This Week in Virology

with Vincent Racaniello, Ph.D.

Episode #203: Mark Challberg, a Cold Room Kind of Guy

Aired 14 October 2012

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http://www.twiv.tv/2012/10/14/twiv-203-mark-challberg-a-cold-room-kind-of-guy/

Vincent: This Week in Virology, the podcast about viruses, the kind that make you sick.

This Week in Virology, episode number 203, recorded on October 12th, 2012.

Hi everybody, I'm Vincent Racaniello and you're listening to TWiV, the podcast all about viruses.

Today I'm in… we are in Alexandria, Virginia. We're back in Alexandria. You may remember we were here some time ago. And yes, we are at Ed Niles' house. But Ed Niles is not with us today.

With me today from north central Florida is Rich Condit.

Rich: Hi Vincent. How are you?

Vincent: Across the room from me.

Rich: Yeah, here we are face-to-face again.

Vincent: In Ed Nile's living room.

Rich: Yeah.

Vincent: After study section. Just like last time.

Rich: Right, exactly.

Vincent: And we are here because we have a guest today, who we're going to speak with.

Rich: Wait a minute.

Vincent: What's that?

Rich: The weather.

Vincent: Before the guest even?

Rich: Yeah.

Vincent: Okay, weather in Alexandria.

Rich: A beautiful day, sunny blue skies, fall is just starting, 65 degrees Fahrenheit… whatever that is in Celsius I don't know.

Vincent: I don't have my phone with me so I can't give you the Celsius. It's a really nice day here.

I have no idea what it's like in New York. It's probably similar.

Okay our special guest today is from the National Institutes of Health, NIAID – National Institute of Allergy and Infectious Diseases, the Virology Branch in the Division of Microbiology and Infectious Diseases. He is a Program Officer for alpha herpes viruses and pox viruses. It's Mark Challberg.

Mark: Hi Vince, good to be here.

Vincent: Welcome to TWiV Mark. We see you all the time, Rich and I, at study sections. And now we get to talk to you. That's great. Thanks for doing this with us.

Mark: Well my pleasure.

Vincent: So we just finished, Rick and I just finished study section and we thought that it would be nice thing to chat with you about your whole life [Laughter] starting from the beginning.

Rich: By the way, study section, I don't know if we've actually talked about this in detail, but for those listeners who don't deal with this every day, what that is a panel of scientists who do peer review on grants. So when a scientist submits a grant to NIH asking for money, that goes through a bunch of administrative stuff that we'll probably hear about a little bit today because Mark is part of that process, that winds up, along with a bunch of other grants, in a peer review process that involves getting together a group of 20 or so scientists to evaluate the scientific merit of these grants and then pass them on with those ranking to the NIH administration for determination whether or not they are going to get any money. So that's what study section is.

Vincent: So I wanted to start from the beginning with you Mark. Let's just start where… where are you from? You have a southern accent, right?

Mark: I don't think so. I hope not.

Vincent: You know… it's not southern, what is it? Is it Brooklyn?

Mark: Well I actually grew up overseas. My parents were missionaries. I grew up in South Korea.

Vincent: Okay.

Mark: And I left Korean when I went to college. I went to college in Maine. So it's hard to see a southern accent coming.

Vincent: I… so what do you think, you don't have any sense of southern accent in it?

Rich: Well he talks a little funny [laughter].

Vincent: [Laughing] He talks a little funny. Okay.

Mark: Must be all the time I've spent in Maryland.

Vincent: Maybe, that maybe it.

Rich: Maryland.

Vincent: Maryland accent. Which college in Maine did you go to?

Mark: Bowdoin College.

Vincent: Bowdoin. Okay.

Rich: So you lived in Korea until you were how old?

Mark: Eighteen.

Vincent: Wow.

Rich: Do you speak Korean or did you?

Mark: I did, a little bit, yeah.

Rich: Huh.

Mark: Although I went to an American high school there.

Vincent: So in Bowdoin what did you major in?

Mark: Chemistry.

Vincent: Okay. And when you graduated were you ready to go on for a PhD?

Mark: Yes. So I went to John Hopkins Medical School where I got my PhD in the Department of Physiological Chemistry at the time.

Vincent: This is roughly what decade?

Mark: Early 70s.

Vincent: Okay.

Rich: And that was with Tom Kelly right?

Mark: No. No that was… got my PhD with Paul Englund.

Rich: Of course. Okay.

Mark: And then I stayed on at Hopkins to do a post doc with Tom Kelly.

Rich: Okay.

Vincent: And for your PhD, what did you do?

Rich: I worked on adenovirus.

Vincent: Wow.

Rich: Adenovirus DNA polymerase.

Vincent: That's why you guys are friends, right?

Rich: I didn't know Mark then. You know, I didn't meet Mark until way into the herpes era, I don't think, which we'll get to. But I must say that my very first PhD student worked on a temperature sensitive mutant that we isolated in the virus DNA polymerase. And to prove that that was a temperature sensitive mutant in the polymerase we went so far as to partially purify the enzyme and show that it was thermal labile in vitro.

And what that really involved… and this scared me to death right, because, you know, I was not a biochemist. But what we did was made a small scale lysates from infected dishes, made lysates and ran them over a DEAE column, and eluded step wise with different salt concentrations using Mark's protocol as our guide because what he'd showed is that the vaccinia polymerase alluded at a different salt concentration than the host polymerase. There's a very clear two peaks. In uninfected cells there's only one peak and in infected cells there's two peaks. We took the second peak and showed its thermal labile.

So thank you Mark.

Mark: You're quite welcome.

Rich: It worked like a charm.

Vincent: So you had purified the vaccinia polymerase by this column?.

Mark: Right.

Rich: And a few others.

Vincent: So then I have a paper here that Rich put up in 1979, Challberg and Englund, *Purification and Properties of the Deoxyribonucleic Acid Polymerase Induced by Vaccinia Virus*. So this is….

Mark: That would be the one.

Vincent: This is part of it, right? And this polymerase was a complex I guess or was it a single polypeptide?

Mark: It was a single polypeptide.

Vincent: Coming right off the column, one polypeptide right?

Mark: Well after several columns it was one polypeptide.

Vincent: And that single polypeptide has activity by itself?

Mark: Yes, it has DNA polymerase activity by itself. It's not competent to replicate vaccinia DNA because the way it actually comes is as a complex with two other proteins but we didn't know that at the time and you know we purified by using an assay that didn't depend on these two other subunits. And so we got a single polypeptide.

Vincent: So at this time, this is 1979, was this the first viral DNA polymerase purified?

Mark: It might have been.

Rich: I'll bet, I'll bet it is.

Mark: I had the first animal virus DNA polymerase. Certainly phage polymerases had been purified.

Rich: Right, right. That makes sense.

Vincent: So you went in to this, this was a PhD program. And he said, Englund said, "You should purify this enzyme." And you did?

Mark: Yep.

Vincent: That's how it works.

Mark: Yes, that's what I did. That pretty much sums it up.

Vincent: Nice.

Rich: Did you in fact, like HeLa and spinner or something like that?

Mark: And spinner, yeah.

Rich: Okay. So… a lot of stuff, right?

Mark: Yeah.

Rich: Fairly large volume?

Mark: I think the standard prep involved about 30 liters of HeLa cells.

Rich: Okay.

Vincent: You say in this abstract that it co-purifies with an exonuclease.

Mark: Right.

Vincent: Is that part of the….

Mark: That's an activity that's intrinsic to that polypeptide and it's typical of many DNA polymerases, the exonuclease acts as an anodyne [?] function to get rid of mis-incorporated nucleotides.

Vincent: So if you took this polypeptide and you added… how did you assay polymerase activity, what was your assay?

Mark: Using what was called at the time activated DNA, which was just cat thymus DNA that was incubated with a little bit of magnesium for a while so it could get all nicked up. It had gaps and so forth and then the polymerase… sort of a minimal activity of a DNA polymerase is to be able to add a few nucleotides to expose three prime terminals.

Vincent: Cat thymus DNA, wow.

Rich: So the activated DNA basically is in effect a template with a primer on it.

Mark: Right.

Rich: And that's what the polymerase works on.

