Our second speaker today is Derrick Tom Ray, who's professor of cell biology and director of the Yale Cinema Microscopy Lab, received his PhD from University. California is an expert on quantitative live cell imaging, and he specializes in innovative approaches to microscopy, including fluorescent probes, data analysis, and spatial mapping. Received an NIH director's New Innovator Award and a coffee fellow, today is there talk about his recent work,
NOTE Confidence: 0.7802432
00:00:26.460 --> 00:00:28.542 which is primarily focused on spatial
NOTE Confidence: 0.7802432
00:00:28.542 --> 00:00:29.930 control of membrane trafficking
NOTE Confidence: 0.7802432
00:00:29.980 --> 00:00:31.788 during cell morphogenesis migration.
NOTE Confidence: 0.7802432
00:00:31.790 --> 00:00:32.954 In cilia formation.
NOTE Confidence: 0.7802432
00:00:32.954 --> 00:00:35.270 So Derek, the floor is yours.
NOTE Confidence: 0.8684666
00:00:48.600 --> 00:00:49.010 So.
NOTE Confidence: 0.8072154
00:00:51.670 --> 00:00:55.340 Derek, your music. Yeah
NOTE Confidence: 0.8615084
00:00:55.340 --> 00:00:58.810 OK, great. Let me go back a slide or two.
NOTE Confidence: 0.8072154
00:01:02.760 --> 00:01:05.488 Great, so I’ll be talking bout cilia which
NOTE Confidence: 0.8072154
00:01:05.488 --> 00:01:08.480 have some relevance to cancer and signaling.
NOTE Confidence: 0.8072154
00:01:08.480 --> 00:01:10.965 I’ll be talking basically about a new
NOTE Confidence: 0.8072154
00:01:10.965 --> 00:01:13.298 pathway that we’re discovering and tell
NOTE Confidence: 0.8072154
00:01:13.298 --> 00:01:15.704 you about the ramifications of this.
NOTE Confidence: 0.8072154
00:01:15.710 --> 00:01:18.377 I not talking anybody work on SARS?
NOTE Confidence: 0.8072154
00:01:18.380 --> 00:01:21.992 Kobe 2 but I do have some work on that and
NOTE Confidence: 0.8072154
my conflict of interest is declared there.

So is Sherman sort of a primary?

Let me just tell you the main message in

the main message is that we’re proposing

and characterizing a novel pathway.

Basically the central paradigm is

and then go on to sort of describe to

you that I think that that basically

our data suggests that we have

an alternate pathway here.

OK. Just. OK, it’s clicking differently,

so we first start off with a quick

primer here about primary cilia so you

know really sort of two aspects here.
What are they? And I suppose sort of related here, why do you care? Why should we care? So in terms of the first part, that’s relatively easy there. They’re basically present on nearly all cells. There’s a. The exceptions being red blood cells and T cells. If you go by electron microscopy. End both sort of light microscopy. You see their long and slender. The width is maybe 152 nanometers and they can extend about 10 microns long.
There’s solid Terry organelles their primary cilia and let me tell you a little bit about some of the known functions. So you can sort of think of them as a specialized sensory antenna, so you have an adaptation of this for vision of your rod cells. I guess relevant to right now and and coronavirus they also important for smell. And there is a loss of cilia which is causing the Syrian dysfunction of that and as well as signaling aspects. And we sort of mentioned a little bit more on that because it’s really relevant to this audience. Especially in regards to cancer.
So primary slip, so why?

Why do you care in this?

And the easiest way of sort of thinking of this is thinking in terms of ciliopathies.

OK, there’s a number of diseases that are basically dependent was actually sort of discovered retroactively these diseases have been known for quite a long time, and then only in the last couple of decades was it really realized, in part from pioneering work at Yale.

About the sort of link between
these diseases and disruptive ciliary function so you can see this in sort of the Joubert syndrome or liver cysts kidneys. It’s quite multi organ.

