WEBVTT

NOTE Confidence: 0.862013406666666

00:00:00.000 --> 00:00:02.367 First of all, I'd like to thank you all

NOTE Confidence: 0.862013406666666

00:00:02.367 --> 00:00:04.717 for attending our grand rounds today.

NOTE Confidence: 0.862013406666666

00:00:04.720 --> 00:00:09.402 It is my pleasure to present this special

NOTE Confidence: 0.862013406666666

00:00:09.402 --> 00:00:13.555 event, which is sponsored by the Chenevert

NOTE Confidence: 0.862013406666666

00:00:13.555 --> 00:00:17.485 family brain Tumor Center at Yale.

NOTE Confidence: 0.862013406666666

00:00:17.490 --> 00:00:20.752 And today we have the pleasure to

NOTE Confidence: 0.862013406666666

00:00:20.752 --> 00:00:22.920 welcome Doctor Sigrid Natural.

NOTE Confidence: 0.862013406666666

00:00:22.920 --> 00:00:25.630 And. She isn't an assistant

NOTE Confidence: 0.862013406666666

00:00:25.630 --> 00:00:27.256 professor of molecular,

NOTE Confidence: 0.862013406666666

00:00:27.260 --> 00:00:30.408 cellular and developmental biology.

NOTE Confidence: 0.862013406666666

00:00:30.410 --> 00:00:32.756 She received her PhD from Stanford

NOTE Confidence: 0.862013406666666

00:00:32.756 --> 00:00:35.203 University and during her graduate work

NOTE Confidence: 0.862013406666666

00:00:35.203 --> 00:00:37.645 in the laboratory of Rajat Rochetti,

NOTE Confidence: 0.862013406666666

00:00:37.650 --> 00:00:39.500 she developed novel chemical tools

00:00:39.500 --> 00:00:41.350 to study the Hedgehog signaling,

00:00:41.350 --> 00:00:43.280 uncovering new modes of steroid

00:00:43.280 --> 00:00:44.824 mediated regulation of this

00:00:44.824 --> 00:00:46.520 critical developmental pathway.

00:00:46.520 --> 00:00:48.350 She was a postdoc fellow which

00:00:48.350 --> 00:00:50.280 run hay at University of Chicago,

00:00:50.280 --> 00:00:52.626 where she set out to identify

00:00:52.626 --> 00:00:54.644 novel chemical modifications in M

00:00:54.644 --> 00:00:56.569 RNA and uncovered their functions.

00:00:56.570 --> 00:00:58.646 Her lifestyle is working on numerous

00:00:58.646 --> 00:01:00.513 aspects of RNA mediated regulation

00:01:00.513 --> 00:01:01.770 of cell signaling.

00:01:01.770 --> 00:01:03.792 And their current focus is on

00:01:03.792 --> 00:01:05.599 uncovering the regulation and function

00:01:05.599 --> 00:01:07.599 of chemical modifications on RNA.

NOTE Confidence: 0.862013406666666

00:01:07.600 --> 00:01:11.191 She was awarded a demo reunion postdoc

NOTE Confidence: 0.862013406666666

00:01:11.191 --> 00:01:13.980 Fellowship and subsequently a demo reunion.

NOTE Confidence: 0.862013406666666

00:01:13.980 --> 00:01:16.380 They fray award for breakthrough

NOTE Confidence: 0.862013406666666

00:01:16.380 --> 00:01:18.138 scientists for this work.

NOTE Confidence: 0.862013406666666

00:01:18.140 --> 00:01:19.388 And more recently,

NOTE Confidence: 0.862013406666666

00:01:19.388 --> 00:01:23.316 she was awarded the Distinguished Scientist

NOTE Confidence: 0.862013406666666

00:01:23.316 --> 00:01:28.244 Award from the Sontag Foundation in

NOTE Confidence: 0.862013406666666

00:01:28.244 --> 00:01:31.956 in to reward her work in brain tumors.

NOTE Confidence: 0.862013406666666

00:01:31.960 --> 00:01:33.409 So please welcome.

NOTE Confidence: 0.946551173333333

00:01:41.450 --> 00:01:44.830 Thank you so much for having me.

NOTE Confidence: 0.946551173333333

00:01:44.830 --> 00:01:46.426 Look, I need a phone book

NOTE Confidence: 0.946551173333333

00:01:46.426 --> 00:01:50.320 behind this podium. One second.

NOTE Confidence: 0.757745015

00:01:50.320 --> 00:01:52.060 Get rid of this.

NOTE Confidence: 0.928924572222222

00:01:53.230 --> 00:01:55.408 All right. Thank you so much for having me.

NOTE Confidence: 0.928924572222222

00:01:55.410 --> 00:01:57.702 And I'm really, really thrilled to

NOTE Confidence: 0.928924572222222

00:01:57.702 --> 00:01:59.480 be here particularly because of,

NOTE Confidence: 0.928924572222222

00:01:59.480 --> 00:02:01.576 I think part of the reason this came

NOTE Confidence: 0.928924572222222

00:02:01.576 --> 00:02:03.732 about is that because of the santag

NOTE Confidence: 0.928924572222222

00:02:03.732 --> 00:02:06.010 word application that I was submitting,

NOTE Confidence: 0.928924572222222

00:02:06.010 --> 00:02:07.906 I guess a couple of years ago now,

NOTE Confidence: 0.928924572222222

00:02:07.910 --> 00:02:09.390 Doctor Romero kindly not only

NOTE Confidence: 0.928924572222222

00:02:09.390 --> 00:02:11.051 answered my e-mail, but even agreed

NOTE Confidence: 0.928924572222222

00:02:11.051 --> 00:02:13.150 to chat over zoom for for a bit,

NOTE Confidence: 0.928924572222222

00:02:13.150 --> 00:02:14.854 which you know was incredibly generous

NOTE Confidence: 0.928924572222222

00:02:14.854 --> 00:02:17.109 as I prepared for for that application.

NOTE Confidence: 0.928924572222222

00:02:17.110 --> 00:02:18.643 And So what I'm excited to share

NOTE Confidence: 0.928924572222222

00:02:18.643 --> 00:02:20.350 with you today is a little bit about

NOTE Confidence: 0.928924572222222

00:02:20.350 --> 00:02:22.071 what our lab is doing with respect

NOTE Confidence: 0.928924572222222

00:02:22.071 --> 00:02:23.407 to gene expression regulation.

NOTE Confidence: 0.928924572222222

00:02:23.410 --> 00:02:26.084 By Arna modifications and I'm going to

NOTE Confidence: 0.928924572222222

00:02:26.084 --> 00:02:29.423 try to use the cursor on the screen so

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NOTE Confidence: 0.928924572222222

00:02:29.423 --> 00:02:33.020 that the folks on zoom can also follow along.

NOTE Confidence: 0.928924572222222

00:02:33.020 --> 00:02:34.260 Now to start with,

NOTE Confidence: 0.928924572222222

00:02:34.260 --> 00:02:36.120 I don't really have any disclosures,

NOTE Confidence: 0.928924572222222

00:02:36.120 --> 00:02:37.308 but I will perhaps start with

NOTE Confidence: 0.928924572222222

00:02:37.308 --> 00:02:38.700 a bit of a disclaimer.

NOTE Confidence: 0.928924572222222

00:02:38.700 --> 00:02:40.908 You may have already realized that I'm a

NOTE Confidence: 0.928924572222222

00:02:40.908 --> 00:02:44.000 basic scientist, I am not a clinician.

NOTE Confidence: 0.928924572222222

00:02:44.000 --> 00:02:46.616 And you know, while I think,

NOTE Confidence: 0.928924572222222

00:02:46.620 --> 00:02:47.080 you know,

NOTE Confidence: 0.928924572222222

00:02:47.080 --> 00:02:48.920 our work is really rooted in sort of

NOTE Confidence: 0.928924572222222

00:02:48.970 --> 00:02:50.620 fundamental biological questions,

NOTE Confidence: 0.928924572222222

00:02:50.620 --> 00:02:53.371 I do consider myself sort of cancer

NOTE Confidence: 0.928924572222222

00:02:53.371 --> 00:02:54.157 biology adjacent.

NOTE Confidence: 0.928924572222222

00:02:54.160 --> 00:02:56.176 And I have been for quite a while.

NOTE Confidence: 0.928924572222222

00:02:56.180 --> 00:02:58.350 So my PhD work focused on the

NOTE Confidence: 0.928924572222222

00:02:58.350 --> 00:02:59.280 Hedgehog signaling pathway.

NOTE Confidence: 0.928924572222222

00:02:59.280 --> 00:03:00.778 So I sort of became quite familiar

NOTE Confidence: 0.928924572222222

00:03:00.778 --> 00:03:02.009 with the sort of hedgehog.

NOTE Confidence: 0.928924572222222

00:03:02.010 --> 00:03:03.282 Even medulloblastoma field and

NOTE Confidence: 0.928924572222222

00:03:03.282 --> 00:03:05.580 then during my postdoc I sort of

NOTE Confidence: 0.928924572222222

00:03:05.580 --> 00:03:07.225 collaborated with a few different

NOTE Confidence: 0.928924572222222

00:03:07.225 --> 00:03:09.287 cancer biologists sort of uncovered the

NOTE Confidence: 0.928924572222222

00:03:09.287 --> 00:03:11.357 roles of RNA modifications and cancer.

NOTE Confidence: 0.928924572222222

00:03:11.360 --> 00:03:14.088 So you know while we are very much

NOTE Confidence: 0.928924572222222

00:03:14.088 --> 00:03:16.119 a fundamental basic science lab,

NOTE Confidence: 0.928924572222222

00:03:16.120 --> 00:03:16.390 I,

NOTE Confidence: 0.928924572222222

00:03:16.390 --> 00:03:18.280 I really do not only enjoy but

NOTE Confidence: 0.928924572222222

00:03:18.280 --> 00:03:20.260 really sort of motivate the lab to

NOTE Confidence: 0.928924572222222

00:03:20.260 --> 00:03:22.090 think about sort of the applications

NOTE Confidence: 0.928924572222222

00:03:22.090 --> 00:03:24.430 and implications of our work for

NOTE Confidence: 0.928924572222222

00:03:24.430 --> 00:03:25.600 for cancer biology.

00:03:25.600 --> 00:03:26.656 But all of that being said,

00:03:26.660 --> 00:03:29.585 I rely a lot on collaborators and sort of

00:03:29.585 --> 00:03:32.018 dedicated dedicated clinicians to sort of.

00:03:32.020 --> 00:03:33.424 Would have you know helped motivate

00:03:33.424 --> 00:03:34.884 our and drive our work forward

00:03:34.884 --> 00:03:35.828 in in that respect.

00:03:35.830 --> 00:03:38.107 So today I'll give you a little bit of

00:03:38.107 --> 00:03:40.768 an introduction on our new modifications.

00:03:40.770 --> 00:03:42.807 It's not always sort of the the

00:03:42.807 --> 00:03:44.764 first thing you think about when

00:03:44.764 --> 00:03:46.464 it comes to cancer biology.

00:03:46.470 --> 00:03:48.934 So I will then sort of segue directly

00:03:48.934 --> 00:03:51.855 into some of the work that's already been

00:03:51.855 --> 00:03:54.139 done on RNA modifications and cancer.

00:03:54.140 --> 00:03:56.138 And then I'm going to describe

00:03:56.138 --> 00:03:57.750 actually the current limitations of

NOTE Confidence: 0.928924572222222

00:03:57.750 --> 00:03:59.526 a lot of that work including my own

NOTE Confidence: 0.928924572222222

00:03:59.526 --> 00:04:01.393 and and try to describe for you the

NOTE Confidence: 0.928924572222222

00:04:01.393 --> 00:04:03.084 sort of new approaches that the lab

NOTE Confidence: 0.928924572222222

00:04:03.084 --> 00:04:05.299 is taking to try to sort of improve

NOTE Confidence: 0.928924572222222

00:04:05.299 --> 00:04:07.249 our ability to really understand how

NOTE Confidence: 0.928924572222222

00:04:07.249 --> 00:04:08.909 these chemical marks might actually

NOTE Confidence: 0.928924572222222

00:04:08.909 --> 00:04:10.504 sort of regulate gene expression

NOTE Confidence: 0.928924572222222

00:04:10.504 --> 00:04:13.235 and how we might actually be able

NOTE Confidence: 0.928924572222222

00:04:13.235 --> 00:04:15.970 to translate that into the clinic.

NOTE Confidence: 0.928924572222222

00:04:15.970 --> 00:04:17.058 And so with that,

NOTE Confidence: 0.928924572222222

00:04:17.058 --> 00:04:18.418 I'll just dive right in.

NOTE Confidence: 0.928924572222222

00:04:18.420 --> 00:04:18.978 And I think,

NOTE Confidence: 0.928924572222222

00:04:18.978 --> 00:04:19.350 you know,

NOTE Confidence: 0.928924572222222

00:04:19.350 --> 00:04:21.331 when we think of the central dogma

NOTE Confidence: 0.928924572222222

00:04:21.331 --> 00:04:22.980 in terms of its sort of,

NOTE Confidence: 0.928924572222222

00:04:22.980 --> 00:04:23.662 you know,

NOTE Confidence: 0.928924572222222

00:04:23.662 --> 00:04:24.344 the fund,

NOTE Confidence: 0.928924572222222

00:04:24.344 --> 00:04:26.049 the foundations of gene expression

NOTE Confidence: 0.928924572222222

00:04:26.049 --> 00:04:26.390 regulation,

NOTE Confidence: 0.928924572222222

00:04:26.390 --> 00:04:27.850 this probably looks quite familiar.

NOTE Confidence: 0.798394385625

00:04:27.850 --> 00:04:29.266 This is what I teach the

NOTE Confidence: 0.798394385625

00:04:29.266 --> 00:04:30.210 undergraduates that I teach

NOTE Confidence: 0.798394385625

00:04:30.264 --> 00:04:31.950 biochemistry over on the main campus.

NOTE Confidence: 0.798394385625

00:04:31.950 --> 00:04:33.294 And so when we think about

NOTE Confidence: 0.798394385625

00:04:33.294 --> 00:04:33.966 gene expression regulation,

NOTE Confidence: 0.798394385625

00:04:33.970 --> 00:04:35.395 really what we're thinking about

NOTE Confidence: 0.798394385625

00:04:35.395 --> 00:04:37.139 is the flow of information from

NOTE Confidence: 0.798394385625

00:04:37.139 --> 00:04:38.705 the genome and DNA through RNA

NOTE Confidence: 0.798394385625

00:04:38.705 --> 00:04:40.269 molecules and then on to proteins.

NOTE Confidence: 0.798394385625

00:04:40.270 --> 00:04:41.630 But of course, you know,

NOTE Confidence: 0.798394385625

00:04:41.630 --> 00:04:44.406 this is a rather simplified view and of

NOTE Confidence: 0.798394385625

00:04:44.406 --> 00:04:47.389 course you know DNA needs to be replicated.

NOTE Confidence: 0.798394385625

00:04:47.390 --> 00:04:49.448 We know that a lot of interesting

NOTE Confidence: 0.798394385625

00:04:49.448 --> 00:04:50.807 reverse transcript cases exist to

NOTE Confidence: 0.798394385625

00:04:50.807 --> 00:04:52.511 sort of move back from RNA to DNA

NOTE Confidence: 0.798394385625

00:04:52.561 --> 00:04:54.229 and then of course protein based

NOTE Confidence: 0.798394385625

00:04:54.229 --> 00:04:55.816 enzymes and factors of course

NOTE Confidence: 0.798394385625

00:04:55.816 --> 00:04:58.146 regulate this at many steps.

NOTE Confidence: 0.798394385625

00:04:58.150 --> 00:04:59.744 But on top of that, you know,

NOTE Confidence: 0.798394385625

00:04:59.744 --> 00:05:01.226 even within each of these processes,

NOTE Confidence: 0.798394385625

00:05:01.230 --> 00:05:02.640 of course there are many

NOTE Confidence: 0.798394385625

00:05:02.640 --> 00:05:03.768 sort of intervening steps.

NOTE Confidence: 0.798394385625

00:05:03.770 --> 00:05:05.120 And so as an RA biologist,

NOTE Confidence: 0.798394385625

00:05:05.120 --> 00:05:06.596 I'm going to bias your view

NOTE Confidence: 0.798394385625

00:05:06.596 --> 00:05:07.870 and say that the RNA,

NOTE Confidence: 0.798394385625

00:05:07.870 --> 00:05:09.250 so to sort of protein transition

NOTE Confidence: 0.798394385625

00:05:09.250 --> 00:05:10.170 is the most interesting.

NOTE Confidence: 0.798394385625

00:05:10.170 --> 00:05:11.955 But of course some of my basic

NOTE Confidence: 0.798394385625

00:05:11.955 --> 00:05:13.630 biology friends will disagree with me.

NOTE Confidence: 0.798394385625

00:05:13.630 --> 00:05:14.890 But that being said, you know,

NOTE Confidence: 0.798394385625

00:05:14.890 --> 00:05:17.543 even just to go from a functional

NOTE Confidence: 0.798394385625

00:05:17.543 --> 00:05:19.750 MRI MRA molecule to a protein,

NOTE Confidence: 0.798394385625

00:05:19.750 --> 00:05:21.916 we have to undergo many intermediate

NOTE Confidence: 0.798394385625

00:05:21.916 --> 00:05:23.870 steps including things like capping,

NOTE Confidence: 0.798394385625

00:05:23.870 --> 00:05:26.850 splicing, processing of other types,

NOTE Confidence: 0.798394385625

00:05:26.850 --> 00:05:28.494 then translation into protein

NOTE Confidence: 0.798394385625

00:05:28.494 --> 00:05:29.727 and then eventually.

NOTE Confidence: 0.798394385625

00:05:29.730 --> 00:05:30.920 RNA decay.

NOTE Confidence: 0.798394385625

00:05:30.920 --> 00:05:33.300 And as you know,

NOTE Confidence: 0.798394385625

00:05:33.300 --> 00:05:34.880 you have probably already quite

NOTE Confidence: 0.798394385625

00:05:34.880 --> 00:05:37.080 familiar at each of these steps DNA,

NOTE Confidence: 0.798394385625

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00:05:37.080 --> 00:05:37.956 RNA and protein.

NOTE Confidence: 0.798394385625

00:05:37.956 --> 00:05:39.708 There are many different chemical marks

NOTE Confidence: 0.798394385625

00:05:39.708 --> 00:05:41.449 that can regulate these processes.

NOTE Confidence: 0.798394385625

00:05:41.450 --> 00:05:44.420 This is sort of a fundamental paradigm of of,

NOTE Confidence: 0.798394385625

00:05:44.420 --> 00:05:45.316 you know,

NOTE Confidence: 0.798394385625

00:05:45.316 --> 00:05:47.556 signaling and gene expression regulation.

NOTE Confidence: 0.798394385625

00:05:47.560 --> 00:05:49.303 And so we know for instance that

NOTE Confidence: 0.798394385625

00:05:49.303 --> 00:05:51.022 DNA can be methylated and that

NOTE Confidence: 0.798394385625

00:05:51.022 --> 00:05:52.840 this is sort of a critical,

NOTE Confidence: 0.798394385625

00:05:52.840 --> 00:05:53.678 you know,

NOTE Confidence: 0.798394385625

00:05:53.678 --> 00:05:55.773 regulator of gene expression at

NOTE Confidence: 0.798394385625

00:05:55.773 --> 00:05:58.358 the level of the genomic DNA.

NOTE Confidence: 0.798394385625

00:05:58.360 --> 00:06:00.208 And then all the way on the other

NOTE Confidence: 0.798394385625

00:06:00.208 --> 00:06:02.316 side we of course know that enzymes.

NOTE Confidence: 0.798394385625

00:06:02.320 --> 00:06:03.840 Think of things like kinases,

NOTE Confidence: 0.798394385625

00:06:03.840 --> 00:06:05.540 of course phosphorylated and modified,

NOTE Confidence: 0.798394385625

00:06:05.540 --> 00:06:07.500 with many other post translational

NOTE Confidence: 0.798394385625

00:06:07.500 --> 00:06:09.068 modifications that really dictate

NOTE Confidence: 0.798394385625

00:06:09.068 --> 00:06:10.678 their activity and function,

NOTE Confidence: 0.798394385625

00:06:10.680 --> 00:06:13.150 and in some cases localization.

NOTE Confidence: 0.798394385625

00:06:13.150 --> 00:06:14.650 What some people are sometimes

NOTE Confidence: 0.798394385625

00:06:14.650 --> 00:06:16.549 less familiar with is that in

NOTE Confidence: 0.798394385625

00:06:16.549 --> 00:06:18.571 fact RNA molecules are also very

NOTE Confidence: 0.798394385625

00:06:18.571 --> 00:06:19.582 heavily chemically modified.

NOTE Confidence: 0.798394385625

00:06:19.590 --> 00:06:22.481 And this can sometimes be a little

NOTE Confidence: 0.798394385625

00:06:22.481 --> 00:06:24.148 bit counterintuitive because when

NOTE Confidence: 0.798394385625

00:06:24.148 --> 00:06:26.272 we think of our name molecules

NOTE Confidence: 0.798394385625

00:06:26.272 --> 00:06:27.670 and particularly M RNA,

NOTE Confidence: 0.798394385625

00:06:27.670 --> 00:06:29.525 we think of very transient

NOTE Confidence: 0.798394385625

00:06:29.525 --> 00:06:31.009 sort of chemical molecules.

NOTE Confidence: 0.798394385625

00:06:31.010 --> 00:06:32.826 So why would you sort of want to

NOTE Confidence: 0.798394385625

00:06:32.826 --> 00:06:34.590 fine tune something that is such,

NOTE Confidence: 0.798394385625

00:06:34.590 --> 00:06:35.184 you know,

NOTE Confidence: 0.798394385625

00:06:35.184 --> 00:06:36.966 such a transient molecule in nature,

NOTE Confidence: 0.798394385625

00:06:36.970 --> 00:06:39.546 but it turns out that RNA actually

NOTE Confidence: 0.798394385625

00:06:39.546 --> 00:06:43.250 carries over 150 now known RNA.

