

This Week in Virology

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

Episode 49: Viral genomes

September 9th, 2009

<http://www.twiv.tv/2009/09/13/twiv-49-viral-genomes/>

Vincent Racaniello:

Hey everybody, it's Vincent Racaniello, back with Dick Despommier!

Dick Despommier:

Hey Vince!

Vincent:

To tell you more about viruses, more Virology 101. And you know Dick, it was about a year ago that we started this.

Dick:

Right, seems like yesterday!

Vincent:

And here it is, just you and I again, which is the way it was a year ago.

Dick:

Right.

Vincent:

We've changed a lot, we've done a lot of things, but the goal remains the same, right?

Dick:

True. Absolutely.

Vincent:

To teach people about viruses.

Dick:

To inform.

Vincent:

And I still have a great deal of fun, and what I was just thinking of not too long ago, how easy it is to get material for this show!

Dick:

Hah hah hah, it's true!

Vincent:

I mean it just takes a brief scan of either the news, or...

Dick:

Yep.

Vincent:

... as with today, we want to inform people about fundamental issues, it's quite easy. So I guess as scientists...

Dick:

That's because you're a Virologist, Vince! [laughing]

Vincent:

You are a scientist.

Dick:

Yeah, I'm a scientist.

Vincent:

And that's what we do!

Dick:

That's correct.

Vincent:

We ask and answer questions.

Dick:

We do that.

Vincent:

So this is a perfect forum for us.

Dick:

Yep!

Vincent:

So I hope we continue to grow in the next year.

Dick:

Sure!

Vincent:

There'll be lots of interesting things to talk about, I'm sure. Before we get into today's main topic, I want to point out to everyone that Dick was on Radiolab.

Dick:

Yeah.

Vincent:

Which is a podcast put out by WNYC, right?

Dick:

Correct.

Vincent:

And he was on an episode called "Parasites".

Dick:

Yup!

Vincent:

And we'll put a link for that in the show notes. He's actually in the second segment, it's in three or four segments I think. And his is best, he talks about hookworms.

Dick:

[chuckling]

Vincent:

It's a great story about how conquering the hookworm helped raise the south...

Dick:

Yep!

Vincent:

... that's a fabulous story!

Dick:

Yeah! And it's a true story, those are the best stories, that are true.

Vincent:

Well I didn't think you were lying to those guys.

Dick:

No, but I wasn't exaggerating either.

Vincent:

It's a great story, I'm not going to tell you anything, because you need to go listen to it.

Dick:

Ha ha ha!

Vincent:

Another thing that emerged from one of the other stories that they did was that perhaps hookworm can suppress people who have terrible allergies...

Dick:

Right.

Vincent:

... suppress their immune responses.

Dick:

Well, actually, it redirects their immune response.

Vincent:

It redirects?

Dick:

Yeah because..., you know if you take away the targets for the immune response, I think we had a show about this once, where we were talking about... uhm..., what does the immune system actually see? Alright? And we've talked about this in terms of viruses and protozoa and even for helminths, big large worms, secrete targets that have elicited a set of immune responses that have been selected over time to actually interfere with the Biology of those organisms. And, so, in the terms of worms, I mean in the 20s when the immune system was first being explored and exploited, uh, it was not a convincing argument to tell somebody who had spent all their life looking at viruses or bacteria, that, now here's a big giant worm that can also be defeated by the same immune system. But as time evolved and as people began to dissect the immune system more and more and to find out more about what an eosinophil does, for instance, and what IgE might be doing, it became apparent that those two arms of the immune system were pointed directly towards these worms. And the worms were being defeated, but in a way that you couldn't really understand because you can't really kill a big giant worm with an antibody. Or can you? Maybe you can, but you know, you can poke a hole in it! Especially if it's complement-dependent!

Vincent:

We're... vaccines are being developed, right?

Dick:

Sure, but it turns out that worms secrete things into their hosts to enable them to get things back, like food.

Vincent:

I see.

Dick:

So if you can inhibit the substance that the worm uses..., now what does that sound like to you, Vince, in terms of a bacterial infection? This is a quiz for you now. Ha ha ha ha ha!

Vincent:

Inhibit the substance that the worm requires?

Dick:

So there's a bacteria that secretes something into its environment, that we react to, and in doing so, we decrease the pathogenicity of the microbe, so *Clostridium* is like that, right? They produce exotoxins...

Vincent:

... toxins, yes.

Dick:

And the antibodies are against those, and Diphtheria, it's the same thing.

Vincent:

Right. Right.

Dick:

So, it's not an unusual system to imagine, just bigger, that's all, that these worms are secreting similar substances, that if you interfere with them, you get rid of the worm. Now, by sanitation, we've gotten rid of all of our worms! But we still have the guns, so to speak. And they still have to practice, because if they don't, they'll get stale. Apparently nature has to continually use it, or lose it. So... [laughing], we all know what that means. So in the instance of somebody who now lives their entire lives in the absence of worms...

Vincent:

Mhmm.

Dick:

... it's possible that the immune system takes target practice at something that looks a little bit like a worm.

Vincent:

Sure.

Dick:

And I can mention one of those diseases, Crohn's, and I think we've discussed this in the past, right?

Vincent:

Yeah. Sure.

Dick:

Well it turns out that there are a lot of food allergens that mimic parasite products. So what the last segment of that show was all about, was to use worms to give the targets back to their hosts, and leave...

Vincent:

Leave the host alone.

Dick:

... leave the rest of it alone.

Vincent:

This is a great idea, but don't you think it's probably better to understand the mechanism and deliver a molecule rather than the whole parasite?

Dick:

No question about this. No question.

Vincent:

Because this gentleman in that show was infecting himself, and that's just not the way to go.

Dick:

I know. [laughing]

Vincent:

Because as you said, in the South, it may have been lethargic and stupid.

Dick:

But of course. But that's with an overabundance of the worm.

Vincent:

Yes.

Dick:

These are controlled numbers.

Vincent:

So, so... yeah.

Dick:

There's another thing wrong with that, Vince. Even if it works, and even if it's harmless, your immune system will eventually prevent that infection from establishing forever, it will kick it out, and you will never be able to use that strategy again. And so that's an armamentarium that's only, uh..., partially good.

Vincent:

This is very interesting stuff Dick. And I'm going to propose to you that we do a monthly podcast, called "This Week in Parasites", or "Parasitology Today", you can pick the title, I'll do all the engineering, I'll do the recording, I'll ask the questions, you pick the topic...

Dick:

Fabulous.

Vincent:

... just you and I once a month, because I think that would be valuable.

Dick:

Yeah, I do think so!

Vincent:

Get it on iTunes.

Dick:

Yep!

Vincent:

Alright! Let's talk..., now today I want to talk about the viral genome.

Dick:

Oh!

Vincent:

This is going to be fun because we're going to go way back to the 1940s.

Dick:

Ha ha ha!

Vincent:

Yeah, we're going to talk about what kinds of viral genomes there are, and we're going to give you an easy way to sort through the thousands and thousands of different viruses into very easy bits.

Dick:

Vince, I'm going to learn a lot on this show.

Vincent:

Excellent. First, we have to revisit what we used to call - when I was young anyway - the Central Dogma of Molecular Biology.

Dick:

Aha!

Vincent:

Do you remember what that is?

Dick:

I actually do remember what that is. Yes.

Vincent:

Tell us what it is.

Dick:

Well it says that DNA is the molecule that governs the genetics of an organism, and it produces another molecule called RNA, which then goes into the cytoplasm, and encodes for a protein of your choice...

Vincent:

Right.

Dick:

... and there are many, many, many varieties of RNAs that come from the DNA...

Vincent:

Ok.

Dick:

It says that DNA is first.

Vincent:

Right. Well we know that later on that can be modified, we'll talk about that. But that's the Central Dogma, first really established in 1944 by those guys at Rockefeller.

Dick:

Interesting.

Vincent:

Oswald Avery and MacLeod.

Dick:

And MacLeod. That's right.

Vincent:

And they showed that if you took DNA from a bacterium and put it into another bacterium, you could transfer some phenotypic traits.

Dick:

Yup!

Vincent:

It's called transformation.

Dick:

Exactly.

Vincent:

And up until then people had thought protein was the genetic material.

Dick:

Alfred Mirsky was the biggest proponent for that.

Vincent:

Because they were much more complex than nucleic acids.

Dick:

That's right.

Vincent:

Nucleic acids had four different chemicals, how could they possibly be...

Dick:

Exactly.

Vincent:

... you know, our inheritance?

Dick:

So Vince, who was the... uhm..., what was the linchpin for deciding that DNA was the molecule of choice? Before Watson and Crick?

Vincent:

Oswald Avery and MacLeod.

Dick:

Yeah but then the next one, after that.

Vincent:

Hershey-Chase?

