This Week in Virology

with Vincent Racaniello, Ph.D. and Dickson Despommier, Ph.D.

Episode 60: Making viral RNA

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VR: This week in virology, episode 60, for November 27, 2009. Hi everybody, I'm Vincent Racaniello and I'm with Dick Despommier .

DD: Hey Vince.

VR: How's it going, Dick?

DD: It's going very well, Vince.

VR: It's the day after..

DD: This is the day after turkey day, the day after Thanksgiving and we should continue to give thanks.

VR: Sure, we're always thankful. Did you have a good meal?

DD: I had a wonderful meal, and you?

VR: You know, I cooked the turkey in a paper bag.

DD: En papillote?

VR: En papillote. It's incredibly moist. You should try it sometime. It's the one thing that I've always not liked about turkey.

DD: It's dry.

VR: It's dry. But this made it incredibly moist. It's very simple, you just have to make sure the bag doesn't have chemicals in it.

DD: Perhaps we should have given this presentation last week rather than this week.

VR: For all of those in the U.S. who celebrate Thanksgiving, Happy Thanksgiving. What are you doing here on Friday after Thanksgiving?

DD: Hey, listen, I'm a dedicated scientist.

VR: I came here to do a podcast.

DD: I came here to do a podcast, that's what I should have said.

VR: Cause you see, we're pretty dedicated to this.

DD: We actually enjoy this.

VR: We love it but we also want everyone to have their TWiV fix.

DD: This is all true, this is all true.

VR: And today is another in our continuing series of Virology 101. So far we've talked about classification, we've talked about how viruses get into cells. And now what I want to start for the next couple of Virology 101's is how viruses duplicate their nucleic acid. But before we start I would like to acknowledge our support because I'm not going to remember half way through the lecture, as it were.

DD: Good point.

VR: So let's acknowledge the support...By the way, do you like that glass thing?

DD: I love it, whatever it is.

VR: I'm cleaning up my lab because I'm moving across the hall and I found this and it's beautiful.

DD: It's attaching to a vacuum thing...

VR: I think it's probably to dry...You put a little calcium pellets in it and it dries but I thought you could use it as a nice vase for flowers.

DD: You could, you could.

VR:Of course, it also looks like a pipe...

DD: You could use it as a water feature...should you design gardens...

VR: Ok, so we'd like to first acknowledge the support of by Citrix, the makers of Goto myPC. And here's the problem, you're spending more time at the office than at home and that means missing out on your personal life. Do you feel that way, Dick?

DD: No.

VR: That's because you're Professor Emeritus.

DD: Not personally. That's because I have 2 computers- one's at home and one's here but this sounds like a solution to that problem.

VR: Well, your work life and personal life are out of balance if you're spending too much time at the office and that leads to stress and stress leads to high blood pressure. And that can kill you.

DD: All kinds of other bad things.

VR: So you don't want high blood pressure. Did you know every time you get stressed your blood pressure goes up like, 10 or so or more.

DD: I didn't know that.

VR: Not good. Here' s a solution - you could stop working, which is not viable. Or you could use Gotomy pc. Because we all know what this stressful stuff is. You can access your office computer from home so you have more control over when and where you work. Here's an example - log onto your office computer before the kids wake up so you're not rushing to the office in the morning. Or leave work early to take the kids out and then finish your work after dinner. That's very nice.

Our listeners can try gotomy pc free for 30 days. It's a month of unlimited remote access for free. For this special offer visit go to www.Gotomypc.com/podcast, That's www.gotomypc.com/podcast to get a free 30 day trial. And we thank Citrix for their support of this week in virology. It's a good thing to support, it's educational.

DD: Absolutely.

VR: And we should say that the small amount of income we accrue from Citrix goes towards paying our costs. We're not getting wealthy, we're not making any money, we do this because we love to teach.

DD: This is true.

VR: Dickson is the ultimate teacher, penultimate. Not penultimate, the ultimate.

DD: The penned ultimate.

VR: So let's go to nucleic acid synthesis. We're going to call this RNA synthesis. And let's explain why. We need to do a little bit of background first. All life forms have DNA as their informational molecule. It's a great informational molecule because as we've said before the code of life is in triplets of nucleotides, each triplet coding for an amino acid. But of course as our cells divide, that DNA has to be duplicated and that's a process worked out by Watson and Crick many years ago. DNA or deoxyribonucleic acid is a great molecule to duplicate because it has this base equivalency, A=T , C=G which Erwin Chargaff here figured out many years ago .. that means if you have.. DNA, as we know, is a double stranded molecule And each strand is a sequence of bases and if on one strand you have ATT then the other will be TAA. We call that "complementarity". And it makes for an easy way to duplicate that molecule. So let's say you have a double stranded DNA and just before your cells divide it has to duplicate.The strands separate and each strand is then duplicated. There's an enzyme that duplicates it, it's called an DNA polymerase and it looks at the base, "Ah, it's an A, then I need to put a T to make a new strand."

DD: How does it know to do that, by the way, Vince? What's the mystery of that? That sounds like a mystery to me.

VR: Well, the polymerase, the enzyme, is sliding along this DNA, and it's been unwound by another enzyme and it has an active site which we'll talk about in the enzyme , and the base that's about to be copied sits in that active site and actually it's a hit or miss, it tries a couple of different ones and only the right one works.

DD: So the final say is by the base pair not by the enzyme?

VR: Yeah, so the base pair, the highest energy, the right pair A-T or C-G , boom, goes ahead. The other ones , the enzyme did not look good. These 4 triphosphates ATP, CTP, GTP, and TTP, the precursors of DNA, they're just floating around and they're popping in and out of the active site very, very quickly. I mean, DNA replication zips along.

DD: That's right. Faster than you can sequence it.

VR: That's right. And so it's really these things are popping in and out and the best one goes forward. So DNA is nice to duplicate but viruses, now, have either RNA or DNA genomes, if you recall.

DD: Of course.

VR: Now, many of the principles for replication are the same... So we're going to use this word replication. So I've said “duplication” of DNA so far but really we call it “replication” so that's the term I'd like you to be familiar with - we need to replicate our DNAs and viruses need to replicate their DNA or RNA. So viruses can have DNA or RNA as nucleic acids. Today we're going to focus on viruses with RNA because it's too much to do it all in one session.

DD: But you remember we also had another session where we discussed the end result of how the virus uses its genome in order to replicate and that is it all has to stream towards messenger RNA.

VR: Absolutely, and in fact we're going to see that again today. We're going to use it as a rubric because it's incredibly powerful.

DD: So, it leads me to ask the question. I intended to ask it last time we were discussing this and that is - if it's easiest for a virus to walk in with its own RNA that's already the message why haven't all viruses adopted that as their strategy? Why hasn't nature selected that for the main way viruses conduct business?

VR: Well, that's a great question. Because it all...

DD: Because the rest looks clumsy to me.

