This Week in Virology

with Vincent Racaniello, Ph.D.

Episode #206: Viral turducken

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[0:9:36]

Discussion of Paper about Dedifferentiating Cells to Become Stem Cells

Vincent: We have two cool papers today. The first one is actually very topical because it has to do with a discovery for which the Nobel Prize this year in physiology or medicine was awarded. We did not mention because this was for de-differentiating cells to become stem cells. Are you aware of this Dickson?

Dickson: Vaguely.

Vincent: So Shinya Yamanaka was one of two people to get the Nobel Prize this year and that is what this paper is founded on. He found that you could put four transcription proteins—people call them factors, but they are proteins—into cells and they de-differentiate and become stem cell like.

Dickson: Just inject them into the cell?

Vincent: No, he used a retroviral vector to deliver them.

Alan: This is critical to understanding this next paper.

Vincent: So this is a paper that deals with this. He got the Nobel Prize for this appropriately because this is amazing that you can make stem cells and you do not have to get them from embryos.

You must have followed this Alan.

Alan: Oh intensively. This comes up now all the time these induced pluripotent stem cells.

We have mentioned it in passing a couple of time on TWiV I think because they are such an amazing technology. They have the properties of embryonic stem cells but you can take skin cells from somebody

and reprogram them and you get stem cells of that person, which raises that whole new possibility of maybe growing somebody a new liver made out of their own cells so they would not reject it.

That is still a ways off but the technology itself is really, really cool. And the fact that you only need four genes to do it is jaw dropping.

Vincent: I know it is amazing. I do not know how he came upon to do this, but it is really remarkable.

These stem cells you can make them from any kind of fibroblast and then they are pluripotent, they can become any kind of cell.

Alan: Yes, you can grow neurons, you can grow heart cells; you can turn them into whatever you want just by altering their set of growth factors that are associated with them.

Vincent: And you cannot outlaw them, the use of these cells.

Alan: Right because they are adult… well you could, but it would be pretty absurd, not that the other thing is not, but…. They are not derived from an embryo, which is crucial.

Vincent: Embryonic stem cells.

[0:12:22]

Activation of Innate Immunity Is Required for Efficient Nuclear Reprogramming

So this paper is called, Activation of Innate Immunity Is Required for Efficient Nuclear Reprogramming. That is great. It is connecting the production of these stem cells with native immunity. The key, as Alan says, is the viral vector.

Kathy: And the authors are Lee, Sayed, Hunter, Au, Wong, Mocarski, Pera, Yakubov, and Cooke, mostly from Stanford.

Vincent: The only one I know is Ed Mocarski, as you probably know also. He is a herpes virologist.

Using retroviruses is not ideal. Why Dickson?

Dickson: Because they integrate into the nucleus.

Vincent: Good job.

Alan: Yes.

Vincent: If you wanted to do this so that you could build a liver, as Alan said, you would not want to use retroviruses because it might integrate somewhere and disrupt an essential gene or make a make a tumor.

Alan: The good news is you have a new liver. The bad news is it has cancer.

Dickson: That is right you have a lot of new liver.

Vincent: How would you like to wake up to that Alan?

People have been trying to find out other ways to do this that does not involve using a retroviral vector. That is where this paper comes in.

Alan: It turns out the obvious thing, just injecting the proteins does work, kind of but really badly.

Dickson: Right.

[0:13: 56]

The Four Proteins

Vincent: The four proteins are called Oct4, Sox2, Klf4, c-Myc.

Dickson: c-Myc is a famous one.

Vincent: O-S-K-M, remember that four letter acronym, OSKM.

Dickson: And they will tell you. What does that stand for Vince?

Vincent: The four factors – Oct4, Sox2….

Dickson: No, what do the letters stand for?

Vincent: They are transcription proteins.

Dickson: They have names though, no?

Vincent: OCT 4.

Dickson: Oh, I am sorry. I thought they named them after, like….

Alan: I am sure those are abbreviations.

Dickson: Exactly, that is… just for a complete explanation.

Vincent: Oh, like OCT is octamer binding protein.

Dickson: See, that is what I am saying. That makes sense.

Vincent: Okay, but we do not need to go into that. That is like obscure stuff. Get people uninterested in this wonderful story.

Dickson: Ha, ha, ha.

Vincent: I think other people have found that you can put the proteins in to cells, the four proteins, and you can get production of stem cells but it is very inefficient.

Alan: Orders of magnitude less efficient.

Dickson: Was this work predicated on the fact that when you examine what stem cells produce, these four proteins always come up?

Vincent: No.

Dickson: So they did not have a hint as to how to… you said that you did not know how they came up with this idea.

Vincent: Alan, do you know how he stumbled upon these four proteins?

Alan: I skimmed through this story. At some point there were some hints that some of these were involved, and he started putting things in and getting some results that were suggestive. It was more or less one of these that you just, persistence type of effort that eventually narrowed it down to these four proteins that you really needed.