But again, for this audience, I think it was particularly relevant in the context of cancer, because you actually can release vesicles from a cell which can transmit signals, and then your cilia being part of that rule that would turn things on and off. It gets a little more complex here because you actually can release vesicles from a cell which can transmit signals,
so that’s one aspect of it, but you can
sort of think of it in a simple way.
Simplified way of controlling
this signaling on or off.
Now, just to sort of just highlighting
that complexity was sort of a review,
that sort of indicates that it
can go either way.
You could have a certain degree
of activation you could turn on
of activation you could turn on
hedgehog by activation of this
receptor level and sort of pathway B.
Or you could actually do it by
going independent that.
So if you look at cancer is summer activated by the presence of silly, and some are by the repression. OK, but if we sort of. Go beyond that aspect and say well, what is sort of the normal paradigm. Well, the normal paradigm here is that cilia occur once per cell cycle. OK, so you generate them and during G and G1G0 there sort of present throughout and then they have to be assembled during division. So in that sense, if you think of it from that lens, you’re really not controlling except for the point of cell division.
And maybe any delays on whether you would have slid Genesis or not, and I’d like to say, change that paradigm view. So remind you again. I do a lot of imaging as you sort of heard in the intro, and this is just one example of super resolution image Ng where we can actually see this very long structure of cilia, which is, like I said, it could be you know 10 microns or so long with the resolution down to sort of 20 nanometers where you
00:06:18.430 --> 00:06:20.495 could actually see it as a tube.
NOTE Confidence: 0.8284338
00:06:20.500 --> 00:06:22.396 So that’s one sort of technique,
NOTE Confidence: 0.8284338
00:06:22.400 --> 00:06:24.374 but I’m going to show you another
NOTE Confidence: 0.8284338
00:06:24.374 --> 00:06:25.890 source of imaging techniques,
NOTE Confidence: 0.8284338
00:06:25.890 --> 00:06:27.474 and in short of.
NOTE Confidence: 0.8284338
00:06:27.474 --> 00:06:29.850 Exemplify that can provide new insight.
NOTE Confidence: 0.8284338
00:06:29.850 --> 00:06:30.620 Here again,
NOTE Confidence: 0.8284338
00:06:30.620 --> 00:06:32.930 this is sort of the structural
NOTE Confidence: 0.8284338
00:06:32.930 --> 00:06:34.799 aspects of this accident.
NOTE Confidence: 0.8284338
00:06:34.800 --> 00:06:37.696 Sort of in the side, which is,
NOTE Confidence: 0.8284338
00:06:37.696 --> 00:06:39.348 it’s just relatively relevant,
NOTE Confidence: 0.8284338
00:06:39.350 --> 00:06:42.150 is that they can present with this
NOTE Confidence: 0.8284338
00:06:42.150 --> 00:06:44.681 pocket Macy put the laser pointer
NOTE Confidence: 0.8284338
00:06:44.681 --> 00:06:47.183 on and in the ciliary pocket.
NOTE Confidence: 0.8284338
00:06:47.190 --> 00:06:47.593 Here,
NOTE Confidence: 0.8284338
00:06:47.593 --> 00:06:50.414 and this is the sort of shown
extra short here, but this can be a very deep imagination. In fact it can almost be. Nearly entirely evaginated. OK, So what is sort of the key process of how they form? So the general paradigm, in fact there's one for epithelial cells, which I will not sort of discuss, but for most of the cells. In fact the majority, if they start with this silly or vesicle which forms,
and then you have this double membrane

And then you actually have this, really think of it as a super large vesicle. It’s a vesicle.

This vesicle could be 7 microns long, and then as an event it actually has

to infuse the plasma membrane produce. This is a standard paradigm.

