WEBVTT

NOTE duration:"00:54:09.5640000"

NOTE language:en-us

NOTE Confidence: 0.83569926

00:00:00.000 --> 00:00:01.810 Everyone Jeannie Hendrickson I'm one

NOTE Confidence: 0.83569926

00:00:01.810 --> 00:00:04.511 of the Co directors of the enrichment

NOTE Confidence: 0.83569926

00:00:04.511 --> 00:00:06.566 program of the Yale Cooperative

NOTE Confidence: 0.83569926

00:00:06.566 --> 00:00:08.729 Center of Excellence in Hematology.

NOTE Confidence: 0.83569926

00:00:08.730 --> 00:00:11.257 Very excited to have our first virtual

NOTE Confidence: 0.83569926

00:00:11.257 --> 00:00:13.695 speaker sponsored by the Yale Cooperative

NOTE Confidence: 0.83569926

00:00:13.695 --> 00:00:15.840 Center of Excellence in Hematology,

NOTE Confidence: 0.83569926

00:00:15.840 --> 00:00:18.465 but hopefully broadcast to a lot of

NOTE Confidence: 0.83569926

00:00:18.465 --> 00:00:20.480 the other cooperative centers of

NOTE Confidence: 0.83569926

00:00:20.480 --> 00:00:22.946 excellence in hematology across the US.

NOTE Confidence: 0.83569926

00:00:22.950 --> 00:00:25.362 Whether you're looking at us Live

NOTE Confidence: 0.83569926

00:00:25.362 --> 00:00:28.245 Today or virtually on the video that

NOTE Confidence: 0.83569926

00:00:28.245 --> 00:00:30.633 we will eventually post of Mirage.

NOTE Confidence: 0.83569926

00:00:30.640 --> 00:00:32.775 Talk, we're happy that you could join

NOTE Confidence: 0.83569926

00:00:32.775 --> 00:00:34.932 us today and we're extremely grateful

NOTE Confidence: 0.83569926

00:00:34.932 --> 00:00:37.278 to Doctor Moore of Sokolovsky for

NOTE Confidence: 0.83569926

00:00:37.278 --> 00:00:39.570 being our kind of inaugural speaker,

NOTE Confidence: 0.83569926

00:00:39.570 --> 00:00:41.490 virtually for the YCCEH she's a

NOTE Confidence: 0.83569926

00:00:41.490 --> 00:00:43.258 professor at the University of

NOTE Confidence: 0.83569926

00:00:43.258 --> 00:00:44.560 Massachusetts Medical Center,

NOTE Confidence: 0.83569926

00:00:44.560 --> 00:00:47.059 she has had quite a distinguished career,

NOTE Confidence: 0.83569926

00:00:47.060 --> 00:00:48.432 really discovering fundamental insights

NOTE Confidence: 0.83569926

00:00:48.432 --> 00:00:51.350 into Areth row poesis in the process thereof.

NOTE Confidence: 0.83569926

00:00:51.350 --> 00:00:53.130 So again, we're very grateful.

NOTE Confidence: 0.83569926

00:00:53.130 --> 00:00:55.629 But she's our inaugural speaker this year.

NOTE Confidence: 0.83569926

00:00:55.630 --> 00:00:59.200 The way the web and R is going to work.

NOTE Confidence: 0.83569926

00:00:59.200 --> 00:01:01.336 She's going to give her talk.

NOTE Confidence: 0.83569926

00:01:01.340 --> 00:01:03.839 Then at the end of her talk,

NOTE Confidence: 0.83569926

00:01:03.840 --> 00:01:05.036 if you have questions.

NOTE Confidence: 0.83569926

00:01:05.036 --> 00:01:07.194 The questions in the chat or in

NOTE Confidence: 0.83569926

00:01:07.194 --> 00:01:08.709 the question and answer box.

NOTE Confidence: 0.83569926

00:01:08.710 --> 00:01:10.481 I also think I have the option

NOTE Confidence: 0.83569926

00:01:10.481 --> 00:01:11.780 to allow you to talk,

NOTE Confidence: 0.83569926

00:01:11.780 --> 00:01:13.724 so if you put in the chat you

NOTE Confidence: 0.83569926

00:01:13.724 --> 00:01:15.367 would like to talk in person.

NOTE Confidence: 0.83569926

00:01:15.370 --> 00:01:16.645 I can unmute your microphone

NOTE Confidence: 0.83569926

00:01:16.645 --> 00:01:17.665 and you can talk.

NOTE Confidence: 0.83569926

00:01:17.670 --> 00:01:19.385 So without further ado we will turn

NOTE Confidence: 0.83569926

00:01:19.385 --> 00:01:21.507 it over to Rob and thanks again Rob.

NOTE Confidence: 0.83569926

00:01:21.510 --> 00:01:22.790 We're excited to have you.

NOTE Confidence: 0.9007227

00:01:24.720 --> 00:01:27.130 Great, thank you so much.

NOTE Confidence: 0.9007227

00:01:27.130 --> 00:01:31.466 I'm truly honored to be invited to this

NOTE Confidence: 0.9007227

00:01:31.466 --> 00:01:35.427 forum and excited to tell you about

NOTE Confidence: 0.9007227

00:01:35.427 --> 00:01:39.300 the work that we've been doing so.

NOTE Confidence: 0.9007227

00:01:39.300 --> 00:01:41.538 I will attempt to start straightaway.

NOTE Confidence: 0.9007227

00:01:41.540 --> 00:01:44.168 Here we go so.

NOTE Confidence: 0.9007227

00:01:44.170 --> 00:01:47.628 When we think about the cell cycle,

NOTE Confidence: 0.9007227

00:01:47.630 --> 00:01:50.330 we think about a generic program

NOTE Confidence: 0.9007227

00:01:50.330 --> 00:01:53.308 whose purpose is to generate more

NOTE Confidence: 0.9007227

00:01:53.308 --> 00:01:56.028 cells to increase cell number.

NOTE Confidence: 0.9007227

00:01:56.030 --> 00:01:59.474 But when we look at a tissue,

NOTE Confidence: 0.9007227

00:01:59.480 --> 00:02:01.950 the cells that are cycling

NOTE Confidence: 0.9007227

00:02:01.950 --> 00:02:03.926 I usually transient cell.

NOTE Confidence: 0.9007227

00:02:03.930 --> 00:02:07.155 So long term teacher residents

NOTE Confidence: 0.9007227

00:02:07.155 --> 00:02:09.090 like terminally differentiated

NOTE Confidence: 0.9007227

00:02:09.090 --> 00:02:11.490 cells or stem cells.

NOTE Confidence: 0.9007227

00:02:11.490 --> 00:02:12.774 Do not cycle.

NOTE Confidence: 0.9007227

00:02:12.774 --> 00:02:16.536 So what does it mean that only transient

NOTE Confidence: 0.9007227

00:02:16.536 --> 00:02:20.538 cell states are undergoing cell cycle?

NOTE Confidence: 0.9007227

00:02:20.540 --> 00:02:22.540 These cells are continuously

NOTE Confidence: 0.9007227

00:02:22.540 --> 00:02:25.540 changing the genes that they express

NOTE Confidence: 0.9007227

00:02:25.622 --> 00:02:28.484 and our work suggests that they

NOTE Confidence: 0.9007227

00:02:28.484 --> 00:02:30.392 are also continuously changing

NOTE Confidence: 0.9007227

00:02:30.473 --> 00:02:32.825 the kind of cell cycle program

NOTE Confidence: 0.9007227

00:02:32.825 --> 00:02:35.764 that is expressed by these cells.

NOTE Confidence: 0.9007227

00:02:35.764 --> 00:02:39.174 It's likely that linear development.

NOTE Confidence: 0.9007227

00:02:39.180 --> 00:02:41.428 Had really coevolved with

NOTE Confidence: 0.9007227

00:02:41.428 --> 00:02:44.238 modifications in the cell cycle,

NOTE Confidence: 0.9007227

00:02:44.240 --> 00:02:46.875 so cell cycle programs probably

NOTE Confidence: 0.9007227

00:02:46.875 --> 00:02:50.218 adapted to specific tissue and cell

NOTE Confidence: 0.9007227

00:02:50.218 --> 00:02:52.670 cycles and developmental stage,

NOTE Confidence: 0.9007227

00:02:52.670 --> 00:02:56.149 and it is possible that in order

NOTE Confidence: 0.9007227

00:02:56.149 --> 00:02:59.045 to truly understand how a

NOTE Confidence: 0.9007227

00:02:59.045 --> 00:03:01.657 developmental process is regulated,

NOTE Confidence: 0.9007227

00:03:01.660 --> 00:03:04.900 we need to understand the

NOTE Confidence: 0.9007227

00:03:04.900 --> 00:03:08.140 specifics of its cell cycle.

NOTE Confidence: 0.9007227

00:03:08.140 --> 00:03:10.605 So here we're looking at

NOTE Confidence: 0.9007227

00:03:10.605 --> 00:03:12.577 the erythroid linear edge.

NOTE Confidence: 0.9007227

00:03:12.580 --> 00:03:15.040 The erythroid developmental trajectory

NOTE Confidence: 0.9007227

00:03:15.040 --> 00:03:18.115 has two phases earlier through

NOTE Confidence: 0.9007227

00:03:18.115 --> 00:03:21.159 pieces and terminal differentiation.

NOTE Confidence: 0.9007227

00:03:21.160 --> 00:03:23.939 What I'll show you today is that

NOTE Confidence: 0.9007227

00:03:23.939 --> 00:03:26.466 the cell cycle throughout this

NOTE Confidence: 0.9007227

00:03:26.466 --> 00:03:29.591 process varies in logs lockstep

NOTE Confidence: 0.9007227

00:03:29.591 --> 00:03:32.378 with differentiation stage both in

NOTE Confidence: 0.9007227

00:03:32.378 --> 00:03:34.850 terms of the cell cycle length,

NOTE Confidence: 0.9007227

00:03:34.850 --> 00:03:38.860 which here is represented vertically.

NOTE Confidence: 0.9007227

00:03:38.860 --> 00:03:41.625 And in terms of the ratio of

NOTE Confidence: 0.9007227

00:03:41.625 --> 00:03:44.429 S phase to the gap phases.

NOTE Confidence: 0.9007227

00:03:44.430 --> 00:03:49.155 So how did we set out on this question?

NOTE Confidence: 0.9007227

00:03:49.160 --> 00:03:51.790 We started out by asking.

NOTE Confidence: 0.82816005

00:03:54.040 --> 00:03:57.010 One question and that was how is a recruit

NOTE Confidence: 0.82816005

00:03:57.010 --> 00:03:59.089 terminal differentiation activated?

NOTE Confidence: 0.82816005

00:03:59.090 --> 00:04:01.813 We know that in earlier it releases

NOTE Confidence: 0.82816005

00:04:01.813 --> 00:04:04.399 we have progenitors that are already

NOTE Confidence: 0.82816005

00:04:04.399 --> 00:04:07.087 committed to their way through drainage,

NOTE Confidence: 0.82816005

00:04:07.090 --> 00:04:10.386 but that are not expressing any of the

NOTE Confidence: 0.82816005

00:04:10.386 --> 00:04:13.408 jeans that are present in red cells.

NOTE Confidence: 0.82816005

00:04:13.410 --> 00:04:16.068 At some point these progenitors undergo

NOTE Confidence: 0.82816005

00:04:16.068 --> 00:04:18.690 a selfhacked decision that switches on

NOTE Confidence: 0.82816005

00:04:18.690 --> 00:04:21.406 this specific program of red cell genes,

NOTE Confidence: 0.82816005

00:04:21.410 --> 00:04:25.505 and so the question is how does that happen?

NOTE Confidence: 0.82816005

00:04:25.510 --> 00:04:28.312 Of course, many labs are addressing

NOTE Confidence: 0.82816005

00:04:28.312 --> 00:04:31.794 this question, and the angle that

NOTE Confidence: 0.82816005

00:04:31.794 --> 00:04:34.974 we had was to ask.

NOTE Confidence: 0.82816005

00:04:34.980 --> 00:04:37.836 Can we identify the cell in

NOTE Confidence: 0.82816005

00:04:37.836 --> 00:04:39.740 which this activation happens?

NOTE Confidence: 0.82816005

00:04:39.740 --> 00:04:44.540 Our model system is the mouse fetal liver.

NOTE Confidence: 0.82816005

00:04:44.540 --> 00:04:46.192 And and that was,

NOTE Confidence: 0.82816005

00:04:46.192 --> 00:04:49.141 we also have experiments that show you

NOTE Confidence: 0.82816005

00:04:49.141 --> 00:04:52.245 later on in the mouse adult bone marrow.

NOTE Confidence: 0.82816005

00:04:52.250 --> 00:04:54.658 The fetal liver is a great system for

NOTE Confidence: 0.82816005

00:04:54.658 --> 00:04:57.339 working in original places because over 90%

NOTE Confidence: 0.82816005

00:04:57.340 --> 00:05:00.628 of the cells of the erythroid Lenny Edge.

NOTE Confidence: 0.82816005

00:05:00.630 --> 00:05:04.356 So we have two cells of his markers cities.

NOTE Confidence: 0.82816005

00:05:04.360 --> 00:05:06.796 Have anyone until 119 with just

NOTE Confidence: 0.82816005

00:05:06.796 --> 00:05:09.692 these two markers we can divide the

NOTE Confidence: 0.82816005

00:05:09.692 --> 00:05:12.478 fetal liver into a number of subsets

NOTE Confidence: 0.82816005

00:05:12.558 --> 00:05:15.288 that form a developmental sequence.

NOTE Confidence: 0.82816005

00:05:15.290 --> 00:05:18.722 So we find that in the S node

NOTE Confidence: 0.82816005

00:05:18.722 --> 00:05:20.769 subset and S1 subset,

NOTE Confidence: 0.82816005

00:05:20.770 --> 00:05:23.055 that's where we see colony

NOTE Confidence: 0.82816005

00:05:23.055 --> 00:05:23.969 forming progenitors.

NOTE Confidence: 0.82816005

00:05:23.970 --> 00:05:27.578 So this is where we see earlier with

NOTE Confidence: 0.82816005

00:05:27.578 --> 00:05:30.830 paresis and then in subsets as 22S5,

NOTE Confidence: 0.82816005

00:05:30.830 --> 00:05:33.634 we see progressively more

NOTE Confidence: 0.82816005

00:05:33.634 --> 00:05:35.737 differentiated erythroid precursors.

NOTE Confidence: 0.82816005

00:05:35.740 --> 00:05:37.966 And you can see this also here.

NOTE Confidence: 0.82816005

00:05:37.970 --> 00:05:40.688 The colonies that are formed by the S note

NOTE Confidence: 0.82816005

00:05:40.688 --> 00:05:43.077 cells and S1 cells are pretty similar.

NOTE Confidence: 0.82816005

00:05:43.080 --> 00:05:46.616 We wouldn't be able to tell them apart.

NOTE Confidence: 0.82816005

00:05:46.620 --> 00:05:48.300 But two important findings

NOTE Confidence: 0.82816005

00:05:48.300 --> 00:05:51.200 suggested to us over 10 years ago.

NOTE Confidence: 0.82816005

00:05:51.200 --> 00:05:54.520 Now that the S not see if you,

NOTE Confidence: 0.82816005

00:05:54.520 --> 00:05:56.600 we are really quite different

NOTE Confidence: 0.82816005

00:05:56.600 --> 00:05:58.680 to the those in S1.

NOTE Confidence: 0.82816005

00:05:58.680 --> 00:06:00.966 So the first finding was when

NOTE Confidence: 0.82816005

00:06:00.966 --> 00:06:03.032 we examined the fetal livers

NOTE Confidence: 0.82816005

00:06:03.032 --> 00:06:05.337 of a preceptor knockout cells.

NOTE Confidence: 0.82816005

00:06:05.340 --> 00:06:08.668 So here we're looking at two litter mates.

NOTE Confidence: 0.82816005

00:06:08.670 --> 00:06:10.095 This was published.

NOTE Confidence: 0.82816005

00:06:10.095 --> 00:06:13.420 The knockout was first published by Home

NOTE Confidence: 0.82816005

00:06:13.502 --> 00:06:16.670 Grow in the Lodish lab back in 1995 and.

NOTE Confidence: 0.82816005

00:06:16.670 --> 00:06:20.170 When we did flow cytometry on the

NOTE Confidence: 0.82816005

00:06:20.170 --> 00:06:23.308 fetal livers of embryos from a.

