WEBVTT

NOTE duration:"00:18:16.5360000"

NOTE language:en-us

NOTE Confidence: 0.701435923576355

00:00:00.030 --> 00:00:02.320 Can speaker today is Joanne Sweetie?

NOTE Confidence: 0.861575365066528

00:00:02.850 --> 00:00:23.300 And again Joanne, someone who started in a different area. She's an enzyme ologist studying DNA polymerase is they got interested in the whole question of DNA mutagenesis and repair its effect on cancer and even more respect an autoimmune disease. So it's a quite a broad spectrum issues. The instant professor of therapeutic radiology in one of our.

NOTE Confidence: 0.882764220237732

00:00:23.860 --> 00:00:45.790 Many leadership roles in the Cancer Center, which is why this is sad, 'cause Joanne is leaving. She is dis. Dis recently announced that she is going to University of Arizona to take on a major leadership position at their Cancer Center and Joanne. Despite that is agreed to talk today. But this is a chance for us to thank you. Again, for all their service to yell and best of luck going forward. Thank you.

NOTE Confidence: 0.927065134048462

00:00:47.690 --> 00:01:01.310 Alright, it's a pleasure to give a talk to you today and I'm actually going to go back to my biochemical roots. I'm going to talk about some mechanistic insights into a mutator phenotype and cancer.

NOTE Confidence: 0.900517225265503

00:01:01.900 --> 00:01:21.370 And this is just to say that were funded a little bit by Abby but what I'll be talking about today. In The Talk has nothing to do with the app V funding. So this is one of my favorite molecules. I have 2 favorite molecules. One of them is rec. A protein and the second. One is DNA polymerase beta. This has more to do with cancer probably than rec a protein.

NOTE Confidence: 0.890543758869171

00:01:21.920 --> 00:01:53.490 So this is the molecule that we've been studying in the lab for a long time. DNA polymerase beta has 2 activities. It has a DRP lyase activity that can remove a DRP group from a 5 prime end of DNA and it also has a polymerase activity. And here you'll see the DNA moving through the polymerase with the substrate in this case of DCTP and you can see this is the Lias Domain. This is the thumb domain, which binds to DNA modulates interactions with DNA the Palm.

NOTE Confidence: 0.897063732147217

00:01:53.490 --> 00:02:15.230 Sub domain, which is where the the active site of the enzyme is and then we have the fingers, which sort of modulate the binding of the D NTP. The nomenclature is based on our dear friend and colleague Tom Stites. When he first crystallized Clennell Fragmente. He'd likened it to a right hand gripping a rod the rod being DNA.

NOTE Confidence: 0.874414205551147

00:02:16.400 --> 00:02:47.230 Pobeda functions in basic scission repair it. This is a very important cellular process mainly removes a small base damage 3. The Romano functional or by functional glycosylation pathway monofunctional glycosylase is remove the base of leaving in a basic site, which is in size. The back bonus. Then in size. By AP and a nuclease, leaving a single nucleotide gap with the 3 prime hydroxyl and a 5 prime phosphate that 5 prime. Phosphate is what's removed by Paul data.

NOTE Confidence: 0.889046430587769

00:02:47.230 --> 00:03:17.760 Call beta fills in the gap and then down here off the slide is where Ligase Aseels. The neck by functional glycosylase is removed. Both the base and cut the backbone, leaving these awful DNA ends that need to be remodeled and again. All roads lead to DNA polymerase beta So what I'm saying here and this is a lot of people work on this. Surprisingly, a lot of people work on pool beta. It's actually competitive field, but this is just our work showing that basically.

NOTE Confidence: 0.90142810344696

00:03:17.760 --> 00:03:51.590 Errors committed by DNA polymerase beta during single nucleotide gap filling can actually lead to mutations and the reason I'm saying that is because of this basic scission repair operates on 20,000 to 15,000 lesions. Purcell per day so it's a major genome maintenance pathway. It operates and processes base is damaged by reactive oxygen and nitrogen species uracil indie amination products methyl lated bases, especially here with chemotherapy so if you have basic scission repair mutations.

NOTE Confidence: 0.929811418056488

00:03:51.590 --> 00:03:56.070 Our lab has actually shown that you can be resistant to alkylating agents.

NOTE Confidence: 0.8917076587677

00:03:56.570 --> 00:04:02.410 Active demethylation and transcription Semantic Harbor Mutation and this is other work that we do in the lab.

