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1 00:00:00.000 --> 00:00:02.810 Yale podcast network.

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3 00:00:05.240 --> 00:00:09.833 Hello and welcome to another episode of the Yale Journal Biology and medicine podcast

4 00:00:09.833 --> 00:00:10.784 YJBM is a pub.

5 00:00:10.784 --> 00:00:20.234 Med index quarterly Journal edited by Yale medical graduate and professional students and peer reviewed by experts in the fields of biology and medicine each issue of the Journal is

6 00:00:20.234 --> 00:00:23.560 devoted to a focus topic an through the YJBM podcast.

7 00:00:23.560 --> 00:00:25.461 It would take you through the past,

8 00:00:25.461 --> 00:00:28.102 present, and future of the issues subject matter.

9 00:00:28.102 --> 00:00:34.226 This episode is part of our series devoted to our September 2019 issue on Organelles, I'm your co-host Kelsie Cassell,

10 00:00:34.226 --> 00:00:36.719 a second year graduate student Epidemiology.

11 00:00:36.719 --> 00:00:45.615 And I'm also your cohost my name is Wesley Lewis and I'm a first year in computational biology and Bioinformatics and I'm your 3rd and final cohost,

12 00:00:45.615 --> 00:00:50.570 Emma Carley. I'm a second year student in the Department of cell biology and today.

13 00:00:50.570 --> 00:00:53.317 We're joined by Doctor Megan King and doctor.

14 00:00:53.317 --> 00:01:01.018 Patrick Lusk Doctor King and Doctor Lusk are Associate Professors in the cell biology Department here at Yale and Full disclosure.

15 00:01:01.018 --> 00:01:03.406 They happened to be my wonderful PI's,

16 00:01:03.406 --> 00:01:05.853 so thank you. Both for being here today,

17 00:01:05.853 --> 00:01:08.700 you're welcome. Happy to be here.

18 00:01:08.700 --> 00:01:15.000 OK, we'll be talking over each other through most of this sounds good.

19 00:01:15.000 --> 00:01:18.271 So Doctor King and Doctor Lusk both study the nucleus.

20 00:01:18.271 --> 00:01:28.855 One of the many organelles featured in the organelles issue of this Journal so briefly the nucleus is a large double membrane organelle found in eukaryotic cells that houses the

21 00:01:28.855 --> 00:01:32.780 genome. But this organelle is not simply a storage space for DNA.

22 00:01:32.780 --> 00:01:39.203 It's actually a densely packed highly dynamic cellular compartment involved in many key cellular processes.

23 00:01:39.203 --> 00:01:45.930 So we're excited to learn a lot more about this awesome organelle from Doctor King and Doctor Lusk.

24 00:01:45.930 --> 00:01:46.915 I'm so to start of-

25 00:01:46.915 --> 00:01:52.670 can you please introduce yourselves and tell us about how you became interested in studying the nucleus?

26 00:01:52.670 --> 00:01:57.067 Absolutely so my inspiration for studying so biology in general,

27 00:01:57.067 --> 00:02:01.329 actually happened during my undergraduate education at.

28 00:02:01.329 --> 00:02:04.938 It's wonderful school, called the University of Alberta in Alberta,

29 00:02:04.938 --> 00:02:19.759 Canada. And essentially uh you know up until probably my 3rd year at science had been taught primarily is sort of a by road kind of memorization type of.

30 00:02:19.759 --> 00:02:25.415 Teaching, which was not that inspiring but actually it was actually a cell biology class in.

31 00:02:25.415 --> 00:02:31.375 I think my junior year where I was finally introduced to what science is all about and of course,

32 00:02:31.375 --> 00:02:39.281 that's sort of the capacity to make new discoveries right and to uncover something that nobody else is understood or seen before.