NOTE Confidence: 0.82816005

00:06:23.310 --> 00:06:26.061 Some this knockout we found that whilst

NOTE Confidence: 0.82816005

00:06:26.061 --> 00:06:29.525 in the wild type number we already see

NOTE Confidence: 0.82816005

00:06:29.525 --> 00:06:32.420 cells populating most of these subsets.

NOTE Confidence: 0.82816005

00:06:32.420 --> 00:06:35.280 This is Embedded Day 12.5.

NOTE Confidence: 0.82816005

00:06:35.280 --> 00:06:36.844 In the perception knockout,

NOTE Confidence: 0.82816005

00:06:36.844 --> 00:06:39.562 we see an absolute block at the

NOTE Confidence: 0.82816005

00:06:39.562 --> 00:06:41.613 transition from S No 2 S one,

NOTE Confidence: 0.82816005

00:06:41.620 --> 00:06:44.420 so that tells us that the transition

NOTE Confidence: 0.82816005

00:06:44.420 --> 00:06:47.400 is dependent on a preceptor signaling.

NOTE Confidence: 0.82816005

00:06:47.400 --> 00:06:50.304 These few cells that you see over here

NOTE Confidence: 0.82816005

00:06:50.304 --> 00:06:53.057 that tagline positive in the IP receptor

NOTE Confidence: 0.82816005

00:06:53.057 --> 00:06:55.800 knockout belong to the yolk SAC limit,

NOTE Confidence: 0.82816005

00:06:55.800 --> 00:06:57.710 and they're not part of

NOTE Confidence: 0.82816005

00:06:57.710 --> 00:06:58.474 definitive erythropoiesis.

NOTE Confidence: 0.82816005

00:06:58.480 --> 00:07:00.598 Now the second kind of experiment

NOTE Confidence: 0.82816005

00:07:00.598 --> 00:07:03.255 that we did that told us there's

NOTE Confidence: 0.82816005

00:07:03.255 --> 00:07:05.589 something different about S1 and S

NOTE Confidence: 0.82816005

00:07:05.589 --> 00:07:08.029 Note was a cell cycle experiment,

NOTE Confidence: 0.82816005

00:07:08.030 --> 00:07:11.150 and here we have a cartoon that illustrates

NOTE Confidence: 0.82816005

00:07:11.150 --> 00:07:13.478 typical cell cycle experiment that I'll

NOTE Confidence: 0.82816005

00:07:13.478 --> 00:07:16.809 show you a number of times during my talk,

NOTE Confidence: 0.82816005

00:07:16.810 --> 00:07:17.336 so.

NOTE Confidence: 0.82816005

00:07:17.336 --> 00:07:19.440 We take a mouse.

NOTE Confidence: 0.82816005

00:07:19.440 --> 00:07:23.520 In this case it's a pregnant female and

NOTE Confidence: 0.82816005

00:07:23.520 --> 00:07:27.812 injected with beyond you and then harvest

NOTE Confidence: 0.82816005

00:07:27.812 --> 00:07:30.987 hematopoietic tissue 30 minutes later.

NOTE Confidence: 0.8135519

00:07:30.990 --> 00:07:33.378 What we find is 2 things.

NOTE Confidence: 0.8135519

00:07:33.380 --> 00:07:36.159 First, we find which cells are positive.

NOTE Confidence: 0.8135519

00:07:36.160 --> 00:07:38.150 For Bru, these cells have

NOTE Confidence: 0.8135519

00:07:38.150 --> 00:07:39.344 incorporated be audio,

NOTE Confidence: 0.8135519

00:07:39.350 --> 00:07:42.526 which is a finding analog into their DNA.

NOTE Confidence: 0.8135519

00:07:42.530 --> 00:07:45.855 So these cells are in S phase

NOTE Confidence: 0.8135519

00:07:45.855 --> 00:07:49.710 during the 30 minutes of the pulse.

NOTE Confidence: 0.8135519

00:07:49.710 --> 00:07:51.742 A second finding. Is.

NOTE Confidence: 0.8135519

00:07:51.742 --> 00:07:55.309 Allows us to determine the speed of

NOTE Confidence: 0.8135519

00:07:55.309 --> 00:07:58.270 aspects and so we can compare the

NOTE Confidence: 0.8135519

00:07:58.270 --> 00:08:01.807 level of the audio incorporation in

NOTE Confidence: 0.8135519

00:08:01.807 --> 00:08:05.047 the cells of different populations.

NOTE Confidence: 0.8135519

00:08:05.050 --> 00:08:07.876 Cells that have incorporated less beardi.

NOTE Confidence: 0.8135519

00:08:07.880 --> 00:08:09.848 You must be synthesizing.

NOTE Confidence: 0.8135519

00:08:09.848 --> 00:08:12.800 DNA slower and have a longer

NOTE Confidence: 0.8135519

00:08:12.891 --> 00:08:15.441 essays than cells that incorporate

NOTE Confidence: 0.8135519

00:08:15.441 --> 00:08:18.540 the audio at a faster pace,

NOTE Confidence: 0.8135519

00:08:18.540 --> 00:08:21.245 and we've confirmed that with

NOTE Confidence: 0.8135519

00:08:21.245 --> 00:08:23.950 direct experiments that look that

NOTE Confidence: 0.8135519

00:08:24.042 --> 00:08:26.874 use a double family impulse to

NOTE Confidence: 0.8135519

00:08:26.874 --> 00:08:29.630 Measure S phase duration directly.

NOTE Confidence: 0.8135519

00:08:29.630 --> 00:08:31.933 And so when we did this experiment

NOTE Confidence: 0.8135519

00:08:31.933 --> 00:08:34.449 in the mouse fetal liver here we're

NOTE Confidence: 0.8135519

00:08:34.449 --> 00:08:37.066 looking at cells from each of the

NOTE Confidence: 0.8135519

00:08:37.066 --> 00:08:39.579 subsets and at the cell cycle status.

NOTE Confidence: 0.8135519

00:08:39.580 --> 00:08:44.158 We found 2 interesting things first.

NOTE Confidence: 0.8135519

00:08:44.160 --> 00:08:47.358 Whilst about 60% of the cells

NOTE Confidence: 0.8135519

00:08:47.358 --> 00:08:51.550 in S nought so these cells are.

NOTE Confidence: 0.8135519

00:08:51.550 --> 00:08:53.328 In S phase, at any one time.

NOTE Confidence: 0.8135519

00:08:53.330 --> 00:08:55.990 So this is a highly replicative tissue.

NOTE Confidence: 0.8135519

00:08:55.990 --> 00:08:58.356 When we look at S1 over here,

NOTE Confidence: 0.8135519

00:08:58.360 --> 00:09:02.890 nearly all of the cells, 90% in this case.

NOTE Confidence: 0.8135519

00:09:02.890 --> 00:09:05.305 Are in S phase of the cycle.

NOTE Confidence: 0.8135519

00:09:05.310 --> 00:09:08.748 So that's interesting finding number one.

NOTE Confidence: 0.8135519

00:09:08.750 --> 00:09:11.330 And the second finding was that

NOTE Confidence: 0.8135519

00:09:11.330 --> 00:09:14.409 the speed of S phase is about

NOTE Confidence: 0.8135519

00:09:14.409 --> 00:09:16.770 50% faster in S phase.

NOTE Confidence: 0.8135519

00:09:16.770 --> 00:09:21.329 Cells that are in S1 compared with S phase

NOTE Confidence: 0.8135519

00:09:21.329 --> 00:09:24.934 cells in the preceding as node subset.

NOTE Confidence: 0.8135519

00:09:24.940 --> 00:09:27.080 And so a number of.

NOTE Confidence: 0.8135519

00:09:27.080 --> 00:09:30.192 We've done a lot of work on this

NOTE Confidence: 0.8135519

00:09:30.192 --> 00:09:33.069 and I'll give you the summary.

NOTE Confidence: 0.8135519

00:09:33.070 --> 00:09:36.689 The way that we've interpreted our data.

NOTE Confidence: 0.8135519

00:09:36.690 --> 00:09:40.461 Is that the transition from S note to S1

NOTE Confidence: 0.8135519

00:09:40.461 --> 00:09:44.403 can only happen in S phase of the cycle.

NOTE Confidence: 0.8135519

00:09:44.410 --> 00:09:47.338 And this is based on a number of

NOTE Confidence: 0.8135519

00:09:47.338 --> 00:09:50.046 experiments where we have arrested as space,

NOTE Confidence: 0.8135519

00:09:50.050 --> 00:09:51.925 either genetically or using drugs

NOTE Confidence: 0.8135519

00:09:51.925 --> 00:09:53.425 that inhibit DNA polymerases.

NOTE Confidence: 0.8135519

00:09:53.430 --> 00:09:55.310 And when we do that,

NOTE Confidence: 0.8135519

00:09:55.310 --> 00:09:57.560 we totally prevent the transition

NOTE Confidence: 0.8135519

00:09:57.560 --> 00:09:59.810 from S note to S1.

NOTE Confidence: 0.8135519

00:09:59.810 --> 00:10:00.170 And.

NOTE Confidence: 0.8135519

00:10:00.170 --> 00:10:02.330 What we prevent isn't simply the

NOTE Confidence: 0.8135519

00:10:02.330 --> 00:10:04.098 upregulating I population of city 71,

NOTE Confidence: 0.8135519

00:10:04.100 --> 00:10:06.424 which is the marker of this transition,

NOTE Confidence: 0.8135519

00:10:06.430 --> 00:10:08.422 but all of the events that

NOTE Confidence: 0.8135519

00:10:08.422 --> 00:10:09.750 are associated with it,

NOTE Confidence: 0.8135519

00:10:09.750 --> 00:10:12.648 which is the induction activation of

NOTE Confidence: 0.8135519

00:10:12.648 --> 00:10:14.580 the array through transcriptional.

NOTE Confidence: 0.8135519

00:10:14.580 --> 00:10:16.988 Program their research differentiation

NOTE Confidence: 0.8135519

00:10:16.988 --> 00:10:17.590 program.

NOTE Confidence: 0.8739649

00:10:19.750 --> 00:10:22.526 The second thing that we found and I

NOTE Confidence: 0.8739649

00:10:22.526 --> 00:10:25.409 will talk about that a bit more later,

NOTE Confidence: 0.8739649

00:10:25.410 --> 00:10:28.641 running the talk is that S phase at the

NOTE Confidence: 0.8739649

00:10:28.641 --> 00:10:31.534 time of this transition is much shorter

NOTE Confidence: 0.8739649

00:10:31.534 --> 00:10:34.639 than S phase of preceding cycles.

NOTE Confidence: 0.8739649

00:10:34.640 --> 00:10:39.661 OK. And that the actual length of S

NOTE Confidence: 0.8739649

00:10:39.661 --> 00:10:43.249 phase is really only about four hours,

NOTE Confidence: 0.8739649

00:10:43.250 --> 00:10:47.597 which is pretty short for MA million S phase.

NOTE Confidence: 0.83275175

00:10:49.690 --> 00:10:52.280 Now what else is happening at the

NOTE Confidence: 0.83275175

00:10:52.280 --> 00:10:54.954 time of this suite at the time

NOTE Confidence: 0.83275175

00:10:54.954 --> 00:10:57.500 of transition from S note to S1,

NOTE Confidence: 0.83275175

00:10:57.500 --> 00:10:59.796 it turns out that there is a time

NOTE Confidence: 0.83275175

00:10:59.796 --> 00:11:01.814 when we see reconfiguration of

NOTE Confidence: 0.83275175

00:11:01.814 --> 00:11:04.562 chromating at the beta globin locus.

NOTE Confidence: 0.83275175

00:11:04.570 --> 00:11:07.315 We see a change in the timing of replication

NOTE Confidence: 0.83275175

00:11:07.315 --> 00:11:10.149 of the locals histone tail modifications.

NOTE Confidence: 0.83275175

00:11:10.150 --> 00:11:13.158 We see a loss in DNA methylation beginning

NOTE Confidence: 0.83275175

00:11:13.158 --> 00:11:16.294 at the time of this switch and more

NOTE Confidence: 0.83275175

00:11:16.294 --> 00:11:19.287 recent work from Dark Hexes Lab done by.

NOTE Confidence: 0.83275175

00:11:19.290 --> 00:11:22.212 Rob Bakery and others have shown

NOTE Confidence: 0.83275175

00:11:22.212 --> 00:11:25.728 that eight acsec and using tiled see,

NOTE Confidence: 0.83275175

00:11:25.730 --> 00:11:28.820 we see either changes in chromatin

NOTE Confidence: 0.83275175

00:11:28.820 --> 00:11:30.880 Accessibility promoter enhancer Contacts

NOTE Confidence: 0.83275175

00:11:30.951 --> 00:11:33.146 that begin with this transition,

NOTE Confidence: 0.83275175

00:11:33.150 --> 00:11:36.120 so this is clearly a key

NOTE Confidence: 0.83275175

00:11:36.120 --> 00:11:37.110 developmental switch.

NOTE Confidence: 0.81380343

00:11:40.210 --> 00:11:43.899 I haven't explained a color scheme here.

NOTE Confidence: 0.81380343

00:11:43.900 --> 00:11:47.320 What we think is happening is

NOTE Confidence: 0.81380343

00:11:47.320 --> 00:11:50.324 that CF uer undergoing expansion

NOTE Confidence: 0.81380343

00:11:50.324 --> 00:11:53.990 whilst in the S North subset.

NOTE Confidence: 0.81380343

00:11:53.990 --> 00:11:58.142 And then the very last generation of CFUE

NOTE Confidence: 0.81380343

00:11:58.142 --> 00:12:02.476 starts its life in the S note subset in G1.

NOTE Confidence: 0.81380343

00:12:02.480 --> 00:12:05.798 When it enters this specialized short space,

NOTE Confidence: 0.81380343

00:12:05.800 --> 00:12:09.229 it is at it up. Regulates city 71.

NOTE Confidence: 0.81380343

00:12:09.229 --> 00:12:11.700 And it is at that point that

NOTE Confidence: 0.81380343

00:12:11.783 --> 00:12:13.589 it undergoes commitment.

NOTE Confidence: 0.81380343

00:12:13.590 --> 00:12:14.962 The progeny of DCF.

NOTE Confidence: 0.81380343

00:12:14.962 --> 00:12:17.330 UE will no longer be safe UE.

NOTE Confidence: 0.81380343

00:12:17.330 --> 00:12:19.160 They will be priority through

NOTE Confidence: 0.81380343

00:12:19.160 --> 00:12:22.324 blast in the race for blast that

NOTE Confidence: 0.81380343

00:12:22.324 --> 00:12:24.217 undergo terminal differentiation.

NOTE Confidence: 0.81380343

00:12:24.220 --> 00:12:27.100 So when we asked next is is this

NOTE Confidence: 0.81380343

00:12:27.100 --> 00:12:30.168 CFU E2E TD transition true sweet?

NOTE Confidence: 0.81380343

00:12:30.170 --> 00:12:33.354 So it's all very well and good if

NOTE Confidence: 0.81380343

00:12:33.354 --> 00:12:36.896 we take all of us not cells and all

NOTE Confidence: 0.81380343

00:12:36.896 --> 00:12:40.368 of this one sells and compare them,

NOTE Confidence: 0.81380343

00:12:40.370 --> 00:12:42.680 large differences suggest to switch but

NOTE Confidence: 0.81380343

00:12:42.680 --> 00:12:45.188 is not is a heterogeneous population

NOTE Confidence: 0.81380343

00:12:45.188 --> 00:12:48.748 of cells and by taking all of them

NOTE Confidence: 0.81380343

00:12:48.831 --> 00:12:52.089 together we could be masking incremental

NOTE Confidence: 0.81380343

00:12:52.089 --> 00:12:54.261 changes within this population.

NOTE Confidence: 0.81380343

00:12:54.270 --> 00:12:57.320 The problem with addressing this

NOTE Confidence: 0.81380343

00:12:57.320 --> 00:13:01.560 question was that we really had no

NOTE Confidence: 0.81380343

00:13:01.560 --> 00:13:05.354 reliable way of taking apart this subset.

NOTE Confidence: 0.81380343

00:13:05.360 --> 00:13:07.256 And in fact,

NOTE Confidence: 0.81380343

00:13:07.256 --> 00:13:09.784 the entire trajectory starting

NOTE Confidence: 0.81380343

00:13:09.784 --> 00:13:12.434 with hematopoietic stem cells

NOTE Confidence: 0.81380343

00:13:12.434 --> 00:13:16.340 and ending at this point where

NOTE Confidence: 0.81380343

00:13:16.340 --> 00:13:18.293 terminal differentiation starts,

NOTE Confidence: 0.81380343

00:13:18.300 --> 00:13:20.888 this trajectory was really

NOTE Confidence: 0.81380343

00:13:20.888 --> 00:13:22.829 only partially understood,

NOTE Confidence: 0.81380343

00:13:22.830 --> 00:13:26.883 and so about four years ago the

NOTE Confidence: 0.81380343

00:13:26.883 --> 00:13:31.637 technology of single cell RNA SEQ had

NOTE Confidence: 0.81380343

00:13:31.637 --> 00:13:34.533 advanced massively by microfluidic.

