So we're going to talk about prions today and prion diseases which is a fascinating subject and one, again, of potential medical significance. We'll see how it plays out in time, but there's a great deal of concern out there about prions and the diseases that they cause. Before we do, I mentioned last time at the end of the lecture about the hope that we can develop vaccines for HIV. And there's a great deal of effort going on in this country and around the world to do that. That would clearly be the best thing to do, to prevent HIV rather than trying to treat it. And there are a number of strategies that are underway. Some have been tested in animal models of the disease. So far nothing has worked. So there are no vaccines or effective vaccines for HIV. And this just gives you a list of what's being tried. And I put this up in part to remind you of the traditional vaccine strategies. More or less everything that can be tried is being tried for HIV, including the development of what we call attenuated strains. Strains of the virus which, in theory at least, should be able to replicate but might not be pathogenic. We can take the virus, cut out some essential genes. It will still replicate but it won't cause disease. In theory that would be helpful. One could also take the inactivated HIV approach. Take some live virus, kill it either with heat or with, say, formaldehyde, and use that as the immunogen. Once could take the component vaccine strategy, that is using recombinant DNA technology, purify particular bits of HIV like the envelope glycoproteins, GP120 or GP41, and inject those in the hopes that somebody makes an antibody response against those. One could use heterologous vaccines like the old vaccinia smallpox example. Because, in fact, there is a related virus to HIV called SIV. It infects monkeys so it's called simian immunodeficiency virus. It's quite similar. And it's been tried. Again, not so far successfully, but it's been tried as a potential immunogen for HIV. And finally recombinant vaccines. To put HIV genes into some other virus like vaccinia in the hopes that, again, the virus will replicate and make some HIV proteins to which the body makes an immune response which might be protective. These have not worked. And there are certain problems that have held things up. Firstly is safety. There's a lot of worry out there that if any of these strategies aren't fool-proof, you might actually expose somebody to a dangerous virus. Let's say you think you're attenuating HIV but you're really not or you think you're killing HIV but you're really not. That might be very, very dangerous. So there is a lot of worry that goes into these strategies. But more importantly is efficacy. So far nothing has worked. And the reason we think it doesn't work is the same reason that we've become resistant to, rather become sensitive to flu virus that we reviewed last time. HIV, like flu, changes very rapidly. And there are a lot of HIV strains out in the world. And so if you get vaccinated against one, it might be protective against that one, but it's not going to be protective against all the other ones. So, so far nobody has come up with something that will lead to resistance sort of across the board, across many, many strains. OK. So the subject for today is prion diseases. This is a photograph of a cow being slaughtered because it carries a disease called bovine spongiform encephalopathy or Mad Cow Disease. And it's an example of a class of diseases which are called Prion Disease or transmissible spongiform encephalopathies, clearly the longest and most difficult to pronounce word we have used in this class. Transmissible, it can be infectious, or they can be infectious. Although, as you'll see, not all of this class of diseases is infectious. Spongiform, because it causes sponge-like pathology. And where does it cause the sponge-like pathology? In the brain. OK? The amazing part of this story is the agent which causes this disease. Everything else we've taught you about in this course follows the standard central dogma of molecule biology. The organisms has a genome which can be made out of DNA or, as you saw in the context of some viruses, RNA. The genome then gives rise to RNA in the context of transcription and protein. And the protein then assists in the replication of the genome. OK? Prions, which cause these diseases, have no genome. They have no genome. No RNA. No DNA. No nothing. They're protein only. And yet they replicate. This is an amazing story, which is really rather new, largely ignored and treated with some derision by the field, but now known to be true, at least in many respects. And the diseases are not just of cows. The original disease or at least the first one that was studies is a disease of sheep. It's called Scrapie. And I'll tell you a little bit more about it. There's another disease very similar of cows. Again, bovine spongiform encephalopathy or BSE, commonly known as Mad Cow Disease. The cows go a little crazy and die. And then there's another disease, actually a class of disease of humans. This one is called Creutzfeldt-Jakob Disease, but it's not the only one. And, again, they have the same characteristics of sponge-like morphology pathology in the brain. So these are important diseases agriculturally. They're important diseases of humans, although rather rare, but the biggest worry is it appears that you can get Mad Cow Disease from an infected