VR: Well, if you put this in Baltimore scheme with the seven types of genomes you could say well, why doesn't just one...

DD: Exactly.

VR: We don't have an answer to this but my feeling is they're all evolutionarily satisfactory. They all work. One doesn't have any advantages. So you might say, well, retroviruses, you start with an RNA where you can make lots of mistakes and have diversity which is good for evolution and then you make it into DNA which becomes a permanent part of the cell. So why doesn't that last forever? I'm sorry, why doesn't that predominate? It doesn't. So the answer must be, and it's not a satifactory answer, I know, that all the forms of genomes must work.

DD: I used to think of evolution as equivalent to a safecracker who was sitting at a safe in which there were no police. And someone was just sticking food under the door and the safecracker was sitting there at the safe just trying to open it, but given enough time of course it will, so every one of these viruses is an example of a successful safecracker and they all did it differently , you see, because the safe number was different.

VR: You're absolutely right and they all were successful or else we wouldn't be talking about them.

DD: What do they say? You put all the viral genomes together from the ocean and they stretch out 200 light years. 200 light years, Vince! That's an enormous length of space. I can't get my head around that one.

VR: That's a whole other story, that's why work on viruses.

DD: Hey, and that's why I'm totally mystified by this whole area.

VR: Now here are a couple of things we need to establish. First of all, as we've already said, cells, mammalian cells, any kind of living cell, can replicate its own DNA. It has enzymes to do that. Cells can also make RNA from DNA, as you know, messenger RNA and other kinds of RNA. They have enzymes to do that but cells cannot make RNA from RNA. At least they cannot take a long RNA that's like a viral genome and make a copy of it so the enzymes to do that are unique to viruses. So that's really something to keep in mind as we talk about this because that means that every virus that has RNA as its genetic information has to have its own enzyme to copy that RNA. That's a bit of a burden.

DD: Yup, it's an expense.

VR: Because as we'll see in a future virology 101 there are some viruses with DNA genomes that don't have to encode any enzymes because they use all of the enzymes of the cell. They can be very small and efficient but these RNA viruses can't do that. Alright, let's go over just a tiny bit of RNA history.

1935 - Tobacco mosaic virus was crystallized. Wendell Stanley made little crystals. You know you can take salt and water and saturate the solution to make crystals? You can do the same with viruses.

In 1936 it was found that in those crystals there's 5% RNA. Nobody knew what to make of that because people didn't even , at that point, understand that DNA was genetic material. That wasn't something that came about until 1944. Avery-MacLeod- Mccarty experiment showing that DNA was the genetic material ..the structure of DNA in 1953

And it wasn't until 1956 when I was a wee 3 years old that the RNA in tobacco mosaic virus was shown to be infectious. In other words, you could purify that RNA from the virus , insert it into cells and it would give rise to an infectious cycle. So it showed that RNA can be genetic material. And now of course we know this, it's obvious, but it's important to know that at some point in the past we didn't understand this.

DD: And huge controversies and lots of contentious behaviour and secretive...the things... I mean I'm sure you've read the book, "The Double Helix" - it's got lots of gossip and...

VR: “Double Helix”, I don't think we've ever picked that. That might be a great pick for today, Dick. Excellent. Who wrote that?

DD: It was written by Watson and Crick.

VR: Yeah, I did read that when I was a kid.

DD: By the way, I was 16 when you were 3. I should have known better, I should have been studying this.

VR: Were you the bully? Pushing me around?

DD: No, I wasn't the bully actually. No, I wasn't that kid. Come on, you know better than that. I was a 98 lb weakling.

VR: From Hoboken?

DD: Dumont, New Jersey.

VR: Dumont - I had a good friend in Dumont. Ok,let's now start with our Baltimore scheme again. Do you remember this Dick?

DD: I do but you're showing it clearly on the screen and I'm sure that everyone will see this once our website goes up.

VR: Seven classes of viral genomes.

DD: It looks simple when you express it like this.

VR: That's the beauty of rubrics such as this.

DD: Yes, well, once you see the pattern.

VR: Patterns are important. What do we call that - reductionism? Not necessarily - seeing patterns.

DD: And there is a pattern to all this so that's the beauty of that part of it.

VR: That's the neat thing with viruses, there's so many and people get bewildered but in fact there are patterns that you can use to really sort it out and make it quite simple.

DD: You could make a Venn diagram out of this.

VR: You could use a Venn diagram.

DD: And you could weight the Venn diagram according to the number of viruses that we know of that use each of these strategies. Has anybody ever done that?

VR: I'm not aware but that's a great idea.

DD: That gives you the overall evolutionary picture of the way life evolves, in terms of viruses at least.

VR: Are you saying viruses are living?

DD: No, but they do evolve, don't they?

VR: They do evolve, yes. Alright. Today we're going to talk about 3 of these 7 classes. Remember, mRNA is in the middle. We are going to talk about viruses with positive strand, single stranded RNA genomes like polio virus. We're going to talk about viruses with single stranded, negative sense RNA genomes, like influenza virus.

DD: Two very, very important viral groups.

VR: And finally we'll talk about viruses with double stranded RNA genomes. Also important,you know the rotaviruses - very important causes of gastroenteritis- have genomes like that. But you may see here that's theres another class of viruses with plus stranded RNA and those are the retroviruses.

DD: Those are the conundrums of virology.

VR: Those viruses go through a DNA intermediate and that's a whole different ballgame.We're going to do a whole Virology 101 on them.

DD: Excellent.

VR: Here are two rules that are extremely important when we're talking about RNA replication. First, an RNA must be copied end to end with no loss of sequence. Dick, you may say, “That's obvious, Vince, why are you telling me this?” Well, sometimes the obvious parts are what we don't consider. Got a cold Dick? You haven't got *T. spiralis*, do you?

DD: If I did I wouldn't hacking like this.

VR: I cooked my pork really well yesterday.

DD: Oh good. You had pork on Thanksgiving?

VR: No, actually, no one ate it.

DD: You should have brought it in, we would have had it for lunch.

VR: I'm going to go home and have it for lunch later. Alright, the RNA genome must be copied end to end with no loss of sequence so the enzymes have to start at the ends, not in the middle, or not near the ends , you can't lose a base, or two or three.

DD: If you do, of course, you won't succeed as a viral particle.

VR: So there's obviously evolutionary pressure to maintain an enzyme that can do that.

DD: So how does it know when it's made a complete sequence?

VR: My view is, Dick, that there's nothing else to copy.

DD: It runs out of space and there's nothing left, synthesis stops and that's the end.

VR: It runs to the end, it falls off and that's it. How does it know where to begin? Well, we'll talk about that. That's an important one too. That's more regulatory. The second issue is we have to be able to make mRNAs . So you have to duplicate the genome without loss of sequence and you have to make mRNAs. All viruses need to to do that, no exception. And now, here's just a tiny bit of history again because this is actually fascinating. It turns out that, a man named David Baltimore was among the first to discover that viruses could duplicate their RNAs. He showed it biochemically, as we say. I'm going to show you an experiment, Dick. It's a very old experiment from the early 1960's when David Baltimore was a graduate student at Rockefeller.

