

WEBVTT

NOTE duration:"00:27:20.3650000"

NOTE language:en-us

NOTE Confidence: 0.871407806873322

00:00:00.040 --> 00:00:06.450 For the opportunity to share with you, some of our my labs new findings and.

NOTE Confidence: 0.896198391914368

00:00:07.300 --> 00:00:39.610 My lab is basically interested in this recently discovered complex, which is a transcription pre initiation complex that regulates transcription of a specific class of viral genes through understanding this complex. We can actually inhibit production of Epstein Barr Virus, which is production of new virus particles of Epstein. Barr virus, which is as you all know is an uncle. Genic herpes virus this actually these findings are also applicable to another.

NOTE Confidence: 0.853259205818176

00:00:39.610 --> 00:00:51.300 Hope this virus, which is capozzi sarcoma herpes virus that has the same complex so keep that in mind. As I'm presenting the data and I'll show you some of these.

NOTE Confidence: 0.820711076259613

00:00:54.300 --> 00:00:56.850 OK, I'll just use my.

NOTE Confidence: 0.564940333366394

00:00:58.590 --> 00:00:59.070 OK.

NOTE Confidence: 0.880438804626465

00:00:59.930 --> 00:01:32.540 So as you all know herpes viruses are sweet subfamilies or purpose. Viruses Alpha Beta and Gamma and oncogenic Opus Viruses fall into the gamma herpes virus so you have the Kaposi Sarcoma herpesvirus against HV and the evv like all herpes viruses. They all have these latent state where very few jeans are expressed and this is the dominant phase of the predominant phase of the viral lifecycle, but there's also this lytic phase that we're trying to understand where the virus, which is from this.

NOTE Confidence: 0.806329131126404

00:01:32.540 --> 00:01:48.240 Latent to the lytic phase and during the lytic phase that's a phase where most of the viral jeans are expressed virus particles are assembled and their release to infect new cells and new tissue and.

NOTE Confidence: 0.861587405204773

00:01:49.500 --> 00:02:14.560 In EV must of the avian facts basically epithelial cells and Diesels. These herpes viruses are actually large compared to other

viruses or there. About 100 and EBV. It's about 172 kilo basepairs and almost 90% of individuals or more than 90% of the human adult human populations. In fact, it was EBV.

NOTE Confidence: 0.856634140014648

00:02:16.410 --> 00:02:31.130 So a BB is associated with several diseases couple of these are images. Etiological agents like Asian for these diseases. Like Infectious, Conan excuses and over here, we look like here, but it's also associated with.

NOTE Confidence: 0.72877562046051

00:02:31.790 --> 00:02:37.950 Uh was several Lynn Formazan, Carcinoma, such as Burkitt Lymphoma.

NOTE Confidence: 0.847778081893921

00:02:38.890 --> 00:03:00.450 Such as Burkitt Lymphoma Hodgkin's disease nozle for Angel Carcinoma Gastric Carcinoma. PTLDN is associated. Lynn former some of these informers such as Burkitt Lymphoma and user Fraser. Carcinoma has this interesting geographical distribution that we still don't understand why exactly this is the case.

NOTE Confidence: 0.884762346744537

00:03:00.970 --> 00:03:32.090 Basically my lab is interested in studying the lytic cycle and we strongly believe that the lytic cycle is a major contributor to the development of cancers associated or malignancies associated with EBD and so let me just present some couple of points that highlight why we are interested in the lytic cycle. For example, you'll see that lytic infection proceeds development of ABV associated diseases that's true.

NOTE Confidence: 0.841763198375702

00:03:32.090 --> 00:03:58.080 The case of Hodgkin's disease in the case of of Burkitt Lymphoma Nasopharyngeal Carcinoma and definitely for PTLN and in the case of MPC or nature venture carcinoma and PTLN you'll see that we use high viral load and elevated antibody titles, too, as predictive markers for the development of these tumors.