sheep and you might be able to get Creutzfeldt-Jakob Disease from an infected cow. And you might remember a few years ago there was a slaughter. And I just showed you one picture of hundreds of thousands of cows in Great Britain because there was an outbreak of Mad Cow Disease, and there was an emergence of some patients who developed Creutzfeldt-Jakob Disease. The worry being that you could transmit from the infected cow the development of this disease. OK. This just shows you some pathology. Again, one similarity between the diseases of sheep, cows, humans, and here's another human disease, is this sort of sponge-like change in the context of the brain. These vacuoles which are caused by the death of cells. And, obviously, if you have dying cells in your brain that going to lead to motor defects, as well as cognitive defects, both of which occur in this disease. You might also notice, especially here and here, there are buildups of plaques. These are protein aggregates. And we actually think it's the buildup of those protein aggregates that kills the cells. And they turn out to be important in the story. OK. So let me tell you a little bit about the history of this, which is itself fascinating. So, as I said, the first disease that was studied was Scrapie. And it was observed in around 1900. The animals had this phenotype of scraping their skin and in scraping off their fur. That's why it's called Scrapie. But in general they also had reduced coordination. And it was eventually fatal. And importantly it seemed to occur in herds. So something in the environment of the herd, perhaps the agent that was responsible was transmitting the disease from one effected sheep to another. And it had, as I showed you up there, characteristic morphology in the brain. So this was studied. And it was considered to be an important disease in the farming community, especially in Europe, most particularly in the United Kingdom. But it wasn't largely known. More or less confined to

especially in Europe, most particularly in the United Kingdom. But it hadn't largely moved more or less confined to that community. Until some investigators began studying a disease of humans called Kuru. This was a disease of a particular tribe of peoples called the Four Highlanders in Popua, New Guinea. These individuals developed a disease which was rather similar to Scrapie. Reduced coordination. They also developed dementia, which may well have happened in the sheep but you wouldn't have known it. It was also fatal. And it had the very same brain pathology. And it also seemed to be transmissible because it occurred within tribes. So investigators now started to study this disease. And they discovered, in fact, that the individuals who developed this disease had engaged in -- -- ritualistic cannibalism. Individuals who died of this disease were prepared for burial. And, in the process of preparing the body for burial, parts of the body was eaten. That was the way they celebrated the death of people in this tribe. And they were able to link the ingestion, specifically of brains of infected or affected individuals, with the subsequent development of this disease. And that suggested that there was an agent that was being passed from the affected individual to the individual who became affected, suggesting again that there was some sort of agent responsible. When the etiology of this disease was figured out, the tribe was informed and the process was stopped. So in the late 1950s there was no more ritualistic cannibalism and indeed the disease went away, but it continued to be studied by investigators. And in 1966 they succeeded in transmitting it to an animal species, to monkeys. It took a long time, several years for the monkeys to exhibit the symptoms of the disease. And it was then attributed to what was called a slow virus. It was known to be very small because you could filter the material before injecting it into the animal. So it was smaller than a cell and it was assumed to be a virus. And, actually, the investigators who made that discovery went on to win the Nobel Prize for their Ã¬discovery of the slow virusÃ® responsible for this and possibly related diseases. Well, that was all well and good, but it was very difficult to study this disease using this experimental system. It took a long time. It's not easy to do experiments in monkeys and so on. And so it was necessary to find new experimental systems. And this was pioneered by an investigator at the University of California, San Francisco by the name of Stan Prusiner. And his goal was to develop an assay for the agent that caused Scrapie. He called it the Scrapie agent. And he probably started by assuming that this was some sort of virus, one of these slow viruses, which has not been seen by anybody before but just been inferred to exist. And so what he did was to see whether he could transfer the agent from a sheep by taking an extract of the brain of an infected sheep and injecting it into the brain of a hamster. And sure enough that led, in time, to disease. And the disease manifestations looked very much like Scrapie itself. So now he had an experimental system. And the time it took to get the disease in the hamsters was relatively short, just a couple of years as opposed to maybe ten years in the case of the monkeys. He went further and he took a brain extract from the infected hamsters and he injected it into mice. And they, too, eventually developed