NOTE Confidence: 0.81380343

00:13:34.540 --> 00:13:35.884 Approaches were introduced by

NOTE Confidence: 0.81380343

00:13:35.884 --> 00:13:37.228 a number of labs,

NOTE Confidence: 0.81380343

00:13:37.230 --> 00:13:40.310 including the lab of a long climb.

NOTE Confidence: 0.81380343

00:13:40.310 --> 00:13:42.476 And we were very fortunate in

NOTE Confidence: 0.81380343

00:13:42.476 --> 00:13:44.944 that he agreed to collaborate with

NOTE Confidence: 0.81380343

00:13:44.944 --> 00:13:46.836 us on this question.

NOTE Confidence: 0.81380343

00:13:46.840 --> 00:13:49.206 And so we took kit positive cells

NOTE Confidence: 0.81380343

00:13:49.206 --> 00:13:51.652 from the bone marrow or from fetal

NOTE Confidence: 0.81380343

00:13:51.652 --> 00:13:54.142 liver and we also took kid positive

NOTE Confidence: 0.81380343

00:13:54.142 --> 00:13:56.816 cells from the bone marrow of mice

NOTE Confidence: 0.81380343

00:13:56.816 --> 00:13:58.948 that were injected with Ipoh.

NOTE Confidence: 0.81380343

00:13:58.948 --> 00:14:02.140 Although I will not discuss that here.

NOTE Confidence: 0.81380343

00:14:02.140 --> 00:14:05.452 And we've undertaken single cell RNA

NOTE Confidence: 0.81380343

00:14:05.452 --> 00:14:08.187 sequencing on these projectors and

NOTE Confidence: 0.81380343

00:14:08.187 --> 00:14:11.246 what you're looking at here are two

NOTE Confidence: 0.81380343

00:14:11.246 --> 00:14:13.985 D projections of K nearest neighbor

NOTE Confidence: 0.81380343

00:14:13.985 --> 00:14:16.655 graphs of gene expression in the

NOTE Confidence: 0.81380343

00:14:16.660 --> 00:14:21.000 fetal liver and in the bone marrow.

NOTE Confidence: 0.81380343

00:14:21.000 --> 00:14:23.988 Topologically, these graphs are very similar.

NOTE Confidence: 0.81380343

00:14:23.990 --> 00:14:26.978 Each dot is a single cell,

NOTE Confidence: 0.81380343

00:14:26.980 --> 00:14:29.578 and the proximity of dots suggests

NOTE Confidence: 0.81380343

00:14:29.578 --> 00:14:32.933 a proximity in terms of their

NOTE Confidence: 0.81380343

00:14:32.933 --> 00:14:35.246 transcriptome similarity transcriptomes.

NOTE Confidence: 0.81380343

00:14:35.250 --> 00:14:37.812 And what you can see is that

NOTE Confidence: 0.81380343

00:14:37.812 --> 00:14:39.503 these transcriptomes are form

NOTE Confidence: 0.81380343

00:14:39.503 --> 00:14:41.057 one continuous structure.

NOTE Confidence: 0.81380343

00:14:41.060 --> 00:14:42.736 It's a branching structure.

NOTE Confidence: 0.81380343

00:14:42.736 --> 00:14:46.269 And in the fetal liver and bone marrow,

NOTE Confidence: 0.81380343

00:14:46.270 --> 00:14:49.420 the branches are pretty similar.

NOTE Confidence: 0.81380343

00:14:49.420 --> 00:14:51.688 Except that here we see a very

NOTE Confidence: 0.81380343

00:14:51.688 --> 00:14:53.678 large bulge compared with a much

NOTE Confidence: 0.81380343

00:14:53.678 --> 00:14:55.263 smaller bulge in the marrow.

NOTE Confidence: 0.81380343

00:14:55.270 --> 00:14:57.545 This bulge in fact contains the CFUE,

NOTE Confidence: 0.81380343

00:14:57.550 --> 00:14:59.170 as I will show you.

NOTE Confidence: 0.8530163

00:15:01.880 --> 00:15:06.250 I won't dwell on it today, but.

NOTE Confidence: 0.8530163

00:15:06.250 --> 00:15:09.148 We've used GNU algorithm that was

NOTE Confidence: 0.8530163

00:15:09.148 --> 00:15:12.100 developed by the client laboratory,

NOTE Confidence: 0.8530163

00:15:12.100 --> 00:15:14.605 especially Caleb Weinreb and some

NOTE Confidence: 0.8530163

00:15:14.605 --> 00:15:17.947 Wallach in the client lab called

NOTE Confidence: 0.8530163

00:15:17.947 --> 00:15:20.179 Population Balance analysis.

NOTE Confidence: 0.8530163

00:15:20.180 --> 00:15:22.410 And this algorithm allowed us

NOTE Confidence: 0.8530163

00:15:22.410 --> 00:15:25.246 to assign each cell within this

NOTE Confidence: 0.8530163

00:15:25.246 --> 00:15:27.836 structure with a fate probability.

NOTE Confidence: 0.8530163

00:15:27.840 --> 00:15:31.193 In fact, with a set of seven

NOTE Confidence: 0.8530163

00:15:31.193 --> 00:15:32.630 self check probabilities,

NOTE Confidence: 0.8530163

00:15:32.630 --> 00:15:36.291 which told us what is the probability

NOTE Confidence: 0.8530163

00:15:36.291 --> 00:15:40.479 of each cell to ultimately attain a cell

NOTE Confidence: 0.8530163

00:15:40.479 --> 00:15:45.610 fate in one of these seven branches and that?

NOTE Confidence: 0.8530163

00:15:45.610 --> 00:15:48.274 Really can be the result of our analysis

NOTE Confidence: 0.8530163

00:15:48.274 --> 00:15:51.137 can be collapsed into this structure,

NOTE Confidence: 0.8530163

00:15:51.140 --> 00:15:53.120 which is a hierarchical structure,

NOTE Confidence: 0.8530163

00:15:53.120 --> 00:15:54.696 not unlike the classical

NOTE Confidence: 0.8530163

00:15:54.696 --> 00:15:55.878 structure of hematopoiesis.

NOTE Confidence: 0.8530163

00:15:55.880 --> 00:15:57.855 The main difference is that

NOTE Confidence: 0.8530163

00:15:57.855 --> 00:15:59.830 we don't see discrete stages.

NOTE Confidence: 0.8530163

00:15:59.830 --> 00:16:03.094 We see a continuum which is meant to

NOTE Confidence: 0.8530163

00:16:03.094 --> 00:16:05.757 be represented by this kind of cloud.

NOTE Confidence: 0.80456686

00:16:08.910 --> 00:16:11.678 And one more point that I'd like to

NOTE Confidence: 0.80456686

00:16:11.678 --> 00:16:13.976 make is subsequent subsequent work

NOTE Confidence: 0.80456686

00:16:13.976 --> 00:16:17.525 of the client lab together with Luca

NOTE Confidence: 0.80456686

00:16:17.611 --> 00:16:20.481 Biosca Slab looking at single cell RNA

NOTE Confidence: 0.80456686

00:16:20.481 --> 00:16:23.620 seq of human marrow showed that the

NOTE Confidence: 0.80456686

00:16:23.620 --> 00:16:25.995 structure and topology is obtained

NOTE Confidence: 0.80456686

00:16:25.995 --> 00:16:28.989 from human bone marrow is actually very

NOTE Confidence: 0.80456686

00:16:28.989 --> 00:16:32.227 similar to that of the mouse and in

NOTE Confidence: 0.80456686

00:16:32.227 --> 00:16:36.460 terms of gene expression for each gene.

NOTE Confidence: 0.80456686

00:16:36.460 --> 00:16:37.522 Steam is aro.

NOTE Confidence: 0.80456686

00:16:37.522 --> 00:16:39.646 We see a very similar pattern

NOTE Confidence: 0.80456686

00:16:39.646 --> 00:16:42.156 in the mouse and in the human,

NOTE Confidence: 0.80456686

00:16:42.160 --> 00:16:45.975 which are represented here as mirror images.

NOTE Confidence: 0.80456686

00:16:45.980 --> 00:16:48.199 And so we can conclude that the

NOTE Confidence: 0.80456686

00:16:48.199 --> 00:16:51.227 mouse is a pretty good model for

NOTE Confidence: 0.80456686

00:16:51.227 --> 00:16:53.227 human hematopoiesis in general.

NOTE Confidence: 0.80456686

00:16:53.230 --> 00:16:56.128 Of course, that we know of some

NOTE Confidence: 0.80456686

00:16:56.128 --> 00:16:58.069 very clear differences as well.

NOTE Confidence: 0.80456686

00:16:58.070 --> 00:17:00.582 So now we were in a position to

NOTE Confidence: 0.80456686

00:17:00.582 --> 00:17:03.308 look at the erythroid trajectory,

NOTE Confidence: 0.80456686

00:17:03.310 --> 00:17:05.054 starting with multipotential progenitors

NOTE Confidence: 0.80456686

00:17:05.054 --> 00:17:07.670 in black and continuing along the

NOTE Confidence: 0.80456686

00:17:07.736 --> 00:17:10.034 array thread branch with the color

NOTE Confidence: 0.80456686

00:17:10.034 --> 00:17:12.340 representing every thread fate probability.

NOTE Confidence: 0.80456686

00:17:12.340 --> 00:17:15.777 We can use this probability to align

NOTE Confidence: 0.80456686

00:17:15.777 --> 00:17:19.280 the cells along a linear axis starting

NOTE Confidence: 0.80456686

00:17:19.280 --> 00:17:23.308 with MPP and ending with the end of ETD.

NOTE Confidence: 0.80456686

00:17:23.310 --> 00:17:28.670 And just to see that things look good.

NOTE Confidence: 0.80456686

00:17:28.670 --> 00:17:31.829 You can look at cells that we know our

NOTE Confidence: 0.80456686

00:17:31.829 --> 00:17:33.296 President Multipotential progenitor's

NOTE Confidence: 0.80456686

00:17:33.296 --> 00:17:36.929 like cities 34 gotta one which is

NOTE Confidence: 0.80456686

00:17:36.929 --> 00:17:39.580 expressed by the entire array thread

NOTE Confidence: 0.80456686

00:17:39.580 --> 00:17:43.118 branch and Alpha globin which is induced.

NOTE Confidence: 0.80456686

00:17:43.118 --> 00:17:46.838 Only with the activation of

NOTE Confidence: 0.80456686

00:17:46.838 --> 00:17:49.070 Arethra terminal differentiation.

NOTE Confidence: 0.80456686

00:17:49.070 --> 00:17:52.798 In order to be able to do experiments

NOTE Confidence: 0.80456686

00:17:52.798 --> 00:17:54.717 with transcriptome information we

NOTE Confidence: 0.80456686

00:17:54.717 --> 00:17:57.860 needed a way of learning how to

NOTE Confidence: 0.80456686

00:17:57.860 --> 00:18:00.197 isolate cells from specific regions

NOTE Confidence: 0.80456686

00:18:00.197 --> 00:18:03.342 of our single cell RNA SEQ graph.

NOTE Confidence: 0.80456686

00:18:03.342 --> 00:18:06.436 And so we've developed a fax approach

NOTE Confidence: 0.80456686

00:18:06.436 --> 00:18:09.497 that gives us five populations and then

NOTE Confidence: 0.80456686

00:18:09.497 --> 00:18:13.040 we sorted each of these five populations,

NOTE Confidence: 0.80456686

00:18:13.040 --> 00:18:16.267 repeated the single cell RNA SEQ work,

NOTE Confidence: 0.80456686

00:18:16.270 --> 00:18:19.096 and then projected them onto our.

NOTE Confidence: 0.80456686

00:18:19.100 --> 00:18:22.173 Original map and what you can see

NOTE Confidence: 0.80456686

00:18:22.173 --> 00:18:24.862 and what's relevant to today's talk

NOTE Confidence: 0.80456686

00:18:24.862 --> 00:18:27.872 is that the P1 and P2 subpopulations

NOTE Confidence: 0.80456686

00:18:27.962 --> 00:18:30.277 project pretty cleanly into this

NOTE Confidence: 0.80456686

00:18:30.277 --> 00:18:33.141 very narrow neck at the beginning

NOTE Confidence: 0.80456686

00:18:33.141 --> 00:18:36.928 of the urethra branch and then into

NOTE Confidence: 0.80456686

00:18:36.928 --> 00:18:40.570 this sort of bulge that follows up.

NOTE Confidence: 0.80456686

00:18:40.570 --> 00:18:43.702 And so we now have a way of isolating

NOTE Confidence: 0.80456686

00:18:43.702 --> 00:18:45.937 cells that correspond to these

NOTE Confidence: 0.80456686

00:18:45.937 --> 00:18:48.187 two regions of the graph.

NOTE Confidence: 0.80456686

00:18:48.190 --> 00:18:51.067 And the P5 population is also pretty

NOTE Confidence: 0.80456686

00:18:51.067 --> 00:18:53.932 good at giving us the multipotential

NOTE Confidence: 0.80456686

00:18:53.932 --> 00:18:56.512 progenitor cells right at the

NOTE Confidence: 0.80456686

00:18:56.512 --> 00:18:59.318 beginning of the erythroid branch.

NOTE Confidence: 0.80456686

00:18:59.320 --> 00:19:01.740 So with these three subpopulations,

NOTE Confidence: 0.80456686

00:19:01.740 --> 00:19:06.458 we can isolate cells from the entire.

NOTE Confidence: 0.80456686

00:19:06.460 --> 00:19:12.494 If we throw brunch and so now we're ready to

NOTE Confidence: 0.80456686

00:19:12.494 --> 00:19:17.739 do some assays and we find that P1 and P2.

NOTE Confidence: 0.80456686

00:19:17.740 --> 00:19:19.870 Almost my stroke of luck.

NOTE Confidence: 0.80456686

00:19:19.870 --> 00:19:25.455 Kivas populations at the highly enriched

NOTE Confidence: 0.80456686

00:19:25.455 --> 00:19:28.690 for CFUE. In fact, P1 contains.

NOTE Confidence: 0.80456686

00:19:28.690 --> 00:19:32.336 Almost all of the CFO is some

NOTE Confidence: 0.80456686

00:19:32.336 --> 00:19:35.744 small number also present in P2.

NOTE Confidence: 0.80456686

00:19:35.750 --> 00:19:39.530 And P2 is the only subpopulation that

NOTE Confidence: 0.80456686

00:19:39.530 --> 00:19:43.850 contains BFUE, so P2 is from the neck.

NOTE Confidence: 0.80456686

00:19:43.850 --> 00:19:46.550 Here represents cells with be

NOTE Confidence: 0.80456686

00:19:46.550 --> 00:19:48.170 a few potential.

NOTE Confidence: 0.80456686

00:19:48.170 --> 00:19:52.566 BF uer colonies that are multifocal either.

NOTE Confidence: 0.80456686

00:19:52.570 --> 00:19:54.450 Small bunches of small foci.

NOTE Confidence: 0.80456686

00:19:54.450 --> 00:19:57.274 Deezer called late be a few E and

NOTE Confidence: 0.80456686

00:19:57.274 --> 00:20:00.186 we see them around day four of.

NOTE Confidence: 0.80456686

00:20:00.190 --> 00:20:03.094 Culture or like might contain much

NOTE Confidence: 0.80456686

00:20:03.094 --> 00:20:06.032 larger column they might have might

NOTE Confidence: 0.80456686

00:20:06.032 --> 00:20:08.786 give rise to much larger colonies

NOTE Confidence: 0.80456686

00:20:08.786 --> 00:20:09.704 after about

NOTE Confidence: 0.789408

00:20:09.792 --> 00:20:11.676 a week or even 10 days.

NOTE Confidence: 0.789408

00:20:11.680 --> 00:20:15.216 See if you eat, give rise to 1.

NOTE Confidence: 0.789408

00:20:15.220 --> 00:20:17.430 Focus of colonies that contain

NOTE Confidence: 0.789408

00:20:17.430 --> 00:20:19.640 around 30 differentiated red cells,

NOTE Confidence: 0.789408

00:20:19.640 --> 00:20:24.057 about two to three days after plating.

NOTE Confidence: 0.789408

00:20:24.060 --> 00:20:25.890 OK, so we now have.