NOTE Confidence: 0.798394385625

00:06:43.250 --> 00:06:43.990 Qualifications,

NOTE Confidence: 0.798394385625

00:06:43.990 --> 00:06:45.700 and I'm showing you just a

NOTE Confidence: 0.798394385625

00:06:45.700 --> 00:06:47.509 very small subset of them here,

NOTE Confidence: 0.798394385625

00:06:47.510 --> 00:06:50.390 but these range from everything from

NOTE Confidence: 0.798394385625

00:06:50.390 --> 00:06:53.460 the addition of methylation marks to

NOTE Confidence: 0.798394385625

00:06:53.460 --> 00:06:56.140 isomerization ins around different bonds.

NOTE Confidence: 0.798394385625

00:06:56.140 --> 00:06:57.265 And you know.

NOTE Confidence: 0.798394385625

00:06:57.265 --> 00:06:58.765 On the other end,

NOTE Confidence: 0.798394385625

00:06:58.770 --> 00:07:00.606 we have sort of double methylations,

NOTE Confidence: 0.798394385625

00:07:00.610 --> 00:07:01.804 but this is actually a very

NOTE Confidence: 0.798394385625

00:07:01.804 --> 00:07:02.600 sort of limited view.

NOTE Confidence: 0.798394385625

00:07:02.600 --> 00:07:04.844 And there's all kinds of incredible

NOTE Confidence: 0.798394385625

00:07:04.844 --> 00:07:06.739 chemistry that can happen on

NOTE Confidence: 0.798394385625

00:07:06.739 --> 00:07:08.389 RNA and particularly T RNA's

NOTE Confidence: 0.798394385625

00:07:08.389 --> 00:07:11.170 to regulate gene expression.

NOTE Confidence: 0.798394385625

00:07:11.170 --> 00:07:13.074 And so if we sort of overlay

NOTE Confidence: 0.798394385625

00:07:13.074 --> 00:07:13.890 this on what

NOTE Confidence: 0.857960633125

00:07:13.961 --> 00:07:16.217 we actually encounter in the cell,

NOTE Confidence: 0.857960633125

00:07:16.220 --> 00:07:18.488 which is not a very linear pathway,

NOTE Confidence: 0.857960633125

00:07:18.490 --> 00:07:20.688 we get a very complex picture like

NOTE Confidence: 0.857960633125

00:07:20.688 --> 00:07:23.013 this where we have modifications that

NOTE Confidence: 0.857960633125

00:07:23.013 --> 00:07:25.641 are in fact regulating literally every

NOTE Confidence: 0.857960633125

00:07:25.641 --> 00:07:28.377 step of an M RNA during its life cycle.

NOTE Confidence: 0.857960633125

00:07:28.380 --> 00:07:30.390 And so many of these chemical

NOTE Confidence: 0.857960633125

00:07:30.390 --> 00:07:31.395 modifications are added,

NOTE Confidence: 0.857960633125

00:07:31.400 --> 00:07:33.336 sort of as the RNA is being made.

NOTE Confidence: 0.857960633125

00:07:33.340 --> 00:07:35.600 Not all of them are, but many of them are.

NOTE Confidence: 0.857960633125

00:07:35.600 --> 00:07:38.786 And so the RNA modification machinery

NOTE Confidence: 0.857960633125

00:07:38.786 --> 00:07:41.450 will in fact interact with.

NOTE Confidence: 0.857960633125

00:07:41.450 --> 00:07:42.586 My cursor is frozen.

NOTE Confidence: 0.857960633125

00:07:42.586 --> 00:07:43.926 Well, actually interact with

NOTE Confidence: 0.857960633125

00:07:43.926 --> 00:07:45.334 the transcription machinery as

NOTE Confidence: 0.857960633125

00:07:45.334 --> 00:07:47.049 the RNA is being made.

NOTE Confidence: 0.857960633125

00:07:47.050 --> 00:07:47.840 The RNA.

NOTE Confidence: 0.857960633125

00:07:47.840 --> 00:07:50.210 Most MRA's carry a 5 prime

NOTE Confidence: 0.857960633125

00:07:50.210 --> 00:07:52.936 cap that is in fact derived of

NOTE Confidence: 0.857960633125

00:07:52.936 --> 00:07:54.828 numerous modifications and then

NOTE Confidence: 0.857960633125

00:07:54.828 --> 00:07:57.904 every step along the way from

NOTE Confidence: 0.857960633125

00:07:57.904 --> 00:08:00.108 splicing to polyadenylation export,

NOTE Confidence: 0.857960633125

00:08:00.110 --> 00:08:01.970 all of this sort of,

NOTE Confidence: 0.857960633125

00:08:01.970 --> 00:08:02.436 you know,

NOTE Confidence: 0.857960633125

00:08:02.436 --> 00:08:03.368 modifications have been implicated

00:08:03.368 --> 00:08:04.720 in many of these processes.

00:08:04.720 --> 00:08:05.770 They're exact functions.

00:08:05.770 --> 00:08:07.170 We don't always know,

00:08:07.170 --> 00:08:09.550 but we have sort of evidence to

00:08:09.550 --> 00:08:10.968 suggest that modifications can

00:08:10.968 --> 00:08:12.978 regulate every step of this process.

00:08:12.980 --> 00:08:15.720 Once an MRA gets exported,

00:08:15.720 --> 00:08:17.800 modifications can impact the

00:08:17.800 --> 00:08:19.880 association with translation machinery.

00:08:19.880 --> 00:08:22.309 So the ribosome itself can in fact

00:08:22.309 --> 00:08:23.350 detect sometimes modifications

00:08:23.405 --> 00:08:24.899 out or in your start codons,

00:08:24.900 --> 00:08:26.465 and then something that's going

00:08:26.465 --> 00:08:28.879 to come up a few times today.

00:08:28.880 --> 00:08:30.372 Our name modifications can

00:08:30.372 --> 00:08:32.237 also regulate M RNA decay.

NOTE Confidence: 0.857960633125

00:08:32.240 --> 00:08:33.578 And now this picture is actually

NOTE Confidence: 0.857960633125

00:08:33.578 --> 00:08:34.760 only focused really on M RNA.

NOTE Confidence: 0.857960633125

00:08:34.760 --> 00:08:36.979 It turns out really all RNA's are

NOTE Confidence: 0.857960633125

00:08:36.979 --> 00:08:39.178 modified really across all kingdoms of life,

NOTE Confidence: 0.857960633125

00:08:39.180 --> 00:08:40.620 but for the purposes of today,

NOTE Confidence: 0.857960633125

00:08:40.620 --> 00:08:44.247 we're mostly going to be focused on M RNA.

NOTE Confidence: 0.857960633125

00:08:44.250 --> 00:08:46.007 And so when you zoom in very,

NOTE Confidence: 0.857960633125

00:08:46.010 --> 00:08:46.850 very far down,

NOTE Confidence: 0.857960633125

00:08:46.850 --> 00:08:48.810 really at the sort of chemical level,

NOTE Confidence: 0.857960633125

00:08:48.810 --> 00:08:50.644 really what's going on here is that,

NOTE Confidence: 0.857960633125

00:08:50.650 --> 00:08:51.226 you know,

NOTE Confidence: 0.857960633125

00:08:51.226 --> 00:08:52.378 these small little chemical

NOTE Confidence: 0.857960633125

00:08:52.378 --> 00:08:54.006 moieties can in fact dramatically

NOTE Confidence: 0.857960633125

00:08:54.006 --> 00:08:55.678 change base pairing patterns.

NOTE Confidence: 0.857960633125

00:08:55.680 --> 00:08:57.488 And so just as an example at the

NOTE Confidence: 0.857960633125

00:08:57.488 --> 00:08:59.132 top here I'm showing you an AU

NOTE Confidence: 0.857960633125

00:08:59.132 --> 00:09:01.007 base pair and a few examples of

NOTE Confidence: 0.857960633125

00:09:01.007 --> 00:09:02.375 different modifications and how

NOTE Confidence: 0.857960633125

00:09:02.375 --> 00:09:04.458 they would sort of impact base

NOTE Confidence: 0.857960633125

00:09:04.458 --> 00:09:06.228 pairing at the chemical level.

NOTE Confidence: 0.857960633125

00:09:06.230 --> 00:09:09.282 And so the addition of a methylation

NOTE Confidence: 0.857960633125

00:09:09.282 --> 00:09:12.430 group here an N6 methyl adenosine,

NOTE Confidence: 0.857960633125

00:09:12.430 --> 00:09:14.822 which is abbreviated M6A.

NOTE Confidence: 0.857960633125

00:09:14.822 --> 00:09:18.410 Can slightly destabilize this base pair.

NOTE Confidence: 0.857960633125

00:09:18.410 --> 00:09:20.205 If you actually shift that

NOTE Confidence: 0.857960633125

00:09:20.205 --> 00:09:22.330 methylation group over just a bit,

NOTE Confidence: 0.857960633125

00:09:22.330 --> 00:09:24.255 you actually get something that

NOTE Confidence: 0.857960633125

00:09:24.255 --> 00:09:25.795 completely impedes base pairing

NOTE Confidence: 0.857960633125

00:09:25.795 --> 00:09:27.689 altogether and then so on and so forth.

NOTE Confidence: 0.857960633125

00:09:27.690 --> 00:09:29.178 You can imagine that as you

NOTE Confidence: 0.857960633125

19

00:09:29.178 --> 00:09:30.170 increase the chemical diversity,

NOTE Confidence: 0.857960633125

00:09:30.170 --> 00:09:32.414 you can really impact on RNA

NOTE Confidence: 0.857960633125

00:09:32.414 --> 00:09:34.308 base pairing and structure and

NOTE Confidence: 0.857960633125

00:09:34.308 --> 00:09:36.288 a really wide variety of ways.

NOTE Confidence: 0.857960633125

00:09:36.290 --> 00:09:37.907 But at the sort of functional level,

NOTE Confidence: 0.857960633125

00:09:37.910 --> 00:09:39.308 zooming back out a little bit,

NOTE Confidence: 0.857960633125

00:09:39.310 --> 00:09:40.980 what does this really mean?

NOTE Confidence: 0.857960633125

00:09:40.980 --> 00:09:43.716 And So what do these modifications

NOTE Confidence: 0.857960633125

00:09:43.716 --> 00:09:44.628 actually do?

NOTE Confidence: 0.857960633125

00:09:44.630 --> 00:09:46.460 So we're going to focus mostly

NOTE Confidence: 0.857960633125

00:09:46.460 --> 00:09:48.205 today on one RNA methylation

NOTE Confidence: 0.857960633125

00:09:48.205 --> 00:09:50.480 mark and six methyl adenosine,

NOTE Confidence: 0.857960633125

00:09:50.480 --> 00:09:53.008 which I'm showing on the top left here.

NOTE Confidence: 0.857960633125

00:09:53.010 --> 00:09:54.102 And it's a great example for

NOTE Confidence: 0.857960633125

00:09:54.102 --> 00:09:54.830 a lot of reasons,

NOTE Confidence: 0.857960633125

00:09:54.830 --> 00:09:57.142 but one of them is that we can

NOTE Confidence: 0.857960633125

00:09:57.142 --> 00:09:58.945 actually ascribe some very specific

NOTE Confidence: 0.857960633125

00:09:58.945 --> 00:10:00.493 M RNA regulatory mechanisms

NOTE Confidence: 0.857960633125

00:10:00.493 --> 00:10:02.030 to this specific mark.

NOTE Confidence: 0.857960633125

00:10:02.030 --> 00:10:03.805 So this methylation Mark is

NOTE Confidence: 0.857960633125

00:10:03.805 --> 00:10:05.580 installed by a complex of

NOTE Confidence: 0.822163327333333

00:10:05.655 --> 00:10:07.800 a methyl transferase complex that

NOTE Confidence: 0.822163327333333

00:10:07.800 --> 00:10:09.945 contains metal three and metal

NOTE Confidence: 0.822163327333333

00:10:10.018 --> 00:10:12.601 14 proteins as well as some other

NOTE Confidence: 0.822163327333333

00:10:12.601 --> 00:10:15.670 accessory factors and it can be removed.

NOTE Confidence: 0.822163327333333

00:10:15.670 --> 00:10:17.140 By demethylase SES.

NOTE Confidence: 0.822163327333333

00:10:17.140 --> 00:10:18.477 Two of which I'm showing you here,

NOTE Confidence: 0.822163327333333

00:10:18.480 --> 00:10:20.555 called FTO&amp;L PH-5, essentially making

NOTE Confidence: 0.822163327333333

00:10:20.555 --> 00:10:23.230 this sort of a reversible process.

NOTE Confidence: 0.822163327333333

00:10:23.230 --> 00:10:24.436 You can add a methylation mark,

NOTE Confidence: 0.822163327333333

00:10:24.440 --> 00:10:27.464 you can remove it back and forth.

NOTE Confidence: 0.822163327333333

00:10:27.470 --> 00:10:29.225 But the consequences of of

NOTE Confidence: 0.822163327333333

00:10:29.225 --> 00:10:30.278 this particular methylation,

NOTE Confidence: 0.822163327333333

00:10:30.280 --> 00:10:32.044 particularly near the three prime end

NOTE Confidence: 0.822163327333333

00:10:32.044 --> 00:10:34.243 of M RNA's is is really interesting

NOTE Confidence: 0.822163327333333

00:10:34.243 --> 00:10:36.497 because in fact that mark alone is

NOTE Confidence: 0.822163327333333

00:10:36.563 --> 00:10:38.807 sufficient to sort of recruit the

NOTE Confidence: 0.822163327333333

00:10:38.807 --> 00:10:41.210 adenylation and decay machinery to M RNA's.

NOTE Confidence: 0.822163327333333

00:10:41.210 --> 00:10:43.010 And so essentially what happens is

NOTE Confidence: 0.822163327333333

00:10:43.010 --> 00:10:45.633 the this methyl mark is specifically

NOTE Confidence: 0.822163327333333

00:10:45.633 --> 00:10:47.888 recognized by multiple different proteins,

NOTE Confidence: 0.822163327333333

00:10:47.890 --> 00:10:50.098 but in many cases this protein

NOTE Confidence: 0.822163327333333

00:10:50.098 --> 00:10:51.616 called YTHD F2.

NOTE Confidence: 0.822163327333333

00:10:51.616 --> 00:10:53.908 And in that process,

NOTE Confidence: 0.822163327333333

00:10:53.910 --> 00:10:56.418 YTHD F2 then recruits the DND

NOTE Confidence: 0.822163327333333

00:10:56.418 --> 00:10:58.090 adenylation and decay complex,

NOTE Confidence: 0.822163327333333

00:10:58.090 --> 00:10:59.334 which effectively means that

NOTE Confidence: 0.822163327333333

00:10:59.334 --> 00:11:01.200 this M RNA is destabilized not

NOTE Confidence: 0.822163327333333

00:11:01.256 --> 00:11:03.046 necessarily at the chemical level,

NOTE Confidence: 0.822163327333333

00:11:03.050 --> 00:11:05.774 but because the actual decay machinery

NOTE Confidence: 0.822163327333333

00:11:05.774 --> 00:11:08.238 is getting actively recruited to this

NOTE Confidence: 0.822163327333333

00:11:08.238 --> 00:11:10.534 M RNA to decay it more quickly than

NOTE Confidence: 0.822163327333333

00:11:10.606 --> 00:11:12.874 it would if it was not methylated.

NOTE Confidence: 0.822163327333333

00:11:12.880 --> 00:11:13.999 And so this,

NOTE Confidence: 0.822163327333333

00:11:13.999 --> 00:11:15.118 it turns out,

NOTE Confidence: 0.822163327333333

00:11:15.120 --> 00:11:17.880 can then coordinate with other modifications.

NOTE Confidence: 0.822163327333333

00:11:17.880 --> 00:11:20.360 So what I'm showing you here is an

NOTE Confidence: 0.822163327333333

00:11:20.360 --> 00:11:22.079 example actually from development.

NOTE Confidence: 0.822163327333333

00:11:22.080 --> 00:11:23.768 This is for those of you that are

NOTE Confidence: 0.822163327333333

00:11:23.768 --> 00:11:25.230 interested in developmental biology,

NOTE Confidence: 0.822163327333333

00:11:25.230 --> 00:11:27.166 the maternal zygotic transition

NOTE Confidence: 0.822163327333333

00:11:27.166 --> 00:11:28.618 and zebrafish development.

NOTE Confidence: 0.822163327333333

00:11:28.620 --> 00:11:30.906 But really this is applicable to

NOTE Confidence: 0.822163327333333

00:11:30.906 --> 00:11:32.840 many other situations as well.

NOTE Confidence: 0.822163327333333

00:11:32.840 --> 00:11:35.130 And So what can happen is you can have for

NOTE Confidence: 0.822163327333333

00:11:35.187 --> 00:11:37.363 instance in this case a subset of genes,

NOTE Confidence: 0.822163327333333

00:11:37.370 --> 00:11:39.687 in this case the the maternal genes

NOTE Confidence: 0.822163327333333

00:11:39.687 --> 00:11:41.950 that are marked by this M6A mark.

NOTE Confidence: 0.822163327333333

00:11:41.950 --> 00:11:44.200 That decay machineries and recruited at

NOTE Confidence: 0.822163327333333

00:11:44.200 --> 00:11:46.220 the appropriate time and development,

NOTE Confidence: 0.822163327333333

00:11:46.220 --> 00:11:48.719 causing a drop in the maternal the

NOTE Confidence: 0.822163327333333

00:11:48.719 --> 00:11:51.580 levels of these maternal transcripts at

NOTE Confidence: 0.822163327333333

00:11:51.580 --> 00:11:53.820 the time at which the zygotic genes are

NOTE Confidence: 0.822163327333333

00:11:53.820 --> 00:11:56.537 the ones that are supposed to be activated.

NOTE Confidence: 0.822163327333333

00:11:56.540 --> 00:11:58.660 So to facilitate this transition,

NOTE Confidence: 0.822163327333333

00:11:58.660 --> 00:12:00.495 essentially maternal genes are being

NOTE Confidence: 0.822163327333333

00:12:00.495 --> 00:12:02.980 decayed by the presence of this mark.

NOTE Confidence: 0.822163327333333

00:12:02.980 --> 00:12:04.882 But there's a subset of transcripts

00:12:04.882 --> 00:12:06.586 in this context that actually

00:12:06.586 --> 00:12:08.481 need to be maintained and

00:12:08.481 --> 00:12:09.997 stabilized through this process,

00:12:10.000 --> 00:12:13.037 and it turns out that these the subset of.

00:12:13.037 --> 00:12:14.233 Transcripts is actually marked

00:12:14.233 --> 00:12:15.830 by a different modification,

00:12:15.830 --> 00:12:18.026 in this case 5 methyl cytidine.

00:12:18.030 --> 00:12:20.510 And in this case in fact the modification

00:12:20.510 --> 00:12:22.517 is recruiting a different set of

00:12:22.517 --> 00:12:24.177 machinery that is preventing these

00:12:24.177 --> 00:12:25.969 these transcripts from being decayed

00:12:25.969 --> 00:12:28.487 and allowing for sort of longer half

00:12:28.487 --> 00:12:30.989 lives and stability of those transcripts

00:12:30.989 --> 00:12:32.940 through this developmental transition.

00:12:32.940 --> 00:12:33.260 Now,

00:12:33.260 --> 00:12:35.180 it's important to note actually that

NOTE Confidence: 0.822163327333333

00:12:35.180 --> 00:12:37.532 we have arrived at this sort of

NOTE Confidence: 0.822163327333333

00:12:37.532 --> 00:12:39.192 very simplified picture by numerous

NOTE Confidence: 0.822163327333333

00:12:39.192 --> 00:12:41.097 studies in many different contexts,

NOTE Confidence: 0.822163327333333

00:12:41.100 --> 00:12:42.732 all studying different modifications

NOTE Confidence: 0.822163327333333

00:12:42.732 --> 00:12:43.956 on their own,

NOTE Confidence: 0.822163327333333

00:12:43.960 --> 00:12:44.334 separately.

NOTE Confidence: 0.822163327333333

00:12:44.334 --> 00:12:46.952 So we have sort of synthesized this

NOTE Confidence: 0.822163327333333

00:12:46.952 --> 00:12:48.503 information together to postulate

NOTE Confidence: 0.822163327333333

00:12:48.503 --> 00:12:50.717 a model whereby you could have

NOTE Confidence: 0.822163327333333

00:12:50.717 --> 00:12:51.860 different modifications coordinating

NOTE Confidence: 0.822163327333333

00:12:51.860 --> 00:12:53.936 these events all in one system.

NOTE Confidence: 0.822163327333333

00:12:53.940 --> 00:12:56.164 But I will point out that we actually

NOTE Confidence: 0.822163327333333

00:12:56.164 --> 00:12:58.234 don't have the power for the most

NOTE Confidence: 0.822163327333333

00:12:58.234 --> 00:13:00.223 part yet to actually detect all of

NOTE Confidence: 0.822163327333333

00:13:00.223 --> 00:13:02.388 this sort of in real time, you know,

NOTE Confidence: 0.822163327333333

00:13:02.388 --> 00:13:03.480 with multiple modifications.

NOTE Confidence: 0.822163327333333

00:13:03.480 --> 00:13:03.998 At once.

NOTE Confidence: 0.822163327333333

00:13:03.998 --> 00:13:06.070 But you can imagine that this kind of

NOTE Confidence: 0.860175690952381

00:13:06.127 --> 00:13:08.221 decay mechanism would not only be

NOTE Confidence: 0.860175690952381

00:13:08.221 --> 00:13:10.350 important for something like development,

NOTE Confidence: 0.860175690952381

00:13:10.350 --> 00:13:12.630 but also in cases of disease.

NOTE Confidence: 0.860175690952381

00:13:12.630 --> 00:13:15.406 And so as soon as this sort of

NOTE Confidence: 0.860175690952381

00:13:15.406 --> 00:13:17.310 decay mechanism was discovered,

NOTE Confidence: 0.860175690952381

00:13:17.310 --> 00:13:20.126 we had an explosion of work in many,

NOTE Confidence: 0.860175690952381

00:13:20.130 --> 00:13:22.430 many different areas, but particularly

NOTE Confidence: 0.860175690952381

00:13:22.430 --> 00:13:25.539 in various sort of forms of cancer.