Dick:

That's a little bit the answer that I was seeking. But what about one of our homegrown heroes? Erwin Chargaff.

Vincent:

Erwin Chargaff. Right here at Columbia.

Dick:

Yeah!

Vincent:

Tell us what he did.

Dick:

Well he broke apart the molecule and determined that there were equal numbers of As...

Vincent:

Ahhh!

Dick:

... and Ts, and Gs and Cs.

Vincent:

Yeah.

Dick:

There is a dimerism associated with that.

Vincent:

As were equal to T...

Dick:

That's right!

Vincent:

And Gs were equal to C.

Dick:

And Gs were equal to C. In terms of the abundance of each.

Vincent:

And did he know what that meant?

Dick:

Of course not, he completely threw up his hands...

Vincent:

But Watson and Crick when they said "hmmm, this is how the molecule is put together..."

Dick:

Well..., and Rosalind Franklin.

Vincent:

And Rosalind Franklin, of course.

Dick:

That was the big tip off, I thought.

Vincent:

That's good, yeah, Chargaff was here, actually.

Dick:

Yep!

Vincent:

And, I remember seeing some of his centrifuges...

Dick:

Yeah?

Vincent:

With his name on it, he was long gone when I got here in 1982.

Dick:

He was here when I was here.

Vincent:

Really?

Dick:

Yeah! I was here in 1962.

Vincent:

He is a very bitter man.

Dick:

Indeed.

Vincent:

Did you read his, uhm...

Dick:

Yeah. Yeah.

Vincent:

... Biography?

Dick:

Yep, I did.

Vincent:

Yeah, he feels he didn't get credit. And maybe he didn't, but that's the way science is, it's very rough, in terms of giving credit.

Dick:

This is true.

Vincent:

So in 1944, Oswald Avery, MacLeod, then Chargaff in the 50s? Early 50s did he do that?

Dick:

Yeah.

Vincent:

Chemistry, Biochemistry?

Dick:

Yeah.

Vincent:

Now, this is for living cells. Do you know what experiment showed that DNA is the genetic material of viruses?

Dick:

Oooh! That's a good question, Vince.

Vincent:

That's the Hershey-Chase experiment.

Dick:

Ok!

Vincent:

And that was done in 1952, a year before I was born. And what they did, was they grew bacteria in medium containing either ^{32}P , radioactive phosphate, or ^{35}S , radioactive sulfur. The phosphate goes into DNA, the sulfur goes into protein. So they have bacteria with hot DNA, or hot prot... Sorry.

Dick:

[chuckling] I know what you meant. [laughing]

Vincent:

They grew up phages with either ^{35}S or ^{32}P , so you have phages with hot DNA or hot protein, and they took these phages and they added them to bacteria, and they attached. They let them attach for just a short time..., long enough just to initiate an infection. And then they put them in a blender, and they turned the blender on, which would separate the bacteria from the bacteriophage...

Dick:

Sure. By shearing them off.

Vincent:

And then they asked "which radioactivity, ^{32}P or ^{35}S went into the bacteria?" And what do you think they found?

Dick:

Hmmm. I would guess the P, rather than the S...

Vincent:

^{32}P went in, yes. And they said "Aha! That means that the DNA is the genetic material of viruses!" Classic experiment! Incredible.

Dick:

Where did they get the ^{32}P from, Vince?

Vincent:

Ahh! That's a good question. Because nowadays we order it...

Dick:

[laughing]

Vincent:

... and I don't know where they got it. There must have been a company who made it.

Dick:

I..., no no no!

Vincent:

You make it?

Dick:

No, you just go out to the Brookhaven National Labs. It's all over the place.

Vincent:

Yeah, back in those days that's what you did. What about the ^{35}S ?

Dick:

That's where Van Slyke worked also.

Vincent:

Ahh, good point!

Dick:

In fact, that jump started the whole field of Ecology, because the Savannah River project..., ahh..., at the University of Georgia took advantage of the first nuclear power plant.

Vincent:

Aha.

Dick:

Which made lots of sloppy isotopes.

Vincent:

That's cool.

Dick:

And what they could do is then follow all of those things in nature as well.

Vincent:

You know what you pay nowadays for a small amount of ^{32}P ? Hundreds and hundreds of dollars.

Dick:

Really?

Vincent:

They probably gave it away.

Dick:

They probably gave it away.

Vincent:

Ah, good point. Anyway, 1952..., so DNA viruses are..., DNA bacteriophages, that's the genetic information.

Dick:

Mhmm.

Vincent:

And of course, the structure of DNA was solved 1953.

Dick:

Right.

Vincent:

Which happens to be my birth year.

Dick:

Aha!

Vincent:

So maybe that meant I was destined to be a scientist.

Dick:

[laughing]

Vincent:

And then all..., what about RNA viruses?

Dick:

Mmmm.

Vincent:

You know, in the 1930s it had been found that about 5% of the tobacco mosaic virus virion was RNA, but no one knew what to make of that.

Dick:

Mhmm.

Vincent:

Then in 1956 someone took the RNA out of the virion and put it into plant cells and showed that it was infectious. In other words, it's the genetic material. So the first demonstration that RNA could be genetic material of a virus was in 1956 with tobacco mosaic virus.

Dick:

Hmm.

Vincent:

And again, another of the stream of important discoveries made with that plant virus.

Dick:

Plants have played a huge role in Genetics in the history of Genetics, hasn't it?

Vincent:

Huge!

Dick:

Enormous.

Vincent:

And we are now beginning to realize that, just in the last few months of TWiV, we ignored them initially...

Dick:

Ha ha ha!

Vincent:

... but now we're appreciating all the stuff that's going on.

Dick:

I mean there was Gregor Mendel's work, and then there was another guy here at Columbia, his name is Olive..., of all names. Olive! That was his name. Dr. Olive..., and...

Vincent:

As in olive oil.

Dick:

Indeed. And he was one of the first viewers of chromosomes.

Vincent:

Hmm!

Dick:

In plants. First.

Vincent:

Ok.

Dick:

At any rate, that's just...

Vincent:

So by 1959 we had many, many RNA viruses. We had human viruses, we had bacteriophages, and then in the 60s everything continues. So that's sort of what we need to know for this..., uhm..., little Virology 101.

Dick:

Mhmm! Are there any mixed viruses?

Vincent:

RNA and DNA?

Dick:

Yeah.

Vincent:

I can't think of any, but...

Dick:

Ok.

Vincent:

I can't think of...yeah..., OH! Sorry, yes! [laughing]

Dick:

He's got one, folks!

Vincent:

I've got one and we're going to talk about it today.

Dick:

Ok!

Vincent:

But before we go on, a word from the sponsors of this episode. Citrix. For most of you, or many of you, all of your important files are on your office computer. And if you want to go home and work, or if you need to go on a business trip and work, or any kind of trip, you have to bring your files with you, you have to copy them to a CD, a keychain drive, or you have to do syncing, or whatever..., it's really a hassle. And you're going to forget something, believe me, I know, I've forgotten many things. The solution is incredibly simple. You use GoToMyPC brought to you by Citrix. It's the easy and secure way to access your computer from anywhere. You go to their website, you install a small program on the computer that you want to share, you get a login ID and a password, and then from any internet connected computer in the world, you can then access your computer in your office. You can get the files, you can check your email. Totally painless and secure. You can try GoToMyPC free right now. Now. For 30 days. Visit GoToMyPC.com/podcast, that's GoToMyPC.com/podcast, you'll get a free 30 day trial, and we thank them for their support. I forgot to mention, Dick, uhm..., the Hershey-Chase experiment was...

Dick:

Yes.

Vincent:

... benefitted from serendipity.

Dick:

Aha.

Vincent:

Because we now know there are phages, uh, whose protein gets into the cell.

Dick:

Interesting! [laughing]

Vincent:

Along with the DNA. So what if they had had that result, it would have confused everything, right?

Dick:

You bet.

Vincent:

So often serendipity plays a big role in science, doesn't it?

Dick:

No question.

Vincent:

Well, that's nothing new. Alright, so today now, we're going to talk about how we use the nucleic acid to classify viruses. This is actually a scheme thought of by a Virologist by the name of David Baltimore.

Dick:

I'm familiar with David Baltimore.

Vincent:

And it's called the Baltimore scheme.

Dick:

Ha!

Vincent:

There are thousands of different viruses, but there are only a finite number of viral genomes. In fact, there are seven different kinds of viral genomes.

Dick:

Seven? I'm writing this down as you speak, because I am a nn.. novitiate with regards to viruses.

Vincent:

Yeah, you want to go through this?

Dick:

Let's go through it! Seven! Count them!

Vincent:

We're going to tell you how this Baltimore scheme makes life easy for students of Virology. It's going to enable you to take thousands and thousands..., tens of thousands of virions and put them in seven types.

Dick:

Cool.