33 00:02:39.281 --> 00:02:48.828 And so this is something that I hadn't really been taught but finally understood the power of that and I got involved with research at that time and sort of

34 00:02:48.828 --> 00:03:00.813 got involved with research. Looking into the transport portal so that control all molecular communication between the nucleus or at the most important organelle in the cell and the cytoplasm,

35 00:03:00.813 --> 00:03:08.445 which which encompasses the rest of the organizers of the cell so these portals of the time were very poorly understood,

36 00:03:08.445 --> 00:03:18.728 but they sort of are the one of the defining features features of the nucleus because the nucleus is such a large organelle and you have to have tremendous amount

37 00:03:18.728 --> 00:03:20.620 of molecular traffic to allow.

38 00:03:20.620 --> 00:03:30.798 Gene expression and so we became very interested in understanding essentially how these portals work and that's sort of continued actually over the last have to say 2 decades,

39 00:03:30.798 --> 00:03:36.050 though, to my work as an independent investigator my own laboratory.

40 00:03:36.050 --> 00:03:38.487 So actually came to cell biology much later.

41 00:03:38.487 --> 00:03:41.088 I really was much more fascinated by chemistry.

42 00:03:41.088 --> 00:03:42.931 When I was a high school student.

43 00:03:42.931 --> 00:03:49.108 But I also found chemistry little bit dry and so when I discovered that there was something called bio chemistry.

44 00:03:49.108 --> 00:03:51.003 That was really interesting to me.

45 00:03:51.003 --> 00:03:53.116 The idea that I could study chemistry.

46 00:03:53.116 --> 00:03:56.801 That was carried out by biological molecules was really intriguing.

47 00:03:56.801 --> 00:04:05.080 And so that's what I set out to study as an undergraduate and I really loved the field of biochemistry particular particular protein chemistry.

48 00:04:05.080 --> 00:04:08.649 And that even when I went to work on for my PhD work.

49 00:04:08.649 --> 00:04:16.447 I my PhD is actually in biochemistry and biophysics and I would say the biophysics training is one of my motivations.

50 00:04:16.447 --> 00:04:28.014 Now, for my current work because one of the things that were very interested in our cellular forces and that interest in in forces really came from thinking about biophysical

51 00:04:28.014 --> 00:04:30.990 questions as a graduate student.

52 00:04:30.990 --> 00:04:40.029 In terms of focusing on the nucleus that really came from rediscovering a love of mine from probably when I was 9 or 10 years old,

53 00:04:40.029 --> 00:04:42.997 and that was looking through a microscope.

54 00:04:42.997 --> 00:04:54.867 I like many young budding scientists was fascinated by taking pond scum and putting it on a microscope and getting out a book and identifying all of the different critters

55 00:04:54.867 --> 00:04:57.319 that were flying around and.

56 00:04:57.319 --> 00:05:03.925 It was during my pH D that I finally was able to take advantage of GFP green fluorescent protein.

57 00:05:03.925 --> 00:05:15.048 This is the protein tag that we put on molecules were interested in so that we can watch them dynamically in live cells and that technology was actually only really

58 00:05:15.048 --> 00:05:19.495 put into the laboratory setting for asking fundamental questions.

59 00:05:19.495 --> 00:05:23.406 When I was an undergraduate and so as a graduate student.

60 00:05:23.406 --> 00:05:31.089 It was continuing to become more popular and I would say my first foray into looking through a microscope at AGFP.

61 00:05:31.089 --> 00:05:41.262 Tagged protein in a microscope was really kind of revolutionary for me and really made me appreciate the kind of open approach that cell biology takes and that is that

62 00:05:41.262 --> 00:05:50.345 if you're looking at something for the very first time 'cause you're the first person to make a jeffy Fusion protein of this really exciting protein.

63 00:05:50.345 --> 00:05:59.367 You're going to be able as as doctor last mentioned to see something that no one else has ever seen before and you have no idea what that's going to

64 00:05:59.367 --> 00:06:05.259 be so kind of a prepared mind and setting up an interesting system or assay can reveal anything.

65 00:06:05.259 --> 00:06:16.225 And I think that's what really won me over to cell biology as compared to kind of very structured biochemistry tons analogy that I had done up until that point.

66 00:06:16.225 --> 00:06:19.016 It turned out that one of the molecules.

67 00:06:19.016 --> 00:06:27.870 I decided to study surprisingly actually associated with these nuclear pore complex is the portals of transport that doctor loss.

