

WEBVTT

NOTE duration:"00:22:28.610000" language:en-us

NOTE Confidence: 0.766857743263245

00:00:00.040 --> 00:00:05.980 In the mail and Department of genetics just wanna get my timer starting.

NOTE Confidence: 0.890445470809937

00:00:07.680 --> 00:00:21.810 So I'm going to talk to you about some recent work. We've done on human papilloma virus infection and I want to turn the lights down so if you want it fall asleep that's OK try not to snore.

NOTE Confidence: 0.356753319501877

00:00:28.230 --> 00:00:29.430 OK.

NOTE Confidence: 0.804810762405396

00:00:30.700 --> 00:00:39.250 So papilloma vocal first off I should say have a disclosure. I'm inventor on a patent application related to this work.

NOTE Confidence: 0.873317360877991

00:00:40.410 --> 00:01:00.410 So papilloma viruses are important human pathogens of these are small and simple viruses with a single DNA molecule genome. The only encode about 10 proteins and like most DNA viruses. They replicate in the nucleus. The very important medically 'cause they cause cancer. In fact about 5% of all human cancer deaths are.

NOTE Confidence: 0.907617568969727

00:01:01.230 --> 00:01:05.980 Cancers caused by these viruses in the most prominent of which is cervical cancer.

NOTE Confidence: 0.91402930021286

00:01:06.940 --> 00:01:24.370 However, if you heard last week, Doug Lewis. There are wonderful vaccines that prevent infection by many of these viruses and we're hopeful that within the next few decades, will be able to eliminate the major cancers caused by these viruses. But we have a long way to go, but many people are not vaccinated still.

NOTE Confidence: 0.835977792739868

00:01:25.380 --> 00:01:45.380 As I said the simple viruses. Here's a picture tomorrow. Particle does not have a membrane envelope is in an envelope virus there. Only 2 proteins in the capsid both coded by the virus. The L one major protein and then the L 2 protein which is so called minor capsid protein's I'll convince you I hope it's actually a very import.

NOTE Confidence: 0.777538001537323

00:01:46.180 --> 00:01:47.880 As I said a DNA genome.

NOTE Confidence: 0.871713519096375

00:01:48.900 --> 00:02:08.900 The bars particles actually a very efficient DNA delivery machine. It does many different things that protects the genome from the elements when it's outside cells, but it binds to cells gets taken up by cells deliveries of genome to the nucleus aside of replication disassembles to release the genome so it can replicate then innovate.

NOTE Confidence: 0.871441185474396

00:02:09.700 --> 00:02:29.700 I'm going to tell you about one particular aspect. We've been focusing on and that is the delivery of the viral genome to the nucleus when we began this work almost nothing was known about this process. In fact, our knowledge to be summarized by this cartoon HD into the endosome the miracle occurs and it gets to the nucleus.

NOTE Confidence: 0.851350963115692

00:02:30.500 --> 00:02:50.500 Here we felt we should be a little bit more explicit in step 2, so we decided to undertake a genetic analysis of HIV entry so Elvis Lapaz. KUSA grass seed in my laboratory conducted in SIRNA whole genome screen to identify cellular proteins required for infection and based on Alex is results.

NOTE Confidence: 0.866280555725098

00:02:51.300 --> 00:03:11.300 Studies done by ways hang in the laboratory of very unexpected picture emerged and that is the virus takes a very unusual. In fact, unprecedented wrote to the nucleus after sinus. I toast it travels through transport vesicles to the Trans Golgi Network. They need the Golgi apparatus. They did to the ER before it finally gets into the nucleus.

NOTE Confidence: 0.8641477227211

00:03:12.100 --> 00:03:32.100 So the viruses is inside membrane bound organelles his entire route to the nucleus. This is probably a good immune evasion strategy avoids cytoplasmic commute sensors, but really is a quite unusual path. This pathway is called a retrograde transport pathway of refer to it as that we got interested in this first step.