Vincent:

And you'll be able to look at any virus from now on on TWiV, any virus that you hear, you'll be able to know exactly how it replicates.

Dick:

Wow.

Vincent:

With this scheme. So here's the key fact that makes life easier for students of Virology. Viral genomes must make mRNA that can be read by host ribosomes.

Dick:

Otherwise, how can they reproduce?

Vincent:

Exactly. All viruses on the planet follow this rule. There's no exception. Alright, so the virus has to make messenger RNA, and that is translated into protein by the cell.

Dick:

Right!

Vincent:

So David Baltimore said, "hmm, let's put mRNA right in the middle of the page, and then we'll see how every kind of viral genome gets to it".

Dick:

Great idea.

Vincent:

And he found seven different ways.

Dick:

How long ago was that, Vince?

Vincent:

It was in the 1970s, early 1970s. One of the first things I learned as a graduate student.

Dick:

I'll be darned. And you did some work with him, didn't you?

Vincent:

I did a postdoctoral fellowship with him, right.

Dick:

That's remarkable.

Vincent:

It's remarkable that I worked with him?

Dick:

No, it's remarkable that you don't trout fish. [laughing]

Vincent:

Because he does?

Dick:

He's a very good trout fisherman.

Vincent:

Trying to get him on TWiV.

Dick:

Ah, you're hoping he accepts. He's probably not fishing. [laughing]

Vincent:

We should go out to the fishing area and record. It'd be great.

Dick:

Yup! Yup!

Vincent:

Does he know you?

Dick:

Yup, he does!

Vincent:

So, that would be a plus in getting him on TWiV then.

Dick:

Hah hah hah.

Vincent:

So you guys can talk about fishing.

Dick:

We can do that.

Vincent:

So David Baltimore used this insight, again, you make mRNA.

Dick:

Alright!

Vincent:

And the host..., so..., we're going to have a TWiV way in the future on translation...

Dick:

Right.

Vincent:

But, we have DNA, which is made into mRNA, which is translated into protein, and for viruses..., viruses cannot carry out the translating mRNA into protein, the cell has to do that. It's a very complicated machinery that's needed, and no virus encodes all that machinery.

Dick:

Otherwise it would be almost independent.

Vincent:

Yeah, it wouldn't be a virus anymore, right?

Dick:

Correct.

Vincent:

You're absolutely right. So viruses are translational parasites among other things.

Dick:

Translational parasites.

Vincent:

[laughing]

Dick:

Have you ever seen that movie "Lost in Translation", Vince? [laughing]

Vincent:

I didn't. Should I see it?

Dick:

It's a wonderful movie with Bill Murray, it's absolutely charming.

Vincent:

Alright, I'll go see it.

Dick:

Because some viruses don't end up completing their replication cycle inside the cell, so they must be lost in translation.

Vincent:

Some of them are definitely lost in translation, and others at other steps. But I've seen that word, that movie title...

Dick:

Yeah. Right.

Vincent:

... used for review articles and so forth.

Dick:

Ahh, you'd love the movie. It's very funny.

Vincent:

Right, now before we go into the seven classes of genome, let's make some definitions. We have done this before, but we'll assume that no one listening has ever listened to TWiV before.

Dick:

Right!

Vincent:

Which is always a good assumption. When I teach, I always assume that no one has heard any of it before.

Dick:

This benefits me more than anybody. [laughing]

Vincent:

It doesn't hurt, right? To assume...

Dick:

Doesn't hurt.

Vincent:

Plus polarity, or plus strand.

Dick:

PLUS strand.

Vincent:

Now polarity doesn't mean electrical polarity or anything like that, it's just a convention.

Dick:

Right.

Vincent:

Plus strand simply means the same strand as the mRNA. That which is translated.

Dick:

Right.

Vincent:

And that could be an RNA or a DNA, so the DNA could be plus-stranded, which simply means that its bases are in the same..., are the same as the mRNA. So let's say ACGAATATG, if that's the plus strand of the mRNA, then the DNA would have the same sequence. I just made a mistake, what did I do wrong?

Dick:

Well, I think what you really meant to say was that it's the complement to the strand.

Vincent:

Well I said ATG for RNA, it's AUGUAA, etc.

Dick:

Ahh!

Vincent:

DNA has Ts where RNA has Us.

Dick:

Ok.

Vincent:

Anyway, the point is that it's the same sequence in the RNA and the DNA if we're talking about plus strand. And you're right, the minus strand is the complement! That's all it is. Plus strand is the same strand as mRNA, the minus strand is the complement.

Dick:

Which you need in order to make the next plus strand.

Vincent:

Exactly!

Dick:

Ok!

Vincent:

Exactly. As you know some viruses...

Dick:

Like velcro, I look at this like velcro. [laughing]

Vincent:

[laughing] Sure, why not? Chemical velcro! Exactly. Now Dick, can the plus strand of mRNA be translated?

Dick:

Uh, I would guess that the plus strand of RNA..., yes!

Vincent:

Sure, it's by definition.

Dick:

It starts out as a messenger.

Vincent:

Yeah, by definition.

Dick:

It's called jump starting, isn't it?

Vincent:

Can the minus strand be translated?

Dick:

No.

Vincent:

Can the plus strand of DNA be translated?

Dick:

It has to be translated.

Vincent:

No. DNA is not translated, you have to make mRNA first. I tricked you.

Dick:

No no, I thought you meant "transcribed". I'm sorry.

Vincent:

Yeah. DNA is never translated.

Dick:

No no no, not translated. I meant..., I mistook the word "translation" for "transcribed".

Vincent:

You're forgiven. DNA is never translated. [laughing]

Dick:

No no no. [laughing]

Vincent:

RNA is translated.

Dick:

I do know this, actually. [laughing] Honest, I swear to God I know this!

Vincent:

Ok, sorry! Do you want me to cut this out?

Dick:

No no, leave it in, leave it in. We all make that mistake.

Vincent:

Plus strand mRNA is translated, minus strand RNA is not, DNA is never translated, it has to be transcribed...

Dick:

... to be transcribed.

Vincent:

... or copied to form an mRNA. Right.

Dick:

It's a shame that those two words start the same.

Vincent:

I understand.

Dick:

[laughing]

Vincent:

Ok, so with the Baltimore system...

Dick:

Mhmm.

Vincent:

... all you have to do is know the nature of the viral genome, and you'll be able to tell what needs to take place to make mRNA.

Dick:

Hear hear.

Vincent:

That's the beauty of this Baltimore scheme.

Dick:

That's fantastic!

Vincent:

I have to rearrange my office, so I can face you all the time, don't you think?

Dick:

It would be nice to look at you.

Vincent:

Yeah, I've got to rearrange.

Dick:

Eye contact is good for conversation. [laughing]

Vincent:

I agree, I don't like this, but..., alright, there are seven classes of viral genomes, you ready?

Dick:

Here we go.

Vincent:

First is double-stranded DNA. Then we have..., Dick is writing.

Dick:

I am! I've got to take notes here.

Vincent:

Then we have gapped double-stranded DNA.

Dick:
Gapped!

Vincent:
And we have single-stranded DNA.

Dick:
Wait, you're going too fast.

Vincent:
We have double-stranded RNA.

Dick:
Right.

Vincent:
We have single-stranded plus RNA, and we have single-stranded minus RNA.

Dick:
Ahhh!

Vincent:
And finally, single-stranded plus RNA with a DNA intermediate. That's seven. Seven classes of viral genomes.

Dick:
I'll be darned!

Vincent:
Let's just go through each one, and give some examples and talk about how they work. Now let's start with double-stranded DNA genomes.

Dick:
Right!

Vincent:
Can you name a virus with a double-stranded DNA genome?

Dick:
My guess is that it would be a large virus. Ahhh... Poxvirus.

Vincent:
Yes, Poxvirus has a double-stranded DNA genome.

Dick:
How did I know that? That was just a wild guess folks, believe me.

Vincent:

So, double-stranded DNA, it has a plus strand and a minus strand...

Dick:

Right!

Vincent:

... forming a double helix, right?

Dick:

Yep!

Vincent:

There are 22 families of viruses with double-stranded DNA genomes.

Dick:

Wow!

Vincent:

And you're right, Poxviruses, Adenoviruses, Herpes...

Dick:

Wow!

Vincent:

... Papillomaviruses, Polyomaviruses. Now Dick, how do you make mRNA from a double-stranded DNA genome?

Dick:

Well you have to unwind the double strand first, right? And the positive-stranded DNA then makes negative strand RNA, and the...., the other way! Negative-stranded DNA makes a positive strand RNA, and that's the message.

Vincent:

That's the message, right! It's pretty simple.

Dick:

Right!

Vincent:

You go from DNA to mRNA...

Dick:

And the other strand makes the other copy of the DNA that makes the...

Vincent:

Yes.

Dick:

... messenger RNA. So you make more DNA, and you make more messenger RNA...

