Dr. Sue Priola Interview

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Maya Ponte: If you can first tell me where you grew up and how you first got interested in science.

Susan Priola: Okay. I grew up in Albuquerque, New Mexico and got interested in science through science fairs in high school. My dad was a scientist. He always did interesting things.

MP: What kind of a scientist?

SP: He was a physiologist, he was a cardiovascular physiologist. So a completely different field, we spoke entirely different scientific languages. But I was interested in it because of that and I got to do high school science fairs and won some scholarships and some money to go to school. I basically decided on science because I liked it and it was more of a challenge than a lot of other things. It was harder – it was actually the hardest subject I had in school, as an undergraduate.

MP: Where did you go for your undergraduate degree?

SP: University of New Mexico.

MP: Once you were there, how did you decide what field within science to specialize in?

SP: Well, I originally wanted to be an archeologist, but then realized that I didn't think I could stand the uncertainty of it all - never really knowing for sure what was going on. I wanted something that I could do more experimentally and I was always interested in biology and microbiology, so I ended up focusing on – I guess the degree is in biology – but I focused on the molecular side.

MP: And did you learn a lot of molecular techniques when you were an undergraduate or did you learn those more in grad school?

SP: No, basically in graduate school. I had never done anything like cloning or that as an undergraduate. I did have one job as an undergraduate where I did some protein work, so I learned how to run protein gels and cleave proteins and stuff. So I had a little bit of experience there, but otherwise it was just the sort of lab stuff that you get when you take undergraduate biology courses. So I didn't learn, really, anything until I got to graduate school about that.

MP: And where did you go to graduate school and whose lab did you work in?

SP: I went to UCLA and I worked in Jack Stevens' lab. He has retired now, but he was a herpes virus guy. He's the guy who first identified that when a person is infected witha herpes virus the virus kind of disappears and goes latent. He figured out what was being expressed from the virus and where the latent virus was located. He was a really big guy in the herpes field. And I did my PhD with him studying a pig herpes virus called pseudo rabies virus.

MP: And how did that background, do you feel, prepare you for working in the TSE research field?

SP: It did not. No, it did because I had some basic techniques behind me, but in terms of dealing with something where you're not entirely sure what it is, you don't have a genome to mutate, manipulate and recombine and study what it does. It doesn't really prepare you for thinking about it, too much. You can start thinking about it along virological lines, which is what I do. But when it comes to doing experiments, you have to start thinking about it in terms of protein experimentation. So it's a bit of a challenge that way, but on the other hand it leaves things so wide open for figuring out new ways to do things and think about things, and that's much more fun than having to stick to some script with a virus, because there's really no script with prion proteins. You make it up as you go.

MP: So in graduate school you were working on a pseudorabies virus. From there where did you go?

SP: Well, from there I came right to here. I got my degree and I didn't actually know what I wanted to do. I knew I was interested in neurodegeneration and neurological diseases, so Dr. Stevens gave me a list of suggested places to go: Rocky Mountain Labs was one of them, there was a lab in Minnesota. Those were the two that I looked into. And he mentioned Dr. Oldstone's [Micheal B.] lab at Scripps. But I sort of liked the idea of studying scrapie, so I interviewed at Ashley Haase's lab in Minnesota and at here. I thought there was more opportunity here for me to do different things. I came out and talked to Bruce and there were a lot of tools that he'd set up that were waiting to be used.

MP: Like what?

SP: They had just put this epitope tag into the mouse PrP molecule so the mouse 3F4 PrP; they had the scrapie-infected cells ready; and they had hamster PrP clones so there was an opportunity there to do recombinant mouse-hamster PrP work and see what happens. There was another thing - he had set up the basic cloning vector which was to look at these insertional mutations, these extra repeat mutations in human and murine TSE. So that was sitting there waiting to be done. So when I came out and interviewed and I saw all this, he said, "Well, I've got three projects," and I couldn't make up my mind, so I said, "Okay, well I'll just fiddle with all of them." And that's what I ended up doing when I came here.

MP: Tell me about the three projects that you initially worked on.

SP: One of them was to take the epitope tagged mouse 3F4 PrP gene and hamster PrP, recombine them, express them in cells, and see if I could then infect those cells with both mouse and hamster scrapie. That was the initial project. When I started, since I knew nothing about scrapie, the first thing I had to do was read the literature and try to figure out what to do. It was immediately apparent from the literature that infecting cells was extremely difficult. So I thought that the odds of that working were probably pretty slim. So instead, I took those recombinants and put them into cells that were already infected and asked if they were converted to abnormal PrP. I used that to map the important regions – the regions that are necessary for PrPres formation.

MP: So you used the SN2A cells that Rick Race had already developed?

SP: Right, had already put together. And I used the system that Bruce had where these recombinants are packaged into this retrovirus, which means that you can get almost 100% of the cells expressing your construct. So it's much more efficient than transfection and it's stable. And that turned out to work. So that's where that project went and I've pursued that in various forms actually ever since.

MP: And is that the experiment where you were able to show that the hamster PrP will protect against –

SP: Right, will interfere with mouse PrPres formation . Exactly. Now Stanley Prusiner's group had published a paper about a year or two after I got here where they did sort of the same thing and showed that the middle part of the PrP was important in PrPres formation, which we also eventually found. But what they hadn't seen was what I saw with the hamster PrP interfering with mouse PrPres formation. And that was kind of cool because it provided an explanation for why it was difficult to infect an animal that was expressing a different PrP molecule. Or why a transgenic animal that had both mouse and hamster PrP was slower – had a longer incubation time because the two competed and interfered. They were both able to bind but both were not able to convert with equal efficiency, so it just slows the whole process down. So that was a bonus from that. The second project had to do with the insertional repeats. The idea there was just to take hamster PrP, which had five of these octapeptide repeats, and increase the number – and this was the vector he'd made, so I could just insert as many extra copies as I wanted. It was really cool.

MP: Bruce had made that?

SP: Bruce had made that before I came. I basically just got to make the expression vectors and the little cassettes to put in – the little extra repeat cassettes to put in. So I made those, I went from up to 15 repeats, and then asked how those numbers of repeats changed the properties.

MP: And how did they?

SP: Well, they – one of the big questions was, as you increase the numbers of repeats could you in fact get spontaneous formation of PrPres? And the answer to that is – Nope!

MP: Okay.

SP: But as you increase the number of repeats they become a little more protease-resistant, but nowhere near – I mean, like 500 times less than PrPres or PrPsc. So nowhere near the resistance we were looking for.

MP: Did you ever try a bioassay?

SP: I actually didn't do that, in part because David Harris' lab actually scooped me on this, I think not once, not twice, but maybe three times. And so by the time I was getting to the point where I could have done that he had a transgenic mouse and was looking at that issue. So it was like, "Nah, I'm not going to do that."

MP: I see, I see. Okay, that's maybe why I'm not so familiar with that, because then Harris then did that –

SP: Yeah, it's really – he's done – yeah, I've only actually published – we ended up publishing a paper on it, and I've actually got enough data for a second paper that shows something that no one's seen. But yeah, so I didn't do that experiment because David Harris was already well along way. But what I was able to show was the increase in protease resistance – it aggregates more as the number of repeats go up. What happens kind of differs between whether or not it's in a fibroblast cell or a neuroblastoma cell, so the properties can change a bit.

MP: What happens?

SP: Well, in neuroblastoma cells – let's see if I can remember this now. So PrP is expressed on the cell surface by this PPI anchor and you can clip it off with this impossible to pronounce enzyme [laughter] and it floats up into the medium. It turns out that when you put on the extra repeats it's much harder to clip off the cell surface with this enzyme at this PIPLC in neuroblastoma cells. But in fibroblast cell it comes off fine. So there was some difference in the way it was being expressed on the cell surface, for example.

And it was a little bit more – even the wild-type was a bit protease resistant coming out of the fibroblast cells. So there were subtle differences like that, which just said the cell type was going to influence how these properties change – it was going to modulate how the properties change.

MP: So this is an early indication to you that cell type -

SP: Oh yeah, that cell type was going to influence things. Yeah, for sure. So that's what we basically ended up publishing, and that was the second project. That was the one project that Bruce gave me that I actually took to completion in the way in which he saw it. The other one, I made a left hook.

MP: Right. [laughs]

SP: The third one was to look at P19 cells which are a differentiable mouse cell lines, and see what happens to PrPsen expression when you differentiate these cells. And it turns out that you can differentiate them to neuronal-like cells, to epithelial-type cells, and there's a third cell type – good lord, I forget.

MP: [laughs]

SP: So obviously this one [laughs] – I did the experiments and it turned out that when you differentiated the cells, PrPse expression went way up.

MP: Okay.

SP: And I actually put together a little paper within a year of when I arrived here and sent it off and it was rejected, and it's still in my file cabinet –

MP: You're kidding!

SP: No, because when it came back rejected I was thinking, "Oh, I can do more stuff with it," but by that time I had other things going –

MP: Right. Because the first one you were describing had already taken off -

SP: Well, that one had taken off, for sure, and I had something else that I'd found that I was dealing with. So I put it in the file cabinet.I keep thinking it's my spare paper for when I have the time [laughs] It's been like 12 years, so. And other people have, shortly after ours was rejected, published on other differentiable cell types in which that happens. So again, it's an instance of, well it probably won't ever see the light of day now because it's really not contributing anything new.

MP: Sometimes they get left in the dust. [laughs]

SP: It got left in the dust and still sits there, and I've forgotten the cells, they're in the back of my mind about what could you do with them in the future.

MP: And what year did you come here again?

SP: '91.

MP: '91? Summer? Fall?

SP: February 5th, 1991.

MP: Wow. [laughs] And you've been here ever since?

SP: I've been here ever since. I came initially as Bruce's postdoc and didn't expect to be here more than five years. So I just got extended, converted to tenure track and what not.

MP: One of the lucky ones.

SP: Yeah, extremely.

MP: Did you go through the whole through the Title 42 system?

SP: Yeah, Title 42. Yeah, because I guess now the policy at NIAID is that tenure track and tenured people are on this Title 42 track. So now I am.

MP: Okay, so what would you say are the most important techniques that you adapted, worked with, or developed in the lab?

SP: Definitely using this retroviral expression system and recombinants to assay PrPres formation in tissue culture, that's a big one that we still use. What other techniques? I guess a lot of the techniques are pretty basic. This more recent one, this cell-overlay one had been a big one. Those are really the two biggest ones I think. Yeah, because I'm not so much new technique-oriented, unless I have to be, as I am just trying to think of what to do with what's available, yeah, and it's not necessarily because I don't like to develop new techniques, but there has to be a really good reason for doing it.

MP: Tell me more about the retroviral expression system.

SP: Well, Bruce had the PrPs cloned, he had them in the retroviral vector. They knew how to produce viruses with them and they'd already done all that. And what I remember, the way I remember it happening, is that when I realized I probably wasn't going to be able to infect these cells with hamster or mouse scrapie - thinking what else could I do with it? - and then wondering could I just use the retroviruses as a delivery system to put the construct in and use that to map where the important determinants are. At that time Byron and I, we shared an office way off in the corner somewhere they'd stuck us. And I mentioned this to him and he thought it was a really good idea and that Bruce had just put mouse 3F4 into scrapie positive cells before I came, which I hadn't known. But that what happened is it shut everything down. So that was the interference thing, another version of this interference. And so they hadn't really pursued it past that.

MP: And they didn't realize what was happening, they just saw that it wasn't working and they kind of dropped it.

SP: Right, they just saw that it wasn't working. They tried to clone a scrapie positive cell that was also mouse 3F4 positive, and they couldn't get one. So it turned out that even though I'd had this thought to do it that they'd already given it – not quite in the way I was thinking of it - but they'd already done something similar. So when I brought the idea up to Byron he said, "Oh yeah, that would probably work." And so that's why I started going that way. So it was just taking the tools that were there and putting them together, to use, in a way that would help me answer my question. So it's not as though I invented all the tools and did it, but I used –

MP: You adapted them.

SP: I adapted, yeah, that's exactly right, that's exactly right.

MP: And then with the cell overlay assay, can you tell me about how that came about - where the idea came from and how you optimized it?

SP: Yeah sure, that was my former postdoc, Ina Vorberg, who was really, really good.

MP: Where is she now?

SP: She's in Herman Schetzel's lab in Munich, in Germany, so she left about a year and a half ago now. She had seen this paper by Christian Korth, who was in Stan Prusiner's lab, where what they had done is they had taken 3F4 tagged PrP, in which they had inactivated the glycosylation sites in a way that would still allow it to be expressed on the cell surface but with no sugars. And they put this unglycosylated mouse 3F4 PrP into cells, and they actually did the brain homogenates thing, put it on top and looked for 3F4 positive PrPres. And, what they found was that they could see 3F4 positive PrPres. They overlayed multiple, different strains. They always saw this, and they concluded that you really needed unglycosylated PrPsen to do this efficiently, and that it didn't work as well with some strains as with others. They made these kinds of conclusions which, at the time, I thought the system was nice – I just disagreed with the conclusions. But she wanted to do this in the lab, and I had no interest in it, because I didn't want to repeat what they had done. So we had this back and forth for a few months where she kept wanting to try it and I'd go, "I don't want to try that!" Because you've got to make the deglycosylated stuff, and what are we going to do with it after that?

So then I was getting really interested in strains. “Okay, now there's something,” I said, "why don't we do this? Don't go making any new clones, just take our mouse 3F4 that's fully glycosylated and see what we can do.” I'd made some cell clones with this, so we had cells that expressed constitutively at high levels, which they didn't do in the Prusiner paper - they just used transient transfection, I believe.

MP: And they weren't able to get it to work?

SP: Well, it worked, but they couldn't get it to work with the glycosylated. So one of the big things they concluded was "unglycosylated is best", which I think is actually right.

So I said, "Well, let's do this: use the fully glycosylated mouse 3F4 PrPsen in the neuroblastoma cells, and put on top of it brain homogenates from these different mouse strains and let's see what their glycosylation pattern looks like."