68 00:06:27.870 --> 00:06:29.980 Garrity introduced and for me.

69 00:06:29.980 --> 00:06:34.747 It was actually watching the nucleus during mitosis or cell division,

70 00:06:34.747 --> 00:06:45.678 so the nucleus. Is in human cells completely breaks down as the cells are segregating their chromosomes and that has to be re established in the following cell cycle and

71 00:06:45.678 --> 00:06:47.610 the dynamics of that process.

72 00:06:47.610 --> 00:06:58.677 As I watched it taking movies of cells using a microscope was just incredibly fascinating and that's what really sparked my interest in the nucleus as an organelle was the

73 00:06:58.677 --> 00:07:04.110 fact that it went through this incredible cycle every time cells divide.

74 00:07:04.110 --> 00:07:09.326 Awesome so as I previously mentioned the nucleus is a very complicated.

75 00:07:09.326 --> 00:07:19.350 Compartment so can you talk to us specifically about what your research in your lab is focused on in this very complex organelle?

76 00:07:19.350 --> 00:07:28.805 So we actually we try to not enter too far into the nucleus were very interested in actually the bounding membranes and again.

77 00:07:28.805 --> 00:07:41.091 These portals that nuclear pore complex is that control all the molecular traffic and we've been particularly interested in emerging concepts over the last I'd say,

78 00:07:41.091 --> 00:07:50.399 5 years or so and the idea that the nucleus isn't sort of this static organelle Megan mention this idea during mitosis where.

79 00:07:50.399 --> 00:07:52.901 It completely breaks down and is rebuilt,

80 00:07:52.901 --> 00:07:55.163 but in most of the cells in our body.

81 00:07:55.163 --> 00:07:58.617 It actually this doesn't their terminally differentiated,

82 00:07:58.617 --> 00:08:02.487 particularly if you think about cells in your brain for example,

83 00:08:02.487 --> 00:08:09.396 they have intact nuclei that don't break down and yet the nuclei are considered sort of these static working hours,

84 00:08:09.396 --> 00:08:18.983 but they're actually quite dynamic on the molecular level and one of the things that we've discovered is not just us but other groups in the field is that these

85 00:08:18.983 --> 00:08:28.882 organelles can actually break. Have it let's say micro fractures if you will small tears in the nuclear envelope that can actually disrupt this compartmentalization,

86 00:08:28.882 --> 00:08:36.708 which is critical for organelle identity where we define organelles by their bio chemical constituents and so the segregation of for example,

87 00:08:36.708 --> 00:08:45.803 transcription where you make a message or RNA in the nucleus from translation from where you make proteins in the set is all is established by the integrity of this

88 00:08:45.803 --> 00:08:49.938 critical barrier, which is the barrier itself is built from the membranes,

89 00:08:49.938 --> 00:08:53.179 but also by the functioning of these nuclear pores.

90 00:08:53.179 --> 00:09:00.725 And So what we've been very interested in understanding is essentially because this barrier can breakdown in particular,

91 00:09:00.725 --> 00:09:13.446 with different disease states. I'm trying to understand if there are cellular mechanisms that sells employed to essentially protect the cell and protect the nucleus from this loss of compartmentalization

92 00:09:13.446 --> 00:09:23.750 and we have discovered pathways that actually are able to recognize when the nucleus nuclear membranes are breached over the nuclear pores aren't working properly.

93 00:09:23.750 --> 00:09:33.441 And start to mitigate that damage may think this is important for mitigating an disease actually in the context of human disease?

94 00:09:33.441 --> 00:09:41.044 Could you talk a little bit about how disruption of this nuclear envelope could lead to disease like?

95 00:09:41.044 --> 00:09:45.293 What sorts of diseases are related to these disruptions,

96 00:09:45.293 --> 00:09:48.573 yeah, so I think that there's 2 categories.

97 00:09:48.573 --> 00:09:50.662 One is no general diseases,

98 00:09:50.662 --> 00:09:54.240 where it's not very clear that in diseases like.