Vincent:

Exactly.

Dick:

... and you make protein.

Vincent:

That's replication, right.

Dick:

Got it, got it, got it.

Vincent:

So...

Dick:

Yeah, I knew that, actually.

Vincent:

... mRNA is produced from the DNA. Some viruses encode an enzyme that can do that, others use a host enzyme, a DNA-dependent RNA polymerase, it's called, to do that.

Dick:

Right.

Vincent:

You cannot make mRNA from single-stranded DNA.

Dick:

No.

Vincent:

You can only make it from double-stranded DNA. Now that simple fact will be very useful when we talk about viruses with single-stranded DNA genomes, right?

Dick:

[laughing] Because they first have to make more DNA.

Vincent:

Exactly!

Dick:

In order to make a double strand, to then make RNA.

Vincent:

Do you see how beautiful this scheme is? [laughing]

Dick:

I do! [laughing]

Vincent:

Ok. So double-stranded DNA genomes come in a couple of different flavors, or should I say configurations...

Dick:

Right.

Vincent:

... they can be linear...

Dick:

Ah.

Vincent:

... Poxvirus DNA, Adenovirus DNA..., Poxvirus..., if Rich Condit is listening, don't fall off your bicycle Rich!

Dick:

[laughing]

Vincent:

I know it's not really linear, I know the ends are joined in the Pox DNA, right? And so it's not really linear, but we call it linear configuration. And then there are some viruses with circular DNA, and so it's double-stranded DNA where the ends are covalently joined, so you form a circle. So if you started riding this circle of DNA, eventually you'd get back to where you started, ok?

Dick:

I understand.

Vincent:

Polyomaviruses...

Dick:

Polyoma.

Vincent:

... Papillomaviruses, which, uh..., human Papillomaviruses are the cause of genital cancers...

Dick:

And warts.

Vincent:

Warts.

Dick:

Ok!

Vincent:

Ok, that's one class: double-stranded DNA.

Dick:

Right!

Vincent:

So it's easy to get to mRNA, you just transcribe the double-stranded DNA, ok?

Dick:

Well you have to unwind it first.

Vincent:

Got to unwind it, sure. Details, right. This is just the overview. We're going to talk about that, we're going to have a TWiV on transcription. It's going to be so geeky...

Dick:

Ha ha ha!

Vincent:

But on the other hand, we want people to understand, so if we're being...

Dick:

This is not geeky!

Vincent:

... too complicated, tell us and we'll simplify it.

Dick:

Vince, you know what this is? This is the biological equivalent to the show that I watch at 3 o'clock in the morning when I can't sleep. It's called "How it's made".

Vincent:

I've never seen that.

Dick:

Your listeners all know this show, it's a very popular show.

Vincent:

Really?

Dick:

Yeah, and it comes out of Canada and the host of this show goes: "Today, on "How it's made", we're going to show you how guitars, nuclear weapons and douchebags are made", and they do! They take you to the assembly line, they do popcorn, they do candy apples, they do high end guitars, they do..., why don't we have a "How it's made" show for viruses?

Vincent:

Well that's what this is, right?

Dick:

Of course that's what this is. It's just another version of "How it's made"!

Vincent:

Ok.

Dick:

I love it!

Vincent:

Yeah, you're right!

Dick:

I love it.

Vincent:

Except, we're a little more..., no, I won't say that.

Dick:

Ha, so the "geeky" has nothing to do with it, you're just curious as to the inner workings of life itself.

Vincent:

Right.

Dick:

That's what this is.

Vincent:

Well, a geek is someone who..., is very passionate about one thing and focuses on it a lot, extensively.

Dick:

Yeah, probably.

Vincent:

So it doesn't have a defined subject, it could be anything, so we're geeky about viruses.

Dick:

I think "geeky" means something more than that though, I think those are people who pay attention to the details..., how many sprockets on the wheel, and...

Vincent:

Yeah.

Dick:

... how the hands of a clock work.

Vincent:

Yeah, you're right.

Dick:

So, I'm a geek. I'm definitely a geek.

Vincent:

Alright, the next class of genomes, are gapped DNA genomes.

Dick:

You know, you have to explain that one to me Vince, because this is a specialized term.

Vincent:

It's very simple.

Dick:

Gapped, single-stranded did you say?

Vincent:

No, it's double-stranded but there are pieces missing.

Dick:

Ohh....

Vincent:

So there are parts where it's actually single-stranded, most of it is double-stranded, but there are pieces missing from one part, so you have a gap, right?

Dick:

What's the purpose of that?

Vincent:

Oh, that's a good question. Why would a virus evolve to be this way?

Dick:

Exactly!

Vincent:

And I don't have an answer...

Dick:

Oh!

Vincent:

Just to say that it works.

Dick:

[laughing] Does David have an answer?

Vincent:

We could ask him, but these are the genomes of...

Dick:

Yeah, let's talk about them!

Vincent:

Hepadnaviruses, Hepatitis B virus...

Dick:

Oh, Hep.

Vincent:

So it's got a double-stranded DNA, but it has a gap, and it also has a small piece of RNA in there as well. So there's the answer to your question.

Dick:

The gap is filled with RNA?

Vincent:

No it's not, there's a piece of RNA stuck somewhere in there, and there's also a gap. So it's got two features.

Dick:

Wow! Every gapped double-stranded DNA has a little smidgeon...

Vincent:

Yeah, there's a little piece of RNA, it's got a gap...

Dick:

And what is that RNA?

Vincent:

There's also a protein attached to the RNA..., to the DNA, but we don't need to get into that.

Dick:

Wait. So I need to ask you a question here.

Vincent:

Yes.

Dick:

Is this RNA single-stranded?

Vincent:

Yes, it's kind of hanging off.

Dick:

And is it plus or minus?

Vincent:

It's plus.

Dick:

It is!

Vincent:

Yes, it's just a short RNA.

Dick:

Does it encode for a peptide, by any chance?

Vincent:

No. No.

Dick:

Does it encode for anything?

Vincent:

I don't believe it does, it has a role in the replication of this genome.

Dick:

Because if you eliminate it, something bad happens to the virus.

Vincent:

I'm sure, but we're not going to go there today, because I'm not ready for that.

Dick:

Ha ha ha!

Vincent:

Ha ha ha!

Dick:

You mean, I've stumped the stars?

Vincent:

Mmmm, maybe, let's see. So half of the strand is not double-stranded, so you've got half double-stranded and the rest is single-stranded in this genome. And then you have a little piece of RNA hanging off as well.

Dick:

But the full strand must be the negative strand of DNA then because it has to replicate to make a double strand to make RNA then, right?

Vincent:

You're right! It is the negative strand!

Dick:

That's David Baltimore's rule!

Vincent:

Yeah, exactly! So this genome actually cannot be transcribed into mRNA.

Dick:

No.

Vincent:

Because single strands can't be transcribed. So what do you think is the first thing that happens when this genome gets into a cell?

Dick:

It unwinds.

Vincent:

And then what happens?

Dick:

And then it replicates itself.

Vincent:

Yeah, it fills the gap.

Dick:

Yeah.

Vincent:

We mind the gap. Hepadnaviruses mind the gap.

Dick:

That's incredible.

Vincent:

And they fill it in and then you can make mRNA from it.

Dick:

Wow.

Vincent:

Ok, there's actually another step in the replication of these viruses, that we're not going to talk about today.

Dick:

No.

Vincent:

It involves reverse transcription...

Dick:

Ahhhh!

Vincent:

... and that's where that little RNA comes from.

Dick:

And that's what David Baltimore got his Nobel prize for.

Vincent:

Yeah, reverse transcriptase, with a different virus, but this came along actually after he discovered his reverse transcriptase.

Dick:

Wow.

Vincent:

Anyway, that's another class. Hepatitis B viruses...

Dick:

Ok!

Vincent:

... cause liver infections in humans...

Dick:

And cancer.

Vincent:

... and cancer, hepatocellular carcinoma, when you have a Hep B infection for many years, you end up getting cancer.

Dick:

That's right. It was the first virus for a vaccine that actually that prevented cancer.

Vincent:

That's right! Ok!

Dick:

So do all of the Hepatitis series behave similarly?

Vincent:

No, Hepatitis virus is a very broad term.

Dick:

Understood.

Vincent:

This means any virus that can infect the liver.

Dick:

Right, so this A, B, C, D, E...

Vincent:

E...

Dick:

... all the way out to G, or F, now, right?

Vincent:

G. Yeah.

Dick:

So are all those gapped...

Vincent:

No, they're all different.

Dick:

They're all different.

Vincent:

Only the Hepatitis B viruses, which have the double-stranded gapped DNA.

Dick:

But they all use the same attachment site?

Vincent:

No. Absolutely not.

Dick:

But they all home in on the liver. Ha ha!