NOTE Confidence: 0.8126656

00:20:28.350 --> 00:20:30.865 Pretty complete set of tools

NOTE Confidence: 0.8126656

00:20:30.865 --> 00:20:32.877 to do our investigation.

NOTE Confidence: 0.8126656

00:20:32.880 --> 00:20:35.690 We can look at multipotential

NOTE Confidence: 0.8126656

00:20:35.690 --> 00:20:37.938 progenitors BFUS&CFUS at the

NOTE Confidence: 0.8126656

00:20:37.938 --> 00:20:40.763 transcriptome levels and we've given

NOTE Confidence: 0.8126656

00:20:40.763 --> 00:20:42.947 them transcriptome related names.

NOTE Confidence: 0.8126656

00:20:42.950 --> 00:20:45.704 You can correlate them with faith

NOTE Confidence: 0.8126656

00:20:45.704 --> 00:20:48.190 assays and isolate them by fax.

NOTE Confidence: 0.8126656

00:20:48.190 --> 00:20:51.925 And so we are in a position to ask,

NOTE Confidence: 0.8126656

00:20:51.930 --> 00:20:55.682 are we looking at a true switch when

NOTE Confidence: 0.8126656

00:20:55.682 --> 00:20:58.586 we transition from S Note 2 S one?

NOTE Confidence: 0.8126656

00:20:58.590 --> 00:21:01.502 Is this a few ITA ET transition

NOTE Confidence: 0.8126656

00:21:01.502 --> 00:21:02.750 at truth Switch?

NOTE Confidence: 0.8126656

00:21:02.750 --> 00:21:04.906 And so here we are looking at

NOTE Confidence: 0.8126656

00:21:04.906 --> 00:21:06.489 genes that are differentially

NOTE Confidence: 0.8126656

00:21:06.489 --> 00:21:09.409 expressed during the linear access.

NOTE Confidence: 0.8126656

00:21:09.410 --> 00:21:12.469 The linear suit I'm going from MPP

NOTE Confidence: 0.8126656

00:21:12.469 --> 00:21:14.211 to terminal differentiation and

NOTE Confidence: 0.8126656

00:21:14.211 --> 00:21:16.365 they arrange arrange story in terms

NOTE Confidence: 0.8126656

00:21:16.365 --> 00:21:19.070 of the peak expression along this.

NOTE Confidence: 0.8126656

00:21:19.070 --> 00:21:21.245 Access and without any really

NOTE Confidence: 0.8126656

00:21:21.245 --> 00:21:23.898 fancy analysis you could see that

NOTE Confidence: 0.8126656

00:21:23.898 --> 00:21:26.280 they form kind of three cohorts.

NOTE Confidence: 0.8126656

00:21:26.280 --> 00:21:29.297 There is a cohort of gene expression

NOTE Confidence: 0.8126656

00:21:29.297 --> 00:21:32.210 that happens during a very rapid change.

NOTE Confidence: 0.8126656

00:21:32.210 --> 00:21:35.610 Many streams are being switched on or off.

NOTE Confidence: 0.8126656

00:21:35.610 --> 00:21:38.935 Then we enter a period of relative

NOTE Confidence: 0.8126656

00:21:38.935 --> 00:21:42.134 stability of the ceep progenitors of

NOTE Confidence: 0.8126656

00:21:42.134 --> 00:21:46.047 correspond functionality to see a few E.

NOTE Confidence: 0.8126656

00:21:46.050 --> 00:21:48.768 Jeans and not many genes of

NOTE Confidence: 0.8126656

00:21:48.768 --> 00:21:50.580 turning on or off.

NOTE Confidence: 0.8126656

00:21:50.580 --> 00:21:52.794 Although you can see that there

NOTE Confidence: 0.8126656

00:21:52.794 --> 00:21:54.830 is a progressive slow change

NOTE Confidence: 0.8126656

00:21:54.830 --> 00:21:57.265 and this probably represents an

NOTE Confidence: 0.8126656

00:21:57.265 --> 00:21:59.213 amplification stage where there

NOTE Confidence: 0.8126656

00:21:59.290 --> 00:22:01.450 is little transcriptome change.

NOTE Confidence: 0.8126656

00:22:01.450 --> 00:22:04.650 And then we see.

NOTE Confidence: 0.8126656

00:22:04.650 --> 00:22:07.402 Rapid change or in fact I should say

NOTE Confidence: 0.8126656

00:22:07.402 --> 00:22:10.717 about sharp change from the CFD program

NOTE Confidence: 0.8126656

00:22:10.717 --> 00:22:12.757 to terminal differentiation program.

NOTE Confidence: 0.8126656

00:22:12.760 --> 00:22:14.945 Very few cells expressed genes

NOTE Confidence: 0.8126656

00:22:14.945 --> 00:22:16.693 of both terminal differentiation

NOTE Confidence: 0.8126656

00:22:16.693 --> 00:22:18.310 and sea Fury program.

NOTE Confidence: 0.8126656

00:22:18.310 --> 00:22:20.669 So that tells us that we're looking

NOTE Confidence: 0.8126656

00:22:20.669 --> 00:22:23.440 at a sharp transcriptional switch.

NOTE Confidence: 0.8126656

00:22:23.440 --> 00:22:25.570 So the answer is yes,

NOTE Confidence: 0.8126656

00:22:25.570 --> 00:22:27.600 the transition from this Cepheus

NOTE Confidence: 0.8126656

00:22:27.600 --> 00:22:29.224 Stage 2 terminal differentiation

NOTE Confidence: 0.8126656

00:22:29.224 --> 00:22:31.548 is a sharp transcriptional switch.

NOTE Confidence: 0.8126656

00:22:31.550 --> 00:22:35.886 So what is the context of that switch?

NOTE Confidence: 0.8126656

00:22:35.890 --> 00:22:37.852 And we're in the position to

NOTE Confidence: 0.8126656

00:22:37.852 --> 00:22:39.610 look at that as well.

NOTE Confidence: 0.8126656

00:22:39.610 --> 00:22:41.300 This is a busy slide,

NOTE Confidence: 0.8126656

00:22:41.300 --> 00:22:43.996 but let me take you through it slowly.

NOTE Confidence: 0.8126656

00:22:44.000 --> 00:22:46.415 So if we look at the medial

NOTE Confidence: 0.8126656

00:22:46.415 --> 00:22:48.060 medium panel first in red,

NOTE Confidence: 0.8126656

00:22:48.060 --> 00:22:50.756 we're looking at the expression of CD 71.

NOTE Confidence: 0.8126656

00:22:50.760 --> 00:22:52.664 This is the market we used by

NOTE Confidence: 0.8126656

00:22:52.664 --> 00:22:55.254 fax and we can see that there is

NOTE Confidence: 0.8126656

00:22:55.254 --> 00:22:57.465 a gradual increase in cities of

NOTE Confidence: 0.8126656

00:22:57.465 --> 00:22:59.209 71 throughout the trajectory.

NOTE Confidence: 0.8126656

00:22:59.210 --> 00:23:01.576 But at the time of the switch

NOTE Confidence: 0.8126656

00:23:01.576 --> 00:23:02.590 to terminal differentiation,

NOTE Confidence: 0.8126656

00:23:02.590 --> 00:23:04.956 this becomes a very rapid up regulation,

NOTE Confidence: 0.8126656

00:23:04.960 --> 00:23:07.536 and so the upregulation of CD 71.

NOTE Confidence: 0.8126656

00:23:07.540 --> 00:23:10.330 Which we previously took as a

NOTE Confidence: 0.8126656

00:23:10.330 --> 00:23:13.250 marker of activation of the switch.

NOTE Confidence: 0.8126656

00:23:13.250 --> 00:23:14.966 Acts as a marker of that,

NOTE Confidence: 0.8126656

00:23:14.970 --> 00:23:18.449 also at the single cell RNA level.

NOTE Confidence: 0.8126656

00:23:18.450 --> 00:23:21.124 When we look at either cells of

NOTE Confidence: 0.8126656

00:23:21.124 --> 00:23:23.841 his markers here we're looking at

NOTE Confidence: 0.8126656

00:23:23.841 --> 00:23:26.326 Ipoh receptor expression in blue.

NOTE Confidence: 0.8126656

00:23:26.330 --> 00:23:28.505 EPO receptor is expressed pretty

NOTE Confidence: 0.8126656

00:23:28.505 --> 00:23:31.425 early on in the trajectory in

NOTE Confidence: 0.8126656

00:23:31.425 --> 00:23:33.417 increases gradually subsequently.

NOTE Confidence: 0.8126656

00:23:33.420 --> 00:23:35.988 We can now look at transcription

NOTE Confidence: 0.8126656

00:23:35.988 --> 00:23:39.366 factors and we see in Grey gotta one

NOTE Confidence: 0.8126656

00:23:39.366 --> 00:23:43.000 is high initially and then is downregulated.

NOTE Confidence: 0.8126656

00:23:43.000 --> 00:23:46.787 Gotta sorry got it too in grey.

NOTE Confidence: 0.8126656

00:23:46.790 --> 00:23:49.508 Gotta one is low initially in

NOTE Confidence: 0.8126656

00:23:49.508 --> 00:23:51.854 multipotential parameters and then is

NOTE Confidence: 0.8126656

00:23:51.854 --> 00:23:54.584 expressed induced early in the trajectory.

NOTE Confidence: 0.8126656

00:23:54.590 --> 00:23:57.635 I'm sorry Ann is maintained at pretty

NOTE Confidence: 0.8126656

00:23:57.635 --> 00:24:00.100 high levels throughout the trajectory.

NOTE Confidence: 0.8126656

00:24:00.100 --> 00:24:02.668 Maybe going up a little bit

NOTE Confidence: 0.8126656

00:24:02.668 --> 00:24:05.610 at the time of the switch.

NOTE Confidence: 0.8359557

00:24:05.610 --> 00:24:06.838 Generally speaking,

NOTE Confidence: 0.8359557

00:24:06.838 --> 00:24:09.908 none of the key transcriptional

NOTE Confidence: 0.8359557

00:24:09.908 --> 00:24:12.549 regulators that we know of.

NOTE Confidence: 0.8359557

00:24:12.550 --> 00:24:15.655 Reporters of the timing of

NOTE Confidence: 0.8359557

00:24:15.655 --> 00:24:19.390 the switch from CFUE to ETD.

NOTE Confidence: 0.8359557

00:24:19.390 --> 00:24:21.385 When we look at the cell cycle,

NOTE Confidence: 0.8359557

00:24:21.390 --> 00:24:23.090 however, we see something that

NOTE Confidence: 0.8359557

00:24:23.090 --> 00:24:25.148 does seem to correlate very well

NOTE Confidence: 0.8359557

00:24:25.148 --> 00:24:26.906 with the timing of the switch.

NOTE Confidence: 0.8359557

00:24:26.910 --> 00:24:29.976 So what we're looking at here each

NOTE Confidence: 0.8359557

00:24:29.976 --> 00:24:32.419 each color denotes the average

NOTE Confidence: 0.8359557

00:24:32.419 --> 00:24:36.066 expression of genes in each that are

NOTE Confidence: 0.8359557

00:24:36.066 --> 00:24:38.737 characteristic of each cell cycle phase,

NOTE Confidence: 0.8359557

00:24:38.740 --> 00:24:40.628 either S Phase G,

NOTE Confidence: 0.8359557

00:24:40.628 --> 00:24:42.516 2MG1S and so on,

NOTE Confidence: 0.8359557

00:24:42.520 --> 00:24:45.824 and there is very little difference there.

NOTE Confidence: 0.8359557

00:24:45.830 --> 00:24:47.722 Sensually, flat or uninteresting

NOTE Confidence: 0.8359557

00:24:47.722 --> 00:24:50.087 for most of the trajectory.

NOTE Confidence: 0.8359557

00:24:50.090 --> 00:24:53.120 And this really tells us that

NOTE Confidence: 0.8359557

00:24:53.120 --> 00:24:55.140 cells are cycling asynchronously

NOTE Confidence: 0.8359557

00:24:55.231 --> 00:24:57.756 through most of the trajectory.

NOTE Confidence: 0.8359557

00:24:57.760 --> 00:25:01.252 But as we approach the time of the switch,

NOTE Confidence: 0.8359557

00:25:01.260 --> 00:25:03.210 which is this dashed line,

NOTE Confidence: 0.8359557

00:25:03.210 --> 00:25:05.751 you can see the formation of a

NOTE Confidence: 0.8359557

00:25:05.751 --> 00:25:08.260 number of peaks starting with G1,

NOTE Confidence: 0.8359557

00:25:08.260 --> 00:25:11.100 S and then S in red and orange

NOTE Confidence: 0.8359557

00:25:11.100 --> 00:25:12.540 and then G2NG2M.

NOTE Confidence: 0.8359557

00:25:12.540 --> 00:25:15.634 So what we have here in fact

NOTE Confidence: 0.8359557

00:25:15.634 --> 00:25:17.740 is the cell cycle.

NOTE Confidence: 0.8359557

00:25:17.740 --> 00:25:23.160 And the first peak that is formed is S phase.

NOTE Confidence: 0.8359557

00:25:23.160 --> 00:25:26.984 And so it appears that the earliest event

NOTE Confidence: 0.8359557

00:25:26.984 --> 00:25:31.250 at the Switch from Seaview to ET D is

NOTE Confidence: 0.8359557

00:25:31.250 --> 00:25:35.116 marked by cells in S phase of the cycle.

NOTE Confidence: 0.8359557

00:25:35.120 --> 00:25:38.340 So this confirms our earlier functional data.

NOTE Confidence: 0.8359557

00:25:38.340 --> 00:25:41.850 That activation of the TD happens

NOTE Confidence: 0.8359557

00:25:41.850 --> 00:25:45.460 during S phase of the cycle.

NOTE Confidence: 0.8359557

00:25:45.460 --> 00:25:48.916 And So what we asked next is OK.

NOTE Confidence: 0.8359557

00:25:48.920 --> 00:25:51.810 Transcription factors are not good.

NOTE Confidence: 0.8359557

00:25:51.810 --> 00:25:53.370 Taught don't really report

NOTE Confidence: 0.8359557

00:25:53.370 --> 00:25:55.320 the timing of this switch.

NOTE Confidence: 0.8359557

00:25:55.320 --> 00:25:57.270 At least their expression doesn't.

NOTE Confidence: 0.8359557

00:25:57.270 --> 00:25:59.540 It's quite possible that post

NOTE Confidence: 0.8359557

00:25:59.540 --> 00:26:00.902 transcriptional post translational

NOTE Confidence: 0.8359557

00:26:00.902 --> 00:26:02.806 modifications of these do correlate

NOTE Confidence: 0.8359557

00:26:02.806 --> 00:26:04.672 with the timing of this switch,

NOTE Confidence: 0.8359557

00:26:04.680 --> 00:26:07.350 and that's an open question.

NOTE Confidence: 0.8359557

00:26:07.350 --> 00:26:08.970 Um?

NOTE Confidence: 0.8359557

00:26:08.970 --> 00:26:11.535 Can we find something else

NOTE Confidence: 0.8359557

00:26:11.535 --> 00:26:13.587 that might tell us?

NOTE Confidence: 0.8359557

00:26:13.590 --> 00:26:16.628 Something about the timing of the switch.

NOTE Confidence: 0.8359557

00:26:16.630 --> 00:26:19.843 So to do that we went back to the

NOTE Confidence: 0.8359557

00:26:19.843 --> 00:26:23.138 jeans that are expressed during this.

NOTE Confidence: 0.8359557

00:26:23.140 --> 00:26:26.100 See if you E program and we asked

NOTE Confidence: 0.8359557

00:26:26.100 --> 00:26:28.884 whether there are the slow progressive

NOTE Confidence: 0.8359557

00:26:28.884 --> 00:26:31.818 change that happens during this time,

NOTE Confidence: 0.8359557

00:26:31.820 --> 00:26:34.858 which streams are changing during that time?

NOTE Confidence: 0.8359557

00:26:34.860 --> 00:26:36.940 Which teams change their expression

NOTE Confidence: 0.8359557

00:26:36.940 --> 00:26:40.174 in a way that is correlated with

NOTE Confidence: 0.8359557

00:26:40.174 --> 00:26:42.859 progression along suit of time?

NOTE Confidence: 0.8359557

00:26:42.860 --> 00:26:48.400 And when we did that, the top five go.

NOTE Confidence: 0.8359557

00:26:48.400 --> 00:26:50.992 Terms that we got were essentially

NOTE Confidence: 0.8359557

00:26:50.992 --> 00:26:54.229 all to do with DNA replication.

NOTE Confidence: 0.8359557

00:26:54.230 --> 00:26:58.118 The cell cycle, S phase, and so on.