NOTE Confidence: 0.860175690952381

00:13:25.540 --> 00:13:27.444 And so I'm showing you just a subset

NOTE Confidence: 0.860175690952381

00:13:27.444 --> 00:13:29.340 of papers here, most of them not mine,

NOTE Confidence: 0.860175690952381

00:13:29.340 --> 00:13:31.473 but one of them is and I will sort

NOTE Confidence: 0.860175690952381

00:13:31.473 --> 00:13:33.711 of use that as an example, but.

NOTE Confidence: 0.860175690952381

00:13:33.711 --> 00:13:35.555 Essentially by studying these

NOTE Confidence: 0.860175690952381

00:13:35.555 --> 00:13:36.938 different modifications in

NOTE Confidence: 0.860175690952381

00:13:36.938 --> 00:13:38.839 multiple different types of cancer,

NOTE Confidence: 0.860175690952381

00:13:38.840 --> 00:13:40.888 it was sort of realized that it might

NOTE Confidence: 0.860175690952381

00:13:40.888 --> 00:13:43.321 be that these modifications are also

NOTE Confidence: 0.860175690952381

00:13:43.321 --> 00:13:45.185 regulating transcript stability of

NOTE Confidence: 0.860175690952381

00:13:45.185 --> 00:13:47.258 critical oncogenic or tumor suppressor

NOTE Confidence: 0.860175690952381

00:13:47.258 --> 00:13:49.418 transcripts in the context of cancer.

NOTE Confidence: 0.860175690952381

00:13:49.420 --> 00:13:51.680 And So what I'm going to do today is sort

NOTE Confidence: 0.860175690952381

00:13:51.740 --> 00:13:54.053 of use one of these studies as an example,

NOTE Confidence: 0.860175690952381

00:13:54.060 --> 00:13:55.086 this one here,

NOTE Confidence: 0.860175690952381

00:13:55.086 --> 00:13:57.138 which is actually done in AML,

NOTE Confidence: 0.860175690952381

00:13:57.140 --> 00:13:58.790 but for reasons that hopefully

NOTE Confidence: 0.860175690952381

00:13:58.790 --> 00:14:00.778 will become clear are sort of

NOTE Confidence: 0.860175690952381

00:14:00.778 --> 00:14:02.600 relevant to some of the brain cancer

NOTE Confidence: 0.860175690952381

00:14:02.600 --> 00:14:04.359 work we're hoping to do as well.

NOTE Confidence: 0.860175690952381

00:14:04.360 --> 00:14:06.068 But really this is just sort of

NOTE Confidence: 0.860175690952381

00:14:06.068 --> 00:14:07.942 to give you a snapshot of sort

NOTE Confidence: 0.860175690952381

00:14:07.942 --> 00:14:09.297 of what we have learned,

NOTE Confidence: 0.860175690952381

00:14:09.300 --> 00:14:10.815 but also the significant limitations

NOTE Confidence: 0.860175690952381

00:14:10.815 --> 00:14:12.909 that we still encounter in this field.

NOTE Confidence: 0.910400546

00:14:15.090 --> 00:14:16.790 And so this story started,

NOTE Confidence: 0.910400546

00:14:16.790 --> 00:14:19.250 this is a collaborative effort.

NOTE Confidence: 0.910400546

00:14:19.250 --> 00:14:21.545 I was sort of, I was a postdoc in

NOTE Confidence: 0.910400546

00:14:21.545 --> 00:14:23.898 Juan he's lab and John Jin Chen was

NOTE Confidence: 0.910400546

00:14:23.898 --> 00:14:26.088 working on a big project essentially

NOTE Confidence: 0.910400546

00:14:26.088 --> 00:14:28.173 trying to characterize the roles

NOTE Confidence: 0.910400546

00:14:28.173 --> 00:14:30.618 of N6 methyl adenosine in AML.

NOTE Confidence: 0.910400546

00:14:30.618 --> 00:14:34.174 And they stumbled on sort of a really

NOTE Confidence: 0.910400546

00:14:34.174 --> 00:14:36.298 interesting observation with respect

NOTE Confidence: 0.910400546

00:14:36.298 --> 00:14:38.960 to R2 HYDROXYBUTYRATE or R2 HG,

NOTE Confidence: 0.910400546

00:14:38.960 --> 00:14:41.053 which at the time was sort of

NOTE Confidence: 0.910400546

00:14:41.053 --> 00:14:43.408 thought to be an uncle metabolite.

NOTE Confidence: 0.910400546

00:14:43.410 --> 00:14:44.698 And that was, there was sort of

NOTE Confidence: 0.910400546

00:14:44.698 --> 00:14:45.990 various lines of evidence for this.

NOTE Confidence: 0.910400546

00:14:45.990 --> 00:14:48.881 But essentially there was sort of a

NOTE Confidence: 0.910400546

00:14:48.881 --> 00:14:51.075 long history of of this metabolite

NOTE Confidence: 0.910400546

00:14:51.075 --> 00:14:52.905 being described as an Aqua metabolite.

NOTE Confidence: 0.910400546

00:14:52.910 --> 00:14:55.059 But there were some sort of weird

NOTE Confidence: 0.910400546

00:14:55.059 --> 00:14:57.264 results that I'll get into that sort

NOTE Confidence: 0.910400546

00:14:57.264 --> 00:14:59.290 of suggested that that might not be

NOTE Confidence: 0.910400546

00:14:59.290 --> 00:15:01.789 the case in every in every situation.

NOTE Confidence: 0.910400546

00:15:01.790 --> 00:15:04.250 And so this metabolite is actually

NOTE Confidence: 0.910400546

00:15:04.250 --> 00:15:06.575 derived from a mutant form

NOTE Confidence: 0.910400546

00:15:06.575 --> 00:15:08.390 of isocitrate dehydrogenase.

NOTE Confidence: 0.910400546

00:15:08.390 --> 00:15:11.374 And so I DH isn't the enzyme that's

NOTE Confidence: 0.910400546

00:15:11.374 --> 00:15:13.950 responsible for transforming isocitrate.

NOTE Confidence: 0.910400546

00:15:13.950 --> 00:15:14.907 To Alpha Ketoglutarate,

NOTE Confidence: 0.910400546

00:15:14.907 --> 00:15:16.821 which is a critical factor for

NOTE Confidence: 0.910400546

00:15:16.821 --> 00:15:18.438 many many different enzymes,

NOTE Confidence: 0.910400546

00:15:18.440 --> 00:15:19.850 including RNA modification

NOTE Confidence: 0.910400546

00:15:19.850 --> 00:15:21.730 enzymes in the cell.

NOTE Confidence: 0.910400546

00:15:21.730 --> 00:15:24.030 But it turns out that

NOTE Confidence: 0.910400546

00:15:24.030 --> 00:15:25.410 with specific mutations,

NOTE Confidence: 0.910400546

00:15:25.410 --> 00:15:26.649 as many of you might be aware,

NOTE Confidence: 0.910400546

00:15:26.650 --> 00:15:29.234 I DH can generate sort of a different

NOTE Confidence: 0.910400546

00:15:29.234 --> 00:15:31.729 a different form of this molecule card,

NOTE Confidence: 0.910400546

00:15:31.730 --> 00:15:35.426 excuse me, called R2 hydroxy glutarate.

NOTE Confidence: 0.910400546

00:15:35.430 --> 00:15:38.418 So it turns out that this

NOTE Confidence: 0.910400546

00:15:38.418 --> 00:15:41.630 particular form of this metabolite,

NOTE Confidence: 0.910400546

00:15:41.630 --> 00:15:44.255 R2 HD can in fact compete with

NOTE Confidence: 0.910400546

00:15:44.255 --> 00:15:46.538 alpha ketoglutarate at many of the

NOTE Confidence: 0.910400546

00:15:46.538 --> 00:15:48.704 sort of enzyme sites that require

NOTE Confidence: 0.910400546

00:15:48.704 --> 00:15:51.049 alpha ketoglutarate for activity.

NOTE Confidence: 0.910400546

00:15:51.050 --> 00:15:53.565 This includes cofactors for many

NOTE Confidence: 0.910400546

00:15:53.565 --> 00:15:55.577 different enzymes including histone

NOTE Confidence: 0.910400546

00:15:55.577 --> 00:15:57.828 DNA and RNA demethylase hes,

NOTE Confidence: 0.910400546

00:15:57.830 --> 00:15:59.288 which, you know,

NOTE Confidence: 0.910400546

00:15:59.288 --> 00:16:01.734 if you've encountered any sort of

NOTE Confidence: 0.910400546

00:16:01.734 --> 00:16:03.374 gene expression analysis of cancers,

NOTE Confidence: 0.910400546

00:16:03.380 --> 00:16:05.980 we know that many of these are dysregulated.

NOTE Confidence: 0.910400546

00:16:05.980 --> 00:16:08.510 And in many different cancers.

NOTE Confidence: 0.910400546

00:16:08.510 --> 00:16:10.410 And so sort of combined,

NOTE Confidence: 0.910400546

00:16:10.410 --> 00:16:12.797 we can sort of imagine a scenario

NOTE Confidence: 0.910400546

00:16:12.797 --> 00:16:14.680 where the presence of suddenly,

NOTE Confidence: 0.910400546

00:16:14.680 --> 00:16:15.234 you know,

NOTE Confidence: 0.910400546

00:16:15.234 --> 00:16:17.173 a much higher level of this metabolite

NOTE Confidence: 0.910400546

00:16:17.173 --> 00:16:19.331 R2 HG could significantly impact gene

NOTE Confidence: 0.910400546

00:16:19.331 --> 00:16:21.502 expression patterns based on the fact

NOTE Confidence: 0.910400546

00:16:21.502 --> 00:16:23.518 that it would sort of compete with

NOTE Confidence: 0.910400546

00:16:23.518 --> 00:16:25.423 these enzymes that are modulating

NOTE Confidence: 0.910400546

00:16:25.423 --> 00:16:27.628 all of these different groups.

NOTE Confidence: 0.910400546

00:16:27.630 --> 00:16:29.800 And so it was interesting about this

NOTE Confidence: 0.910400546

00:16:29.800 --> 00:16:31.518 particular study and when they sort

NOTE Confidence: 0.910400546

00:16:31.518 --> 00:16:33.561 of like roped myself and a few other

NOTE Confidence: 0.910400546

00:16:33.561 --> 00:16:35.737 people in my postdoc lab and on this

NOTE Confidence: 0.910400546

00:16:35.737 --> 00:16:37.795 project was the sort of interesting

NOTE Confidence: 0.910400546

00:16:37.795 --> 00:16:39.580 observation that when they just

NOTE Confidence: 0.910400546

00:16:39.650 --> 00:16:41.526 took a panel of AML cell lines.

NOTE Confidence: 0.910400546

00:16:41.530 --> 00:16:43.432 So these are grown in dishes

NOTE Confidence: 0.910400546

00:16:43.432 --> 00:16:45.040 and culture in the lab.

NOTE Confidence: 0.910400546

00:16:45.040 --> 00:16:47.476 There was a pretty striking difference

NOTE Confidence: 0.910400546

00:16:47.476 --> 00:16:49.945 in how sensitive they were to

NOTE Confidence: 0.910400546

00:16:49.945 --> 00:16:51.880 the presence of this metabolite.

NOTE Confidence: 0.910400546

00:16:51.880 --> 00:16:53.608 And so there was actually a

NOTE Confidence: 0.910400546

00:16:53.608 --> 00:16:55.402 panel of about maybe three times

NOTE Confidence: 0.910400546

00:16:55.402 --> 00:16:56.857 the size in the paper.

NOTE Confidence: 0.910400546

00:16:56.860 --> 00:16:58.150 And because this is published,

NOTE Confidence: 0.910400546

00:16:58.150 --> 00:16:58.966 I'm not going to go through

NOTE Confidence: 0.910400546

00:16:58.966 --> 00:16:59.700 sort of all the data,

NOTE Confidence: 0.910400546

00:16:59.700 --> 00:17:01.079 but I'm going to try to give

NOTE Confidence: 0.910400546

00:17:01.079 --> 00:17:02.660 you sort of the snapshots to

NOTE Confidence: 0.910400546

00:17:02.660 --> 00:17:04.460 sort of give you the idea.

NOTE Confidence: 0.910400546

00:17:04.460 --> 00:17:07.554 So in just this subset of cells,

NOTE Confidence: 0.910400546

00:17:07.560 --> 00:17:10.339 we can see that there is quite

NOTE Confidence: 0.910400546

00:17:10.339 --> 00:17:12.745 a few that are sensitive in the

NOTE Confidence: 0.910400546

00:17:12.745 --> 00:17:14.670 sense that the size of the circle

NOTE Confidence: 0.887607190909091

00:17:14.729 --> 00:17:16.482 means. How many cells are still viable

NOTE Confidence: 0.887607190909091

00:17:16.482 --> 00:17:18.950 and so at an early time point which is in

NOTE Confidence: 0.887607190909091

00:17:18.950 --> 00:17:21.329 Gray you can see all the cells are viable.

NOTE Confidence: 0.887607190909091

00:17:21.330 --> 00:17:23.162 But when you treat with R2 HG the

NOTE Confidence: 0.887607190909091

00:17:23.162 --> 00:17:24.449 the different colors are different

NOTE Confidence: 0.887607190909091

00:17:24.449 --> 00:17:26.255 time points and you can see that

NOTE Confidence: 0.887607190909091

00:17:26.313 --> 00:17:28.155 dramatically the circle gets really small

NOTE Confidence: 0.887607190909091

00:17:28.155 --> 00:17:30.010 really quickly and so this suggests

NOTE Confidence: 0.887607190909091

00:17:30.010 --> 00:17:32.490 a cell line that when you add this

NOTE Confidence: 0.887607190909091

00:17:32.555 --> 00:17:34.879 uncle metabolite a lot of cells die.

NOTE Confidence: 0.887607190909091

00:17:34.880 --> 00:17:36.000 But on the flip side,

NOTE Confidence: 0.887607190909091

00:17:36.000 --> 00:17:37.624 you can see in the bottom row here

NOTE Confidence: 0.887607190909091

00:17:37.624 --> 00:17:39.291 that there are actually quite a few

NOTE Confidence: 0.887607190909091

00:17:39.291 --> 00:17:40.919 that are also quite resistant to this.

NOTE Confidence: 0.887607190909091

00:17:40.920 --> 00:17:42.894 So despite using the same time course,

NOTE Confidence: 0.887607190909091

00:17:42.900 --> 00:17:45.010 the same dosing we have, you know,

NOTE Confidence: 0.887607190909091

00:17:45.010 --> 00:17:46.060 different cell lines that have

NOTE Confidence: 0.887607190909091

00:17:46.060 --> 00:17:47.120 ostensibly are the same thing,

NOTE Confidence: 0.887607190909091

00:17:47.120 --> 00:17:49.336 but of course we know that they're not.

NOTE Confidence: 0.887607190909091

00:17:49.340 --> 00:17:50.628 They're responding quite differently

NOTE Confidence: 0.887607190909091

00:17:50.628 --> 00:17:52.560 to the presence of this metabolite.

NOTE Confidence: 0.887607190909091

00:17:52.560 --> 00:17:54.569 And so this sort of started us

NOTE Confidence: 0.887607190909091

00:17:54.569 --> 00:17:57.164 off on a quest to sort of figure

NOTE Confidence: 0.887607190909091

00:17:57.164 --> 00:17:59.270 out why that might be true.

NOTE Confidence: 0.887607190909091

00:17:59.270 --> 00:18:00.702 And through this process,

NOTE Confidence: 0.887607190909091

00:18:00.702 --> 00:18:03.359 the Chen Lab noticed that one of

NOTE Confidence: 0.887607190909091

00:18:03.359 --> 00:18:05.544 the enzymes that was particularly

NOTE Confidence: 0.887607190909091

00:18:05.544 --> 00:18:07.748 disregulated across cell lines that

NOTE Confidence: 0.887607190909091

00:18:07.748 --> 00:18:10.094 were sort of sensitive and resistant

NOTE Confidence: 0.887607190909091

00:18:10.094 --> 00:18:12.598 to this metabolite was this enzyme FTO,

NOTE Confidence: 0.887607190909091

00:18:12.598 --> 00:18:15.436 which you might recall was one of the

NOTE Confidence: 0.887607190909091

00:18:15.436 --> 00:18:17.386 demethylase hes that I highlighted.

NOTE Confidence: 0.887607190909091

00:18:17.390 --> 00:18:20.478 So FTO removes the M6A mark from many

NOTE Confidence: 0.887607190909091

00:18:20.478 --> 00:18:23.414 different M RNA's and the sort of

NOTE Confidence: 0.887607190909091

00:18:23.414 --> 00:18:26.291 predicted outcome of this would be that

NOTE Confidence: 0.887607190909091

00:18:26.291 --> 00:18:29.154 you would have more M6A left on transcripts.

NOTE Confidence: 0.887607190909091

00:18:29.154 --> 00:18:30.598 Because you've sort of

NOTE Confidence: 0.887607190909091

00:18:30.598 --> 00:18:32.100 inhibited the demethylase.

NOTE Confidence: 0.887607190909091

00:18:32.100 --> 00:18:34.220 So that's a relatively simple thing to test.

NOTE Confidence: 0.887607190909091

00:18:34.220 --> 00:18:36.452 And so we can measure these

NOTE Confidence: 0.887607190909091

00:18:36.452 --> 00:18:38.360 modifications by various different means.

NOTE Confidence: 0.887607190909091

00:18:38.360 --> 00:18:39.851 The data I'll show you is a

NOTE Confidence: 0.887607190909091

00:18:39.851 --> 00:18:41.140 mass spec based measurement,

NOTE Confidence: 0.887607190909091

00:18:41.140 --> 00:18:44.230 but we did this by by other means as well.

NOTE Confidence: 0.887607190909091

00:18:44.230 --> 00:18:46.666 And so when you measure the

NOTE Confidence: 0.887607190909091

00:18:46.666 --> 00:18:48.830 levels of this M6A modification,

NOTE Confidence: 0.887607190909091

00:18:48.830 --> 00:18:50.710 we always normalize relative

NOTE Confidence: 0.887607190909091

00:18:50.710 --> 00:18:52.590 to the unmodified a.

NOTE Confidence: 0.887607190909091

00:18:52.590 --> 00:18:54.654 So that's sort of what this

NOTE Confidence: 0.887607190909091

00:18:54.654 --> 00:18:57.099 ratio here on the Y axis shows,

NOTE Confidence: 0.887607190909091

00:18:57.100 --> 00:18:59.584 can see that when you treat

NOTE Confidence: 0.887607190909091

00:18:59.584 --> 00:19:01.810 with drug for sensitive lines,

NOTE Confidence: 0.887607190909091

00:19:01.810 --> 00:19:04.354 you see this small but sort of reproducible

NOTE Confidence: 0.887607190909091

00:19:04.354 --> 00:19:06.726 increase in M6A levels like we predicted,

NOTE Confidence: 0.887607190909091

00:19:06.730 --> 00:19:08.970 whereas it was much more variable in

NOTE Confidence: 0.887607190909091

00:19:08.970 --> 00:19:11.006 the resistant lines and there was

NOTE Confidence: 0.887607190909091

00:19:11.006 --> 00:19:13.028 certainly no sort of consistent effect.

NOTE Confidence: 0.887607190909091

00:19:13.030 --> 00:19:14.580 So this is potentially intriguing,

NOTE Confidence: 0.887607190909091

00:19:14.580 --> 00:19:16.405 but it still didn't really

NOTE Confidence: 0.887607190909091

00:19:16.405 --> 00:19:17.135 explain everything.

NOTE Confidence: 0.887607190909091

00:19:17.140 --> 00:19:19.777 And so we sort of turned to like what,

NOTE Confidence: 0.887607190909091

00:19:19.780 --> 00:19:22.180 why would the presence of this

NOTE Confidence: 0.887607190909091

00:19:22.180 --> 00:19:24.520 metabolite actually have such an impact?

NOTE Confidence: 0.887607190909091

00:19:24.520 --> 00:19:27.032 And we got a big clue from this

NOTE Confidence: 0.887607190909091

00:19:27.032 --> 00:19:29.269 Western blot that was done in the

NOTE Confidence: 0.887607190909091

00:19:29.269 --> 00:19:31.618 Chen lab in which they showed that

NOTE Confidence: 0.887607190909091

00:19:31.618 --> 00:19:34.215 the levels of this demethylase FT O

NOTE Confidence: 0.887607190909091

00:19:34.215 --> 00:19:36.655 were vastly different in the sensitive

NOTE Confidence: 0.887607190909091

00:19:36.655 --> 00:19:38.720 lines versus the resistant lines.

NOTE Confidence: 0.887607190909091

00:19:38.720 --> 00:19:41.710 And conversely levels of Mick

NOTE Confidence: 0.887607190909091

00:19:41.710 --> 00:19:43.504 were dramatically lower.

NOTE Confidence: 0.887607190909091

00:19:43.510 --> 00:19:44.982 In the sensitive lines,

NOTE Confidence: 0.887607190909091

00:19:44.982 --> 00:19:46.822 then in the resistant lines.

NOTE Confidence: 0.887607190909091

00:19:46.830 --> 00:19:49.889 And that the levels of these two

NOTE Confidence: 0.887607190909091

00:19:49.889 --> 00:19:52.230 key factors actually change when

NOTE Confidence: 0.887607190909091

00:19:52.230 --> 00:19:54.585 you add this metabolite RHG.

NOTE Confidence: 0.887607190909091

00:19:54.590 --> 00:19:56.576 So this was intriguing because of

NOTE Confidence: 0.887607190909091

00:19:56.576 --> 00:19:58.635 course Mick is a critical factor

NOTE Confidence: 0.887607190909091

00:19:58.635 --> 00:20:01.283 that drives a lot of not just AML,

NOTE Confidence: 0.887607190909091

00:20:01.290 --> 00:20:02.790 but a lot of other cancers.