Vincent: And you put a radioactive triphosphate in and you measured incorporation, right?

Mark: Right.

Vincent: You used a scintillation counter probably, right?

Mark: A scintillation counter, absolutely.

Vincent: See, remember this work still?

Mark: Oh yeah.

Vincent: It come right back, that's good.

Mark: Absolutely.

Vincent: That's cool. So then after you finished… do you want to do anything more before we move onto…?

Rich: I would just make one other comment about this polymerases… we've talked a little bit about it, and that is, I mean Mark didn't know this at the time but, it looks like this polymerase is the enzyme that does recombination in vaccinia as well. So it catalyzes a strand exchange which I think is a little unusual for polymerases. So I think it's weird and interesting in that regard.

Vincent: And this is the DNA polymerase of vaccinia virus, there's not another one, it's not a family of different polymerases? This is it, right?

Mark: This is it. And of course, unlike many DNA viruses the vaccinia does all of what it does in the cytoplasm of the host cell. So there aren't any other DNA polymerases around there.

Vincent: That's right, that's right. It makes its own factory in the host cell.

Mark: Right.

Vincent: Right. So you finished this and you already told us you stayed at Hopkins to do a post doc, right?

Mark: Right.

Vincent: With Tom Kelly.

Mark: With Tom Kelly, yeah.

Vincent: And why did you stay there and not go somewhere else?

Mark: Well this is sort of a long story. I actually had arranged to go somewhere else.

Vincent: Okay.

Mark: But my wife was finishing up some post doctorial work at Hopkins as well, and so I decided after I got my PhD to stick around for a year and, you know, just waits for her to finish up. We were both supposed to go out to Stanford at the same time.

So I started working on this project with Tom and things just worked out so wonderfully well I just couldn't leave it. So I stayed.

Vincent: That's good.

Mark: Yeah.

Vincent: Can't beat that. So you went to Tom Kelly and said, "Can I work for you?"

Mark: Yep.

Vincent: And he knew you had experience with DNA polymerase and that was his interest at the time, right?

Mark: Right.

Vincent: Except he was working on a different virus.

Mark: He was working on adenovirus rather than vaccinia virus.

Vincent: Right.

Mark: And then he said, “Yeah, I want to get into the biochemistry of adenovirus replication and you obviously have some experience with DNA polymerases so why don’t we do something?”

Vincent: So at that point would you have considered yourself a biochemist or a virologist?

Mark: Certainly a biochemist.

Vincent: Yeah, sounds like it.

Mark: Yeah I was just very gradually reeled into the world of virology.

Vincent: So what did Kelly tell you to do?

Mark: He said, “Well, you know what, what we need to do is get an in vitro system that faithfully recapitulates DNA replication in a cell-free system.” And he said, “Nobody has been able to do that but you’ve got a year. Doesn’t matter if it works or doesn’t work, give it a whirl.”

Rich: And how did he choose adenovirus, because as it turns out adenovirus is a pretty weird system to….

Mark: It was but Tom was working on adenovirus so….

Vincent: Why is it weird?

Rich: Ah because of the terminal protein priming mechanism.

Vincent: Okay.

Rich: Plus as I recall, and we can get into this if you want, as I recall, it took a while to figure out that adenovirus actually encoded its own DNA polymerase. I recall that being kind of a mystery.

Mark: Right although that was actually known. That was known.

Vincent: So he said, “Develop an in vitro system.” Was the polymerase purified by that time?

Mark: No, the polymerase wasn’t purified.

Rich: Was the terminal protein priming thing known?

Mark: No, although it was suspected.

Rich: Because I remember that starts out with actually Tony Robinson, okay, working on CELO virus figured out that there was a terminal protein. And then Rekosh and Robinson and Willy Russell took that over and really refined that.

I guess you had some work on that down the road as well.

Mark: Well that's right. So I guess what I did was to say… as you say just about that time it was becoming obvious that the ends of adenovirus DNA, the five prime end was covalently connected to a protein. So that's how it comes out of the Freon [?]. If you purify adenovirus DNA without proteases there is a protein attached to each 5-prime end.

So I said, well that DNA with the protein covalently bound has to be the template for DNA replication in vivo, so we should start with that in vitro.

So I just made extracts of adenovirus infected cells and purified the protein, as I say, in a way that would not involve the use of proteases to preserve the integrity of the terminal protein, and use that as a template for in vitro DNA replication.

The first experiment I did worked. It was amazing.

Vincent: Did you purify the polymerase or was it an extract?

Mark: It was just extract.

Vincent: Extracts of infected cells?

Mark: Infected cells, right.

Vincent: So you are adding purified adeno-DNA, so and then you got replication of the… so how could you distinguish it from endogenous DNA in the extract?

Mark: Well….

Vincent: Maybe you couldn't, right?

Mark: Well you could because you could run it on a sucrose gradient.

Vincent: Okay.

Mark: Adenovirus has a discrete length, and so that was one way.

Vincent: Okay.

Mark: And also there were several controls build into this. So it only worked with infected cell extracts. And it also only worked if you kept the integrity of the terminal protein on the template.

Vincent: Okay. You did that experiment? You cut it off and you showed it wouldn't replicate it any longer.

Mark: Right.

Vincent: Huh. And so here we have a 1979 paper, Challberg and Kelly, *Adenovirus replication in vitro*. And you did that in that first year?

Mark: I did that in about the first week.

Vincent: Wow. That's great. And the last sentence is, "The system provides an assay that should be useful for the purification and subsequent characterization of viral and cellular proteins involved in DNA replication." So is that what you did next?

Mark: Yes.

Vincent: So you stayed in Tom's lab?

Mark: I stayed in Tom's lab for the next three years.

Vincent: And did you purify some proteins that were involved?

Mark: Uh… purified some proteins, we actually, I actually spent more time on the… using the in vitro system to demonstrate that the mechanism of initiation, the protein priming mechanism.

Rich: So how does that work? Let's lock this down.

Mark: So DNA polymerases are known to only add nucleotides to 3-prime ends hybridized to a template. So unlike RNA polymerases, DNA polymerases in general cannot start with a 5-prime triphosphate and add the next nucleotide to that. Something has to be there already.

So it was always an issue in the case of linear DNA, double-stranded DNA viruses, how DNA replication could initiate at the ends of a molecule. How does that first nucleotide…?

So the way that adenovirus solved it was to use the hydroxyl group of a serine residue and a protein as a stand in for the 3-prime hydroxyl terminus of a DNA strand.

So the first nucleotide that adenovirus DNA polymerase polymerizes is it polymerizes to a… it covalently adds it to a protein. And then DNA replication starts from there.

Vincent: So the polymerase actually adds the nucleotide to the protein.

Mark: Yes, right.

Vincent: This is called terminal protein, right?

Mark: Right.

Vincent: And that acts in effect as a primer?

Mark: Right.

Vincent: For the rest of the replication. So you worked on aspects of that then, right?

Mark: Right.

Vincent: So this was 1979. I guess by then the sequence of the genome was known?

Mark: Ah… a little after that. I think it came out in the 80s.

Vincent: And then you could see the coding region for your protein that you'd been studying.

Now adeno is different from pox in that all the DNA replication takes place in the nucleus of the cell, right?

Mark: Right. And one of the issues that came up, so it turned out that we did an experiment to determine the size of the protein that is used as a primer for DNA replication. The idea was to prove that it was the terminal protein that it is attached to virion DNA.

Well, we did the experiment and it turned out to be a different size. So that was a little bit puzzling and a little bit disconcerting. And so I spent maybe a year trying to sort that out. And what it was the terminal protein, the primary form of the terminal protein, was quite a bit bigger than the terminal protein on virion. The way it worked was that protein, the larger version of the protein was processed down to the smaller version of the protein during the process of encapsidation, proteolytic processing during encapsidation.

Rich: Is this protein priming of DNA replication, is that unique to adenovirus or has that shown up anywhere else?

Mark: Well, I don't think there are any other animal viruses that do that. There is a bacteriophage that does it exactly the same way.

Rich: Okay.

Vincent: Is that V-29 I think?

Mark: Ah no….

Vincent: It doesn't matter. There is a phage.