NOTE Confidence: 0.8359557

00:26:58.118 --> 00:27:00.548 And here are some examples.

NOTE Confidence: 0.8359557

00:27:00.550 --> 00:27:03.158 We're looking at cycling

NOTE Confidence: 0.8359557

00:27:03.158 --> 00:27:06.418 8 two cycling E1 R&R.

NOTE Confidence: 0.8359557

00:27:06.420 --> 00:27:06.921 Units.

NOTE Confidence: 0.8359557

00:27:06.921 --> 00:27:08.925 Proteins that are associated

NOTE Confidence: 0.8359557

00:27:08.925 --> 00:27:11.430 with the origin of replication.

NOTE Confidence: 0.8359557

00:27:11.430 --> 00:27:14.503 All of these are ramping up their

NOTE Confidence: 0.8359557

00:27:14.503 --> 00:27:16.351 expression throughout the trajectory

NOTE Confidence: 0.8359557

00:27:16.351 --> 00:27:18.586 right through the BFUE&CFU stage

NOTE Confidence: 0.8359557

00:27:18.586 --> 00:27:22.312 and reach a peak at the time of the

NOTE Confidence: 0.8359557

00:27:22.312 --> 00:27:26.840 transcription of switch from CF UE2ET D.

NOTE Confidence: 0.8359557

00:27:26.840 --> 00:27:30.968 Now what does that mean functionally?

NOTE Confidence: 0.8359557

00:27:30.970 --> 00:27:33.682 So to understand the significance of

NOTE Confidence: 0.8359557

00:27:33.682 --> 00:27:35.933 this really quite impressive ramping

NOTE Confidence: 0.8359557

00:27:35.933 --> 00:27:38.544 up in cell cycle X gene expression,

NOTE Confidence: 0.8359557

00:27:38.550 --> 00:27:41.497 we went back to a functional experiment.

NOTE Confidence: 0.8359557

00:27:41.500 --> 00:27:45.042 So here we're looking at the same

NOTE Confidence: 0.8359557

00:27:45.042 --> 00:27:48.031 old experiment where we take a

NOTE Confidence: 0.8359557

00:27:48.031 --> 00:27:50.326 mouse injected with beyond you

NOTE Confidence: 0.8359557

00:27:50.326 --> 00:27:53.252 and then check the cell cycle

NOTE Confidence: 0.8359557

00:27:53.252 --> 00:27:56.120 status and the speed of space.

NOTE Confidence: 0.8359557

00:27:56.120 --> 00:27:59.296 But now we were armed with some more

NOTE Confidence: 0.8359557

00:27:59.296 --> 00:28:01.709 information about early erythropoiesis.

NOTE Confidence: 0.8395177

00:28:01.710 --> 00:28:05.574 We used the slow upregulation of CD 71

NOTE Confidence: 0.8395177

00:28:05.574 --> 00:28:10.479 as a way of measuring sudo time by fax.

NOTE Confidence: 0.8395177

00:28:10.480 --> 00:28:14.290 And we were able to also.

NOTE Confidence: 0.8395177

00:28:14.290 --> 00:28:17.674 Staying for the P1 and P2

NOTE Confidence: 0.8395177

00:28:17.674 --> 00:28:19.930 subpopulations which mark there

NOTE Confidence: 0.8395177

00:28:20.031 --> 00:28:23.097 be a few Ian CF Louise subsets.

NOTE Confidence: 0.8395177

00:28:23.100 --> 00:28:25.612 And so we sort of these cells and

NOTE Confidence: 0.8395177

00:28:25.612 --> 00:28:28.192 looked at the beardi you incorporation

NOTE Confidence: 0.8395177

00:28:28.192 --> 00:28:31.000 in here we're looking at individual

NOTE Confidence: 0.8395177

00:28:31.076 --> 00:28:33.710 cells Bru positive and Bru negative.

NOTE Confidence: 0.8395177

00:28:33.710 --> 00:28:36.993 We arrange them along the CD 71

NOTE Confidence: 0.8395177

00:28:36.993 --> 00:28:40.370 expression suit of time and if I did

NOTE Confidence: 0.8395177

00:28:40.370 --> 00:28:43.520 this sort of time into 14 different

NOTE Confidence: 0.8395177

00:28:43.520 --> 00:28:46.465 Gates 7 seven percentile gates.

NOTE Confidence: 0.8395177

00:28:46.470 --> 00:28:49.566 And then we can look at each of

NOTE Confidence: 0.8395177

00:28:49.566 --> 00:28:52.162 these gates and analyze cell cycle

NOTE Confidence: 0.8395177

00:28:52.162 --> 00:28:55.390 status as well as S phase speed.

NOTE Confidence: 0.8395177

00:28:55.390 --> 00:28:57.916 So the first really quite clear.

NOTE Confidence: 0.7805582

00:29:00.680 --> 00:29:02.865 Finding is that cells in

NOTE Confidence: 0.7805582

00:29:02.865 --> 00:29:05.050 S phase of this cycle.

NOTE Confidence: 0.7805582

00:29:05.050 --> 00:29:06.454 Increased markedly with progression

NOTE Confidence: 0.7805582

00:29:06.454 --> 00:29:08.560 along the earlier we throw trajectory,

NOTE Confidence: 0.7805582

00:29:08.560 --> 00:29:11.008 so maybe 20% of this other INS phase

NOTE Confidence: 0.7805582

00:29:11.008 --> 00:29:13.829 at the early parts of the trajectory,

NOTE Confidence: 0.7805582

00:29:13.830 --> 00:29:15.580 and as we approach this,

NOTE Confidence: 0.7805582

00:29:15.580 --> 00:29:18.002 which essentially all the cells are in

NOTE Confidence: 0.7805582

00:29:18.002 --> 00:29:20.846 space and you could see this right here.

NOTE Confidence: 0.7805582

00:29:20.850 --> 00:29:24.180 So at the time of this which nearly all the

NOTE Confidence: 0.7805582

00:29:24.257 --> 00:29:27.506 cells are in a space where is around here,

NOTE Confidence: 0.7805582

00:29:27.510 --> 00:29:30.948 most of the cells are not.

NOTE Confidence: 0.7805582

00:29:30.950 --> 00:29:33.715 When we look at S phase speed,

NOTE Confidence: 0.7805582

00:29:33.720 --> 00:29:35.690 we see there is there.

NOTE Confidence: 0.7805582

00:29:35.690 --> 00:29:37.230 There is an increment.

NOTE Confidence: 0.7805582

00:29:37.230 --> 00:29:39.540 There is an increase in the

NOTE Confidence: 0.7805582

00:29:39.618 --> 00:29:41.618 speed of West phase early,

NOTE Confidence: 0.7805582

00:29:41.620 --> 00:29:44.406 but then it stays quite stable until

NOTE Confidence: 0.7805582

00:29:44.406 --> 00:29:47.150 the point of the switch to ATD.

NOTE Confidence: 0.7805582

00:29:47.150 --> 00:29:50.310 And so this quite stable speed of essays.

NOTE Confidence: 0.7805582

00:29:50.310 --> 00:29:51.711 In other words,

NOTE Confidence: 0.7805582

00:29:51.711 --> 00:29:54.046 quite stable S faced length

NOTE Confidence: 0.7805582

00:29:54.046 --> 00:29:56.238 can't explain the change in

NOTE Confidence: 0.7805582

00:29:56.238 --> 00:29:58.584 the number of cells in space.

NOTE Confidence: 0.7805582

00:29:58.590 --> 00:30:01.214 And so our interpretation.

NOTE Confidence: 0.7805582

00:30:01.214 --> 00:30:04.494 Is that what's happening is?

NOTE Confidence: 0.7805582

00:30:04.500 --> 00:30:08.124 Shortening in G and in the G1 phase.

NOTE Confidence: 0.7805582

00:30:08.130 --> 00:30:08.906 Of course,

NOTE Confidence: 0.7805582

00:30:08.906 --> 00:30:10.846 this massive increase in the

NOTE Confidence: 0.7805582

00:30:10.846 --> 00:30:13.535 number of S phase cells explains

NOTE Confidence: 0.7805582

00:30:13.535 --> 00:30:16.415 why S phase genes are increased

NOTE Confidence: 0.7805582

00:30:16.415 --> 00:30:18.120 throughout the trajectory.

NOTE Confidence: 0.84984

00:30:20.630 --> 00:30:22.946 And we suspect that the reason

NOTE Confidence: 0.84984

00:30:22.946 --> 00:30:25.530 for that is G1 shortening,

NOTE Confidence: 0.84984

00:30:25.530 --> 00:30:28.372 and so as a proportion the number

NOTE Confidence: 0.84984

00:30:28.372 --> 00:30:31.310 of cells in S phase increases.

NOTE Confidence: 0.84984

00:30:31.310 --> 00:30:34.326 The later we are in the Seaview stage

NOTE Confidence: 0.84984

00:30:34.326 --> 00:30:37.539 as we approach the actual switch.

NOTE Confidence: 0.84984

00:30:37.540 --> 00:30:39.772 G1 is pretty short.

NOTE Confidence: 0.84984

00:30:39.772 --> 00:30:43.120 The switch itself we think involves

NOTE Confidence: 0.84984

00:30:43.230 --> 00:30:47.550 S phase shortening. So.

NOTE Confidence: 0.84984

00:30:47.550 --> 00:30:51.030 To summarize what I've told you so far.

NOTE Confidence: 0.84984

00:30:51.030 --> 00:30:54.450 We use single cell RNA sequencing

NOTE Confidence: 0.84984

00:30:54.450 --> 00:30:56.730 to identify the erythroid

NOTE Confidence: 0.84984

00:30:56.828 --> 00:31:00.498 developmental trajectory in the mouse.

NOTE Confidence: 0.84984

00:31:00.500 --> 00:31:03.896 We were able to match specific

NOTE Confidence: 0.84984

00:31:03.896 --> 00:31:07.633 stages that are identified based on

NOTE Confidence: 0.84984

00:31:07.633 --> 00:31:10.373 transcriptomes to fax populations

NOTE Confidence: 0.84984

00:31:10.373 --> 00:31:13.113 and to functional subsets.

NOTE Confidence: 0.84984

00:31:13.120 --> 00:31:15.912 Functional progenitors based on

NOTE Confidence: 0.84984

00:31:15.912 --> 00:31:18.704 confirmation potential and using

NOTE Confidence: 0.84984

00:31:18.704 --> 00:31:22.037 these tools we began to analyze.

NOTE Confidence: 0.84984

00:31:22.040 --> 00:31:23.592 The factors that control

NOTE Confidence: 0.84984

00:31:23.592 --> 00:31:25.920 the switch from CFUE to ET.

NOTE Confidence: 0.84984

00:31:25.920 --> 00:31:29.028 We found that this is a

NOTE Confidence: 0.84984

00:31:29.028 --> 00:31:30.582 true transcriptional switch.

NOTE Confidence: 0.84984

00:31:30.590 --> 00:31:35.310 And that there is no real clear change

NOTE Confidence: 0.84984

00:31:35.310 --> 00:31:38.502 in transcription factor levels that

NOTE Confidence: 0.84984

00:31:38.502 --> 00:31:42.294 reports the timing of this switch.

NOTE Confidence: 0.84984

00:31:42.300 --> 00:31:45.660 By contrast, we do see really quite

NOTE Confidence: 0.84984

00:31:45.660 --> 00:31:48.550 marked changes in the cell cycle.

NOTE Confidence: 0.84984

00:31:48.550 --> 00:31:54.230 Initially we see a gradual shortening in G1.

NOTE Confidence: 0.84984

00:31:54.230 --> 00:31:57.070 And at the time of the switch we

NOTE Confidence: 0.84984

00:31:57.070 --> 00:32:00.676 see a further shortening in S phase.

NOTE Confidence: 0.84984

00:32:00.680 --> 00:32:03.088 So that at the time of this which

NOTE Confidence: 0.84984

00:32:03.088 --> 00:32:05.526 we have a very short cell cycle,

NOTE Confidence: 0.84984

00:32:05.530 --> 00:32:07.125 our measurements indicate that this

NOTE Confidence: 0.84984

00:32:07.125 --> 00:32:09.399 cell cycle is about 6 hours long,

NOTE Confidence: 0.84984

00:32:09.400 --> 00:32:13.194 with S phase being only four hours.

NOTE Confidence: 0.84984

00:32:13.200 --> 00:32:13.837 So.

NOTE Confidence: 0.84984

00:32:13.837 --> 00:32:18.933 What we next asked his first of all,

NOTE Confidence: 0.84984

00:32:18.940 --> 00:32:21.250 what regulates this really quite

NOTE Confidence: 0.84984

00:32:21.250 --> 00:32:24.070 dramatic remodeling of the cell cycle?

NOTE Confidence: 0.84984

00:32:24.070 --> 00:32:26.410 And the second question is,

NOTE Confidence: 0.84984

00:32:26.410 --> 00:32:28.740 is this cell cycle remodeling

NOTE Confidence: 0.84984

00:32:28.740 --> 00:32:30.604 relevant to linear development?

NOTE Confidence: 0.84984

00:32:30.610 --> 00:32:33.536 Does it play a role in these

NOTE Confidence: 0.84984

00:32:33.536 --> 00:32:35.750 important cell fate decisions?

NOTE Confidence: 0.84984

00:32:35.750 --> 00:32:39.478 For example, this switch from CFUE to ETD.

NOTE Confidence: 0.84984

00:32:39.480 --> 00:32:44.808 Is it correlate or does it determine it?

NOTE Confidence: 0.84984

00:32:44.810 --> 00:32:46.738 And so to begin to look at that.

NOTE Confidence: 0.885927

00:32:49.280 --> 00:32:51.776 Just before I get to that,

NOTE Confidence: 0.885927

00:32:51.780 --> 00:32:55.272 I just wanted to show you some of the

NOTE Confidence: 0.885927

00:32:55.272 --> 00:32:57.881 expression of cell cycle regulators

NOTE Confidence: 0.885927

00:32:57.881 --> 00:33:00.551 during the original trajectory and

NOTE Confidence: 0.885927

00:33:00.551 --> 00:33:03.270 what's quite interesting is that.

NOTE Confidence: 0.885927

00:33:03.270 --> 00:33:05.646 Our different shifting shape of the

NOTE Confidence: 0.885927

00:33:05.646 --> 00:33:07.865 cell cycle is probably regulated

NOTE Confidence: 0.885927

00:33:07.865 --> 00:33:10.655 through changing cell cycle regulators.

NOTE Confidence: 0.885927

00:33:10.660 --> 00:33:13.831 And of course we have no idea

NOTE Confidence: 0.885927

00:33:13.831 --> 00:33:16.668 how that happens at this point,

NOTE Confidence: 0.885927

00:33:16.670 --> 00:33:19.436 but we know that for example,

NOTE Confidence: 0.885927

00:33:19.440 --> 00:33:22.224 the dominant E2F four transcription factor

NOTE Confidence: 0.885927

00:33:22.224 --> 00:33:25.907 during most of the trajectory is E2F four,

NOTE Confidence: 0.885927

00:33:25.910 --> 00:33:29.592 but at the time of the switch

NOTE Confidence: 0.885927

00:33:29.592 --> 00:33:33.060 to ETD it becomes E2F2.

NOTE Confidence: 0.885927

00:33:33.060 --> 00:33:35.180 Other regulators, for example,

NOTE Confidence: 0.885927

00:33:35.180 --> 00:33:39.918 when we look at the cycling dies cycling D2,

NOTE Confidence: 0.885927

00:33:39.920 --> 00:33:41.872 is present early on.

NOTE Confidence: 0.885927

00:33:41.872 --> 00:33:43.824 But it is gradually

NOTE Confidence: 0.885927

00:33:43.824 --> 00:33:45.530 downregulated whilst cycling.

NOTE Confidence: 0.885927

00:33:45.530 --> 00:33:49.436 D3 takes over at the time of this switch

NOTE Confidence: 0.885927

00:33:49.436 --> 00:33:53.210 so it is quite possible that these.

NOTE Confidence: 0.885927

00:33:53.210 --> 00:33:54.503 Part of them.

NOTE Confidence: 0.885927

00:33:54.503 --> 00:33:59.649 So how do I that is able to generate these

NOTE Confidence: 0.885927

00:33:59.649 --> 00:34:04.339 quite different shapes of the cell cycle?

NOTE Confidence: 0.885927

00:34:04.340 --> 00:34:07.098 So the first thing that we asked

NOTE Confidence: 0.885927

00:34:07.098 --> 00:34:10.084 is what are the mechanisms that

NOTE Confidence: 0.885927

00:34:10.084 --> 00:34:12.939 underlie this very short space?