Now I want to bring in one other player here and this is one that we’ve worked with quite a bit,
which is called the exocyst complexes, the tethering complex. It was first discovered actually at Yale in yeast, and basically it is known to basically drive the upstream monsters near Fusion machinery to allow spatial temporal control of vesicle exocytosis. This is you can sort of see in. This review has been thought to for quite some while play a role in cilia and mainly ciliogenesis and and stabilization by targeting the vesicles right here where they would
fuse and then basically drive let’s
say control within the accident
that is the standard paradigm.
And while I don’t want to say that is wrong, I think it’s actually missing.
Let’s say 80% of the picture OK.
So we sort of go back to,
you know why we have this paradigm and and
and what sort of the underlying underpinning?
Well, is it? You know I want to
basically first say that is Dre. It’s based on indirect evidence.
We’ve actually not seen the
vesicles their fusing there,
nor actually seen much of the Exorcist.
And I would say that you’re missing something
NOTE Confidence: 0.8069569
00:09:08.490 -- 00:09:10.527 that’s really important and it’s incomplete.
NOTE Confidence: 0.8069569
00:09:10.530 -- 00:09:12.930 But let’s sort of get beyond that and
NOTE Confidence: 0.8069569
00:09:12.930 -- 00:09:15.425 show you what I may be talking about.
NOTE Confidence: 0.8069569
00:09:15.430 -- 00:09:17.677 But first I have to tell you.
NOTE Confidence: 0.8069569
00:09:17.680 -- 00:09:19.655 Why, if we’re claiming to
NOTE Confidence: 0.8069569
00:09:19.655 -- 00:09:20.840 see something different,
NOTE Confidence: 0.8069569
00:09:20.840 -- 00:09:24.542 why and how are we able to do so when people
NOTE Confidence: 0.8069569
00:09:24.542 -- 00:09:27.944 been looking at Syria for quite awhile?
NOTE Confidence: 0.8069569
00:09:27.950 -- 00:09:30.526 And so there are a number of
NOTE Confidence: 0.8069569
00:09:30.526 -- 00:09:32.690 technical aspects to the solution.
NOTE Confidence: 0.8069569
00:09:32.690 -- 00:09:35.578 The first one is we’re using a technique
NOTE Confidence: 0.8069569
00:09:35.578 -- 00:09:38.217 called total internal reflection for us.
NOTE Confidence: 0.8069569
00:09:38.220 -- 00:09:39.214 Since microscopy,
NOTE Confidence: 0.8069569
00:09:39.214 -- 00:09:41.202 let’s say axial superresolution
NOTE Confidence: 0.8069569
00:09:41.202 -- 00:09:44.199 technique and it allows us to image
NOTE Confidence: 0.8069569
just the lower surface of the cell and actually see silly’s emergence OK. But that actually even without superresolution, technique is not enough to be unequivocal there. The distance between cilia and the surface at times is very low, and it’s actually not clear if they are inside the cell or actually have emerged in or outside and think of it again like your antenna, your antenna. If you sort of. Just give an analogy, is going to respond to signals quite differently if it’s actually outside.
00:10:16.168 --> 00:10:18.766 your car truck, what, whatever it be.

00:10:18.766 --> 00:10:20.486 Versus pulled it inside where

00:10:20.486 --> 00:10:22.618 it cannot receive the signals.

00:10:22.620 --> 00:10:25.140 OK, at least not the same signals.

00:10:25.140 --> 00:10:27.373 The other technical aspect is is we

00:10:27.373 --> 00:10:30.034 used a a McLeod clever pH switching

00:10:30.034 --> 00:10:33.059 to identify when cilia are in or out.

00:10:33.060 --> 00:10:35.348 We’re using this as an impulse way and

00:10:35.348 --> 00:10:38.458 we do molecular replacement of the Exorcist.

00:10:38.460 --> 00:10:42.012 The latter was important because for a long

00:10:42.012 --> 00:10:45.167 time people were image in the Exorcist.

00:10:45.170 --> 00:10:47.214 Or just simply overexpressing it and they

00:10:47.214 --> 00:10:49.370 would sort of see localizations like

00:10:49.370 --> 00:10:51.400 this everywhere or some accumulations,

00:10:51.400 --> 00:10:53.638 but when we did this replacement

NOTE Confidence: 0.8069569
strategy we could see it in these
discrete punkte OK and this is now
types of cells looking at vesicle
excess cytosis and we could see in
different graphs distinct events
and I just show you one trace where
vesicle has arrived as we see with the
Exorcist and then with another Reporter.
Any Reporter here which is a floor in
with the pH sensor we can actually
unequivocally identify the Fusion event,
so this is sort of a constitutive pathway.
We know it’s coming from recycling vesicles
and we can identify and study that.
OK, and we can tell the events about when The Exorcist appears and when you have the Fusion fit. This is relevant to the how we're going to be looking at things with the cilia.

So what is it that we see? In I'm really just.