Rich: Cells don't do this, right?

Mark: No.

Vincent: Of course polio replicates with a primer but it's an RNA virus. There's a little VPg… Both of the picornas have VPg on their RNA and that's a primer for replication.

Rich: Right.

Vincent: In fact I remember years ago when people worked on the polio problem they were always comparing the adeno priming situation because it was it was the only other, besides the phage, it was the only other situation.

Rich: By the way, if people go back to virology 101, TWiV 96 is on DNA replication and TWiV 106 is on DNA replication.

Vincent: That's right we did two of them.

Rich: We did two of them and we talk about this issue with not being able to replicate linear molecules and we talk about the adenovirus DNA replication. So if you are interested in seeing all the drawings that come with it, those two TWiVs have slides with them and everything else.

Vincent: The reason I mentioned the nucleus… so adenovirus makes its own DNA polymerase but it probably uses other proteins for replication in the nucleus, right?

Mark: I don't actually think it uses any host proteins.

Vincent: No.

Mark: There are also… adenovirus also encodes a single-stranded binding protein.

Vincent: Okay.

Rich: It is an unusual mechanism for DNA replication.

Vincent: Right.

Rich: It's not your standard replication form because of this strand displacement, right?

Mark: Right, it just goes from one end to the other, it displaces the non-template strand.

Rich: So you don't have… so there's no lagging strand?

Mark: Right, exactly.

Rich: So it doesn't require all of the junk that might ordinarily be required to do that.

Vincent: So you displace a strand, you make a new duplex and the displaced strand will eventually get replicated as well.

Mark: Right, it uses it.

Vincent: Yeah, template. So there's a double-stranded template and then there's a single-stranded templates. They both work with the polymerase?

Mark: Well actually it's a little more complicated than that because adenovirus has an inverted terminal repeat so the two ends of the displaced single strand hybridize the form, a duplex. It looks exactly like the duplex on the fully duplexed molecule.

Rich: So that serves to initiate the second strand synthesis.

Mark: Right.

Vincent: Right. So after this post doc then, I guess you set out on your own to start a lab, right?

Mark: Yes.

Vincent: Where did you do that?

Mark: At Tufts Medical School.

Vincent: Who hired you?

Mark: Elio.

Vincent: Oh, you're kidding.

Mark: No.

Vincent: Oh he's going to love this. I do a podcast with Elio every other week called This Week in Microbiology. I know he was chair at Tufts. He's now retired in San Diego but he still keeps up.

Rich: This is Elio Schaechter?

Vincent: Elio Schaechter. He hired John Coughin. You guys must overlap, he's still there. But Elio is always talking about his old department so I'll have to tell him that.

He'll listen to this anyway. He does listen to TWiVs, so he'll get a kick out of it. So he hired you?

Mark: Yes, he did.

Vincent: Good job Elio.

Rich: When you go to give a job seminar, you've got a portfolio right?

Mark: Right.

Rich: And I know what's coming up here, but what did you tell him you were going to do?

Mark: I told him I was going to continue to work on adenovirus replication.

Rich: Ah ha, okay, good.

Vincent: You know something that I don't. Oh, of course….

Rich: Did you continue to work on adenovirus?

Mark: I did in fact.

Rich: For how long?

Mark: Until I left Tufts actually.

Rich: Oh, okay.

Mark: After four years.

Rich: So herpes doesn't happen until down the road?

Mark: Right.

Rich: Okay.

Vincent: So you were at Tufts for four years.

Mark: Right.

Vincent: Working at similar aspects of that in the DNA replication.

Mark: Yeah, basically continuing on.

Vincent: Okay. And then you left Tufts to go where?

Mark: NIH.

Vincent: And there is where you took up herpes.

Mark: Right.

Vincent: Why did you go there?

Mark: For family reasons.

Vincent: Okay. Did you like Tufts?

Mark: I did.

Vincent: Was it a good environment for doing research?

Mark: Yeah, no, it was great. Tufts was great and you know, as you could imagine, I mean any department headed by Elio was a very convivial place.

Vincent: Were there any virologists there that you…?

Mark: John Coughin.

Vincent: John Coughin, right, okay. And at NIH you went to the Laboratory of Viral Diseases, right?

Mark: Right.

Vincent: Who was that head of that at the time?

Mark: Bernie Moss.

Vincent: And still is, is that correct?

Mark: And still is, yep.

Rich: And will be indefinitely.

Vincent: He probably loved the idea that you were doing DNA replication.

Mark: I had actually known Bernie because when I worked on vaccinia DNA polymerase as a graduate student at Hopkins, I drove down to NIH and got my stock of vaccinia virus and HeLa cells to propagate it in from Bernie's lab.

Because I was not all that great at keeping HeLa cells going, I would go down there every few months to replenish my supply.

Vincent: So there you continued initially adeno and then eventually switched?

Mark: I decided that I needed to switch and so I thought that moving to NIH was a good chance to do that.

Vincent: Why did you want to switch?

Mark: Well because I thought that the adeno problem was basically solved at that point or at least at the level that I wanted to work on it.

Vincent: And so then you thought herpes was not?

Mark: Right.

Vincent: So no one had purified the polymerase from herpes?

Mark: People had purified the polymerase from herpes but there was a lot of uncertainty as to what the rest of the participants of replication were at that point.

Rich: At that point was there genome sequence for herpes?

Mark: It was just being worked on.

Rich: Okay. So you didn't really even know what the genetic content was?

Mark: Right. I mean there was a few DNA minus mutants of herpes that had been mapped that weren't mapped to the polymerase so it was clear there was additional genes involved in DNA replication but they really weren't all known.

[28:15]

Vincent: So what was your goal with herpes, what did you want to accomplish?

Mark: Well, what I really wanted to do was do what I had done with adeno—setup an in vitro system, purify all the proteins, then see how it worked. But for the first six months I was at NIH I spent all that time trying to make extracts from HSV infected cells and I was hoping things would work out just the same way they did with adeno but unfortunately they didn't.

So I had to take a different approach.

Vincent: Eventually it did work out, right?

Mark: Well, I never did—and I think to this day has gotten an in vitro system that actually replicates HSV DNA.

Vincent: It's all done in cells?

Mark: Right.

Rich: So what was the approach? I love this.

Mark: Well, so I thought since I couldn't get an in vitro system with extracts, the next best thing would be, for a different approach, would be to systematically find out all of the HSV proteins that are necessary and then over express those proteins and put them together and get an in vitro system that way.

So the first thing that I had to do was figure out what all the viral gene products were that participated. And so we set up a transfection assay in which we basically divided the HSV genome, which is a pretty big piece of DNA (150 kb), and showed that you could transfect in pieces…, the entire genome in pieces and that there was enough gene expression that occurred from the transfected DNA that all the proteins necessary for replicating HSV DNA, and at the time the origin of DNA replication of HSV was known, so the assay was to ask for the replication of a plasmid containing the origin of replication.

So once we showed that once you could do that with fragmented DNA, then it was just a matter of grinding through and cloning and getting and whittling it down to the unique set of genes.

And fortunately at that point, the HSV1 sequence, DNA sequence came out. So that made life a lot easier.

[0:31:17]

Rich: So then you knew kind of where the start and stop for individual open reading frames…?

Mark: Right.

Rich: So then you had a candidate open reading frame and it made it easier to clone it.

Mark: Right, exactly.

Rich: So actually something just occurred to me about this and that is that during a normal herpes virus infection cycle there is immediate early gene expression that then activates early gene expression and the DNA replication proteins are early genes. But in your transfection assay, ultimately, you don't have that first step of activation by immediate early genes necessarily, ultimately, once you've….

Mark: Well actually we do because in the transfection DNA replication assay you needed to include the immediate early genes to activate the early genes.

Rich: Okay.

Mark: But that paradigm was already known so….

Rich: Okay. So that those immediate early genes just kind of become background in the assay?

Mark: Right.

Rich: So then ultimately, you must have cloned all these downstream from some other promoter or something like that?

Mark: Right, did that.

Rich: Alright.

Vincent: So how many gene products did you need to replicate a herpes origin containing plasmid?

Mark: Seven.