Vincent:

They all home in on the liver, that's why they're called Hepatitis viruses.

Dick:

But they all use a different attachment molecule, and yet they still get to the parenchyma cells?

Vincent:

Oh yeah.

Dick:

That's incredible!

Vincent:

Different cell receptor, that's what we're talking about.

Dick:

Vince..., isn't that incredible?

Vincent:

It is amazing, yeah! Of course they infect other cells as well, but the pathology is in the liver.

Dick:

Ok. Do any of those others cause cancer?

Vincent:
Absolutely!

Dick:
They do!

Vincent:
Hepatitis C virus.

Dick:
Aha!

Vincent:
That's the Flavivirus family, right?

Dick:
Really? Related to the West Nile.

Vincent:
And then we have Hepatitis A, which is a Picornavirus...

Dick:
Related to the Poliovirus.

Vincent:
Hepatitis D is a viroid.

Dick:
Oooohh!

Vincent:
We'll talk about that, we actually had a show about viroids, we talked about it, it only encodes one protein.

Dick:
Exactly.

Vincent:
Not even a capsid.

Dick:
Bizarre.

Vincent:
And Hepatitis C, and so on.

Dick:

Yeah yeah.

Vincent:

So they're all different families of viruses.

Dick:

Remarkable.

Vincent:

Alright. Family number three. Can we move on, or do you want to...

Dick:

No no!

Vincent:

There are Hepatitis B viruses of humans and ducks, and squirrels..., yeah. All kinds of animals. Alright, the third class is single-stranded DNA genomes.

Dick:

Ok!

Vincent:

Five families.

Dick:

Five. Ok!

Vincent:

Circoviruses, which we've mentioned cause disease in pigs, last time we talked about a Circovirus vaccination of pigs.

Dick:

Indeed.

Vincent:

Parvoviruses, which cause...

Dick:

Parvo!

Vincent:

... disease in dogs and cats, right?

Dick:

Yep!

Vincent:

So Dick, what is the basic problem with a single-stranded DNA genome?

Dick:

Well, it's not a double strand, is it?

Vincent:

Yeah, exactly.

Dick:

[laughing]

Vincent:

So when these viruses get into cells...

Dick:

You know, that's like who is buried in Grant's tomb, come on Vince, make it tougher than this one, will you please?

Vincent:

Well that's the first step,... that's the first step.

Dick:

Well you got to replicate yourself to make another strand!

Vincent:

Yeah. You have to make a double strand, yeah, and then you can make mRNA.

Dick:

That's right.

Vincent:

Because if you just think of making mRNA as what you need to achieve, you will understand how every virus replicates.

Dick:

So, the biggest biological reason though, for not being able to replicate as a single strand of DNA, is..., that you'd only make RNA at a certain low, low, low level and you'd never be able to make your DNA to get back out of the cell. That's...

Vincent:

Well unless you had a reverse transcriptase, right?

Dick:

Unless you had a reverse transcriptase, but it's obvious why you'd need to make DNA first, because you need to make both parts of the virus.

Vincent:

Well I think there's a structural reason why you'd have to have double strands versus single strands. The enzymes don't work on single strand templates. And for some reason no enzyme has evolved to do that, to copy a single-stranded DNA template into messenger RNA.

Dick:

And into DNA at the same time.

Vincent:

Well that's a separate enzyme, right?

Dick:

Well you'd have to do both, in order for it to replicate...

Vincent:

Yeah, you're right, that too, it's very unusual for it to do both.

Dick:

It's crazy. Could you get it to do both, though in the test tube?

Vincent:

The Hepadna enzymes can make both kinds of nucleic acid.

Dick:

So in the laboratory you can replicate a viral DNA strand that's single if you had all the right reagents in the tube.

Vincent:

Yes, you could do it *in vitro*, sure.

Dick:

And then it would self-assemble?

Vincent:

What would self-assemble?

Dick:

The viral particle.

Vincent:

There are some examples of that, sure.

Dick:

Ok!

Vincent:

For simple viruses, they will self-assemble, yeah.

Dick:

Alright.

Vincent:

Tobacco mosaic virus, years ago, they took the RNA and the proteins, and they mixed them and they assembled. Fraenkel-Conrat, the whole concept of self-assembly encoded in the proteins, very important concept, yeah.

Dick:

Ok!

Vincent:

Single-stranded DNA genomes can be linear or circular...

Dick:

Ah!

Vincent:

... just like the double-stranded guys, so the Circoviruses are circles, they're very small, one to two kilobases of DNA, and then we have the Parvos, they're linear...

Dick:

So if they're circular and they go into the cell, then what makes them linear in order for them to replicate?

Vincent:

They probably replicate as circles.

Dick:

Oh, do they?

Vincent:

Yeah.

Dick:

They don't have to linearize first, the enzyme...

Vincent:

No, the enzyme can fill it in.

Dick:

...so they must...

Vincent:

In some cases you do get..., there are what's called "rolling-circle" mechanisms of DNA replication where a single double strand will come off the circle, but it's not obligatory, you can also replicate as a circle.

Dick:

Ok.

Vincent:

Yep, alright. Single-stranded DNA genomes come in the cell, become double-stranded...

Dick:

Right.

Vincent:

... and then they make mRNA. Now these viruses tend to be very small, their genomes are small, so the enzymes that make the DNA double-stranded and that make mRNA from the genome are usually cell enzymes. It's only the larger viruses, Adenoviruses, Poxviruses, Herpesviruses, that encode their own polymerases.

Dick:

I forgot to mention Rabies. That's a big virus, which it is.

Vincent:

It's coming up!

Dick:

Ok!

Vincent:

Because now, we finished with the DNA genomes, we're moving to RNA genomes. Dick, did you know that the RNA genome is the most abundant kind of viral genome on the planet?

Dick:

I..., you know, I'm not surprised to hear that.

Vincent:

Most viruses are RNA viruses.

Dick:

Based on the concept that it's jump starting the replication cycle and the protein cycle at the same time...

Vincent:

Really, that makes sense to you?

Dick:

Yeah, it does actually.

Vincent:

Well you're being teleological...

Dick:

I'd rather go in as a message and then make the DNA afterwards to be honest with you.

Vincent:

Now here's a few things about RNA that you need to know Dick.

Dick:

Ok!

Vincent:

Given an RNA viral genome, a cell cannot do anything with it, unless it's an mRNA.

Dick:

Right.

Vincent:

Some viral genomes are in fact mRNAs, but for the most part a cell cannot copy a viral RNA, it cannot make an RNA copy, because the cell doesn't have an RNA-dependent RNA polymerase that's capable of making such long RNAs. So the viruses have to encode all their own enzymes to make more genomes. But again, in the end Dick, what do we need to make?

Dick:

We need to make double-stranded DNA first, and then we need to make messengers.

Vincent:

No, not for all of these, only for one family. RNA can go to mRNA directly.

Dick:

[laughing] Ok.

Vincent:

I'm sorry I asked you that.

Dick:

Ha ha ha! It was the standard answer that I was correcting before, that's all.

Vincent:

You're right, I'm sorry. It's not fair.

Dick:

Well somehow you have to replicate your genome as well as the protein.

Vincent:

Well you have to replicate that RNA genome...

Dick:

Yeah exactly.

Vincent:

... but again, as with the DNA viruses, Dick, the goal is to make mRNA.

Dick:

Right.

Vincent:

Just keep focused on that.

Dick:

No no no, I should have said that and nothing else.

Vincent:

So, I... in fact, for RNA, double-stranded, it cannot be transcribed [laughing]. You cannot make mRNA from a double-stranded RNA. No, it's..., I'm wrong. You cannot translate a double-stranded RNA.

Dick:

You can edit that out. [laughing]

Vincent:

I could be wrong. At least I corrected myself.

Dick:

Vince, you wrote a whole book on this!

Vincent:

Sometimes I forget, I'm getting old.

Dick:

Ha ha ha ha! Well what excuse do I have?

Vincent:

I'm sorry, double-stranded RNA...

Dick:

So tomorrow this will all be new information to me. [laughing]

Vincent:

So let's review. Single-stranded DNA cannot be made into messenger RNA.

Dick:

No. You have to make two strands first.

Vincent:

You can make message from single strand or double strand RNA though. But you can't make protein from double-stranded RNA. Because you know, double-stranded RNA has a plus and a minus strand, but you can't translate that plus strand, you got to melt the duplex first, you have to melt the duplex. Ok?

Dick:

Melt the duplex.

Vincent:

So the first family is double-stranded RNA viruses. Now these viral genomes have two strands, a plus and a minus strand of RNA annealed. Do you know an example, a famous example, and I'll give you a clue, it's from a town in Ohio.

Dick:

Ha ha ha ha ha! Uh, let me think. Norovirus probably falls into that...

Vincent:

No, I'm wrong again! [laughing] It's a different family.

Dick:

You know what, folks...