NOTE Confidence: 0.885927

00:34:12.940 --> 00:34:14.980 And there are two ways of

NOTE Confidence: 0.885927

00:34:14.980 --> 00:34:16.340 getting a short space.

NOTE Confidence: 0.885927

00:34:16.340 --> 00:34:19.329 One is to have more origins of

NOTE Confidence: 0.885927

00:34:19.329 --> 00:34:21.554 replication and the other is

NOTE Confidence: 0.885927

00:34:21.554 --> 00:34:24.116 to have folks that move faster.

NOTE Confidence: 0.885927

00:34:24.120 --> 00:34:27.108 We know from model organisms that

NOTE Confidence: 0.885927

00:34:27.108 --> 00:34:29.720 have extremely short as phase.

NOTE Confidence: 0.885927

00:34:29.720 --> 00:34:32.933 Is that the way they manage to

NOTE Confidence: 0.885927

00:34:32.933 --> 00:34:35.592 replicate the genome very fast is

NOTE Confidence: 0.885927

00:34:35.592 --> 00:34:38.364 by having all of the origins of

NOTE Confidence: 0.885927

00:34:38.457 --> 00:34:41.430 replication firing synchronously,

NOTE Confidence: 0.885927

00:34:41.430 --> 00:34:44.478 and they're all very closely spaced.

NOTE Confidence: 0.885927

00:34:44.480 --> 00:34:47.918 So having efficient firing of origins

NOTE Confidence: 0.885927

00:34:47.918 --> 00:34:51.250 of replication would clearly be one

NOTE Confidence: 0.885927

00:34:51.250 --> 00:34:53.610 mechanism that has a president.

NOTE Confidence: 0.885927

00:34:53.610 --> 00:34:57.327 At the time that we were doing this work,

NOTE Confidence: 0.885927

00:34:57.330 --> 00:34:59.320 we started to collaborate with

NOTE Confidence: 0.885927

00:34:59.320 --> 00:35:01.870 Nick Rind at UMass Medical School.

NOTE Confidence: 0.885927

00:35:01.870 --> 00:35:04.348 Who is studies replication in yeast?

NOTE Confidence: 0.885927

00:35:04.350 --> 00:35:07.646 And we through discussion with him for that.

NOTE Confidence: 0.885927

00:35:07.650 --> 00:35:10.555 That is likely we will find because

NOTE Confidence: 0.885927

00:35:10.555 --> 00:35:14.321 there was really no precedent for folks

NOTE Confidence: 0.885927

00:35:14.321 --> 00:35:16.757 speed being regulated physiologically.

NOTE Confidence: 0.885927

00:35:16.760 --> 00:35:19.316 But so to approach this question,

NOTE Confidence: 0.885927

00:35:19.320 --> 00:35:21.450 we learned from the rind.

NOTE Confidence: 0.885927

00:35:21.450 --> 00:35:24.000 Leiber technique called DNA combing here.

NOTE Confidence: 0.885927

00:35:24.000 --> 00:35:25.278 You take cells,

NOTE Confidence: 0.885927

00:35:25.278 --> 00:35:27.408 pulse them with finding analogs.

NOTE Confidence: 0.885927

00:35:27.410 --> 00:35:29.858 We pass them with two distinct

NOTE Confidence: 0.885927

00:35:29.858 --> 00:35:32.420 finding analogs that we could stay

NOTE Confidence: 0.885927

00:35:32.420 --> 00:35:34.958 in with two different colors during

NOTE Confidence: 0.885927

00:35:34.958 --> 00:35:37.629 the pulse with bio deoxy uridine,

NOTE Confidence: 0.885927

00:35:37.630 --> 00:35:42.208 we were, we are able to follow.

NOTE Confidence: 0.885927

00:35:42.210 --> 00:35:45.540 Into a incorporation of Iodo.

NOTE Confidence: 0.885927

00:35:45.540 --> 00:35:47.163 The that by.

NOTE Confidence: 0.885927

00:35:47.163 --> 00:35:49.868 Later on stretching DNA fibers

NOTE Confidence: 0.885927

00:35:49.868 --> 00:35:52.339 along a cover sleep,

NOTE Confidence: 0.885927

00:35:52.340 --> 00:35:56.524 the green regions are the regions that were

NOTE Confidence: 0.885927

00:35:56.524 --> 00:36:00.010 replicating at the time of the iota pals.

NOTE Confidence: 0.885927

00:36:00.010 --> 00:36:01.864 Then we followed that 10 minutes

NOTE Confidence: 0.885927

00:36:01.864 --> 00:36:03.790 later with a pass of chloro,

NOTE Confidence: 0.885927

00:36:03.790 --> 00:36:04.192 uh.

NOTE Confidence: 0.885927

00:36:04.192 --> 00:36:07.006 Deoxy uridine and here we see the

NOTE Confidence: 0.885927

00:36:07.006 --> 00:36:09.863 red regions are being labeled that

NOTE Confidence: 0.885927

00:36:09.863 --> 00:36:12.815 this is where Clara gets incorporated

NOTE Confidence: 0.885927

00:36:12.902 --> 00:36:15.654 and by doing that we get both the

NOTE Confidence: 0.885927

00:36:15.654 --> 00:36:18.340 speed of the fork and its direction.

NOTE Confidence: 0.885927

00:36:18.340 --> 00:36:21.076 Ality because we know that green

NOTE Confidence: 0.885927

00:36:21.076 --> 00:36:22.900 replication proceeds read replication

NOTE Confidence: 0.885927

00:36:22.968 --> 00:36:25.504 and with that we can place the origins.

NOTE Confidence: 0.885927

00:36:25.510 --> 00:36:28.296 So here we have a fork moving

NOTE Confidence: 0.885927

00:36:28.296 --> 00:36:29.490 in One Direction,

NOTE Confidence: 0.885927

00:36:29.490 --> 00:36:31.665 another fork in another service

NOTE Confidence: 0.885927

00:36:31.665 --> 00:36:33.840 must be a replication bubble

NOTE Confidence: 0.885927

00:36:33.921 --> 00:36:35.806 with origin in the center.

NOTE Confidence: 0.885927

00:36:35.810 --> 00:36:37.702 And with this approach.

NOTE Confidence: 0.885927

00:36:37.702 --> 00:36:41.560 We discovered it was actually no difference.

NOTE Confidence: 0.885927

00:36:41.560 --> 00:36:43.965 No significant difference in the

NOTE Confidence: 0.885927

00:36:43.965 --> 00:36:46.370 distance between origins of replication,

NOTE Confidence: 0.8463134

00:36:46.370 --> 00:36:48.860 but there was a marked difference

NOTE Confidence: 0.8463134

00:36:48.860 --> 00:36:52.139 in the speed of replication Forks.

NOTE Confidence: 0.8463134

00:36:52.140 --> 00:36:55.325 So on average we're looking

NOTE Confidence: 0.8463134

00:36:55.325 --> 00:36:58.510 globally in the genome here.

NOTE Confidence: 0.8463134

00:36:58.510 --> 00:37:01.470 The replication folks in S1

NOTE Confidence: 0.8463134

00:37:01.470 --> 00:37:05.029 move at 50% faster speed than

NOTE Confidence: 0.8463134

00:37:05.029 --> 00:37:07.994 replication folks in S note,

NOTE Confidence: 0.8463134

00:37:08.000 --> 00:37:12.110 and that really entirely accounts for

NOTE Confidence: 0.8463134

00:37:12.110 --> 00:37:16.690 this speed of four S8 shortening.

NOTE Confidence: 0.8463134

00:37:16.690 --> 00:37:19.434 And So what might be regulating that?

NOTE Confidence: 0.8463134

00:37:19.440 --> 00:37:22.584 And is it really relevant to self it?

NOTE Confidence: 0.8463134

00:37:22.590 --> 00:37:25.726 Well, the answer is still not fully clear,

NOTE Confidence: 0.8463134

00:37:25.730 --> 00:37:27.776 but we know of examples where

NOTE Confidence: 0.8463134

00:37:27.776 --> 00:37:29.678 esfe shortening is at least

NOTE Confidence: 0.8463134

00:37:29.678 --> 00:37:32.018 associated with cell fate decisions.

NOTE Confidence: 0.8463134

00:37:32.020 --> 00:37:33.980 Here we're looking at gastrulation,

NOTE Confidence: 0.8463134

00:37:33.980 --> 00:37:36.878 in mammals where we know that

NOTE Confidence: 0.8463134

00:37:36.878 --> 00:37:39.348 there is dramatic space shortening

NOTE Confidence: 0.8463134

00:37:39.348 --> 00:37:42.456 from 7 hours to 2 1/2 hours.

NOTE Confidence: 0.8463134

00:37:42.460 --> 00:37:45.240 We know more recent examples

NOTE Confidence: 0.8463134

00:37:45.240 --> 00:37:48.020 running Yale from Shank Xingguo,

NOTE Confidence: 0.8463134

00:37:48.020 --> 00:37:49.688 where ultrafast cycles.

NOTE Confidence: 0.8364228

00:37:51.710 --> 00:37:54.888 Mark the cells that are most likely

NOTE Confidence: 0.8364228

00:37:54.888 --> 00:37:57.738 two week program into I PS cells,

NOTE Confidence: 0.8364228

00:37:57.740 --> 00:38:00.757 so this is clearly worth following up.

NOTE Confidence: 0.8364228

00:38:00.760 --> 00:38:03.940 So what might be regulating

NOTE Confidence: 0.8364228

00:38:03.940 --> 00:38:05.848 as they shortening?

NOTE Confidence: 0.8364228

00:38:05.850 --> 00:38:09.986 We looked at one key regulator P 57.

NOTE Confidence: 0.8364228

00:38:09.990 --> 00:38:15.678 Keep two. This is a CD K inhibitor.

NOTE Confidence: 0.8364228

00:38:15.680 --> 00:38:18.284 An IT regulates all it inhibits all

NOTE Confidence: 0.8364228

00:38:18.284 --> 00:38:21.856 city case, except I think City K 6.

NOTE Confidence: 0.8364228

00:38:21.860 --> 00:38:25.388 Um? What we found is it that

NOTE Confidence: 0.8364228

00:38:25.388 --> 00:38:28.820 it is present in S, not cells,

NOTE Confidence: 0.8364228

00:38:28.820 --> 00:38:32.420 but is rapidly downregulated in S1.

NOTE Confidence: 0.8364228

00:38:32.420 --> 00:38:34.928 And here you can see that,

NOTE Confidence: 0.8364228

00:38:34.930 --> 00:38:37.185 unusually, it's expressing S phase

NOTE Confidence: 0.8364228

00:38:37.185 --> 00:38:39.960 of those cells rather than in G1,

NOTE Confidence: 0.8364228

00:38:39.960 --> 00:38:42.000 suggesting it play some kind

NOTE Confidence: 0.8364228

00:38:42.000 --> 00:38:44.570 of role in S phase here.

NOTE Confidence: 0.8364228

00:38:44.570 --> 00:38:47.496 Looking at our Western and at Q.

NOTE Confidence: 0.8364228

00:38:47.500 --> 00:38:50.440 PCR, which both indicated the same thing,

NOTE Confidence: 0.8364228

00:38:50.440 --> 00:38:53.366 and so we looked at the P57.

NOTE Confidence: 0.8364228

00:38:53.370 --> 00:38:55.042 Keep two deficient embryos

NOTE Confidence: 0.8364228

00:38:55.042 --> 00:38:57.132 is in the imprinted gene,

NOTE Confidence: 0.8364228

00:38:57.140 --> 00:39:00.972 so we can look at the heterozygous and

NOTE Confidence: 0.8364228

00:39:00.972 --> 00:39:03.850 have essentially a knockout phenotype.

NOTE Confidence: 0.8364228

00:39:03.850 --> 00:39:07.186 And we see that these mice are anemic.

NOTE Confidence: 0.8364228

00:39:07.190 --> 00:39:09.698 They die in the prenatal stage.

NOTE Confidence: 0.8364228

00:39:09.700 --> 00:39:11.790 I have multiple developmental abnormalities.

NOTE Confidence: 0.8364228

00:39:11.790 --> 00:39:14.298 We found that they were anemic.

NOTE Confidence: 0.8364228

00:39:14.300 --> 00:39:16.736 They had fewer cells in the fetal

NOTE Confidence: 0.8364228

00:39:16.736 --> 00:39:20.032 liver and when we looked at the

NOTE Confidence: 0.8364228

00:39:20.032 --> 00:39:22.208 differentiation they had trouble

NOTE Confidence: 0.8364228

00:39:22.208 --> 00:39:24.048 generating differentiated and we

NOTE Confidence: 0.8364228

00:39:24.048 --> 00:39:26.414 throw blasts from the early CF you.

NOTE Confidence: 0.8364228

00:39:26.420 --> 00:39:29.976 So what's going on here Weekly looked

NOTE Confidence: 0.8364228

00:39:29.976 --> 00:39:33.037 thought to look at the S phase.

NOTE Confidence: 0.8364228

00:39:33.040 --> 00:39:35.230 So here we're looking at S

NOTE Confidence: 0.8364228

00:39:35.230 --> 00:39:36.690 phase again in CFUE.

NOTE Confidence: 0.8364228

00:39:36.690 --> 00:39:39.090 It is maintained as along as

NOTE Confidence: 0.8364228

00:39:39.090 --> 00:39:41.689 phase and we in wild type.

NOTE Confidence: 0.8364228

00:39:41.690 --> 00:39:44.210 Projectors see shortening at the

NOTE Confidence: 0.8364228

00:39:44.210 --> 00:39:47.729 time of the switch from C FE2ET D.

NOTE Confidence: 0.8364228

00:39:47.730 --> 00:39:49.992 And here we're looking at RC71

NOTE Confidence: 0.8364228

00:39:49.992 --> 00:39:52.928 so that I'm in the wild type

NOTE Confidence: 0.8364228

00:39:52.928 --> 00:39:55.153 and in knockout littermate and

NOTE Confidence: 0.8364228

00:39:55.153 --> 00:39:58.799 what you can see is that S phase

NOTE Confidence: 0.8364228

00:39:58.799 --> 00:40:00.579 speed is consistently faster.

NOTE Confidence: 0.8364228

00:40:00.580 --> 00:40:03.210 Prematurely fast in the CF,

NOTE Confidence: 0.8364228

00:40:03.210 --> 00:40:06.360 UE of the P57 knockout embryos,

NOTE Confidence: 0.8364228

00:40:06.360 --> 00:40:10.560 so we have a premature shortening of space.

NOTE Confidence: 0.8180986

00:40:12.590 --> 00:40:14.450 When we did DNA combing,

NOTE Confidence: 0.8180986

00:40:14.450 --> 00:40:17.236 we found that the folks were moving

NOTE Confidence: 0.8180986

00:40:17.236 --> 00:40:20.068 faster in their knockout in S not

NOTE Confidence: 0.8180986

00:40:20.068 --> 00:40:22.408 fork speed was already almost as

NOTE Confidence: 0.8180986

00:40:22.488 --> 00:40:24.984 fast as it would be in the wild

NOTE Confidence: 0.8180986

00:40:24.984 --> 00:40:28.060 types of in the water plasma cells.

NOTE Confidence: 0.8180986

00:40:28.060 --> 00:40:31.534 And in the X one of the P57 deficient

NOTE Confidence: 0.8180986

00:40:31.534 --> 00:40:33.769 embryo S1 cells will break,

NOTE Confidence: 0.8180986

00:40:33.770 --> 00:40:35.402 virtually breaking the speed

NOTE Confidence: 0.8180986

00:40:35.402 --> 00:40:37.034 limit on fork speed,

NOTE Confidence: 0.8180986

00:40:37.040 --> 00:40:39.889 and so we have very fast folks.

NOTE Confidence: 0.8180986

00:40:39.890 --> 00:40:44.126 What's the significance of that functionally?

NOTE Confidence: 0.8180986

00:40:44.130 --> 00:40:45.390 Anemia is 1,

NOTE Confidence: 0.8180986

00:40:45.390 --> 00:40:48.330 but why exactly do we get anemia?

NOTE Confidence: 0.8180986

00:40:48.330 --> 00:40:51.431 We didn't find that out until we

NOTE Confidence: 0.8180986

00:40:51.431 --> 00:40:55.330 looked at CF self renewal in the dish.

NOTE Confidence: 0.8180986

00:40:55.330 --> 00:40:59.738 So it turns out that CF UE can

NOTE Confidence: 0.8180986

00:40:59.738 --> 00:41:03.847 undergo self renewal in the dish

NOTE Confidence: 0.8180986

00:41:03.847 --> 00:41:08.155 for quite prolonged periods of time.