She said: "Well, that's not going to work! It's only supposed to work with unglycosylated."

But, if it's going to convert - I mean, it happens in scrapie-infected cells! Why wouldn't it work? And she did it, and by god, it worked. And it worked really well. We're like, "Whoa, that worked really well, I wonder why they didn't see that?" I think it's because they didn't use cloned cells. So we went, "Ah ha! Now we have a way of looking at strain-specific glycosylation in cells." And that was really cool. We thought, "Well, as long as it's forming PrPres, let's just passage them." And so that's why we started passaging it – it wasn't to look for newly infected cells, it was just, you know, as long as it's going, let's do it.

So that was in the neuroblastoma cells, and we saw that we could do the kinetics and we could follow PrPres formation from just a few hours after exposure. I thought, "Well" – I had something else I wanted her to try. I had almost all of these recombinants that I'd made for this project that I had started with Bruce in fibroblast cells. They were in these retroviral packaging cell lines. I had very few of these constructs in neuroblastoma cells.

So I told Ina, "Why don't you try this technique on fibroblast cells?"

She goes, "Well that's not going to work!"

And this is the one I should have bet Ina on, because this one, I got her on this one. I said, "Well why wouldn't it work? It's PrPsen."

And again, she said, "because fibroblast cells aren't infectable!"

"We're just looking for acute PrPres formation."

"Oh, all right." So she did it, and it worked! [laughs]

MP: [laughs]

SP: So I wanted to try that because now I knew all the dozens of constructs I made were in these fibroblast cells; I didn't have to make new clones, I could just use those. So when we saw that we got PrPres formation in fibroblast cells, again, we thought, "Well, we'll just passage them." And lo and behold, they were infectable! We were completely

astonished. She came back – she'd done this initial experiment, she started passaging them. She checked the tenth pass or something for PrPres and she gets this blazing signal in fibroblast cells exposed to mouse 22L. And we were like, "You must have made a mistake." No, no, no! She didn't think so. She repeated it and all, but it was there, and it repeated like clockwork –

MP: Were these initial ones done with recombinant fibroblasts or the regular fibroblast cell lines?

SP: These initial ones were done with the fibroblasts that overexpressed mouse 3F4 because the whole point, we were looking at acute PrPres formation.

MP: And then after that, was it then you went back and did the regular fibroblasts and all the different fibroblasts?

SP: Then we – all the other – yeah. So we started thinking, "Well where are these from?" And we figured out they were from NIH3T3 cells so we went across the hall to Pug Evans [spelled phonetically] who is a retrovirologist, "Do you have any NIH3T3 cells?" He said, "Yep." And I infected those and then she decided to try mouse L cells because of this old report from almost 30 years ago that I had heard people say they didn't believe even though I'd read it and it seemed like they'd infected what they called mouse L cells.

MP: What are mouse L cells?

SP: They're fibroblast cells. I forget what they're derived from, but they're mouse fibroblast cells, and they put Chandler scrapie in them. And when I had mentioned this to people they'd say, "Oh, well, that's probably just inoculum." But they gone out 15 passes! So she ordered L929 cells from ATCC and they turned out to be really susceptible – 60% of those cells will be positive after infection, with 22L. So we're like, "Oh my God!" [laughs]. So, unfortunately, I can't claim that it was some linear, very logical way to go, it was more we started with one little idea and it's just ballooned.

MP: And you happened to have certain things in the lab and –

SP: That's right. Let's try this and see –

MP: [laughs]

SP: And so what happens with this is it's made me realize after the last few years – because there were these dogmas, like you have to have neuronal cells or nervous system dervied cells, and you need overexpression of PrP, and it only works well with unglycosylated – all that stuff's wrong. It's completely wrong! So I'm finally at the point where I'm like – if someone tells me it has to be a certain way, I'll just file it away as a maybe and see what happens.

MP: You're not going to take it as dogma.

SP: Exactly. I have no belief in dogma anymore because every time you start pursuing dogma – or it seems, often when you pursue dogma it sort of disintegrates along the way. So it was really fun, it was just a blast to – it was so funny when it worked in fibroblast cells. I should have bet her. I was going to bet her and I didn't. I would have made some money…

MP: And then what are the implications of that? I mean, you talk a little bit about this in the paper, but from a disease management point of view, what are some of the implications of this realization that other cell types can be infectable?

SP: Yeah, for me the biggest one is that - my feeling right now, based upon what we've seen, and we've done this on a couple of other cell types - that no matter what the cell type, if it has PrPsen it will make PrPres acutely. So if you expose it to an infected brain homogenate – which has a lot, admittedly a lot of PrPres activity - you're going to get new PrPres formation. So that is a process that can occur in any cell type. The specificity comes in whether or not the cell becomes persistently infected.

MP: Right, upon –

SP: Upon continuous passage will it become persistently infected, and I think right now it appears that that is going to be dependent upon the cell type and/or the strain in ways that I don't understand. Well, I have ideas about it, but it could be specific factors that are necessary, it could just be that the cell has to be of a particular metabolic type or be doing certain things better – maybe endocytosis more frequently than other cells, I don't know. But there are some cell and/or strain-specific factors that will determine whether or not the acute exposure goes to persistence. And so, in the body, it's possible that the cells that do that the best are neuronal cells, glial cells in the brain, spleen cells, follicular dendritic cells, things like that. The other cell types, maybe they do make PrPres for a spot, but it doesn't catch on and they don't become infected.

So that's what it implies to me in vivo, which is – it's great to say there are cellular and strain specific factors, but we really don't have a clue as to what they might be.

MP: In the future, do you have any plans to try to elucidate what might be going on?

SP: I would like to, but it gets – it's a bit of a fishing expedition. So I'm not quite ready to go – to take the trip. And there are a couple of things, like I'm really interested in the idea that endocytosis is very, very important to infect cells in vitro, not necessarily in vivo but in vitro, because those cells have to be passaged and maintained and if something gets into the endocytic – into some endolysosomal or endocytic compartment - it can be transmitted from mother to daughter cell more efficiently than if it is stuck out on the cell's surface.

So I think that that could be important. And there are ways to look at how well cells endocytose and what happens when you block it. The earlier work from Byron all says that endocytosis is important, right?

MP: Yes.

SP: So this newer stuff that I've got going with hamster PrPres formation cells - I'm getting set to do an experiment hopefully this week - that if I'm right will show that endocytosis isn't important for hamster PrPres formation, because I think all that occurs on the cell surface, and so it never really gets down into the cell. And that would be the first demonstration of that. So, see how I'm approaching it and kind of picking at it, until I take the plunge, which I have not yet done.

MP: All right. And you also mentioned there's something – I think in the end of the paper you talk about the potential relevance for that to the production of vaccines and –

SP: Oh, the fibroblast stuff?

MP: Right, yes, the fibroblast stuff. Do they use fibroblast often for producing vaccines?

SP: Mostly they've used NIH3T3 cells as feeder cells for another vaccine cell whose name escapes me. But for the most part no, they don't. The point that we're trying to make in what was, hopefully in a non-inflammatory way, just because it's not a neuron and just because it has low levels of PrPsen does not mean it's resistant. You really can't predict that until you test it. And I was actually just at a vaccine meeting a couple of weeks ago, a vaccine cell substrate committee, where they had, at the very end, one session on TSE diseases. And they're very concerned, the vaccine producers, even though it's a remote possibility, that if these cells were exposed to fetal bovine serum that might have BSE in it, could they become infected. And so they're very interested in the susceptibility of these cells to TSE.

And so the point isn't necessarily that your vaccine cell lines are contaminated, it's more that you can't say they're resistant until you test them. Because frankly, we could have put all the possible strain cell combinations in a hat, pulled one out [claps], and been just as good at guessing as what we got, because it was completely unpredictable, it was utterly unpredictable.

MP: With the MoPrP 3F4, how is it tagged? Exactly what do you do to tag it?

SP: Okay, what Bruce did is there are two residues at positions 108 and 111 that differ from hamster PrP. So in hamster PrP those residues are methionines, and in mouse I think they're valine and a leucine. And it turns out, all you have to do is change the valine and leucine to methionines and you get the 3F4 epitope.

MP: So that's all that was done in that case?

SP: Yep. That's right, so it's all mouse PrP except for those two amino acids.

MP: And then that allows it to bind 3F4?

SP: Yep, really well.

MP: So the interesting thing about that is that MoPrP with the 3F4 epitope still interacts better with other mouse PrP strains –

SP: Than hamster.

MP: – than it does with hamster PrP. So it allows you to do these experiments but still to be able to detect it, with the 3F4.

SP: Yeah, that's right.

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if you have too high a level, it shuts everything down. If you have some kind of balance between your mouse 3F4 expression and your normal mouse PrP, then both are converted – you see both. But it's sort of this weird kind of balance going on. So it's a little bit confusing in those papers.

MP: Because one could inhibit the other.

SP: Yeah, because it can inhibit, but it can still convert. And if you take the 3F4 epitope, it clearly works, because now we have transgenic mice that have the 3F4 epitope, David Harris made those, and other people have neural 2A cells that express mouse 3F4 that are infected. So, it's just a question of finding that balance, I think, where the inhibition –

MP: So always these cells in these animals are producing some regular mouse PrP?

SP: It depends. Nowadays everybody makes them on a mouse PrP knockout background.

MP: Okay, so now they only have MoPrP 3F4?

SP: Well, or whatever recombinant they've put in. So yeah, the original transgenic experiments that Prusiner's lab published in the late '80s, those first mice he made that had hamster PrP also had mouse PrP. That was where he found that when the hamster PrP was present and you put mouse scrapie in those guys, incubation times extended. But that once mouse PrP knockouts were available, that problem goes away. Because now you've just got one PrP, right? But in these cells, you've got the two of them, and that's just the way it is, and so you have to get to a point where you've got enough mouse

PrPres being produced that it feeds off the mouse PrPsen and things just go and then it doesn't matter.

MP: And then it'll just stay infected –

SP: That's the way I think of it.

MP: I see. So with Prusiner, when he had made the MoPrP 3F4, he had done it in the same way, or – had anyone made it pretty much in the same way?

SP: Yeah, mmm-hmm, yeah.

MP: And you'd mentioned that when he'd made this unglycosylatable form and said that this was easier to infect, that he was right but for the wrong reasons, what were the reasons that were wrong?

SP: I said it was for the wrong reason because the system he was using, he was implying that you couldn't do this if you had fully glycosylated PrP because it worked so much better with the unglycosylated. And that's not true because we were able to do it.

MP: Right. But because he was using this other system he made this jump about the ability to even do it, whereas what you found is when you have something that was constitutively expressed –

SP: - at high levels there was no problem. Right. And so, I think he's right that unglycosylated PrPsen is converted best, not because of that paper, but because – it's been sort of suggested for years through Byron's work, he was one of the first ones to suggest it - because in the scrapie infected cells, it's the unglycosylated forms that are most dominant in the PrPres. But if you think about it - and this is one of the things that set me all off on strains - when you think about a strain like hamster 263K, which has a PrPres profile where the dominant form is the fully glycosylated – so why, if the unglycosylated is always favored, do you have these strains like BSE and CJD – or variant CJD, where it's the fully glycosylated upper band that is dominant? It makes no sense at all. So that's where this whole idea of glycosylation being more a consequence of what pool of PrPsen the PrPres is seeing comes in. Because you can get around that problem – if the unglycosylated works best why is it represented least in many different strains? - by simply saying that well, it's because it doesn't see much of the unglycosylated form, and what it mostly sees is the fully glycosylated form. And because 90% of what it sees is that, that's going to predominate in the newly formed PrPres.

MP: And so there might be certain cell types or compartments within the cell -

SP: Exactly, that favor - Yeah, that's what this new hamster stuff is about, because everything I do points the same way. So what I have to show now is that, in fact, formation is occurring on the cell surface where I get most of this fully glycosylated stuff.

So it's that dichotomy where on the on hand, I believe it, but on the other hand, it makes no sense; when you look at other strains you have to invoke something else.

MP: Right.

SP: And people have known that. They've invoked other things, like: scrapie infectivity alters the glycosylation machinery of the cell - which the fibroblasts stuff says no, all the cell free stuff says no, that's not important. Or that it's the pattern of PrPsen glycosylation in the cell that determines it – I have a piece of data now that says no. So is the unglycosylated stuff more PK-sensitive, so that you might digest all of it at some PK but maybe the fully glycosylated stuff is more resistant? But I have an experiment that says no, not really, that they're all about the same. It's not the world's greatest experiment, but you know.

So then you're stuck with okay, if it can't be any of those, what else could it be then? So that's – yeah.

MP: Has your idea about what the agent might be evolved at all as you've been in the field? Are you still kind of –

SP: Well, yeah, sure it's evolved in the way I think it might be working and what might be required and what might not be.

MP: What would you say your starting point was? How did you think it worked when you first got here and you were talking to Bruce and you started reading the literature?

SP: I didn't think there was any compelling evidence that it was a protein-only agent because I didn't think that PrPres had really been purified. I just had an experience here a few months ago where we purified PrPres from scrapie-infected mouse brains to give to Byron to do IR, and we had this huge pellet in the bottom of the tube – and they told us there was only three micrograms of protein in there? It was this humongous visible pellet! Turns out, there is only three micrograms of protein, and it is PrPres, but I have no clue what the rest of that pellet is.

MP: And the protocol you used to purify it is the sort of standard –

SP: Is the sort of standard one, so all sorts of other stuff is coming down. So purified PrPres is a tricky deal – it's really not. Protein-wise maybe, but god knows what else is in that pellet.

So when I came, I didn't think there was any compelling evidence to think it was a protein. There was no compelling evidence to think it was a virus except that that's what everything has always turned out to be – a virus or a bacteria. So I sat on the fence. And I still sit on the fence, although I've now heard Prusiner's giving these talks saying that they've generated de nouveau PrPres infectivity. And from what I've seen, they may very well have done that, and if the controls are all good and tight and it's not a contaminant,

and this and that, and if it gets repeated in their labs, great, then the questions is finally settled. If you can generate infectivity de nouveau with just recombinant protein you really can't argue.