OK, good. OK, so here's a short movie of what we're seeing in terms of cilia, sort of called the Biogenesis aspects, where we see basically.
00:12:03.474 --> 00:12:05.038 exorcist recruited to cilia.
NOTE Confidence: 0.77788836

00:12:05.040 --> 00:12:07.910 So that's sort of standard that itself
NOTE Confidence: 0.77788836

00:12:07.910 --> 00:12:10.268 is is basically showing it going
NOTE Confidence: 0.77788836

00:12:10.268 --> 00:12:13.250 through the base of a long 80 cilia,
NOTE Confidence: 0.77788836

00:12:13.250 --> 00:12:15.515 but there's actually another phenomenon
NOTE Confidence: 0.77788836

00:12:15.515 --> 00:12:18.605 that we observed which is quite different
NOTE Confidence: 0.77788836

00:12:18.605 --> 00:12:21.613 and so now you actually see this cilia
NOTE Confidence: 0.77788836

00:12:21.689 --> 00:12:24.055 with this is the Reporter here is.
NOTE Confidence: 0.77788836

00:12:24.060 --> 00:12:26.524 Smooth and flooring or we can also
NOTE Confidence: 0.77788836

00:12:26.524 --> 00:12:29.260 use smooth and GFP so that looks
NOTE Confidence: 0.77788836

00:12:29.260 --> 00:12:31.260 to sort of characteristic curve.
NOTE Confidence: 0.77788836

00:12:31.260 --> 00:12:33.808 Linear silly and this would be in
NOTE Confidence: 0.77788836

00:12:33.808 --> 00:12:35.809 the dimensions several microns long.
NOTE Confidence: 0.77788836

00:12:35.810 --> 00:12:38.456 And what I'd like you to note,
NOTE Confidence: 0.77788836

00:12:38.460 --> 00:12:41.862 and I'm going to play this more than once,
NOTE Confidence: 0.77788836

00:12:41.870 --> 00:12:44.998 is that we actually see the red signal

21
NOTE Confidence: 0.77788836
00:12:44.998 --> 00:12:47.177 getting recruited to this silly boom,
NOTE Confidence: 0.77788836
00:12:47.180 --> 00:12:49.454 right there off on again off
NOTE Confidence: 0.77788836
00:12:49.454 --> 00:12:50.970 again have several times.
NOTE Confidence: 0.77788836
00:12:50.970 --> 00:12:54.638 This is the time one last time.
NOTE Confidence: 0.77788836
00:12:54.640 --> 00:12:58.213 Is in hours OK, so there’s no signal there.
NOTE Confidence: 0.77788836
00:12:58.220 --> 00:12:59.984 It appears it disappears.
NOTE Confidence: 0.77788836
00:12:59.984 --> 00:13:02.630 It appears again over the course
NOTE Confidence: 0.77788836
00:13:02.708 --> 00:13:04.990 of in this case of this movie,
NOTE Confidence: 0.77788836
00:13:04.990 --> 00:13:06.980 which is basically 4 hours.
NOTE Confidence: 0.77788836
00:13:06.980 --> 00:13:09.278 So in appearing there in this
NOTE Confidence: 0.77788836
00:13:09.278 --> 00:13:11.360 sort of the minutes range,
NOTE Confidence: 0.77788836
00:13:11.360 --> 00:13:14.174 this does not fit with what you
NOTE Confidence: 0.77788836
NOTE Confidence: 0.77788836
NOTE Confidence: 0.77788836
00:13:18.522 --> 00:13:21.310 One is, it’s along the entire cilia,
not just the base.

Two is it appears there.

Goes there and then vanish

is and then comes back.

You know an hour or two later again.

So what’s going on there

and what do we you know?

How can we sort of probe into that with?

I guess you can intend to punt.

So we do this by using.

Again,

we’re using this exorcist and

we look after after stimulation.

See something interesting

happening here and you actually

see it quite really in the movie.
00:13:53.670 --> 00:13:57.250 Let me just play it again and you see Pam.

00:13:57.250 --> 00:13:58.321 Let me just.

00:13:58.321 --> 00:14:00.463 I realize it’s hard to catch.

00:14:00.470 --> 00:14:02.618 Bam you see that green object?