DD: I was there while he was a graduate student.

VR: He worked with Richard Franklin - is that the man's name? Do you remember that name? Anyway, what he did... He was working with polio virus and he would infect cells and then he would add to those cells -sorry. He would infect cells and at different times after infection he broke the cells open and made what we call an extract.

DD: What virus was he...?

VR: Polio virus. It has an RNA genome. He would break the cells open and make an extract and then he would add to that extract the 4 triphosphates ATP, GTP, CTP and TTP. And one of them was radioactively labeled so that he could trace whatever was made from them. That's how we used to do things, we used to use radioactivity to trace molecules in cells because we didn't have any other ways to do it. So he made an extract of infected cells at different times after infection and he added these triphosphates, one of which was radioactively labelled, and then he incubated the extract and said, "What's being made?". And he saw this RNA being made and he called it RNA polymerase activity. It started at about 2-3 hours after infection, and it peaked and then it went away. And at the same time infectious polio virus was being made in the cells - that's this little dotted line here and he said, "Hmmm. This means that there's RNA is being made but what's making it?" At this point, people didn't know if the cell is making it or if the virus was. In fact what they thought was that the cell was converting the viral RNA into DNA and then making more RNA.Which turned out to be wrong but he did an experiment which shed light on it. What he did was he put a drug called actinomycin D in these extracts. Do you know what that does?

DD: I do actually.

VR: It prevents prevents RNA synthesis by DNA dependent RNA polymerases of the cell.

DD: Yes, I've actually used that drug myself in some experiments that I've conducted.

VR: So this is specific for cellular enzymes and so he reasoned if this polio virus RNA is being made by the cell enzyme then this drug should inhibit it. What do you think he found? Polio virus RNA synthesis - it still went on, it was not affected by this drug - so he said , "The host's not doing it, must be a viral enzyme." That was the beginning and then he went further. He took that to discover reverse transcriptase many years later because he was thinking about viral enzymes. So this was the first evidence that some RNA viruses can have this kind of enzyme. Later it was discovered in other RNA viruses as well. Now we actually have crystal structures of these polymerases. We know in incredible detail how they work.

Here's some terminology, Dick. The "replicase"- it's what people used to say was the enzyme that copies viral RNA to produce genomes. Replicase, because it replicates the genome.

Here's another one -"Transcriptase". 'Ase' coming from enzyme , the enzyme that makes mRNA. Transcription actually is copying DNA into RNA. So for RNA viruses we don't use that word, but many people do - not correct.

And a "promoter" - it's the sequence controlling the transcription of DNA templates so "promoter" and "transcription" we don't use for RNA viruses, I'm sorry to say. But many people will.

DD: C'est la vie.

VR: So we have both plus and negative strand RNA viruses and knowing just that will get us a lot of information. Now, plus stranded viral genomes can be translated because they're positive stranded -they are the message. So viruses with that polarity genome, they don't have to carry an enzyme in the cell with them because it's encoded in the genome. So a virus like polio virus, which is positive stranded, it has the capsid and the RNA inside and that's it. When that virus infects the cell the first thing that

happens is the RNA is translated into protein and one of those proteins is the enzyme that will copy the genome.

DD: Then the host machinery does all the work. So the boss walks in and hires the help and the next thing you know, they're getting their own product out.

VR: And you may say Dick, that sounds like the simplest way, why doesn't that predominate? But the other way which is if you have a negative strand genome, like influenza virus, you have to make another strand. When that negative strand gets into the cell, and infects it, the cell has no clue what to do with this because it can't be translated, it's not message and there's no enzyme in the cell to copy that negative strand into a plus strand.

DD: Now, it's too bad that there's no host enzyme sitting there ready to destroy it because then the virus wouldn't succeed.

VR: There are and we shall talk about those in the future but the viruses have evolved to abrogate that. Otherwise they wouldn't exist. So negative strand viruses, Dick, if the negative strand virus has to be first copied, what does it carry into the cell in the virion?

DD: Well, it must have a replicase that does this.

VR: Exactly, it actually has enzymes that will copy that negative strand RNA into a plus strrands

DD: An RNA polymerase.

VR: This is a rule- if your virus is a plus stranded RNA it doesn't take anything into the cell with it. If its negative strand brings in an enzyme. Now, what about double stranded RNA, Dick?

DD: Gee, it has an option of going both ways, doesn't it?

VR: Well, it has got the plus strand and the minus strand. You would think it could be just translated.

DD: The plus might be translated or...

VR: If you thought that, though, you'd be wrong.

DD: I knew you'd say that. It was a trick question, everybody.

VR: Because double stranded RNA cannot be translated, only single stranded RNA . The cell doesn't know what to do with double stranded RNA.

DD: So it comes in with its own polymerase also.

VR: Exactly, and it makes messenger RNA.

DD: What about the RNA polymerases? Are they similar for all these other stranded viruses?

VR: Dick, I'm glad you asked that. We will get to that. They are all similar. So here's some universal rules for RNA synthesis- it begins and ends at specific places, that makes sense. Here's an interesting one...Maybe this is a little complicated but I'm going to throw it out there. RNA polymerases, the enzymes that copy RNA, can initiate the synthesis with or without a primer. Now, Dick, you know DNA

synthesis needs a primer. You need a little molecule to get the enzyme going. It's actually a small piece of nucleic acid that sits down on the DNA and the enzyme uses it to get going.

DD: It's like starting your zipper.

VR: It's like starting your zipper and it tells the enzyme where to start, basically. So RNA polymerases can work with or without those. Some need primers and some don't, as we will see. And then you make nucleic acid, in this case RNA, from 5'-3' so nucleic acids have a polarity , not just plus or minus, but a direction, 5' and 3' ends. It's a chemical issue, probably more than we can get into here. But these nucleic acids ares always synthesized from 5'-3' and the template is always copied in the 3'-5' direction. So it's read from 3'-5' and it's made, the new material is made from a 5'-3' direction. Now your question is excellent. There are 4 different kinds of nucleic acid polymerases : there's DNA dependent DNA polymerase, there's DNA dependent RNA polymerase, there's RNA dependent RNA polymerase which is the subject of this TWiV, and then there's RNA dependent DNA polymerase, reverse transcriptase

DD: Sounds like a Punnett square.

VR: What is a Punnett square?

DD: Its a way to express the genetic possibilities of 2 different organisms that cross.

VR: You're right, I knew that.

DD: I knew you knew that.

VR: So these four polymerases are all related. They have sequence homologies in their amino acid sequences. Their crystal structures have all been determined and they're all looking like a right hand. So the analogy is they're a right hand because they have what's called a palm domain which is where all the activity occurs. And they have thumb, a distinct thumb.

DD: The palm is the active site?