Dickson: Trial and error, wow.

Vincent: I think in stem cells they are very transcription quiescent, right? They are very different from most other cells. So they may have looked in these cells and gotten some ideas of what is produced. Or maybe when induce them to differentiate [you look for] what is produced. I do not really know. We could find out?

Dickson: Okay.

Vincent: That is a good question Dickson.

[0:16:03]

Cell-Permeant Proteins

So one of the ways you can put proteins in is by linking them to short sequences, peptide sequences that can get into cells on their own. These are called cell-permeant proteins.

Dickson: Without pinocytosis?

Vincent: Yes, they get right through the membrane.

Dickson: Interesting.

Vincent: The one I know originally is a repeat 22 protein from herpes simplex virus. It was, I think, one of the first discovered. You can just put the protein onto cells in a dish and it gets in. Since then there have been other proteins, there are quite a few other ones. There is a whole industry now based… diffuse that base sequence, 11 amino acids, with whatever protein you want to get into the cell and it will get into cells. It is amazing.

Dickson: How about that?

Vincent: So they found in this paper that this work but, as Alan says, it is really less efficient then using the retroviral vectors. So these four transcription proteins activate certain target genes in the host cell.

So they either assay those target genes, their expression, or the production of colonies, which indicate you are getting these stem cells produced. With just the proteins they got very inefficient nuclear reprogramming.

[0:17:23]

Intrinsic Feature of Viral Particle Involved

Then they made this big leap which is really interesting. They say, 'We hypothesized that an intrinsic feature of viral particles is somehow involved.' Because remember, they introduced them using retroviruses.

Alan: Right. And I think they did some gene expression profiling, right?

Vincent: Yes.

Alan: So they found that when you introduce these using retroviruses you got one of these big gene expression profiles and you compare that with a profile you get when you just put the proteins in with these cell permeating peptides, there were differences that suggested that maybe the viruses were doing something.

Vincent: Right.

[0:18:07]

The Role for Innate Immunity

Kathy: Right. In fact in the very beginning of the discussion they say that, 'We serendipitously discovered a role for innate immunity and our salient observation,' the first one that they mentions is, 'a consistent difference in the temporal characterization of gene expression between cells exposed to these factors whether they get retroviral vectors versus the cell-permeant proteins.'

Vincent: They made an interesting experiment. They made a mutant of the vector that could not integrate into the host genome and that was still very effective at converting the cells to a pluripotent state. So integration is not needed.

Alan: So something about just the virus going in seems to be helping.

Vincent: Yes, and as you said, they looked and they found that a lot of innate immune response genes seemed to be activated when they put these retroviral vectors into cells, like toll-like receptors, NF-kappa B, interferons. So that made them think that maybe the innate immune response is important.

Alan: These are genes that are turned on during an inflammatory response.

Vincent: So the retroviral vector was recognized as foreign. That is the RNA produced from the vector, most likely.

Alan: Right.

Vincent: And that starts a whole program of innate responses, including interferon production, but the sensing is the first step of these vectors. They said well maybe one of the sensor proteins. They honed in on this toll-like receptor 3 pathway because this is a sensor for viral double-stranded RNA. I think we must have talked about this before.

Alan: Yes, that has definitely come up.

[0: 19:50]

TLR-3

Vincent: So basically, if you knock down the TLR-3 or one of the intermediate proteins in the signaling pathway… so you put in the four retroviral vectors encoding the four proteins into cells. If you do that and at the same time knock down TLR-3, you do not get good conversion to pluripotent stem cells.

Dickson: Right.

Vincent: Directly implicating TLR-3.

Alan: Right.

Vincent: And they looked at some of the other pathways, but it is just TLR-3 that is involved. They show that expression of the target genes, of each of the transcription proteins, requires TLR-3. Also making these stem cells requires TLR-3. That is amazing.

Dickson: So Vince, do you think a virus-like particle would do the same thing without its coding regions?

Vincent: No, I think you need the genome because that is what is triggering TLR-3.

Dickson: I see it is not the proteins.

Vincent: So you have to make RNA.

Dickson: Okay, that was a naïve question.

Vincent: No it is fine. You make good questions all the time.

Dickson: It means that I have forgotten something along these….

Vincent: No, no, we did not mention it. There is no reason to believe that… we did not tell you so there is no reason to….

Dickson: Okay.

Vincent: You can stimulate TLR-3 with agonists. One of them would be PolyIC, which mimics double-stranded RNA. So they did a nice experiment where they put the four proteins in a permeable immersion into cells. Remember, that that transforms the cells to pluripotency very inefficiently. But if you put PolyIC in, it is better because it is stimulating TLR-3. So in the absence of a retroviral vector you

can substitute by stimulating the TLR-3. Practically speaking that is good because maybe that points to a way to get away from using the retroviral vectors.

Dickson: Absolutely.