00:14:02.620 --> 00:14:04.410 That’s the vesicle flying off.

00:14:04.410 --> 00:14:06.958 OK, so we see the release of

00:14:06.958 --> 00:14:08.709 the vesicle happening as well.

00:14:08.710 --> 00:14:10.495 Now that’s actually you know

00:14:10.495 --> 00:14:11.923 that part is known,

00:14:11.930 --> 00:14:13.715 but later we actually see

00:14:13.715 --> 00:14:15.143 then the Exorcist here.

00:14:15.150 --> 00:14:18.590 OK, after that we dropped off that signal.

00:14:18.590 --> 00:14:21.206 So basically the SEK 8 which is an

00:14:21.206 --> 00:14:23.388 extra quarter decorate psyllium after

00:14:23.388 --> 00:14:26.298 serum stimulation in vesicle release OK.
And by the way I mean you know we artificially to generate cilia interesting culture, starve them. Of course the normal situation would be in syrup. OK, so. You know, per that sort of model, well, is there any evidence for this? Yes, there have been some papers here and you called it decapitation OK, where it plays off that vesicle? We actually think that the mechanism is going to happen is different? OK they are releasing it,
but you’ll see in our cartoon in the end we’re thinking that it’s happening by a different mechanism.

You can obviously see sort of the importance of that on signal transduction as I was.

Early, basically indicating earlier so.

What do we happen to our RXS reporters after we add cereal?

In this case, the FBS.

And and So what we can see here, you’re sort of the overview slide.

We’re using X-70 as as our Our Exorcist Reporter I MP5 E is a silly Reporter and a couple of different things.
One is now I should mention
for soul this is endogenous.
*** is no longer replaced.
OK so this is sort of native conditions
and what you can see is you can very
clearly see it localising to cilia,
but if you look carefully and I
think you can see in this case here.
And in this case here it actually.
It localizes there but also forms these
additional tubes which are pulling
about look to pee in most movies
would know are pulling out of it.
So what’s going on there?
I will propose to you and submit to
you is that this silly is actually
inside the cell and is actually being remodeled by the X assist to pull off other membranes and remodel it through. Actually have the role of Exorcist being to tether and help fuse this monster huge, you know 510 Micron long vesicle. We you know we basically see this in here sort of another view of that. And again there would be flooring And you smooth in IPP in 5E and these large tubules that are pulling off now. Does this happen when you stimulate with steering? And the answer is yes and if you
look at percent acilia you had

serum they they drop down a bit.

That’s that’s expected.

What is particularly interesting and exciting to us?

Is that the colocalization with extra 70, which is usually quite low, goes up dramatically by adding serum.

So serum is going to turn over this. Also with the cell cycle.

But the important thing is that’s actually driving that recruitment. Now we do some controls here. If we knocked out the other exocyst complex members OK.
00:17:38.360 --> 00:17:39.356 Set an RX-70.

00:17:39.356 --> 00:17:42.147 We do not see that another way of

00:17:42.147 --> 00:17:44.457 saying this is if we destabilize

00:17:44.457 --> 00:17:47.234 The Exorcist so you don’t have your

00:17:47.234 --> 00:17:49.544 entire 8 protein complex and we

00:17:49.550 --> 00:17:51.420 no longer see that localization.

00:17:51.420 --> 00:17:54.396 OK, so that’s sort of a control experiment.

00:17:54.400 --> 00:17:56.260 Then you might well ask.

00:17:56.260 --> 00:17:58.130 Well, OK, so it’s recruited.

00:17:58.130 --> 00:18:01.106 But where is? Where is it being recruited?

00:18:01.110 --> 00:18:02.975 Relative to the Exorcist relative

00:18:02.975 --> 00:18:04.094 to the cilia?

00:18:04.100 --> 00:18:06.844 And I kind of reminded you early on

00:18:06.844 --> 00:18:09.568 that you have this ciliary sheath.