NOTE Confidence: 0.830751836299896

00:03:32.900 --> 00:03:52.900 Paris goes from the endosome were many viruses are to the retrograde pathway with nothing. No other viruses known to utilizing this way, and in particular on approaching call retromer, which is a cellular proteins that normally carries other subtle proteins into the retrograde pathway and what's the role of red shimmer.

NOTE Confidence: 0.550272822380066

00:03:53.700 --> 00:03:54.160 Affection.

NOTE Confidence: 0.815885007381439

00:03:55.140 --> 00:04:05.000 So we study this we did some image NG Studies. Initially and ask what's the consequences not be bound rectum are for infection.

NOTE Confidence: 0.832778692245483

00:04:06.210 --> 00:04:26.210 We use them as a Co PLA for proximity ligation assay in this a resident signal is generated if the virus is in a particular organelle. So here we're looking at virus in the end assault as you can see at 8 hours after infection. Awhile type cells virus gets into the endosome acentos. I toast, but by 8 by 16 hours is left in that store.

NOTE Confidence: 0.692673921585083

00:04:27.010 --> 00:04:29.750 Did the endosome and gone to the trans golgi net?

NOTE Confidence: 0.847869396209717

00:04:30.660 --> 00:04:47.980 However, if you look in knockdown cells where he knocked down the rectum are you see a very different picture of the virus gets in just fine, so richer knockdown does not affect internalization. However, it never leaves the endosome. In fact, it accumulates here. So when you knock down the rectum are the virus is impaired horrendous on exit.

NOTE Confidence: 0.803288161754608

00:04:50.260 --> 00:04:59.310 So that's schematically here we knocked on the rectum are the virus accumulation the endosome. It never makes it to these more distal compartments.

NOTE Confidence: 0.849421918392181

00:05:01.380 --> 00:05:21.380 So that suggests that perhaps the the viruses using the rectum are to enter cells in some way we wondered is the virus. A actual cargo of the rectum are so we asked does record bind to the virus. If so I'm going to pay my laboratory looked at the sequence of this L 2 minor capsid protein and found a short sequence here.

NOTE Confidence: 0.832286953926086

00:05:22.180 --> 00:05:42.180 To look a little bit like a rectum are binding site. So we decided to test set, she constructed peptides that covered 3 different sections of the L 2 protein label. The peptides with Barrett in as in did a pull down experiments so she added. Each one of these peptides. This whole extracts pull down with Jeff evident and then blooded forever and as you can.

NOTE Confidence: 0.821504533290863

00:05:42.980 --> 00:06:02.980 Featural peptide that contains this computer binding site. In fact, Lions rectum are very well by these other peptides failed to bind rectum are suggesting that in fact, the virus this segment. In fact does

bind to the sailor trafficking molecule. She then are constructed. Mutations in this binding site or in this site and asked that black.

NOTE Confidence: 0.853111803531647

00:06:03.780 --> 00:06:23.780 And it does so that site is required for binding and with Chris Byrd, a colleague in cell biology. We show the finest direct using recombinant proteins. She then inserted this mutation into virus and ask does it block infection and in fact, it does block this binding site is required for entry and in fact if you take this site the virus Accum.

NOTE Confidence: 0.811010122299194

00:06:24.580 --> 00:06:44.580 Just like what happens if you knockdown rectum are so if you knockdown retromer or mutate the binding site get the same phenotype, namely the virus is trapped in the endosome an infection can progress so this says that in fact, the rectum are deliveries HPV if the retrograde pathway.

NOTE Confidence: 0.867889285087585

00:06:45.380 --> 00:06:52.610 To the nucleus that then raises the question? How does this happen? How does the richer access of HPV.

NOTE Confidence: 0.853076457977295

00:06:53.680 --> 00:07:13.680 This represents the particle we see mostly L, one which forms the shell. The part of the virus and maybe this little nubbin avail 2 sticks out so combine the retromer, but there's a problem and the problem is if the membrane in the way so the viruses in the end is in the lumen of the endosome whereas Arete Rumor is in the cytoplasm.