NOTE Confidence: 0.861665487289429

00:03:59.170 --> 00:04:29.200 In terms of lab and research in a humanized mice. If you in fact, a mice with a wild type virus our versus a virus at where you knock out the gender thing that encodes a protein responsible for transitioning device from the latent to the lytic state. You'll notice that the knockout. The gene was a knockout virus actually has significantly less Bissell Lymphoma Development in these mice so suggesting that.

NOTE Confidence: 0.860156714916229

00:04:29.200 --> 00:04:35.090 The transition into the lytic state is important for B cell lymphoma development.

NOTE Confidence: 0.883924961090088

00:04:36.030 --> 00:05:07.080 And we don't really understand why exactly the lytic cycle is important. So far, but there's several hints that we can deduce and one of these that if you produce a lot of virus suppliers is likely to infect more cells and that increases the possibility that device will one of these cells will turn Logan become new plastic. Afterwards, the other possibility is that these lytic infection or a lytic jeans, they encode.

NOTE Confidence: 0.830419421195984

00:05:07.080 --> 00:05:38.150 They actually induce expression or activation of several cited kinds and In addition, they also express site to cancer in the case of ABV for example, it ABB expresses viral. I'll turn which is immune suppression and the case of cases V it expresses via line 6. So there are several proteins or products of this little this little product that could.

NOTE Confidence: 0.88739150762558

00:05:38.150 --> 00:06:02.410 Be potential for progression and so forth and so we really interested in understanding the lytic lytic infection will interested in understanding how the virus produces new virus particles and if we can actually block that then I think we have achieved something interesting so.

NOTE Confidence: 0.904368042945862

00:06:03.030 --> 00:06:21.780 This is just a quick summary and the point of the cascade of events that take place during the lytic cycle and what the reason I'm showing this is to show you is to talk about these what we refer as structural proteins so.

NOTE Confidence: 0.914831042289734

00:06:22.420 --> 00:06:34.890 Structural proteins are expressed in this particular stage of the viral lifecycle so the events that take place during political faction. You have these 2 transcription factors one is called zebra.

NOTE Confidence: 0.865618109703064

00:06:35.450 --> 00:06:38.220 And the other one is called RTA.

NOTE Confidence: 0.849413573741913

00:06:38.800 --> 00:06:39.820 Have another.

NOTE Confidence: 0.774729549884796

00:06:40.950 --> 00:06:41.940 Pointer here.

NOTE Confidence: 0.856616199016571

00:06:48.030 --> 00:07:23.040 Not so 2 transcriptions factors one is called Zebra and the other was other one is called RTS. These 2 transcription factors activate transcription. An expression of downstream jeans, which we refer to as early jeans. These origins encode mainly encode proteins that are involved in in viral DNA application as a result virus replicates its genome and then you get the stage of lay gene expression. This is a stage that results in expression of these capsid proteins and glycoproteins.

NOTE Confidence: 0.901971399784088

00:07:23.040 --> 00:07:33.660 Which is really essential for the virus to progress and and produce new virus particles and that's a stage we're really interested in so.

NOTE Confidence: 0.869727432727814

00:07:44.200 --> 00:08:14.440 What you can see here in this diagram basically this is hope is virus particle and this part here? Are these all these capsid proteins that are that protects the DNA inside of it. So it's it forms like a capsule with the DNA inside of it and then on the outside. Here are these glycoproteins and the region between the capsule and the envelope here is a stagnant area, which basically.

NOTE Confidence: 0.889877676963806

00:08:14.440 --> 00:08:34.580 Pax certain proteins at the virus when it enters a cell it unloads these proteins and it basically takes control over so most of these proteins that you're looking at you are proteins encoded during the late fees or we refer to them as legions and this is the face of the viral lifecycle that we're really interested in because.

NOTE Confidence: 0.916492998600006

00:08:35.390 --> 00:09:05.570 There's a lot of research done on the early events that are happening, but very little research done on the late fee. So the mechanisms regulate transcription of legends is really not well understood and that support that we're trying to understand so about. Maybe it years ago. I became interested in this QT and it's called visual of 4. This protein is a viral protein kinase and it's important for the process and we don't know what the function of this.