disease. And importantly, over the course of this protocol, the time to disease development -- -- reduced each time. It took less and less time for the disease to be seen. And so by this point you could do assays in a couple of months. And that's illustrated here. So, again, Prusiner took the brains of infected sheep, transferred them to the hamsters initially. It took him one to two years to develop the brain pathology. And then he took extracts from them, transferred them to mice, and now it was only six months to develop the same sort of lesions. In doing this he was also able to demonstrate that whatever the agent was, whatever it was, in this assay system it could replicate. So how did he know that? Well, he took -- -- a certain amount of diseased brain extract, certain number of milligrams of diseased brain extract, and he extracted from it a certain infectious dose. And that amount was enough to cause disease in the injected animal. If you used less than that it wouldn't cause disease. If he used more than that, that was more than enough. So he injected it into the brain of a mouse, he waited six months. He then extracted the diseased brain, and asked how much material was there? And the answer was at least a thousand-fold the initial infectious dose. So the only way that could happen is if Ã¬the stuff' that was responsible for this, in this time period, was able to replicate. You had more at the end than you had at the beginning, so it was able to produce more of itself. Again, this thinking was that it was probably some sort of virus, the virus was replicating inside the brains of these animals, and at the end of the disease process there was a heck of a lot more virus than when you started. Not a big surprise in that respect. Well, he then used this to try to purify further the agent that was responsible to get at this virus, if that's what it was. So he used this assay. He took fractions of diseased brain and split them up in different ways using different protocols for purification in order to find the thing that was responsible. So he took, for example, an un-pure diseased brain. And he ran that out on a protein gel. Now, of course, in a brain you're going to have lots and lots of proteins. Some will be more abundant than others, but there will be lots. In fact, there will be a big smear of proteins here. You really won't be able to see a pattern. If you took that stuff and purified it somewhat, and he knew that this fraction, this partially purified fraction carried this Scrapie agent because if he transferred into a mouse, that mouse developed disease. And then he looked at the protein composition of that. Now some of the bands went away and some of them stayed. And then he took that stuff and he purified it still further. And now he could see only one band, one protein band. He took that out and it was found to be infectious. It would appear that the protein was enough to cause infection. Now, the skeptics said that's ridiculous. Maybe the protein co-purifies with the thing that causes infection, but there has got to be a virus in there or a bacterium. There has got to be something in there, not just a protein. Proteins cannot replicate themselves. But Prusiner persevered. He purified this to the point where he could sequence it. And, much to his surprise, when he purified that protein -- -- he found that it was derived from the mouse genome. The protein, which he now believed was responsible for the development of the disease, was encoded by the host organism, not by some as yet uncharacterized infectious agent. So that was an important breakthrough. There were still lots of skeptics who said again maybe so, maybe it's there, maybe it's coming from the mouse, but there is something else going on here. And I have to tell you that right around this time I took a class from Stan Prusiner. He was at UCSF. I was a graduate student at UCSF. And he presented us with his findings right around this same time. And we basically laughed at him. We literally did. We mocked him. We thought it was a joke that you could suggest that some protein was causing the disease. And moreover it was replicating. It seemed ridiculous to us. Again, he ignored us. A good idea. And he asked all right, is this agent which I can purify so well, does it carry a nucleic

ignored dogma. A good idea. And he asked all right, is this agent which I can purify so well, does it carry a nucleic acid? One way to do that is to expose the agent to radiation. Radiation damages DNA and RNA. If this organisms, or whatever the hell it is, has a DNA or RNA genome it should be affected. Bacteria are affected when you expose them to UV. Bacterial viruses are affected. Genes in your cells get mutated. The Scrapie agents laughs, doesn't care. You can radiate it till the cows come home. It doesn't matter. Again, suggestive that it didn't have a genome. So he's got a protein that he thinks is causing disease but he has no idea how. He then did another important experiment. He found that the Scrapie-associated form of this protein was actually different somehow from the normal form of the protein made in the cells of the organism. And he found that by looking at the resistance of the protein to protease. So he purified the protein now not just from Scrapie-infected brains but also from normal brains. So we have the normal version of the protein and the Scrapie version of the protein. He called these proteins PrP