NOTE Confidence: 0.845494270324707

00:07:14.480 --> 00:07:34.480 Molecules can't see one another so that then let us to propose an unusual model that basically this L 2 protein which is normally part of this non envelope virus is actually able to stick through the the initial membrane protrude into the cytoplasm where combine rectum are and this is a very unusual thing protein.

NOTE Confidence: 0.904893219470978

00:07:35.280 --> 00:07:41.610 This so we thought it was important to test whether or not the L 2 protein actually protrudes into the cytoplasm.

NOTE Confidence: 0.847679197788239

00:07:42.900 --> 00:08:02.900 To do that pink anyways hang in my laboratory set up a split GFP S 8 and the principle is the following or you could take GFP, which is a fluorescent protein, but cut it into 2 parts a short part of only 16 amino acids and then the rest of the protein. Both of these segments are they don't do not fluoresce on their own, however, if they're expr.

NOTE Confidence: 0.864166021347046

00:08:03.700 --> 00:08:23.700 Apartment they can self associate reformed reconstitute GFP and reconstitute fluorescence, so this could be used as a measure for whether these 2 halves of GFP or in the same cellular compartment, so the idea. Then is to fuse this little bit of GFP to the L 2 approaching the end of the L 2 protein that we think for truth.

NOTE Confidence: 0.839676856994629

00:08:24.760 --> 00:08:44.760 And express GFP one to 10 in the cytoplasm, then ask whether or not. We can generate fluorescence. So Pangu made virus particles that contain GFE a fuse to L 2 infected cells. These cells have an engineer to express GFP went to 10, only in the cytoplasm so if L 2.

NOTE Confidence: 0.797439813613892

00:08:45.560 --> 00:09:05.560 Endosome there's no fluorescence, but if this end of L 2 sticks through the end of swimming to the cytoplasm. It can reconstitute GFP and generate fluorescence. So we did. The experiment and in fact, it worked. This shows if we infect cell if you use a reporter cells with GP one to 10 in them if we in fact with?

NOTE Confidence: 0.85010427236557

00:09:06.360 --> 00:09:26.360 Doesn't have GFP 11 there's no fluorescence? But if you fuse L 2 to the end of GFP 11 then you get this nice cytoplasmic fluorescence exactly as you would predict if the L 2 protein is protruding into the cytoplasm. This is true for HPV types 16, which is the most common type in human cancers and what I've talked about so far.

NOTE Confidence: 0.820361912250519

00:09:27.160 --> 00:09:34.260 Valve 16, however, it's also true for HP 5, which the quite distinct HIV type that insects skin.

NOTE Confidence: 0.846405804157257

00:09:35.850 --> 00:09:55.850 So then how does this protrusion occur again as it is a very unusual reaction? Well Penguin look at the sequence of the end of L 2 and here's this rectum are binding site that I mentioned before this al foil sequence represents directly to this, but she notices another sequence downstream here. It's very basic R Ray.

NOTE Confidence: 0.88646924495697

00:09:55.890 --> 00:10:15.890 Presents are getting K Rivers and slicing to basic amino acids and this sequence or a close variant of it is absolutely conserved. In all 400 sequence papilloma viruses, so this emerged. Hundreds of millions of years ago and has been maintained in papilloma viruses. So we wonder. Perhaps that this sequence of might be important for infect.

NOTE Confidence: 0.885495126247406

00:10:17.640 --> 00:10:37.640 In fact, it looks to us to our eyes it looked like a secretly already knew something about with recall cell penetrating peptides. So so penetrating peptides are short protein sequences that can deliver proteins into cells. They were first discovered over 30 years ago, the most common type. In fact, the first identified, was an HIV.

NOTE Confidence: 0.86524909734726

00:10:38.440 --> 00:10:47.500 Similar sequence to the papilloma viruses and these are called cationic. In fact, even probably are Janine can suffice to deliver proteins into cells.

NOTE Confidence: 0.866132259368896

00:10:48.530 --> 00:11:08.530 Uh sell picturing peptides have been widely explored's drug delivery tools to deliver peptides into cells, though, as far as I know they're not actually in clinical use. Yeah, however, despite 30 years of study really don't know what they do in biology. The natural role of CPS is basically very poorly understood and we thought, maybe we stop.