NOTE Confidence: 0.87303215265274

00:04:03.630 --> 00:04:38.100 OK, so a while back we started to work on this particular polymerase. Mutation K289M to methionine so lysing to methionine at the tip of Helix in Helix end is very important for positioning that DCT pier. the D NTP in the active site. And this was identified by a group is being

present in a Colon Carcinoma. It's a somatic mutation. Our group and I'll show you some evidence for this showed that it induces cellular transformation if we express it in cells.

NOTE Confidence: 0.884504437446594

00:04:38.100 --> 00:05:09.130 But it's a sequence context specific mutator polymerase and I'm going to show you evidence for that and we know that it acts very nicely in basic scission repair completely support. Basic scission repair. It's quite an active DNA polymerase. This is an old slide from an earlier study and here's where we're just expressing K289M in these are in actually in mouse cells that are non transformed but immortal we can now do the same. But in human but I'm just showing you the mouse cells so if we don't express it.

NOTE Confidence: 0.898164451122284

00:05:09.130 --> 00:05:38.710 We don't see foci. These are old fashion faux side, there, not the first side that we talk about today. The rad 51 foci. These ourselves growing on top of each other and if we express' it. We see lots of foci or cellular transformation and this is what they look like up close. I can tell you that we can do the same with wild type protein the normal protein where K is at 289. We don't see this phenotype at all, and we're not over expressing this at all were expressing similar levels to the endogenous polymerase.

NOTE Confidence: 0.868146598339081

00:05:39.210 --> 00:05:44.580 We've also shown that it can induce Anchorage independent growth so these are colonies growing insoft auger.

NOTE Confidence: 0.884232640266418

00:05:45.850 --> 00:06:16.180 Now it's really cool about this polymerase is that it makes mutations and in Vivo. We looked at mutations made by this polymerase in vivo and in Vivo. It mutates this very famous gene the APC gene? Which of course, is a cancer gene mutated and a lot of tumors and it mutates it in the hot spot where we usually find mutations in a PC and you can see that if we just we can do this in Vivo. We can do an in vitro. We can just copy DNA in vitro, but in vivo we know that.

NOTE Confidence: 0.8830526471138

00:06:16.180 --> 00:06:24.640 This polymerase actually has a mutator phenotype about 16 fold higher mutation frequency for mutations just within that hotspot sequence.

NOTE Confidence: 0.891262471675873

00:06:25.550 --> 00:06:52.970 So how we surmised that that in this hot spot sequence if the K289M DNA polymerase. Putinci opposite templates easel. Show you evidence for we would have an increase in mutations in a PC which is going to we're going to lead to phenotypes like cell migration. It'll hibbett a

pop ptosis activate cell growth and of course, lead to cancer. This is of course, not, our work. This is work for many, many, many labs.

NOTE Confidence: 0.923611104488373

00:06:53.830 --> 00:07:10.200 So this is quite an opportunity. We actually have a handle on a molecule that might be causing these mutations in a PC and so we're very interested in the mechanism of mutagenesis by K289M in the sequence context.

NOTE Confidence: 0.891609787940979

00:07:10.970 --> 00:07:42.540 So we know that it's specifically this sequence context. Lots of other sequences. We don't see mutations. We only see we predominantly see it in this sequence context. So if we go back to basics. What do we need to have to obtain a CD G transversion? Well, we need the polymerase to miss incorporate D. CTP opposite template see polymerase is really don't like to do this for some reason this polymerase K289M doesn't mind doing it. So here we're going to bring in G opposite template see.

NOTE Confidence: 0.904876530170441

00:07:42.540 --> 00:08:14.130 We're looking at the rate of polymerization here and you can see that the rate of polymerization for this particular polymerase is slow. This is just fine. It it can fill gaps just fine in the cell an in vitro when we bring in C opposite template. See there's not much of a difference between the rate for correct versus incorrect meaning that the Fidelity. The ability of this polymerise to make mistakes within the sequence contact is very high. The Fidelity is very low so it's an error prone polymerase within the sequence context.

NOTE Confidence: 0.887459695339203

00:08:14.130 --> 00:08:45.520 So we wanted to know why so we thought, well it's a polymerase. It has to there has to be some substrate selection or miss selection during covalent bond formation during the transition. State something called chemistry. And so Charles Mccanna oops. Charles Mckennon Myron. Goodman have built these nuclear analogs where they're decorating. The Beta Gamma Phosphate with different moieties and these actually actually tend to alter the PK.

NOTE Confidence: 0.906758010387421

00:08:45.520 --> 00:09:18.250 So they're changing the PK and we have a whole toolkit of these that we can use to just probe. Trent the transition state so let's look at we won't go too deep into biochemistry, but let's look at the transition state. So here we have our single nucleotide gap. An now we're going to we're going to fill that gap with one of these with one of these analogs. So we've got this chemical transition state, which is the pentavalent transition state with the leaving group, so you can see what happens if we can if we can decorate the beta gamma phosphate here with CF2.