Alan: Right, it is a much more surgical approach.

Kathy: Yes, making it safer, potentially.

Vincent: What else is important here?

[0:22:05]

A Good Reagent

Kathy: They have a couple of cool reagents. One of the experiments they do is with a cell line that has the four reprogramming factors [transcription factors] in it that are DOX inducible. So then they do not even have to put the proteins in, they are already there. That leads to figure 6.

They can do the same kind of experiments with and without polyIC or with and without a retroviral vector that does not encode any of the factors, it is a GFP, almost like an empty vector kind of thing.

So that was a cool reagent.

[0:22:48]

Why Do You Need an Innate Immune Response to Get Nuclear Reprogramming?

Vincent: The next step is that they do a few experiments to try and understand what is going on. Why do you need an innate immune response to get nuclear reprogramming to make these pluripotent cells?

Dickson: They are starting with fibroblasts which are undifferentiated cells to begin with.

Vincent: Yes and they back them up even further, all the way to the beginning.

Dickson: I have another question that I will save until later but I want to come back to that point.

[0:23:17]

Innate Immune Response Triggered by TLR-3 Opens Chromatin

Vincent: They then have this hypothesis that the innate immune response, triggered in this case by TLR-3, is helping to open up the chromatin. This is a concept you must know about Dickson, right?

Dickson: Yes.

Vincent: Closed chromatin is transcriptionally quite, open chromatin….

Dickson: The nucleosomes are over the coding regions so this takes off….

Alan: Much of the DNA in a cell gets packed into a sort of storage form that is compressed chromatin and then actively transcribed regions get unpacked so they can be actively transcribed.

Vincent: One of the signatures for the opening of chromatin is methylation of what, Dickson… histones.

Dickson: Correct.

Vincent: I thought you were going to say it, your eyes were lighting up.

Dickson: I almost did. I once knew this.

Vincent: Methylation of histones is associated in very specific residues of histones and this is associated with the opening of the chromatin. In fact they find if they put their retroviral vectors into cells, they get associated histone methylation patterns, specific ones they can look at in a variety of ways. So this is an epigenetic modification because it is a linkage of a methyl group to the histone protein.

So then they say, ah ha, signaling through the innate pathway is opening the chromatin because we see these histone methylations and that is what is associated with that. In fact, histone methylation is carried out by a variety of enzymes and they show that these [enzymes] are induced when TLR-3 signaling occurs. Is that incredible?

Dickson: It is like a Chinese puzzle—when you put it all together it seems to fit perfectly.

Vincent: Yes, I think that is really the story, that you have an innate response to the vector, the innate response helps to open up the chromatin, and that allows better transcription of the targets of these four proteins, otherwise it is closed and it does not work so well.

It is really a fortuitous thing that he used a retroviral vector. If he had not….

Alan: It would not have worked.

Vincent: It would not have worked, but he might have gotten really inefficient transformation and maybe he would have followed it up and eventually figured this out. I read somewhere that everybody missed this; all the key players missed this.

The one thing I am thinking here is that this is of course totally accidental that the retroviral vector is opening up the chromatin to make this really efficient. But, what I found interesting is that the innate response leads to this.

If you think about it, when TLR-3 recognizes a pathogen it has to turn on the synthesis of a lot of genes, so it has to open up the chromatin. So this makes perfect sense.

I do not think about transcription all that much, I have not really ever. I mean we work on innate responses but I never thought of that. So I find this really cool.

[0:26:25]

A Fundamentally Important Discovery

Alan: There is another important parallel here too. The process of reprogramming a stem cell is a lot like some of the processes that a cell goes through to become tumorigenic.

Vincent: That is right.

Alan: What you are looking at here are the steps that a cell apparently has to go through in order to start differentiating and multiplying in a different way from what its environment would otherwise suggest. That is a fundamentally important discovery and this might be something you could look at in cancer cell lines and maybe eventually target.

[0:27:02]

Article Summary

Vincent: The last paragraph is really a great summary. "Innate immunity appears to favor an open chromatin state which increases cell plasticity in response to a pathogen. We speculate that this state may enhance induction of pluripotentiality, transdifferentiation, or even malignant transformation." That is what Alan just said.

They call it 'transflomation.' [?]

Dickson: I like that.

Kathy: I am not so fond of that yet.

Vincent; We will see.

Alan: Yeah.

[0:27:34]

Silencing of Endogenous Retrotransposons Characteristic of Fully Reprogrammed Pluripotent State

Vincent: One thing they mentioned in the discussion which I wanted to point out, they say, "… [?] Silencing of endogenous retrotransposons is characteristic of the fully reprogrammed pluripotent state." In other words, in these pluripotent stem cells, any endogenous retroviral-like sequences are silent, they are not made.

I asked Steve Geoff about this because they site one of his papers here. So in ES cells (embryonic stem cells), which are pluripotent, if you make them from mice, the endogenous retroviruses are silent. I asked him why.