NOTE Confidence: 0.887607190909091

00:20:02.790 --> 00:20:04.494 And so we wondered whether we

NOTE Confidence: 0.887607190909091

00:20:04.494 --> 00:20:05.630 could sort of turn

NOTE Confidence: 0.840944913846154

00:20:05.694 --> 00:20:07.680 a sensitive line into a resistant

NOTE Confidence: 0.840944913846154

00:20:07.680 --> 00:20:09.368 1 purely by increasing the

NOTE Confidence: 0.840944913846154

00:20:09.368 --> 00:20:10.848 levels of making the cell.

NOTE Confidence: 0.840944913846154

00:20:10.850 --> 00:20:12.230 And it turns out that you

NOTE Confidence: 0.840944913846154

00:20:12.230 --> 00:20:13.150 can actually do that.

NOTE Confidence: 0.840944913846154

00:20:13.150 --> 00:20:14.470 And so I'm just showing you.

NOTE Confidence: 0.840944913846154

00:20:14.470 --> 00:20:16.619 One snapshot here, but there's no more.

NOTE Confidence: 0.840944913846154

00:20:16.620 --> 00:20:19.176 One cell line was one sensitive

NOTE Confidence: 0.840944913846154

00:20:19.176 --> 00:20:21.346 cell line where essentially the

NOTE Confidence: 0.840944913846154

00:20:21.346 --> 00:20:23.686 red lines represent just no sort

NOTE Confidence: 0.840944913846154

00:20:23.686 --> 00:20:26.158 of additional mix just at baseline.

NOTE Confidence: 0.840944913846154

00:20:26.160 --> 00:20:28.808 And when you add the R2HG,

NOTE Confidence: 0.840944913846154

00:20:28.808 --> 00:20:30.952 you can see that there's a drop in

NOTE Confidence: 0.840944913846154

00:20:30.952 --> 00:20:33.480 the ability of the cells to proliferate.

NOTE Confidence: 0.840944913846154

00:20:33.480 --> 00:20:35.556 When you exogenously just add a

NOTE Confidence: 0.840944913846154

00:20:35.556 --> 00:20:37.829 bunch of mic expression to the cells,

NOTE Confidence: 0.840944913846154

00:20:37.830 --> 00:20:40.175 you can see that you can make

NOTE Confidence: 0.840944913846154

00:20:40.175 --> 00:20:41.660 them more resistant to it.

NOTE Confidence: 0.840944913846154

00:20:41.660 --> 00:20:43.478 So this is just one snapshot,

NOTE Confidence: 0.840944913846154

00:20:43.480 --> 00:20:45.330 but this is sort of.

NOTE Confidence: 0.840944913846154

00:20:45.330 --> 00:20:46.746 The model that we sort of,

NOTE Confidence: 0.840944913846154

00:20:46.750 --> 00:20:47.066 you know,

NOTE Confidence: 0.840944913846154

00:20:47.066 --> 00:20:48.534 took out from this and I will say that

NOTE Confidence: 0.840944913846154

00:20:48.534 --> 00:20:49.854 there was a whole bunch of sort of

NOTE Confidence: 0.840944913846154

00:20:49.854 --> 00:20:51.349 mouse work done as well that I was not

NOTE Confidence: 0.840944913846154

00:20:51.349 --> 00:20:53.190 involved in and that I'm not showing.

NOTE Confidence: 0.840944913846154

00:20:53.190 --> 00:20:54.027 If you're interested,

NOTE Confidence: 0.840944913846154

00:20:54.027 --> 00:20:56.350 you are welcome to check out the paper.

NOTE Confidence: 0.840944913846154

00:20:56.350 --> 00:20:59.059 But all together we sort of settled

NOTE Confidence: 0.840944913846154

00:20:59.059 --> 00:21:01.748 on this model where perhaps the

NOTE Confidence: 0.840944913846154

00:21:01.748 --> 00:21:04.158 relative levels of the demethylase

NOTE Confidence: 0.840944913846154

00:21:04.158 --> 00:21:06.611 and Mick were sort of essentially

NOTE Confidence: 0.840944913846154

00:21:06.611 --> 00:21:09.838 dictating where on a spectrum of R2

NOTE Confidence: 0.840944913846154

00:21:09.838 --> 00:21:12.470 HG sensitivity these cell lines were.

NOTE Confidence: 0.840944913846154

00:21:12.470 --> 00:21:14.934 So the idea would be if you have.

NOTE Confidence: 0.840944913846154

00:21:14.940 --> 00:21:18.195 A lot of mixed transcripts floating around.

NOTE Confidence: 0.840944913846154

00:21:18.200 --> 00:21:22.380 You add R2 HG that inhibits FTO.

NOTE Confidence: 0.840944913846154

00:21:22.380 --> 00:21:25.260 You have less demethylation.

NOTE Confidence: 0.840944913846154

00:21:25.260 --> 00:21:28.116 You get a mic target gene expression

NOTE Confidence: 0.840944913846154

00:21:28.120 --> 00:21:32.020 because essentially M6A levels are lower.

NOTE Confidence: 0.840944913846154

00:21:32.020 --> 00:21:33.708 Transcript decay is lower,

NOTE Confidence: 0.840944913846154

00:21:33.708 --> 00:21:35.818 meaning overall mic levels are

NOTE Confidence: 0.840944913846154

00:21:35.818 --> 00:21:38.507 high and so the mic itself and its

NOTE Confidence: 0.840944913846154

00:21:38.507 --> 00:21:40.679 target genes are highly expressed,

NOTE Confidence: 0.840944913846154

00:21:40.680 --> 00:21:41.119 however.

NOTE Confidence: 0.840944913846154

00:21:41.119 --> 00:21:43.753 In the opposite scenario where maybe

NOTE Confidence: 0.840944913846154

00:21:43.753 --> 00:21:46.849 you just have a lot of mic present or

NOTE Confidence: 0.840944913846154

00:21:46.849 --> 00:21:49.589 FTO is able to demethylase and remove a

NOTE Confidence: 0.840944913846154

00:21:49.589 --> 00:21:52.644 lot of that methyl that methylation mark,

NOTE Confidence: 0.840944913846154

00:21:52.644 --> 00:21:56.035 you can overcome this by essentially just

NOTE Confidence: 0.840944913846154

00:21:56.035 --> 00:21:58.759 boosting the amount of Mick transcript.

NOTE Confidence: 0.840944913846154

00:21:58.760 --> 00:22:00.743 So essentially the

NOTE Confidence: 0.840944913846154

00:22:00.743 --> 00:22:02.726 methylation facilitates decay,

NOTE Confidence: 0.840944913846154

00:22:02.730 --> 00:22:04.386 loss of methylation facilitates

NOTE Confidence: 0.840944913846154

00:22:04.386 --> 00:22:06.456 stability and essentially by toggling

NOTE Confidence: 0.840944913846154

00:22:06.456 --> 00:22:08.297 both of these variables you're able

NOTE Confidence: 0.840944913846154

00:22:08.297 --> 00:22:10.584 to get sort of the spectrum of R2

NOTE Confidence: 0.840944913846154

00:22:10.584 --> 00:22:12.300 HD sensitivity even in what is.

NOTE Confidence: 0.840944913846154

00:22:12.300 --> 00:22:13.915 Ostensibly cell lines from sort

NOTE Confidence: 0.840944913846154

00:22:13.915 --> 00:22:15.207 of the same cancer.

NOTE Confidence: 0.840944913846154

00:22:15.210 --> 00:22:18.426 Now there's a lot of limitations to this.

NOTE Confidence: 0.840944913846154

00:22:18.430 --> 00:22:19.588 I'm going to go into some

NOTE Confidence: 0.840944913846154

00:22:19.588 --> 00:22:20.730 of them in great detail.

NOTE Confidence: 0.840944913846154

00:22:20.730 --> 00:22:22.759 But of course a big one is that a lot of

NOTE Confidence: 0.840944913846154

00:22:22.759 --> 00:22:24.766 this is done in cell lines and mouse models.

NOTE Confidence: 0.840944913846154

00:22:24.770 --> 00:22:27.052 And we're sort of zooming in on

NOTE Confidence: 0.840944913846154

00:22:27.052 --> 00:22:28.589 one particular aspect of this.

NOTE Confidence: 0.840944913846154

00:22:28.590 --> 00:22:30.746 We know that this metabolite does many,

NOTE Confidence: 0.840944913846154

00:22:30.750 --> 00:22:33.078 many other things including regulation of

NOTE Confidence: 0.840944913846154

00:22:33.078 --> 00:22:35.510 like way more metabolites than you know.

NOTE Confidence: 0.840944913846154

00:22:35.510 --> 00:22:37.095 We could even particularly cover

NOTE Confidence: 0.840944913846154

00:22:37.095 --> 00:22:38.046 in this talk.

NOTE Confidence: 0.840944913846154

00:22:38.050 --> 00:22:39.818 But I hope this is sort of highlighted

NOTE Confidence: 0.840944913846154

00:22:39.818 --> 00:22:41.689 one way in which sort of the presence

NOTE Confidence: 0.840944913846154

00:22:41.689 --> 00:22:43.240 or absence of this methylation.

NOTE Confidence: 0.840944913846154

00:22:43.240 --> 00:22:45.520 They can sort of dictate where you are,

NOTE Confidence: 0.840944913846154

00:22:45.520 --> 00:22:48.610 particularly in the context of cancers

NOTE Confidence: 0.840944913846154

00:22:48.610 --> 00:22:52.120 in which there are ID H mutations.

NOTE Confidence: 0.840944913846154

00:22:52.120 --> 00:22:53.029 And you know,

NOTE Confidence: 0.840944913846154

00:22:53.029 --> 00:22:56.240 this sort of set me on a path of a

NOTE Confidence: 0.840944913846154

00:22:56.240 --> 00:22:58.585 little bit of a rabbit hole and.

NOTE Confidence: 0.840944913846154

00:22:58.590 --> 00:22:59.997 It turns out that I didn't know

NOTE Confidence: 0.840944913846154

00:22:59.997 --> 00:23:00.600 this at the

NOTE Confidence: 0.900877240909091

00:23:00.646 --> 00:23:02.776 time. I'm sure all of you already know this,

NOTE Confidence: 0.900877240909091

00:23:02.780 --> 00:23:04.940 that you know glioblastoma is another

NOTE Confidence: 0.900877240909091

00:23:04.940 --> 00:23:07.227 instance in which ID H mutation

NOTE Confidence: 0.900877240909091

00:23:07.227 --> 00:23:08.791 status can dramatically impact

NOTE Confidence: 0.900877240909091

00:23:08.791 --> 00:23:10.980 sort of prognosis for patients.

NOTE Confidence: 0.900877240909091

00:23:10.980 --> 00:23:12.120 And one of my students in

NOTE Confidence: 0.900877240909091

00:23:12.120 --> 00:23:13.279 the lab is working on this.

NOTE Confidence: 0.900877240909091

00:23:13.280 --> 00:23:15.650 Emily actually found this sort

NOTE Confidence: 0.900877240909091

00:23:15.650 --> 00:23:18.020 of lovely table that summarizes

NOTE Confidence: 0.900877240909091

00:23:18.103 --> 00:23:20.722 a lot of what we know about N 6

NOTE Confidence: 0.900877240909091

00:23:20.722 --> 00:23:23.078 methyl adenosine in glioblastoma,

NOTE Confidence: 0.900877240909091

00:23:23.080 --> 00:23:25.385 not even taking into account

NOTE Confidence: 0.900877240909091

00:23:25.385 --> 00:23:27.229 ID H mutation status.

NOTE Confidence: 0.900877240909091

00:23:27.230 --> 00:23:29.126 And if you just read a few lines,

NOTE Confidence: 0.900877240909091

00:23:29.130 --> 00:23:30.390 you can already start to see.

NOTE Confidence: 0.900877240909091

00:23:30.390 --> 00:23:33.414 There's a lot of conflicting data on this.

NOTE Confidence: 0.900877240909091

00:23:33.420 --> 00:23:35.526 So for instance depending on you

NOTE Confidence: 0.900877240909091

00:23:35.526 --> 00:23:37.972 know which paper we want to look

NOTE Confidence: 0.900877240909091

00:23:37.972 --> 00:23:39.916 at all sort of published within

NOTE Confidence: 0.900877240909091

00:23:39.916 --> 00:23:42.197 the same approximate time frame.

NOTE Confidence: 0.900877240909091

00:23:42.200 --> 00:23:44.265 We have many different M6A

NOTE Confidence: 0.900877240909091

00:23:44.265 --> 00:23:46.640 related factors we can look at.

NOTE Confidence: 0.900877240909091

00:23:46.640 --> 00:23:48.998 But even when we're looking at the same ones,

00:23:49.000 --> 00:23:51.248 we can find papers that say the presence

00:23:51.248 --> 00:23:53.800 of that or absence of and says oncogenic.

00:23:53.800 --> 00:23:55.888 Another one says it's tumor suppressive.

00:23:55.890 --> 00:23:56.744 We different,

00:23:56.744 --> 00:23:58.879 you know different target genes

00:23:58.879 --> 00:24:00.900 are being explored mix socks.

00:24:00.900 --> 00:24:02.946 To Fox M1, essentially there's very

00:24:02.946 --> 00:24:04.850 little concordance among any of these.

00:24:04.850 --> 00:24:07.050 And that's not to say that they're wrong,

00:24:07.050 --> 00:24:09.226 but I think it sort of reflects that.

00:24:09.230 --> 00:24:10.049 Depending on exactly,

00:24:10.049 --> 00:24:11.687 let's say what cell line or

00:24:11.687 --> 00:24:13.028 what system you're using,

00:24:13.030 --> 00:24:14.716 if you have something like a

00:24:14.716 --> 00:24:16.258 spectrum of sensitivity or just

00:24:16.258 --> 00:24:17.726 differences in gene expression,

NOTE Confidence: 0.900877240909091

00:24:17.730 --> 00:24:19.774 you can arrive at this very confusing

NOTE Confidence: 0.900877240909091

00:24:19.774 --> 00:24:22.147 pattern of data that I am not a clinician,

NOTE Confidence: 0.900877240909091

00:24:22.150 --> 00:24:22.729 like I said.

NOTE Confidence: 0.900877240909091

00:24:22.729 --> 00:24:24.080 But I would assume that this doesn't

NOTE Confidence: 0.900877240909091

00:24:24.126 --> 00:24:25.426 exactly scream confidence to you,

NOTE Confidence: 0.900877240909091

00:24:25.430 --> 00:24:26.400 that this is something that,

NOTE Confidence: 0.900877240909091

00:24:26.400 --> 00:24:26.982 you know,

NOTE Confidence: 0.900877240909091

00:24:26.982 --> 00:24:28.437 maybe would be interesting to

NOTE Confidence: 0.900877240909091

00:24:28.437 --> 00:24:29.750 pursue in the clinic.

NOTE Confidence: 0.900877240909091

00:24:29.750 --> 00:24:31.974 And so this sort of motivated me to

NOTE Confidence: 0.900877240909091

00:24:31.974 --> 00:24:34.455 think a little bit more about why we

NOTE Confidence: 0.900877240909091

00:24:34.455 --> 00:24:37.379 might be getting it not necessarily so wrong,

NOTE Confidence: 0.900877240909091

00:24:37.380 --> 00:24:39.068 but why are we so confused by this.

NOTE Confidence: 0.900877240909091

00:24:39.070 --> 00:24:40.630 And I think a lot of this comes

NOTE Confidence: 0.900877240909091

00:24:40.630 --> 00:24:42.314 down to the approaches that we're

00:24:42.314 --> 00:24:43.869 using to study these problems.

00:24:43.870 --> 00:24:46.686 So M6A in particular has been a very

00:24:46.686 --> 00:24:48.681 popular modification to study because

00:24:48.681 --> 00:24:50.721 it's actually relatively simple to

00:24:50.721 --> 00:24:53.270 sequence where it is in the transcriptome,

00:24:53.270 --> 00:24:53.844 you know,

00:24:53.844 --> 00:24:55.853 in any cell linear system you want.

00:24:55.860 --> 00:24:57.522 The reason for that is that

00:24:57.522 --> 00:24:58.985 there's an antibody based approach

00:24:58.985 --> 00:25:00.465 to to sequence M6A sites.

00:25:00.470 --> 00:25:02.696 And essentially what you do is

00:25:02.696 --> 00:25:05.107 you take an antibody and your

00:25:05.107 --> 00:25:07.237 favorite RNA sample of interest,

00:25:07.240 --> 00:25:09.192 you mix them together,

00:25:09.192 --> 00:25:12.120 you pull down your M6A containing

00:25:12.120 --> 00:25:14.220 RNA's on your antibody and then you

NOTE Confidence: 0.900877240909091

00:25:14.220 --> 00:25:15.748 use high throughput sequencing which

NOTE Confidence: 0.900877240909091

00:25:15.748 --> 00:25:17.212 is now so run-of-the-mill that you

NOTE Confidence: 0.900877240909091

00:25:17.212 --> 00:25:18.640 can really do this with even very,

NOTE Confidence: 0.900877240909091

00:25:18.640 --> 00:25:21.718 very small amounts of input RNA.

NOTE Confidence: 0.900877240909091

00:25:21.720 --> 00:25:23.832 So this has been fantastic in terms of

NOTE Confidence: 0.900877240909091

00:25:23.832 --> 00:25:25.797 driving the field forward and sort of.

NOTE Confidence: 0.900877240909091

00:25:25.800 --> 00:25:27.634 Giving us the ability to sequence and

NOTE Confidence: 0.900877240909091

00:25:27.634 --> 00:25:29.969 say in a lot of different contexts,

NOTE Confidence: 0.900877240909091

00:25:29.970 --> 00:25:32.750 but it's essentially driven people

NOTE Confidence: 0.900877240909091

00:25:32.750 --> 00:25:36.562 to to this sort of top down approach

NOTE Confidence: 0.900877240909091

00:25:36.562 --> 00:25:38.282 of trying to identify unifying

NOTE Confidence: 0.900877240909091

00:25:38.282 --> 00:25:39.699 sequence features and gene groups

NOTE Confidence: 0.900877240909091

00:25:39.699 --> 00:25:41.715 and use that to try to sort of

NOTE Confidence: 0.900877240909091

00:25:41.715 --> 00:25:42.888 generate functional themes.

NOTE Confidence: 0.900877240909091

00:25:42.890 --> 00:25:44.965 So let's identify the most

00:25:44.965 --> 00:25:47.040 dysregulated sort of genes when

00:25:47.115 --> 00:25:49.108 we, you know lose M6A and and see

00:25:49.108 --> 00:25:50.852 if that tells us anything about

00:25:50.852 --> 00:25:52.694 how it might be functioning in

00:25:52.694 --> 00:25:54.717 our favorite system of interest.

00:25:54.720 --> 00:25:57.024 But it turns out that this can be

00:25:57.024 --> 00:25:59.320 a little bit tricky to interpret.

00:25:59.320 --> 00:26:00.616 So when you do an experiment like this,

00:26:00.620 --> 00:26:02.034 you will get many, many genes and

00:26:02.034 --> 00:26:03.678 you will do all kinds of analysis.

00:26:03.680 --> 00:26:06.200 And that's sometimes very illuminating.

00:26:06.200 --> 00:26:08.576 But in some cases it's also worth thinking

00:26:08.576 --> 00:26:11.015 about what exactly do those data look like?

00:26:11.020 --> 00:26:11.900 And so it turns out,

00:26:11.900 --> 00:26:13.755 when you do an experiment like this,

00:26:13.760 --> 00:26:14.640 you get, let's say,

NOTE Confidence: 0.884128847272727

00:26:14.640 --> 00:26:15.960 a section of your favorite gene.

NOTE Confidence: 0.884128847272727

00:26:15.960 --> 00:26:16.800 It could be Mick,

NOTE Confidence: 0.884128847272727

00:26:16.800 --> 00:26:17.640 could be anything else.

NOTE Confidence: 0.884128847272727

00:26:17.640 --> 00:26:19.968 And you will get a whole bunch of

NOTE Confidence: 0.884128847272727

00:26:19.968 --> 00:26:21.907 sequencing reads at different places

NOTE Confidence: 0.884128847272727

00:26:21.907 --> 00:26:23.635 along your favorite transcripts.

NOTE Confidence: 0.884128847272727

00:26:23.640 --> 00:26:26.176 But what this does not tell you is

NOTE Confidence: 0.884128847272727

00:26:26.176 --> 00:26:28.313 whether you have a situation like

NOTE Confidence: 0.884128847272727

00:26:28.313 --> 00:26:30.893 this where you have multiple M RNA's

NOTE Confidence: 0.884128847272727

00:26:30.893 --> 00:26:33.399 where half of them have multiple M6A

NOTE Confidence: 0.884128847272727

00:26:33.399 --> 00:26:35.790 sites and half of them have none.

NOTE Confidence: 0.884128847272727

00:26:35.790 --> 00:26:37.734 Or you could have a situation

NOTE Confidence: 0.884128847272727

00:26:37.734 --> 00:26:39.704 like this where actually all of

NOTE Confidence: 0.884128847272727

00:26:39.704 --> 00:26:41.546 your transcripts have M6A on them,

NOTE Confidence: 0.884128847272727

00:26:41.550 --> 00:26:44.060 but in slightly different locations.

00:26:44.060 --> 00:26:45.524 You might think that seems like a bit

00:26:45.524 --> 00:26:47.240 of a detail, like, why do we care?

00:26:47.240 --> 00:26:49.102 But it turns out that that can

00:26:49.102 --> 00:26:50.320 actually have very profound

00:26:50.320 --> 00:26:51.756 functional consequences in terms

00:26:51.756 --> 00:26:53.880 of how you interpret the data.

00:26:53.880 --> 00:26:54.944 And so for instance,

00:26:54.944 --> 00:26:55.476 you know,

00:26:55.480 --> 00:26:57.755 this might actually represent functionally

00:26:57.755 --> 00:26:59.575 two different transcript pools.

00:26:59.580 --> 00:27:02.019 It could mean that one of these pools is

00:27:02.019 --> 00:27:03.939 getting localized to a specific place

00:27:03.939 --> 00:27:06.259 or getting decayed and one pool is not.