NOTE Confidence: 0.915393769741058

00:09:18.250 --> 00:09:52.210 We can actually make that leaving group more negative and if we make that leaving more negative. It moves chemistry forward and So what happens is that we get an increase in the rate of Chemistry. We can also make the leaving group horrible so that chemistry is very slow and so then we can go on to fill in the gap alright so we're going to probe were going to probe the transition state with these analogs and by the way these analogs can also be polymerase. Inhibitors so we're going to use these various sequences. This is the control sequence and it's a sequence we know.

NOTE Confidence: 0.887649178504944

00:09:52.210 --> 00:10:06.350 The K289M does not mutate it perfectly perfectly accurate in the sequence. It happens to be the favorite sequence of X Ray crystallographers for Paul data. There are thousands of crystals of Pobeda in the PDB and they always use this sequence.

NOTE Confidence: 0.891219198703766

00:10:07.190 --> 00:10:38.470 A PC here's the APC sequence and then what we can do is we can take. These you notice there. All drawl going to be polymerizing. We're all going to be inserting opposite template see it's always template see but the base is around that templating base or different so for a PC. Here's the APC sequence, then what we can do is we can take this chunk in the control and stick it into the APC sequence and we could do the same thing we can take this APC chunk and stick it and stick it into the control sequence so we have 4 different sequences.

NOTE Confidence: 0.937973976135254

00:10:39.400 --> 00:10:40.960 All Right This is a little busy.

NOTE Confidence: 0.910230040550232

00:10:41.530 --> 00:11:03.870 Ah, but this is we can look at linear free energy relationships. We don't need to worry about that. We can just look at at the log of polymerization or the rate of polymerization as a function of the leaving group as a function of the PK so you can see for the control sequence with wild type as we increase the PK polymerase rate goes down and we have a negative slope.

NOTE Confidence: 0.902098178863525

00:11:04.600 --> 00:11:20.320 Fine we can do this also with the APC sequence and we see this negative slope and we can do it with the APC in control, and the control in APC sequence all of those we see the same negative slope.

NOTE Confidence: 0.912195980548859

00:11:20.860 --> 00:11:35.310 But if we look at K289M. It's absolutely remarkable, so we see here with with the control sequence and we put that little piece

of control in the APC. We see these negative slopes, for K289M.

NOTE Confidence: 0.89080137014389

00:11:36.250 --> 00:11:43.730 But with the APC sequence whether it's the APC sequence straight or if we just plop the APC sequence into that control sequence.

NOTE Confidence: 0.931393444538116

00:11:44.380 --> 00:12:00.320 We almost have a flat line, suggesting that in this particular sequence context only for a PC. Do we have a real problem and that chemistry transition state chemistry covalent bond formation is not really.

NOTE Confidence: 0.902457535266876

00:12:00.820 --> 00:12:04.880 Where specificity is occuring it's gotta be occuring someplace else?

NOTE Confidence: 0.889550805091858

00:12:05.490 --> 00:12:35.860 So where is it well so the conclusions here are the rate limiting step of the wild type is sequence context independent but K289M exhibit sequence context specificity at the transition state. Now we've known for years that polymerase is exhibits sequence context specificity, but now we think we might have a handle on the mechanism. So K289M is limited by chemistry with the control sequence. But we think a pre catalytic step limits K289M with the APC sequence.

NOTE Confidence: 0.88363242149353

00:12:35.860 --> 00:12:43.040 And that the four nucleotides flanking the templating base are sufficient for the trenches transition state specificity alright.

NOTE Confidence: 0.8843954205513

00:12:43.680 --> 00:13:15.870 So now again we have a right hand gripping Arad, except right now, it looks like the left one. But that's OK, So what we're going to do now is we're going to look at force are residents. Energie transfer to look at pre catalytic conformational changes. And here we put a donor which fluoresces and ask the fingers close? What I didn't tell you is when the D. NTP binds the fingers close so if we have a donor sequence here and a quench on the DNA as the finger when the D. NTP binds as the fingers close we see quench inflorescence.

NOTE Confidence: 0.920914232730865

00:13:15.870 --> 00:13:34.830 And then the fingers reopen and I'll show you what that looks like right here. So we can monitor. This is a function of time, the fluorescent. Here's the fingers closing at different concentrations of the DNT. They close and then they open back up so we can actually now. These are thousands and thousands of points and we can model this.

