. They might look like these p53 minus-minus mice. In fact, most tumor suppressor genes are required for normal development. So you cannot do that experiment. The embryo would never survive because the gene is important for proliferation control all over the place. So the embryo doesn't make it. So most of the time you cannot do that experiment. And, finally, I'd mentioned last time and I just want to emphasize that the genes that we've focused on for you in this class are genes that involve proliferation control and cell death. And we'd mentioned briefly DNA damage control. But there are many other processes that are important in tumorigenesis that are responsive to mutations in other kinds of genes like the process of invasion and angiogenesis and the all important process of metastasis. So I'll stop there.