MP: One of the arguments I've heard from Bruce and others is that they want to see what they're calling infectivity titered to show that it's not just a toxic effect or something, that there's actually something replicating.

SP: Right, right, that it goes up over time. Yeah, and I don't think that they've done that, from what I've heard. What they have done is they've transmitted it again, from one animal to the next, right.

MP: To convince you, would they need to show you that it was titerable? Or would you be convinced if you saw the proper controls and proper –

SP: Well, would it have to be titerable? Yeah, you would have to show replication, you would. But given that I don't know of any other instance except for that weird Karen Tsiao stuff from the '80s or the early '90s where this has been done, and that was just one transgenic mouse strain, my bet would be that since they were able to transmit it on a second pass, and apparently the incubation time dropped, that that's probably an indication that they did have replication and so there is something going on there, which suggests to me – and it's because of the incubation time drop, which I realize is a really crude method, but if you're going from whatever it was – over 500 days to 150 or 200 or whatever - that's a drop.

MP: That is. [laughs]

SP: And I would argue because this is – that's replication, it's not a formal proof of it but it's a good indication, which would mean that in those experiments the only other possibility would be contamination. And I'm sure they have whatever PBS controls or whatever that they inject at the same time, but I wonder – the appropriate control would be to take whatever the fibrilized stuff is, however they made it, and whoever's lab did it or whatever - to run that in parallel, without protein or with protein, take it through the whole process, treat it exactly the same way, and then put that in animals so that you're using all the same buffers, all the same reagents. My understanding is Ilea Baskakov did the fibrilization – I'm pretty sure in Prusiner's lab they did the injections. So if they do a PBS control for Prusiner's lab that's a control for his lab only.

MP: Well I think that Ilea did the fibrilization when he was at Prusiner's lab.

SP: At Prusiner's lab?

MP: At Prusiner's lab, but I don't – I actually don't believe that they had controls that they ran all the way through.

SP: That's a big deal. Well, I'm sure he's going to have to have some sort of media or solution only control, but that's not enough. If they're only using PBS, that's not what he fibrilizes under. So the PBS is a control for Prusiner's lab's ability to keep contamination out of their injections, and that's good, but it's not showing that you're keeping contamination out of the entire process. Because when these guys came back and told me they heard this talk, one of the things they said was, well, but all the animals got sick and died. Well, okay, usually when it's a contaminant, if it's a low level contaminant, you might just have a couple die. If it's a significant contaminant – and significant could be 10^{-6} worth of infectivity, they will all die and they'll do probably what these guys did – they probably had a few die early and everybody else was later. We see that at low dilutions.

So those controls have to be particularly vigorous because it is a problem. But I'm really curious to see that. If all that's done cleanly and it's repeatable, then I'm off the fence.

MP: Right. So that, to you, would be *the* convincing experiment.

SP: It would be, because then you're getting to the – and showing that it's titerable, I agree, is very, very important. But it seems to me that the early implications, if my understanding is correct, are kind of pointing that way. So we'll have to see. It'll be really interesting to see if other people can repeat it because it's not as though people haven't tried this many, many, many times before.

So that's – so my thinking could evolve very quickly over the next year if that turns out to be okay.

MP: [laughs] How would that change – okay, let me ask – this is forcing you to think into the future.

SP: Uh oh.

MP: Let's say this is all shown to be the case and everything and then it's finally proven that it's a protein-only agent. Do you think that would produce any significant changes in the way science is done in the field, or do you feel that the way things are done already, already operates in such a way that it wouldn't really make much of a difference?

SP: I don't think it would make much of a difference because, really, even though you can be a person who says it's a virus or a person who sits on the fence, you still deal with it. You're assuming it's the protein, because we're all fiddling with the protein.

MP: Because that's what you have.

SP: Because that's what we have, right, we have a 254 amino acid proteins. So it probably wouldn't change much and, in fact, it probably wouldn't change the way I think about species-barriers, or incubation times, or what the minimal – the best - PrPres

inducing unit is – it wouldn't change any of that because you don't have to invoke viruses to think about it. Except for strains. Yeah, so it probably wouldn't change too much.

MP: On the other hand, if it were shown to be a virus, all of the sudden –

SP: Yeah, well, then it's pretty – then you would have to start shifting. That's true, there would probably have to be a shift because now you've got something else as a cofactor at the very least. There would still be things that would – I mean, so many people have shown the dependence of species barriers on the PrPsen sequence that I think it's clear, no matter what, that PrPsen is absolutely essential and that conversion of PrPres is a very important part of the disease process.

MP: Well, I thought a lot of Jean Manson's more recent work points to the idea that sequence – at least primary amino acid sequence - might not really be the determining factor.

SP: Well, more the folding if you're –

MP: So you're thinking it's probably more likely the three-dimensional structure –

SP: Right, so the – yeah I guess that's true because it's easy to forget about that. Yeah, it's not a linear sequence, it's folded into a 3-D structure. But the primary sequence is going to affect how that fold occurs. So actually I agree with that, yeah.

MP: Okay, let me ask you just one more broad question. What do you think have been the most important developments in the field or new or adapted tools or techniques – just broadly, not just now your lab – things that have been put into use like antibodies or whatever – things that might have made a big difference to how people have done research in this field?

SP: Transgenic mice. Transgenic mice have been huge. The development of scrapie-infected cells because that allows you to look at the biochemistry and synthesis of protein. In very broad ways, antibodies – 3F4 antibodies are God's gift to scrapie. So that has been tremendously important, as has the hamster – the 263k model of infectivity because it was such a rapid model, relatively speaking until the transgenics came along.

From a strictly biochemical point of view, hmm…see there's a lot more biological things, but I tend to think more along those lines. But I think from the biochemical point of view, the use of IR; I think infrared spectroscopy has helped with just trying to think of overall conformations of PrPres, I think that's been important. As has isolating PrPres in the first place, showing how it would be done, that was a tremendous – obviously that's huge. And going along with that, realizing – although this isn't necessarily a tool so much, this is more something that was important to know - that the protein was actually a host protein and not some foreign protein that had been introduced. So being able to clone and sequence PrP from mice and hamsters was a big deal.

But transgenic mice have made a huge difference. I think in some ways good and bad.

SP: What do you mean by that?

MP: Well, because they come with their own little glitches, most of them, except for Jean Manson's mice, which are knock-in mice. Those are really nice.

MP: How is that different from most transgenics?

SP: Well, the vector is randomly inserted throughout the genome, so every transgenic mouse is different. And you have to worry about where it's been inserted and what else it might be affecting and expression levels can vary, depending upon how many inserts you have. So what that means is that Jean Manson's mice, she puts whatever her mutant PrP allele is right back into the PrP position on the chromosome. So it's expressed the way it's supposed to be expressed and where it's supposed to be expressed, at the level it should be, so she's got kind of a natural transgenic PrPres. All the others, ours included, the ones that Bruce and Rick had made, are random insertions with variable levels of expression, all of which you have to think about.

MP: Can you go back and look and see where it's inserted and how many times it's inserted?

SP: There are ways to do that but I don't think most people do. They just look at expression level.

MP: Okay, okay, so is it sort of a crude way of telling –

SP: Exactly, what's going on.

MP: And why don't more people do knock-ins?

SP: I'm not entirely sure. I don't know if anybody other than Jean Manson does it. Because it's – well, she has the mice, I mean she gives away the mice. I'm not sure.

MP: Is it more difficult?

SP: It's probably more difficult and you can't – you know, I'm not a transgenic person, I try to avoid them like the plague –

MP: [laughs]

SP: – because they're just so hard to maintain. Or to keep track of. I'm not entirely sure why except that most transgenic facilities are set up to do it the standard way. And so you can basically order your transgenic mouse, as opposed to doing the more specific sort of knock-in stuff that Jean Manson would be best to have. Even then all you need is

the mouse and the right vector, so I'm not entirely sure why people haven't picked up on it more.

MP: And when you say keeping track of, what do you have to do to keep track of transgenics? Why is it so complicated?

SP: Well you have to breed them and then follow the transgene, so you have to make sure – because when the transgene is first introduced you have a founder, so that male or female mouse has the transgene and you mate and you get an F1, a first generation, and one or two of those guys might have the transgene, so you have to assay all these mice by PCR. They're doing it right now for some mice that Rick and Bruce are making, and the guy who's doing it just got done with 400 and he's got another 400 to do, because you have to check every litter to breed the line and get – in our case, get the transgene on a knockout background and make sure that that you really have a homogenous genetic background except for the transgene and – man, it's just a total pain. It takes a lot of time.

MP: Do you tend to work more with cells than with animals or do you work with both?

SP: I do a lot of work with cells. The work I've done with animals has been mostly inhibitor stuff, and that's primarily because Rick has been doing – historically he's done a lot of the pathogenesis stuff. So even though I was trained as a viral pathogenesist, so that's where – all my stuff at UCLA was on animals. And I'd like to do that and I have, actually, some thoughts about what I'd like to do that's different from what Rick and Bruce are doing to get back more to animal work. That's based partly upon what I see in the cells and for other reasons.

MP: Right, and it seems like with what they're producing there are a lot of tools available at this laboratory to do that.

SP: Completely. We're one of the few labs – we're probably the only lab because I know Prusiner's lab doesn't do the cell- free conversion assay that Byron developed – which has been a big bonus for our lab. I didn't put it in the list of things that you talked about because not many labs do it because it takes some special setups to do, in terms of disposing of the radioactivity and whatnot.

MP: But you feel like it has been important to the field –

SP: Oh, completely, and I know that many people in the field – some people in the field - Prusiner's one of them, and there are others – think that it's just random, nonspecific binding and protection. That's just totally wrong and makes no sense. It's the most specific non-specific binding and protection I've ever seen. So we have – we're the only lab - we can go from that test tube level, the cell-free conversion level, up to transgenic mice and everything in between, and I don't think there are many labs in the world that can do that. So we do have this huge range of tools open to us.

MP: So the only thing you can't do is the actual level of the livestock or the deer or whatever?

SP: Not really, although we've talked about it. But we can do everything up to rodent – I mean, everything from cell-free – from molecular interactions to rodent models.

SP: Right. So from in vitro to in vivo but little in vivo. [laughs] Not deer, and elk, and sheep, and stuff…

MP: Yes.

SP: And you talked to Dr. Hadlow too, right?

MP: Yeah, I did.

SP: All the sheep stuff, and whatnot?

MP: Yeah, yeah, goats and [laughs]

SP: Yeah, astonishing what they all did. But we talked about it not too long ago, a couple years ago, about trying to get some of that back, but practically it's so difficult now – you have to keep everything indoors and on cement and it takes a lot of space.

MP: And I've seen those facilities like Compton, where – I mean, it's huge, but you're right, that's how it is, it's all indoors, it's all in concrete, I mean gigantic barns for housing these things.

SP: Enormously expensive. The USDA has it set up at Ames. So in instances like that, if you have something that you want to test then the thing to do is to collaborate with somebody at the USDA or at Compton or somewhere who has the livestock – or Edinburgh, who has the livestock capability.

MP: Right, right, so few places actually have that.

SP: Right, so we – but there is no lab in the US, I don't think, that can do all of it.

MP: But you've got most of it covered, from the in vivo all the way down. And like you said, there aren't a lot of other places that do the cell-free conversion because, as you were saying, it's difficult to handle the radioactivity –

SP: The mix of radioactivity and infectivity. Right.

MP: So having a lab that can dispose of both and handle both is a complicated issue.

SP: Right, right. Because the assay itself is very easy to deal with – there hasn't been a single person who's passed through Byron's lab or even my lab, although I don't do it as

often as his lab, there hasn't been a single person who hasn't been able to do it. So the technique is not hard. With Soto's technique, the PMCIA or whatever it is – now a lot of labs have tried that and very few have been successful with it; there's a touchiness to it. That could have been another huge one, and it may still be, for people to do, because you don't need radioactivity and you can get the amplification and whatnot, but that hasn't really been picked up either, by other labs, because it's been so hard. I know Bob Rowher's lab repeated it, but it's been really tough. They tried it here – Ina gave it one shot – a couple shots and we abandoned it like that. We had a better way to do it. We didn't need the amplification.

MP: But with both the cell-free assay that Byron developed and also Soto's, it uses a non-homogeneous solution - the PrPres that's used is purified from the homogenate.

SP: Yeah, so it's pure protein-wise. 95%.

MP: But there's other stuff in there.

SP: There's other stuff in there, yeah.

MP: And so there's always that open question of what else –

SP: What else could be contributing. Although it's certainly better than anything else that's come before it. You know, Soto's stuff is just the whole homogenate. So the cell free is a step further than that.

MP: Right, because the cell free you actually could go through the steps – the usual steps of purification of PrPres before you add –

SP: Right, exactly.

MP: Whereas with Soto's it is just the –

SP: it is just the brain homogenate with a bit of detergent added, yes. So yeah, with Byron's stuff, he had a postdoc here a while back Moto Hito Horyiuchi [spelled phonetically] who – yes – who tried to look for other factors in that PrPres prep. And Dave had done this too, Dave Kocisko. And Moto never found anything. Dave thought he had found something; it actually increased conversion significantly and it turned out to be Sarkosyl, which was added as part of the prep. [laughs]

MP: Right, right. Interesting, that's so funny –

SP: Yeah, it was pretty cool, so that's why Sarkosyl [?] – It's really important, a little bit of detergent.

MP: And what's the theory on that? Why –

SP: Oh, this is a chemical thing. It has to do with solublization – maybe it needs sort of a membrane micell-like environment, I don't know. You don't have to have it because Moto developed the detergent-free, cell-free, which is much less efficient. It does not work well with a lot of different strains. So detergent's always a little bit better. Yeah.