00:18:09.570 --> 00:18:13.168 This sort of a membrane which kind

NOTE Confidence: 0.7813586
00:18:13.168 --> 00:18:16.897 of does this put the pointer on.
NOTE Confidence: 0.7813586
00:18:16.900 --> 00:18:19.717 You know we have the membrane here and then.
NOTE Confidence: 0.7813586
00:18:19.720 --> 00:18:21.280 It’s a double membrane, right?
NOTE Confidence: 0.7813586
00:18:21.280 --> 00:18:23.345 So kind of goes like that and
NOTE Confidence: 0.7813586
00:18:23.345 --> 00:18:25.039 curves all the way around.
NOTE Confidence: 0.7813586
00:18:25.040 --> 00:18:28.210 OK so or you can kind of see it here
NOTE Confidence: 0.7813586
00:18:28.304 --> 00:18:30.356 in this cartoon. And is it here?
NOTE Confidence: 0.7813586
00:18:30.356 --> 00:18:32.547 If this is there or there now?
NOTE Confidence: 0.7813586
00:18:32.550 --> 00:18:34.734 As I there’s sort of different reports,
NOTE Confidence: 0.7813586
00:18:34.740 --> 00:18:36.305 there’s obviously the ones that
NOTE Confidence: 0.7813586
00:18:36.305 --> 00:18:37.557 have done the base.
NOTE Confidence: 0.7813586
00:18:37.560 --> 00:18:39.125 There have been some reports
NOTE Confidence: 0.7813586
00:18:39.125 --> 00:18:40.377 of about being inside,
NOTE Confidence: 0.7813586
00:18:40.380 --> 00:18:42.168 but we actually believe it is
NOTE Confidence: 0.7813586
00:18:42.168 --> 00:18:43.820 actually here along the pocket.
NOTE Confidence: 0.7813586
00:18:43.820 --> 00:18:45.989 And how do we know that by using a
super resolution imaging modality?

Construction limination microscopy and I think you can sort of see it easily here.

You’d have smooth and so this would be basically your cilia Reporter,

Turn off my paper airplane

On the inner membrane,

if you like to look at it that way and we see SEK 8 or X-70 is a wider distribution.