for prion precursor. We'll come to the definition of prion in a moment. And they could either be normal or Scrapie associated. And the Scrapie associated he gave the abbreviation PrPSc. And, as I said, he exposed these to different conditions looking for a difference, and particularly exposing to proteases. If he takes the purified protein and runs it out on a protein gel, in the absence of protease he sees the band. OK? Like we saw over there or up there. In the presence of protease, the cellular version of the protein is sensitive so there is no band. It gets digested by the protease. The Scrapie associated version of the protein in the absence of protease is there, but in the presence of the protease is still there. So the Scrapie associated form is protease resistant. It is somehow different from the normal. And maybe it was that difference, whatever it was, that caused it to be disease-causing. So based on this collection of evidence and a little bit more, Prusiner set out the Prion Hypothesis that Scrapie, as well as other TSE, transmissible spongiform encephalopathies, are caused by a protein-only infectious agent, which he renamed prion. The disease causing protein is an altered form a cellular protein which can cause the cellular protein to adopt the altered conformation, and in this way the prion can replicate. He could explain the production of more of this stuff by suggesting that the interaction of the altered form with the normal form could turn the normal form in the altered form. And, as I've told you, he called the normal protein PrP and the Scrapie associated PrPSc. So his hypothesis was that there is a normal cellular protein which has a conformation. And this is PrP. And there was some evidence, based on analysis of the structure, although not 3-dimensional structure of this protein, that it was largely alpha-helical. And it was protease sensitive, as I said. And if you ever need to remember which form is which, another mnemonic, it's alpha-helical, remember helix=happy. He suggested that this form could convert, it could change it conformation to a form that was associated with Scrapie. It had a different structure. It was more of a beta pleated sheet. It was protease resistant. And, again, if you want to remember the beta pleated sheet is bad. OK? So there were two forms. But more importantly than that he suggested that if you have this form it can convert this form into this form. So the model was that if you have in the same cell, PrP in the Scrapie form, plus normal PrP, the normal gets converted to Scrapie. And diagrammatically -- -- perhaps through some sort of intermediate in which the two proteins interact, the abnormal form can change the conformation of the normal form giving rise to two abnormal forms. And if this continues and continues and continues in an infected brain, you're going to have a buildup of this abnormal form which maybe aggregates and maybe causes the brain's cells to die. This is a little more detail. Again, this is a theoretical 3-dimensional structure of the cellular form, the normal form of PrP. See the alpha-helical structure? And this is the proposed beta pleated sheet structure of the PrP Scrapie form. Note, the beta pleated sheets. And the idea was that the abnormal form, shown in blue, could interact with the normal form, shown in red, and convert the normal to the abnormal. And in so doing perhaps created these aggregates which built up in the brain's brain cells and caused them to die. So that seems reasonable. And, as I said, a great deal of evidence went on to support it. There was one additional definitive experiment about the nature of the agent which comes a little bit later in the story. And it was really after that, that Prusiner went on to win the Nobel Prize. So, as I said, there are several human diseases. This is not just a disease of sheep. There are several human diseases that look similar, and we now believe are all caused by this exact same mechanism. There's Kuru which is infectious. If you eat the brain of somebody with Kuru you will get Kuru, which is not a good thing to get. It actually stands for, I believe, this name means Ã¬laughing deathÃ®. These individuals become demented and then die. So Kuru is infectious. The one that's been in the news more recently is Creutzfeldt-Jakob Disease. And this can be caused by different mechanisms. You can get it through iatrogenic exposure. Does anybody have any idea what iatrogenic means? This is one of my favorite words in medical terminology. It means a disease you get in the hospital. OK? You go in with one problem, you come out with a worse problem. OK? Iatrogenic. And specifically the way that some of these patients, and it's a very, very small number, I assure you, developed Creutzfeldt-Jakob Disease is they went in for an electroencephalogram where they put a brain probe into your head. Unfortunately, take, can I help you? Hello? Someone's watching me. [LAUGHTER] Not a good topic to have someone watching you teach. Brain probe in the head. OK? Several years later the person develops Creutzfeldt-Jakob Disease. Go back, as who used that brain probe last? Turns out it was somebody with Creutzfeldt-Jakob Disease. The brain probes are stored in formalin, a fixative, it doesn't kill the agent. It's remarkably resistant to almost anything you could do to it. And so these patients, regrettably, got exposed to that thing. Another way is through corneal