NOTE Confidence: 0.908840119838715

00:13:35.690 --> 00:14:07.400 This is really catalysis for Paul Beta, it binds the D. NTP the fingers close. We have some non covalent step. We know it's there by modeling and by checking our models very carefully and we see it here. We see it with NMR as well. It's a non covalent stuff we have no idea what it does and then we have chemistry. So we can look at these pre catalytic. Conformational changes through florescence or Forster resonance energy transfer, and then we can look at the chemical transition state with the toolkit.

NOTE Confidence: 0.872422099113464

00:14:07.400 --> 00:14:10.870 And I've shown you now for K289M with the toolkit.

NOTE Confidence: 0.907397925853729

00:14:11.540 --> 00:14:42.410 This is not really rate limiting back here is where the rate limiting step of the reaction is and so these are just the traces for while typing for the mutant if you get used to doing these experiments like Khadija. This is Khadija Al Najjar's work. If you do these experiments often enough you see that this is greatly different than this. But it's hard to see when you haven't done a lot of these experiments, but then we model them and then we check them statistically. So now I'm going to show you this is just these are just the chemical pathways that kinetic pathways.

NOTE Confidence: 0.920182466506958

00:14:42.410 --> 00:15:04.030 So we have the enzyme binding. the D NTP and then we have the fingers closing step. That's very quick 173 per second. The non covalent step, which we really don't know what's happening here is 24 per second and then phospho diester bond formation. Chemistry is 16 for 2nd. We have no idea what this post chemistry step is but we think it has to do with fingers reopening.

NOTE Confidence: 0.915490090847015

00:15:05.110 --> 00:15:15.140 This is for that control sequence and so you can see for K289M. First of all that the fingers close a little more slowly but the chemistry is rate limiting its rate determining.

NOTE Confidence: 0.89085465669632

00:15:15.670 --> 00:15:32.220 Just as it is for wild time with the control sequence. Now let's look at the APC sequence. So we still see that fingers closing is slow but now we see that this non covalent stuff we have no idea what it is has become rate determining.

NOTE Confidence: 0.898519992828369

00:15:33.450 --> 00:15:43.420 And chemistry is no longer rate determining so this polymerase is perturbed at this non covalent step leading to mutations within the APC dream.

NOTE Confidence: 0.894254624843597

00:15:44.750 --> 00:16:01.770 If we put that control sequence within the APC larger sequence. We again see that chemistry is rate determining so the polymerase is only really dealing with these 6 base pairs. It's only really seeing those 6 base pairs when it talks about sequence context specificity.

NOTE Confidence: 0.883916318416595

00:16:02.610 --> 00:16:10.740 But then if we take the APC sequence and pop it into the control sequence again this non covalent step is rate determining.

NOTE Confidence: 0.895960569381714

00:16:12.520 --> 00:16:43.350 And so our conclusions are that the K 289, now is a sequence context. Dependent mutator polymerase and that the non covalent step is actually becoming rate determining for K289M in the present only of the APC sequence and that the nature of the substrate and of the polymerase are critically important for the Fidelity of DNA synthesis. And we think that the non covalent step is important for substrate selection by Paul beta. Now we've got to figure out what it is So what do we think it is So what we think?

NOTE Confidence: 0.881766140460968

00:16:43.350 --> 00:16:57.730 Is that we know that the D NTP binds right here? This is this? Is Argentine 183. We have it? Depends Argentine 183 is critical for interaction with this D NTP there's the magnesium.

NOTE Confidence: 0.917425155639648

00:16:58.590 --> 00:17:31.700 183 interacts with one 82, which are also interacts with 316 and propagates its effects over to 3:24 and here's K 289 sitting on the tip of Helix and we think this whole thing. There's some evidence that this. This whole structure of the sub domain the stability of this sub domain is absolutely critical for the positioning of Helix N and the positioning of Helix end is critical for positioning. This D NTP absolutely correctly within the active site, so that there's not a missing Corporation.

NOTE Confidence: 0.921556055545807

00:17:31.700 --> 00:17:53.630 So this is what we think is going on. It's about as good as it gets right now, but we're going to continue to try and figure out precisely what this non covalent step is we think it's actually minor adjustments of K2 of Helix and what we actually think is going on is that he'll extent is somehow talking to these large 183 in 182 and we're working on that now.

NOTE Confidence: 0.904314756393433

00:17:54.220 --> 00:18:13.270 And so with that Khadija did most of this work. This is the part of the team that the kinetic studies in the lab here all of our collaborators. We have wonderful funding of that. We're grateful for from the National Cancer Institute and we know that Paul date is the best thanks.