[0:32:42]

Vincent: Okay, and that's this paper here in 1988, Wu, Nelson, McGeoch, and Challberg. Duncan McGeoch, was he a post doc of yours?

Mark: No, he was at the Institute of Virology in Glasgow. He did the DNA sequence. So he made DNA sequence available before it was published.

Vincent: So people are going to cringe that I called him your post doc.

Mark: That's okay.

Vincent: It seven proteins. I remember this paper. I was just six years at Columbia and I remember seeing this and people talking about it in journal clubs.

What were the seven proteins? You know at the time because the sequence had been done.

Mark: The seven proteins were two subunits of the DNA polymerase. There is a catalytic subunit and a processivity subunit for DNA polymerase.

Vincent: Okay.

[0:33:36]

Mark: Three subunits of a helicase primase complex, a single strand of DNA binding protein, and an origin binding protein.

Vincent: Wow. That's beautiful isn't it?

Mark: Yeah.

Rich: Well I remember this as a tour de force, you know. Of course, in my conception, it happened over night, because I was just sitting around having a good time and then all of a sudden this paper comes out that's got all of this stuff in it.

But it really was, it's a novel approach to identifying these things and it gives all of this information in one big dump. It was amazing. It's really great.

Vincent: This starts a new field because then everybody else then starts to look at individual proteins and they could alter them and look at the activity using your system, right?

Mark: Right.

Vincent: I presume you did that as well.

Mark: Right. I was actually more interested in—I mean we did some of that—but I was more interested in the enzymology.

Rich: How long did that take you from getting the idea of the transfection assay?

Mark: It was amazingly fast. I mean it was under a year.

Rich: Okay.

Vincent: Was that mainly the first author here, Wu?

Mark: She was my first post doc, but I had actually done a lot of it. It went way quicker when we got the DNA sequence, because what I was doing for a while was to take it down to a small restriction fragment and then do exonuclease—I mean digestions—that was kind of tedious.

Rich: What this makes me think of, which I'm sure you'll appreciate as a biochemist, is that it is all in the assay, right?

Mark: Right.

Rich: If you have a really good solid assay to do something, that's reliable and reproducible, once you've got that, things can move really fast.

Vincent: Hersey Heaven. Remember Hersey Heaven.

Mark: Absolutely, Hersey Heaven. One good assay and just do the crank.

Vincent: You had an origin containing fragment cloned on a plasmid and put this into cells. Was this a stable cell line that had this plasmid or would you put it in each time?

Mark: No, it was part of the transfection and then the assay makes use of this restriction enzyme anomaly, the Dpn1.

[0:36:18]

Vincent: Why don't you explain that because the listeners may not know it, even the virology listeners may not know it.

Mark: Boy, it's been such a long time….

Rich: So a plasmid grown in e coli is methylated by methylase and that makes it resistant to…, no, sensitive to Dpn1, a restriction enzyme.

Mark: Right.

Rich: But if it's replicated in eukaryotic cells you don't get that methylation, so you look for DPN resistances of the replicated plasmid. [?]

Vincent: You extract plasmid DNA, probably did a hertz supernatant, right? That's how we did….

Mark: Yeah. Actually you can't do quite a hertz because HSV DNA gets replicated by some kind of rolling circle mechanism so it ends up quite big.

Vincent: Okay, yeah. So you just extract DNA and cut it with Dpn1 and look for something that could be cut and that's been replicated.

Mark: Right.

Vincent: Beautiful. Many, many people use that assay.

Mark: Yeah.

Vincent: That's great. How many years did you spend at NIH in your lab?

Mark: Seventeen.

Vincent: Okay.

Rich: Wow.

Vincent: What other things did you work on, mostly herpes replication?

Mark: Exclusively herpes replication. As I said, we were, I was mainly interested in the enzymology and what these… because we didn't, when we define these genes by transfection we didn't know what most of them did.

Vincent: Sure.

Mark: So the first thing was to try to figure out what they did by over expressing them. So each protein was over expressed in bacterial virus expression systems and assayed for various activities relevant for DNA replication.

Vincent: I seem to remember you also defining the number of origins in the genome, was that your work?

Mark: No.

Vincent: There's more than one isn't there?

[0:38:23]

Mark: There are three actually.

Vincent: Okay. Did other people buy into this system and start doing work on it?

Mark: Oh absolutely.

Vincent: To this day, do people still study the simplex DNA replication?

Mark: Yes. As I said, it has proved to be, for reasons that just aren't clear an intractable problem to get it to take these seven proteins. You know you can purify them and demonstrate that they have enzymatic activity and you put them together with an origin containing plasmid and nothing happens.

Vincent: In vitro you mean?

Mark: Right.

Vincent: Ah, so that was your original goal, right?

Mark: Right.

Vincent: Still hasn't been achieved.

Mark: Still hasn't been achieved.

Vincent: You don't know why it doesn't work, right?

Mark: No.

Rich: Do you know that those are actually the origins that are used in cells?

Mark: Uh.

Rich: He's thinking by the way.

Vincent: That's okay, we don't mind pauses. That's alright.

Mark: Well what would be the proof that that was the case?

Rich: I don't know. I mean can you mutagenize them and… well, I don't know how you'd do that because you'd wind up with something that's dead, right? [?]

Mark: Right, you can only ask those questions not in the intact genome but in a transfected plasmid. I guess you know the best evidence… well not the best… the way that it, the way these sequences were originally discovered was if you passaged herpes virus at a high multiplicity in tissue culture you end up getting defective genomes. The defective genomes turn out to be repeats of a sequence which is the origin of replication.

[0:40:36]

Rich: So it turns out, right, that there's a lot of organization… I mean when herpes infects a cell it sets up these replication centers in the nucleus, there are host proteins that are recruited to these, so it strikes me, thinking about this that it could be that a lot of what's missing here is host proteins and some sort of marginal structural components, scaffolding of some sort, chaperones, and this kind of stuff, right?

Mark: Yeah.

[0:41:14]

Vincent: Do you keep up with this field now?

Mark: A little bit, I mean now that… when I started in extramural as a program officer I didn't have any responsibility for herpes viruses at all, it was all pox viruses, and so I didn't really keep up that well but I've recently gotten back or gotten part of the herpes virus portfolio.

Rich: So let's, if we're ready, do that transfer.

Vincent: After 17 years you decided you didn't want to have a lab anymore?

Mark: Yeah, I decided that I really needed to do something different, if I wanted to stay in the lab I needed to do something very different then herpes replication because it just wasn't… it seemed like the questions I was asking in my lab just weren't that interesting to me anymore. So I needed to do something different. But then I decided to do something very different and just get out of the lab.

Rich: So what year is this?

Mark: Let's see… it would have been 2001.

Rich: So eleven years ago?

Mark: Yeah.

Rich: Okay.

Vincent: I remember seeing you at a study section. I said, “What are you doing here?”

Mark: That was not an uncommon reaction.

Vincent: And he said, “Well I decided to make a big change.” I remember this, I don’t know if you remember, probably everyone said that to you but I distinctly remember. That was my first tour on virology study section you had said that.

So you decided to become a program officer is that when you came right out of your lab?

Mark: Right.

Rich: Did you say, “Oh I want to be a program officer.”

Mark: Well I didn’t even know what a program officer did.

Rich: Yes because you had been at NIH.

Mark: Right. When I started the job I really didn’t know what a program officer did. But I knew it had something to do with administering grants and so forth.

Rich: So did you… I mean in your mind was it sort of like, “I want to stay geographically where I am, I’d like to maybe do… try out some science administration, oh here’s this job I’ll try that.”

Mark: Exactly.

Rich: Okay.

Mark: And it worked out because it was 2001-2002 so the whole bio-defense thing exploded.

Rich: As it were.

Mark: As it were and they needed somebody to run the pox virus program because nobody in the DMID extramural knew much about pox viruses. Of course, I didn’t really work on pox viruses but I was in Bernie Moss’s lab which gave me a certain amount of credibility for pox viruses.

Vincent: Right. So you became a program officer. So tell us what a program officer does because probably most listeners don’t know.

Rich: Yeah, this is key to the whole thing.