NOTE Confidence: 0.8180986

00:41:08.160 --> 00:41:11.928 Up to a month for adult.

NOTE Confidence: 0.8180986

00:41:11.930 --> 00:41:15.538 See a few ehad is documented in the

NOTE Confidence: 0.8180986

00:41:15.538 --> 00:41:19.294 literature in our hands up to about 2 weeks.

NOTE Confidence: 0.8180986

00:41:19.300 --> 00:41:21.316 And this contrasts with CF you,

NOTE Confidence: 0.8180986

00:41:21.320 --> 00:41:23.888 even they differentiate their form rec

NOTE Confidence: 0.8180986

00:41:23.888 --> 00:41:27.167 cells within a matter of two to three days.

NOTE Confidence: 0.8180986

00:41:27.170 --> 00:41:29.823 And what stops a fuse from differentiating

NOTE Confidence: 0.8180986

00:41:29.823 --> 00:41:32.659 it keeps them in itself for in

NOTE Confidence: 0.8180986

00:41:32.659 --> 00:41:34.283 your state are glucocorticoids.

NOTE Confidence: 0.8180986

00:41:34.290 --> 00:41:37.998 And so when we placed glucocorticoid

NOTE Confidence: 0.8180986

00:41:37.998 --> 00:41:41.410 57 knock hard to safely.

NOTE Confidence: 0.8180986

00:41:41.410 --> 00:41:44.100 In this self renewal cocktail.

NOTE Confidence: 0.8180986

00:41:44.100 --> 00:41:47.262 We found that they failed to

NOTE Confidence: 0.8180986

00:41:47.262 --> 00:41:49.370 undergo efficient self renewal.

NOTE Confidence: 0.8180986

00:41:49.370 --> 00:41:51.908 And so that is very likely

NOTE Confidence: 0.8180986

00:41:51.908 --> 00:41:54.360 the reason for the anemia.

NOTE Confidence: 0.8180986

00:41:54.360 --> 00:41:56.808 So for some reason they fail

NOTE Confidence: 0.8180986

00:41:56.808 --> 00:41:58.440 to sell from you,

NOTE Confidence: 0.8180986

00:41:58.440 --> 00:42:01.192 and the reason we can clear when we

NOTE Confidence: 0.8180986

00:42:01.192 --> 00:42:04.204 looked at the expression of P57 in cells

NOTE Confidence: 0.8180986

00:42:04.204 --> 00:42:07.010 in the presence of glucocorticoids.

NOTE Confidence: 0.8180986

00:42:07.010 --> 00:42:09.458 Here we're looking at using Dex,

NOTE Confidence: 0.8180986

00:42:09.460 --> 00:42:11.500 which is a synthetic glucocorticoid,

NOTE Confidence: 0.8180986

00:42:11.500 --> 00:42:14.436 so cells express P57 and then P 57

NOTE Confidence: 0.8180986

00:42:14.436 --> 00:42:17.354 isn't used further by DEX and so it

NOTE Confidence: 0.8180986

00:42:17.354 --> 00:42:19.333 seems that dex and glucocorticoids

NOTE Confidence: 0.8180986

00:42:19.333 --> 00:42:22.267 in general probably work at least

NOTE Confidence: 0.8180986

00:42:22.267 --> 00:42:24.768 in part by inducing P.

NOTE Confidence: 0.8180986

00:42:24.768 --> 00:42:25.282 57.

NOTE Confidence: 0.8180986

00:42:25.282 --> 00:42:27.852 Inhibiting Assface CK activity and

NOTE Confidence: 0.8180986

00:42:27.852 --> 00:42:30.996 that in turn allows a stabilizes

NOTE Confidence: 0.8180986

00:42:30.996 --> 00:42:33.521 and long essays and promotes

NOTE Confidence: 0.8180986

00:42:33.521 --> 00:42:36.519 the safe UE self renewal state.

NOTE Confidence: 0.8180986

00:42:36.520 --> 00:42:39.178 So here we see a connection

NOTE Confidence: 0.8180986

00:42:39.178 --> 00:42:42.317 between along S phase and self

NOTE Confidence: 0.8180986

00:42:42.317 --> 00:42:45.487 renewal or persistence of a

NOTE Confidence: 0.8180986

00:42:45.487 --> 00:42:47.389 particular transcriptional state.

NOTE Confidence: 0.8180986

00:42:47.390 --> 00:42:50.786 Suggesting that maybe a fast S

NOTE Confidence: 0.8180986

00:42:50.786 --> 00:42:53.050 phase is somehow destabilizing

NOTE Confidence: 0.8180986

00:42:53.144 --> 00:42:55.649 to transcriptional program.

NOTE Confidence: 0.8180986

00:42:55.650 --> 00:42:59.406 Now we were able to rescue.

NOTE Confidence: 0.8180986

00:42:59.410 --> 00:43:01.374 The situation by adding

NOTE Confidence: 0.8180986

00:43:01.374 --> 00:43:03.829 to the knockout cells P.

NOTE Confidence: 0.8180986

00:43:03.830 --> 00:43:07.034 57 knockout cells acidic A2 inhibiting

NOTE Confidence: 0.8180986

00:43:07.034 --> 00:43:10.679 drugs so this will reduce CD K

NOTE Confidence: 0.8180986

00:43:10.679 --> 00:43:13.024 activity will inhibit CK activity

NOTE Confidence: 0.8180986

00:43:13.024 --> 00:43:16.757 in S phase cells and you can see

NOTE Confidence: 0.8180986

00:43:16.757 --> 00:43:19.444 that compared to the city 57.

NOTE Confidence: 0.8180986

00:43:19.444 --> 00:43:21.654 Knock ourselves the knockout cells

NOTE Confidence: 0.8180986

00:43:21.654 --> 00:43:24.683 treated with the drug almost completely

NOTE Confidence: 0.8180986

00:43:24.683 --> 00:43:27.398 resume normal self renewal activity.

NOTE Confidence: 0.57940215

00:43:29.500 --> 00:43:34.495 And so, um. You can we have a

NOTE Confidence: 0.57940215

00:43:34.495 --> 00:43:36.736 bit more data here, so yeah,

NOTE Confidence: 0.57940215

00:43:36.736 --> 00:43:39.720 so here we're looking at wild type cells.

NOTE Confidence: 0.57940215

00:43:39.720 --> 00:43:41.580 We can take this paradigm,

NOTE Confidence: 0.57940215

00:43:41.580 --> 00:43:42.980 feather and say, well,

NOTE Confidence: 0.57940215

00:43:42.980 --> 00:43:45.690 OK we have CF uees long essays.

NOTE Confidence: 0.57940215

00:43:45.690 --> 00:43:47.178 Stable self renewal can

NOTE Confidence: 0.57940215

00:43:47.178 --> 00:43:49.038 make it even more stable.

NOTE Confidence: 0.57940215

00:43:49.040 --> 00:43:51.581 Can we enhance the sea of yourself

NOTE Confidence: 0.57940215

00:43:51.581 --> 00:43:53.919 on your potential by adding city

NOTE Confidence: 0.57940215

00:43:53.919 --> 00:43:56.265 K inhibitors to the medium and

NOTE Confidence: 0.57940215

00:43:56.265 --> 00:43:57.987 prolonging S phase even more?

NOTE Confidence: 0.57940215

00:43:57.990 --> 00:43:59.970 And that indeed happens so you

NOTE Confidence: 0.57940215

00:43:59.970 --> 00:44:02.245 can see that compared to control

NOTE Confidence: 0.57940215

00:44:02.245 --> 00:44:04.021 cells undergoing self renewal

NOTE Confidence: 0.57940215

00:44:04.021 --> 00:44:05.353 in just dexamethasone.

NOTE Confidence: 0.57940215

00:44:05.360 --> 00:44:08.488 When we add the city K2 inhibitor with,

NOTE Confidence: 0.57940215

00:44:08.490 --> 00:44:10.440 these cells are completely blocked.

NOTE Confidence: 0.57940215

00:44:10.440 --> 00:44:12.990 They don't up regulator 119.

NOTE Confidence: 0.57940215

00:44:12.990 --> 00:44:16.063 When the S phase is slower and

NOTE Confidence: 0.57940215

00:44:16.063 --> 00:44:19.240 we can amplify them much further,

NOTE Confidence: 0.57940215

00:44:19.240 --> 00:44:22.576 so in red at the cells that have

NOTE Confidence: 0.57940215

00:44:22.576 --> 00:44:25.990 sydicate 2 inhibitors added on so we

NOTE Confidence: 0.57940215

00:44:25.990 --> 00:44:29.829 can amplify the CFL stage even further.

NOTE Confidence: 0.57940215

00:44:29.830 --> 00:44:32.710 And clearly this may have some

NOTE Confidence: 0.57940215

00:44:32.710 --> 00:44:33.670 translational applications.

NOTE Confidence: 0.57940215

00:44:33.670 --> 00:44:36.556 Maybe Syndicate two inhibitors will assist,

NOTE Confidence: 0.57940215

00:44:36.560 --> 00:44:39.580 or perhaps replace glucocorticoids in

NOTE Confidence: 0.57940215

00:44:39.580 --> 00:44:43.080 therapeutic approaches that target the CFUE.

NOTE Confidence: 0.57940215

00:44:43.080 --> 00:44:45.485 But it also for fundamental

NOTE Confidence: 0.57940215

00:44:45.485 --> 00:44:47.409 biology point of view.

NOTE Confidence: 0.57940215

00:44:47.410 --> 00:44:50.278 It tells us that stabilizing and

NOTE Confidence: 0.57940215

00:44:50.278 --> 00:44:53.179 prolonging S phase delays the switch.

NOTE Confidence: 0.831401939999999

00:44:55.740 --> 00:44:58.232 So I will. I'm running a little

NOTE Confidence: 0.831401939999999

00:44:58.232 --> 00:45:01.510 bit out of time so I will quickly

NOTE Confidence: 0.831401939999999

00:45:01.510 --> 00:45:04.106 summarize what we what I've shown

NOTE Confidence: 0.831401939999999

00:45:04.106 --> 00:45:07.194 you in the second part of the talk.

NOTE Confidence: 0.831401939999999

00:45:07.200 --> 00:45:09.170 See if you self renewal.

NOTE Confidence: 0.831401939999999

00:45:09.170 --> 00:45:12.280 Depends on a long space.

NOTE Confidence: 0.831401939999999

00:45:12.280 --> 00:45:14.920 And when that is.

NOTE Confidence: 0.831401939999999

00:45:14.920 --> 00:45:17.035 Impaired, for example,

NOTE Confidence: 0.831401939999999

00:45:17.035 --> 00:45:20.560 in the P57 knockout mouse.

NOTE Confidence: 0.831401939999999

00:45:20.560 --> 00:45:22.360 There are insufficient cycles of

NOTE Confidence: 0.831401939999999

00:45:22.360 --> 00:45:24.868 self renewal, and that causes anemia.

NOTE Confidence: 0.831401939999999

00:45:24.868 --> 00:45:29.078 And we can rescue that and in fact enhance.

NOTE Confidence: 0.831401939999999

00:45:29.080 --> 00:45:32.120 See if you see few suffering you even

NOTE Confidence: 0.831401939999999

00:45:32.120 --> 00:45:35.441 in wild type progenitors by inhibiting

NOTE Confidence: 0.831401939999999

00:45:35.441 --> 00:45:38.536 SDK activity and prolonging essays.

NOTE Confidence: 0.831401939999999

00:45:38.540 --> 00:45:41.360 So it appears that exercise shortening

NOTE Confidence: 0.831401939999999

00:45:41.360 --> 00:45:44.380 may be causally related to the switch,

NOTE Confidence: 0.831401939999999

00:45:44.380 --> 00:45:46.740 although the underlying mechanism

NOTE Confidence: 0.831401939999999

00:45:46.740 --> 00:45:49.690 of course isn't clear yet.

NOTE Confidence: 0.831401939999999

00:45:49.690 --> 00:45:53.460 And then in the last few minutes of my talk,

NOTE Confidence: 0.831401939999999

00:45:53.460 --> 00:45:57.036 I'll take just five minutes to tell you

NOTE Confidence: 0.831401939999999

00:45:57.036 --> 00:46:00.259 knew story that isn't yet published.

NOTE Confidence: 0.831401939999999

00:46:00.260 --> 00:46:03.278 Again, that seems to highlight the

NOTE Confidence: 0.831401939999999

00:46:03.278 --> 00:46:06.567 importance of the cycle in this

NOTE Confidence: 0.831401939999999

00:46:06.567 --> 00:46:08.939 time in terminal differentiation.

NOTE Confidence: 0.831401939999999

00:46:08.940 --> 00:46:11.369 So we know that E praeceptor becomes

NOTE Confidence: 0.831401939999999

00:46:11.369 --> 00:46:13.370 essential during terminal differentiation.

NOTE Confidence: 0.831401939999999

00:46:13.370 --> 00:46:16.814 Although it begins to be expressed much

NOTE Confidence: 0.831401939999999

00:46:16.814 --> 00:46:19.820 earlier in earlier with the Preseas.

NOTE Confidence: 0.831401939999999

00:46:19.820 --> 00:46:20.783 And with that,

NOTE Confidence: 0.831401939999999

00:46:20.783 --> 00:46:23.030 the power sector I've shown you earlier,

NOTE Confidence: 0.831401939999999

00:46:23.030 --> 00:46:26.159 we have no cells in terminal differentiation.

NOTE Confidence: 0.831401939999999

00:46:26.160 --> 00:46:27.448 And so we asked.

NOTE Confidence: 0.831401939999999

00:46:27.448 --> 00:46:30.808 And this is work by Daniel Hidalgo in my lab.

NOTE Confidence: 0.831401939999999

00:46:30.810 --> 00:46:31.800 We asked whether.

NOTE Confidence: 0.7722785

00:46:34.520 --> 00:46:36.310 The emperor sector has added

NOTE Confidence: 0.7722785

00:46:36.310 --> 00:46:37.742 functions other than survival

NOTE Confidence: 0.7722785

00:46:37.742 --> 00:46:39.539 during terminal differentiation,

NOTE Confidence: 0.7722785

00:46:39.540 --> 00:46:42.200 and two answer that we developed a

NOTE Confidence: 0.7722785

00:46:42.200 --> 00:46:45.241 genetic model in which we take the

NOTE Confidence: 0.7722785

00:46:45.241 --> 00:46:47.516 perceptive nokut fetal livers and

NOTE Confidence: 0.7722785

00:46:47.516 --> 00:46:50.064 introduce back either the EPO receptor

NOTE Confidence: 0.7722785

00:46:50.064 --> 00:46:52.758 obiesie ELEX to stop them from dying.

NOTE Confidence: 0.7722785

00:46:52.758 --> 00:46:54.792 And with this model system we

NOTE Confidence: 0.7722785

00:46:54.792 --> 00:46:57.270 were able to see that in fact

NOTE Confidence: 0.7722785

00:46:57.270 --> 00:46:59.000 you can get full differentiation

NOTE Confidence: 0.7722785

00:46:59.067 --> 00:47:01.329 without any equal receptor at all,

NOTE Confidence: 0.7722785

00:47:01.330 --> 00:47:05.700 as long as you keep the cells from dying by.

NOTE Confidence: 0.7722785

00:47:05.700 --> 00:47:09.510 D. Transgenic expression of BCLX.

NOTE Confidence: 0.7722785

00:47:09.510 --> 00:47:11.460 However, there were various abnormalities,

NOTE Confidence: 0.7722785

00:47:11.460 --> 00:47:14.050 and the biggest one was that the

NOTE Confidence: 0.7722785

00:47:14.050 --> 00:47:16.448 Sea Fury colonies that were formed

NOTE Confidence: 0.7722785

00:47:16.448 --> 00:47:18.866 were much smaller than the area,

NOTE Confidence: 0.7722785

00:47:18.870 --> 00:47:21.658 which is much smaller.

NOTE Confidence: 0.7722785

00:47:21.660 --> 00:47:24.186 And the second finding was that

NOTE Confidence: 0.7722785

00:47:24.186 --> 00:47:26.390 S phase wasn't as fast,

NOTE Confidence: 0.7722785

00:47:26.390 --> 00:47:28.970 so it was still pretty fast,

NOTE Confidence: 0.7722785

00:47:28.970 --> 00:47:32.170 but not as fast as in cells that

NOTE Confidence: 0.7722785

00:47:32.170 --> 00:47:34.558 were expressing the E prospectors.

NOTE Confidence: 0.7722785

00:47:34.560 --> 00:47:36.198 So clearly E.