NOTE Confidence: 0.886725187301636

00:09:05.570 --> 00:09:35.750 Viral protein kinase, but I did this experiment and what you can see here is let me. Just go through the gel itself, so if you provide an empty vector, which is a CMV here denotes the promoter that we're using nothing happens. These are latent cells. So you don't get any activation. If you provide this protein called zebra, which is one of the transcription factors. I showed you at the beginning, they were activates a lytic cycle and it activates expression of.

NOTE Confidence: 0.859654009342194

00:09:35.750 --> 00:09:45.310 Visual for which is put in tinys. I'm talking about and it activates expression of this quoting called Fr. 3. This is a late protein is part of the viral capsid.

NOTE Confidence: 0.845863699913025

00:09:46.110 --> 00:10:00.030 If you knock down expression of visual of 4 using what I called here is SIN A4G4 or 4B GL 4. You see that the bill for protein is not as Markley expression of the visual for protein is Markley reduced.

NOTE Confidence: 0.905077457427979

00:10:00.700 --> 00:10:17.980 And there's the reduction here also results in significant reduction in the amount of protein of late protein and this was basically the first indication that we've seen where we can see an effect by knocking down one protein and you see an effect on Legion expression.

NOTE Confidence: 0.884619772434235

00:10:18.530 --> 00:10:51.400 We did this trick, which I'm going to be using the rest of the talk where we demonstrate that the S Army is really specific we mutate the sequence in the visual for sequence on a plasma so that we provide synonymous mutations and these mutations are not recognized describe the capacity of the SRD to recognize the actively expressed visual 4 and now we can rescue the defect we can provide these assign a together with their existence.

NOTE Confidence: 0.863353967666626

00:10:51.400 --> 00:11:22.060 Form of visual for here and you will store expression of legions so from this we concluded that visual for is really important in the process of transcription or synthesis of late products, but that's only one product. So we did an RNA seq experiment and uses SIRNA that we developed and looked at the whole viral genome and in this area and this figure. I've labeled the legions.

NOTE Confidence: 0.854152858257294

00:11:22.060 --> 00:11:34.980 In red and you can see that most of the jeans that were affected. When you knock down visual of 4 are actually legions except for these 2 jeans here to Tran Scripps here? Which.

NOTE Confidence: 0.885746955871582

00:11:37.130 --> 00:11:59.520 We're also affected and these 2 transcript. They belong to a gene called visual of 3 and another one called busy out of 3.5 for the sake of time, 3.5 is not involved in Legion expression. But visual 3 is involved in Legion expression. Let me show you the data so again if you provide zebra. This is an early gene.

NOTE Confidence: 0.857586205005646

00:12:00.050 --> 00:12:23.910 You don't you activate early jeans and you activate Fr 3:00. If you not down visual of 3. This other protein. You don't get Legion expression. But you get early gene expression so at this point, the phase. The phase before viral DNA replication. And including why the inner application. Everything is happening, except the late phase of the lytic cycle.

NOTE Confidence: 0.867622673511505

00:12:25.360 --> 00:12:48.070 And again to confirm that this is really this Sr nail specific we use this assign a resistant form of visual 3 and you provide this form of visual treat that cannot be degraded by the SRNA and you will store the gene expression one more time so just to summarize at this point we have identified 2 proteins.

NOTE Confidence: 0.89626282453537

00:12:48.780 --> 00:13:12.930 These are virally encoded proteins. This was the first time to show that these proteins are regulators of Legion expression. One of these proteins is called BG LS3 and visual 3 has no identifiable domains at all. The other protein is called visual of 4, which is a protein kinase and we were the 1st to show that these 2 proteins are important in the process of the gene expression.

NOTE Confidence: 0.885208010673523

00:13:13.640 --> 00:13:43.730 Now at the same time, several other groups have identified other Legion regulators and from this slide. I just want to quickly give you an update of what we know about the process of Legion expression or this complex and our new finding which I won't have time to go through all of them so the first protein that binds to late promoter Soleil promoters has this unique structure, it has this TATT.

NOTE Confidence: 0.807026922702789

00:13:43.730 --> 00:14:01.600 Sequence. So, your regular data boxes a TI-80 aids have T att. These are predominant in legions that Y att sequence was actually identified here and at yell by George Miller's lab and by student called Trisha Cereal.