He said pluripotent cells are generally transcriptionally really very different from all other cells. They tend to be quiet, but whatever is expressed is different. So there is a lot of global silencing and probably the retroviral DNA sequences, which are integrated, also got silenced along the way.

He wonders why the retroviruses have not evolved to evade that silencing because they would like to replicate and spread. So that is a question we do not know the answer to.

[0:28:51]

What Cell Types Can You Reprogram?

Dickson: Now I will ask my question. You start with fibroblasts, which is a relatively undifferentiated cell to begin with in the body. For instance, if you make two gene changes you can take a fibroblast and change it into a striate skeletal muscle cell. It is not a big deal to do that. You just knock out ID and the next thing you know is that you have a muscle cell.

Vincent: Okay.

Dickson: How far down the line of differentiation can you go and get this system to work? Besides fibroblasts, can you do it with fully differentiated cells, like nerve cells, muscle cells, pancreas cells, islet cells? How far down the road can you reverse it?

Alan: I do not know how many different types it has been tried with but I know it has been tried with at least some quote-unquote terminally differentiated cells.

Dickson: It works?

Alan: Yes, this appears to be…. Again, I do not know how many it has been tried with, and there may have been some where people tried it and it did not work so it was not published. So there may be some preference for what works and what does not. But, this appears to be a mechanism that you can just do.

Dickson: Wow.

Vincent: Are you looking for another brain Dickson?

Dickson: Some people would suggest that I could use one. Ah, no, not another brain.

Alan: The real point with IPS cells is that you can use easily obtainable cell types and produce then a stem cell line from anybody you choose or any animal you choose.

Dickson: That is remarkable. That is truly remarkable.

Kathy: Getting back to your earlier question Dickson, it looks like based on knowing if you fused cells together or if you just took the nuclear material that would be enough to induce pluripotency. And then it was known that certain factors were involved in that and then they evidently narrowed in on those four.

Dickson: Wow.

Vincent: That is a very nice paper. It was actually sent to Rich by Grant McFadden. Rich is not here, I bet he would have liked to have been here, but I thought it was too cool to put off.

Alan: It was very cool.

[0:31:10]

Second Paper: **Provirophages and Transpovirons of Giant Viruses**

Vincent: The second paper came out in PNAs not too long ago. It is entitled, *Provirophages and transpovirons as the diverse mobilome of giant viruses*.

Dickson: Oh come on.

Alan: I am glad that people are finally making titles descriptive and approachable to the general public. There is too much use of specialized jargon.

Dickson: My god.

Vincent: It is quite a title isn't it?

Dickson: Your honor I object!

Vincent: It is by Desnues, La Scola, Yutin, Fournous, Roberta, Azza, Jardot, Monteil, Campocasso, Koonin, and Raoult. All French and NIH.

So this is a group that has worked on the giant viruses, a member of the mimiviruses and all their relatives. This is quite interesting, title aside.

Alan: Yes, please.

Kathy: So we can start with their definition of mobilome.

Alan: It has nothing to do with trailer parks.

Kathy: The mobilome is mobile genetic elements. They talk about roughly four classes—transposable elements, plasmids, viruses, and self-splicing elements. So that is what they mean by the mobilome.

Alan: Because of course, if you have more than one of anything in biology these days it has to be an 'ome.'

Dickson: It's got to be an 'ome,' that's right, which is of course a Zen concept.

Vincent: Did anyone hear mobilome before? Have you ever heard that used?

Kathy: No.

Alan: I had never come across it.

Vincent: Yeah, this is new for me.

Alan: But apparently… they cite a reference in using it. Somebody else may be to blame for that one.

Vincent: The provirolphage and transpoviron they've invented in this paper as you will see.

Kathy: I think it is trans 'pō vi rons.

Vincent: I don't know. If it is made up who knows. What did I say, trans 'pō vī rons? Yeah.

Kathy: Yeah, that is sort of a viral-centric way.

Alan: Trans ‘pō vi ron sounds like something you would adjust on your space ship.

Vincent: Kathy, could you say it again I need to get it right.

Kathy: Well I don’t know but I think it might be, trans ‘pō vi ron. That’s the problem with English, we don’t have rules about where accents go.

Vincent: Transpovirons and provirophages, and mobilomes.

What they have done here is to discover another one of these giant viruses. To refresh everyone and I think we have talked about all of these on TWiV. The first is the mimivirus of course, and then shortly after that another large virus; and these are large capsids with huge genomes, over a million bases of double-strand DNA.

Alan: There genomes are comparable to some of the smallest free living micro-organisms.

Vincent: Mamavirus was another one. When mamavirus was discovered they found a virophage that could only replicate in cells infected with that virus. They coined the name virophages. The hosts of these are amebae— acanthamoeba, castellanii or polyphagia.