[0:44:30]

Mark: Right. So I think program officers have maybe three inter-related jobs that are, as I say, inter-related but differ. The first is what you might call portfolio management. We administer a certain area of science, a program. In my case it's pox viruses and alpha herpes viruses.

Vincent: These are extramural, that is stuff NIH funds on the outside of NIH?

Mark: Right.

Vincent: Because when you worked in your lab you were an intramural program, right?

Mark: Right. As Rich alluded to, people who work in NIH intramural know nothing about NIH extramural, because people who work in NIH intramural get their money from a completely different mechanism. They don't have to apply for grants.

Rich: So extramural is what we… where we started with this conversation.

Vincent: That's right.

Rich: People like you and I Vincent submitting grant applications, those are extramural grants, and program officers deal with that stuff.

Vincent: So you have a portfolio of grants. So initially you started with pox viruses and if grant applications would come in they would be part of your portfolio. So what does that actually mean in… you just oversee them?

Mark: Well, I guess the core job of a program officer is to oversee these grants and make sure that the investigators comply with all the relevant laws and policies regarding the use of grant money. So, for example, you know before somebody actually… before their grant gets paid, I have to go through a checklist of things that ask various questions – does this person comply with the policies regarding human subjects; is the animal work all okay, is it approved by the IACUC; and their facility, does it involve select agents—all the sort of things that are a little bit too scientific for an accountant to be able to figure out but on the other hand are not that difficult.

Vincent: Right. So when we are at study section, program officers usually sit in the room.

Mark: Right.

Vincent: You were there today. What are you doing while you're sitting there?

Mark: Okay well so one of the other big jobs of program officer's is to deal with various peer review issues. So once the review is over then the SRO and the people who run the review don't really have anything to do with the applicants because funding decisions for individual grants are not made by the review people, they are made by institutes.

[0:48:00]

Rich: So we are told that all the time explicitly, you are not making funding decisions, you are evaluating the scientific merit of these applications. And they get passed on to the people who actually hold the purse strings.

Mark: Right.

Rich: Which is you?

Mark: Right. Well we don't… program officers don't make the funding decisions, the institutes do.

So why do I go to study sections? One is that after the study section takes place and the reviews are out, then I get a lot of calls from applicants, whose grant was reviewed, wanting to know what happened. You know, why their grant didn't get a good score basically. And they're looking for some extra insight that's not conveyed in the summary statement. And I can't usually give them any additional insight but sometimes. You know, sometimes I'll say well there's all this stuff in the summary statement but the thing that really drove the score was this one issue and that's the issue you need to take of in your revision.

Rich: One of the things that I think happens, and I'd be interested in your insights to this, is that lots of times grants come through and the problem is that they're boring, right? But the reviewers don't know how to say this is boring. And so they pick a bunch of stuff to say to justify the fact that they're giving this a lousy score, rather than saying this is boring.

Mark: Right. And one of the difficult jobs that I have is I try to, I'm not very good at saying this gently, I think, but you know I try to give people an honest assessment of what really went on. I've told many people, look you know you can read the summary statement and read those criticisms all you want but that's not the main issue. The main issue is that they are just not excited by this and they are never going to be excited about this.

Rich: That's really an important job, to communicate that.

Mark: Yep.

Vincent: Yes, because it's not written down.

Mark: Right.

Rich: Well, as a reviewer, if that's my problem I try and write that down as nicely as I can so that they get the idea but I don't know how common that is.

Mark: You know these sub-scores have actually not helped the whole problem because, especially new investigators, new applicants, they go through and they read the criterion scores and they say, "You know they really thought that the investigator was great and my institution was great…" I said you know, don't read anything in that summary statement that is positive, and just ignore it, because they are just throwing you some bones. You need to focus on the negatives here because that is what you need to change. "Oh well… it was only the approach."

Vincent: Hmm, only.

Mark: Only.

Vincent: So in the old days you just gave one score to a proposal and you're referring to the fact that now we have subcategories that include not just the approach but the investigator, the environment….

Mark: Right.

Vincent: Quite often most of them are similar for most grant applications, right? It's really the approach and then maybe significance is what's really differentiating.

So I interrupted you. You said you had three jobs as a program officer, and the one is to deal with the administrative science that can't be dealt with [by an accountant].

Mark: Right. The other is to deal with peer review issues and another aspect of that is, and this varies from institute to institute, but NIAID mostly funds applications strictly on the basis of score. So we establish a pay line, if your score is better than the pay line you get funded, if it's worse than the pay line you don't get funded.

[0:52:34]

But we do make some discretionary awards and that's based on program officer recommendations. So we try to… you know, if we think there's an area of research that really needs to be funded and the applicant and we are going to lose this investigator because he's going to have to dismantle his lab if he doesn't get a little extra money we'll make a discretionary award.

Rich: So that's special pay.

Mark: That's special pay, select pay, bridges, that kind of thing.

Vincent: It's probably the most interesting part of the job, right?

Mark: Right, exactly. Although there is not… at NIAID, as I say, there is not that much money for discretionary awards so it's very competitive.

Vincent: Has it changed over time? Did there used to be more?

Mark: No.

Vincent: But this depends on your scientific judgment, so it's based on your experience which is good because you have that experience and that's why you get assigned certain viruses, right?

Mark: Right.

Vincent: And you now have pox and herpes, alpha herpes. Not all herpes, just alpha.

Mark: Well herpes is a big field.

Vincent: Right. So you have people who do the other herpes viruses, program officers?

Mark: Right.

Rich: So there's another function that I think of for program officers and that is that, as investigators we're often encouraged when we are considering writing a grant application to contact the appropriate program officer and say, "I've got this idea, I'm thinking of writing a grant, am I wasting my time or whatever," and get some spin from the program officer. I'd be interested in your perspective on that.

Mark: Yeah, I don't find that I really have much to say to people who ask me that because if… just the way we are setup is that there is so little discretionary money if an application comes and gets a good score, you know, if it's assigned to NIAID and if assigned to my program and it gets a score below the pay line we pay it. So it's pretty cut and dry.

One other thing that we do that somewhat relates to that is dealing with the whole area of initiatives. So program officers are sort of the front line in the development of funding initiatives and that's really a special pot of money that I kind of think of as jump starting a certain area of research. So that if we think there is something lacking, there is an area of research where there is just not enough people working on that really needs to be pushed then we'll say well we need an initiative in this area. There's this whole process for us doing that. But if it's approved by the upper management at NIAID then we write it up that goes out to the community and it says we are looking….

So a typical initiative would be what's called an RFA, request for application. So, we are looking for applications in this certain area and we are setting aside a certain amount of money that is devoted to that area so please send us those applications.

[0:56:25]

Rich: So when you say 'we' administratively is this like a gaggle of program officers in NIAID or what?

Mark: I think mostly when I say 'we' there I was referring to NIAID. But as I say, initiatives are started by program officers.

Vincent: So if you had an idea you could start it?

Mark: Right.

Vincent: It doesn't have to originate in a meeting with a bunch of people, right?

Mark: Right. We, NIAID has this whole big process, so in the Division of Microbiology and Infectious Disease we, you know,… program officers bring initiatives forward, they're talked about, chewed over, and then they kind of move up the chain of command, eventually they be signed off by somebody in the directive… by the Director. Then it goes to council, the council approve any initiative concepts that are brought up. Then if council approves it, it comes back to the program officer whose idea it was initially to actually write the initiative, write the RFP, and implement the whole thing.

But it all starts with program officers. And that's really the third area I think program officers do, they are kind of advocates for a particular area of science. You know for pox viruses and herpes viruses I'm the person in NIAID who has to say this is important, we should be doing more of this.

Rich: And here is where I am really grateful for your 20, 30 years of genuine academic science background because I know there is a guy at the helm at NIH who knows what it's like and knows what's going on, whose helping make these decisions and guide things.

Vincent: In the interest of full disclosure, Mark has been your program officer for many, many years, right?

Rich: Yes that's right. He has been my program officer for…, since 2001.

Vincent: Right, since he was starting.

So do you mainly deal with the investigator initiated proposals or do you do program projects, U grants, all those… you do everything?

Mark: Yeah, I do everything.

Vincent: Anything within NIAID you could do. Of course each institute has its own program officers and they are all separate from you as well. Is there any cross talk between institutes?