OK, so it is on for going.
00:19:19.590 --> 00:19:21.605 here for going back.
NOTE Confidence: 0.7546144
00:19:21.605 --> 00:19:24.815 It would be on the sillari
NOTE Confidence: 0.7546144
00:19:24.815 --> 00:19:26.770 pocket membrane OK.
NOTE Confidence: 0.7546144
00:19:26.770 --> 00:19:29.720 Maybe switch to pen?
NOTE Confidence: 0.7546144
00:19:29.720 --> 00:19:32.896 So we we see this is tribulus emerging.
NOTE Confidence: 0.7546144
00:19:32.900 --> 00:19:35.716 As I showed you an example and we
NOTE Confidence: 0.7546144
00:19:35.716 --> 00:19:38.418 actually see it in live cell imaging
NOTE Confidence: 0.7546144
00:19:38.418 --> 00:19:40.885 as well were these tubules are
NOTE Confidence: 0.7546144
00:19:40.885 --> 00:19:43.245 dynamically pulling out of Syria?
NOTE Confidence: 0.7546144
00:19:43.250 --> 00:19:46.094 OK so we think it’s actually
NOTE Confidence: 0.7546144
00:19:46.094 --> 00:19:47.990 important for the remodeling.
NOTE Confidence: 0.7546144
00:19:47.990 --> 00:19:48.540 ****
NOTE Confidence: 0.8248625
00:19:48.540 --> 00:19:52.455 It’s a little funny and
NOTE Confidence: 0.8248625
00:19:52.455 --> 00:19:53.388 giving this presentation.
NOTE Confidence: 0.8248625
00:19:53.390 --> 00:19:55.567 Switching back between the pens or not,
NOTE Confidence: 0.8248625
00:19:55.570 --> 00:19:57.818 but let me just tell you to sort
NOTE Confidence: 0.8248625
00:19:57.818 --> 00:20:00.341 of focus on here on the point of
NOTE Confidence: 0.8248625
00:20:00.341 --> 00:20:01.998 the Arrowhead where we actually
NOTE Confidence: 0.8248625
00:20:01.998 --> 00:20:04.252 think of that as a Fusion event
NOTE Confidence: 0.8248625
00:20:04.252 --> 00:20:06.132 so you have this internal cilia,
NOTE Confidence: 0.8248625
00:20:06.132 --> 00:20:08.320 the cilia which is inside the cell.
NOTE Confidence: 0.5048806
00:20:10.130 --> 00:20:14.110 Should. And is is here and you
NOTE Confidence: 0.5048806
00:20:14.110 --> 00:20:16.080 have SEK eight there on it.
NOTE Confidence: 0.8060176
00:20:16.080 --> 00:20:18.048 We think it's important for them,
NOTE Confidence: 0.8060176
00:20:18.050 --> 00:20:19.142 the tethering function,
NOTE Confidence: 0.8060176
00:20:19.142 --> 00:20:21.690 and at this point right here where
NOTE Confidence: 0.8060176
00:20:21.757 --> 00:20:23.906 the membrane it looks like to expand
NOTE Confidence: 0.8060176
00:20:23.906 --> 00:20:26.278 you see that little bit of a burst.
NOTE Confidence: 0.8060176
00:20:26.280 --> 00:20:28.254 At that point, the SEK 8
NOTE Confidence: 0.8060176
00:20:28.254 --> 00:20:29.570 collapses to the base,
NOTE Confidence: 0.8060176
00:20:29.570 --> 00:20:32.266 which has been sort of reported in the
NOTE Confidence: 0.8060176
literature to exist most of the time,
NOTE Confidence: 0.8060176
again at a single snapshot you
NOTE Confidence: 0.8060176
mainly see it at the base,
NOTE Confidence: 0.8060176
but actually if you look at it live over,
NOTE Confidence: 0.8060176
you know it’s called longitudinal.
NOTE Confidence: 0.8060176
You would actually see it.
NOTE Confidence: 0.8060176
Earlier on these internal cilia,
NOTE Confidence: 0.8060176
so we actually think it’s driving that process out.
NOTE Confidence: 0.7073801
OK.
NOTE Confidence: 0.8034458
So well, how can we actually
NOTE Confidence: 0.8034458
sort of prove this unequivocal?
NOTE Confidence: 0.8034458
Blee and the way that we’ve done this is
NOTE Confidence: 0.8034458
basically using a pH switching experiment,
NOTE Confidence: 0.8034458
and when the cilia are outside,
NOTE Confidence: 0.8034458
if we switch the pH,
and we have this pH sensitive Reporter,
every time we switch it, it goes on and off.
OK, so we make acidify, we can turn it off,
and then it goes back on again.
So we basically.
we can tell if it’s outside here,
switching, going up and down,
but as you can see in this context, is not.
It’s actually resistant.
And why is it resistant?
Because inside the cell.
So it is basically inside inside,
indicating this now outside.

OK, so there we can really say.

Quickly that it is is actually merging overtime.

And you can sort of see these experiments were switching pH,

the intensity goes up and down,

Here is a case where is actually not changing much,

the thing fuses.

Right, so it’s not switching much.

It fuses now start switching and then a little bit later you see the

The Exorcist is actually changing

part of it and then it eventually
00:22:23.007 --> 00:22:26.645 drops off and fully into the base. OK,

00:22:26.645 --> 00:22:29.965 so we can start to identify with machinery.

00:22:29.970 --> 00:22:32.938 Is there sort of looking at who might

00:22:32.938 --> 00:22:36.210 be the players that might be engaging

00:22:36.210 --> 00:22:39.482 with The Exorcist and there is some

00:22:39.482 --> 00:22:42.450 interesting ones such as Rab 10 is a

00:22:42.450 --> 00:22:45.677 likely player and actually we see Rab

00:22:45.677 --> 00:22:48.485 10 localising there which is actually

00:22:48.485 --> 00:22:51.600 different from some of the other apps

00:22:51.688 --> 00:22:54.928 so I’d like to sort of end now and their

00:22:54.930 --> 00:22:57.070 remaining minutes with basically are.

00:22:57.070 --> 00:22:59.188 The working model and just walk

00:22:59.188 --> 00:23:01.200 you through that very briefly;

00:23:01.200 --> 00:23:03.450 so the working model is that.