Vincent:

I'm thinking of Rotaviruses, the ones that cause diarrhea...

Dick:

We're having fun, but we're not getting very far here. [laughing]

Vincent:

Yeah, Rotaviruses have double-stranded RNA genomes.

Dick:

Ok. Rotaviruses are very prevalent.

Vincent:

Very! They're big causes of gastroenteritis, but they're not from Norwalk, Ohio.

Dick:

Unfortunately they result in a lot of infant mortality.

Vincent:

Should we leave all the fooling around in this Dick,? I can edit it out.

Dick:

Sure! No, no.

Vincent:

Just to let people realize that we're human...

Dick:

No, but not only that, it's fun to learn this stuff too. Because you learn by your mistakes.

Vincent:

So, Reoviruses are interesting, because they have a segmented genome of double-stranded RNA pieces.

Dick:

Reoviruses.

Vincent:

The point is, that double-stranded RNA cannot be translated, so, uhm..., Dick, what do you think could happen..., you have a double-stranded RNA virus...

Dick:

Right. It gets into the cell..., so it has to unwind...

Vincent:

Ok.

Dick:

And then one of those strands has to encode for the next strand of RNA, right? If it's negative-stranded it encodes for the positive strand, if it's positive-stranded it encodes for the negative strand...

Vincent:

Right.

Dick:

... so you have to make more RNA first...

Vincent:

Right.

Dick:

That's the next step, and then some of that, half of it, becomes message.

Vincent:

Alright, so that's theoretically possible, but in reality these viruses don't bother to denature that double-stranded RNA. Those viruses actually have an enzyme in the capsid that copies and makes a plus strand from the duplex RNA.

Dick:

Without unwinding it?

Vincent:

I presume that it does unwind during the process, but it doesn't melt it out so that when...

Dick:

It zips and unzips it as the enzyme goes..., I gotcha.

Vincent:

Yeah, it zips and unzips.

Dick:

Yeah, I gotcha, I gotcha.

Vincent:

So you actually make a plus strand.

Dick:

Ok. And the virus has that protein. So it's an RNA polymerase then...

Vincent:

It's an RNA polymerase. And the virus has to have it because as we said, the cell can't handle that RNA.

Dick:

So it's RNA polymerase that makes the message.

Vincent:

Yes, and so for these viruses, a double-stranded RNA viruses, the enzyme has to be in the particle. The RNA polymerase is in the particle.

Dick:

Got it. So, are there drugs that inhibit that enzyme?

Vincent:

There could be.

Dick:

Are there?

Vincent:

There aren't any on the market, no.

Dick:

[laughing] And which viruses are we talking about here?

Vincent:

So the Rotaviruses are one.

Dick:

We would love to have a drug against the Rotaviruses.

Vincent:

We have vaccines of course, but a drug would be good.

Dick:

So, Vince then that raises the next question: how do you make the double-stranded RNA? How does the double-stranded RNA get made in order to leave the host cell once the capsid proteins get made?

Vincent:

Well, the single-stranded mRNAs that are made to be translated, at some point during replication, those get encapsidated, made double-stranded, and then they leave the cell.

Dick:

Well that..., yeah but that says what happens, it didn't say how it happened.

Vincent:

Well we'll have to do that in the replication episode.

Dick:

Excellent. I'm jumping ahead though.

Vincent:

Yeah, you're jumping ahead, but that's good, it shows you're curious.

Dick:

Well..., sure! So much for double-stranded RNA.

Vincent:

The next family..., we're almost through our families here.

Dick:

That's true!

Vincent:

That was double-stranded RNA.

Dick:

Right.

Vincent:

Single-stranded RNA. The rest of our viruses are all single-stranded RNA.

Dick:

Right.

Vincent:

The first class is plus strand.

Dick:

Ah!

Vincent:

Now, Dick, in theory, if a virus has a plus strand RNA and that RNA gets into the cell, what's the first thing that could happen, in theory?

Dick:

It could make some protein.

Vincent:

It could, because it's plus strand.

Dick:

It could make some protein.

Vincent:

So many of these viruses do not have enzymes in the virion because the first step is translation, at which point the enzyme can be made by the cell. The viral enzyme can be made by translation.

Dick:

Got it. So how many proteins does this single-stranded RNA encode?

Vincent:

Well let's take a typical example. Picornaviruses are plus-stranded RNA viruses. Their RNA gets into the cell and it's translated into protein. About twelve proteins, ten to twelve proteins.

Dick:

Twelve. Ok, fine.

Vincent:

The point is, if you have a plus-stranded, single-stranded RNA genome, that RNA can be translated as soon as it gets into the cell.

Dick:

Yup.

Vincent:

So you don't need to have the enzyme in the virion as do the double-stranded RNA viruses.

Dick:

Got it.

Vincent:

Ok, so examples of this...

Dick:

Well, Vince!

Vincent:

Picornavirus, Polio, Rhinoviruses, Coronaviruses, the SARS virus, West Nile, Yellow Fever, Hepatitis C virus...

Dick:

All the Flavis.

Vincent:

All the Flavis. The Togaviruses, and even the Retroviruses, they have plus strand RNA genomes...

Dick:

Togoviruses!

Vincent:

... although we're going to put those in a different class because they do something very different.

Dick:

Should I guess that the Togovirus comes from Togo?

Vincent:

No, Toga comes from when they were first seen in the electron microscope, it looked like they had a toga around them.

Dick:
Dumb.

Vincent:
Dick when I used to...

Dick:
Is that true?

Vincent:
... I used to teach togas to medical students...

Dick:
Hah hah hah!!

Vincent:
... and, uhm...

Dick:
I thought you said "Togo" first of all.

Vincent:
No, TOGA, t-o-g-a.

Dick:
Toga! Like a toga party, ok.

Vincent:
Yeah, so when I used to teach medical students about Togaviruses, which I don't any longer...

Dick:
Yeah.

Vincent:
... the first time I mentioned "toga", they would start chanting. To-ga! To-ga!

Dick:
From John Belushi's "Animal House", that's crazy stuff.

Vincent:
So that's what medical students do.

Dick:
Oh dear.

Vincent:

Rubella is another one.

Dick:

Rubella!

Vincent:

Ok. So these, again, are plus-stranded, they can go right into the cell and be translated into protein.

Dick:

Those are pretty famous viruses there.

Vincent:

You see how easy it is, to figure all this out? You can tell even which viruses have to have an enzyme in the virion, by the nature of the genome. And one last family. These are the Retroviruses, they have a plus strand RNA, but they replicate through a DNA intermediate.

Dick:

Say the category again?

Vincent:

Plus strand RNA.

Dick:

So that..., you just said that before though.

Vincent:

This actually..., yes, I know. Now this is where the medical students come up to me afterwards...

Dick:

[laughing] That's right.

Vincent:

... and say "wait a minute, this doesn't make sense, you said that a plus strand RNA should be translated directly", and yeah..., pretty much all the time except for Retroviruses. [laughing] You know...

Dick:

Which is where they get their name from, obviously.

Vincent:

Yeah. That's where David Baltimore and Howard Temin got their Nobel prize from, discovering the enzyme that does this.

Dick:

Two wonderful Virologists.

Vincent:

So these viruses have a plus strand RNA genome. Dick, this plus strand RNA in theory could be translated into protein, but it's not!

Dick:

And so what stops it from being translated?

Vincent:

Now that's a great question. I don't know, I get asked this all the time.

Dick:

Is there a leader sequence that doesn't translate because it doesn't attach to the ribosome or something?

Vincent:

That might be part of it, but these RNAs are translatable *in vitro*, so I don't think that explains it.

Dick:

Ah!

Vincent:

But anyway, what happens is these viruses, contrary to all the logic I just told you, do have an enzyme in their particle called reverse transcriptase...

Dick:

[laughing]

Vincent:

... and when this RNA gets into the cell, this enzyme makes a DNA copy of it.

Dick:

What was the experiment that led up to that discovery, Vince?

Vincent:

Gosh, you know Dick it was such a simple experiment. The rumor is that David was in the lab for six weeks and he finished all the experiments that led to the Nobel prize.

Dick:

Wow.

Vincent:

Well, you want me to really go over it?

Dick:

Sure, because I think the audience needs to know..., how would you ever..., you're dealing with a single plus-stranded RNA virus, it should be doing what the other RNA viruses do...

Vincent:

Right.

Dick:

... and it doesn't do it...

Vincent:

Ok.

Dick:

... so where do you start...

Vincent:

So let's..., have we talked about negative strand..., no we haven't yet.

Dick:

We have not.

Vincent:

Let's do that, and we'll come back to it.

Dick:

Ok.

Vincent:

I don't know if that's confusing, but we need to do negative strands.

Dick:

Right.