Mark: Very little between institutes, yeah. I mean as you probably know, many grant could potentially be of interest to more than one institute and so they get doubly assigned, dually assignments. In practice that doesn't really make much difference.

Rich: So how many program officers are in NIAID?

Mark: I think there is in the neighborhood of 50.

Rich: Okay.

Vincent: I know mine, you know. Other people, other viruses, they have their own officers.

Rich: And this is the National Institutes of Health. How many institutes are there?

Mark: There are more than ten, eleven I think.

Rich: So that adds up to a fair number of program officers. Okay.

Vincent: So in these years that you have been… over ten years you've been doing this, have you seen any trends in the way science is done in this country?

[1:00:22]

Mark: Well, you know, clearly over time science has been getting bigger. You know the DNA, the -omics efforts, whatever it is, there's certainly a lot more that kind of collaborative thing, or multi-PI operations. I would say that's the biggest change over time.

Vincent: Does NIH like that, that NIH prefer that to little grants?

Mark: I'm not sure we prefer it, but we certainly go with the flow on that.

Vincent: So people… I mean you do have mechanisms that allow these multi-university grants. If it's good you will go with it.

Mark: Right.

Vincent: So here is something that probably young people in the field would like to hear about. It's no secret that it's getting tougher and tougher to get an NIH grant and it's probably going to continue to get tougher. What do you tell people who are just coming into science? Should they not come in to science? We don't want to dismantle our wonderful thing here.

Mark: It's very tough; it's hard to know what to tell people. I think… I tell people, you know, unless you have to be doing science you probably shouldn't do it. I mean there has to be an overwhelming desire on your part to do it or it just is too hard. And it's still hard.

Things are getting tougher and tougher and there are programs that we have, that all the institutes have, to make things a little better for new investigators, but in many ways I'm a little concerned about those because we're kind of delaying the hard part. We're pushing off the hard part until the first renewal. You know, when they are no longer a new investigator. So I don't think we can give… it's good to give new investigators a little bit extra help but too much is doing them a disservice I think.

You know, is it better to decide after your post doc that you need to do something else or wait until you can't get your first grant renewed five years later?

Rich: By doing science really it's not necessarily don't do science at all but don't try and do academic science.

Mark: Academic science, don't be a PI in an academic institute.

Rich: This reminds me of the conversation we had with David Baltimore, who said, this is very difficult. And it's the same advice that my daughters got when they were thinking of going into theater, okay. And the advice there is, if you can imagine yourself being happy doing anything else, do that, because this is hard.

Mark: Yeah.

Rich: But if you can't be happy doing anything else, if this is what you have to do, do it.

Well, it makes sense, because that's the kind of passion that's required to make it work.

Mark: Yeah, absolutely. You know things sound…; it's certainly way harder to make a career as an academic scientist now than when I was starting out. I mean, you know, when I put in my first grant application I fully expected it to get funded and it was. It just wasn't that hard. And so I think it takes more work now. I think new investigators have to have some expectation that their first application is not going to succeed. But you know I think for people who are persistent and keep after it, you know… we… it's still possible to make a career.

Vincent: So when I came into science, when I started my own lab in '82, you had about a 30 percent chance of getting your first grant, which was pretty good. The pay line was about in the 30s. It's slowly gotten lower. So why is that? Is that because science is expanding, more people are doing it and the money is the same or… I don't understand why we are in this position.

[1:05:12]

Mark: Well, yeah I mean it's sort of out of my… I certainly don't have a complete grasp of why it's gotten quite so bad but clearly there's a lot more people doing it now than use to because if you actually, if you look at the amount of money that goes into investigator initiated grants over time, it's gone steadily up.

There was the doubling back in the 90s, but what the doubling did was to convince a lot of academic institutions that they needed to expand their faculty. So with the doubling of the NIH budget we got a big increase in the number of applications and investigators.

Then when funding leveled-off the pay lines dropped precipitously because we'd made out-year commitments to all these new awardees. Grants are for five years. And we have no idea what the budget is going to be five years from now. So it makes life very difficult for the financial planners.

Vincent: I think the universities need to commit more money to supporting basic science. As you said, when a… it used to be a university would put up a new building and assume they could fill it with people who had RO1s.

Mark: Right.

Vincent: As everybody knows, when you get a grant that you also get money, indirect costs for the institution, and that's how universities have thrived. But they have to change that way of doing business because it doesn't work anymore.

If mean if universities could contribute a substantial salary for all there faculty this would free up research money. I don't know if that's a naïve view but that's what I've always thought.

Rich: I would agree with that. I think the universities have become enormously reliant on NIH to help the universities do their thing and that just can't go on forever.

[1:07:30]

Mark: I think universities would say, well, if you are going to ask us to do that, what is our mission and then how do we make money? We teach students and we pay for that by tuition. So if we are going to

pay for you faculty, we are not going to be able to have as many as we do and you're not going to be to spend time doing research, you're going to have to spend more of your time teaching.

Vincent: A great university is great because it has a research faculty, right?

Mark: Right.

Vincent: Who are doing stuff, no matter what field it is, but let's pick science, they are doing research supported by NIH, that's what makes them a good faculty, they can teach right from the heart, right? So, it hooves the university to have those kind of people doing research, you can support from NIH, but also contribute to them.

Now, yeah, the money situation is difficult, tuition won't pay for all the professors at the big universities. It's a problem. I don't know how to solve it, that's for sure.

Do you know Rich?

Rich: No, and fortunately I'm not in a position of having to come up with an answer.

[1:08:43]

Vincent: But I think going forward the people that are getting in the field have to think about this and figure out ways to get around it because you and I are reaching the end of our careers, right, at least research careers. I would love to contribute something but I don't know how to solve it. I am not in any position to do that.

Rich: Well we can ask the questions and broadcast them. Maybe stimulate some discussion.

Vincent: But you, I'm sure you agree that doing research is essential for the world the way it is now.

Mark: Absolutely.

Rich: So how do you like being a program officer?

Mark: I like it a lot. It's different than going into the lab every day.

Rich: What do you like about it?

Mark: I like that I get a much more global view of at least one area of science. You know it's the bird's-eye view rather than the worm's-eye view. And I do a lot of different things.

Rich: You been involved in just the activities you've talked about, right? What other sorts of things do you do?

Mark: NIH also, in some areas for example, particularly in the bio-defense area actually has contracts to develop products. The area that I am in, for example, is new small pox vaccines and drugs, anti-small-pox drugs. Since there is no small pox in the world right now we are only looking at a possible terrorist scenario. The only customer for small-pox drugs, potentially, is the government. So we can't really ask

pharmaceutical companies to develop a drug on their own dime and then have it sitting around in the event that we might need it someday. So basically NIH is in the position in that sort of arena where we have to be the driving force for developing new vaccines and drugs and other therapeutics and so forth. So I am involved in a lot of that, which is also interesting.

[1:11:24]

Vincent: Do you work with BARDA?

Mark: I work with BARDA, yeah.

Vincent: So you must know Ed Niles, right?

Mark: I do know Ed.

Vincent: So it's perfectly appropriate that we are in his house. Thanks Ed.

Rich: That's right, absolutely.

Mark: In fact Ed and I work on the same projects.

Vincent: Well we talked to Ed a year ago maybe?

Rich: I can look it up; it's the Ed Niles, a Km Vmax kind of guy [TWiV 120]. I'll look that up.

Vincent: That's what we called Ed, a Km Vmax kind of guy.

Rich: That's how he described himself.

Vincent: How would you describe yourself, you're not a Km Vmax kind of guy?

Mark: Not really, no. I don't know what I would call myself.

Rich: A man for all seasons.

Mark: In my heart of hearts I think I'm still a cold room kind of guy.

Vincent: That's great.

Rich: Excellent, a cold room kind of guy. Excellent.

Vincent: You didn't smoke did you?

Mark: No.

Vincent: I knew a guy who was a big biochemist and he use to do columns in the cold room and he'd stand outside the cold room smoking a cigarette watching it drip and then periodically run in and change it. He didn't want to stand inside and get cold. A cold room kind of guy.