00:27:06.260 --> 00:27:07.036 And conversely,

00:27:07.036 --> 00:27:09.364 this could actually just mean that,

00:27:09.370 --> 00:27:09.960 you know,

NOTE Confidence: 0.884128847272727

00:27:09.960 --> 00:27:12.320 the exact site of the M6A doesn't matter,

NOTE Confidence: 0.884128847272727

00:27:12.320 --> 00:27:14.390 it just has to be somewhere in this vicinity

NOTE Confidence: 0.884128847272727

00:27:14.390 --> 00:27:16.258 and then it'll have the same outcome.

NOTE Confidence: 0.884128847272727

00:27:16.260 --> 00:27:18.552 But we can't sort of disentangle

NOTE Confidence: 0.884128847272727

00:27:18.552 --> 00:27:21.115 these two things with a simple

NOTE Confidence: 0.884128847272727

00:27:21.115 --> 00:27:22.558 IP sequencing experiment.

NOTE Confidence: 0.884128847272727

00:27:22.560 --> 00:27:24.384 And that's not to say we haven't made

NOTE Confidence: 0.884128847272727

00:27:24.384 --> 00:27:25.699 improvements to this these methods.

NOTE Confidence: 0.884128847272727

00:27:25.700 --> 00:27:27.828 We have made a lot of improvements to

NOTE Confidence: 0.884128847272727

00:27:27.828 --> 00:27:29.952 IP based sequencing that allow you to

NOTE Confidence: 0.884128847272727

00:27:29.952 --> 00:27:32.359 get more precise sort of location data,

NOTE Confidence: 0.884128847272727

00:27:32.360 --> 00:27:35.141 but a lot of those are not easily applicable

NOTE Confidence: 0.884128847272727

00:27:35.141 --> 00:27:36.718 necessarily to very low abundance.

NOTE Confidence: 0.884128847272727

00:27:36.720 --> 00:27:38.372 Examples which might sort

NOTE Confidence: 0.884128847272727

00:27:38.372 --> 00:27:40.024 of be clinically relevant.

NOTE Confidence: 0.884128847272727

00:27:40.030 --> 00:27:41.840 So.

NOTE Confidence: 0.884128847272727

00:27:41.840 --> 00:27:43.544 This sort of leads me to sort of

NOTE Confidence: 0.884128847272727

00:27:43.544 --> 00:27:45.112 posit to you that part of the

NOTE Confidence: 0.884128847272727

00:27:45.112 --> 00:27:46.531 reason we get so much confusing

NOTE Confidence: 0.884128847272727

00:27:46.531 --> 00:27:48.456 data is because we are actually sort

NOTE Confidence: 0.884128847272727

00:27:48.456 --> 00:27:51.110 of the data itself is is not as

NOTE Confidence: 0.884128847272727

00:27:51.110 --> 00:27:53.480 clarifying as maybe we would think.

NOTE Confidence: 0.884128847272727

00:27:53.480 --> 00:27:55.097 And so how do we change our

NOTE Confidence: 0.884128847272727

00:27:55.097 --> 00:27:56.483 approaches to sort of deconvolve

NOTE Confidence: 0.884128847272727

00:27:56.483 --> 00:27:57.755 some of these variables.

NOTE Confidence: 0.884128847272727

00:27:57.760 --> 00:27:59.821 And this is really sort of at the crux

NOTE Confidence: 0.884128847272727

00:27:59.821 --> 00:28:02.237 of essentially everything my lab studies,

NOTE Confidence: 0.884128847272727

00:28:02.240 --> 00:28:04.220 but we we sort of apply this in a

NOTE Confidence: 0.884128847272727

00:28:04.220 --> 00:28:05.568 lot of different ways.

NOTE Confidence: 0.903440462333334

00:28:07.720 --> 00:28:09.652 So just to sort of summarize the

NOTE Confidence: 0.903440462333334

00:28:09.652 --> 00:28:11.598 approach that I just gave you because

NOTE Confidence: 0.903440462333334

00:28:11.598 --> 00:28:13.858 I'm going to try to sort of convince

NOTE Confidence: 0.903440462333334

00:28:13.858 --> 00:28:15.688 you that our approach sort of

NOTE Confidence: 0.903440462333334

00:28:15.688 --> 00:28:17.020 provides an interesting alternative.

NOTE Confidence: 0.903440462333334

00:28:17.020 --> 00:28:19.540 The approach that I just described is

NOTE Confidence: 0.903440462333334

00:28:19.540 --> 00:28:21.734 what I would call a top down approach.

NOTE Confidence: 0.903440462333334

00:28:21.740 --> 00:28:24.116 So we do transcriptome wide sequencing

NOTE Confidence: 0.903440462333334

00:28:24.116 --> 00:28:26.580 usually for one specific modification.

NOTE Confidence: 0.903440462333334

00:28:26.580 --> 00:28:28.242 We, you know, sequence everything and

NOTE Confidence: 0.903440462333334

00:28:28.242 --> 00:28:30.687 then we try to pick out features that

NOTE Confidence: 0.903440462333334

00:28:30.687 --> 00:28:32.607 are common across all the transcripts

NOTE Confidence: 0.903440462333334

00:28:32.660 --> 00:28:34.580 that have our favorite modification.

NOTE Confidence: 0.903440462333334

00:28:34.580 --> 00:28:36.758 And this is in fact what led to our,

NOTE Confidence: 0.903440462333334

00:28:36.760 --> 00:28:38.636 you know, the identification of this decay.

NOTE Confidence: 0.903440462333334

00:28:38.640 --> 00:28:40.030 Mechanism that I described earlier.

NOTE Confidence: 0.903440462333334

00:28:40.030 --> 00:28:42.206 So it is definitely not a useless approach.

NOTE Confidence: 0.903440462333334

00:28:42.210 --> 00:28:44.050 It has been very productive,

NOTE Confidence: 0.903440462333334

00:28:44.050 --> 00:28:45.769 but I will argue that we sort of hit

NOTE Confidence: 0.903440462333334

00:28:45.769 --> 00:28:48.200 a wall in terms of really getting a

NOTE Confidence: 0.903440462333334

00:28:48.200 --> 00:28:49.769 mechanistic understanding of of how

NOTE Confidence: 0.903440462333334

00:28:49.769 --> 00:28:51.209 some of these modifications work,

NOTE Confidence: 0.903440462333334

00:28:51.210 --> 00:28:54.598 which has led to some confusing results.

NOTE Confidence: 0.903440462333334

00:28:54.600 --> 00:28:57.295 So what I have tried to do,

NOTE Confidence: 0.903440462333334

00:28:57.300 --> 00:28:59.036 you know in setting up the research

NOTE Confidence: 0.903440462333334

00:28:59.036 --> 00:29:00.878 program in our lab is to try to

NOTE Confidence: 0.903440462333334

00:29:00.878 --> 00:29:02.457 develop what I would call more of

NOTE Confidence: 0.903440462333334

00:29:02.457 --> 00:29:03.417 a bottom up approach.

NOTE Confidence: 0.903440462333334

00:29:03.420 --> 00:29:05.340 So we identify specific transcripts and

NOTE Confidence: 0.903440462333334

00:29:05.340 --> 00:29:08.158 then we do a deep dive to figure out,

NOTE Confidence: 0.903440462333334

00:29:08.160 --> 00:29:10.512 OK, what are all the different

NOTE Confidence: 0.903440462333334

00:29:10.512 --> 00:29:12.080 modifications on this transcript.

NOTE Confidence: 0.903440462333334

00:29:12.080 --> 00:29:13.372 Once we know that,

NOTE Confidence: 0.903440462333334

00:29:13.372 --> 00:29:14.987 can we identify the regulatory

NOTE Confidence: 0.903440462333334

00:29:14.987 --> 00:29:15.940 enzymes are there,

NOTE Confidence: 0.903440462333334

00:29:15.940 --> 00:29:18.236 you know variations across

NOTE Confidence: 0.903440462333334

00:29:18.236 --> 00:29:19.958 different diseases etcetera.

NOTE Confidence: 0.903440462333334

00:29:19.960 --> 00:29:21.647 My argument is sort of that if

NOTE Confidence: 0.903440462333334

00:29:21.647 --> 00:29:22.740 we can do this,

NOTE Confidence: 0.903440462333334

00:29:22.740 --> 00:29:24.855 you can then go and look in your next

NOTE Confidence: 0.903440462333334

00:29:24.855 --> 00:29:26.405 favorite transcript and say it does

NOTE Confidence: 0.903440462333334

00:29:26.405 --> 00:29:28.042 it also apply here and essentially

NOTE Confidence: 0.903440462333334

00:29:28.042 --> 00:29:30.016 build out the network from there.

NOTE Confidence: 0.903440462333334

00:29:30.020 --> 00:29:32.207 So the idea is to sort of not limit

NOTE Confidence: 0.903440462333334

00:29:32.207 --> 00:29:33.526 ourselves necessarily to these

NOTE Confidence: 0.903440462333334

00:29:33.526 --> 00:29:35.216 RNA that we're interested in,

NOTE Confidence: 0.903440462333334

00:29:35.220 --> 00:29:37.096 but to use them to identify rules

NOTE Confidence: 0.903440462333334

00:29:37.096 --> 00:29:39.266 and then try to figure out if

00:29:39.266 --> 00:29:40.574 those rules apply elsewhere.

00:29:40.580 --> 00:29:42.652 And so I'm going to drive describe

00:29:42.652 --> 00:29:44.608 sort of the two approaches we're

00:29:44.608 --> 00:29:47.340 using to sort of make maps like this.

00:29:47.340 --> 00:29:50.182 The first one is a mass spectrometry

00:29:50.182 --> 00:29:50.994 based approach,

00:29:51.000 --> 00:29:52.519 and this has really been pioneered in

00:29:52.519 --> 00:29:54.470 my lab by a student, Lauren Wilson,

00:29:54.470 --> 00:29:57.235 aided by many other people in the

00:29:57.235 --> 00:30:00.218 lab including Josh and undergraduate.

00:30:00.220 --> 00:30:02.324 But the essence essentially the idea is this.

00:30:02.330 --> 00:30:04.418 So what if we just took our favorite

00:30:04.418 --> 00:30:06.176 transcript and we designed a whole

00:30:06.176 --> 00:30:07.952 bunch of probes that are complementary

00:30:08.012 --> 00:30:09.512 to that transcript and we just

00:30:09.512 --> 00:30:10.964 purified it out of cells?

NOTE Confidence: 0.903440462333334

00:30:10.964 --> 00:30:12.296 This sounds crazy,

NOTE Confidence: 0.903440462333334

00:30:12.296 --> 00:30:15.619 but Lauren has actually gotten this to work,

NOTE Confidence: 0.903440462333334

00:30:15.620 --> 00:30:17.460 arguably for more abundant transcripts.

NOTE Confidence: 0.903440462333334

00:30:17.460 --> 00:30:18.640 But nevertheless, you know,

NOTE Confidence: 0.903440462333334

00:30:18.640 --> 00:30:20.115 it's actually working quite well,

NOTE Confidence: 0.903440462333334

00:30:20.120 --> 00:30:21.527 as I'll show you in a second.

NOTE Confidence: 0.903440462333334

00:30:21.530 --> 00:30:22.550 And the idea here is,

NOTE Confidence: 0.903440462333334

00:30:22.550 --> 00:30:22.872 OK,

NOTE Confidence: 0.903440462333334

00:30:22.872 --> 00:30:24.482 let's just hypothetically say that

NOTE Confidence: 0.903440462333334

00:30:24.482 --> 00:30:26.263 we can purify the transcripts

NOTE Confidence: 0.903440462333334

00:30:26.263 --> 00:30:27.967 that we're interested in.

NOTE Confidence: 0.903440462333334

00:30:27.970 --> 00:30:30.508 And then digest them into individual

NOTE Confidence: 0.903440462333334

00:30:30.508 --> 00:30:32.659 nucleosides down to sort of

NOTE Confidence: 0.903440462333334

00:30:32.659 --> 00:30:34.599 the individual module level and

NOTE Confidence: 0.903440462333334

00:30:34.599 --> 00:30:37.017 then analyzed by mass spec sort

NOTE Confidence: 0.903440462333334

00:30:37.017 --> 00:30:38.997 of everything that's in there.

NOTE Confidence: 0.903440462333334

00:30:39.000 --> 00:30:40.883 And so I'm happy to discuss the

NOTE Confidence: 0.903440462333334

00:30:40.883 --> 00:30:42.897 details of the mass spec with

NOTE Confidence: 0.903440462333334

00:30:42.897 --> 00:30:44.040 anyone who's interested.

NOTE Confidence: 0.903440462333334

00:30:44.040 --> 00:30:46.301 But the crux of it is that

NOTE Confidence: 0.903440462333334

00:30:46.301 --> 00:30:47.270 essentially between the

NOTE Confidence: 0.861260623333333

00:30:47.336 --> 00:30:49.778 the fragmentation patterns and the retention

NOTE Confidence: 0.861260623333333

00:30:49.778 --> 00:30:52.700 time on a specific column that we use,

NOTE Confidence: 0.861260623333333

00:30:52.700 --> 00:30:55.172 we can distinguish between even very

NOTE Confidence: 0.861260623333333

00:30:55.172 --> 00:30:56.820 closely related chemical species.

NOTE Confidence: 0.861260623333333

00:30:56.820 --> 00:30:59.263 So that we can distinguish even two

NOTE Confidence: 0.861260623333333

00:30:59.263 --> 00:31:00.679 different singly methylated species

NOTE Confidence: 0.861260623333333

00:31:00.679 --> 00:31:02.849 like and one methyl adenosine and and

NOTE Confidence: 0.861260623333333

00:31:02.849 --> 00:31:04.804 six methyl adenosine and we can go

NOTE Confidence: 0.861260623333333

00:31:04.804 --> 00:31:07.492 down the line and look at sort of any

NOTE Confidence: 0.861260623333333

00:31:07.492 --> 00:31:09.610 modifications we might be interested in.

NOTE Confidence: 0.861260623333333

00:31:09.610 --> 00:31:11.970 And so this has turned out to be

NOTE Confidence: 0.861260623333333

00:31:11.970 --> 00:31:13.808 relatively fruitful as I just alluded to.

NOTE Confidence: 0.861260623333333

00:31:13.810 --> 00:31:15.679 And so we're starting to do this

NOTE Confidence: 0.861260623333333

00:31:15.679 --> 00:31:17.590 admittedly with very abundant transcripts.

NOTE Confidence: 0.861260623333333

00:31:17.590 --> 00:31:19.270 And so the data I'll show you

NOTE Confidence: 0.861260623333333

00:31:19.270 --> 00:31:21.508 is for a very abundant but very,

NOTE Confidence: 0.861260623333333

00:31:21.510 --> 00:31:24.948 very big long non coding RNA called neat one.

NOTE Confidence: 0.861260623333333

00:31:24.950 --> 00:31:27.368 It's got some very interesting biology

NOTE Confidence: 0.861260623333333

00:31:27.368 --> 00:31:30.588 that I won't really go into at the moment.

NOTE Confidence: 0.861260623333333

00:31:30.590 --> 00:31:33.971 But essentially we can purify neat 1

NOTE Confidence: 0.861260623333333

00:31:33.971 --> 00:31:36.551 transcripts to really high enrichment

NOTE Confidence: 0.861260623333333

00:31:36.551 --> 00:31:39.796 relative to sort of baseline total RNA.

NOTE Confidence: 0.861260623333333

00:31:39.796 --> 00:31:42.300 So the exact numbers that you get for

NOTE Confidence: 0.861260623333333

00:31:42.366 --> 00:31:44.634 enrichment depend a little bit on

NOTE Confidence: 0.861260623333333

00:31:44.634 --> 00:31:46.696 what you're comparing it to, right?

NOTE Confidence: 0.861260623333333

00:31:46.696 --> 00:31:48.984 So are you comparing to an M RNA

NOTE Confidence: 0.861260623333333

00:31:48.984 --> 00:31:51.388 or a very abundant ribosomal RNA?

NOTE Confidence: 0.861260623333333

00:31:51.390 --> 00:31:52.083 But for instance,

NOTE Confidence: 0.861260623333333

00:31:52.083 --> 00:31:54.162 if we look at sort of a favorite

NOTE Confidence: 0.861260623333333

00:31:54.162 --> 00:31:55.350 housekeeping M RNA.

NOTE Confidence: 0.861260623333333

00:31:55.350 --> 00:31:58.535 We can enrich this long non coding

NOTE Confidence: 0.861260623333333

00:31:58.535 --> 00:32:01.123 RNA many thousand fold over what

NOTE Confidence: 0.861260623333333

00:32:01.123 --> 00:32:03.867 we would have in just sort of the

NOTE Confidence: 0.861260623333333

00:32:03.867 --> 00:32:05.160 baseline pool of RNA.

NOTE Confidence: 0.861260623333333

00:32:05.160 --> 00:32:05.824 And importantly,

NOTE Confidence: 0.861260623333333

00:32:05.824 --> 00:32:08.897 Lauren can now do this to sort of a

NOTE Confidence: 0.861260623333333

00:32:08.897 --> 00:32:11.333 level of abundance that we can actually,

NOTE Confidence: 0.861260623333333

00:32:11.340 --> 00:32:11.896 you know,

NOTE Confidence: 0.861260623333333

00:32:11.896 --> 00:32:13.842 get enough RNA out of this to

NOTE Confidence: 0.861260623333333

00:32:13.842 --> 00:32:15.756 digest and do mass spec analysis.

NOTE Confidence: 0.861260623333333

00:32:15.760 --> 00:32:19.480 And so the idea here is that we take this,

NOTE Confidence: 0.861260623333333

00:32:19.480 --> 00:32:19.968 you know,

NOTE Confidence: 0.861260623333333

00:32:19.968 --> 00:32:21.676 RNA of interest and we just look

NOTE Confidence: 0.861260623333333

00:32:21.676 --> 00:32:23.419 for our favorite modifications.

NOTE Confidence: 0.861260623333333

00:32:23.420 --> 00:32:25.715 I'm just showing you a few of them here.

NOTE Confidence: 0.861260623333333

00:32:25.720 --> 00:32:27.274 You can look at different cell lines,

NOTE Confidence: 0.861260623333333

00:32:27.280 --> 00:32:27.940 you know,

NOTE Confidence: 0.861260623333333

00:32:27.940 --> 00:32:29.920 whatever cell lines you might be

NOTE Confidence: 0.861260623333333

00:32:29.920 --> 00:32:32.007 interested in and then basically

NOTE Confidence: 0.861260623333333

00:32:32.007 --> 00:32:33.823 profile the relative abundance

NOTE Confidence: 0.861260623333333

00:32:33.823 --> 00:32:35.185 of different modifications.

NOTE Confidence: 0.861260623333333

00:32:35.190 --> 00:32:38.928 In this sample. In different cell lines.

NOTE Confidence: 0.861260623333333

00:32:38.928 --> 00:32:41.900 And so for instance here we have a 549

NOTE Confidence: 0.861260623333333

00:32:41.900 --> 00:32:45.260 and HeLa cells and you can see that

NOTE Confidence: 0.861260623333333

00:32:45.260 --> 00:32:48.200 the M1A levels are a little bit different,

NOTE Confidence: 0.861260623333333

00:32:48.200 --> 00:32:49.300 which maybe it's interesting,

NOTE Confidence: 0.861260623333333

00:32:49.300 --> 00:32:50.125 maybe it's not.

NOTE Confidence: 0.861260623333333

00:32:50.130 --> 00:32:52.040 We'll have to find out.

NOTE Confidence: 0.861260623333333

00:32:52.040 --> 00:32:53.965 The M6A levels are relatively

NOTE Confidence: 0.861260623333333

00:32:53.965 --> 00:32:56.659 stable and so on and so forth.

NOTE Confidence: 0.861260623333333

00:32:56.660 --> 00:32:59.026 And so this is just a snapshot,

NOTE Confidence: 0.861260623333333

00:32:59.030 --> 00:33:00.812 but I hope you can sort of appreciate that,

NOTE Confidence: 0.861260623333333

00:33:00.820 --> 00:33:01.292 you know,

NOTE Confidence: 0.861260623333333

00:33:01.292 --> 00:33:03.567 this is one way to get a much more

NOTE Confidence: 0.861260623333333

00:33:03.567 --> 00:33:05.523 unbiased picture of what might be

NOTE Confidence: 0.861260623333333

00:33:05.523 --> 00:33:07.659 present in your long and encoding.

NOTE Confidence: 0.861260623333333

00:33:07.660 --> 00:33:09.501 RNA of interest I will say in

NOTE Confidence: 0.861260623333333

00:33:09.501 --> 00:33:10.957 the interest of Full disclosure

NOTE Confidence: 0.861260623333333

00:33:10.957 --> 00:33:13.065 we cannot do this yet for MRA's.

NOTE Confidence: 0.861260623333333

00:33:13.065 --> 00:33:14.590 MRI's are much less abundant.

NOTE Confidence: 0.861260623333333

00:33:14.590 --> 00:33:16.870 Maybe someday we will get there,

NOTE Confidence: 0.861260623333333

00:33:16.870 --> 00:33:18.398 but at the moment we can at least

NOTE Confidence: 0.861260623333333

00:33:18.398 --> 00:33:20.093 take a stab at taking our favorite

NOTE Confidence: 0.861260623333333

00:33:20.093 --> 00:33:21.951 long non coding our names of interest

NOTE Confidence: 0.861260623333333

00:33:21.951 --> 00:33:23.583 and trying to see what modifications

NOTE Confidence: 0.861260623333333

00:33:23.583 --> 00:33:24.943 are sort of sprinkled throughout.

NOTE Confidence: 0.861260623333333

00:33:24.943 --> 00:33:26.840 And it will say another RNA we

NOTE Confidence: 0.861260623333333

00:33:26.898 --> 00:33:28.428 have applied this to is Merlot,

NOTE Confidence: 0.861260623333333

00:33:28.430 --> 00:33:31.166 one which many of you might be familiar with.