VR: The palm is the active site. And they have thumb and finger domains which do various other things.

DD: Physical structure...So that lends the question then if they have a similar active site and there's sequence homologies between them, then certain drugs might affect a whole group of viruses, if it could act at the level.

VR: Yes, but it turns out not to be the case because they're sufficiently different that each one needs its own drug.

DD: But there are drugs that do interact at that level?

VR: There are drugs that inhibit polymerases, absolutely there are. Now of course if your virus uses the host polymerase then you can't inhibit...

DD: No, but I'm talking about RNA viruses.

VR: RNA viruses - in theory, that should work, that's a very good point. Because these are unique enzymes to the virus then you should be able to be able to inhibit them with drugs . And there are... people have come up with inhibitors. For the retroviruses there are many inhibitors of the reverse

transcriptase. Those are beautiful antivirals- they're the nucleoside and the non-nucleoside reverse transcriptase inhibitors. But for RNA viruses there are just aren't that many. I believe there's one in development for influenza virus at the moment. I believe there's one in development for hepatitis C virus but that's it. There's nothing licenced for any... no RNA polymerase inhibitors licenced for any virus, RNA virus, except the retroviruses.

DD: Seems like a good target.

VR: It is a great target. Part of the problem Dick, of course, is that for most of these RNA virus infections you can't diagnose them until it's too late. The flu is an exception because now we have rapid diagnostic kits even though they don't have very good accuracy. But for common cold for other viruses, it's difficult when they're over so quickly. But anyway, that's a great target.

So let's go through just a couple of these different configurations and talk about how the genome replicates. Let's start with viruses with a plus stranded genome. We're going to look first at the flavi- and picornaviruses- two viruses close to us. The flaviviruses being yellow fever type, West Nile, and the dengue group and picornaviruses being polio and rhinoviruses. These are very simple.

DD: Those poor rhinos, I keep thinking about them every time every time I hear this.

VR: Why are they poor?

DD: Rhinos. They've got their own viruses. Now that's just a bad joke everybody.

VR: Dick, what does rhino mean?

DD: I guess "nose".

VR: Why do they call rhinoceros...?

DD: Because it has a horn on its nose, but that doesn't figure. Take away its horn and it's not a nosy-like animal. It's not like one of those proboscis monkeys you can see with a giant nose. Whatever.

VR: They have a plus stranded genome- it's one piece of RNA. And all it has to do is...remember, when it gets in the cell, because it's a plus strand, it's translated first.

DD: Right off.

VR: Right off. And among the proteins that are made is the RNA dependent RNA polymerase.

DD: Among the proteins that are made -it's not just a single protein.

VR: It makes about a dozen...

DD: What about TMV, the simplest of all of them?

VR: TMV does the same thing, it encodes its own polymerase, gets into the cell, it's translated...

DD: How many proteins?

VR: I don't know the number, Dick. We could look it up. Polio makes a dozen proteins. And one of those is the polymerase which will eventually make more RNA but the first thing that happens is translation

and the genome is copied. Now Dick, if you have a plus stranded RNA and you have to make a complete copy of it, what's the polarity of that copy?

DD: I guess it goes from the 5' to the 3'.

VR: What's the polarity though?

DD: Negative.

VR: So you make a negative copy of the whole thing, which is nothing but a copy. It doesn't do anything - it's not made into protein, it doesn't go into virions for these viruses. It's just a template. It's interesting that you have to do this but that's the cost.

DD: I love to watch these home building shows and they make these jigs to make a certain angle cut and Norm is out there with his circular saw and you throw the jig away when you finish.

VR: Same thing. The negative strand is of no use as far as we know. Who knows for what we know... But you're right, this is a jig and that's all it's for. And then you take that and you copy it and you make a plus strand. And then you can put those into viruses, you can translate it...

DD: Now you're off and running.

VR: And it's very simple. Now there are other plus stranded RNA viruses that work a little bit differently. Like alphaviruses. Do you know what they are? They're part of this Togavirus family which are generally insect-borne viruses, these are Western equine encephalitis virus, Eastern equine encephalitis, Venezuelen equine encephalitis. Chikungunya is an alphavirus. Very similar structures but different enough that they're placed in a different group. This is actually a group of alphaviruses. They have a plus stranded RNA genome which can be translated as soon as it gets into a cell and that's made more of by going through a negative strand complement, just like the picornaviruses. And then the negative strand is used to make more genomes.

DD: So I sense a stoichiometry to all of this. As the viral particle attaches, decoats, enters, the viral replication process begins..In the beginning you get a lot of template made and then you start to get a lot of positive stranded RNA molecules made and along with that the proteins that are going to self-assemble at the end of this thing to let the virus out. So when David Baltimore conducted his original research he was dealing with huge numbers of populations of both positive and negative stranded RNA as the virus starts its replication cycle. So how did he sort those two things out?

VR: That's a good question. First, the interesting thing is that, in those viruses, you make much more plus strand RNA in cells than minus strands.

DD: So you don't need a lot of template to make more plus...

VR: So that minus strand is a jig, so you don't want to make more than you need.

DD: That's the point then, how does it know how much is enough?

VR: This is a good question. How does it regulate how much of minus...

DD: Because it can't regulate anything - the host has got the machinery...

VR: That's the question that is extant. One of the many questions...

DD: It's a mystery.

VR: It's a mystery. And if you look in my textbook there's a section on how do we regulate plus versus minus and we really don't know. One of the obvious answers is that...There's some sequence at the end of the genome that regulates copying by this enzyme and one of the ideas is that in the plus strand it's not very efficient to make minus strands but in the minus strand it's very efficient so it's sort of like it's easier to make a lot of plus from minus than minus from plus but it's a simplistic answer and we don't actually know how it works. For some other viruses it's been looked at, but not for these.

So those are two families that make RNAs. They don't have an enzyme in the particle but they're translated and then some of the proteins made go on to replicate the genomes. And I'm just showing you a picture here Dick of the picornavirus genome to show it's a big RNA and it's made into a bunch of proteins.

Now one interesting...

DD: No introns, right?

VR: No introns, no intervening sequences.

DD: Viruses don't have those, do they?

VR: Some DNA viruses do.

DD: Do they really? So where did they get those from? That's another question, of course. But we always assume that ours came from incomplete viral replications (inaudible) or retroviruses or stuff ...all of our introns...

VR: I don't know.

DD: And there are viruses that have introns? Viruses have viruses?

VR: Actually, some RNA viruses...Influenza virus does do splicing - doesn't actually have an intron but it does splice.

DD: This raises several other spurious questions that have nothing to do with this presentation but it might. So you have two plus stranded RNA viruses, different, and you throw an equal amount into the cell mix and they both get taken up by the same cell, now their RNA polymerases start to compete with each other. Which one wins?

VR: Wow, that's a good question. It depends on the virus because viruses do other things to cells to...

DD: I mean polio and influenza are found side by side in many places throughout the world.