Sputnik, that’s one of the virophages. Then another virophage called Mavirus, which we also talked about on TWiV, it was found to require the Cafeteria roenbergensis virus. Cafeteria roenbergensis (CroV) are these proteus [protei], very numerous proteus [protei] in the ocean. They are infected by this virus CroV and they have a virophage that is called Mavirus. Ma comes from mariner because these virophages have homology to a transposable element called Mariner… sorry, Maverick. Not Mariner, I’m so sorry, very confusing.

Then there is a third one, the Organic Lake virophage. I think we talked about that, I certainly talked about it in my course. Organic Lake is an Antarctic lake, hyper-saline. They found the sequence of this virophage in it. They don’t know what the host, or the helper virus is but they assume, because the lake is full of these large DNA viruses that infect algae (those are the ones Jim Van Etten work on), they assume that this virophage infects them but they don’t know that.

So three virophages.

Kathy: I just got by email from my brother a picture of the Antarctic lake, Organic Lake and its map location. I’ll send those to you at some point.

Vincent: You must have told him we were talking about this paper.

Kathy: Two minutes before the show started.

Vincent: Does he live in Antarctica?

Kathy: He has lived in Antarctica.

Vincent: We have a letter from him later.

Kathy: Yep, if we get that far.

Vincent: Thanks.

Kathy: Organic Lake is a couple hundred meters, it's hyper-saline, it's only about 7.5 meters deep.

Dickson: Is that why it doesn't freeze is because it has too much salt?

Kathy: I don't know that it doesn't freeze but you said that and it's probably right.

Vincent: We have big viruses and then we have virophages that infect only cells infected with these big viruses.

Alan: Right.

Vincent: I would like to register a complaint at this point. The first sentence of the abstract, "A distinct class of infectious agents, the virophages that infect giant viruses of the *Mimiviridae* family…." This is not right, they don't infect the virus, they infect the virus infected cell. Now we all know what they mean but you know the press, the public lay-press or whatever, has picked up on how virus infecting a virus and they repeat it. I think we talked about this a couple weeks ago. These authors are responsible for that; I didn't realize it.

They say it again later. "…demonstrating for the first time that a giant virus could be infected by another…." Come on! Do you all harbor the same problem with that or you don't care?

Dickson: We care deeply.

Kathy: I tried to notice them, I missed the very first one, because I thought whenever I was looking it would say that they are replicating in the factories… and I knew you were sensitive to that. I missed the ones you've pointed out.

Vincent: So the factories, right it is in an infected cell. In fact this is not new; we have known for many, many years that there are helper viruses and viruses that depend on the helpers to replicate in the same cell. So that in itself is not new but the idea that it is replicating in the virus is not correct.

Dickson: How can it be?

Alan: Yeah, I totally see where you are coming from and I agree that ideally they would use the better explanatory term, that it infects… uses this as a helper virus; but it does kind of… it is kind of a parasite of the larger virus.

Vincent: Depends what you mean… I guess if you say virus meaning virus infected cell that would be okay, but I don't think that this science writer said that.

Dickson: Let me get this straight. I am the resident Parasitologist here; however lacking in knowledge I am in regards to viruses. You have two competing organisms for the same resource which is the host cell. Is that correct?

Vincent: Correct.

Dickson: Alright, all it is is a competition for resources then. Does the little virus slow down the replication of the large virus?

Vincent: Yes it does.

Dickson: Okay so it is like an ecological setting inside the machinery of the host cell.

Vincent: It does slow down the big one. And the little one cannot replicate without the big one.

Dickson: That means that it not only infecting the other virus it is competing with it for the same resource.

Vincent: It is infecting the same cell.

Dickson: So I think that is a misrepresentation of the molecular mechanism.

Alan: Now does any of that change, because we are going to come up to this point in a moment, does any of that change if we are talking about a virus that integrates into the larger virus and travels along with it?

Vincent: Yeah, that is a good point. We can discuss it when we get to that.

Alan: Because then that becomes a little more of ….

Vincent: It is a little dicey but I think replicate is still a different thing. Integration is… let's talk about it later.

So in this paper they discover a new big virus and a new virophage that infects cells infected with that big virus. The new virophage is called Sputnik 2, the fourth virophage.

Dickson: there was only one Sputnik, sorry.

Vincent: The virus is called Lentille virus. Do you know what Lentille in French means, Dickson?

Dickson: Beans.

Vincent: No. You are thinking of lentil.

Dickson: I am.

Vincent: It is not bean.

Dickson: Well I don't know what it means.

Vincent: It is lens. They isolated it from contact lens fluid from a patient with keratitis.

Alan: I would assume lentils are called that because they are lens shaped.

Vincent: Yeah, that's right.

Alan: Rather than the other way around.

Dickson: Wow.

Vincent: So Dickson, it's from contact lens. Aren't these French clever?

Dickson: They are.

Vincent: Lentille, I think that's great. You name a virus from where it comes from and this came from a contact lens.