NOTE Confidence: 0.870209693908691

00:14:02.130 --> 00:14:13.020 And many years later, French group identified this protein that binds to the T att so this. This is basically 8 at a box like protein.

NOTE Confidence: 0.846022307872772

00:14:13.720 --> 00:14:39.400 Uh we found that among all these proteins, so this is some of our new findings that BCR Fund is only protein that has the highest affinity to RNA polymerase 2. So we believe the BCR formula codes are the problem is too. But we also found that this protein called BG LLC that we discovered actually recruits when it interacts with BCR front. There's a lot more of only polymers to that comes down.

NOTE Confidence: 0.879949688911438

00:14:40.900 --> 00:14:53.800 And then we found that visual suites phosphorylated and phosphorylation is important for recruitment of two other proteins in the complex protein called BF Two and another one called VL F1.

NOTE Confidence: 0.848328590393066

00:14:54.540 --> 00:15:06.250 And the equipment of these 2 proteins results in the recruitment overt sort or another protein called BDLF 3.5. These are all Cormier presentation experiments that we've done.

NOTE Confidence: 0.798908531665802

00:15:07.060 --> 00:15:12.910 And then this other protein vidiella for NOX with busy with VGL of 3.

NOTE Confidence: 0.849108159542084

00:15:14.080 --> 00:15:49.090 If you just look at this complex, you notice that BG LS. We is a hot protein or scaffold cooking. It basically organizes the interaction of all these other proteins and one more protein that basically interacts with visual suite is the kinase. The PGL of 4 kinds and we found that this kind is can actually facilely. The C terminal only polymers too. And we all know that the C terminal of only polymer is too fast violation of the system of RNA polymers do is essential for the process of transcriptions are 2 kinds.

NOTE Confidence: 0.832550466060638

00:15:49.090 --> 00:16:19.960 Is a CD K 7:00 and CD K9 that are involved in fast violation of on a promise to and these kinases inhibitors for these kindnesses had been the subject of the topic of several trials in cancer and cancer surfy so the question now is the interesting experiments are currently ongoing in the lab, but the interesting part is that if you have visual for expressing the cell.

NOTE Confidence: 0.884933650493622

00:16:19.960 --> 00:16:48.420 And you're trying to inhibit CDK 9:00 or CD7 may be busy or 4 can actually substitute for the functions of these cyclin dependent kinase is and at this point we don't know if this inhibitors would inhibit visual for not so it's just one of the interesting observations that we currently have so this is just a summary for what I'm going to be not. I'm going to. I'm not going to be talking about all of this 'cause it's a lot but.

NOTE Confidence: 0.8757364153862

00:16:49.040 --> 00:17:16.010 We refer to this group of proteins at Legion regulators and I'm not talking about all these proteins. I'm only going to focus on one point, which is that visual 3 is actually phosphorylated and fast, foolish is important for equipment of these 2 proteins. So we have a single phosphorylation site. If you abolish this single fast violations basically abolish expression of legions and production of new virus particle.

NOTE Confidence: 0.878792703151703

00:17:17.610 --> 00:17:21.780 So the question, we have does phosphorylation regulate senses of Legion products.

NOTE Confidence: 0.857626020908356

00:17:22.730 --> 00:17:51.770 And we started this project by doing mass back. We purified visual 3 from evv infected cells in the politically infected cells and then we did first Wayne Richmond followed by mass back and we identified the 3 mean here. It's winning 42 as a first violated residue the experiment was done, 7 times and each of them we identified fast relation at 2042.

NOTE Confidence: 0.884341716766357

00:17:52.340 --> 00:18:02.540 This residue is actually quite interesting because it's conserved in all herpes viruses. So you see here. The training and it's swinging 42 and all herpes viruses.

NOTE Confidence: 0.870576620101929

00:18:03.470 --> 00:18:09.080 And particularly in this uh another uncle genic herpes virus capozzi sarcoma herpes virus.