NOTE Confidence: 0.854511022567749

00:11:09.330 --> 00:11:29.330 A real activity of these sequences to deliver the papilloma virus into the retrograde pathway, so first. We asked whether or not the sequence acquired for infection. So he just simply replacing with 6. Allah names and this totally abolishes infection. Not surprising as I said, This is a very conservative sequence. Similarly, 3 Arjun.

NOTE Confidence: 0.842098653316498

00:11:30.130 --> 00:11:50.130 Active in 3, Arginines is not long enough to serve as a good CPP so then we then decided to actually test directly is the sequence does it have cell penetrating activity using the standard assets? Which is diffuse it GFP purify Fusion proteins in bacteria than out of the cells.

NOTE Confidence: 0.847844541072845

00:11:50.930 --> 00:12:10.930 The wildtype sequence with the wild type basic region and then a couple of mutants. And as you can see when you add them to Hackett keratinocytes. The Wild type protein is very efficiently taken up by cells, causing the cells to florists and we're looking here at intracellular fluorescence on the other hand, the 6 a mutant is totally defective for uptake.

NOTE Confidence: 0.837891221046448

00:12:11.730 --> 00:12:31.730 Mutant is almost totally defective is quite in period for uptake in this parallel is exactly the phenotype of viruses that carry these mutations in at the wild type virus. I was the infect cells well. It's a mutant is totally effective for infection in the 3 R Mutant is quite impaired for infection. His Susan with the idea that this segment might be important.

NOTE Confidence: 0.815774917602539

00:12:32.530 --> 00:12:46.690 Infection by acting as itself penetrating peptides, so, based on this we hypothesize the L to self care training peptide drives. The C Terminus of L 2 through the end of cell membrane into the cytoplasm where it's in a position to bind read trimmer.

NOTE Confidence: 0.811687052249908

00:12:47.770 --> 00:13:07.770 We can test that will display GPS so here this shows a split GF. PSA the wild type protein generates fluorescence as shown here. The Westerwelle type virus generates lessons is shown here. But if you take this cell penetrating peptide sequence. You almost entirely block protrusion. So you actually do need the self penetrating peptide sequence in or.

NOTE Confidence: 0.849756121635437

00:13:08.570 --> 00:13:24.790 Rain into the cytoplasm and if you build these mutations into virus at the self penetrating mutant fails to bind rectum are in infected cells as we predicted this in the wrong place doesn't get into the cytoplasm and Furthermore dispute accumulates in the end assault.

NOTE Confidence: 0.884335517883301

00:13:26.640 --> 00:13:46.640 So based on all this week and propose a somewhat coherent model for how this step of infection takes place when the viruses innisai toast. It initially goes into the lumen of the endosome then under the action of the cell penetrating peptide. The protein protrude through the membrane into the cytoplasm where.

NOTE Confidence: 0.850974857807159

00:13:47.440 --> 00:14:07.440 Amber and other trafficking factors that are essential for infection. Furthermore, once this happens. We think there may be a trend membrane domain at the end N terminus of the L 2 protein that Sam Campos described that perhaps anchors L 2 into the internal membrane so this is really is. Remarkable reaction if you think about it.

NOTE Confidence: 0.842905402183533

00:14:08.240 --> 00:14:28.240 Tombola virus that's composed a sizable proteins under some trigger perhaps low PH. The CPP becomes activated allows us to 2 sticks of the membrane bina. Central factors that be converted from a cyber protein into a transmembrane protein so that character in that first cartoon was right. This is a miracle that happens during insect.

NOTE Confidence: 0.840007066726685

00:14:30.120 --> 00:14:50.120 So to summarize this part of the talk HPV traffic through the retrograde pathway to the nucleus of RL sell pantry in peptide transfers. A segment of L 2 from the end of some into the cytoplasm once it's in the cytoplasm. The Seidel Plasmic. Rectum are combined to the L 2 protein and transfer it into the retrograde pathway, therefore issues and uh.