VR: That's a very good question.

DD: And here you've got both of them competing for the same host machinery. Which one overrides the system?

VR: It really depends because polio can do a lot of things to the cell which ends up blocking other viruses.

DD: What I'm driving at is that maybe there's a virus sitting out there someplace that actually shuts off the invader virus and maybe you can make use of that to actually prevent...

VR: In fact there are...

DD: Like viral therapy to cure viruses...

VR: For some retroviruses...For example, when one retrovirus gets in, it then does things to cell to prevent other retroviruses from infecting because it wants the sole use of the cell to itself and people study this for the reason you point out, so we can learn how to interfere with multiplication.

DD: Maybe an incomplete virus might be perfect for this situation.

VR: Absolutely, and we'll probably get to that because we're going to be doing this in perpetuity.

DD: In perpetuity. That means for as long as we can speak.

VR: Now one thing that most people will probably not be aware of that is that when RNA viruses make RNA using these viral enzymes, RNA dependent RNA polymerases, they don't just do it floating around in the cytoplasm.

DD: Cytoplasm doesn't look like that anyway, Vince.

VR: Yeah, I know you're this big proponent of a highly structured cytoplasm.

DD: It's an ecosystem of molecules.

VR: It is. So these things aren't just floating around but they're on very specific structures.

DD: Clusters...

VR: And these are actually membrane vesicles. So, for polio, what happens is when the virus infects the cells it totally messes up the membrane system of the cell and it makes all these little vesicles...

DD: We would call these “microsomes” in another world.

VR: In another world, you could call them but they're distinctly different and it's on the surface of those vesicles that the viral RNA polymerase is duplicating the viral RNAs.

DD: How does it do that?

VR: What part of it?

DD: How does it create all those tiny vesicles out of, what is it coming from - the endoplasmic reticulum?

VR: For polio this is a bit contentious. The origin of these vesicles...in the cell you have the transport of the vesicles from the ER to the Golgi apparatus and then to the cell surface.

DD: And lysosomes, don't forget those.

VR: And some people believe that the vesicles originate from that pathway.

DD: What does the membrane look like?

VR: It's actually a double membrane vesicle which more resembles autophagosomes. I don't know if you know what those are.

DD: Of course I do.

VR: So when cells are stressed they form these double membraned autophagosomes which basically are to try to digest the cell and recycle contents so they can be used by another cell. It's sort of a defense mechanism. And the idea is that..and I'm more convinced that this is the origin of these vesicles. My good friend, Carla Kierkegaard is a proponent of this idea that they originate...

DD: Any relationship to the other Kierkegaard?

VR: No, not that I know of , but maybe actually...

DD: That's an unusual name.

VR: Yes, actually, I've asked her that but I've forgotten the answer.

DD: She gave you some philosophical answer. Some existential...

VR: These originate from autophagosomes. So polio infects the cell, it stimulates the formation of autophagosomes and then the virus uses them to replicate its genome on the surface. It's brilliant ...

DD: Now, at the same time, can host synthesis of normal proteins go on?

VR: Well, polio, in fact, and all the picoRNAs shuts that off.

DD: And this might be the way.

VR: No, it's different, different from this. We'll have to get to that in another session. But all RNA viruses modify the membranes in the cell to some extent to do this. They don't always use autophagosomes, they use other membranes. In fact, for plant viruses there's a very nice study where they've shown that you can make the RNA polymerase go to a different kind of vesicle and it will still work. So, apparently it's not the particular composition of the vesicle, but it's just that it's a membrane surface.

DD: We used to refer to this as a virus factory. Is that still a term that you could still use?

VR: You could still use it because you see there are lots of virus particles that are made here. Now this will be a little obscure to many readers...

DD: And listeners...

VR: And listeners...

DD: Hopefully, listeners that also read...

VR: The idea is that, basically, in a cell the RNA synthesis that viruses undertake doesn't happen randomly. It happens on the surface of very, very small vesicles and we'll put a picture of this on the show

notes so you can see what we're talking about. You may say, "Who cares where it happens?" Well, if you really understand the mechanisms, the fundamentals, how it works, then you can always think about interfering .That's what we're always working towards with viruses. The best knowledge comes from knowing everything and then you can design better drugs. So let's move on to another kind of virus now. We've gone through the plus strand viruses. These alphaviruses are a bit different but I don't want to get into it because we want to move on to the negative strand viruses now. This includes two kinds - there are negative strand RNA viruses where the RNA is one molecule like rabies virus and then there are ones with segmented RNAs like influenza virus. Influenza has a segmented RNA genome. But it's always the same, the genome is negative stranded RNA, it can not be translated. It's got to be copied into mRNA and that's done by a viral enzyme that the virus brings into the cell with it. Always.

DD: So it's part of the viral capsid?

VR: It's inside the viral capsid.

DD: Inside the viral capsid.

VR: So rabies vesicular stomatitis virus... within the capsid there's a viral enzyme associated with the RNA. Influenza, within that influenza particle that's infecting you, not only is there a segmented RNA genome but there is RNA polymerase in each virus particle. Otherwise, it wouldn't work. And that RNA polymerase, as soon as the RNA gets into the cell , begins churning out RNAs.

Now in these two viruses, the mRNAs are slightly different from the full length copy of the minus strand. Now that's a bit hard, I know. But, let's just look at rabies virus or measles, or these viruses with negative strand genomes .You have a long - strand RNA . So what could happen is this negative strand could be copied to plus strand...

DD: So that's a 3'-5' rather than a 5'-3' as we were just talking about before. Because this is the opposite strand. It's the mirror image, so to speak.

VR: Now this could be copied into a full length plus strand and then it could be made into a long protein like polio which could be chopped up. But it doesn't work like that with these viruses. These viruses make one RNA for each protein. Rabies has five different proteins.

DD: This is more host-cell like...

VR: This is more host cell like and it makes individual messenger RNAs for each of them.

DD: So it doesn't have to worry about chopping it up afterwards.

VR: It doesn't have to worry about chopping it up. So it takes that negative strand RNA that comes in the cell with its enzyme , the enzyme makes little mRNAs from each one. It just starts at one end , and it goes ,boom, down the RNA and it makes one mRNA, then it stops, then it starts and makes another and it makes another all the way down the line. And each of those mRNAs make proteins. And uses proteins to make new enzymes...

DD: It's like looking at a simplified host cell.

VR: It is sort of. This is much more host-cell like because these messenger RNAs are very much like those that occur in a cell.

DD: Can you get all this to work in cell-free extract?

VR: Yes, you can. That's how a lot of understanding of this has been done. It can be done in cell-free extracts. If you take purified vesicular stomatitis or influenza virions and you put them in vitro with some triphosphates and a little magnesium, it will make RNA. Absolutely. So Dick, you see, we make all these messenger RNAs , they're not complete copies of the negative strand. But what's one of the rules that we have to do when we're replicating RNA?