NOTE Confidence: 0.85713404417038

00:18:09.810 --> 00:18:39.860 So we wanted to know what's the importance of this first violation and here I'm using a we're using 2 different cell lines. A Burkitt Lymphoma Salina. Jessicas enormous online and if you these are naturally infected cells and if you knock down the endogenous visual 3. You see a reduction in this viral capsid protein. BFR 3, but no effect on the early gene so it's specific to legions.

NOTE Confidence: 0.872410833835602

00:18:40.420 --> 00:19:03.890 Uh you can you ask you the suppress effect of the SRNA by providing this resistant form of visual 3, which is wild type form. But if you muted. The Wild type from the introduced screening 42. A mutation you see that there is significant reduction in the amount of effort, we put here. This is also true in these gassy carcinoma cells.

NOTE Confidence: 0.878084123134613

00:19:07.190 --> 00:19:37.810 As I mentioned this complex works in transcription, so it's a viral communication complex that regulates transcription of Lee jeans and a specific for Legion. So if you look at at this early age in here. This is viral lytic jeans express at the early stage. You can change screen in 42 to Allen in but nothing happens. If you look at these for late transcripts, changing 3 and 42, ETA Allen in significantly reduces expression.

NOTE Confidence: 0.870918333530426



00:19:37.810 --> 00:20:04.040 For transcription of these jeans, so we've identified a single phosphorylation site that basically abolish it seems to abolish transcription of late jeans. We wanted to look at viral DNA replication, so as I mentioned events that take place before DNA before DNA replication early events an to check whether all the early events are intact. We look at viral DNA replication. That's one of the ways we do it.

NOTE Confidence: 0.883547842502594

00:20:04.570 --> 00:20:29.620 And you can see that when you knock down T42A basically unlimited T42A nothing happens. But if you look at the amount of virus produced when you change the 3:00 need to 42 to 2042 to alanine. You basically abolish production of new virus particles so it's single phosphorylation site abolish its production of new virus particles.

NOTE Confidence: 0.88248598575592

00:20:30.570 --> 00:21:00.580 We wanted to know why, why is this single phosphorylation important for transcription of these jeans and mechanistically? What exactly is is is happening and the first thing we started doing was to the first thing we started doing was basically to do these pairwise communication between visual 3. The Wild type and the mute and ask can interact with other components of the complex.

NOTE Confidence: 0.823204100131989

00:21:00.580 --> 00:21:06.510 But all these Pairwise Camino presentation experiments did not work and we

NOTE Confidence: 0.903844714164734

00:21:07.260 --> 00:21:26.080 Basically came up with this idea, which I think is an interesting idea would trying to rescue the defect. Anti 42 by overexpressing the other components of the complex and see whether the other components of the complex can actually shift equivalent to.

NOTE Confidence: 0.856399714946747

00:21:26.830 --> 00:21:32.110 Result in some kind of partial rescue of the T42A Mutant.

NOTE Confidence: 0.897425055503845

00:21:32.650 --> 00:21:36.590 And so, if you look at the at this experiment here.

NOTE Confidence: 0.900021970272064

00:21:37.630 --> 00:22:07.640 This is a T42A mutant you don't get any hardly get any viral capsid protein expressed the BFR 3 protein. But if you overexpress the other components of the complex. You start seeing significant expression of of Legion, suggesting that these components. Additional components can suppress the effect of the T42-AB and so that was very interesting because we wanted to know which component.

NOTE Confidence: 0.816328585147858

00:22:07.640 --> 00:22:11.230 Can do it can basically rescue the defect?

NOTE Confidence: 0.868478536605835

00:22:11.740 --> 00:22:41.710 And we did all these experiments with I'm showing you some of them. So here in this experiment, we again. We expressed all the neat lady and regulators here, but now we started excluding one at a time and we notice that if we exclude vidiella 4 BCF. One nothing happens. But if you exclude these 2 proteins. BFF too, and BVL F1. You don't get the rescue, so we asked a question whether.

NOTE Confidence: 0.866882622241974

00:22:43.540 --> 00:22:46.420 Can these 2 proteins by themselves?