MP: Interesting. Well is there anything else that you can think of?

[interview ends]

[Previous interview, conducted 7/20/04]

MP: Okay, so first of all, I'm really interested in the basis of strains. How would you describe a scrapie strain?

SP: Well, it's defined in two ways: by how it acts in the animal, and by what the abnormal prion protein isolated from that animal looks like. So the key thing about strains is that in the same animal, so one particular type of mouse, say a mouse that's called like a C57 black mouse, a very specific strain of mouse, you can propogate three different scrapie strains in that mouse even though it only has one prion protein sequence. So what that means is that when you put strain A into that mouse it gives you a certain pathology in the brain, it gives you a certain incubation time, it gives you certain clinical symptoms. When you take the PrPres – or abnormal prion protein out of that mouse, that protein looks a particular way biochemically.

MP: And when you say it gives you a particular incubation time is that dependent on the dose?

SP: Yes, it'll be dependent on the dose, so the higher the dose the shorter the incubation time.

MP: Okay, so when you say that it's reproducible, that the strain – the incubation time of the strain is reproducible, that's entirely dependent on the dose of the strain that you're putting in there?

SP: Oh, yeah.

MP: How do you know that you're putting enough in?

SP: There's something in virology called "titering", so when you titer infectivity you basically take your source of infectivity and you dilute it, say tenfold the whole time, so tenfold, a hundredfold, a thousandfold. And you put each of those dilutions into the animal and then you wait for the animal to get sick. And a titer is basically the dilution at which 50% of your animals get sick, it's called an infectious dose 50. So what you do is

you titer all these strains in the same animal and that gives you a dose and you can then put in exactly – well, based on these numbers you can put in the amount of infectivity you want to get to a certain incubation time. So if you were to compare three different strains, ideally you'd want to put them into the mouse all at the same titer. That means that you have the same amount of infectivity in each one. So any difference in incubation time is because of the strain, not because you're putting in more or less.

MP: How long does it take to titer the infectious agent?

SP: Oh, it takes a long time. In virology, it'll take you a day to titer something. In scrapie and TSE research, it'll take minimally a year.

MP: Wow.

SP: Yeah. So this is one reason you don't often see titers in TSE papers because they take just an enormously long time.

MP: That sounds like that would be a real complication.

SP: It is. So what we do, we have multiple mouse strains that we work with and we're in the process of just finishing up tittering each one so we know how much is in the infectious dose we give them, if we give them into the brain intracranially or peripherally or orally, we know what the titer is on each route and so we know how much we want to give them.

MP: So you'll have stocks that you'll have diluted to a certain amount so that you know exactly what the titer –

SP: Right, we'll have a stock that's as concentrated as we usually make them, and then we'll have a bunch of vials of those stocks and we'll titer it once. And the one advantage that the prion diseases have – the TSE diseases have over other infectious diseases is that that titer is very, very, very stable over time, when it's stored – you know, frozen. And that's not true of a lot of viruses, so.

MP: Right, so even though it takes longer to do the experiments, if you store something it's stable for a longer period of time. So once you've calculated the amount that you have in storage, you're pretty certain it's going to stay that way.

SP: Sure, and we've actually done – or I haven't, but someone here at the lab has actually taken something that they titered 15 years ago and titered it again and it's like within a tenth of a point of what it was. So it's very stable.

MP: Are all strains equally stable, like is RML as stable as 22A?

SP: No, they're not, they're not.

MP: Okay. And how do you guys choose which strains to work with?

SP: Well, a big concern is practicality. So one of the more interesting strains is a strain called 87B which is a long incubation time strain. It's very interesting biochemically but it takes 260 days in an animal experiment, so it's impractical that way. So for that reason most people use shorter incubation time strains. In mice, the primary ones are the RML strain, 22A, a strain called Me7 – because they're short.

MP: Okay, so those have short incubation periods? And so those will be chosen.

SP: Yeah. It's a huge, it's a huge concern – Yeah.

MP: Okay. What is so interesting about 87V? Why would someone want to use that experimentally?

SP: It's interesting because it's a long incubation time strain. And it has a slightly different prion protein sequence to it, so there's an interest there in studying why – why and or if that protein sequence influences its incubation time, how it does that.

MP: Wait, when you say a different protein sequence, I mean they're all the same amino acid sequence right –

SP: I mean they're all prion proteins but this one has two amino acid changes verse strains that are short incubation times. There's two differences in the prion proteins. So you put the strain 87V into a strain of mouse that has this PrP sequence that has these two changes. So there's been interest on what effect those two changes have on incubation time.

MP: And so those are actually two amino acids that are different?

SP: That are different, yeah. Two minor changes – it's still prion protein because you know you have changes, you can have mutations – one or two changes between these proteins, they're called polymorphisms and it's not at all unusual.

MP: I read a paper that you had done, I think fairly recently, where you were talking about the molecular basis for strains, and I thought that was really interesting, because having been to some conferences and thought about it a little bit, it's really confusing.

SP: It is.

MP: How would you explain the relationship of a strain to the measured glycoform ratios?

SP: For me, the glycoform ratio is kind of a flag that may be telling us where in the cell the abnormal prion protein is being made – where the PrPres is being made.

MP: Okay, because that was one of the options I remember you touched on in the paper, but it sounded like multiple things were affecting what you're getting. Do you think that has something to do with what Collinge and Gambetti are measuring in their different systems for glycoform typing?

SP: Potentially, yeah. And so that's exactly it. Very subtle changes either in the cell type or in the normal PrP that's expressed in that cell type are reflected in the glycoform ratio. So that, to me, explains one of the conundrums of strains, and that is how can you have a strain of something if you've just got a protein and no nucleic acid? And if you take into account the possibility that some of the properties of the abnormal prion protein are a consequence of how the cell is dealing with it, then that makes it a biochemical issue. So strain A looks a particular way because it's on the cell surface when it's made.

MP: Right, and I remember in the paper you talked about RML particularly being different from the other strains because it looked like where it was meeting with the PrPc in terms of the cell process was affecting what you would get in terms of the glycoform ratio?

SP: That's right.

MP: How would, potentially, different strains of PrP scrapie come into contact with different parts of the cell, in terms of the conversion?

SP: That's a great question. Well, all the data has always pointed to the fact that PrPsc formation is on the cell surface or along what's called the endocytic pathway, so when stuff is trafficked into the cell. And the idea is that for certain strains the biochemical conditions for conversion are most favorable closer to the cell surface, and for others they're more favorable along the endocytic pathway. So they may all get taken up into the cell, but for some they can't to the abnormal form because it might be slightly too acidic, the environment might be too acid, it might be too basic.

MP: And that might subtly alter the conformation, or the way that it's contacting –

SP: Exactly, it might alter the – oh possibly, yeah. It might subtly affect the conformation, certainly it might affect the stability, so you might have something – the conversion happening but it's not very stable and it might fall apart or be taken apart by the cell. There's a lot of things that you could envision, I guess, biochemically happening. It might be more sensitive to cellular proteases and might be chewed away faster. So if that's the case then it's not too big of a leap to say that if a cell is really endocytically active, so it's always sucking stuff inside, it's really active and dividing and replicating, that you might get a very different glycoform pattern than a cell which is just sitting there.

MP: I see, that's interesting.

SP: Yeah. I really like that idea because it explains a lot – it's really hard to prove but it explains a lot.

MP: In terms of?

SP: In terms of why in certain neurons you might have certain patterns associated with certain cells. So a neuron that's just sitting there not doing much –

MP: Like a – well, like a neuron for instance versus maybe something in the spleen or –

SP: Right, might be very different. There's a recent paper out, we just did it in journal club, where they were looking at variant CJD in the brain versus the tonsil and the spleen, and there are some differences.

MP: Oh really, who's the paper by?

SP: Let's see, it's – I just happen to have it here on my desk. The first author is Mark W. Head and it's the *American Journal of Pathology*. So…

MP: That sounds interesting, I'll definitely check that out.

SP: Yeah, it is interesting, it does look like a definite difference. And so that gets to what you just said, that it might be different in an organism like the spleen versus the brain and that's definitely true for certain rodent strains, I can't remember which ones now, but some of the mouse strains. It looks very different in the spleen than in the brain, but it's the same strain.

MP: Yeah, that's interesting. What does that mean in terms of its function or what it could be doing then in these different cells?

SP: You mean like whether it's pathogenic?

MP: Yeah, exactly, like whether it's going to do anything pathogenic – whether it's going to mess with the sort of cell machinery or not.

SP: Yeah. One possibility – and I don't actually know if this is true or not but it's always been thrown around that it's, as you said, because cells in the spleen are much more active versus neurons, that they can maybe turn over or get rid of the abnormal form before it accumulates to levels that are really going to kill the cells.

MP: And do you have any thoughts on what whether there is an intermediate molecule that modulates pathogenesis? Aguzzi had a paper showing what dimers of PrPc that were held together using some sort of antibody or something, I can't remember exactly, could inhibit the formation of PrP scrapie. Does that indicate that the intermediate molecule is probably a dimer? What state – do you see what I'm getting at?

SP: Yeah, I think so. Well, the Aguzzi paper, it's an artificial dimer. So it's a little hard – I don't – it's always been predicted based on this – do you know about the seeded polymerization model?

MP: Yes

SP: Okay, according to that model, kinetically if you have a dimer or multimeric intermediate that process occurs much more rapidly. So it's always been assumed that there are going to be multimers of PrPsc and that those are actually helpful. Whereas Aguzzi's paper kind of suggests that they're not helpful because they can stop the process. But he's using a completely artificial dimer.

MP: I see.

SP: So we had a paper many years ago where we found a dimer of PrP in the cells, I'm still not sure how it was put together – it's a real mystery – and it converts just fine.

MP: So it doesn't block anything else, it just converts?

SP: Yeah, it doesn't block anything. And when you look at it frequently, and you'll see it sometimes if you're looking for it in many of the published papers, if you look at these gels of PrPres, you can sometimes see a laddering of bands going up the gel.

MP: And what does that mean?

SP: That's a pretty good indicator that you've got a monomer, a dimer, a trimer, a –

MP: Okay, I see what you're saying.

SP: So I don't know that anyone's ever shown it directly but when you see something like that it's just a red flag that you've got some oligomers in there.

MP: Okay. So that's kind of – so those are the little pieces of evidence that point towards that, but it's not really known.

SP: Right, right, exactly. It's not really known but it makes total sense – I mean, everything fits, in terms of the theory, it's just that finding those intermediates is really difficult. They may not have the same properties – I mean, they're intermediates - they may not have the same properties of the normal and abnormal form, so finding them is tough.

MP: I see what you're saying. And then also in the same paper, and this is kind of related, it has to do with the western blots, you mentioned that RML and 22L derived PrPres were not tested because the PrPres was variably truncated and it was difficult to identify the different PrPres glycoforms. I thought that was really interesting.

SP: That's interesting that you picked that up. There's a really strange thing going on. So that's the data using the cell-free conversion system. And it turns out that system only works very, very well, very cleanly, with strains that look – that have the same sort of glycopattern as variant CJD.

MP: Really?

SP: Yeah, so if you go back and you look at these Byron Caughey cell free conversion studies, they use Hamster 263k. If you look at that glycopattern it's very reminiscent of variant CJD. They've used mouse strains like Me7, that works well, H7B – look at the glycoform patterns of those strains, they look like variant CJD! When the patterns look more like what Gambetti or Collinge would call – I don't know, type 1 or type 2 where that middle band tends to be heavier - those don't work well under the cell-free conditions. They give you these real truncated patterns and – you'll see this in the original cell-free paper, in a couple of them - they sort of dodge the issue by saying they're truncated but they look real, and they are, they're real. They're just not in the right spot.

MP: Have there been binding studies where they can show that it's truncated? I'm just curious because that seems like something you could do; go back and check where they're starting or where they're stopping, to see if they're truncated. And how would they be getting truncated if it's cell-free?

SP: Well, there should be no workable enzymes in the cell-free. What might be happening, it gets back to what you mentioned before, what we talked about briefly before about the environment being important, and you had asked could it change the conformation or whatever. What those smaller forms probably mean is that the conditions under which we standardly do this in vitro conversion may not be right for these strains because the molecule might become too unfolded and then get chewed back further by PK than it normally would. Because it's not folded – it's not the same conformation; more of it is accessible to Proteinase K digestion.

MP: Because when it shows up as truncated, that's after PK treatment. For instance, going back to Bessen and Marsh's paper, that was something that's been observed with different conformations, that you get different truncated forms.

SP: Right. So the idea is that, and it seems to be – at least within the range of strains I've had access to - it seems to be limited to those strains that don't look like variant CJD. And my explanation for that goes back to the location of PrPres formation. The conditions we use for this conversion tend to be neutral – pH 6.97, whatever. And those are conditions that are probably going to exist at or near the cell surface, whereas the further you go down the endocytic pathway, the more acidic things get.

MP: So could you, for instance, run the cell-free assay under more acidic conditions?

SP: Yes. I've done that.

MP: And what happens?

SP: Well it's not published, but it turns out – someday it might be published. It turns out that it looks as though those strains that don't work terribly well - they don't work terribly well over a broad range of conditions. You still sort of get the same Gamish of product. So there's something else going on. But for those strains that work very well, they still work best at this neutral pH. As you go lower or higher in pH they tend to peter away.

MP: Which we've indicated is probably a cell surface reaction?

SP: Yeah, that they're more restricted at least in their requirements. Yeah. But see that data sort of sits there.

MP: [laughs] I'm sure a lot of data sits there.

SP: Oh, a lot [laughs] – it's true.

MP: I wanted to ask you a little bit about how you choose which strains to use in a particular experiment. And how do you choose which mice to use, like which type of mice? How do you make those kinds of decisions?

SP: There are certain mice that work better with certain strains. So it turns out that the big thing that pushes what strains we choose are the incubation times and how easy they are to manipulate. All the short incubation time strains can go into one type of mouse.

MP: Which is?