transplants. Every once in a while somebody with a corneal transplant develops Creutzfeldt-Jakob Disease because the cornea came from somebody who had or went on to develop Creutzfeldt-Jakob Disease. There are also sporadic forms. So sometimes people just get Creutzfeldt-Jakob Disease as though spontaneously. Their normal PrP protein flips to the Scrapie form. And once that happens it's catalytic, it keeps happening. And then, interestingly enough, there are familial forms, familial forms of CJD. It runs in some families. They're not very common but there are some. And there are two other diseases, which I'm not going to write out because you don't need to know their names, but one of them is called GSS and it's also familial. And another is called FFH. FF, sorry, FFI. And it's also familial. This is a funny one. Not funny if you have it, but the FFH stands for fatal familial insomnia. Now, I've had insomnia sometimes I feel like dying at the end of it, but these patients actually do. This is a degenerative disease associated somehow with insomnia, and it's ultimately fatal. GSS has some similarities with Creutzfeldt-Jakob Disease but it's a little bit different. And

and it's ultimately fatal. CJD has some similarities with Creutzfeldt-Jakob Disease but it's a little bit different. And so all three of these diseases have similarities, but they also have their own unique features. And interestingly they all have a common cause. All of the familial forms of these diseases have a common cause. Can anybody tell me what it is? Why might you get this disease? Something in your genome, why might you get it? They carry mutations in the PrP gene. So if you sequence the genome of these patients, you find that they carry a PrP gene which is different from the normal one. And the different diseases carry different mutations in the PrP gene. Some of them cause this manifestation. Other mutations cause this manifestation. And still other mutations cause this manifestation. But it's all more or less the same. PrP protein is flipping and causing other like proteins around it, other versions, normal versions to flip, too. The fact that there are differences is intriguing and a little bit hard to explain. I don't think there is a satisfactory explanation for how different mutations in the same gene cause apparently different conformations. And those different conformations can be propagated faithfully upon interaction with the normal protein. So here's a diagram of that. Here's the point mutant, let's say it's CJD. And here's a point mutant FFI. It's not the same point mutation. This one folds in its abnormal conformation into this structure. This one folds in a slightly different structure. And maybe this can propagate its structure, and it can propagate its own structure on interaction with the normal protein. And those different structures may be aggregated different subsets of cells or the different cells are sensitive in some ways to these different structures. So some die in one disease, others die in a different disease, and that's what causes the different manifestations. And so I've given you examples now of these diseases which are caused by the same general mechanism, although with slightly different etiologies. In the case of Kuru, you can ingest an abnormal copy of the protein. It makes its way to the brain, interacts with normal protein and converts it to abnormal. There are probably examples of spontaneous or sporadic conversion of normal protein to abnormal protein. Again, a process that gets propagated. Or you can carry a mutation which will increase the likelihood that this conversion takes place. And when it does it gets propagated inside of you. And maybe sometimes people pick up sporadically a mutation in their PrP gene which causes those cells again to flip this conformation more rapidly. So lots of different mechanisms but the same basic idea, PrP becoming abnormal and causing cells around it, or rather proteins around it to become abnormal, too. Why has this caused increased attention recently? Because of the outbreak of this related disease bovine spongiform encephalopathy or BSE. This is a story from about five years ago. It was just after the height of the fear of the increase in the incidence of this disease in Great Britain. Some of the cows in Great Britain had made their way to other countries and, therefore, were slaughtered. And throughout Great Britain cows were slaughtered in remarkable numbers to try to limit the scope of this disease. Why were people so worried about the outbreak of a disease in cows? Because it turned out, and this just shows you the outbreak. This is the number of BSE cases in this timeframe. And you can see the number of affected cows dramatically increased. The reason that they dramatically increased is that the cows were being fed parts of infected sheep. The sheep had the disease, or some of them, and they passed it onto the cows. And then the cows developed the disease. When they noticed this practice, they started prohibiting the use of other animal products in the feeding of these cows. And so eventually the incidence dropped. And these are various safeguards that were put into place at different times to limit what the cows actually got exposed to. And nowadays there are very strict practices about what you can feed your cows and your sheep to try prevent the spread of this disease. And it's not just a spread of the disease in cows, but