NOTE Confidence: 0.876894772052765

00:22:47.130 --> 00:23:19.540 We store expression of legends and if we provide a different combinations of these. Legion regulators 2 at a time and you can see the BF2MB VL F1 are the only two proteins that we showed earlier important that are the only two proteins that are essential for rescuing or suppressing the phenotype of D42A. So this suggested that maybe the first violation basically interacts with with these 2 Putin's and so we resorted back to.

NOTE Confidence: 0.838019073009491

00:23:19.540 --> 00:23:22.340 Camino presentation, but at this point we?

NOTE Confidence: 0.876977562904358

00:23:23.210 --> 00:23:26.550 Expresa complex altogether over the 3:00 proteins together.

NOTE Confidence: 0.876999020576477

00:23:27.170 --> 00:23:41.390 And just to show you here. If you focus on these 2 lens here. This is a wild type #6 and #7 is the mutant and we expressing the 3:00 proteins BFF to visualize 3 and BVL F1.

NOTE Confidence: 0.832023084163666

00:23:42.320 --> 00:24:14.030 And you'll see here in the wild type when you pull down with visual of sweetie. Pull down BF2 and you pull down BVL F1, but the mutant is defective interacts finals BFF too. But it's defective in pulling down BVL phone and this is a densitometry of these bands and you can see we believe that phosphorylation here is important for recruitment RB elephant. If you meet it aside. The first relation site. Then you don't get VVL fun to be part of the complex. Beeville phone cannot be recruited to this complex.

NOTE Confidence: 0.869182109832764

00:24:16.170 --> 00:24:36.530 So, in summary, we started basically by looking at we interested in these Lee jeans or these jeans that encode structured viral structure proteins capsule proteins. The glycoproteins and these proteins are essential for the novel infection and so forth.

NOTE Confidence: 0.870074570178986

00:24:37.070 --> 00:24:52.070 And I production of virus particle and we identified 2 proteins. One is a kinase an another protein that we refer to as visual three that doesn't really we don't really have understand the function of this proteins or no identifier book domains or anything like that.

NOTE Confidence: 0.863470196723938

00:24:52.770 --> 00:25:24.740 And so we started looking at visual three, was a visual sorry. We did a lot of common occupations. But we wanted to focus on visual sweet and we found the visual suite is phosphorylated and that fast related at a single site 3 name 42 few mitted. This site to alanine. You don't get any transcription of Lee jeans. You don't get any production of this oncogenic herpes virus. This site is also conserved in case HV and we did all these topic expressional complementation experiments to understand.

NOTE Confidence: 0.854418218135834

00:25:24.740 --> 00:25:54.790 The dynamics are mechanism of interaction between the phosphorylated form of visual 3 and other components of the complex and we found that that visual sweet basically interacts with these 2 proteins. VVVVLA fund and BFR F2 and if you abolish phosphorylation, then visual. She doesn't interact with these 2 proteins and so we have several future directions.

NOTE Confidence: 0.852668404579163

00:25:56.040 --> 00:26:28.610 One of these and here is a direction, so T 42 regulate the function of the pick. Another herpes virus. I showed you that case. HV has exact same residue is it fast related or not an is it important in expression of the issues or not the most important question, which kind is first fully visual of 3 and also other components of the complex are they also phosphorylated as a modified somehow is there any other ubiquitin ated or anything like that so that's something?

NOTE Confidence: 0.896633863449097

00:26:28.610 --> 00:26:49.150 Actually, looking at and then we understand that via DNA replication is important. So I didn't really talk much about that. But we know that by the application is essential for the process of Legion expression and the question is what is the link between viral DNA replication and expression of Lee jeans so?

NOTE Confidence: 0.873013138771057

00:26:49.810 --> 00:27:01.550 Was that I would like to thank people? Who did the work and Lynn Leigh Anne Anne Walsh in my lab and previous lab members definitely want to think George Miller for support.

NOTE Confidence: 0.885090112686157

00:27:02.070 --> 00:27:18.740 And other people here at the land outside and I liked saying to thank the American Cancer Society and the Yale Cancer Center for support. I started this project with a pilot grant from the yell counts with something so I'm very grateful for that and thank you for your attention.