SP: The C57 black mouse. The standard laboratory one.

MP: I see, so that's the one that's most commonly used because it produces the short incubation times in those strains.

SP: Exactly, exactly. And the only time where you really have to consider the mouse type, as long as you're talking about wild-type mice, is really to make sure that the PrP gene is a short incubation time gene, versus this long incubation time. So when we study the 87B strain we do have to use a different strain of mouse because this PrP sequence differs by these two amino acids.

MP: I see. And what about antibodies? 3F4?

SP: You use 3F4 with hamster 263k because it was raised to hamster PrP. And you can use it when you study hamster, human, bovine. I think you can use it in those cases. It does not bind to mouse PrP, that's why it's been so heavily used.

MP: Of course, that's why you have to label the PrPsen produced by the cell culture so that you can detect it.

SP: Exactly, exactly.

MP: I see. Do you guys work with transgenics at all?

SP: Yeah.

MP: Do you make transgenics that overexpress the prion protein or are they usually single copy?

SP: Well, we, like just about everybody else, overexpress. Which, of course, makes it that much more artificial. But again, it's an incubation time issue.

MP: Because if it's overexpressing you get a shorter incubation time? So when you have a transgenic mouse that's overexpressing even murine PrP can you get a good reduction in incubation time?

SP: Yeah, there's a mouse called a PG20 mouse and it's – I think it was derived by Charles Weissmann's group a few years ago. And you put mouse scrapie into that mouse, it gets sick and dies in 40-some days.

MP: Wow, that's fast.

SP: Yeah, but there's a weird thing about it. It's a little bit tough to detect PrPsc in it. [laughs]

MP: It is?

SP: Yeah, it's a strange thing but they go very quickly and they express a ton of murine PrP. They overexpress mouse PrP.

MP: That's very interesting, so it overexpresses mouse PrP but you're only able – you're able to detect less PrP scrapie –

SP: Well it's harder to detect, yeah, yeah.

MP: What antibody would you use then to detect that?

SP: Well, it would depend upon the lab. What would we use? We'd use some rabbit polyclonal antibodies we have to detect it. You know, labs have their own thing.

MP: I see, but even across labs that are using different antibodies…

SP: Well, okay, I guess I can't answer that, I'm not sure.

MP: And what is your thinking currently on the infectious agent and the relationship between PrPres and infectivity?

SP: Well, I think there's no question PrP is involved, I think that's pretty clear. I sort of sit on the fence about the virus versus protein-only theory, but I do think that there's something else involved. The data just doesn't fit PrP being able to do everything. It just doesn't. It cannot encode for everything that people say it does.

MP: And in particular what are the main things that stick out to you as the pieces of data that don't fit with it being just PrP?

SP: Well, one is the strain data, and the glycoform data which, since I can take a strain and make it look like anything I want just by changing what I expose it to, that says to me that PrPres doesn't encode the whole thing. That something else is important and that might just be the whole cell. It could be something that broad. There's the fact that – and again, this is unpublished data at the moment - but we have cells that express PrPsen at normal and wildtype levels and we can infect them with certain strains and you'll get PrPres formation every time for the first few days, but then most times it goes away. So here you've got something where you've put the infectious agent on, you're getting more of the protein made, it's supposed to be a process that once it's started it cannot stop - and it goes away.

MP: Weird, and that's without really messing with it at all?

SP: Yeah, it's just letting the cell do its thing.

MP: That's interesting because – like the results Collinge had with the mice that when they turned off the production of PrPc, they were able to show that even vacuolation could go away, right?

SP: Right.

MP: But in that experiment they were at least controlling it, sort of artificially, from outside. But here, there was nothing you were doing, then, to tamper with the levels of PrPc expression?

SP: Nope, just tried to infect it and found out, lo and behold, whoops, yeah you get initial PrPres production a bit and it kind of goes away. [laughs]

MP: That's remarkable.

SP: It's pretty cool. Well it could be simply because of the artificiality of the system, you know, you've got cells that are in there that are dividing and whatnot. But it does go against, once again, this dogma that once it starts it goes on and all you need is PrP for this. Nope, you need something else.

MP: Mmm-hmm. And for instance, in Supattapone's recent paper where he shows mammalian RNA is sort of a catalyst for the reaction, the conversion reaction. Do you think that could be a clue?

SP: It does speak to the issue of nucleic acid being involved. I will say it's not necessarily super surprising since it's been found in the past that these – nucleic acid is basically a polyanion so it's a molecule with multiple negative charges because of the phosphate backbone on nucleic acid, and it's been known for quite awhile from work that Byron Caughey did years and years ago that polyanions tend to stimulate formation of PrPres in tissue culture cells or in test tubes. So here RNA is another sort of polyanion and he finds it can stimulate it. Well I guess what was kind of interesting was that it wasn't just a certain population that did it, but it was a pretty broad population. But it does suggest that nucleic acid could be involved, which raises the specter of, again, some kind of viral involvement.

MP: And what do you think about the prospects now for therapy for CJD or variant CJD?

SP: So late-stage therapy, so like you would need in humans?

MP: Yeah.

SP: Based upon what I've seen done here and what I've done myself and what's in the literature, I think that if the sole criteria for a therapeutic agent is inhibition of PrPres, you're not going to find anything that'll work, unless it's completely by accident. It has to be just luck because the track record is if you find something that inhibits PrPsc in tissue culture cells or in a test tube, it will not work later during disease – or maybe slightly, but it just doesn't go, it doesn't correlate.

MP: Do you think you'd need like multitherapy then?

SP: Yeah, that's sort of the way that I've been thinking for a while, not that I've done much about it, but I've been thinking that way for a while, that what you would need would be multiple – sort of like the HIV approach, a multiple drug therapy. So you might have something that inhibits PrPsc and you might combine it with something that will inhibit, perhaps, oxidative damage that can cause a neurodegeneration or inhibit apoptosis – you know, programmed cells death, things like that. I think you're going to need multiple therapies –

MP: Something that inhibits later parts, or later stages of the disease process as well as PrPres function.

SP: Exactly, exactly. Because it really may be that by the time you're showing symptoms and whatnot PrPres has nothing more to contribute. It's just accumulating into these insoluble blobs and what it has done might have been done well before any clinical signs are manifest. So it might take stopping the neurodegenerative processes that go on that might not be PrP-specific.

MP: Do you think that the therapies that are being tried right now, the quinacrine or the PPS - have much chance of working without an additional therapy?

SP: I would be skeptical that they would. I hope they do, but the evidence would suggest to me that they won't work, and in cases where it looks like they are working – unfortunately because of the small numbers of patients that's meaningless. There's an old Paul Brown review from some book where he actually has a couple of nice tables that detailed up to that point, which I think is 1990 or something, all the clinical – the drugs that had been tried clinically to halt the progression of sporadic, in this case sporadic CJD. It's interesting, if you go through that table you find that, actually, quinacrine was tested before.

MP: You think it actually might have been given to patients at some time in the past?

SP: Well, I'm thinking it was done in hamsters, in that case it was hamsters, and it didn't work. But I could be mistaken. Well anyway, in this table, you go through it and it's interesting because they tested an antiviral drug, like I think it was amantadine. And they had three patients, and one might have shown some improvement and one didn't, and one went faster. What does that mean?

MP: And what is improvement defined as?

SP: It's the progression seemed to slow. It didn't get worse. And the question is did it not get worse because the drug worked or did it not get worse because that patient – for some other reason – did the disease progress slightly differently in that patient? It's impossible to tell with these small numbers you have. So it's very complicated.

MP: Now, what if there were a screening test available and all of a sudden you were able to diagnose people much earlier in the course. Do you think, then, that there are potential therapies?

SP: I think there'd be a better chance. Yes. There are papers from many years ago, from Kimberland, back in the '80s and whatnot, that showed that compounds like dextransulfate, and I think he did pentosan polysulfate, if you gave them a few weeks after infection before clinical signs, under certain conditions they could prevent disease. So yeah, if you had a really good early diagnostic –

MP: Then you might be able to do something.

SP: You could try these interventions. Even the stuff that I've done here, the porforins and thalocyanines, it turns out that they don't work late well at all unless you drop the dose of agent, so it gets back to what you were discussing earlier. If you drop the dose, then you can treat a month later, and you can get some survivors. So that, again, suggests that earlier in the disease course, when there's presumably less infectivity around, you might be able to successfully come in with a therapeutic. But it hinges on a very early diagnostic. That's the key.

MP: Right, right. And since you've entered the field can you think of any laboratory techniques or tools that have really advanced the field?

SP: That have advanced the field – well, for our lab, but it does seem to be mostly our lab that uses it - the cell free conversion has been a huge tool. And I know that there's – there are people in the field who don't like it or don't trust it, and that's fair enough, but it does seem to mimic very well what's going – pretty well what's going on.

MP: And what is the utility – I mean, like what are the main things you'd say it's useful for?

SP: Well, you're looking at a far more purified population of prion protein and it allows you to manipulate the conditions whereby the abnormal form is made so you can start looking at biochemically what needs to happen, what are the conditions that favor it, what are those that don't, you can start asking questions, as we've done, about species barriers. Because you can now mix and match your PrPs you don't have to wait three years for an animal experiment. But you know, again, that technique has primarily taken root in this lab. In general, the transgenic mice, people are just going to town with the transgenic mice.

MP: [laughs] They certainly are. Particularly Aguzzi –

SP: – Yeah, if he can make a transgenic mouse he's gonna! And they've given lots of useful information but they are still transgenic mice, so there's always caveats. The tissue culture systems are getting better –

MP: And are you thinking of like Charles Weissmann's –

SP: Yeah, he's got his – it's not perfect but it's a pretty good system for titering the strains of mouse scrapie. So were gaining more of an understanding of how to infect tissue culture cells and get things going which can be very important for trying to get human model systems – you know, a human persistently infected cell line going. There's the structure from Kurt Wutrich of normal PrP – at least the e. coli version of PrP - that's very important.

MP: And how does that help? On your level of working on things, how does knowing the structure or having the structure of PrPc help?

SP: Well, it's like anything - it gives you a picture. And so if there's a conformational change which has to happen, which seems pretty clear now, for PrPres to be made, you can look at that picture and think, well, where could that be triggered, what could this do – you can manipulate it and move it about space and say, "Oh, this amino acid fits here and that looks important, let's change that." So you can use it to do those sorts of experiments. It's a useful thing to have to think about. So it doesn't by itself solve a ton of problems but it suggests ways that those problems could be solved.

MP: I see. And what do you think are the main problems or difficulties with research to be overcome that would make the field move a lot faster?

SP: Well, it's a small field, and it's difficult to get into because the material is so difficult to work with and handle.

MP: And why is that? What are the main difficulties?

SP: Because it's hard to get rid of. Other scientists kind of freak out about it.

MP: In terms of cleaning things up?

SP: Yeah, decontamination of stuff. I mean, we have people here at Rocky Mountain Labs who work with salmonella and chlamydia and plague - and they don't want TSEs anywhere near their lab! I'm much more worried about their salmonella and plague. But they just freak out because they ask, "Well, can you clean it and make sure it's sterile?" And you go, "Well…kinda." And then they don't want it around their equipment. So it requires, if you want to start up a lab brand new, a pretty good outlay of money for equipment, it requires making sure that you can dispose of material properly at your facility.

MP: Is that expensive? What does it take to do that?

SP: No, it's not expensive if you know how to do it. We have an onsite incinerator, which is just terrific – we can incinerate everything. And even then, we deactivate it the best ways we know how.

MP: With what?

SP: Well, we use the LpH thing, yeah, which is very effective and much less, in many ways, expensive –

MP: How long have you been using that?

SP: Oh years.

MP: Years. Why do you use it in preference to sodium hydroxide?

SP: It's easier to handle, it actually inactivates, as well, if not better than the sodium hydroxide; it's just more convenient. It's obnoxious for other reason, there's always plusses and minuses, but the onsite incinerator is a big issue.

The other thing is that, not just the startup, but in terms of working with these things, I was trained as a virologist – I studied herpes viruses, and there are certain things that you do with viruses – very well-established techniques, ways to study things – that you just can't do in the TSEs. You have to think of new ways to do things all the time.

MP: For instance, like what?

SP: Well, for example, with the SARS virus. SARS hit last year and then in two or three months they found the virus, showed it was responsible, sequenced it and now they've got it. And they can manipulate it, and here I'm thinking, "I've been working in this field for 13 years, I still don't know what I'm working with. Yeah, that's the advantage of having a virus.

MP: There are really good techniques that are fast that you can use.

SP: Exactly, exactly. And so with TSEs, here we've got – sometimes I'm embarrassed when I go talk to other virologists – I've got one protein to work with. One, of 254 amino acids! You know, there's a limit to what you can do, and we still can't figure it out and it's because the techniques that you use for viruses or bacteria do not always apply, and they've been tried. So to break into the field you have to be trained in a lab that knows what it's doing in the field. And there are very few of those labs in the United States, so you don't have a big recruitment, necessarily, of people. Prusiner's lab is huge, I guess.

MP: Yeah, they do a good job of training.

SP: Yeah, well I don't know how many of his former postdocs – not many of them, for the number he trains, seem to go off and found their own labs. I don't know what the numbers are but it doesn't seem that many of them do. But I could – I could be mistaken.

MP: I don't know, I've never really looked. But yeah, I see what you're saying, there's sort of a bottleneck in terms of people being able to get trained and then to get themselves set up.

SP: That's right. And you have to convince the university and whatnot that it's worth the money and time.

MP: In terms of the research, what are the things that make it take so long to do?

SP: In part because if you want to do the in vivo stuff, it's just the time involved. So like the inhibitor studies that we've done here in the transgenic mice, the first experiment,

and it worked – took two years. And then you have to repeat it. So in vivo-wise, that's huge.

MP: Takes a long time, so that's why the benefits of the cell-free conversion assay can stand out. But then at the same time – do you feel like when you're doing a cell-free study that you also have to complement it with a cell study or even an animal study?