NOTE Confidence: 0.861260623333333

00:33:31.170 --> 00:33:32.565 It's another sort of abundant

NOTE Confidence: 0.861260623333333

00:33:32.565 --> 00:33:33.960 long non coding RNA that's

NOTE Confidence: 0.816570879

00:33:34.010 --> 00:33:36.002 been of interest in in a few different

NOTE Confidence: 0.816570879

00:33:36.002 --> 00:33:37.920 cancers and so we're taking a stab

NOTE Confidence: 0.816570879

00:33:37.920 --> 00:33:39.290 at looking at the modifications.

NOTE Confidence: 0.816570879

00:33:39.290 --> 00:33:41.830 From that arena as well.

NOTE Confidence: 0.816570879

00:33:41.830 --> 00:33:43.168 But again, I told you I

NOTE Confidence: 0.816570879

00:33:43.168 --> 00:33:44.060 would tell you limitations.

NOTE Confidence: 0.816570879

00:33:44.060 --> 00:33:48.004 So mass spec is a very powerful tool,

NOTE Confidence: 0.816570879

00:33:48.010 --> 00:33:50.745 particularly to get really specific

NOTE Confidence: 0.816570879

00:33:50.745 --> 00:33:52.386 chemical identity information.

NOTE Confidence: 0.816570879

00:33:52.390 --> 00:33:54.035 But you lose location information

NOTE Confidence: 0.816570879

00:33:54.035 --> 00:33:55.680 because we're digesting all of

NOTE Confidence: 0.816570879

00:33:55.738 --> 00:33:57.154 this up into tiny little pieces

NOTE Confidence: 0.816570879

00:33:57.154 --> 00:33:59.200 so that we can really identify the

NOTE Confidence: 0.816570879

00:33:59.200 --> 00:34:00.935 chemical species that are present.

NOTE Confidence: 0.816570879

00:34:00.940 --> 00:34:02.508 So that's a bit of a problem

NOTE Confidence: 0.816570879

00:34:02.508 --> 00:34:04.399 and as I already alluded to,

NOTE Confidence: 0.816570879

00:34:04.400 --> 00:34:06.560 it's also limited at the moment

NOTE Confidence: 0.816570879

00:34:06.560 --> 00:34:08.000 much more abundant transcript.

NOTE Confidence: 0.816570879

00:34:08.000 --> 00:34:09.374 So things that we can actually

NOTE Confidence: 0.816570879

00:34:09.374 --> 00:34:11.238 purify to a degree to where we can

NOTE Confidence: 0.816570879

00:34:11.238 --> 00:34:12.582 actually do Mass Effect on them.

NOTE Confidence: 0.816570879

00:34:12.590 --> 00:34:15.326 It is also limited in the sense that,

NOTE Confidence: 0.816570879

00:34:15.330 --> 00:34:15.746 you know,

NOTE Confidence: 0.816570879

00:34:15.746 --> 00:34:16.786 we certainly wouldn't be able

NOTE Confidence: 0.816570879

00:34:16.786 --> 00:34:18.130 to do this from something like

NOTE Confidence: 0.816570879

00:34:18.130 --> 00:34:19.486 a biopsy or a patient sample,

NOTE Confidence: 0.816570879

00:34:19.490 --> 00:34:21.290 which I'll get to later.

NOTE Confidence: 0.816570879

00:34:21.290 --> 00:34:22.388 But that's sort of, you know,

NOTE Confidence: 0.816570879

00:34:22.390 --> 00:34:24.390 the dream down the line.

NOTE Confidence: 0.816570879

00:34:24.390 --> 00:34:27.324 So what do we do to complement this approach?

NOTE Confidence: 0.816570879

00:34:27.330 --> 00:34:29.370 So this is where we turn to a

NOTE Confidence: 0.816570879

00:34:29.370 --> 00:34:30.886 sequencing based method and this

NOTE Confidence: 0.816570879

00:34:30.886 --> 00:34:32.782 is actually where I've had the

NOTE Confidence: 0.816570879

00:34:32.782 --> 00:34:34.791 tremendous good fortune to work with

NOTE Confidence: 0.816570879

00:34:34.791 --> 00:34:36.426 another member of the department.

NOTE Confidence: 0.816570879

00:34:36.430 --> 00:34:37.566 And Anna Marie Pyle,

NOTE Confidence: 0.816570879

00:34:37.566 --> 00:34:39.630 her lab is just down the hall

NOTE Confidence: 0.816570879

00:34:39.630 --> 00:34:41.406 and we've got a fantastic sort

NOTE Confidence: 0.816570879

00:34:41.406 --> 00:34:44.028 of RNA wing in the Yale Science

NOTE Confidence: 0.816570879

00:34:44.028 --> 00:34:46.108 Building and they discovered and

NOTE Confidence: 0.816570879

00:34:46.108 --> 00:34:48.333 characterized this interesting reverse

NOTE Confidence: 0.816570879

00:34:48.333 --> 00:34:50.737 transcriptase enzyme called marathon.

NOTE Confidence: 0.816570879

00:34:50.740 --> 00:34:54.044 And Marathon is sort of interesting

NOTE Confidence: 0.816570879

00:34:54.044 --> 00:34:55.284 from a few different aspects.

NOTE Confidence: 0.816570879

00:34:55.290 --> 00:34:57.198 So first, as the name suggests,

NOTE Confidence: 0.816570879

00:34:57.200 --> 00:34:58.688 it can reverse transcribe

NOTE Confidence: 0.816570879

00:34:58.688 --> 00:34:59.804 really long transcripts.

NOTE Confidence: 0.816570879

00:34:59.810 --> 00:35:00.520 I mean,

NOTE Confidence: 0.816570879

00:35:00.520 --> 00:35:03.005 I actually have never seen anything quite

NOTE Confidence: 0.816570879

00:35:03.005 --> 00:35:05.957 as processive as this particular enzyme.

NOTE Confidence: 0.816570879

00:35:05.960 --> 00:35:07.106 The other thing that it does,

NOTE Confidence: 0.816570879

00:35:07.110 --> 00:35:08.820 though that's particularly useful for us,

NOTE Confidence: 0.816570879

00:35:08.820 --> 00:35:10.698 is it tends to install mutations

NOTE Confidence: 0.816570879

00:35:10.698 --> 00:35:12.400 when it encounters a modified

NOTE Confidence: 0.816570879

00:35:12.400 --> 00:35:13.960 modification in the RNA.

NOTE Confidence: 0.816570879

00:35:13.960 --> 00:35:16.074 That's not to say every single modification,

NOTE Confidence: 0.816570879

00:35:16.080 --> 00:35:17.104 but many of them,

NOTE Confidence: 0.816570879

00:35:17.104 --> 00:35:19.679 and even some that are a bit more subtle.

NOTE Confidence: 0.816570879

00:35:19.680 --> 00:35:21.493 So you might already be starting to

NOTE Confidence: 0.816570879

00:35:21.493 --> 00:35:23.112 piece together that this would be

NOTE Confidence: 0.816570879

00:35:23.112 --> 00:35:24.696 relatively useful if we could just

NOTE Confidence: 0.816570879

00:35:24.696 --> 00:35:26.302 reverse transcribe an RNA and use

NOTE Confidence: 0.816570879

00:35:26.302 --> 00:35:28.230 mutations to sort of identify where

NOTE Confidence: 0.816570879

00:35:28.230 --> 00:35:31.380 possible modification sites might be.

NOTE Confidence: 0.816570879

00:35:31.380 --> 00:35:33.207 Now the one caveat here is that

NOTE Confidence: 0.816570879

00:35:33.207 --> 00:35:35.015 we can't always tell the specific

NOTE Confidence: 0.816570879

00:35:35.015 --> 00:35:36.625 modification just based on the

NOTE Confidence: 0.816570879

00:35:36.625 --> 00:35:38.718 fact that there's a mutation there.

00:35:38.720 --> 00:35:40.676 There's a lot of different ways

00:35:40.676 --> 00:35:41.980 you can get mutations,

00:35:41.980 --> 00:35:43.486 but the massive benefit of this

00:35:43.486 --> 00:35:45.135 type of approach is that you can

00:35:45.135 --> 00:35:46.896 do it even for M RNA's that are

00:35:46.896 --> 00:35:48.876 not very abundant because for a

00:35:48.876 --> 00:35:49.866 reverse transcription reaction.

00:35:49.870 --> 00:35:51.950 Need much less than something

00:35:51.950 --> 00:35:53.198 like from aspec.

00:35:53.200 --> 00:35:55.152 So Dorothy in the lab has been the

00:35:55.152 --> 00:35:57.060 one really pioneering this approach.

00:35:57.060 --> 00:35:58.740 And as you can see here,

00:35:58.740 --> 00:36:01.107 so this time I'm using BRCA 2 as sort

00:36:01.107 --> 00:36:03.614 of an example of an MRA of interest.

00:36:03.620 --> 00:36:05.629 And you can see that as you

00:36:05.629 --> 00:36:07.060 reverse transcribe with marathon,

NOTE Confidence: 0.816570879

00:36:07.060 --> 00:36:10.546 you see some specific peaks where

NOTE Confidence: 0.816570879

00:36:10.546 --> 00:36:12.870 you get mutation signatures.

NOTE Confidence: 0.816570879

00:36:12.870 --> 00:36:14.334 We thought we knew what these

NOTE Confidence: 0.816570879

00:36:14.334 --> 00:36:15.310 mutation sites represented were

NOTE Confidence: 0.776843212941176

00:36:15.350 --> 00:36:16.827 actually a little bit less short now,

NOTE Confidence: 0.776843212941176

00:36:16.830 --> 00:36:18.214 which is why I'm not telling you the

NOTE Confidence: 0.776843212941176

00:36:18.214 --> 00:36:19.800 identity, the identity of the modification,

NOTE Confidence: 0.776843212941176

00:36:19.800 --> 00:36:22.024 because we're still trying to work that out.

NOTE Confidence: 0.776843212941176

00:36:22.030 --> 00:36:24.830 But what's interesting is that you can see

NOTE Confidence: 0.776843212941176

00:36:24.830 --> 00:36:27.357 that there's actually different levels of

NOTE Confidence: 0.776843212941176

00:36:27.357 --> 00:36:29.991 mutation depending on the specific site.

NOTE Confidence: 0.776843212941176

00:36:30.000 --> 00:36:31.212 As I said though, an important

NOTE Confidence: 0.776843212941176

00:36:31.212 --> 00:36:32.707 caveat here is you can get mutations

NOTE Confidence: 0.776843212941176

00:36:32.707 --> 00:36:33.997 by a lot of different routes,

NOTE Confidence: 0.776843212941176

00:36:34.000 --> 00:36:35.190 including of course because the

NOTE Confidence: 0.776843212941176

00:36:35.190 --> 00:36:37.031 genomic DNA might be a little bit

NOTE Confidence: 0.776843212941176

00:36:37.031 --> 00:36:38.235 different than your reference.

NOTE Confidence: 0.776843212941176

00:36:38.240 --> 00:36:40.896 And so here you can see that we've

NOTE Confidence: 0.776843212941176

00:36:40.896 --> 00:36:42.809 actually just picked up a snip.

NOTE Confidence: 0.776843212941176

00:36:42.810 --> 00:36:44.903 You can tell that actually you can

NOTE Confidence: 0.776843212941176

00:36:44.903 --> 00:36:46.729 almost predict that based on the

NOTE Confidence: 0.776843212941176

00:36:46.729 --> 00:36:48.463 fact that it's so highly modified.

NOTE Confidence: 0.776843212941176

00:36:48.470 --> 00:36:50.451 But then these others you can see

NOTE Confidence: 0.776843212941176

00:36:50.451 --> 00:36:52.257 there aren't really sort of concordant

NOTE Confidence: 0.776843212941176

00:36:52.257 --> 00:36:54.051 DNA based mutations and so we're

NOTE Confidence: 0.776843212941176

00:36:54.051 --> 00:36:56.240 sort of trying to follow up what

NOTE Confidence: 0.776843212941176

00:36:56.240 --> 00:36:57.460 those modifications might be.

NOTE Confidence: 0.776843212941176

00:36:57.460 --> 00:36:59.252 And so you can see that there's sort

NOTE Confidence: 0.776843212941176

00:36:59.252 --> 00:37:01.567 of an iterative process where you

NOTE Confidence: 0.776843212941176

00:37:01.567 --> 00:37:03.379 could essentially either identify

NOTE Confidence: 0.776843212941176

00:37:03.379 --> 00:37:04.638 potentially interesting modifications

NOTE Confidence: 0.776843212941176

00:37:04.638 --> 00:37:06.982 by mass spec in the favorite RNA and

NOTE Confidence: 0.776843212941176

00:37:06.982 --> 00:37:09.569 then try to use sequencing based on that

NOTE Confidence: 0.776843212941176

00:37:09.569 --> 00:37:11.478 knowledge to identify where they are.

NOTE Confidence: 0.776843212941176

00:37:11.480 --> 00:37:13.616 Or you could start with sequencing,

NOTE Confidence: 0.776843212941176

00:37:13.620 --> 00:37:15.180 identify specific sites on

NOTE Confidence: 0.776843212941176

00:37:15.180 --> 00:37:16.740 a transcript of interest,

NOTE Confidence: 0.776843212941176

00:37:16.740 --> 00:37:18.266 and then try to work out what

NOTE Confidence: 0.776843212941176

00:37:18.266 --> 00:37:19.397 the modification is either by

NOTE Confidence: 0.776843212941176

00:37:19.397 --> 00:37:20.693 mass spec or sort of another,

NOTE Confidence: 0.776843212941176

00:37:20.700 --> 00:37:22.572 you know, orthogonal method.

NOTE Confidence: 0.776843212941176

00:37:22.572 --> 00:37:24.912 But by iterating this process,

NOTE Confidence: 0.776843212941176

00:37:24.920 --> 00:37:25.995 the idea is that basically

NOTE Confidence: 0.776843212941176

00:37:25.995 --> 00:37:27.539 you can get sort of a map of.

NOTE Confidence: 0.776843212941176

00:37:27.540 --> 00:37:29.310 And RNA of interests and really

NOTE Confidence: 0.776843212941176

00:37:29.310 --> 00:37:31.100 that RNA could be anything.

NOTE Confidence: 0.776843212941176

00:37:31.100 --> 00:37:31.976 I'll tell you a little bit

NOTE Confidence: 0.776843212941176

00:37:31.976 --> 00:37:32.880 about the ones that you know,

NOTE Confidence: 0.776843212941176

00:37:32.880 --> 00:37:34.428 we're working on at the moment.

NOTE Confidence: 0.776843212941176

00:37:34.430 --> 00:37:36.182 But the idea is that if you can

NOTE Confidence: 0.776843212941176

00:37:36.182 --> 00:37:37.955 get this sort of more complete

NOTE Confidence: 0.776843212941176

00:37:37.955 --> 00:37:39.863 picture of what's actually on there,

NOTE Confidence: 0.776843212941176

00:37:39.870 --> 00:37:41.206 you can then go in and look at,

NOTE Confidence: 0.776843212941176

00:37:41.210 --> 00:37:42.988 OK, if I do perturbation X or

NOTE Confidence: 0.776843212941176

00:37:42.988 --> 00:37:44.670 if I look in disease Y,

NOTE Confidence: 0.776843212941176

00:37:44.670 --> 00:37:47.782 you can actually start to look at specific

NOTE Confidence: 0.776843212941176

00:37:47.782 --> 00:37:50.468 changes in those specific locations.

NOTE Confidence: 0.776843212941176

00:37:50.470 --> 00:37:53.207 And So what are we actually trying?

NOTE Confidence: 0.776843212941176

00:37:53.210 --> 00:37:53.690 Whoops,

NOTE Confidence: 0.776843212941176

00:37:53.690 --> 00:37:56.570 trying to do with this so.

NOTE Confidence: 0.776843212941176

00:37:56.570 --> 00:37:58.388 There's a lot of different applications

NOTE Confidence: 0.776843212941176

00:37:58.388 --> 00:38:00.741 I envision and this is where I probably

NOTE Confidence: 0.776843212941176

00:38:00.741 --> 00:38:02.373 should have put disclosure at the

NOTE Confidence: 0.776843212941176

00:38:02.427 --> 00:38:04.583 beginning that says you know I would

NOTE Confidence: 0.776843212941176

00:38:04.583 --> 00:38:06.278 love always perspective from clinicians

NOTE Confidence: 0.776843212941176

00:38:06.278 --> 00:38:07.814 and more translational researchers

NOTE Confidence: 0.776843212941176

00:38:07.814 --> 00:38:09.950 sort of interesting targets to look at.

NOTE Confidence: 0.776843212941176

00:38:09.950 --> 00:38:11.470 But I'll tell you a little bit about

NOTE Confidence: 0.776843212941176

00:38:11.470 --> 00:38:13.090 the ones that that we are looking at.

NOTE Confidence: 0.776843212941176

00:38:13.090 --> 00:38:16.065 And so I've mentioned the long non

NOTE Confidence: 0.776843212941176

00:38:16.065 --> 00:38:18.530 coding RNA's neat one and maillot one.

NOTE Confidence: 0.776843212941176

00:38:18.530 --> 00:38:21.934 These are sort of our first model

NOTE Confidence: 0.776843212941176

00:38:21.934 --> 00:38:23.922 transcripts let's say for the mass spec

NOTE Confidence: 0.776843212941176

00:38:23.922 --> 00:38:25.358 approach because they are abundant,

NOTE Confidence: 0.776843212941176

00:38:25.360 --> 00:38:26.740 they are big and there's

NOTE Confidence: 0.776843212941176

00:38:26.740 --> 00:38:27.568 some interesting biology.

NOTE Confidence: 0.776843212941176

00:38:27.570 --> 00:38:28.551 Associated with them,

NOTE Confidence: 0.776843212941176

00:38:28.551 --> 00:38:30.513 which means that once we have

NOTE Confidence: 0.776843212941176

00:38:30.513 --> 00:38:31.499 modifications of interest,

NOTE Confidence: 0.776843212941176

00:38:31.500 --> 00:38:33.435 we can go in and try to perturb them.

NOTE Confidence: 0.776843212941176

00:38:33.440 --> 00:38:36.554 We can go look in hopefully either

NOTE Confidence: 0.776843212941176

00:38:36.554 --> 00:38:39.116 sort of more disease relevant samples

NOTE Confidence: 0.776843212941176

00:38:39.116 --> 00:38:41.482 than just cell culture lines and

NOTE Confidence: 0.776843212941176

00:38:41.482 --> 00:38:43.642 actually go and look at whether

NOTE Confidence: 0.776843212941176

00:38:43.715 --> 00:38:46.120 these changes actually translate to,

NOTE Confidence: 0.776843212941176

00:38:46.120 --> 00:38:46.874 you know,

NOTE Confidence: 0.776843212941176

00:38:46.874 --> 00:38:49.136 samples that are a bit more

NOTE Confidence: 0.776843212941176

00:38:49.136 --> 00:38:49.890 directly translationally

NOTE Confidence: 0.807776348181818

00:38:49.953 --> 00:38:51.376 relevant. We're also interested

NOTE Confidence: 0.807776348181818

00:38:51.376 --> 00:38:53.600 in sort of M RNA's like Braca 2,

NOTE Confidence: 0.807776348181818

00:38:53.600 --> 00:38:55.625 but many others that we can use to sort

NOTE Confidence: 0.807776348181818

00:38:55.625 --> 00:38:57.306 of highlight the sequencing approach

NOTE Confidence: 0.807776348181818

00:38:57.306 --> 00:38:59.794 and essentially again go in and find

NOTE Confidence: 0.807776348181818

00:38:59.794 --> 00:39:01.484 some interesting modifications and try

NOTE Confidence: 0.807776348181818

00:39:01.484 --> 00:39:04.331 to perturb them and monitor how they

NOTE Confidence: 0.807776348181818

00:39:04.331 --> 00:39:06.816 change under under different conditions.

NOTE Confidence: 0.807776348181818

00:39:06.820 --> 00:39:08.636 But I think thinking sort of bigger picture,

NOTE Confidence: 0.807776348181818

00:39:08.640 --> 00:39:10.383 what these approaches sort of allow us

NOTE Confidence: 0.807776348181818

00:39:10.383 --> 00:39:12.346 to think about is to actually monitor

NOTE Confidence: 0.807776348181818

00:39:12.346 --> 00:39:14.092 changes and modifications in real time.

NOTE Confidence: 0.807776348181818

00:39:14.100 --> 00:39:16.548 And so a problem that we're,

NOTE Confidence: 0.807776348181818

00:39:16.550 --> 00:39:18.454 we're sort of starting to think a little

NOTE Confidence: 0.807776348181818

00:39:18.454 --> 00:39:20.458 bit about this is driven really by Emily.

NOTE Confidence: 0.807776348181818

00:39:20.460 --> 00:39:23.408 In our lab is can we actually, you know,

NOTE Confidence: 0.807776348181818

00:39:23.408 --> 00:39:25.431 look at instances of things like drug

NOTE Confidence: 0.807776348181818

00:39:25.431 --> 00:39:27.065 resistance where we're sort of used

NOTE Confidence: 0.807776348181818

00:39:27.065 --> 00:39:28.694 to thinking about DNA based mutations

NOTE Confidence: 0.807776348181818

00:39:28.694 --> 00:39:30.398 that are making a protein resistant

00:39:30.398 --> 00:39:32.288 to the drug that's targeting it.

00:39:32.288 --> 00:39:34.364 But are there other changes beyond

00:39:34.364 --> 00:39:36.611 that that we're missing that we could

00:39:36.611 --> 00:39:38.951 actually sort of use either as biomarkers

00:39:38.951 --> 00:39:41.063 or diagnostics or something else that

00:39:41.063 --> 00:39:44.170 we could actually either you know,

00:39:44.170 --> 00:39:46.599 target or at least use to monitor

00:39:46.599 --> 00:39:48.344 the development of disease sort

00:39:48.344 --> 00:39:49.919 of at the RNA level?

00:39:49.920 --> 00:39:52.260 The consequences of IH1 status are

00:39:52.260 --> 00:39:54.522 really unknown here and this is

00:39:54.522 --> 00:39:56.342 really where I think where we've

00:39:56.342 --> 00:39:58.028 become sort of really interested in

00:39:58.028 --> 00:39:59.699 glioblastoma for a few different reasons.