DD: You've got to do it end to end.

VR: You have to do it end to end. Do you think that the virus then tacks all these mRNAs together to make a full length plus?

DD: Of course not.

VR: There's another set of reactions where this negative strand is copied into a full length plus strand. And that is actually another sort of jig, to use your nice analogy, because that's never used for anything except making more minus strands. So for these viruses, the negative strand is the important thing, it's what is in the virus. The mRNAs are important because that's from which proteins are made but the full length plus strand is a jig. The virus just uses it to make more minus strands.

DD: I need to ask you an ecological question because you've raised the issue of rabies. So, rabies is a normal viral inhabitant in bats. Now, not all species of bats have it, but a lot of them do. In their cells this process is going on all the time without killing the bat. Now does this replicate in bat nervous tissue or does it replicate somewhere else? Because in us, it's a nervous tissue...it's a tropic virus for our nervous system.

VR: Dick, I'm afraid to say I don't know the answer. I would guess that's it's not replicating in bat nervous tissue otherwise they would have issues.

DD: This is correct. So the point that I'm trying to raise here is that in one species a virus is neurotropic in another species, the same virus, in another host species, is not neurotropic. Then if we could discover what the difference was between our two biologies and some way develop a scheme to direct the virus somewhere else besides our nervous tissue...

VR: Absolutely.

DD: Then rabies would not be a lethal...

VR: The whole issue of where viruses grow in different hosts is incredibly important for that reason. Because if there's a difference from one host to another and you can figure out why then that gives you more intervention options.

DD: All this is anthropocentric, everything we're discussing here is from the perspective of humans. So rabies is like 80% fatal in humans.

VR: Most of it is driven by wanting to make us healthier.

DD: That's right, but in nature this is not a lethal virus.

VR: For bats it's not often lethal although some bats do get sick.

DD: Those are the wrong bats.(laugh) It's true.

VR: It's like people are the wrong hosts.

DD: That's exactly right. We're accidental. So how does the normal host control the pathology of this infection?

VR: I don't know the answer. Dick, I think rabies in bats is not a greatly studied...It's probably not easy to keep bats in the lab to do these infections.

DD: Actually, that's not so hard.

VR: People have colonies of bats in the laboratory? It's very hard to study rabies in the lab because it's extremely dangerous.

DD: And also there's a pneumonic version of it that's not so good.

VR: So, I'm not aware of anybody doing that but I know there are some listeners who work on rabies so you could let us know. We do actually have a fellow at University of Maryland who's going to join us in the future and he's looking at what kinds of viruses are in bats in the wild. It's an interesting question why it kills some animals and not others. We just don't know the answers.

DD: I mean, it doesn't want to.

VR: It doesn't want to, of course.

DD: Evolutionarily speaking, I mean.

VR: In general, I think it's reasonable to assume that it doesn't want to eliminate all of its hosts so it reaches some balance which ,sort of, is parasitic. Ok, let's do two more viruses and then we'll wrap this up.

DD: Vince, I must interrupt you for one moment and tell you that if you think I'm a good teacher, I think you're a wonderful teacher and this is the way to learn it. We're doing this one-on-one, actually I'm looking at the same visuals that Vince is looking at as we speak so I'm able to ask questions based on what goes up on the screen. When Vince gives his lectures to the medical students and I've sat in on many of his presentations and they're very very straight forward , extremely easy to understand and yet they include all these very very complex systems that he wants the students to learn. But they don't get this opportunity to interrupt at the moment and say, "You know Vince, what about this over here?" or, "What does this mean in terms of...?" So we're really looking at this as this wonderful opportunity to explore how a lecture is broken down on a specific topic in a way that makes it more interactive. So if I'm not asking the right questions out there people, write in and tell me to ask more stupid questions.

VR: Now this is why the podcast form is good for this...

DD: I love it.

VR: And I don't want to do it by myself, I wanted you to be here because I hear you ask the questions. I'm trying to determine how to incorporate this into teaching because I agree with you that getting up in front of the class isn't the most ideal way to do this.

DD: Of course it isn't. It's very passive.

VR: So, as you know, I'm teaching a new virology course next semester and I would really like to be able to use...Maybe I could do a live webcast and have my students log in and ask questions and interact while I'm doing it. Anyway, what I'm doing here today Dick is I'm recording this screen and it's going to be put up on the web as part of this.

So influenza virus has negative stranded RNAs - but it has eight of them. But really what we just talked happens for each of these eight segments. They're negative strands. As soon as they get into the cell the viral enzyme, the RNA polymerase that comes with them , makes an mRNA and the mRNA is not a complete copy of that negative strand. It's short at one end. So the virus has to make a plus stranded full length copy, a jig, if you will. I love that analogy, that's beautiful because it's really not used for anything except as an intermediate. And from that full length plus strand, it makes more minus strands. So to resummarize, the virus RNA is minus stranded. It comes in the cell, it makes an mRNA which is plus stranded, it can be made into proteins. It's not a complete copy of the genome so then the virus has to also take the negative strand and make a full length plus strand. That's why I made this point early on that you have to make a complete copy of the genome because...

DD: Because otherwise you can't replicate yourself the next time.

VR: And there are some viruses that make mRNAs that are not complete copies.

DD: So how does it divide the labour up between let's make a lot of proteins, let's make a lot of viral genome because that has to self-assemble at the end, doesn't it?

VR: It does. It has to regulate all of this, it has to regulate how much messenger RNA, how much of each messenger RNA is made, how much full length plus strand RNA...

DD: Do you get a lot of incomplete viral synthesis because the regulation process isn't exact?

VR: That's a very good question. We do get defective particles made for many of these viruses but we don't really understand why and it may be that the whole process...

DD: It says, "Screw it, I don't have to regulate this, I'll just make enough to get into the next cell."

VR: Here, look, we're on Lecture 3 or 4 of virology 101 and we still have a dozen more to go just to get through the replicative cycle. So anytime there's a screw up that's it. If you screw up at an early stage nothing else happens. So that's probably why you make defective particles because you have to be perfect at every step. And as you know, Dick, in life, or in viruses, nothing is perfect.

DD: So this virus doesn't care about its waste dump.

VR: It seems not. It's not ecologically sound, Dick.

DD: No, it's just a brute strength approach. You walk in, you make your virus, you get out and you leave all this destruction behind.

VR: Dick, is nature ecologically sound?

DD: It is actually.

VR: Why?

DD: Why? Because it's economic.

VR: I don't think it is but we can talk about it another time.

DD: There's no waste, there's no such thing as waste, it's all recycled. Nothing is recycled here. This is a brute strength approach.

VR: The plus strand that's the jig for these viruses, it may be degraded and reused - who knows. Nobody has really looked into that. It could be Dick, I'll give you that. Alright, let's do the last class of viruses because this is quite illustrative and that is the double stranded RNA viruses. As I said before these viruses have a double stranded RNA in the particle and that can't be translated in the cell. It's only single stranded RNA that can be translated. So these viruses have to bring in with them an enzyme that will make messenger RNA that can be copied into protein and then they also have to duplicate their genomes to make more. So they take the plus strand and they make a negative stranded copy of it and again, this is both by viral enzymes.