So you had left the bench because you wanted to do something totally different. Are you going to want to do that again?

Mark: I think this is the end.

Vincent: Okay.

Rich: TWiV 120, February 13th, 2011. Ed Niles a Km Vmax kind of guy. So, Vincent, that was over a year and a half ago.

Vincent: Yeah. That's the last time we were here in Alexandria I guess.

Well I think that's a good story, don't you Rich?

Rich: Outstanding, I've always thought…, well first of all, I've always had a lot of respect for your science Mark.

Mark: Thank you Rich.

Rich: And I've always appreciated you being there as a program officer and so I'm happy to have this opportunity to kind of tie all that together and tell the rest of the world about it. And I think it's also important, I mean we can provide insight to people as to how this whole process works and give some inside insight into, you know, what goes on and I appreciate the opportunity to do that.

[1:14:00]

Vincent: We never had any program person on TWiV of course. I think you're unique because you made this switch. Would you say to young people or mid-career people that are thinking about it, that that would be a good thing for them to do?

Mark: Yeah, I do. I mean it is way different than doing science but it gives you a certainly different perspective on science. And I would say to follow up on what you said, back when I had a grant and was a young PI, I had really no clue what went on in NIH program or what the whole… you know, I wrote an application and sent it in and a while later they sent me some money or not. But I think it's important, really, to understand… it has gotten so difficult that it's actually behooves investigators to understand the process because it makes a difference.

Rich: As I recall, when I was first starting out, from my perspective the administrative side, the program officers, that kind of side of NIH was always really scary to me. I didn't see them as advocates actually. Okay, now I realize that I was completely wrong.

Mark: Right.

Rich: In fact, the program officers and the administrators are very much advocates. You guys want to see the science advance and want to see it advance in the right way and that's an important thing to communicate.

Actually I have another question. You have a very robust scientific background going into this, is that common for program officers?

Mark: I would say mine is a little more unusual but most program officers have had some experience as investigators on their own, though we don't take that many people as program officers say straight out of a post doc. They are looking for a little bit more experience than that.

Rich: That's good because I think it's reassuring to know that the people who are on the administrative end of this know what it's like to be in the trenches.

Mark: Yeah.

Vincent: I can't resist one more question. So if you were going to go back to the lab today, you found a fountain of youth, but today, what would you work on? Anything in the world, not just viruses, if you want, but I'm sure that's what you know the best.

[1:16:55]

Mark: You know I think it would be something much more involved with… closer to the disease potential of a virus than the basic science I think because I've just gotten more aware of the public health implications of different viruses. It would something like dengue, something like that.

Vincent: Pathogenesis.

Mark: Yeah.

Vincent: Okay. Should we do a couple of emails?

Rich: Yeah.

Vincent: We have some follow up from last time. The first one is from Suzanne:

"Your most recent TWIV in Nebraska made me want to add a few verses to that old kids' song,

'There's a virus in the alga in the paramecium on the speck on the flea on the wart on the frog on the bump on the log in the hole in the bottom of the sea.'

Just thinking and thought it might amuse you guys, too."

So we did the last TWiV in Nebraska and Jim Van Etten was telling us about these algal viruses that he works on. They infect algae and the algae are inside of paramecia, and that's where that came from.

The cool thing is, in my understanding is in the… when the algal is in the paramecium it doesn't get infected. Apparently the virus is compartmentalized in the paramecium. It doesn't infect the algal and then something disturbs it and it gets infected. We never got into it. We have to have him back to talk about that.

Rich: Yes, I have to review this every time I talk to Jim because it's very confusing.

Vincent: We'll get him out to explain. He said that he'd come back and do it.

Jim writes with respect to TWiV 200 video, that's the NEIDL video, "Will we finally see a naked Racaniello?"

Rich: I don't think so.

Vincent: Well he's going to have to wait and find out.

Rich: When is that coming out? Do you know?

Vincent: In a few weeks I guess. I think its half way edited. It takes a long time for them to edit it.

The next one is from Claudio.

Congratulations on surpassing 200 episodes!

"I've been a rabid fan of TWi(V/P/M) since I first stumbled upon TWiP. I look forward to every new episode- they've helped me get through my days as a small electronics technician and have made multiple road trips bearable. This series of podcasts awoke a latent interest in microbiology- time to make something for TWiV! I've attached a picture of some simple plaques I've printed on my DIY 3D printer."

And he made a plaque of bacteriophage and next to it, it says, 'TWiV 200'.

Rich: This is a serious pun, you realize. Those are plaques.

Vincent: Ah, you're right. It is a serious pun. That's excellent.

Rich: They look cool.

Vincent: They look really cool.

"Thanks for all the hard work and time you folks put into these podcasts. I look forward to more! Let me know where to send these plaques and I'll send them on their way!"

So I'll let you know where to send them Claudio and I'll distribute them to you guys because Rich and Alan should get one. Yeah.

Rich: Man I would love to have one of those. I mean…, great.

Vincent: The next one is from John.

"I've been working on a transcript for episode 131: A REOstat for cancer.

I hope I'm not duplicating anyone else's efforts."

So if anyone was thinking about it, don't do that one. Jon's transcribing that one, which we really appreciate.

"Thanks for the wonderful podcasts, I listen to them while I do all manner of stupid things, like cooking, cleaning, ect. I must admit, my apartment got a lot cleaner after I found TWiV and TWIP."

And Jon is a post doc in mathematical physics. How about that, I love the people that we get.

Rich: Yeah.

Vincent: Hey Rich, can you read the next one.

Rich: Sure.

Kathrin writes:

"This may be a bit random, but here it goes: A friend of mine (a virologist) absolutely adores your podcast. She's done me a great favour and I'd love to say thanks with a little gift. I was thinking of a magazine subscription, but am not sure what to go for. I want something on the 'geekiness level' of twiv (so a bit more geeky than National Geographic or New Scientist) that is affordable for a 'normal' person. She's relocating to the States this week, so maybe you guys have some magazines that would be perfect, but that I'm not aware of.

Do you have any suggestions for me?

Many thanks and kind regards,

Kathrin"

Vincent, you have some suggestions.

Vincent: Well I just thought of some science magazines but I don't know if they're geeky enough; you know Wired, Scientific American, Discover magazine, and Science magazine. If she's a scientist she may get that already. There is American Scientist, Seed, Science News, and Popular Science. I've sort of seen every one of those. Those would all be good, probably. Do you know any others Rich?

Rich: No, I think that pretty much covers it. Your first two here are Wired and Scientific American; I think are two good ideas. And my sense is that they are kind of different perspectives of the same thing.

Vincent: They are, yeah. Of course Scientific American has articles mainly by scientists and so it's different from Wired which hires professional science writers. But you can take your pick. They are both having online presences.

Rich: Tell me if I am wrong because… but it seems to me that Wired is kind of hip science.

Vincent: It is.

Rich: And Scientific America is the old stuffy scientist kind of science, alright.

Vincent: I think I'm sure Scientific American would like to change that.

Rich: Yeah, it's cool though. I mean Scientific American has a lot of history to it. It's a great magazine.

Vincent: You know we just got a subscription for our kids. I haven't look at it in years.

Rich: What, Scientific American?

[1:22:57]

Vincent: Yeah. So years ago I use to look at it but I haven't in years and I looked at it… and I actually enjoy it. It's good. It's good to see what's going on in other areas of science in particular.

Rich: Yeah, my father subscribed to Scientific American and I use to look at that. It has very, very good, very readable, but accurate scientific articles. I think probably more in depth stuff than you would find with Wired.

Vincent: Sure, absolutely.

Rich: And a lot of features like history of science and mathematical problems in it and that kind of stuff.

Vincent: So the latest issue I got I opened it up and on page three was a full-page ad by a bio-supplier company that you would know, it says, "The NIH budget may be cut 2.5 billion dollars. Here's why it shouldn't happen." It gave a whole list of things of why this can't happen, you know, shut down productive labs, put scientists out of business, interrupt productivity. Go to the web site for more info. But I thought that was pretty cool. Two and a half billion would be a big deal.

Mark: Well it's what, eight percent.

Vincent: Yeah, wow, that would be a problem.

Should we do one more Rich?