SP: Absolutely.

MP: And is that because it's not accepted by everyone in the world?

SP: No, actually, it's not that, because that's kind of their problem, not mine. I've worked with it enough that I kind of know the – or I have some, I don't know all of it, but I have some concept of where it's good and where it's not and where I trust it and whatnot. It's more because, since I was trained as a viral pathogenicist, anything I do in a test tube or a cell, if I possibly can I want to put it into an animal and see if it's also true.

MP: I see. And is that also something that reviewers like to see, that you're publishing – that you've done all these different steps?

SP: Sure, sure, right, well, for example, the paper that you were talking about, the strain paper in *JVC* that we did, we first sent that to *EMBO*, and it actually got three pretty darn good reviews. But one reviewer said, "Well they've got to do this in animals before I believe it." And it's an impossible experiment to do in animals; I can't do it, it can't be done. We've done it in test tubes and in the cells but with the current technology we can't do it in animals.

MP: Why is it impossible in animals?

SP: You don't have the control.

MP: You don't have the control?

SP: You simply don't have the control. I don't know of a transgenic system available that will allow me to target PrP to a specific set of cells with a known glycopattern that I know exactly what is happening. I can't do that. I have to be able to do that.

MP: Well for instance, like the cells that you use, you develop a cell culture in that paper that there 3F4 epitope labeled. Could you make an animal like that?

SP: Yeah, in fact David Harris has made an animal like that, with that over-expressive mouse with the 3F4 epitope. We can do some stuff with that, but again, when it's a transgenic animal, you've got PrP expressed in all sorts of cells in the brain. I can look at the glycoform patterns and I can even try to micro-dissect them out, but I can't control it anymore than that, I can just observe what the animal gives me. You see, so you don't

have the level of control that you require to come to some of these conclusions. I can't shut off endycoytosis.

MP: Right, I see what you're saying; it would be a messier experiment to do –

SP: Oh, most definitely, I mean you can do it, and I could certainly do it – in fact, he gave us his mice for other reasons – I could do it but I'm not sure that I could interpret it any better than the tissue culture stuff, and it would probably be harder. So we're doing a collaboration with somebody in England where we might be able to work around some of that, but it's an almost impossible critique to deal with. So when that happens - it was bounced from *EMBO*, it got into *JVC*, not a single comma was changed.

MP: Wow, nice. [laughs]

SP: So yeah – that's the first time that has happened to me, it's so cool. My responsibility is done. So yeah, it's a huge issue. You want to go back to the animal but, particularly with these diseases, it's very, very difficult. Yeah.

MP: And what do you think – this is changing the topic a little bit, but with David Harris and Susan Lindquist, their two different interpretations of what's going on in terms of the endocytic pathways – or the endoplasmic reticulum and where things are happening -

SP: Cytosplasmic PrP –

MP: Yeah, exactly. What do you think? Can you describe the dilemma and then can you tell me what you think are sort of like the –

SP: You know, technically I have to admit that when I haven't reread the papers first –

MP: Oh yeah, I understand.

SP: At its most basic, Sue Linquist's theory is that cytosolic PrP is the trigger for everything. So if PrP is misprocessed, then shunted off into the cytosol, it will trigger the events whether it's sporadic or infectious, whatever; it's the key. And I think David Harris is more like "no, it doesn't have to be completely cytoplasmic, first of all, and it's not necessarily the trigger for everything and the reason it happens isn't necessarily because it's transported to the cytoplasm but because it's processed differently."

MP: Didn't he use some results, like the way it was glycosylated, to indicate how it had been processed?

SP: Right. They think it had to do – did it have to with high-mannose sugars, or something like that? It's very complicated. When I wrote about it in that little science paper I was so careful to try to not tick off either Sue or David. But it's a really interesting debate and it's worth pursuing. It may have – you know, I'm not sure that I

agree with Sue Lindquist that it's the basis for everything – I see where it could be but I don't know that the evidence is there yet –

MP: Well, for instance, I think Sue was very concerned for a while about proteasome inhibitors and their use in cancer chemotherapy because they might trigger TSEs.

SP: And that's quite an extrapolation.

MP: So okay, can we switch topics and talk about the TSE Advisory Committee a little bit?

SP: Sure.

MP: How did you get involved? Were you invited to be on it or did you apply?

SP: It was kind of this weird osmosis thing. I was asked to – several years ago – to give a talk at a couple of these TSE advisory committees on some of the – oh gosh, what was it, maybe it was on species barrier stuff. I gave a talk on scrapie and goats versus BSE in cattle –

MP: Using the cell free conversion assay to look at species barrier?

SP: Yeah, exactly. And then I was asked to sort of sit in the audience as someone to go to in case certain questions came up. And then they asked me if I'd be interested in being on the committee, they were going to have on these openings, and I said yeah and went through a process where I, with several other people, attended one of the meetings, sort of sitting on the side there, again, available for questions if they came up, just sort of listening, and then they asked me to be on it. I don't actually understand why they chose me. I think they – you know, I have a range of expertise that they like and I guess I didn't do anything too stupid.

MP: [laughs] Well you probably fit well with the committee.

SP: And I think possibly that too. I don't know exactly how they go about choosing people, but I know, at least I try very hard not to come with any particular agenda or bias. I try very hard to do that.

MP: Do they prepare you pretty well before a meeting – like do you pretty much, do they give you the appropriate papers and prepare you for what's going to happen?

SP: Oh yeah, were you at this? You weren't at the one in July or whatever?

MP: I was, I missed the first day but I was there on the second day.

SP: Okay, was it the gelatin one?

MP: Yes.

SP: Okay. That one, yes, they gave us a three-inch thick binder as just one of the briefing materials. I couldn't believe it, I was afraid I'd have to pay for extra weight for my baggage because it was so huge. And that was in addition to briefing materials, papers, background, whatever, for the other questions the committee was addressing. So they work very, very hard to give you pretty comprehensive written material and then, of course, very hard to bring in the speakers that they think are appropriate, to give you information that way.

So yeah, I always feel very well prepared. Having to go through the three-inch gelatin manufacture thing, that was just – whoa. But it certainly did prepare me.

MP: Yeah, I can imagine. So there's a lot of homework ahead of time.

SP: Oh yeah.

MP: And how much interaction do you have with the staff of the FDA?

SP: Quite a bit before a meeting. They're very good about sending you the agendas and timeframes, who's going to speak, and now since I've been made chairman they do take time to – we have a teleconference where they go over the questions and make sure that I understand what's going on, what exactly they're asking, so I can help keep the committee on track while still giving everybody the opportunity to say what they want to say, because that's the point; it's supposed to be an impartial advisory committee, not something that's being steered.

MP: Exactly. So you want everyone to have a chance to speak but you also want to keep the meeting on target so it doesn't go until 9:00 in the evening. [laughs]

SP: Exactly. Which happened once. And it is so hard to maintain focus –

MP: Right, I was actually just looking at the transcript from – I think it was the first meeting you were at and it said the meeting adjourned at 8:45 PM? [laughs]

SP: That's right, it was my first meeting, and I remember sitting there going, "Oh my God!" I just forced myself into bed, I couldn't even think.

MP: It must have seemed like it was never going to end, because I know when I go and I'm just sitting in the audience it always seems like a really long time.

SP: It is, and you know, it's when you're sitting on there you do have to – you have to focus the whole time, and you do occasionally, your mind can wander occasionally. But the longer it goes on the less productive it becomes.

MP: Exactly, so in a sense you want to keep it – you want to keep on schedule as much as possible or else it gets harder for people to really discuss things.

SP: Exactly, but if questions or problems come up then those just have to be discussed as fully as possible because the FDA wants to hear that. They want to hear the debate and the discussion, it's very important.

MP: And do you feel like they usually – when you guys, based on your discussion and the recommendations or the way that you vote, do they usually take your advice or is it kind of a sometimes/sometimes not situation?

SP: They always take it very, very, very seriously.

MP: So they always take it into consideration?

SP: Oh, most definitely. And most of the time they do what the committee advises. That's been my impression, anyway, because I hear, just like you, after the fact what the FDA does. And so I think they quite often just take the advice as it's given. Because that's, again, what the committee is there for, to provide the expert opinion based upon the data and what they hear, which is, I think, more difficult with the TSE advisory committee than with others. I've sat as a guest participant person on one of the vaccine committees – I forget which one. I was basically there as the TSE expert, and I think I answered one question: "Can that happen?" "No." you know, that was it, and you know I sat there two days.

But they're given a wealth of data to look at because they're dealing with viruses or bacteria or whatever, and they have a ton of data. So often with the TSE advisory committee there's no data. There are theoretical models, there are hypothetical situations, there's this and that but there's no hard data. And so it's a very difficult thing to try to advise based upon hypothetical models and theoretical risks and whatnot, to do so responsibly.

MP: That's one of the things that strikes me with this disease as being the most difficult. When people come and they show you these models about possible prevalence and potential risk. How do you evaluate them and how do you give them weight in terms of your considerations?

SP: Well for me personally, because I'm not a statistician, I can't judge the merit of what they're telling me scientifically, I have to trust for the most – sometimes I can if their assumptions are, I think, not quite right. But I have to trust that they do their job and they do it very well. There's a statistician on the committee now and I think he very well might be there for just that purpose.

MP: Is that Dr. Bailer?

SP: I think it is, yeah.

SP: And so for me, I have to look at what they're saying and do my best to judge it scientifically based upon my limited base of knowledge in that area and then weigh that against what the predicted effect of that would be in the real medical world. So with the blood supply issue; if you put on these sorts of prohibitions will that put our blood supply in danger? Will it put more people at risk than you're trying to protect, that sort of thing?

MP: Exactly. And, for instance, I know that there was a lot of modeling going on over the years to do with the blood supply, calculating this many donors could be lost but this much risk would be reduced, and how do you approach those sort of evaluations? Do those play a role in how you evaluate?

SP: Sure, sure. I remember there's a couple instances, one is they had said that, well, if we put this restriction on the donors, then that'll decrease the risk of variant CJD transmission from a theoretical – 6% to 5%. I'm like, "Wow! 1%!" It's a huge restriction and it doesn't give you much benefit. And I guess this was one of the reasons – my understanding is - that they went ahead with the CJD deferrals in the first place, they could – with a few, initially, simple questions - they could theoretically reduce the risk of transmission by 94% and not significantly impair the blood supply. And that's why they originally did it. So now, as time goes on, you always think, "Okay, if we put on these extra restrictions are we hitting the blood supply harder? And is it really worth it for what we're doing?"

So that's sort of the way I'm always trying to think of, for me, the real risk to the population at large from having an interrupted blood supply or not enough of a surplus or storage or whatever.

MP: So when you say real risk you mean in terms of having to delay surgery or things that can sort of be seen, that can be observed in people.

SP: Exactly. I would just hate to have something like that happen where because of deferrals that have been put on for various reasons, now people are in serious jeopardy of having blood for surgery. To me, that's a real risk; that could happen – no question. It has happened.

MP: Right. So it's a very complicated issue because you are definitely – despite the calculations, you're always weighing a real risk against a theoretical risk, whether or not you put numbers on that theoretical risk. And how do you deal with that issue?

SP: Well, I guess you have to think – it gets back to trying to guess what will happen. If it turns out variant CJD can be transmitted through blood, now there's this instance in England, which, I don't know the details of, but might suggest that it can be, by putting these things in place, you've already got a protection up. You can point to that and say, "Look, we've already done this as responsibly as we could to try to protect against this possibility." It's a precautionary thing.

[break in audio – tape turns over]

SP: …variant CJD is in the blood supply, which you can't detect, you can't find, you can't guard against, you can't treat. Well, then you're sitting there thinking, "We could have done this, possibly prevented that and now we can't." It's like letting the horses out of the barn – it's done.

MP: So when given a choice, you would rather take some precautions –

SP: Take some precautions –

MP: Even I it might never – like for instance, before this UK case – or potential UK case, the only information we had is that sporadic CJD had never been transmitted as far as anyone knew –

SP: Right, sporadic CJD's different from variant CJD. It's very different.

MP: Can you tell me a little bit about what the most important science is that you use – either studies or just pieces of information - that you use when evaluating the risk to the blood? I'm sure in all the meetings that you've sat through there have been a variety of studies that have been discussed, but which ones sort of stick out in your mind as being useful in evaluating the possibility of a risk of new variant CJD being transmitted?

SP: It's the transfusion studies that were done, primarily the newer ones by Nora Hunter, where she showed you could take blood from a BSC-infected cow and transmit infectivity to sheep. There's the Cevernikova studies with the 263K, and I think she did some human stuff as well. There are the old studies by Paul Brown, he did sporadic CJD and showed which tissues had infectivity using monkeys, which is pretty good. Those I give a lot of weight because those are scientific studies that are done pretty well and the data looks pretty solid and consistent.

MP: How do you compare the Hunter/Houston study, or even Paul Brown's primate study to Cevernakova's mouse study? How do you evaluate the utility of each study?

SP: Well yeah, that's a complicated question, I guess for that specific example, when I think about it, I think of the BSE sheep studies because one thing that appears pretty consistent is that when BSE goes into another animal species it does similar things, regardless of the animal species. With the small data pool we have that appears to pretty much hold up, so the BSE sheep experiment is closer to the vCJD – BSE/vCJD human thing than the others. But then some of the studies that Cevernakova or Bob Rowher or those guys have done, you use animal model systems like the 263K system where, you know, 263K scrapie is kind of more restricted in some ways than mouse scrapie. So it's not quite like variant CJD in people, it's not off in the periphery, but they can find the stuff in blood; it might not be concentrated, but they can do it. So that means it's there in a sense even when you have less peripheral involvement. So I give that weight for a

different reason, it means that even without large-scale peripheral involvement it can be there.

MP: That causes another eyebrow to be raised.