00:23:03.450 --> 00:23:04.479 In this starves,
say you have cilia with these sort of deeply emerged pockets.
The Exorcist is in the facilities recycling endosomes and probably have some targeting of that success here.
In the serums to stimulation, that which is actually the normal case, right?
If you sort of thought about sleep in the body.
There, there actually constantly being remodelled.
so the event of releasing the vesicles actually different than sort of was proposed.
We believe of it sort of coming off from
one that’s fully outside this actually, as it starts to pull in, we believe that it was remodeling that can actually promote this vesicle to release. Once inside you have recruitment of the Exorcist to this large and inside. Which is again majority of this sort of internalize cilia which can remodel, pulling off tubules and consequently then can recycle back. So you have this entire pathway here that can modulate the signaling. So with sort of that this is. Really this is really driven by senior
scientist in the lab Felix Riviera Molina. Sort was reported by the people, but really he took the lead here, so that's where I'd like to end and address any comments that you might have. Thank you for very interesting talk. Are there questions from the audience? Let me put that you can just type them in. While we're waiting, yeah. So there are a lot of genetic disorders of cilia formation that have many different phenotypes or anything associated with increased cancer risk. Yes, there there have been and there were sort of where it gets
NOTE Confidence: 0.8162323
00:25:04.707 --> 00:25:07.269 complicated is it depends on sort of
NOTE Confidence: 0.8162323
00:25:07.342 --> 00:25:10.044 what cell types you’re looking at it.
NOTE Confidence: 0.8162323
00:25:10.050 --> 00:25:12.444 Again, again, you know pushing things up
NOTE Confidence: 0.8162323
00:25:12.444 --> 00:25:15.277 and down and sort of mentioning earlier.
NOTE Confidence: 0.8162323
00:25:15.280 --> 00:25:16.824 So yes, there are.
NOTE Confidence: 0.8162323
00:25:16.824 --> 00:25:18.754 Haven’t been sort of investigating
NOTE Confidence: 0.8162323
00:25:18.754 --> 00:25:21.265 this so much personally but but yes.
NOTE Confidence: 0.9135724
00:25:22.970 --> 00:25:23.520 Thank you.
NOTE Confidence: 0.87619084
NOTE Confidence: 0.7930513
00:25:30.430 --> 00:25:31.690 I know this zoom atmosphere
NOTE Confidence: 0.7930513
00:25:31.690 --> 00:25:33.520 makes it a little bit different.
NOTE Confidence: 0.7930513
00:25:33.520 --> 00:25:36.875 Will. So enjoy when we see
NOTE Confidence: 0.7930513
00:25:36.875 --> 00:25:38.180 people face to face again.
NOTE Confidence: 0.7807439
00:25:47.870 --> 00:25:50.350 Another question then should in.
NOTE Confidence: 0.76829845
00:25:51.560 --> 00:25:52.463 In cancer cells,
NOTE Confidence: 0.76829845

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which are obviously many of them

often constantly proliferating,

do you see abnormalities of

cilia formation you could?

Do they have more cilia?

Do they turn over more rapidly?

What happens?

This is a little bit as alluding to

you can kind of push it either way,

so that’s where that’s actually where the

confusion is to cancer is that you would say,

well, do they have more silly?

Do they have less cilia and basically

the paper review had indicated that

there’s sort of the two sides on it,

so in one case you actually hyper
activate by, let’s say adding smoothing and hedgehogs signaling, so the Hedgehog is obviously a key component, as same as sort of PGF would be basically a hyper activated. The other case is where you would actually activate the signaling by the absence of the cilia. So that’s sort of where it’s you know. In some cancers are driven by having cilia, and some are driven by the absence. Sort of given the this sort of the funny paradox. Depending on that nature of which signaling pathway you’re talking about.
Is it a hedgehog sort of smoothing?
And what is the activation at the Basel State?
So that’s why I think it’s given some complexity to the field because you couldn’t just simply say this is only this week, but it does.
There’s evidence for both there.
Are there other any other questions in the audience?
If not, thank you.
Thank you all for coming.
Thank you for two speakers.
It was very interesting and
00:27:39.535 --> 00:27:41.446 everybody you have a 22 extra

00:27:41.446 --> 00:27:43.370 minutes for your day. Thank you.

00:27:43.370 --> 00:27:45.311 Thank you Dan.