Rich: Sure.

Vincent: Why don't you take that next one too?

[1:24:14]

Rich:

"Dear Twivvers,

I'm a 4th year PhD student at Yale University in the Environmental Engineering Department working in an environmental microbiology lab. The weather in New Haven these days is scorching hot with the occasional summer thunderstorm- a great deal for my adviser who can be assured that I spend the majority of my time inside our building as my home lacks air conditioning.

I'd like to comment on your amazing podcasts- I am a regular listener to Twiv and Twim- I especially enjoy your coverage of the human microbiome (get Jo Handelsman on Twim more often!) and the talks

about influenza transmission. My PhD work centers on the influence of the human occupant on the indoor air microbiome and, thus, these topics are at my dear heart.

In Twiv 191, you talked about influenza transmission via the airborne route, droplets and contact. The question about virus inactivation in air was raised and remained unanswered at that point. I'd like to take this opportunity and hint to a great modeling work by Lindsey Marr of Virginia Tech that combines dynamics of influenza virus containing aerosols (influenced by temperature, humidity, as well as varying air exchange rates in the room) with virus inactivation parameters to estimate removal rates from air. Her results indicate that virus inactivation is important for virus removal from air and largely dependent upon humidity (virus inactivation and humidity are positively correlated). The modeled air exchange rate (AER) parameters (1 or 10 in the paper) are very reasonable for mechanically ventilated building as often found in the US. But having some experience in measuring air exchange rates in public buildings and schools, 1 air exchange per hour sometimes is (sadly) high in those common spaces and may partially explain why we catch a cold in places such as classrooms and waiting rooms. Removal by deposition, air exchange and inactivation should be kept in mind when the infectivity of influenza is discussed especially under the recent focus by the academic community.

As Lindsey shows the importance of humidity on virus inactivation, she had also mentioned (not sure if that is listed in this paper) that protein content in saliva influences survival rates of viruses (high and low content are good for survival). Did you know that infants and adults have different saliva compositions with regards to protein and salt content?!? Isn't that mighty cool?!?! So, again, another parameter is added. In summary, one has to look on a much broader scale and might have to take many more factors into account than previously shown when accurately estimating disease transmission via the airborne route.

The paper is published in PlosOne and thus accessible to all listeners. Here is the link: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0021481

I also attach the paper saving you some time.

Great podcast! Keep up the viral work!

Nina"

And she's a Ph.D. candidate at Yale in Environmental Engineering

Well that's cool.

Vincent: So basically we knew that humidity was bad from the Polazi [?] work on transmission in hamsters. So that's why flu probably predominates in the winter when there's low humidity. I didn't know this about the air exchange.

Rich: Right.

Vincent: You know we are always complaining of rooms where the ventilation rate is high but it's probably good for us, especially when… like in a study section when there are a lot of people in a room.

Rich: A lot of virologists in the room.

Vincent: This saliva business is pretty cool. I didn't know that. Alright, thanks for that.

Let's do a couple of picks. What have you got for us today, Rich?

Rich: I've got to get down to the bottom here.

Vincent: A long list of email.

Rich: Okay, I've been wanting to do this for a while. I've been conscious of this…. Almost there, right.

Mattel Toys has a new Hot Wheel, and it's the Curiosity Rover. I don't think it's actually for sale yet. I've seen a couple of articles about how this is going to happen….

Vincent: Oh, way cool.

Rich: I've seen a couple of articles about how this is going to happen and I have looked on the Mattel web site and stuff and I haven't actually seen it so I'm not sure it's in stores yet. But, you know, keep a look out for this. I have to get one of these.

Vincent: These are the new ones, the new one that just went up. Is that right? It says 2012.

Rich: Yeah, absolutely, this is the new mars rover and it's being made by Mattel, or is going to be, as a Hot Wheels car, which I think is just great. Alright to have somebody like Mattel selling state of the art, up to date science toys for kids.

Vincent: Carries tools, such as a drill….

Rich: I want to see my grandson running Curiosity Rover in the living room. As a matter of fact, I ought to buy a few of these and give them out.

Vincent: Carries a drill, camera, and a laser to see if Mars could have ever supported small life forms called microbes.

You know, if there are microbes, there are viruses.

Rich: You bet.

Vincent: That's pretty neat.

My pick is a special issue of mBio which just came out and it has a series of articles debating should the moratorium on research enhancing H5N1 transmissibility end? Has articles by Arturo Casadevall, Ron Fouchier, Anthony Fauci, your ultimate boss right?

Mark: Right.

Vincent: Marc Lipsitch and Barry Bloom, Ian Lipkin, and Stanley Falkow.

Rich: Wow, that's quite a line up.

Vincent: With their own view points on should we allow this to go forward, should we raise the moratorium or what. So I think this is pretty cool for everybody. We might even want to discuss this at some point on TWiV. So here you go, you can check it out.

Rich: Do your homework.

Vincent: I'm linking to a blog post on mBio that links to all the articles so it's easier to find them. I think that's pretty important stuff, especially since we talked about it so much here on TWiV.

[1:31:12]

And we have a listener pick of the week. This is from Claudio who had made those nice plaques for us.

"I'd like to offer up a couple of related reader's picks for you folks. I came across this article in Ars Technica entitled, DIY lab equipment, courtesy of 3D printing. Three-D printing has been around for some time now, mainly in larger industry and academic institutions. The hobbyist and do-it-yourself community has been working hard in bringing these kinds of tools to those willing to do some work. My open source 3-D printer is a Prusa Rep Rap similar to the one linked in the article. I was lucky enough to be a member of the local hacker space/maker space, it's called mid-south makers and with the help of a fellow member with a maker bot we were able to print the parts to put several together for ourselves. To better describe it, it's a solution looking for a problem.

The open source 3D printing community extends to designs as well. There are several online repositories that host user created designs, including many models that can help flesh out a home lab. There are Dremel powered centrifuges and open source orbital shaker, PCR machine replacement parts, and even a portable cell lysis device for DNA extraction.

And for a second readers pick I'd recommend DIY Bio.org. The DIY hackers space movement has been breaking into microbiology, DYIBio.org is an organization dedicated to making biology an accessible pursuit for citizen scientists, amateur biologists, and biological engineers who value openness and safety. Hopefully some of your listeners would be willing to help further amateur science."

This is pretty cool stuff.

Rich: Yeah, I've seen this… I think we had a video on TWiV some time ago from some guy who was using this micro-centrifuge.

Vincent: Yes.

Rich: Where he made the little head for his Dremmel tool on a PCR printer, and it worked just fine.

Vincent: Yeah, these 3D printers are great. I know that structural biologists use them now a lot to make models. You can print viruses out in 3D, proteins, anything. It's very cool. Alright, thanks for that Claudio.

And that will do it for TWiV 203. You'll be able to find this as usual on iTunes at TWiV.tv.

It's the postman. Is it the Journal of Biological Chemistry?

Rich: No it's a Netflix.

Vincent: Last time we were here at Ed's house we got the Journal of Biological Chemistry dropping through the mail post as we were recording. How appropriate was that?

Since you are here Ed we want to thank you for letting us use your house.

Ed Niles: I am happy to have you.

Vincent: Thanks very much. I also want to thank… oh, before I do that, let me remind everyone to send your questions and comments to TWiV at TWiV.tv.

Mark Challberg from NIAID, thank you for joining us today.

Mark: Thank you for having me Vince.

Vincent: Hope you like it.

Mark: I did, great.

Vincent: Are you going to listen to this one?

Mark: Well… I don't know about that.

Vincent: He might not want to listen to it. Rich Condit is at the University of Florida, Gainesville. Thank you, Rich.

Rich: Sure enough, great fun.

Vincent: I understand you're going to hang out here with Ed Niles for the weekend.

Rich: Yep, we'll go visit civil war sites tomorrow.

Vincent: Have fun.

Rich: Yep.

Vincent: I'm Vincent Racaniello; you can find me at my web site, Virology.ws. You've been listening to This Week in Virology. Thanks for joining us. We'll be back next week.

Another TWiV is viral.

Steve Stokowski made this caption file using a YouTube Machine Translation and the online captioning tool Subtitle-Horse.