Alan: But it is not a lentivirus.

Vincent: It is not. That could cause some confusion. This is great, I'd love to talk to the authors about why they did this.

Dickson: It means that whatever you can imagine about life, life has already done.

Vincent: So you know what I think happened, the person probably cleaned their contact lenses with water, it was contaminated with amebae and it had this virus in it.

So they have a new big virus and a new virophage and they actually infect amebae and they show that the virophage replicates within the factories setup by the big virus. They do immunofluorescence to show that very nicely.

Dickson: Is it known that these viruses do or do not integrate into the amebae's genome?

Vincent: I have not seen evidence of integrating into the amebal genome. But, thank you for the lead in Dickson.

What they find is that the virophage DNA, remember these are both double-stranded DNA, integrates into the genome of the Lentille virus. They show it by Southern blotting and by sequencing that it integrates into the genome of the giant virus.

Alan: Does it infect it?

Vincent: Well in the cell it probably does, in the factory right. That is probably where the integration takes place?

Alan: Sure.

Vincent: It is integrated… I would say it is integrating its genome into that… because this is not new, there have been some other examples of this. I think Rich has mentioned a couple time there is a pox virus with a retrovirus in its genome, right?

Alan: That sounds familiar.

Vincent: There is a herpes virus also with another virus in its genome. The two papers are referenced in this paper. I would say it is just integrating its DNA and it's a target for integration.

Alan: Right.

Vincent: If a retrovirus sees double-stranded DNA it will integrate into it. That is probably what happened.

Dickson: Interesting.

Vincent: Now we do not know why it is integrating here.

Kathy: They show it very nicely in a classical method, the Southern blot that is in figure one.

Alan: Yes it was nice to see a Southern blot in a paper.

Vincent: Dickson do you remember Southern blot?

Dickson: Of course I do, I am from New Orleans.

Vincent: What does that mean?

Dickson: It is in the South. I have another question though? When these viruses replicate in the host cell, in the amebae, don't you get two different virus particles?

Vincent: Yes, you do.

Dickson: So doesn't the second little virus particle have the genome of the little virus inside?

Vincent: Yes it does.

Dickson: So why integrate into the larger virus' genome?

Vincent: I don't know. Nobody knows.

Kathy: That is a good question.

Dickson: Another question is then, in the larger virus after replication, does every single viral particle contain the smaller virus as well or is it just partial?

Vincent: Yes, some of them have the small particle within it and some of them don't. I think it is kind of a random event.

Dickson: So it is not probably essential for the co-infection.

Vincent: Well, it is essential… the co-infection is essential for the virophage to replicate.

Alan: Yes, and integrating into the lager virus would drastically increase its chances of getting a co-infected cell.

Dickson: Well that raises the question, then eventually why doesn't it do that instead of making little virus particles also then?

Vincent: Yes, that is a good question.

Dickson: It is a waste, it sounds like it is a wasted metabolic…

Alan: It is stuck in its old ways.

Vincent: Does anyone remember, does every Lentille viral genome have an integrated virophage? I don't know if they could tell. Probably the Southern blot would tell you that it is not equal molar.

Dickson: You could do it by limiting dilutions.

Vincent: I don't remember off hand.

Kathy: I didn't get that feeling.

Dickson: If you have the genome you could do it by ultracentrifugation.

Kathy: They did talk about the hot spot of integration and a presence of a further candidate integration site suggests that it may insert in multiple possibly random sites.

Vincent: I don't think they really address it. If you found it in every genome then maybe it is providing some function to the bigger virus. That's the question.

Dickson: These are questions that you may not have raised but I am certainly going to raise them now. When viruses replicate inside a host cell they obviously produce proteins which aid and abet them to do that. That is a general principle that we even had in Virology 101. Perhaps there is a co-operation necessary here between the viral proteins that the genomes encode in order to allow them both to replicate. Is that known? What proteins do these genomes encode?

Vincent: Many, many proteins.

Dickson: Yeah many, so that's the point, do any of those proteins directly involved in affecting host factors….

Vincent: I am sure a lot of the proteins are totally novel, we don't even know what they are. I mean a lot of them are DNA replication proteins or helicases and polymerases but they are lots that we don't understand. So it is a good question. All I can say is that the virophage inhibits the replication of the larger… of the helper. I would find it difficult to believe that the helper needs the virophage, but it could be, can't rule it out.

They also find another piece of DNA in these big viruses, the Lentille viruses. They find about seven, seven and half kilobase linear double-stranded DNA. It has long inverted terminal repeats. Its structure looks like a transposable element. They call it the transpoviron.

Alan: Because it is a transposable element, a transposon inside a virion, it is a transpoviron.

Vincent: They made it -viron instead of -virion because it's a transposon right?

Alan: Right.

Vincent: Okay, very clever. They like making names in Marseille.

Alan: Yes, this team is very into inventing language.

Vincent: Is there something about tea in Marseille?