DD: Does this have a similar viral factory to the polio virus story?

VR: It does. We know less about it but yes, there are factories that in which viruses are made.

DD: It requires microsomal vesiclesand stuff like that?

VR: It requires vesicles, yes.

DD: So this is not an intranuclear virus?

VR: I'm glad you asked that. All of these viruses are cytoplasmic with the exception of influenza virus. Now, influenza virus is very unusual in that it replicates its RNA in the nucleus of the cell. Do you want to go through why it does that? We have a few slides where we can explain that.

DD: Of course.

VR: Influenza virus RNA synthesis is inhibited by a drug that comes from a deadly mushroom - Amanita. The drug is called alpha-amanitin. Alpha-amanitin is an inhibitor of cellular DNA dependent RNA polymerase. And this is an enzyme that has nothing to do with influenza virus RNA so why should it inhibit influenza virus RNA synthesis? Well, it turns out that to make influenza virus messenger RNAs the viral enzyme needs a primer and that primer is derived from host cell messenger RNAs. It’s made by pol II – DNA dependent RNA polymerase which is inhibited by alpha-amanitin.

DD: Also known as the "Angel of Death".

VR: So the virus needs those mRNAs and hence it's susceptible to this drug. But Dick, it's not as simple as that. There's another family of viruses called bunyaviruses.Those viruses also require priming of their RNAs with host cell mRNAs but they are not inhibited by alpha-amanitin.

DD: They have escaped the control mechanism.

VR: And the reason is that those viruses replicate in the cytoplasm, not the nucleus. They use old pieces of mRNA, they don't have to be freshly made in the nucleus as does influenza virus RNA.

DD: They use a salvage pathway so to speak...

VR: It uses old pieces, where flu needs brand new pieces of mRNAs that are made in the nucleus. The other thing, Dick, is that flu RNA synthesis requires splicing and that may be the real reason...

DD: Because of these eight pieces...

VR: Well, It's just a way of getting two proteins from a single messenger RNA and so it has to occur in the nucleus probably because of that and it's one of the few RNA viruses that replicates in the nucleus. All the others are in the cytoplasm. I think that's enough of RNA synthesis. Shall we summarize?

RNA viruses have to replicate their genomes like everything else. They have to use enzymes that they encode because cells don't know how to copy virus RNA. If the virus is a plus stranded RNA , then it doesn't have to carry an enzyme into the cell because the plus strand can be translated. But if the virus has a negative strand or a double stranded RNA then it has to bring into the cell an enzyme to make the RNA that the cell can recognize. Many viruses make mRNAs that are not complete copies of the genome so they have to make intermediates or jigs, as Dick called them, to serve as templates. Any questions Dick?

DD: Tons of them, but I think I've asked a lot already.

VR: Can I read a few emails?

DD: Absolutely.

VR: I have an email from Ricardo who writes, " Hello Vincent." He sent a link about H1N1 and Guillain-Barre syndrome which we'll post. "It is impressive the amount of hate emails there are on H1N1 vaccines. In Portugal there seems to be no issues about vaccination, at least until now. With all this hate mail people are starting to ask if they should take the vaccines or not. I just hope it doesn't spread to other vaccines. I've heard from a few health-related workers that they won't take the vaccines because they have doubts about their safety. The worst thing is that most of the time the reason for that is just an email they have received. So this is the problem with the internet, it empowers you to learn more but you also get scared because there's a lot of misinformation out there." From Ricardo.

Now this one is from Eric...

"Dear Vince and Dick, First, allow me to thank you for putting together this wonderful program. I'm a medical student at Eastern Virginia medical school and have enjoyed listening to the episodes on evenings when I finally put down my textbooks. Some think it's strange but TWiV is one of the places I turn when I can't study anymore. I've also enjoyed reading "The Coming Plague" as one of my pleasure books. I've long held an interest in virology and infectious diseases and after I graduate I would love to be working in the field. It is my belief that I will be more effective as an infectious disease physician if I am involved with the tracking and study of emergent and infectious diseases. I would be in a position to limit their spread by assisting with information-gathering and epidemiology as well as assisting public health departments. Unfortunately, the daily routine of most infectious disease physicians seems to be filled with lurching from patient to patient in order to manage cases of HIV, hepatitis and antibiotic- resistant organisms. While this is an important role I feel that I could do more by involving myself in the stages prior to the contraction of the infections. I was hoping you two might have some advice on how to bridge the gap between clinical medicine, public health and virology research."

DD: Sure, get a job with CDC. Go for EIS officer. You could do that or you could join some NGO that has health outreach into various countries. You could do volunteer work for Doctors without Borders or you could get connected with WHO go for an MPH degree which gives you entree in to that world. Then donate part of your time to travelling, get involved with some public health programs in places like India or China or Southeast Asia, to name just a few. Of course, in Africa as well. You'll get big overviews of how infectious diseases affect huge numbers of people and that would be great experience for you to gather earlier rather than later on in your experience. Probably after you finish your residency.

VR: Good advice. I also asked Scott Hammer about this. He's a physician in infection disease. He's chief of ID here, and he's done a lot with HIV clinical trials. And he wrote, "Thanks for forwarding this comment. In response I'd inform your interested listener that the career opportunities in infectious disease are broad and include fundamental research, clinical translational research , clinician-teacher role at an academic centre, epidemiology and public health, both domestic and international, private practice and industry. A substantial proportion of infectious disease fellows are pursuing master's degrees in epidemiology at their co-located schools of public health so that they have formal training in both ID and epi, biostats, study design, global health, etc. For more information I'd refer him to the following websites: The Infectious Diseases Society of America, the American Society for Microbiology, and the Association of Schools of Public Health. In addition, he could surf the websites of some of the individual fellowship programs, Columbia partners, MJH and Brigham in Boston, Johns Hopkins , the University of Washington in Seattle and the University of California in San Francisco." Thanks, Scott. We'll put some of those links in the show notes.

DD: There's one other society that I would mention here and that's the American Society for Tropical Medicine and Hygiene. It has a huge outreach component and embraces not only parasitic diseases of a eukaryotic nature but also covers a lot of these viral infections as well.

VR: Bill wrote, "TWiV team, Finally I'm off the reovirus topic. My daughter experienced flu-like symptoms, along with a meaningful percentage of her sorority at U Michigan, high fever (102), body aches, fatigue, cough. My question is this : Given the need to ration the H1N1 vaccine, is it possible to quickly and inexpensively ascertain whether or not she actually had H1N1, after the fact? How hard are antibodies to assay, what is the process and has someone manufactured a test? Altruistically, I would like her vaccine to go to someone who actually needs it."