here in this pink line tracking with the incidence of BSE that was going up around this same time period was the incidence of CJD in the pink. And indeed it was documented that these individuals developed this disease because of prior exposure to infected meat. So you can get this disease through exposure to an animal that carries this disease. However, I need to tell you it's still rather rare. It doesn't happen very often so you don't need to panic. In fact, there was great fear that this would skyrocket after 2000 because it was feared that there was some incubation period taking place and maybe lots more people would develop the disease. That turned out not to be true. If you look at the peak in 2000, it actually drops back down again. So the epidemic of CJD that was feared actually didn't materialize. Still, there are great fears that any exposure could give you some risk of developing this disease. And you may remember just earlier this year, a big scar in this country because two cows in Canada were found to have BSE, and maybe that cow meat had made its way into our food supply. So, again, there's tremendous worry out there. But it's important for you to know that the incidence, even for people known to be infected, is very, very small. And because it often comes up, how do you go from ingesting something to get a disease in the brain? We now think that the cells in the gut, and probably dendritic cells, cells of the immune system pick up the Scrapie protein and transfer it up to the brain. And once there somehow it gets into the neurons and then spreads from neuron to neuron, thereby causing the disease in the brain. OK. The last thing I want to tell you in the last five minutes is that there seems to be some form of species barrier. So if you get some human Scrapie protein, PrP Scrapie, the likelihood that you're going to get disease is pretty high because that protein interacts well with your PrP protein. But if you get a PrP Scrapie protein from a different species, it doesn't interact so well with yours. And this is called a species barrier. And you can see it's shown here. If you take a hamster with an infected brain and give it to a mouse, it takes six months for that mouse to develop the disease. OK? It takes a while for the hamster PrP Scrapie to interact sufficiently with the mouse protein, which is present in the mouse's cells, to give you disease. But if you now take this preparation which is PrP Scrapie from mouse, because it's the mouse protein that's been converted here, and inject it into mouse, now it happens much, much faster. So there's a species barrier. And once it's been passed through one species, you overcome that barrier. Now, how could you test this notion that there's a species-specific difference. Well, the way it was done by Prusiner and others is to introduce a hamster gene into this mouse here. So this mouse now makes not just mouse PrP but also hamster PrP. And what would be the expectation for that experiment? If you now take this transgenic mouse that carries a hamster PrP gene and introduce the Scrapie agent from hamster, what would happen? It would happen faster because now you could template off of the hamster protein, and sure enough this animal develops disease faster. And when you purify the PrP Scrapie from that infected brain, it's composed of both mouse and hamster. OK? Now, what would happen if you made a

that infected brain, it's composed of both mouse and hamster PrP. Now, what would happen if you made a mouse that didn't have a PrP gene? You knocked it out. A, you might be surprised if that mouse lived because you might think that the PrP gene is important for something. Well, it turns out you can make a mouse that lacks PrP all together. You can delete the PrP gene. So let's say you had a PrP deficient mouse and you introduced the Scrapie agent into it, what would happen? Well, you need a PrP protein to be converted in order to develop the disease. If you don't have the gene, you don't have the protein, so it cannot be converted. And sure enough that's the case. If you do this experiment, you propagate some PrP in a mouse and then transfer it into a mouse that's lacking PrP altogether, the animal is disease-free. And this was the experiment that really sealed the deal and led to the Nobel Prize for Prusiner. At this point nobody could really argue that it was the cellular protein being converted in the absence of any genome that was responsible. So the problem doesn't go away. Here's another species which has been observed to have another one of these diseases. It's clearly a Scrapie Disease. It's called Chronic Wasting Disease or CWD. It occurs in herds of deer, as well as in elk. And if you look at the incidence of animals that are affected, there are several herds in our country that are affected. And, again, there's a lot of worry out there, it's a little below the surface now, but there's a lot of worry out there that if you eat that stuff you might develop CJD. Importantly, there are no documented cases, no documented cases so far. There are some reported cases but they've been investigated and found probably not to be associated with exposure to this meet. Nevertheless, there's a fear that the species barrier might be breached in some individuals, and those individuals would develop this disease. So with that I will stop. Thank you for you attention. Good luck on the final. And don't forget office hours. [APPLAUSE]