Alan: Tea?

Dickson: No.

Vincent: That was the Shakespearian thing, the quality of ….

Dickson: You are going to get letters Vince. I know you are going to get some letters.

Kathy: Oh, it's that bad pun.

Alan: Yes, yes. Of mercy is not strained.

Vincent: That's Marseille.

Kathy: That's Marseille, yeah.

Vincent: God I knew there was Marseille in there. So these are transpovirons and this is packaged in the Lentille particles. They also find it integrated into the genome of Lentille virus.

Alan: So it can be packaged by itself or it can integrate.

Vincent: Right.

Kathy: Right.

Vincent: It can also integrate into the viral phage genome.

Kathy: Into the Sputnik.

Dickson: I have yet another question. What part of the amebae is the virus factory, cytoplasm or nucleus?

Vincent: Cytoplasm.

Dickson: Not the nucleus.

Vincent: Not the nucleus. Why do you ask?

Dickson: Well, because I am curious.

Vincent: These are large cytoplasmic DNA viruses right. These are cytoplasmic factories much like you see in Pox virus infect the cells.

Dickson: Is the amebae killed by the virus?

Vincent: Yes it is.

Dickson: Ah ha.

Vincent: In fact these… in Organic Lake on of the ideas was that these viruses helped regulate the algal populations in different seasons.

Alan: Right.

Dickson: So Vince, how would someone with a contact lens have this as part of their culture?

Vincent: Because they used water…

Dickson: Yeah but where did the water come from?

Alan: Tap water.

Vincent: Tap water.

Dickson: You said these came from an Organic Lake in south….

Vincent: No, not this one, Organic Lake is a different one.

Dickson: Oh, I am confusing it.

Vincent: This is a different virus, related but diverse.

Dickson: Okay.

Vincent: It is very confusing, I know. I think that it is tap water that has the amebae in it.

Dickson: But there are other acanthamoeba in this Organic Lake… this other one.

Vincent: Organic Lake doesn't have acanthamoeba; I think it has many algae.

Kathy: Phycodnaviruses that infect green algae, yes.

Vincent: As far as I know Dickson, amebae are not algae, or vice versa right?

Dickson: Correct.

Vincent: I know I learned something from this.

Dickson: Well there are chloroplasts involved in this somewhere along the line.

Vincent: Okay, we will have to do a podcast on that.

Dickson: Those are other infectious agents by the way.

Vincent: Transpovirons, okay they are not present in the amebae. When you infect cells with Lentille viruses, that is amebae, the transpovirons seem to replicate. But if you just infect cells with Sputnik 2 the transpovirons does not replicate. So you need something from Lentille virus to replicate this transpovirons.

They think that maybe Sputnik helps transfer the transpovirons among bigger viruses, it is like a vehicle. So this transpovirons is mobile DNA and maybe the Sputnik moves it around. That is there idea.

They have now a lot of different mimiviridae isolates, 19 different mimiviridae. They look at all the sequences and they find transpovirons in all three groups—they these Mimis are put into three groups based on sequence analysis—and they can find transpovirons in members of all three groups. It is something that is real and it is out there. That is very cool.

The transpovirons, Dickson, if I said we have a 7.5 kilobase DNA, this is a transpovirons, what one pressing question would you ask about it?

Dickson: What does that encode?

Vincent: Exactly, brilliant.

Dickson: Do I get something for this, like a glass of water? Never say that to a man over seventy.

Vincent: Do you want a TWiV mug?

Dickson: Oou, I would love it.

Vincent: Didn't I give you one?

Dickson: I don't have it. I would like a TWiP mug.

Vincent: I don't have a TWiP mug.

Dickson: We will have to make one. But that is an obvious question, what does that encode?

Vincent: Yes, so it encodes six to eight proteins.

Dickson: Six to eight, because they are overlapping sequences?

Vincent: Ah…like there's a DNA helicase that they recognize and a zinc-finger protein, but everything else they say no homology to anything known on the earth, so all new proteins. These presumably help it to replicate but they don't know. Gosh, there is so much to do here. It is really so cool. This is it. You go out there, you isolate new stuff and you have more work to do.

Dickson: Why do we have to spend all that money going to Mars when we all this wonderful material right here on Earth that we don't know about?

Vincent: Well because everyone has good ideas about what kind of science to do.

Alan: And come on, it's Mars, it is really cool. That is an awesome project.

Vincent: I think we should be able to do anything.

Dickson: I am begging certain questions here.

Vincent: I understand and I think we should do any science that we want.

Dickson: Well we don't have the money for that.

Vincent: We should have the money.

Dickson: So we have to triage.

Vincent: We spend four billion dollars on the campaign in this country. We could use that to go to Mars.

Dickson: We ended up in exactly the same place as we were when we started.

Alan: We could put our electrical wiring underground.

Dickson: This is all true.