NOTE Confidence: 0.7722785

00:47:36.198 --> 00:47:38.382 Praeceptor can further accelerate

NOTE Confidence: 0.7722785

00:47:38.382 --> 00:47:39.474 space speed.

NOTE Confidence: 0.7722785

00:47:39.480 --> 00:47:41.226 When we look at cell growth,

NOTE Confidence: 0.7722785

00:47:41.230 --> 00:47:43.670 this is in vitro.

NOTE Confidence: 0.7722785

00:47:43.670 --> 00:47:47.065 And see that cells expressing the ape

NOTE Confidence: 0.7722785

00:47:47.065 --> 00:47:50.230 receptor grow much faster than cells

NOTE Confidence: 0.7722785

00:47:50.230 --> 00:47:53.464 expressing BCLX and the doubling time.

NOTE Confidence: 0.7722785

00:47:53.470 --> 00:47:55.170 Let's look at control cells.

NOTE Confidence: 0.7722785

00:47:55.170 --> 00:47:57.666 That doubling time is only six hours in

NOTE Confidence: 0.7722785

00:47:57.666 --> 00:48:00.267 our cells that express the equal sector,

NOTE Confidence: 0.7722785

00:48:00.270 --> 00:48:03.546 but it climbs to a towers in cells that

NOTE Confidence: 0.7722785

00:48:03.546 --> 00:48:06.268 don't have a protective signaling.

NOTE Confidence: 0.7722785

00:48:06.270 --> 00:48:08.832 We were fortunate to be able to

NOTE Confidence: 0.7722785

00:48:08.832 --> 00:48:11.108 collaborate with Sean James Goose Lab,

NOTE Confidence: 0.7722785

00:48:11.110 --> 00:48:13.966 who developed a beautiful Reporter mice.

NOTE Confidence: 0.7722785

00:48:13.970 --> 00:48:16.973 This mouse reports the length of the

NOTE Confidence: 0.7722785

00:48:16.973 --> 00:48:19.720 cell cycle using a Fusion protein.

NOTE Confidence: 0.7722785

00:48:19.720 --> 00:48:23.168 H2B fluorescence timer Fusion.

NOTE Confidence: 0.7722785

00:48:23.170 --> 00:48:25.260 These Fusion is fluorescent blue

NOTE Confidence: 0.7722785

00:48:25.260 --> 00:48:27.761 when it is first synthesized and

NOTE Confidence: 0.7722785

00:48:27.761 --> 00:48:29.471 then becomes a fluoresces red

NOTE Confidence: 0.7722785

00:48:29.471 --> 00:48:31.599 about an hour or two later.

NOTE Confidence: 0.7722785

00:48:31.600 --> 00:48:35.569 And so cells that have a short cycle IBB

NOTE Confidence: 0.7722785

00:48:35.569 --> 00:48:39.558 lower than cells that have a longer cycle.

NOTE Confidence: 0.7722785

00:48:39.560 --> 00:48:41.610 And so with her lab,

NOTE Confidence: 0.7722785

00:48:41.610 --> 00:48:45.434 we injected mice with either Ipoh or Saline.

NOTE Confidence: 0.7722785

00:48:45.440 --> 00:48:47.762 And found that two mice injected

NOTE Confidence: 0.7722785

00:48:47.762 --> 00:48:49.760 with Saline here in blue.

NOTE Confidence: 0.7722785

00:48:49.760 --> 00:48:52.300 Um?

NOTE Confidence: 0.7722785

00:48:52.300 --> 00:48:53.998 Here we're looking at the ratio

NOTE Confidence: 0.7722785

00:48:53.998 --> 00:48:56.000 of blue to red fluorescence.

NOTE Confidence: 0.7722785

00:48:56.000 --> 00:48:59.290 We see a shift in that ratio when we look

NOTE Confidence: 0.7722785

00:48:59.375 --> 00:49:02.743 at two mice that are injected with people.

NOTE Confidence: 0.7722785

00:49:02.750 --> 00:49:05.263 And here we're looking at a specific

NOTE Confidence: 0.7722785

00:49:05.263 --> 00:49:07.268 erythroblast earlier Race for Life stage,

NOTE Confidence: 0.7722785

00:49:07.270 --> 00:49:09.745 and we can look at a number of our

NOTE Confidence: 0.7722785

00:49:09.745 --> 00:49:11.794 way through glass stages and at

NOTE Confidence: 0.7722785

00:49:11.794 --> 00:49:14.485 each stage we see a clear difference

NOTE Confidence: 0.7722785

00:49:14.485 --> 00:49:16.317 in cell cycle length.

NOTE Confidence: 0.7722785

00:49:16.320 --> 00:49:18.270 So it seems that if receptor

NOTE Confidence: 0.7722785

00:49:18.270 --> 00:49:20.150 can shorten space even further,

NOTE Confidence: 0.7722785

00:49:20.150 --> 00:49:23.390 and in fact shorten the cycle.

NOTE Confidence: 0.7722785

00:49:23.390 --> 00:49:26.358 I'm going to skip this next part,

NOTE Confidence: 0.7722785

00:49:26.360 --> 00:49:27.455 which very briefly,

NOTE Confidence: 0.7722785

00:49:27.455 --> 00:49:30.010 you would think that if a preceptor

NOTE Confidence: 0.7722785

00:49:30.079 --> 00:49:32.509 drives as shortest cycle and more

NOTE Confidence: 0.7722785

00:49:32.509 --> 00:49:35.260 numerous cycles in terminal differentiation,

NOTE Confidence: 0.7722785

00:49:35.260 --> 00:49:37.804 the red cells that would result

NOTE Confidence: 0.7722785

00:49:37.804 --> 00:49:39.076 would be smaller.

NOTE Confidence: 0.7722785

00:49:39.080 --> 00:49:42.464 But in fact we find exactly the opposite.

NOTE Confidence: 0.7722785

00:49:42.470 --> 00:49:45.870 We find that the rattles are formed at

NOTE Confidence: 0.7722785

00:49:45.870 --> 00:49:49.246 larger and I won't go through the data,

NOTE Confidence: 0.7722785

00:49:49.250 --> 00:49:53.156 but we see a larger diameter in cells that.

NOTE Confidence: 0.7722785

00:49:53.160 --> 00:49:55.625 Experienced high ipho and so

NOTE Confidence: 0.7722785

00:49:55.625 --> 00:49:58.090 that's a sort of paradox.

NOTE Confidence: 0.7722785

00:49:58.090 --> 00:50:00.519 This is interesting in its own right.

NOTE Confidence: 0.7722785

00:50:00.520 --> 00:50:02.600 I'm.

NOTE Confidence: 0.7722785

00:50:02.600 --> 00:50:04.445 And so I will summarize

NOTE Confidence: 0.7722785

00:50:04.445 --> 00:50:06.700 what I've told you so far.

NOTE Confidence: 0.7722785

00:50:06.700 --> 00:50:08.112 And summarize my talk.

NOTE Confidence: 0.7722785

00:50:08.112 --> 00:50:10.230 What we find is that during

NOTE Confidence: 0.85460913

00:50:10.305 --> 00:50:12.917 the arethra developmental trajectory,

NOTE Confidence: 0.85460913

00:50:12.920 --> 00:50:15.584 the cell cycle takes up a

NOTE Confidence: 0.85460913

00:50:15.584 --> 00:50:17.360 number of different shapes.

NOTE Confidence: 0.85460913

00:50:17.360 --> 00:50:20.018 If you like, it is long,

NOTE Confidence: 0.85460913

00:50:20.020 --> 00:50:22.595 early in the trajectory with

NOTE Confidence: 0.85460913

00:50:22.595 --> 00:50:26.210 along for a space and along G1.

NOTE Confidence: 0.85460913

00:50:26.210 --> 00:50:28.054 During the gradual progression

NOTE Confidence: 0.85460913

00:50:28.054 --> 00:50:31.397 through to see if you re staged

NOTE Confidence: 0.85460913

00:50:31.397 --> 00:50:34.799 towards the switch to ETD we have

NOTE Confidence: 0.85460913

00:50:34.799 --> 00:50:37.288 initially gradual shortening of G1.

NOTE Confidence: 0.85460913

00:50:37.290 --> 00:50:40.020 And sorry, gradual shortening of D1.

NOTE Confidence: 0.85460913

00:50:40.020 --> 00:50:43.800 And then at the time of this which we have.

NOTE Confidence: 0.85460913

00:50:43.800 --> 00:50:45.800 An abrupt shortening of essays,

NOTE Confidence: 0.85460913

00:50:45.800 --> 00:50:48.775 the shortening West phase is the result

NOTE Confidence: 0.85460913

00:50:48.775 --> 00:50:50.980 of downregulation of P57 Kip too,

NOTE Confidence: 0.85460913

00:50:50.980 --> 00:50:52.580 which is acidic Lee,

NOTE Confidence: 0.85460913

00:50:52.580 --> 00:50:53.380 two inhibitor.

NOTE Confidence: 0.85460913

00:50:53.380 --> 00:50:56.495 It inhibits S phase city K activity

NOTE Confidence: 0.85460913

00:50:56.495 --> 00:50:59.864 and that leads to an increase in

NOTE Confidence: 0.85460913

00:50:59.864 --> 00:51:02.289 the speed of replication Forks.

NOTE Confidence: 0.85460913

00:51:02.290 --> 00:51:05.730 The long ass face in the CFG stage

NOTE Confidence: 0.85460913

00:51:05.730 --> 00:51:08.793 is critical for the stability of that

NOTE Confidence: 0.85460913

00:51:08.793 --> 00:51:12.209 stage in it for its self renewal,

NOTE Confidence: 0.85460913

00:51:12.210 --> 00:51:15.110 and it appears that glucocorticoids

NOTE Confidence: 0.85460913

00:51:15.110 --> 00:51:18.010 utilize that by enhancing expression

NOTE Confidence: 0.85460913

00:51:18.092 --> 00:51:21.638 of of the silicate to inhibit P 57 and

NOTE Confidence: 0.85460913

00:51:21.638 --> 00:51:24.857 prolonging the CFO is suffering you'll stage.

NOTE Confidence: 0.85460913

00:51:24.860 --> 00:51:27.460 And we may be able to use that

NOTE Confidence: 0.85460913

00:51:27.460 --> 00:51:28.586 therapeutically with silicate

NOTE Confidence: 0.85460913

00:51:28.586 --> 00:51:30.218 two inhibitors as well.

NOTE Confidence: 0.85460913

00:51:30.220 --> 00:51:32.686 Once we cross this this late

NOTE Confidence: 0.85460913

00:51:32.686 --> 00:51:33.919 into terminal differentiation,

NOTE Confidence: 0.85460913

00:51:33.920 --> 00:51:36.386 the initial cycles are very short.

NOTE Confidence: 0.85460913

00:51:36.390 --> 00:51:39.846 In fact I forgot to mention this cycles

NOTE Confidence: 0.85460913

00:51:39.846 --> 00:51:43.249 of the earlier throw blasts as some

NOTE Confidence: 0.85460913

00:51:43.249 --> 00:51:46.920 of this shortest in the bone marrow.

NOTE Confidence: 0.85460913

00:51:46.920 --> 00:51:50.964 And these can be even further

NOTE Confidence: 0.85460913

00:51:50.964 --> 00:51:52.986 shorter when we.

NOTE Confidence: 0.85460913

00:51:52.990 --> 00:51:55.797 Use that when we simulate these cells.

NOTE Confidence: 0.85460913

00:51:55.800 --> 00:51:59.064 With people so at high levels of people

NOTE Confidence: 0.85460913

00:51:59.064 --> 00:52:02.688 which we would find in stress situations,

NOTE Confidence: 0.85460913

00:52:02.690 --> 00:52:06.296 high altitude or disease or bleeding.

NOTE Confidence: 0.85460913

00:52:06.300 --> 00:52:09.268 Cycles become even shorter.

NOTE Confidence: 0.85460913

00:52:09.270 --> 00:52:12.342 And that in itself is raises

NOTE Confidence: 0.85460913

00:52:12.342 --> 00:52:15.349 the question of why is that?

NOTE Confidence: 0.85460913

00:52:15.350 --> 00:52:19.913 And I did not have time to discuss that,

NOTE Confidence: 0.85460913

00:52:19.920 --> 00:52:22.664 but all the work shows that the

NOTE Confidence: 0.85460913

00:52:22.664 --> 00:52:25.843 fast cycles of earlier reefer blasts

NOTE Confidence: 0.85460913

00:52:25.843 --> 00:52:29.038 contribute to rapid DNA demethylation,

NOTE Confidence: 0.85460913

00:52:29.040 --> 00:52:31.950 and that interns assist the speed

NOTE Confidence: 0.85460913

00:52:31.950 --> 00:52:34.620 of the ATD transcriptional program.

NOTE Confidence: 0.85460913

00:52:34.620 --> 00:52:37.854 So it's possible that that might

NOTE Confidence: 0.85460913

00:52:37.854 --> 00:52:40.010 be the reason why.

NOTE Confidence: 0.85460913

00:52:40.010 --> 00:52:41.470 This takes place here,

NOTE Confidence: 0.85460913

00:52:41.470 --> 00:52:44.760 but of course we don't know that for sure.

NOTE Confidence: 0.85460913

00:52:44.760 --> 00:52:49.376 So I'd like to thank the people that

NOTE Confidence: 0.85460913

00:52:49.376 --> 00:52:54.856 did the work and these people in my lab.

NOTE Confidence: 0.85460913

00:52:54.860 --> 00:52:57.259 Including include US Warrior, Sami, Nathan.

NOTE Confidence: 0.85460913

00:52:57.259 --> 00:53:00.291 Better bear to see who did a lot

NOTE Confidence: 0.85460913

00:53:00.291 --> 00:53:03.318 of the single cell RNA SEQ work.

NOTE Confidence: 0.85460913

00:53:03.320 --> 00:53:06.505 Melinda Futron who worked in the DNA

NOTE Confidence: 0.85460913

00:53:06.505 --> 00:53:08.626 combing together with young Kwang

NOTE Confidence: 0.85460913

00:53:08.626 --> 00:53:11.322 who did all the work on P57 and

NOTE Confidence: 0.85460913

00:53:11.406 --> 00:53:14.206 Daniel Hidalgo who did the work on

NOTE Confidence: 0.85460913

00:53:14.206 --> 00:53:16.859 the ape receptor signaling and our

NOTE Confidence: 0.85460913

00:53:16.859 --> 00:53:18.791 collaborators were very fortunate

NOTE Confidence: 0.85460913

00:53:18.791 --> 00:53:21.258 in our collaborators and old clients

NOTE Confidence: 0.85460913

00:53:21.258 --> 00:53:23.463 lab with a single cell RNA SEQ.

NOTE Confidence: 0.85460913

00:53:23.470 --> 00:53:25.850 Necrime at UMass Medical School.

NOTE Confidence: 0.85460913

00:53:25.850 --> 00:53:29.203 Who helped us with the DNA combing

NOTE Confidence: 0.85460913

00:53:29.203 --> 00:53:32.035 and shanting Google in Yale who

NOTE Confidence: 0.85460913

00:53:32.035 --> 00:53:34.200 had been a great colleague,

NOTE Confidence: 0.85460913

00:53:34.200 --> 00:53:37.784 was also interested in the cell cycle and

NOTE Confidence: 0.85460913

00:53:37.784 --> 00:53:40.697 we've collaborated with her transgenic mouse.

NOTE Confidence: 0.85460913

00:53:40.700 --> 00:53:42.089 So thank you.

NOTE Confidence: 0.8510252

00:53:45.520 --> 00:53:48.094 Thank you very much for a great talk.

NOTE Confidence: 0.8510252

00:53:48.100 --> 00:53:49.715 We have two questions for

NOTE Confidence: 0.8510252

00:53:49.715 --> 00:53:51.330 you in the Q&A session.

NOTE Confidence: 0.8510252

00:53:51.330 --> 00:53:54.237 I don't know if you can see them wrong,

NOTE Confidence: 0.8510252

00:53:54.240 --> 00:53:56.824 but I can read them to the group.

NOTE Confidence: 0.8510252

00:53:56.830 --> 00:53:59.728 The first is from Doctor Liu says great talk.

NOTE Confidence: 0.8510252

00:53:59.730 --> 00:54:02.054 Any speculation in how CD K2 and

NOTE Confidence: 0.8510252

00:54:02.054 --> 00:54:04.248 P57 regulate fork speed in S phase.

NOTE Confidence: 0.8742839

00:54:05.100 --> 00:54:07.319 We would like to know that yeah,

NOTE Confidence: 0.8742839

00:54:07.320 --> 00:54:09.560 so we don't know there are many.