SP: That's right because that goes to – well now in sporadic CJD there's not as much peripheral involvement. So but then you're stuck with apples and oranges, and you have to, at the end of the day, come to the conclusion that it can happen. We know it can happen in these models, we don't know it can happen in humans, but because it can happen we have to assume that possibility exists, and you have to go from there – the possibility exists. It can happen. You cannot say that there's no data to support it because there is. As peripheral as that data is, it's there.

MP: How would you evaluate that primate study, like say in cynomalogus macaques or squirrel monkeys or whatever, of blood transfusion compared to the sheep studies?

SP: Yeah, well because it's another step closer would I give it any more weight? Hmm. I guess that's an interesting question because if it was transfused – if it did transmit I'd say okay, so it matches the sheep/cow thing, and it would support it. It wouldn't necessarily change my mind, it would just reinforce that it could happen, and it could happen in an animal that's a step closer to humans than a sheep, than a ruminant, is. So it would reinforce it. Would it cement it in my mind that it could definitely happen? No, because it's a monkey model. But it would certainly reinforce the initial observation and just make you think more that you have to guard against the possibility.

MP: What if it did not transmit in primate studies?

SP: I was kind of hoping you wouldn't ask that.

MP: [laughs]

SP: What if it didn't? Ohh, I could hedge and say, "Well it depends upon how the experiment was designed," but if it didn't, well then, you see, you're stuck with a negative result versus a positive. You'd have to give the positive more weight.

MP: Just to be precautionary?

SP: Yes to be precautionary because okay, it didn't go into monkeys, it did go into these other instances. So it's the difference between positive and negative data. Is the data negative because it doesn't work or is the data negative because there's some flaw that's been in the study or that's apparent or inapparent? It might be flawed for a reason we don't know yet. So you would still have to give more weight to the positive data because it's positive data. It seems – it's what you'd have to do, I think.

MP: From a regulatory standpoint?

SP: Yeah, well even from a scientific standpoint you'd still say, well it's negative data which suggests it doesn't go, but it's negative data, so you never know. There's always a problem with negative data.

MP: Negative data, is always more difficult to interpret than positives?

SP: Always, always.

MP: Let's say you had a blood transfusion experiment in transgenic animals – like transgenic mice where they had human prion protein genes.

SP: Yeah, the transgenic thing, it's given us a lot of information, I think, that's been good to apply to other experiments, but the transgenics themselves are artificially – you put a human PrP gene in a mouse, it's still not a human. So I always take the transgenic experiments, actually, with a note of caution because in many ways it's more artificial than not. When you're doing experiments with a strain of TSE that's been adapted to the experimental animals such as a mouse or a hamster, you've got wild-type levels of PrP expression, the animals aren't genetically modified, they're just what they are. When you genetically modify an animal by putting in these transgenes, which can incorporate anywhere in the genome, you way overexpress, you have to take that into consideration, that what you're seeing may not reflect reality. I don't want to in any way put down the transgenic experiments because they're very important and they've given us a lot of information. But you do have to interpret them with that, I think, in the back of your mind.

MP: They're very useful models and they tell us a lot but in terms of trying to extrapolate from them to the human experience…

SP: You have to be kind of careful. So one experiment that comes to mind is the Prusiner one that I know has been bandied about in the press a bit, that you can find prions in skeletal muscle, and that's his PNAS paper. And the experiment that convinces me there is the very first experiment they do, which is RML scrapie into wild-type mice. And they find, very convincingly, infectivity in the skeletal muscle. And that's – you know, we've never looked here. "Ohh, whoa!" And it looks real and it looks good. The rest of the paper is these transgenic manipulations that are kind of difficult to interpret for a lot of reasons; so difficult in some ways that I just don't look at them. I think of that first experiment – that's the one that convinces me.

MP: Right, because you're adding layers of complexity to interpreting –

SP: You're adding layers of complexity, right, exactly. And so that makes it hard for me to wrap my mind around it because I'm thinking, well they didn't do this and that could be true and they don't really know X, and at the end of the day I always go back to that first experiment.

MP: And do they use – I'm trying to remember, did they use special techniques to detect the PrP scrapie in muscle or was it just sort of standard detection?

SP: I think they just looked. I think it was a western blot.

MP: Okay, but they didn't do any special precipitation or anything?

SP: Well they might have done this new phosphotungstic acid precipitation, which is supposed to increase – I tried it one year, it was a dismal failure, I screwed something up.

MP: What happened?

SP: I just didn't get anything. [laughs] Obviously I've done something, right? So I just haven't used it again.

MP: Right, and have you called them and talked to them about it or was this just –

SP: No, it wasn't really necessary for what I wanted to do.

MP: Right, okay, you were just trying to see – test it out in the lab, yeah.

SP: There's someone else here who's done it and thinks it maybe increases his sensitivity a couple fold.

MP: But just a couple fold, it's not a huge difference?

SP: It wasn't a huge difference, but you know, so my experience with it has been bad. But, yeah, I think they might have used that phosphotungstic acid thing. And I don't think we'd ever really looked in tissues like that in a mouse. I know that Rick Race and Bill Hadlow many years ago did these experiments looking at tissues from sheep – they have a paper, they looked at all sorts of sheep tissues. And there are papers where they looked at the same thing in naturally infected goats. And there is in this one paper where they looked at actually infected goats - one out of the fourteen goats they found a trace of infectivity in the skeletal muscle tissue. So there's that little piece of information. There's a new paper that just came out, I think –

MP: In the *New England Journal of Medicine*? Budka?

SP: Oh okay, that's right, Budka. And it's finding abnormal prion protein in muscle of CJD?

MP: Yes, sporadic CJD. And it's something like one out of three that they test – I can't remember how many they test – like 12 or something, but they find it in one out of three

SP: One out of three, I'd have to look that up because that's now only the third paper I know that said this.

MP: So there's the goats, there's the mice and then there's the people –

SP: Yeah, the goats, Prusiner, and this, right. All of that suggests that it can be there. Now whether it can be there in BSE or VCJD is different. BSE, there's no indication that it is. Even when they've taken that tissue and put it back into cows, which should be the most sensitive, as far as I know they've still never found it. So BSE may be much more restricted, and it may be because it was – I was just thinking of this the other day, I was having a talk with one of my technicians here, Ann Raynes, and she was mentioning how it seems BSE is more easily transmitted orally. This has sort of been one of these things that you hear people often say, that it appears to go more easily by an oral route than other TSEs. It occurred to me that that makes perfect sense because if BSE is a consequence of scrapie – sheep scrapie being fed to cows - it was selected orally. So it's evolution in action, you're passing something orally time and time again –

MP: [laughs] Oh that's interesting, yeah. I never thought of it that way.

SP: I had never either and it's been years – oh! It just occurred to me – so I'm probably going to try to do an experiment in mice like that. But the thing is, is it's been selected in a way that as far as I know nothing else we work with has been.

MP: That would be interesting to test in mice –

SP: Yeah, I thought I'd give it a shot. Yeah, it may be possible that when a TSE agent is transmitted and adapted by an oral route, you do get a significantly restricted expression of the abnormal protein.

MP: Right, it might be a consequence.

SP: So I mean, it's worth testing.

MP: Back to the muscle results - how do they affect your thinking about surgery and cleaning of instruments and whatnot?

SP: Well, the Prusiner one wouldn't have affected my thinking much at all because of the fact that the old Paul Brown papers on sporadic CJD said - I think they did test muscle in those old papers and they didn't find it – they tested everything, I think they did test muscle. So I'd have to actually – I'd have to read that newer Aguzzi paper to answer that.

MP: And the Paul Brown ones you're talking about, those are probably inoculation intracerebrally?

SP: IC into monkeys.

MP: The other question is, if they're measuring using western blot, they may be detecting PrPres, but is it infective?

SP: That's true, you do have to show that it has infectivity. Now I will say that as far as I know there's never been an instance where you have bona fide PrPres and no infectivity. So you can have infectivity without detectable PrPres, and there could be lots of technical reasons for that. But if they do see – I'll have to look up this paper – if they do see PrPres then, as much as a scientist can ever say, "I guarantee you," I almost guarantee you they will find infectivity.

Now, that paper – I'd have to read it, but that paper could change one's thinking because it's in the human model system – they're looking where they should, it depends upon how they looked and what they found and –

MP: I think they used the phosphotungstenate precipitation. So they might be detecting very low levels. But then the question is, would they be worrying?

SP: Well you know, it could be. Well now there's something I hadn't thought of – if they're doing it with this phosphotungstic acid thing then maybe they should test that in animals to see if it is infective, because that is a different techniques than is normally used, or has been traditionally used, and I guess you have to deal with the possibility that, under certain conditions, you might be precipitating out something that might not be exactly – might not be bona fide PrPres, it might be – so I suppose technically you should, in that case, since you're using a newer purification process you should probably test it as a matter of course.

MP: Right, right. I'm sure someone will…

SP: Oh sure, and someone might have, I must admit I don't know. But it would change your thinking and it would bring into question, sort of like the restrictions they have for cornea donors, that if you're presented with a person who has died with signs of neurologic dysfunction, that you not take the nervous system tissues - like when they used to do it in dura mater - you don't take the corneas and you might think twice about taking any tissues from them. And it's like with the cornea things, if it turns out that you've taken tissue from someone who is later diagnosed with a TSE, dispose of those instruments, don't ever use them again. And since it's such a rare thing, that isn't as big a financial burden as disposing of instruments every time. That would be just horrendous. But did you have – I'll have to look at that paper because that is a definite –

MP: So back to the committee meetings a little bit, how much context – so when you're thinking about something like the blood supply and how to regulate or even corneal transplants - how much context do you feel like you need in order to advise appropriately on these issues?

SP: How much context?

MP: I guess what I'm getting at here is a little bit of the distinction between risk assessment and risk management, and how in the SEAC committee they're very hesitant to venture into many areas that are outside the science of risk. For instance, with blood – what things do you feel you need to consider other than the risk of transmitting TSE?

SP: Right, right. For me the important context is what would be the potential outcome of some advice we give to restrict something - to add new restrictions to a manufacturing process or new rules to tissue harvesting or blood supply – what would be the possible impact on the normal patient population.

MP: So you want to know, in practical terms, what is this going to look like when it's put into practice, and in terms of consequences other than its effects on TSE risk.

SP: Absolutely, is it realistic. And I remember one meeting, it might have been that first horrendous 8:30 PM meeting that I attended, I think I said one thing during that whole meeting and it was they were talking about putting on new restrictions on the blood supply. And all the blood people were saying "they can probably make up the difference with new recruitment" and whatnot, and the one thing I said was, "Well, why don't you show us that you can do that?" Get the people, show us that you can do that, recruit new people and do so effectively and consistently – and then – because in a way it's like putting the cart before the horse. "Go put on these restrictions and then guarantee us you can get back the population." And it turned out they couldn't, I think, is largely what happened. They had mixed results.

MP: At the February meeting of last year it seems like there were a lot of organizations that came and talked about how they really did lose a lot of donors. But then how do you then take that information into account?

SP: That they couldn't get the donor base back the way they thought?

MP: Right.

SP: Yeah, well, that's again the cart before the horse and you think, "Well great, now we've got these restrictions, and we can't take them away based upon what little science we have because that's why we put them there in the first place." You're kind of stuck. I think what it does do, is it makes you extraordinarily hesitant to potentially put on any new restrictions.

MP: Looking back at this old meeting there was at one point a question that was asked to Alan Williams and he seemed to indicate that it wouldn't be that difficult to take the restrictions off if they later decided that it wasn't a risk…

SP: He did.

MP: And that doesn't seem to be the case, really.

SP: No, because – and I think even at this last gelatin meeting, there was some talk of listing some of – I can't remember all the details on the restrictions – I don't think there are any necessarily restrictions on gelatin – except for the tissue, where they get the tissue. And there was some talk about changing the sourcing and no one was too keen on that – it was like, "Well, do we know – does the new scientific research on inactivation during the gelatin process say we probably don't need it?" "Yeah." "But do we want to take it away?" "No." Because if we're wrong about that, we're screwed by taking away – by reintroducing the source. So, yeah, that's why I think it's much more difficult in many ways to retract because you are sort of, as you said, trying to be protective.

MP: Right, because it's a difference between removing risk and adding risk, and you're then – if you're going to talk about removing restrictions you're being put in a position where you're being asked to –

SP: Add risk, even if it's theoretical or hypothetical. And that's what makes it so difficult and so hard to juggle – I must admit, I get very frustrated sometimes having to think about these things in terms of hypothetical and theoretical limits – "Oh my God!" You know, I don't have any data; I just have to think hypothetically. It's very, very difficult, which is why I was so thrilled that - despite the three-inch thick, 400 lb briefing material - I was so happy that there was data. I said, "Oh my God, look! It's actual stuff I can look at and assess."

MP: In that case there was a lot of data in terms of the process and the inactivation studies that like Robert Sommerville and David Taylor and those guys have been doing.

SP: Oh yeah, and it makes – the positive thing about that is it makes you extraordinarily hesitant to ask for any restrictions on the process because they've got this data that says well, the process probably takes care of what – if anything is there - probably inactivates it. It was reassuring actually, and I know that one question came up that at some point the lady from the corneal transplant got up there and went through her inactivation steps for the instruments. That was really impressive – "Oh my God, they do a better job than we do!" And then some comment came, "Well should we restrict?" And I was like, "No!" They're already doing it, you know, I think reasonably. So you want to try to – you don't want to put an unnecessary burden on these people who, most of the time they seem – I mean, I've always been sort of positively impressed with the efforts that these institutions go to to try to follow the FDA recommendations. I've always thought, at the very least, they make a good effort towards doing that.

MP: How much do you think, in terms of the blood supply stuff, how much do you think the history of HIV and particularly the involvement of certain groups that may have been affected by HIV and hepatitis C, like the hemophiliacs, has played a role in being precautionary in that area?

SP: Oh I think it's definitely because – particularly that group of individuals was directly affected when people were wrong, when they said, "Oh, it probably won't be

transmissible through blood," they were just absolutely wrong. And you don't want to make that mistake again.