00:39:59.700 --> 00:40:02.492 One of them is the sort of huge

00:40:02.492 --> 00:40:04.178 dichotomy with with DH one.

NOTE Confidence: 0.807776348181818

00:40:04.180 --> 00:40:06.100 But another is that, you know,

NOTE Confidence: 0.807776348181818

00:40:06.100 --> 00:40:07.260 it's a disease in which,

NOTE Confidence: 0.807776348181818

00:40:07.260 --> 00:40:08.639 at least based on what I've read

NOTE Confidence: 0.807776348181818

00:40:08.639 --> 00:40:09.958 and I'm happy to be corrected,

NOTE Confidence: 0.807776348181818

00:40:09.960 --> 00:40:11.466 there's actually a fair amount of

NOTE Confidence: 0.807776348181818

00:40:11.466 --> 00:40:13.411 evidence that a lot of drug resistance

NOTE Confidence: 0.807776348181818

00:40:13.411 --> 00:40:14.901 isn't really happening based on

NOTE Confidence: 0.807776348181818

00:40:14.901 --> 00:40:16.608 mutations at the DNA level suggesting

NOTE Confidence: 0.807776348181818

00:40:16.608 --> 00:40:18.198 that there might be some really

NOTE Confidence: 0.807776348181818

00:40:18.200 --> 00:40:20.080 interesting things we can look at at the.

NOTE Confidence: 0.807776348181818

00:40:20.080 --> 00:40:22.780 RNA level including things like modifications

NOTE Confidence: 0.807776348181818

00:40:22.780 --> 00:40:25.408 that aren't really driven by you know,

NOTE Confidence: 0.807776348181818

00:40:25.410 --> 00:40:26.610 DNA mutations specifically.

NOTE Confidence: 0.807776348181818

00:40:26.610 --> 00:40:29.410 And so hopefully I've convinced you that

NOTE Confidence: 0.807776348181818

00:40:29.474 --> 00:40:31.826 that you know what we're doing is sort

NOTE Confidence: 0.807776348181818

00:40:31.826 --> 00:40:33.948 of somewhat feasible and interesting.

NOTE Confidence: 0.807776348181818

00:40:33.950 --> 00:40:35.110 I think moving forward,

NOTE Confidence: 0.807776348181818

00:40:35.110 --> 00:40:35.690 you know,

NOTE Confidence: 0.807776348181818

00:40:35.690 --> 00:40:38.096 we've definitely have a really strong

NOTE Confidence: 0.807776348181818

00:40:38.096 --> 00:40:40.190 interest in glioblastoma and this is

NOTE Confidence: 0.807776348181818

00:40:40.190 --> 00:40:42.731 sort of what the Sontag Foundation has

NOTE Confidence: 0.807776348181818

00:40:42.731 --> 00:40:45.267 sort of funded us to look into Umm,

NOTE Confidence: 0.807776348181818

00:40:45.270 --> 00:40:47.280 thankfully with the brain tumor

NOTE Confidence: 0.807776348181818

00:40:47.280 --> 00:40:49.290 centers as support and guidance,

NOTE Confidence: 0.807776348181818

00:40:49.290 --> 00:40:51.089 but we're really interested in sort of.

NOTE Confidence: 0.807776348181818

00:40:51.090 --> 00:40:52.675 Applying this across all different

NOTE Confidence: 0.807776348181818

00:40:52.675 --> 00:40:54.642 types of disease and other cancers

NOTE Confidence: 0.807776348181818

00:40:54.642 --> 00:40:56.604 in particular because I don't think

NOTE Confidence: 0.807776348181818

00:40:56.604 --> 00:40:58.197 that our approaches are necessarily

NOTE Confidence: 0.807776348181818

00:40:58.197 --> 00:41:00.136 going to be limited to you know,

NOTE Confidence: 0.807776348181818

00:41:00.140 --> 00:41:01.460 glioblastoma specifically.

NOTE Confidence: 0.807776348181818

00:41:01.460 --> 00:41:03.440 But you know,

NOTE Confidence: 0.807776348181818

00:41:03.440 --> 00:41:05.264 I'm hoping that with a little bit of

NOTE Confidence: 0.807776348181818

00:41:05.264 --> 00:41:06.697 discussion or maybe I've peaked some

NOTE Confidence: 0.807776348181818

00:41:06.697 --> 00:41:08.572 interest in some of you here that maybe

NOTE Confidence: 0.807776348181818

00:41:08.572 --> 00:41:10.284 you can give us some great new ideas

NOTE Confidence: 0.807776348181818

00:41:10.290 --> 00:41:12.618 and spark some new collaborations to,

NOTE Confidence: 0.807776348181818

00:41:12.620 --> 00:41:15.800 to work on this moving forward.

NOTE Confidence: 0.807776348181818

00:41:15.800 --> 00:41:17.800 And so with that I would just like

NOTE Confidence: 0.807776348181818

00:41:17.800 --> 00:41:19.377 to acknowledge the people that are

NOTE Confidence: 0.807776348181818

00:41:19.377 --> 00:41:21.170 actually doing the work in our lab.

NOTE Confidence: 0.807776348181818

00:41:21.170 --> 00:41:23.528 And so I think this is a up to

NOTE Confidence: 0.807776348181818

00:41:23.528 --> 00:41:25.820 date picture for the most part.

NOTE Confidence: 0.807776348181818

00:41:25.820 --> 00:41:28.060 So we are mostly graduate student lab,

NOTE Confidence: 0.807776348181818

00:41:28.060 --> 00:41:30.400 but we have undergraduates and

NOTE Confidence: 0.807776348181818

00:41:30.400 --> 00:41:32.740 postgraduate researchers working with us

NOTE Confidence: 0.866562550833333

00:41:32.812 --> 00:41:35.650 as well. We're supported by a tremendous

NOTE Confidence: 0.866562550833333

00:41:35.650 --> 00:41:39.558 team of collaborators both in in a piles lab

NOTE Confidence: 0.866562550833333

00:41:39.558 --> 00:41:42.440 but also Brent gravely and Emmanuel Saliba.

NOTE Confidence: 0.866562550833333

00:41:42.440 --> 00:41:45.580 We have a joint NHR I grant to sort of study.

NOTE Confidence: 0.866562550833333

00:41:45.580 --> 00:41:48.380 Um, you know, develop methods to sort

NOTE Confidence: 0.866562550833333

00:41:48.380 --> 00:41:50.479 of sequence modifications more broadly.

NOTE Confidence: 0.866562550833333

00:41:50.480 --> 00:41:53.049 We're also very grateful to our mass

NOTE Confidence: 0.866562550833333

00:41:53.049 --> 00:41:55.921 spec support and the Yale Chemical

NOTE Confidence: 0.866562550833333

00:41:55.921 --> 00:41:57.619 Biology Instrumentation Center.

NOTE Confidence: 0.866562550833333

00:41:57.620 --> 00:42:00.882 And of course our funding sources through

NOTE Confidence: 0.866562550833333

00:42:00.882 --> 00:42:04.022 the NIH, the Hood Foundation and the Sontag

NOTE Confidence: 0.866562550833333

00:42:04.022 --> 00:42:06.170 Foundation that I already mentioned.

NOTE Confidence: 0.866562550833333

00:42:06.170 --> 00:42:07.875 We've got also great support

NOTE Confidence: 0.866562550833333

00:42:07.875 --> 00:42:09.990 from the RNA center at Yale,

NOTE Confidence: 0.866562550833333

00:42:09.990 --> 00:42:11.670 which is a a great community.

NOTE Confidence: 0.866562550833333

00:42:11.670 --> 00:42:13.008 But of course I would definitely

NOTE Confidence: 0.866562550833333

00:42:13.008 --> 00:42:14.569 like to thank the Cancer Center,

NOTE Confidence: 0.866562550833333

00:42:14.570 --> 00:42:16.082 not only for the invitation to actually

NOTE Confidence: 0.866562550833333

00:42:16.082 --> 00:42:18.084 come in and talk to you a little bit today,

NOTE Confidence: 0.866562550833333

00:42:18.090 --> 00:42:19.118 but I was truly,

NOTE Confidence: 0.866562550833333

00:42:19.118 --> 00:42:20.660 it was the most pleasant surprise

NOTE Confidence: 0.866562550833333

00:42:20.712 --> 00:42:22.284 when I emailed Doctor Amura about

NOTE Confidence: 0.866562550833333

00:42:22.284 --> 00:42:23.949 this sort of like random hey,

NOTE Confidence: 0.866562550833333

00:42:23.950 --> 00:42:25.567 I'm writing a grant about brain cancer.

NOTE Confidence: 0.866562550833333

00:42:25.570 --> 00:42:28.054 Like, would you be willing to chat and and

NOTE Confidence: 0.866562550833333

00:42:28.054 --> 00:42:30.286 maybe talk through some of these ideas?

NOTE Confidence: 0.866562550833333

00:42:30.290 --> 00:42:32.090 It was a fantastic interaction

NOTE Confidence: 0.866562550833333

00:42:32.090 --> 00:42:33.170 and great conversation.

NOTE Confidence: 0.866562550833333

00:42:33.170 --> 00:42:35.434 I hope this is sort of a productive

NOTE Confidence: 0.866562550833333

00:42:35.434 --> 00:42:36.660 collaboration in the future.

NOTE Confidence: 0.866562550833333

00:42:36.660 --> 00:42:37.383 And with that,

NOTE Confidence: 0.866562550833333

00:42:37.383 --> 00:42:39.070 I'll just leave it there and I'll

NOTE Confidence: 0.866562550833333

00:42:39.129 --> 00:42:40.750 take any questions you might have.

NOTE Confidence: 0.761150663333333

00:42:50.230 --> 00:42:51.028 Well, let's check.

NOTE Confidence: 0.8888614675

00:42:53.290 --> 00:42:53.918 I don't think so.

NOTE Confidence: 0.865788147

00:42:53.930 --> 00:42:55.406 In the meantime, let's get some

NOTE Confidence: 0.865788147

00:42:55.406 --> 00:42:56.390 questions from the audience.

NOTE Confidence: 0.73228536625

00:43:02.690 --> 00:43:04.378 I have two questions though kind of like.

NOTE Confidence: 0.726515603529412

00:43:07.830 --> 00:43:09.811 The first one being I was wondering

NOTE Confidence: 0.726515603529412

00:43:09.811 --> 00:43:11.882 if you if your studies especially

NOTE Confidence: 0.726515603529412

00:43:11.882 --> 00:43:14.202 when they looked at like locations

NOTE Confidence: 0.726515603529412

00:43:14.202 --> 00:43:16.132 which does obviously cause an

NOTE Confidence: 0.726515603529412

00:43:16.132 --> 00:43:18.030 increase in strain escalation,

NOTE Confidence: 0.726515603529412

00:43:18.030 --> 00:43:20.295 seeing whether using methylation can

NOTE Confidence: 0.726515603529412

00:43:20.295 --> 00:43:22.560 change body affects or potentially

NOTE Confidence: 0.726515603529412

00:43:22.631 --> 00:43:24.956 changes the amount of modifications

NOTE Confidence: 0.726515603529412

00:43:24.956 --> 00:43:26.649 tomorrow night, I'm not saying.

NOTE Confidence: 0.862024616

00:43:28.230 --> 00:43:29.410 It's a really great question.

NOTE Confidence: 0.862024616

00:43:29.410 --> 00:43:32.530 That's actually a PhD project in our lab

NOTE Confidence: 0.862024616

00:43:32.530 --> 00:43:34.390 essentially not exactly that but yes,

NOTE Confidence: 0.862024616

00:43:34.390 --> 00:43:35.920 so that's a fantastic question.

NOTE Confidence: 0.862024616

00:43:35.920 --> 00:43:36.900 So for the people on zoom that

NOTE Confidence: 0.862024616

00:43:36.900 --> 00:43:38.008 may not have caught the question,

NOTE Confidence: 0.862024616

00:43:38.010 --> 00:43:40.008 the question is essentially about the

NOTE Confidence: 0.862024616

00:43:40.008 --> 00:43:42.370 sort of effects of methylation status of

NOTE Confidence: 0.862024616

00:43:42.370 --> 00:43:45.460 DNA on sort of RNA modification status.

NOTE Confidence: 0.862024616

00:43:45.460 --> 00:43:48.188 So at the moment there's a lot of

NOTE Confidence: 0.862024616

00:43:48.188 --> 00:43:49.846 correlative evidence suggesting that

NOTE Confidence: 0.862024616

00:43:49.846 --> 00:43:52.582 either DNA methylation and or histone

NOTE Confidence: 0.862024616

00:43:52.582 --> 00:43:54.220 methylation could impact sort of

NOTE Confidence: 0.862024616

00:43:54.220 --> 00:43:55.990 the presence or absence of specific

NOTE Confidence: 0.862024616

00:43:56.050 --> 00:43:58.318 modifications and then that will of course.

NOTE Confidence: 0.862024616

00:43:58.320 --> 00:44:00.336 Relate with sort of gene expression,

NOTE Confidence: 0.862024616

00:44:00.340 --> 00:44:01.940 but we have a bit of a chicken

NOTE Confidence: 0.862024616

00:44:01.940 --> 00:44:02.820 and egg problem.

NOTE Confidence: 0.862024616

00:44:02.820 --> 00:44:04.780 I think intuitively you would assume that

NOTE Confidence: 0.862024616

00:44:04.780 --> 00:44:06.946 the DNA level stuff would impact the RNA

NOTE Confidence: 0.862024616

00:44:06.946 --> 00:44:08.990 level stuff and only in One Direction.

NOTE Confidence: 0.862024616

00:44:08.990 --> 00:44:11.279 But it turns out that that's actually

NOTE Confidence: 0.862024616

00:44:11.279 --> 00:44:14.347 maybe not true and that there's sort of

NOTE Confidence: 0.862024616

00:44:14.347 --> 00:44:16.525 interactions between sort of chromatin

NOTE Confidence: 0.862024616

00:44:16.525 --> 00:44:17.890 transcription machinery modification

NOTE Confidence: 0.862024616

00:44:17.890 --> 00:44:20.143 machinery that maybe actually going

NOTE Confidence: 0.862024616

00:44:20.143 --> 00:44:21.659 back and impacting chromatin.

NOTE Confidence: 0.862024616

00:44:21.660 --> 00:44:23.796 So the short answer is yes,

NOTE Confidence: 0.862024616

00:44:23.800 --> 00:44:25.360 it likely is impacting it.

NOTE Confidence: 0.862024616

00:44:25.360 --> 00:44:27.320 We don't exactly know how,

NOTE Confidence: 0.862024616

00:44:27.320 --> 00:44:29.680 but one way that we're trying to study.

NOTE Confidence: 0.862024616

00:44:29.680 --> 00:44:31.185 Just to look at the code transcriptional

NOTE Confidence: 0.862024616

00:44:31.185 --> 00:44:32.046 regulation of different modifications

NOTE Confidence: 0.862024616

00:44:32.046 --> 00:44:33.494 and if we can figure out kind of

NOTE Confidence: 0.862024616

00:44:33.494 --> 00:44:34.418 exactly where they're put on,

NOTE Confidence: 0.862024616

00:44:34.420 --> 00:44:36.140 then we can go and tinker with the

NOTE Confidence: 0.862024616

00:44:36.140 --> 00:44:38.000 DNA and then figure out if it's

NOTE Confidence: 0.862024616

00:44:38.000 --> 00:44:41.180 still happening or. Yeah, yeah.

NOTE Confidence: 0.862024616

00:44:41.180 --> 00:44:44.060 Question is.

NOTE Confidence: 0.862024616

00:44:44.060 --> 00:44:44.710 Retaliating.

NOTE Confidence: 0.7547894

00:44:47.050 --> 00:44:48.170 It's like I don't know.

NOTE Confidence: 0.89287934

00:44:55.830 --> 00:44:57.822 Yes. So we we always try to validate

NOTE Confidence: 0.89287934

00:44:57.822 --> 00:44:59.908 sort of in multiple different ways,

NOTE Confidence: 0.89287934

00:44:59.910 --> 00:45:01.809 not only sort of loss of the enzyme but

NOTE Confidence: 0.89287934

00:45:01.809 --> 00:45:03.888 also loss of the modification it turns out.

NOTE Confidence: 0.89287934

00:45:03.890 --> 00:45:05.842 So the shorter answer is yes, we do.

NOTE Confidence: 0.89287934

00:45:05.842 --> 00:45:07.809 The tricky part is that oftentimes we're

NOTE Confidence: 0.89287934

00:45:07.809 --> 00:45:09.441 dealing with very small changes and

NOTE Confidence: 0.89287934

00:45:09.441 --> 00:45:11.409 actually in the case of metal three,

NOTE Confidence: 0.89287934

00:45:11.410 --> 00:45:13.230 it's even worse because it's an essential

NOTE Confidence: 0.89287934

00:45:13.230 --> 00:45:15.286 gene and if you lose it for too long,

NOTE Confidence: 0.89287934

00:45:15.290 --> 00:45:16.610 cells are dead.

NOTE Confidence: 0.89287934

00:45:16.610 --> 00:45:20.120 So we do our best to sort of.

NOTE Confidence: 0.89287934

00:45:20.120 --> 00:45:21.709 Tune the perturbation so we can get

NOTE Confidence: 0.89287934

00:45:21.709 --> 00:45:23.460 a change in modification but not,

NOTE Confidence: 0.89287934

00:45:23.460 --> 00:45:24.544 you know, lose everything.

NOTE Confidence: 0.89287934

00:45:24.544 --> 00:45:26.474 But I will say that actually one

NOTE Confidence: 0.89287934

00:45:26.474 --> 00:45:27.980 thing that we've been trying to

NOTE Confidence: 0.89287934

00:45:27.980 --> 00:45:29.676 work out though it's been very,

NOTE Confidence: 0.89287934

00:45:29.676 --> 00:45:31.548 very difficult is to use these

NOTE Confidence: 0.89287934

00:45:31.548 --> 00:45:33.604 sort of crisper cast based systems

NOTE Confidence: 0.89287934

00:45:33.604 --> 00:45:35.826 to sort of target enzymes to

NOTE Confidence: 0.89287934

00:45:35.826 --> 00:45:37.248 specific modification sites.

NOTE Confidence: 0.89287934

00:45:37.250 --> 00:45:39.239 So the idea would be you take a dead

NOTE Confidence: 0.89287934

00:45:39.239 --> 00:45:40.915 cast 9 or a dead cast whatever.

NOTE Confidence: 0.89287934

00:45:40.920 --> 00:45:42.696 There's like 10 of them now I think

NOTE Confidence: 0.89287934

00:45:42.696 --> 00:45:44.508 that are essentially trying to tether

NOTE Confidence: 0.89287934

00:45:44.508 --> 00:45:46.770 enzymes using this this machinery to a

NOTE Confidence: 0.89287934

00:45:46.770 --> 00:45:48.429 specific place using a guide RNA and

NOTE Confidence: 0.89287934

00:45:48.429 --> 00:45:50.290 then the enzyme is around and should be.

NOTE Confidence: 0.89287934

00:45:50.290 --> 00:45:52.415 Essentially just be removing a

NOTE Confidence: 0.89287934

00:45:52.415 --> 00:45:54.540 modification at that specific site.

NOTE Confidence: 0.89287934

00:45:54.540 --> 00:45:56.640 We're trying some of these systems.

NOTE Confidence: 0.89287934

00:45:56.640 --> 00:45:58.524 We haven't gotten all of them

NOTE Confidence: 0.89287934

00:45:58.524 --> 00:46:00.120 to work very well yet,

NOTE Confidence: 0.89287934

00:46:00.120 --> 00:46:02.433 but that would be sort of a much more

NOTE Confidence: 0.89287934

00:46:02.433 --> 00:46:04.430 targeted and much better way to look at

90

NOTE Confidence: 0.89287934

00:46:04.430 --> 00:46:06.499 sort of very specific modifications.

NOTE Confidence: 0.89287934

00:46:06.500 --> 00:46:06.920 So,

NOTE Confidence: 0.75333511

00:46:07.240 --> 00:46:09.810 yeah, good questions. Yeah.

NOTE Confidence: 0.748746281923077

00:46:15.420 --> 00:46:17.796 Yes. So yes, so the question is have

NOTE Confidence: 0.748746281923077

00:46:17.796 --> 00:46:20.600 we looked at IMIDAZOLINE and LH3? Yes,

NOTE Confidence: 0.748746281923077

00:46:20.600 --> 00:46:24.520 I actually didn't talk about the M1A work.

NOTE Confidence: 0.748746281923077

00:46:24.520 --> 00:46:26.032 I have a long history with that

NOTE Confidence: 0.748746281923077

00:46:26.032 --> 00:46:28.840 modification from my postdoc. Actually,

NOTE Confidence: 0.748746281923077

00:46:28.840 --> 00:46:30.976 it's been a very tricky one to study.

NOTE Confidence: 0.748746281923077

00:46:30.980 --> 00:46:32.646 And if you're familiar with that literature,

NOTE Confidence: 0.748746281923077

00:46:32.650 --> 00:46:34.282 you might know there's been a lot of

NOTE Confidence: 0.748746281923077

00:46:34.282 --> 00:46:35.480 arguments about sort of prevalence,

NOTE Confidence: 0.748746281923077

00:46:35.480 --> 00:46:36.647 presence, location, etcetera.

NOTE Confidence: 0.748746281923077

00:46:36.647 --> 00:46:39.370 And so I think are a lot

NOTE Confidence: 0.748746281923077

00:46:39.449 --> 00:46:41.465 without the H3 as a postdoc.

NOTE Confidence: 0.748746281923077

00:46:41.470 --> 00:46:43.798 And and the sort of odd thing that

NOTE Confidence: 0.748746281923077

00:46:43.798 --> 00:46:46.035 I stumbled on and I think this is

NOTE Confidence: 0.748746281923077

00:46:46.035 --> 00:46:47.595 like buried in the supplementary

NOTE Confidence: 0.748746281923077

00:46:47.595 --> 00:46:50.199 figure whatever of that paper is.