99 00:09:54.240 --> 00:10:04.049 A male trophic lateral sclerosis or else there's actually a disruption in the integrity of nuclear pores themselves.

100 00:10:04.049 --> 00:10:08.124 You know why that ultimately causes disease isn't well understood.

101 00:10:08.124 --> 00:10:19.313 But we're trying to understand essentially does the does this disruption trigger these surveillance pathways that we've discovered in more fundamental genetic models like for example,

102 00:10:19.313 --> 00:10:27.706 budding yeast, which is been a fantastic model for exploring sort of the fundamental biology behind the nuclear envelope membrane system.

103 00:10:27.706 --> 00:10:29.347 The other thing is cancer.

104 00:10:29.347 --> 00:10:37.759 So one thing that's clear is that when you do lose the disruption or when you disrupt the integrity of the nuclear membranes.

105 00:10:37.759 --> 00:10:41.053 This leads to DNA damage and it's not actually clear.

106 00:10:41.053 --> 00:10:48.922 Um necessarily again with the cause of that damage is there's lots of debate in the field as to what actually causes the damage.

107 00:10:48.922 --> 00:10:57.827 But nonetheless as we all know Genomic integrity or do you know damage is an input to cancer and so we're very interested in understanding again?

108 00:10:57.827 --> 00:11:07.769 How these surveillance mechanisms may have actually mitigate that damage may actually make it much lower so much worse than it needs to be and hopefully slow down.

109 00:11:07.769 --> 00:11:12.522 Cancer progression. So when I first started my group at Yale.

110 00:11:12.522 --> 00:11:21.693 I was really motivated by a very fundamental question and that is that the nuclear envelope is actually part of the endoplasmic reticulum.

111 00:11:21.693 --> 00:11:25.323 So we're talking about as a separate organelle 'cause.

112 00:11:25.323 --> 00:11:27.764 It does have this distinct identity,

113 00:11:27.764 --> 00:11:38.909 but the outer nuclear membrane is continuous with the ER membranes in the lumen between the two memories of the nuclear envelope is contiguous with the ER lumen.

114 00:11:38.909 --> 00:11:46.328 And so one thing we know about membranes from the kind of biophysics side is that they're very what we call compliant.

115 00:11:46.328 --> 00:11:52.313 That means that they were kind of very easily bendable and Shapeable and in the ER for example,

116 00:11:52.313 --> 00:12:02.663 microtubules actually template. the ER tubules and so one of the questions that I've had for a long time is what prevents the nucleus from what allows the nucleus to

117 00:12:02.663 --> 00:12:06.154 maintain its shape because it's not actually an island.

118 00:12:06.154 --> 00:12:13.320 It's actually integrated into the cytoskeleton so those are all of the filaments that give the nucleus structure.

119 00:12:13.320 --> 00:12:20.923 And the cytoskeleton actually is able to deliver for sign of the nucleus and this is important in many contexts for example.

120 00:12:20.923 --> 00:12:27.490 There are many tissues in which control over the position of the nucleus within the cell is very important,

121 00:12:27.490 --> 00:12:31.322 and that's actively determined by these cytoskeletal elements.

122 00:12:31.322 --> 00:12:35.398 So what keeps the nucleus looking in this kind of beautiful round.

123 00:12:35.398 --> 00:12:40.690 Spherical shape that we're used to seeing when it's actually being acted on by forces,

124 00:12:40.690 --> 00:12:43.669 particularly we know that the membranes that are.

125 00:12:43.669 --> 00:12:49.474 Really define the nucleus are very soft and malleable and at the answer that way.

126 00:12:49.474 --> 00:13:02.639 We think about that question is that ultimately the mechanical properties of the nucleus are determined by the chromosomes themselves right so the one other unique aspect of the nucleus

127 00:13:02.639 --> 00:13:13.679 is that it houses are DNA and the DNA is in the form of chromosomes and chromosomes are massive they are actually the largest polymer inside of human cells.

128 00:13:13.679 --> 00:13:25.394 And. Chromosomes also have their own kind of biophysics and so the hypothesis that we've been testing for the past 10 years is the idea that the chromosomes are actually

129 00:13:25.394 --> 00:13:29.375 attached to the membranes an by being attached to the membranes.