Vincent:

So the plus strand Retroviruses, the RNA gets into cells, the viral reverse transcriptase makes a single-stranded, then a double-stranded DNA copy, and then that integrates into the host DNA, and finally these transcribe to form mRNA as part of the host chromosome.

Dick:

Good heavens.

Vincent:

So this is not something that you can predict...

Dick:

No.

Vincent:

... this is the one thing you have to memorize, folks, I'm sorry.

Dick:

[laughing] If you had a visual image it would help.

Vincent:

Well we can supply that, sure. Alright, so let's move to the last category, which is negative strand RNA viruses.

Dick:

Single? Or double?

Vincent:

Single-stranded. Double we've done, we've done the double-stranded.

Dick:

Single-stranded RNA... negative-stranded.

Vincent:

Alright, so can you name a virus with this kind of genome? And I'll give you a hint. [pig sound]

Dick:

It's in pigs..., uh..., hog cholera.

Vincent:

No.

Dick:

But that's a virus.

Vincent:

Yeah, of course it's a virus [laughing]

Dick:

[laughing] At least I knew that part!

Vincent:

Pigs! What have we been talking about for...

Dick:

Swine flu.

Vincent:

Yeah, influenza viruses have negative strand RNA genomes. Can that be translated...

Dick:

They're segmented though...

Vincent:

They're segmented, so there are both kinds, there are viruses with segmented RNAs in pieces like influenza, it has eight, and then there are viruses like Rhabdo-, Rabies virus, where the RNA is one long piece..., or Measles virus.

Dick:

Right.

Vincent:

Now, Dick. Here's the key. If this RNA gets into a cell, can it be translated?

Dick:

The answer is no!

Vincent:

Can the cell make a plus-stranded RNA copy of it?

Dick:

Ahhhh..., he's hinting, he's giving me too much hint here. He's shaking his head no, so I guess I should say no. [chuckling]

Vincent:

Can the cell copy a long RNA? Does the cell have an RNA-dependent RNA polymerase?

Dick:

It does not.

Vincent:

No, it doesn't! So, what has to be in the virion then?

Dick:

Well, it's got to have that enzyme included...

Vincent:

Exactly.

Dick:

... into it. That's why they're so big. Is that right?

Vincent:

No they're not much bigger than many plus strands.

Dick:

Rabies is a pretty big virus though.

Vincent:

You can pack a lot into a capsid.

Dick:

Ok.

Vincent:

But, now you see, you can distinguish a plus and a minus strand virus in that way, one has an enzyme in it...

Dick:

Got it.

Vincent:

The minus strand and the plus generally don't, with exception of the reverse transcriptase-containing viruses.

Dick:

[laughing]

Vincent:

So, Influenza, Measles, Rabies virus, they're negative strand, they have to carry an enzyme into the cell, which will make an mRNA from that negative strand, and then they can make proteins. Ok, so now we can answer your question about David's experiment.

Dick:

Right! What was the insight that allowed...

Vincent:

So he was thinking about this plus and minus strand business and the idea in the 50s and 60s emerged that plus strand viruses didn't have to have a polymerase in the virion and minus strand viruses did. So he did experiments to prove that. He took Vesicular Stomatitis virus, which is related to Rabies virus, he purified virions, he added a little detergent to poke holes in the membrane, and then he added the four nucleoside triphosphates...

Dick:

Mhmmm.

Vincent:

... A, C, U, and G. And one of them was labeled with ^{32}P , and then he said if there's an enzyme in there, it's going to make longer RNAs. And that's what he found. He could just incubate these virions, these cracked virions with some triphosphates and they would make RNA!

Dick:

Hmm!

Vincent:

So he did that for VSV, and it worked, and with Polio you never got RNA because there's no polymerase in the virion!

Dick:

Right.

Vincent:

And then he started thinking about Retroviruses for which there was some evidence that they were getting into the host cell genome.

Dick:

Name another Retrovirus that we didn't know about then.

Vincent:

So he worked on the one that they guy at, uhm..., Einstein discovered in the early 1900s...

Dick:

At Einstein?

Vincent:

Rous. Rous sarcoma...

Dick:

Oh he was at Rockefeller..., Rous was at Rockefeller.

Vincent:

Yeah. Rous sarcoma virus, murine leukemia virus, we didn't have HIV then, which is a Retrovirus.

Dick:

Right, exactly.

Vincent:

That was done with animal Retroviruses.

Dick:

Got it.

Vincent:

So he took purified Retrovirions and he did the same experiment, he cracked them open with detergent, added some triphosphate and boom! he got DNA.

Dick:

Ah!

Vincent:

He didn't get RNA as you did with VSV, you got DNA. So he said this is a reverse transcriptase, published, Nobel prize.

Dick:

I'll be darned!

Vincent:

And, well, we can get him to tell that story better...

Dick:

Well!

Vincent:

But that's basically it.

Dick:

But he had to do a lot of experiments to prove that any..., none of the host components was present in the virus preparation to make sure that host enzyme was not active here.

Vincent:

Yes, absolutely.

Dick:

Which is tough.

Vincent:

Yes, but he did them, and apparently he did them in six weeks...

Dick:

Do did he do a Cesium Chloride gradient isolations of virus...

Vincent:

No I think he used inhibitors that inhibited the cell but not the viral polymerase.

Dick:

Got it.

Vincent:

Ok?

Dick:

Well, you know what Louis Pasteur used to say, don't you? Luck favors the prepared mind.

Vincent:

... the prepared mind, exactly.

Dick:

And David's mind is always prepared.

Vincent:

I like that! Those are our seven classes Dick.

Dick:

Wow!

Vincent:

And now if you just think of mRNA as your goal, you'll know how to get to that.

Dick:

Let's review. Always good for a review. Let me say them and see if I'm correct.

Vincent:

Ok.

Dick:

We have seven different...

Vincent:

Dick, what time do you have to leave?

Dick:

Uhm, soon.

Vincent:

Ten minutes? Because I want to do a few emails.

Dick:

Alright.

Vincent:

But you can review.

Dick:

That's alright. [laughing] I can review some other time. I'll review it the next time I come over.

Vincent:

Because there are...

Dick:

We'll go through emails.

Vincent:

Alright. We have one from Eric.

Dick:

Eric.

Vincent:

"Hi Vincent, Dick, Alan and guest." Well, it's just the two of us today. "I just listened to the latest TWiV and was pleasantly surprised to hear an email from one of my collaborators mentioning our new project to do metagenomic analysis of viromes of North American bats."

Dick:

Ah!

Vincent:

This is Eric who did a podcast with us, remember?

Dick:

Ok, ok!

Vincent:

"In the follow-up from the email from Matt, Vincent and Dick mentioned that limiting the search for viruses to the feces would only provide a partial glimpse of the virome, and you posed the question to us: "Eric and Matt, why are you only looking in the feces?" Well, I am writing to respond to your question. First of all we are not only looking in the feces, but mainly focusing there first. In the past few years several newly identified Coronaviruses have been found in the feces of bat species. And since our background is in Coronavirology, it seemed like a great place to start. In addition, to my knowledge there have not been any published metagenomic studies conducted using bat guano, so we wanted to see what else might be lurking there."

Dick:

Hmm.

Vincent:

"We have also been taking oral swabs, collecting urine and we'll look for viruses in tissues of bats that have died from flying into propellers on wind farms, or as the result of white nose syndrome. Ultimately we would like to characterize the entire virome of different bat species

and begin to determine how viral populations differ between the bat species and understand what changes are required to allow different viruses to spill over into different bat species or jump the trans-mammalian species barrier. We just started collecting samples and our early screens have shown some interesting results. We're planning our first deep sequencing run for later this month. Thanks for continuing to do the show, I love listening, and it seems like the three of you are old friends that I get to hang out with every week."

Dick:
Cool!

Vincent:
"Congratulations to Dick on being appointed Emeritus professor."

Dick:
[chuckling]

Vincent:
"Best Regards, Eric." We have some buddies out there.

Dick:
Hey Eric!

Vincent:
Yeah that's cool, I can't wait to hear about that, Eric, and your collaborator. "Greetings all."
This is from someone called "Axiomatically Atypical".

Dick:
Ha ha ha ha ha!

Vincent:
Do you remember we had an email from Parapatetic Apoplectic?

Dick:
I do.

Vincent:
It's the same person.

Dick:
[laughing] Somebody is in love with words.

Vincent:
Well aren't you?

Dick:
Absolutely!

Vincent:

"On the recent TWiV 46 while discussing virus entry into cells you expressed how great it would be if you could make a video of this phenomenon, but stated that such an endeavor was for another era. Hopefully your video will be of this era." Yeah, we can do it someday, just not now. That's all I meant. "In the mean time perhaps non-Virologists like myself can watch HIV replication 3D-medical animation video on YouTube..." And he provides a link for that.

Dick:

Right.

Vincent:

... "to get a sense of how viruses enter the cells. I wonder if you could comment on the accuracy of this video."