VR: There are no commercial kits at the moment for assaying antibodies to H1N1 or any influenza virus. This can be done in the laboratory quite readily but no one has made a test. We look for either virus or viral genomes by PCR. A number of people have asked me this: If I've already been infected couldn't I go to the doctor and before I get a vaccine, get an antibody test? There is no test, I don't know of any being developed and my feeling is it adds another layer of complexity. It's easier just to give the vaccine rather than to check first and see if you need it. I don't know of any situation where you do that with a vaccine. What do you think about that?

DD: Sorry, I don't know either but a thought occurred to me as the question was being asked and that is- it would be a huge advantage if you could have a recurrent population of known viral origin to serve as a bank for immune serum for patients who enter into a lethal phase of this infection and they're desperate for attempts to try to cure it. Tamiflu doesn't work at that level but maybe immunoglobulin concentrated from these people might.

VR: Yes, I think for the more lethal diseases for which there aren't vaccines that certainly is an option. But, you know Dick, vacccine programs don't involve screening and then...You just do it because that's

the simplest way. Especially if you get into areas of the world that don't have a good health infrastructure, it's easier just to vaccinate. So, Bill there isn't any commercial assay.

DD: But your family doctor maybe willing to send the serum down to the CDC for you.

VR: Ok, one more. " Dear Professor Vincent: Today one of the students at our department came and asked me about how viruses are able to establish an infection and that their RNA is able to evade foreign genetic material detection mechanisms inside the cells. I answered her question with, "There's some sort of balance between infection and immune reactions and that same question could be addressed saying , "How can a body get infected with viruses in the presence of immunity?" The answer is that the outcome is determined by which side, the virus or immune system, wins. God gave viruses mechanisms by which they can evade immune systems in different ways and I gave her some examples, e.g. HIV that infects immune cells. Am I right?"

Wow, this is a topic of another TWiV but there are intracellular detection mechanisms for finding viral RNA. The innate sensors.

DD: Interferon plays a role in this which is why it's called interferon.

VR: For every mechanism of the cell there's a viral counter-mechanism. Otherwise, viruses would not exist. We have a great immune defence system. It has three components. There's an initial intrinsic component, an innate component and there's an adaptive component which we'll talk about in a future episode. And they're beautiful and viruses can antagonize every one of them, otherwise they wouldn't exist and that's the answer.

Ok, Dick. You've already given us a pick of the week - what was that?

DD: It was "The Double Helix".

VR: By Watson and...I don't know if we picked that, did we?

DD: I don't think so. We can go back and look, of course.

VR: Yeah, let's go to TWiV. There's some website called TWiV.tv. There's our weekly picks. Let's search for "helix" - not found. Ok.

DD: How could we have omitted that?

VR: But it's relevant for today because we talked about nucleic acid replication. My pick is a blog called, "Worms and Germs" blog which I found last week. We did a story...you were at Johns Hopkins last week, talking about vertical farms...

DD: This is true but I encountered a TWiV listener while I was there, which was great.

VR: Yeah, I heard. Anyway, last week we talked about a case where they're immunizing wild animals against rabies by putting baits that have vaccinia virus vectors with rabies proteins. A woman was picking blackberries and her dog got one of these and bit it and the virus came out and she picked it up and it got into her lacerations so she got a little vaccinia. Anyway, I found this blog while I was researching that. It's called "Worms and germs blog - promoting safe pet ownership". It's a joint venture of the Ontario Veterinary College's Centre for Public Health and Zoonoses and the City of Hamilton Public Health Department. Anyway, it's good. It has a whole list of diseases, including parasites...

DD: Well, when they say “worms”, they’re all parasites.

VR: Giardia in dog parks...

DD: They should be more worried about some other things though.

VR: Raccoon latrines in Chicago...Raccoon roundworm...

DD: Baylisascaris...

VR: Can’t wait for the next TWiP. So, that's my pick of the week. That should do it for another TWiV. If you're a new listener , please subscribe in iTunes. It’s free and you get every episode automatically. If you don’t , go to .tv and you can download the episode. You can play them and you can see all our show notes. Now today I've done something different, it’s called a screen recording of this episode because I’ve shown a lot of slides to Dick and I’m going to try to post that. So even if you get the audo you might want to check that TWiV.tv for that.

DD: Are there videos in which the viral replication cycle is depicted as a video?

VR: Yes, there are some good ones.

DD: Maybe we should try to put something like that up too to give a visual explanation for...

VR: There’s actually a good one for both HIV and influenza. Would you like me to link to that?

DD: I would love you to because we are visual animals.

VR: I wish we could make them readily but unfortunately we’re not artists. TWiV is part of Microbe world.org. It's a community created by the American Society for Microbiology to help disseminate information about microbes. And you can also find us at Sciencepodcasters.org and at Promed network.com. Of course Dick and I have launched our new podcast– “This Week in Parasitism”. It’s at Microbeworld/TWiP and Episode # 2 will be posted on Monday, November 30, 2009 so check that out. It’s also on on iTunes and that's a lot of fun because I get to ask the questions and I’m really learning from that as well.

DD: Well, this is a mutually beneficial association, Vince. Symbiotic. Well, it can’t be symbiotic because we’re the same species. Synergistic. Symbiosis is a concept that involves two different species and we’ve often misused that word but what we are is synergistic. We could give lectures on these individual subjects but commonalities between any parasite and its host are brought out and then you get the complete spectrum when the two people...We should have a third person here for fungi and then another one for Rickettsia and another one for bacteria and then we could have this wonderful conversation of how microbes across the spectrum of parasitism behave in their hosts, which is the ultimate goal of this podcast.

VR: I think that you and I have grown in this role.

DD: Had a lot of fun.

VR: And that’s why we started the second podcast because we realized we could extend our teaching and teach me something.

DD: That's why we're here the day after Thanksgiving.

VR: You see Dick, I didn't want anyone to miss a podcast and I also enjoyed doing it. It's nice when we have a group of people like Rich Condit and Alan Dove because you get a lot of different viewpoints.

DD: By the way, happy Thanksgiving to both of them.

VR: Happy Thanksgiving to all our listeners, if you celebrate Thanksgiving. I also enjoyed one on one with you because it's a different kind of interaction. So that's why we're here, and we hope you enjoyed this. And of course, as always, send us your questions and comments. TWiV@twiv.tv You can send us an mp3 file or you can call us up on Skype. Our name is twivpodcast and leave us a message or you can go over to MicrobeWorld.org/TWiV. You also can leave comments there and you can also post stories that you'd like us to talk about. Dick, thanks for coming in and doing this, I appreciate it.

DD: Pleasure.

VR: You want to talk about medicalecology.org or Trichinella.org ?

DD: We'll do Trichinella.org

VR: You should check that out and also check out TWiP. You've been listening to "This Week in Virology" - TWiV- the podcast that's all about viruses which are a kind of parasite themselves. Thanks for listening everbody. We'll be back next week. Another TWiV is viral.