130 00:13:29.375 --> 00:13:38.807 They impart their mechanical properties on to this nuclear envelope verify stiffening it and this is important for its ability to maintain its integrity.

131 00:13:38.807 --> 00:13:45.299 So it doesn't undergo these kind of fractures leading to some of the complications that Patrick mentioned.

132 00:13:45.299 --> 00:13:54.746 And so that's the interface that were really focused on were interested in how chromatin contributes to defining the mechanical properties of the nucleus.

133 00:13:54.746 --> 00:13:56.880 That's kind of the yen of the lab.

134 00:13:56.880 --> 00:14:06.023 I would say the Yang of the lab is related to one of those forces are being transduced onto the nuclear envelope and on to the chromatin is that also

135 00:14:06.023 --> 00:14:08.278 important for the chromatin biology.

136 00:14:08.278 --> 00:14:15.409 So how does it actually affect what's happening inside the nucleus and so one context of that is mechanotransduction?

137 00:14:15.409 --> 00:14:24.384 Testing the idea that forces are directly transduced across the nuclear envelope onto the chromatin to regulate jeans in a way that's important,

138 00:14:24.384 --> 00:14:34.595 particularly at the level of tissues and organisms and the other aspect is related to how the kind of dynamics that can be driven by the cytoskeleton or imparted on

139 00:14:34.595 --> 00:14:45.549 to chromatin, which is important for gene regulation and also for mechanisms involved in DNA repair so I appreciate you

talking about some of the major interests that have come

140 00:14:45.549 --> 00:14:53.845 out of your lab. Could you potentially follow up with some of the updates that you're most excited about in your research so classically.

141 00:14:53.845 --> 00:15:00.089 We think of organelles as being membrane bound compartments that's the kind of original identification of them,

142 00:15:00.089 --> 00:15:10.067 but one of the really new concepts in cell biology is that there's a lot of Self Organization of really functional organelles that are not determined by being individual membrane

143 00:15:10.067 --> 00:15:15.196 bound compartments. In fact, the nucleus is really the origin of this kind of organization,

144 00:15:15.196 --> 00:15:17.399 so it's been appreciated for 100 years.

145 00:15:17.399 --> 00:15:28.772 That there are different sub compartments of the nucleus a good example is the nucleolus where all ribosomes are being a generated an assembled and that is again is not

146 00:15:28.772 --> 00:15:34.153 a well. That's a clearly a compartment that you can see an electron micrograph.

147 00:15:34.153 --> 00:15:37.519 For example, it is also not bounded by membranes.

148 00:15:37.519 --> 00:15:47.409 So we've known from studying kind of nuclear organization that there are mechanisms by which cells can self organize reactions even if they're not.

149 00:15:47.409 --> 00:15:57.027 In an individual membrane bound compartment that concept has now broadened out to a whole list of what I would call them.

150 00:15:57.027 --> 00:16:00.417 The kind of modern addition to organelles,

151 00:16:00.417 --> 00:16:05.539 which are really identified by their functional characteristics,

152 00:16:05.539 --> 00:16:16.340 and composition and one of the new concepts is that many of these are organized through a process called liquid liquid phase separation,

153 00:16:16.340 --> 00:16:19.019 so this is an idea that there are.

154 00:16:19.019 --> 00:16:30.806 Intrinsically disordered regions of proteins that are actually functionally very important again this on its own is kind of a revolution historically people felt that the kind of structured ordered

155 00:16:30.806 --> 00:16:36.222 regions of proteins were always doing the work of a protein and carrying out its function,

156 00:16:36.222 --> 00:16:46.520 but instead these intrinsically disordered regions actually allow molecules to organize with themselves in a way that allows him to segregate out from the other components.

157 00:16:46.520 --> 00:16:49.139 So you can think about this as kind of your.

158 00:16:49.139 --> 00:16:52.465 Classic salad dressing you have oil and water and so,

159 00:16:52.465 --> 00:16:54.806 if you shake up salad dressing right.