Dick:

Hmm.

Vincent:

"The video description states that it was posted by Dr. Rajadurai who has a free download of said video on his web page."

Dick:

There was one actually at the TEDTalks that I went to.

Vincent:

By...

Dick:

This guy up in Massachusetts known... I'll try to remember his name for next time. It was very very interesting and very detailed.

Vincent:

I haven't looked at this one, but I'll take a look and if it's not good I won't post it.

Dick:

Mmm.

Vincent:

I know there's another one by Barringer which is very good, you can find it on iTunes, I'll put a link to that.

Dick:

Yeah, they're very intensive in terms of the use of software in order to get it to look realistic.

Vincent:

Ok, Gary wrote "Dear Vincent and Dick, the last podcast on viral entry was just great, just as all your previous podcasts have been. I pick up your podcast announcements through Twitter and follow each note as I really can't wait to listen and take notes. Thank you, thank you, thank you for this offering, the best I know of in the wide podcast world."

Dick:

Wow.

Vincent:

"My question: Do we know what happens to viral particles that do not make it out of endosomes, either following medication-induced acidification inhibition or other fail-mode processes? Again, many thanks."

Dick:

Hmm.

Vincent:

Yeah, you know during viral entry many viruses escape the endosome, so they don't get digested when the lysosome fuses and the viruses that get stuck get degraded by lysosomal enzymes, proteases...

Dick:

Sure!

Vincent:

... and RNases and so forth. Judy, a High School Biology teacher sent us a story about bees, and so did Jim, who wrote "here's something more to add to your apiary file". Sent us a link to a paper that just came out in PNAS where a group did metagenomic analyses of bees with various colony disorders all over the country. And they didn't find anything suggestive of a virus infection, but they did notice that the ribosomal RNA in these bees was being degraded.

Dick:

Hmm!

Vincent:

So they wonder if that's indicative of a virus infection.

Dick:

Or maybe some other pollutant.

Vincent:

Some pollutant. Yeah, I don't know. So there's no conclusion from that, so it's a very extensive study, which we'll link to, but not clear why the bees would be having their ribosomal RNA degraded. Of course, ribosomal RNA is part of the ribosome, which is this machine that we need in the cell to make proteins, right?

Dick:
Right.

Vincent:
And which the virus can't encode, it's too big.

Dick:
Yeah.

Vincent:
One more email Dick?

Dick:
Sure.

Vincent:
Joe wrote "I found your podcast when researching the current state of swine flu this past spring for business continuity planning. I've finally gotten around to listening to the early episodes, it's very interesting and enjoyable, thanks for all the great information. I recently drove by one of the many ever increasing flocks of turkeys that have invaded Northern California in the last few years. I live in the East Bay of the San Francisco Bay area. Having just finished episode 1 on West Nile virus, and how hard it has hit crow populations..."

Dick:
Hmmm.

Vincent:
"... it occurred to me that maybe there's some connection between West Nile and the rapid increase in the number of turkeys in the last five years."

Dick:
Aha!

Vincent:
"I've lived in the Bay area for over 20 years and cannot recall ever seeing a turkey here before the last 4-5 years. Now they're everywhere. In my memory this change was after West Nile became an issue in California."

Dick:
Very interesting.

Vincent:
"So my questions are, 1) Do you know any turkeys are resistant to West Nile or mosquitoes..."

Dick:
Hmmm.

Vincent:

"... since they are an American species, I would think they would not be, but West Nile does not appear to impact their numbers at all. Do we know if the rapid rise in turkey populations is a function of them replacing the missing crows in their niche?"

Dick:

Very interesting. You know, turkeys are roadside diners and so are crows. And crows are very efficient feeders of the bugs that knock off your windshield and the front of your cars, that's what they're eating, basically. And so they're just picking up the scraps that you're creating for them. Uhm..., and you see turkeys along the sides of the road also, but turkeys are more green eaters, than they are carrion eaters. I've never seen a turkey picking over a dead animal that's been squashed. So, I would doubt that turkeys are replacing crows in terms of the same niche, however, maybe crows and turkeys are antagonistic towards each other. And maybe crows tend to herd them off, and so, if you eliminate some antagonist, then it opens up the door for a wider range for the particular animal that you're talking about.

Vincent:

Mhmm.

Dick:

By the way, the United States almost had that as their national bird, I think everybody probably knows this one, but Benjamin Franklin suggested the turkey as the national bird, and... [laughing], this country has had a few of them as presidents, even [laughing].

Vincent:

As far as you know, though, they are not, uhm...

Dick:

No.

Vincent:

... susceptible to West Nile.

Dick:

Oh I didn't say they weren't susceptible to the virus, but I don't think they're being killed off by the virus, let's put it this way, whereas crows not only are susceptible, but they die from it.

Vincent:

Now, so clearly they're not being killed off in this area.

Dick:

No.

Vincent:

So either they're not bitten by the mosquito, which is possible...

Dick:

Very possible.

Vincent:

.. or, the virus doesn't grow in them.

Dick:

That's right.

Vincent:

And, so, I don't know the answer.

Dick:

Or, if it grows in them, they don't die. There's a lot of bird species out there that harbor the virus, but they don't get in..., they don't die.

Vincent:

I'm going to look it up on Google and PubMed, we'll see if we find an answer. Ah, "as somebody who spends his days trying to keep our employees and the environment safe, I really appreciate your show, it's neat to see how much we know, and how much there is left for us to learn. I found your information on H1N1 flu very helpful, and updating our avian flu planning documents to the new situation."

Dick:

Oh, wow!

Vincent:

Thanks Joe. Ok, Dick has to run, so let me just give you my science pick of the week...

Dick:

Aha!

Vincent:

... which is a place called "The Big Think".

Dick:

Oh!

Vincent:

BigThink.com. You ever hear of this?

Dick:

I've been on that show.

Vincent:

So there are a series of videos on H1N1 influenza, and one of them is by Peter Palese.

Dick:

Ok!

Vincent:

So I'm going to give some links to those. They're actually quite good, they're giving talks about the virus. One is Peter Palese, another one is Barry Bloom, who you know...

Dick:

He's terrific, yeah.

Vincent:

He's talking about immunization and a couple of other people, so I'll put...

Dick:

Yeah. And I'm also on the big think for the vertical farm.

Vincent:

You are?

Dick:

I am!

Vincent:

Well, I'll have to find that video dude! You know, I can't get enough of Dick Despommier!

Dick:

Ha ha ha! Yes you can, and so can your audience. Come on, this is...

Vincent:

Do you have a pick?

Dick:

I do, I have a pick!

Vincent:

Do you have a science recommendation?

Dick:

Uhm, I've been watching a lot of the Discovery Channel shows lately, and there's a new channel called Planet Green...

Vincent:

Hmmm.

Dick:

... which I like very much. Ahh..., it's a new cable station, and uhm..., it covers all of the things involved with retrofitting houses to make them more energy efficient, and all the other initiatives that are going on out there using Biofuels for instance, to go across the country, and new insulation materials, I saw something last night on the use of recycled tires to make asphalt-like shingles.

Vincent:

Hmm.

Dick:

They have a life span of 80 years. So if you want to sequester carbon, that's a hell of a way to do it.

Vincent:

Yeah.

Dick:

And you never have to redo your roof again for 80 more years...

Vincent:

Sure.

Dick:

... it's really an amazing thing. People are starting to think about how I can reuse something, which is a great thing. So the book that I mentioned once before, "Cradle to Cradle"...

Vincent:

Mhmm.

Dick:

... which fits right into this whole scenario, it seems as though all of these viruses follow that same pattern.

Vincent:

Sure.

Dick:

Cradle to Cradle..., their cradle, not ours obviously, but they recycle everything and they put themselves back together again.

Vincent:

Ok, that is the Discovery Channel, Planet Green.

Dick:

Yep.

Vincent:

You know, speaking of tires, I was down on a artificial turf field and they fill the artificial grass in with these little black pieces to make it cushion their recycled bits of tires.

Dick:

Oh, ok.

Vincent:

My son, he plays Lacrosse and he fills my car with them...

Dick:

[laughing]

Vincent:

... because he gets in the car afterwards and there are all these black things getting on the floor.

Dick:

Ha ha ha! Great.

Vincent:

Well Dick, thanks for joining me today!

Dick:

It's my pleasure, Vince, as usual.

Vincent:

Dick Despommier, Professor Emeritus, he's at medicalecology.org...

Dick:

Yep!

Vincent:

... and we have a website at twiv.tv where you can find show notes. Do send your questions to twiv@twiv.tv. Thanks for joining us, you've been listening to "This Week in Virology". We'll be back next week. Another TWiV is viral.

Content on This Week in Virology (www.twiv.tv) is licensed under a Creative Commons Attribution 3.0 License.

Transcribed by Gertrud Rey