MP: So even though it's a theoretical risk –

SP: You don't want to make that mistake again.

MP: There's this extra precaution being put there because it's happened before.

SP: Exactly. It's happened – like a couple times before, and even with the West Nile virus where they find evidence that it can be transmitted to blood.

MP: In considering the risk, do you view blood in the same way as plasma products or do you separate them in your mind?

SP: You have to separate them. Because of the way – and I'm sorry, I can't remember all the details - it's because of the way they're treated that the plasma products are far less likely to be a source of infectivity than the others. It's the way they're treated and isolated.

MP: Have you guys been given presentations from plasma fractionators where they talk about the processing in studies that they've done?

SP: Bob Rohwer has done some modeling.

MP: And is it those studies – or rather, what causes you to think that plasma products are probably a lower risk than blood components?

SP: It's those studies, yeah. Paul Brown's done stuff, too – he's got several papers out on that. Yeah, so it's those studies that cause you to separate the two. And I think there's a sourcing issue too, for the US, that plasma can only be US-derived or something like that. So the feeling is there's no need to deal with it. I think that was part of it, because of the sourcing.

MP: And what about some of Bob Rohwer's more recent work where he actually deals with endogenous infectivity?

SP: Endogenous infectivity, what do you mean by that?

MP: Okay, so in a lot – okay, I'll explain it – in a lot of Bob Rower's work, up until very recently he was dealing almost entirely with brain spikes in blood, so that – and the same with Paul Brown and Larissa Cevernikova. They were always dealing with spiking – I mean not always, there were some endogenous ones where the animal was actually infected and they could look at partitioning, but it was really difficult to do because it was such low levels of infectivity, and so what they would do is they would take brain homogenate and add it to the blood and then go through steps, either in a series to see

how a series of steps would affect the partitioning, or just each step doing an additional spike. What do you think about that, the spiking with brain homogenate and blood?

SP: Well, that's the worst possible scenario. So on the one hand, you're weighting the system to almost give you a result where you're not going to clear everything because you're putting in so much to start. So if you're able to clear everything you put in that's great, because you can show you can put in a ton of infectivity and this particular process gets rid of it all. If you don't get rid of it all, which is usually what happens, there's some residual left, it's good information to have but it now makes you question, well, you've put in so much and you shot yourself in the foot. So essentially the process would clear the small amount that would be there normally but can't clear what you've artificially put in, so is that really relevant? So the question of relevance, and – again, it's just trying to weigh the information they give you versus the other science that's out there versus what you need to do. It's very complicated. It's weighting and relevance. I like the spiking studies but it's nicer if you can take infectivity that's there naturally, as long as you can detect how much is there to start. That's the most direct way to do things, if you can say, "We had 10^3 logs to start and we went through this process and we detected nothing," that's pretty good. The process is robust enough to take away what might be there naturally.

MP: And that's the most convincing –

SP: It's the most relevant.

MP: Yeah. But what about the fact that with plasma products you're also batching – I mean you're putting in –

SP: You're batching. So if you get a contaminated batch will it cross over to –

MP: Right. Because for instance with human growth hormone, that was one of the issues that caused – I mean that you ended up, if you had one pituitary extract from someone with sporadic CJD it would then get mixed in with a whole bunch of other –

SP: Yeah, well that's a nightmare scenario. And I know that's come up in relation to blood products and other manufacturing processes – what happens if you do get a contaminated tissue and it contaminates your equipment and then it goes on to contaminate your processing. And, you know, I don't know that there's much data if any on that at all. So it's something that, from a laboratory standpoint, having worked with this stuff for many years I know darn well what can happen. You can grind up a brain with a homogenizer, sterilize it, and you might still detect infectivity on it. So from a practical standpoint, I know what can happen. From a real world standpoint, I don't know that it will happen. So again, there's no data on whether that should be a concern or not. It's something you have to keep in mind though.

MP: Because on the one hand you can look at plasma products as having less risk – first of all, it's going to be dilute, whatever makes it in there, and mixing it would make it

even more dilute. And then you have all the processing steps, which would deactivate or remove some of the infectivity. But then there's also the issue that if there is infectivity put in there, it's mixed around, and that more than one person could be getting exposed. So it's a complicating thing.

SP: Extremely so, and I was thinking about this recently too because of this BSE cow in Washington, because those materials – it was rendered. So now you had something that was positive for BSE that's been rendered and it's been in contact with all this stuff – how big an issue is that?

MP: Yeah, what do you think of the mad cow case in the US?

SP: The one that we're blaming on Canada?

MP: Yeah, the one we're – okay, well do you think it's appropriate to blame it on Canada?

SP: Well it came from Canada, that's what happened. Oh, I think it points out important deficiencies in our system that they're now addressing, and I think it raises an appropriate red flag for a lot of things. It brings up the issue of monitoring – closer monitoring and whatnot. So I'm not, myself, personally concerned that there's going to be a huge BSE epidemic in the United States, but I wasn't even before the cow. I don't know. I think it's done some useful things.

MP: Do you think that there could be cows that are being missed?

SP: Oh I think that could be true – sure, I think that could be true anywhere though. You're going to miss something if you're not looking for it or looking in the wrong place. So, for example, these ideas that we should test every cow in the United States for BSE – okay, what age do you want to test them? Kind of silly to test every cow. So if I test every cow at one year old I can almost guarantee I will find no BSE.

MP: But if you had, say, a more targeted testing program like only animals 30 months and over, something like that – more efficient?

SP: Sure, or any animal – like I think they do in Europe, any animal that goes to slaughter, they do one of these rapid, the Biorad or the Prionics test and they don't put that animal into the food chain until they get the test back. And so there you're targeting the population that's entering directly the human food chain, and that's cool, the 30-month rule is cool. Yeah, I think that's the sort of thing that's most appropriate and I do like that the USDA tests the downer cows. And it's because of Bruno Oesch, I saw him present at a meeting once – where did he present this? I think it was an open meeting – yeah, it was an open FDA meeting, where using the test his company, Prionics, had developed, they looked at populations of cows in Switzerland - the normal population, the slaughterhouse population, and the downer cow population - and they found a higher percentage of BSE positivity in their downer cow population. So the USDA, by testing

that population, I think, is testing a population that gives you the greatest chance of finding a positive.

MP: Are you familiar at all with the Harvard Center for Risk Analysis Study?

MP: Because I was looking through it and I was kind of interested, there was one chart where they show what they're using as their estimates for infectivity in cattle at different stages in the disease, and they show that for a short period of time, like from 6 to 18, months they have a small amount of infectivity in the distal ilium , and then they show that from 18 to almost the time that clinical signs start that there's basically no infectivity anywhere in the cow. And then it shoots way up, exponentially increases until it reaches high levels in the central nervous system. It seems a little strange – okay, let me just ask, so in mice, that you're dealing with, do you know what the infectivity curve would look like in mice?

SP: Yeah. So when you put it in, when you inoculate infectivity IC, directly into the brain, within a couple of hours you can barely detect it anymore; it's gone. And there's a period of time – it's a classic thing in virology, the name of which now escapes me, but it's classic – oh, an eclipse period. It's an eclipse period. You put the infectivity in and it all goes away until it starts to replicate and then you can start to detect it again. So scrapie does have an eclipse period. It all disappears, and it's not clear why – is it because it's just diluted - you stick it in the brain, you are disrupting the brain blood barrier, it kind of gets shot out everywhere – is it just diluted throughout the mouse? Is it because the body can just get rid of most of it? And there are some reports now, recently, that say things like dendritic cells can chew it up and get rid of it. So to have a period like this in a cow isn't unexpected. I don't remember – they probably did those assays in mice. And so there's a question of sensitivity. And so I think what you could say is that at least for the level of sensitivity they have, the infectivity kind of disappears for a period of time.

MP: And in mice – how long does the eclipse period last in mice? Is it on the order of months?

SP: I'm thinking weeks. I'm trying to remember what Rick did that, when he injected something IC the first place he could detect infectivity was actually in the spleen about three weeks later, three/four weeks later. So there was a several week eclipse period.

MP: I guess one of the reasons it's different with a cow also is because you don't have the lymphoreticular system being involved, so that maybe whereas in a mouse you have this replication in the spleen and whatnot occurring in the intermediate timepoints that you're not seeing in a cow?

SP: Right, exactly. Very much so.

MP: So that could be where the difference is coming from, because in sheep, likewise, you would see higher infectivity at earlier points in time, I would imagine, because of the lymphoreticular system.

SP: Right, yeah. So it's acting just like a viral infection would – it gets in, disappears and comes back.

MP: Does having BSE in North America cause you to think differently about the blood supply? For instance, most of our regulation is based on sourcing, and not sourcing from people who may have been exposed.

SP: I don't know yet because what I know about the BSE cow that was found here is what I read in the news. So I don't have enough information about how it was processed and what was done with it and where it went and whatnot to answer that question. I guess I would have to have more information to make that kind of thing. I'll certainly listen carefully if I get that information. Oh, and now you have to take it into consideration because – it's not necessarily because there was a BSE-positive cow here, it's because that BSE-positive cow was rendered. So it's gone other places. And that's the key thing. If it had been a BSE-positive cow that they found that never went anywhere, fine. But now it has entered into wherever it's gone. And the fact that you find one positive cow when you're looking – I forget what number I've read – but that the USDA tested 20,000, maybe, cows last year and they found one positive. So you wonder if they tripled the testing would they find three positives? Is it a rare event or is it something – and that makes a difference, as well, when you start thinking about blood supply issues or food supply issues. Say, "Well now they've looked at 60,000 cows and they found two cases, now you have a prevalence of basically one in every 20,000." And that adds up to a lot of cows very quickly.

MP: Right, exactly. But it's much more difficult when you've only detected one case to figure out what that means –

SP: Were you lucky or unlucky?

MP: Exactly. Yeah, it's the same problem if you think of the tonsil studies in England where they're trying to use those to estimate the prevalence of VCJD and I think they got one positive tonsil out of 8,000.

SP: Yeah, that's what I remember, too.

MP: And what do you do with that number? I mean, is it really – are one in 8,000 people infected or were you lucky?

SP: Exactly, exactly. You know, I was thinking about this in regards to the corneal transplants too, it struck me a few meetings ago when the corneal transplant people got up there and they were talking about the restrictions they had in place to prevent possible transmission of CJD via these transplants. They said, "Well since we've put those rules in

place we've done 600 and some thousand corneal transplants and have not had another instance of transmission." And that's great, but with an incidence of sporadic CJD of one in a million, are your rules effective or are you just right now you're not within the statistics of it? So it's the same issue.

MP: I think that's really what makes this disease so complicated is the rarity and the dealing with the statistics.

SP: And it has an enormous impact on every level, as rare as it is.

MP: What is it about this disease that has such an enormous impact, despite its rarity?

SP: Well I think – I guess one way I always think about it is we've all become accustomed to when a new disease pops up you turn to the scientific world and some guy in a white lab coat gets up there and says, "Oh yes, it's caused by this. It works this way and we now have a therapeutic to it, a vaccine, a diagnostic" – you can see lots of forward progress being made, like with SARS and West Nile – very quickly. And with the TSEs, this hits the news and you go what can you do about it? And you go, "Well, we don't know what it is; we can't diagnose it early; we can't treat it if you've got it; it's always fatal once you have clinical signs." And you can't tell people they can't get rid of it – can you wash your hands to get rid of it? Nope. If you cook your hamburger well can you get rid of it? Nope. So all of the sudden you're not able to give people the answers that they want to hear to be reassured. And in large part – at least, that's the way I think of it. I don't know if that's true or not, but I think psychologically it's different thinking about this, it's this unknown kind of creepy disease versus viruses or bacteria, which everybody at some level knows about. You can go to the doctor and get an antibiotic. So it takes away some of the – I mean I myself am far more nervous about getting West Nile virus or SARS and not recognizing it early enough than I am about getting a TSE.

MP: It seems more likely to you, like these other things, seems scarier and more likely but this is just that there are so few answers –

SP: There are so few answers.

MP: – and we really don't know – in terms of even characterizing its transmission it's so unknown –

SP: It's so hard to do cleanly.

MP: And also I think the problem is, is with the incubation period and the potential for subclinical cases, that you just don't know what's happening. It could be multiplying and all of a sudden become visible and you wouldn't have even known it was there.

SP: That's right. Yeah, it goes back to the diagnostics – you don't really know it's there until late. Which isn't very reassuring to anybody – well what can you do?

MP: All right, well I should probably go but thank you so much for chatting with me. Is there anything else you can think of that I should look at, or that strikes you as being relevant to my –

SP: Boy, no, you've hit on so many topics. No, not offhand, I think you addressed a lot of it actually.

MP: Okay. Well, I will probably see you at the upcoming FDA advisory committee meeting. I'm planning on attending, it seems like it will be very interesting.

SP: Well, you do travel a lot with this project, don't you?

MP: Yeah, actually I do travel a lot. I'll be hopefully going to the CIAC [spelled phonetically] meeting later that month, too.

SP: That's so cool you get to go to the – how long do you spend in the UK when you're there?

MP: Usually, the past three times I've gone, I've spent almost a month there because I was going to Edinburgh and I was going – you know, I was kind of traveling around outside of London –

SP: Wow, great!

MP: And it's been great but I'm also kind of just getting a little bit tired. [laughs]

SP: Yeah, that I can understand. I had – last year I had 13 short trips –

MP: Oh my God!

SP: – I was like oh my God, and it was like once every three weeks almost and I hate that!

MP: Ahh, that's a lot.

SP: That's a lot, I want to stay in on place for more than three weeks.

MP: I know, and I realize sometimes I really like being home. [laughs]

SP: That's right, that's what I realized, too. At first it's cool, then you're like, "Oh my God, I've got to go there!" –

MP: [laughs] All right, well, thanks a lot and have a really good rest of the week and everything –

SP: You too.

MP: Bye.

SP: Bye-bye Maya.

End of transcript