160 00:16:54.806 --> 00:17:01.210 It will it will then come apart and self organize into these 2 domains and you can kind of think about.

161 00:17:01.210 --> 00:17:07.079 That being driven by some protein segregating out from other proteins in the cell.

162 00:17:07.079 --> 00:17:09.796 So we are very interested in that concept,

163 00:17:09.796 --> 00:17:17.250 most recently because a coming back to this idea that chromatin is important for the mechanical properties of nuclei,

164 00:17:17.250 --> 00:17:21.861 particularly the chromatin that is associated with the nuclear envelope.

165 00:17:21.861 --> 00:17:25.903 So if you look at any classic electron micrograph of a nucleus.

166 00:17:25.903 --> 00:17:28.808 You'll see that there's very dense chromatin.

167 00:17:28.808 --> 00:17:37.230 That's associated with the periphery of the nucleus with the inner nuclear membrane and it turns out from a number of recent studies.

168 00:17:37.230 --> 00:17:43.330 That heterochromatin this dance chromatin has has these liquid liquid phase separation properties.

169 00:17:43.330 --> 00:17:53.375 This is initially kind of very disconcerting to us because when you think of a liquid you think a song It's very soft and we had already found the heterochromatin

170 00:17:53.375 --> 00:17:55.778 was important for making nuclei stiff,

171 00:17:55.778 --> 00:18:00.894 but that really is a kind of misnomer of how we think about liquid's most liquids.

172 00:18:00.894 --> 00:18:02.433 We think about our soft,

173 00:18:02.433 --> 00:18:08.720 but in fact glasses. A liquid right and that's actually quite hard and so really in the physics terms.

174 00:18:08.720 --> 00:18:19.428 A liquid is something that has disordered molecules doesn't really tell you anything about its mechanical properties and so the interview kind of come to terms with that and we

175 00:18:19.428 --> 00:18:29.592 are excited about the idea that these phase separated domains don't just organize molecules which is really how they've been studied for the most part in the past 5 or

176 00:18:29.592 --> 00:18:33.343 10 years, but also that they can actually do mechanical work.

177 00:18:33.343 --> 00:18:39.029 That means that the phase separated domains actually want to stay in the shape that they have.

178 00:18:39.029 --> 00:18:41.000 And if you try to deform them,

179 00:18:41.000 --> 00:18:51.640 they don't want to be deformed and so that actually can impart the stiffness to the nucleus that we've observed with respect to heterochromatin so this is a.

180 00:18:51.640 --> 00:19:00.750 Really for us an exciting time to consider the mechanical properties of something that was previously understood to mainly be organizing.

181 00:19:00.750 --> 00:19:06.607 Different regions of the cell and these kind of new concept of what an organelle is are there.

182 00:19:06.607 --> 00:19:12.342 Other examples of liquid liquid phase separation that we might have heard of before or like?

183 00:19:12.342 --> 00:19:15.859 How did the theory originate?

184 00:19:15.859 --> 00:19:21.587 And so the original I think there's been a number of observations over many years.

185 00:19:21.587 --> 00:19:27.040 But as all really interesting and exciting and fundamental aspects of Science.

186 00:19:27.040 --> 00:19:31.181 Things are rediscovered continually an with new techniques.

187 00:19:31.181 --> 00:19:39.670 You really can get to the molecular details and the generalizable principles that apply to all different areas of science.

188 00:19:39.670 --> 00:19:42.637 So I would say the kind of landmark paper.

189 00:19:42.637 --> 00:19:44.915 I was a study by Cliff Brangwyn,

190 00:19:44.915 --> 00:19:56.971 working with Tony Heimann. He was studying P granules in C elegans embryos and it was known that the organization of these P granules was is aligned along the axis

191 00:19:56.971 --> 00:20:05.884 of the embryo early an embryo Genesis and what he did was he basically applied a fundamental cell biological approach,

192 00:20:05.884 --> 00:20:15.622 which is as I just said to GFP tag something so that you can look at that molecule in a cell and then to take a movie and what he

193 00:20:15.622 --> 00:20:27.191 discovered was that. Actually, the molecules that make up these P granules are very dynamic and what allows them to accumulate and one axis of the cell and be depleted

194 00:20:27.191 --> 00:20:31.919 from the other was actually that the molecules are being stabilized,

195 00:20:31.919 --> 00:20:40.799 and that these domains that are the P granules were growing in one region of the embryo and dissolving in the other and.