NOTE Confidence: 0.8725705742836

00:14:50.920 --> 00:14:58.450 Mccargo that utilizes a unique mechanism to access the retrograde pathway use works been published in it in a series of papers.

NOTE Confidence: 0.90244996547699

00:14:59.900 --> 00:15:19.900 It also tells us something about what is the role of self penetrating peptides in biology as I said, since 1988. They've been thought to transfer proteins into cells. That's not what this one does rather it transfers. A protein or protein segment from one intracellular compartment into another and possibly conversa cycle protein into a transmit.

NOTE Confidence: 0.895751118659973

00:15:21.250 --> 00:15:39.830 So maybe the reason, people have been able to figure out what these segments do that are looking for the wrong activity. Maybe they don't serve to take proteins into cells, but rather to move proteins around inside cells and perhaps are misnamed and rather than being considered self penetrating peptides really should be called membrane penetrating peptides.

NOTE Confidence: 0.908500730991364

00:15:40.860 --> 00:15:53.820 So, in the last few minutes. I'm going to buy some unpublished work. How can we use this basic mechanistic understanding to imagine a new way of preventing virus infection and tell you about the progress we made and again this is work by Penguin.

NOTE Confidence: 0.79613196849823

00:15:54.800 --> 00:16:14.800 So here's our model L 2 sticks into the cytoplasm binds rectum. Ernest essential for infection. If that's true. We imagine that if we could flood cells with short peptides that buying Rhett rumor that might they might all by in the rectum and titrate retromer away from incoming virus so the incoming vice.

NOTE Confidence: 0.832124352455139

00:16:14.840 --> 00:16:34.840 First would not be able to accept so the first goal. There was to design a peptide that might do this so we designed a short design a short peptide that contains record binding site. That's essential for this idea to work now. The problem with peptide. Therapeutics and they don't get it sells very well.

NOTE Confidence: 0.819135844707489

00:16:35.640 --> 00:16:55.640 Played into cells where the viruses figure that out for us, namely has itself penetrating peptide. So we simply can take a short bit of L 2 that has the greater binding site. This cell penetrating peptide. We would predict this would drive the pep 8, into the cytoplasm where this goodbye in Gresham are in black infection as controls we?

NOTE Confidence: 0.824064612388611

00:16:56.440 --> 00:17:16.440 Built to mutant peptides one with a mutant in the Richmond binding site and another with a mutation in the penetration sequence. Well, this peptide very efficiently blocks infection here. We're looking at infection by wild type. HPV 16 virus at different concentrations of peptide you're very nice dose dependent inhibition of infection with.

NOTE Confidence: 0.841214001178741

00:17:17.240 --> 00:17:34.610 50 year round for micromolar so the peptide does block infection. It doesn't block SC 4 DLC for is totally unrelated bars that does not use restroom are so that's as predicted, and if these doses of our peptide doesn't display any obvious toxicity.

NOTE Confidence: 0.872216999530792

00:17:35.670 --> 00:17:45.150 The next couple of experience I'll show you experience using but this level of peptide around 14 micromolar, which is quite effective as you see here.

NOTE Confidence: 0.854499757289886

00:17:46.060 --> 00:18:06.060 First off we ask does it also block other HPV types? What I showed you before was type 16 but it also blocks type 18 like 60 in a high risk general type and also type 5, which I mentioned before is a skin type so this peptide effectively blocks several cancer associated HPV types and then look at the mutations here are 2 mutation.

NOTE Confidence: 0.822780847549438

00:18:06.860 --> 00:18:16.720 Trading peptide they both eliminate activity 'cause It can't get into cells and this is a mutation in the rec room binding site. This also eliminates activity 'cause It can't find retro.

NOTE Confidence: 0.864256381988525

00:18:18.340 --> 00:18:38.340 So then we wanted to perform the experiments to confirm this proposed model of action mechanism of action so first. We wanted to say does this peptide really prevent finding of retroverted virus and it does here. This is a PLA experiment. So the wild type virus 8 hours after infection you can see nice finding of.