NOTE Confidence: 0.748746281923077

00:46:50.200 --> 00:46:52.762 It was strange because I when I

NOTE Confidence: 0.748746281923077

00:46:52.762 --> 00:46:54.385 overexpressed out PH3IN cell lines,

NOTE Confidence: 0.748746281923077

00:46:54.385 --> 00:46:55.860 you can see an ink.

NOTE Confidence: 0.748746281923077

00:46:55.860 --> 00:46:57.358 So you see an increase in LH3,

NOTE Confidence: 0.748746281923077

00:46:57.360 --> 00:46:59.664 you would expect a decrease in M1A and

NOTE Confidence: 0.748746281923077

00:46:59.664 --> 00:47:02.420 we did see that but when we knocked down

NOTE Confidence: 0.748746281923077

00:47:02.420 --> 00:47:05.714 LH3 we did not see the sort of reverse.

NOTE Confidence: 0.748746281923077

00:47:05.720 --> 00:47:07.060 But interestingly other labs have

NOTE Confidence: 0.748746281923077

00:47:07.060 --> 00:47:09.105 and so I don't know if this is

NOTE Confidence: 0.748746281923077

00:47:09.105 --> 00:47:10.624 sort of a methods based thing or

NOTE Confidence: 0.748746281923077

00:47:10.681 --> 00:47:12.193 not I will say in an in vitro.

NOTE Confidence: 0.748746281923077

00:47:12.200 --> 00:47:12.758 Experiment,

NOTE Confidence: 0.748746281923077

00:47:12.758 --> 00:47:15.548 it absolutely will demethylated M1A.

NOTE Confidence: 0.748746281923077

00:47:15.550 --> 00:47:17.863 It will do that probably on RNA and DNA.

NOTE Confidence: 0.748746281923077

00:47:17.870 --> 00:47:20.142 The question is more just if in the

NOTE Confidence: 0.748746281923077

00:47:20.142 --> 00:47:21.504 cell itself they're encountering

NOTE Confidence: 0.748746281923077

00:47:21.504 --> 00:47:24.045 each other to the extent that you

NOTE Confidence: 0.748746281923077

00:47:24.045 --> 00:47:26.286 would need for it to regulate M RNA.

NOTE Confidence: 0.748746281923077

00:47:26.290 --> 00:47:28.180 So we're not sure we're we're sort

NOTE Confidence: 0.748746281923077

00:47:28.180 --> 00:47:30.369 of going to still try to work that

NOTE Confidence: 0.748746281923077

00:47:30.369 --> 00:47:32.568 out in a more targeted way if we can.

NOTE Confidence: 0.748746281923077

00:47:32.570 --> 00:47:34.327 We actually would love to target LPH

NOTE Confidence: 0.748746281923077

00:47:34.327 --> 00:47:37.186 3 with a cast system and sort of

NOTE Confidence: 0.748746281923077

00:47:37.186 --> 00:47:38.822 specifically demethylated specific places,

NOTE Confidence: 0.748746281923077

00:47:38.830 --> 00:47:42.208 specific sites but that's been a tricky 1.

NOTE Confidence: 0.748746281923077

00:47:42.210 --> 00:47:46.090 For a lot of reasons, yeah.

NOTE Confidence: 0.748746281923077

00:47:46.090 --> 00:47:47.140 So

NOTE Confidence: 0.884536414

00:47:47.140 --> 00:47:49.140 I was fascinated by the use

NOTE Confidence: 0.884536414

00:47:49.140 --> 00:47:52.350 of antibodies for enrichment.

NOTE Confidence: 0.644027781428572

00:47:52.350 --> 00:47:54.044 Have those antibodies ever been used inside

NOTE Confidence: 0.756750996

00:47:54.060 --> 00:47:56.160 to try to identify location

NOTE Confidence: 0.756750996

00:47:56.160 --> 00:47:57.810 of the marks or any kind of?

NOTE Confidence: 0.890784386

00:47:58.820 --> 00:48:00.040 Yeah, that's a great question.

NOTE Confidence: 0.890784386

00:48:00.040 --> 00:48:04.216 So I don't know about Umm.

NOTE Confidence: 0.890784386

00:48:04.220 --> 00:48:07.694 So yes, a little bit in sort

NOTE Confidence: 0.890784386

00:48:07.694 --> 00:48:09.954 of cell culture based systems,

NOTE Confidence: 0.890784386

00:48:09.960 --> 00:48:12.216 it's often a little bit tricky unless you

NOTE Confidence: 0.890784386

00:48:12.216 --> 00:48:14.478 can get a very specific concentration of

NOTE Confidence: 0.890784386

00:48:14.478 --> 00:48:17.039 a modification in a very specific place.

NOTE Confidence: 0.890784386

00:48:17.040 --> 00:48:18.732 For something like M6A that's sort

NOTE Confidence: 0.890784386

00:48:18.732 --> 00:48:20.659 of diffuse on many different RNA,

NOTE Confidence: 0.890784386

00:48:20.660 --> 00:48:21.860 it can be a little tricky.

NOTE Confidence: 0.890784386

00:48:21.860 --> 00:48:24.380 That being said, for some things,

NOTE Confidence: 0.890784386

00:48:24.380 --> 00:48:26.880 you know particularly if you

NOTE Confidence: 0.890784386

00:48:26.880 --> 00:48:28.732 have very concentrated sort of.

NOTE Confidence: 0.890784386

00:48:28.732 --> 00:48:30.358 You know, a nuclear body or

NOTE Confidence: 0.890784386

00:48:30.358 --> 00:48:31.069 a cytoplasmic body.

NOTE Confidence: 0.890784386

00:48:31.070 --> 00:48:32.127 You might be able to detect them,

NOTE Confidence: 0.890784386

00:48:32.130 --> 00:48:33.204 I don't know.

NOTE Confidence: 0.890784386

00:48:33.204 --> 00:48:34.994 Various people have tried and

NOTE Confidence: 0.890784386

00:48:34.994 --> 00:48:37.120 to sort of varying success.

NOTE Confidence: 0.890784386

00:48:37.120 --> 00:48:38.026 I think it's something we need

NOTE Confidence: 0.890784386

00:48:38.026 --> 00:48:39.239 to look at a little bit more.

NOTE Confidence: 0.890784386

00:48:39.240 --> 00:48:40.854 The the other tricky thing with

NOTE Confidence: 0.890784386

00:48:40.854 --> 00:48:42.498 these antibodies is it turns out

NOTE Confidence: 0.890784386

00:48:42.498 --> 00:48:44.052 they're not all super specific and

NOTE Confidence: 0.890784386

00:48:44.052 --> 00:48:45.873 so then we can't interpret whether

NOTE Confidence: 0.890784386

00:48:45.873 --> 00:48:47.751 the signal we're getting is actually

NOTE Confidence: 0.890784386

00:48:47.760 --> 00:48:50.352 because of that modification or just

NOTE Confidence: 0.890784386

00:48:50.352 --> 00:48:53.279 it's binding to a lot of things.

NOTE Confidence: 0.890784386

00:48:53.280 --> 00:48:55.176 So something we still need to work out

NOTE Confidence: 0.890784386

00:48:55.176 --> 00:48:57.456 but I there's people there have been

NOTE Confidence: 0.890784386

00:48:57.456 --> 00:48:59.206 some interesting methods trying to

NOTE Confidence: 0.890784386

00:48:59.265 --> 00:49:01.260 sort of use proximity based you know,

NOTE Confidence: 0.890784386

00:49:01.260 --> 00:49:02.605 akin to a proximity ligation

NOTE Confidence: 0.890784386

00:49:02.605 --> 00:49:03.950 based strategy where you know

NOTE Confidence: 0.890784386

00:49:04.002 --> 00:49:05.346 if you can bridge two things.

NOTE Confidence: 0.890784386

00:49:05.350 --> 00:49:07.618 Together to kind of combine an antibody

NOTE Confidence: 0.890784386

00:49:07.618 --> 00:49:09.638 with something else to tell you that

NOTE Confidence: 0.890784386

00:49:09.638 --> 00:49:11.620 that Mark is there with some success.

NOTE Confidence: 0.890784386

00:49:11.620 --> 00:49:13.378 But the imaging based methods are

NOTE Confidence: 0.890784386

00:49:13.378 --> 00:49:15.278 a little bit behind I would say,

NOTE Confidence: 0.890784386

00:49:15.280 --> 00:49:15.540 yeah.

NOTE Confidence: 0.834696256666667

00:49:18.810 --> 00:49:19.518 At the beginning.

NOTE Confidence: 0.573482131666667

00:49:23.630 --> 00:49:26.252 I was wondering if that's drastic

NOTE Confidence: 0.573482131666667

00:49:26.252 --> 00:49:29.539 enough to affect the RNA confrontation,

NOTE Confidence: 0.573482131666667

00:49:29.540 --> 00:49:31.488 but you could essentially have proteins

NOTE Confidence: 0.573482131666667

00:49:31.488 --> 00:49:33.364 that couldn't or wouldn't be picked up.

NOTE Confidence: 0.85666396

00:49:34.740 --> 00:49:35.760 Yeah, that's a great question.

NOTE Confidence: 0.85666396

00:49:35.760 --> 00:49:38.300 So the question is for those of you on zoom,

NOTE Confidence: 0.85666396

00:49:38.300 --> 00:49:40.064 whether the impact of modifications on

NOTE Confidence: 0.85666396

00:49:40.064 --> 00:49:41.915 base pairing would be sufficient to

NOTE Confidence: 0.85666396

00:49:41.915 --> 00:49:43.769 maybe even impact essentially decoding I

NOTE Confidence: 0.85666396

00:49:43.769 --> 00:49:46.175 guess is what you're asking and and sort

NOTE Confidence: 0.85666396

00:49:46.175 --> 00:49:47.930 of introduce mutations into proteins.

NOTE Confidence: 0.85666396

00:49:47.930 --> 00:49:51.300 There is certainly evidence that the

NOTE Confidence: 0.85666396

00:49:51.300 --> 00:49:53.400 presence or absence of modifications

NOTE Confidence: 0.85666396

00:49:53.400 --> 00:49:56.010 can impact at least at the very least

NOTE Confidence: 0.85666396

00:49:56.010 --> 00:49:58.388 sort of start code on usage and to

NOTE Confidence: 0.85666396

00:49:58.388 --> 00:50:00.740 some extent a little bit of decoding.

NOTE Confidence: 0.85666396

00:50:00.740 --> 00:50:02.065 Not as familiar with the

NOTE Confidence: 0.85666396

00:50:02.065 --> 00:50:02.595 translation literature.

NOTE Confidence: 0.85666396

00:50:02.600 --> 00:50:04.287 There's certainly evidence for it in sort

NOTE Confidence: 0.85666396

00:50:04.287 --> 00:50:06.532 of in vitro translation systems in vivo.

NOTE Confidence: 0.85666396

00:50:06.532 --> 00:50:08.674 It's a bit harder to tease apart,

NOTE Confidence: 0.85666396

00:50:08.680 --> 00:50:09.656 but if you're interested

NOTE Confidence: 0.85666396

00:50:09.656 --> 00:50:10.876 in that literature at all,

NOTE Confidence: 0.85666396

00:50:10.880 --> 00:50:12.944 actually Shalini Oberdorfer's lab

NOTE Confidence: 0.85666396

00:50:12.944 --> 00:50:15.192 has done some really amazing work

NOTE Confidence: 0.85666396

00:50:15.192 --> 00:50:18.180 on a C4C and how it sort of dictates

NOTE Confidence: 0.85666396

00:50:18.180 --> 00:50:20.580 start code on usage through sort

NOTE Confidence: 0.85666396

00:50:20.580 --> 00:50:22.420 of base pairing interactions.

NOTE Confidence: 0.85666396

00:50:22.420 --> 00:50:23.540 So that's not our work,

NOTE Confidence: 0.85666396

00:50:23.540 --> 00:50:24.698 but it's beautiful and I would

NOTE Confidence: 0.85666396

00:50:24.698 --> 00:50:26.054 encourage you to take a look at

NOTE Confidence: 0.85666396

00:50:26.054 --> 00:50:26.774 it if you're interested,

NOTE Confidence: 0.85666396

00:50:26.780 --> 00:50:27.354 because yes,

NOTE Confidence: 0.85666396

00:50:27.354 --> 00:50:28.789 there there's certainly some evidence

NOTE Confidence: 0.85666396

00:50:28.790 --> 00:50:29.110 for that.

NOTE Confidence: 0.851628805833333

00:50:38.830 --> 00:50:40.937 So the question is the time scale

NOTE Confidence: 0.851628805833333

00:50:40.937 --> 00:50:42.289 of our modifications and the

NOTE Confidence: 0.851628805833333

00:50:42.290 --> 00:50:44.145 and how that sort of relates to

NOTE Confidence: 0.851628805833333

00:50:44.145 --> 00:50:45.509 chromatin and things like that.

NOTE Confidence: 0.851628805833333

00:50:45.510 --> 00:50:48.012 So this is something that we're

NOTE Confidence: 0.851628805833333

00:50:48.012 --> 00:50:50.330 still trying to work out so.

NOTE Confidence: 0.851628805833333

00:50:50.330 --> 00:50:52.052 We've been working on actually a totally

NOTE Confidence: 0.851628805833333

00:50:52.052 --> 00:50:53.678 different strategy I haven't talked about.

NOTE Confidence: 0.851628805833333

00:50:53.680 --> 00:50:55.353 We're trying to work out a dual

NOTE Confidence: 0.851628805833333

00:50:55.353 --> 00:50:56.530 labeling strategy in our labs.

NOTE Confidence: 0.851628805833333

00:50:56.530 --> 00:50:57.946 This is a different graduate student,

NOTE Confidence: 0.851628805833333

00:50:57.950 --> 00:50:59.720 Luke, who is working on this

NOTE Confidence: 0.851628805833333

00:50:59.720 --> 00:51:00.900 where we're essentially trying

NOTE Confidence: 0.851628805833333

00:51:00.950 --> 00:51:02.550 to combine nascent RNA labeling,

NOTE Confidence: 0.851628805833333

00:51:02.550 --> 00:51:05.160 something like an EU approach or

NOTE Confidence: 0.851628805833333

00:51:05.160 --> 00:51:07.270 a fourth European labeling with

NOTE Confidence: 0.851628805833333

00:51:07.270 --> 00:51:09.365 essentially trying to both Mark

NOTE Confidence: 0.851628805833333

00:51:09.365 --> 00:51:11.460 naison RNA and nascent methylation

NOTE Confidence: 0.851628805833333

00:51:11.529 --> 00:51:13.374 with a deuterated Sam analog.

NOTE Confidence: 0.851628805833333

00:51:13.374 --> 00:51:15.666 We haven't been able to pin

NOTE Confidence: 0.851628805833333

00:51:15.666 --> 00:51:17.230 down the kinetics.

NOTE Confidence: 0.851628805833333

00:51:17.230 --> 00:51:18.325 I have some very preliminary

NOTE Confidence: 0.851628805833333

00:51:18.325 --> 00:51:19.884 data from like way back in my

NOTE Confidence: 0.851628805833333

00:51:19.884 --> 00:51:20.934 postdoc that this can happen.

NOTE Confidence: 0.851628805833333

00:51:20.940 --> 00:51:21.698 Within minutes.

NOTE Confidence: 0.851628805833333

00:51:21.698 --> 00:51:23.972 But it's very hard to catch

NOTE Confidence: 0.851628805833333

00:51:23.972 --> 00:51:25.828 the dual sort of label.

NOTE Confidence: 0.851628805833333

00:51:25.830 --> 00:51:27.365 And so that's something that

NOTE Confidence: 0.851628805833333

00:51:27.365 --> 00:51:28.593 we're still working out.

NOTE Confidence: 0.851628805833333

00:51:28.600 --> 00:51:30.864 We're hoping that that will be working soon,

NOTE Confidence: 0.851628805833333

00:51:30.870 --> 00:51:32.538 but that's exactly the question we

NOTE Confidence: 0.851628805833333

00:51:32.538 --> 00:51:34.524 want to answer because we don't know

NOTE Confidence: 0.851628805833333

00:51:34.524 --> 00:51:36.162 if the timescales are relevant yet

NOTE Confidence: 0.851628805833333

00:51:36.162 --> 00:51:37.810 because we can't pin them down yet,

NOTE Confidence: 0.851628805833333

00:51:37.810 --> 00:51:38.960 if that makes any sense.

NOTE Confidence: 0.10927865

00:51:45.690 --> 00:51:46.290 Hmm.

NOTE Confidence: 0.21503004

00:51:50.710 --> 00:51:51.150 The.

NOTE Confidence: 0.861928434285714

00:51:56.790 --> 00:51:58.267 Yeah. So the question is whether the,

NOTE Confidence: 0.861928434285714

00:51:58.270 --> 00:52:00.392 the mutation rate sort of essentially

NOTE Confidence: 0.861928434285714

00:52:00.392 --> 00:52:01.680 correlates with what's actually

NOTE Confidence: 0.861928434285714

00:52:01.680 --> 00:52:03.290 happening at the modification level.

NOTE Confidence: 0.861928434285714

00:52:03.290 --> 00:52:04.650 So we're literally working with

NOTE Confidence: 0.861928434285714

00:52:04.650 --> 00:52:06.628 Anna Kyle's lab to try to generate

NOTE Confidence: 0.861928434285714

00:52:06.628 --> 00:52:08.326 the standards to figure that out.

NOTE Confidence: 0.861928434285714

00:52:08.330 --> 00:52:10.941 So what we essentially need to make

NOTE Confidence: 0.861928434285714

00:52:10.941 --> 00:52:13.367 is a calibration curve with known

NOTE Confidence: 0.861928434285714

00:52:13.367 --> 00:52:15.373 sort of modified oligos because

NOTE Confidence: 0.861928434285714

00:52:15.373 --> 00:52:17.914 based on some previous work with a

NOTE Confidence: 0.861928434285714

00:52:17.914 --> 00:52:19.550 different reverse transcriptase,

NOTE Confidence: 0.861928434285714

00:52:19.550 --> 00:52:21.468 we know that there is some correlation

NOTE Confidence: 0.861928434285714

00:52:21.468 --> 00:52:23.129 between the level of modification.

NOTE Confidence: 0.861928434285714

00:52:23.130 --> 00:52:25.706 And sort of the mutation rate in terms

NOTE Confidence: 0.861928434285714

00:52:25.706 --> 00:52:28.379 of it's somewhat accurate reflection

NOTE Confidence: 0.861928434285714

00:52:28.380 --> 00:52:29.695 because you know it's reverse

NOTE Confidence: 0.861928434285714

00:52:29.695 --> 00:52:31.010 transcribing not just the modified

NOTE Confidence: 0.861928434285714

00:52:31.059 --> 00:52:32.457 pool but also the unmodified pool.

NOTE Confidence: 0.861928434285714

00:52:32.460 --> 00:52:34.218 So there should be some concordance.

NOTE Confidence: 0.861928434285714

00:52:34.220 --> 00:52:36.040 It is however probably not perfect and

00:52:36.040 --> 00:52:38.752 so we kind of need to make a calibration

00:52:38.752 --> 00:52:40.384 curve essentially getting you know

00:52:40.384 --> 00:52:42.340 known modified oligos at zero percent,

00:52:42.340 --> 00:52:42.944 25 percent,

00:52:42.944 --> 00:52:45.360 50% and generating a curve to try to

00:52:45.428 --> 00:52:47.253 correlate where those where those

00:52:47.253 --> 00:52:49.743 are based on some really early work

00:52:49.743 --> 00:52:51.990 in our first M1A paper that for

00:52:51.990 --> 00:52:54.240 M1A we know that mutations.

00:52:54.240 --> 00:52:56.970 Um are relatively usually relatively strong.

00:52:56.970 --> 00:52:59.607 And in that case M1A tended to occur at

00:52:59.607 --> 00:53:02.310 about 20% of its given transcript pool.

00:53:02.310 --> 00:53:03.941 So like if you had a favorite

00:53:03.941 --> 00:53:05.418 transcript that was 1A methylated on

00:53:05.418 --> 00:53:06.938 average it was about 20% methylated.

00:53:06.938 --> 00:53:09.322 We never figured out what that means though.

NOTE Confidence: 0.861928434285714

00:53:09.330 --> 00:53:10.646 We don't know if that means most

NOTE Confidence: 0.861928434285714

00:53:10.646 --> 00:53:12.068 of it's being decayed or you know,

NOTE Confidence: 0.861928434285714

00:53:12.070 --> 00:53:12.904 anything like that,

NOTE Confidence: 0.861928434285714

00:53:12.904 --> 00:53:14.572 but we're still working that out.

NOTE Confidence: 0.763393186666667

00:53:20.400 --> 00:53:22.140 Thank you very much.

NOTE Confidence: 0.763393186666667

00:53:22.140 --> 00:53:24.315 Sacred support is amazing talk.

NOTE Confidence: 0.763393186666667

00:53:24.320 --> 00:53:26.648 It's really very rewarding to see

NOTE Confidence: 0.763393186666667

00:53:26.648 --> 00:53:28.954 this high end science coming to

NOTE Confidence: 0.763393186666667

00:53:28.954 --> 00:53:31.313 visit her here on the other side.

NOTE Confidence: 0.763393186666667

00:53:31.320 --> 00:53:33.448 Let's hope that this will be the

NOTE Confidence: 0.763393186666667

00:53:33.448 --> 00:53:35.639 beginning of many more collaborations.

NOTE Confidence: 0.763393186666667

00:53:35.640 --> 00:53:37.196 And on that note,

NOTE Confidence: 0.763393186666667

00:53:37.196 --> 00:53:39.994 I would like to remind you all

NOTE Confidence: 0.763393186666667

00:53:39.994 --> 00:53:42.962 that we do have an RFA out for

NOTE Confidence: 0.763393186666667

00:53:42.962 --> 00:53:44.088 funding laboratory research.

NOTE Confidence: 0.763393186666667

00:53:44.088 --> 00:53:46.706 There has more of a translational character.

NOTE Confidence: 0.763393186666667

00:53:46.710 --> 00:53:48.110 So you might want to check it out.

NOTE Confidence: 0.763393186666667

00:53:48.110 --> 00:53:48.898 Thank you very much.