Vincent: There idea is the mimiviruses, the virophages, and the transpovirons are all related. These sequences go amongst each other by recombination, they are mobile elements. There is a lot of work to figure that out.

Alan: They point out that this is not completely unprecedented. I learned it as phagemids, they call them phasmids—virus associated plasmids in bacteria, and apparently in archaea as well. Now it has been found in a eukaryotic cell, now all three domains of life have these transposable elements that ride along with or as viruses.

Vincent: That is a very cool story.

Kathy: As far as we know, is this only the case when the larger host virus, if you will, is a DNA virus?

Vincent: As far as I know, yeah. Let's see, this….

Kathy: I think all of these are DNA viruses.

Vincent: bacterial phage and the archaeal virus are DNA. Well, I wouldn't rule out RNA, why not? We just haven't found it, right?

Kathy: I am just thinking about the cytoplasmic replication made me think about it… I don't know.

Vincent: But in the known… let's say the known mammalian DNA viruses, I am not aware of any such transpovirons, are you, Kathy?

Kathy: No.

Vincent: I guess we would have noticed it, right?

Kathy: Well, again if it… if the cytoplasmic replication is a key then maybe the Pox virus is what….

Alan: Yeah, that would be the place to look.

Vincent: Interesting.

Alan: I guess, I mean intuitively it seems like it might have to be a DNA virus to be able to get the kinds of integration events that you would need for this, right?

Vincent: Maybe, yeah.

Alan: But I wouldn't rule out finding it in something else.

Vincent: Or you could have it reverse transcribing an RNA virus, right?

Alan: Sure.

Vincent: I think that might be able to do it. I just feel we are going to find these in other viruses were we just missed them, but we'll see.

Dickson: This raises another interesting question, those mimiviruses that infect amebae, you would assume because it has to have some receptor attachment mechanism.

Vincent: We would assume, yeah.

Dickson: So algae are quite different because they have cell walls. So that has like totally different attachment mechanism to get inside. So we have no concept of how these get between host cells.

Vincent: You know I asked Jim Van Etten that when I was out in Nebraska, and they don't know. They have wonderful pictures of these algal viruses attaching to algal cells and there is even what looks like a portal and DNA is going through but they don't know how it is attaching.

We are going to get him on TWiV and he can tell us all about it. See if you can make that episode. You would like him.

Dickson: I am sure I would.

Vincent: He is about your age.

Dickson: Decrepit.

Vincent: No, not at all.

Dickson: So you can use a florescent green protein tracer to actually watch this happen.

Vincent: Yes, they do.

Dickson: In real time.

Vincent: And they use green algae so you don't have to put GFP into them.

Dickson: That is a ridiculous statement, even I know the difference.

Vincent: He showed me a plague assay, it is a monolayer of cells, algal cells, chlorella they use, and it's green and the virus makes these beautiful plagues and you don't have to stain them… just holes.

Alan: That's great.

Vincent: Have you seen these Kathy, you must have right?

Kathy: Seems like I have but yeah… yeah.

Vincent: It is just beautiful. He said he'd come back on and talk about it.

Dickson: By the way this has some practical importance here because algae are becoming more and more important for producing bio-fuels and contamination with one of these viruses could raise havoc with that industrial process, if that ever scales up to that level.

Alan: Also as they pointed out in one of their earlier papers, these viruses can have a profound effect on the ecology of any ecosystem where algae or amebae are dominant.

Vincent: I asked Jim whether these big viruses could infect people, these big algal viruses. He said, I'm not ready to talk about that yet. He said it in TWiV.

Dickson: He might be ready some time later, is that the deal?

Vincent: Yeah, I think so. We will see, stay tuned.

Alan: I guess technically this paper is one that indirectly… one of these giant viruses has infected something that has infected somebody.

Vincent: Maybe. Dickson, if you put an amebae into your eye, what would happen? Do you get keratitis?

Alan: It depends on the ameba doesn't it?

Dickson: No you don't because you need a point at which the ameba interfaces between some inanimate object and your cornea. Otherwise it becomes just a part of the lacrimal fluid and then it gets ejected by your eyes watering. If you trap it up underneath the lens—it comes in the form of a cyst, it migrates, it is trying to get out of this lens and that's why you get this beautiful ring of keratitis that goes around where the lens interfaces onto the cornea itself.

It doesn't erode away the cornea between the two edges of the lens; it is only at the edge at the interface of the edge of the lens. That is where the action is. So there must be something physical about its ability to do that.

Vincent: I just looked at the Organic Lake photo that your brother sent, Kathy. It is quite nice.

Dickson: How does that compare to your Organic Lakes on Titan? From the moment you said Organic Lake I thought about that because there are lakes of methane on Titan.

Vincent: Do you think there is good fishing there, Dickson?

Alan: I would probably be ice fishing.

Dickson: The ice is as solid as a brick.

Vincent: Let's do some email.

[0:59:40]

Transcribed by Steve Stokowski