NOTE Confidence: 0.806079924106598

00:18:39.140 --> 00:18:54.360 To the L one purple actually to be able to protein, but you measure L one here, however, if you had the peptide that inhibits infection. You see you eliminate binding of the virus to to the rector, so as predicted just peptide displaces retamar from virus.

NOTE Confidence: 0.826608002185822

00:18:55.560 --> 00:19:15.560 We've also done expresses a wild type peptide. We've also done experiments with mutant peptide with automatic binding site. And this does not displace director as predicted well. This is blocking the way

we think it does. We have a prediction where the virus gets stuck in that is issued accumulate in the endosome just like happens when you take the record binding site and again that's exactly.

NOTE Confidence: 0.801040768623352

00:19:16.360 --> 00:19:28.140 18 hours after infection by this time, the wild type virus has less the endosome. However, if you had the peptide. You prevent endosome exit in the virus that accumulates in the endosome.

NOTE Confidence: 0.838922381401062

00:19:29.060 --> 00:19:49.060 So just as I told you before. This sort of is sort of common Kathleen when you block infection. At this step. We can cause and assemble accumulation and prevent the virus and getting into these distal compartments by knocking down rectum are by mutating the rectum binding site by mutating that cell penetrating peptide so the binding site never guessed into the cytoplasm or now.

NOTE Confidence: 0.819131433963776

00:19:49.210 --> 00:20:01.640 By treating cells with a peptide has a self penetration sequence to get the record by new sites in the cytoplasm so this is sort of. As I said it's a common pathway inhibition.

NOTE Confidence: 0.851376712322235

00:20:03.020 --> 00:20:16.470 So to summarize this why I showed you as at AC terminal L 2 peptide specifically inhibits infection by several HPV types, but not unrelated viruses. In addition, requires an attack binding site and install penetration sequence.

NOTE Confidence: 0.833694398403168

00:20:17.870 --> 00:20:37.870 The L 2 peptide inhibits the interaction of incoming HPV to the restroom are in this causes HPV to accumulate in the end. It's all so we think this may at some point be really a new way of preventing virus infections new paradigm for antiviral activity. There are very effective antiviral.

NOTE Confidence: 0.839960992336273

00:20:38.670 --> 00:20:58.670 No most of them work the vast majority by inhibiting viral enzymes so for example, in case of HIV. We have inhibitors up block reverse transcriptase protease and integrates they're very effective in combination therapy. There's some inhibitors that block binding viruses to cells. There are some that block disassembly viruses such as amantadine with influenza virus.

NOTE Confidence: 0.854802191257477

00:20:59.470 --> 00:21:10.450 That this may be a new way to block infection, namely to inhibit intracellular trafficking the virus. So it doesn't get too excited replication by blocking the binding to an essential trafficking factor.

NOTE Confidence: 0.823685944080353

00:21:11.910 --> 00:21:31.910 So this is a Roach motel approach the virus checks in the endosome that doesn't check out so just close by acknowledging that people did all the work Alex took the leap into HIV entropy never study entry before Alex SIRNA screen using eminent biology graduate student that is terrific job and work with ways.

NOTE Confidence: 0.793131828308105

00:21:32.710 --> 00:21:52.710 To illuminate the retrograde pathway, Andrea Post Doc in the lab showed that HPV HBL 2 bikes replica rectum are nest important for Anderson, exit an infection, then really a lot of praise to ping ways. Ang post doc in the lab. Currently, who discovered this cell penetrating peptide develop display GPS say.

NOTE Confidence: 0.856883525848389

00:21:53.510 --> 00:22:05.290 I told you I had envisioned a terrific job. I haven't had time to talk about John's work. But he is now looking at more mechanistic detail and identified proteins that regulate rectum are function and block infection.

NOTE Confidence: 0.827716410160065

00:22:06.450 --> 00:22:25.090 Gabriel work limit with paying way do some sequence analysis and we did a terrific collaboration with Chris Burden. Some of our retro more work and this is supported by grants in the NCI in the National Institute of allergy infectious disease. So I'll stop here and I'm very happy answer questions. Thank you.