196 00:20:40.799 --> 00:20:46.413 The dynamics of those molecules really revealed for the first time these ideas of phase separation.

197 00:20:46.413 --> 00:20:55.228 So there's a couple of principles that underlies this and one of them is that you have this condensation of molecules and the P granules and any kind of the

198 00:20:55.228 --> 00:21:05.444 first example that spend well characterized of this and also that the molecules themselves are actually dynamic so molecules are moving into the granular and out of the granules and

199 00:21:05.444 --> 00:21:12.359 because he was studying the growth in the disappearance of the granules in different parts of the embryo it allowed him to.

200 00:21:12.359 --> 00:21:22.566 Really quantitatively describe that behavior that has now been generalized to lots of different aspects of biology spanning from T cell.

201 00:21:22.566 --> 00:21:30.612 Receptor signaling a something that is studied by our colleague in the Cell Biology Department Doctor Zhao.

202 00:21:30.612 --> 00:21:41.040 Lei su who is examining the role that face separation plays in the immune system and number of bodies inside the nucleus that involve rnas,

203 00:21:41.040 --> 00:21:44.170 including things like stress granules and.

204 00:21:44.170 --> 00:21:54.573 Other organelles that are important for the ability of cells to rapidly respond to stress by changing their protium involve the regulation of how rnas are compartmentalized and I think

205 00:21:54.573 --> 00:22:02.782 it only keeps growing. I would say in the Dina repair field one of the things that we're interested in there is now the idea that the two ends of

206 00:22:02.782 --> 00:22:09.699 a double strand break are held together by molecules so have the ability to form this kind of phase so it's only going to,

207 00:22:09.699 --> 00:22:12.990 I think keep showing up as a concept.

208 00:22:12.990 --> 00:22:21.450 So it sounds like the role of phase separation and epigenomics chromatin structure might be more complicated than we previously thought.

209 00:22:21.450 --> 00:22:29.700 Could you speak to that and how it may be interfaces with epigenomics and genome sequencing in general.

210 00:22:29.700 --> 00:22:38.792 Yes, so I would say phase separation has emerged as not being just important for the kind of physical properties of heterochromatin.

211 00:22:38.792 --> 00:22:45.903 But there's also the idea now that a lot of kind of classic concepts of Watt regulates gene expression.

212 00:22:45.903 --> 00:22:47.680 I'll give you an example.

213 00:22:47.680 --> 00:23:00.395 One of the modifications. We know that it's essential for productive transcription is the phosphorylation of the C terminal domain of RNA polymerase 2 and it's now appreciated that that

214 00:23:00.395 --> 00:23:09.730 phosphorylation. Probably drives a phase transition so we've again this is like prior knowledge that we've known a lot about the modification.

215 00:23:09.730 --> 00:23:20.130 But the assumption was that that modification was related to kind of classic biochemistry of assembling all the right factors to get productive transcription.

216 00:23:20.130 --> 00:23:31.182 Now that's been kind of re envisioned as actually determining a phase and that phase may be a mechanism of incorporating all of the factors that might like to partition

217 00:23:31.182 --> 00:23:34.809 into that phase and exclude factors that might inhibit.

218 00:23:34.809 --> 00:23:43.557 The productive transcription, so it's interesting to watch really classic knowledge be kind of rethought into this concept of phase separation,

219 00:23:43.557 --> 00:23:52.061 which may explain behaviors that we thought we understood but we understood that may be more in a test tube or more from chip seq so right.

220 00:23:52.061 --> 00:23:59.532 One thing that people do is they could use an antibody to phosphorylated C terminal domain and if you were to do genomics.

221 00:23:59.532 --> 00:24:06.769 You would see that that modification is enriched and all the actively transcribing jeans so it was a characteristic.

222 00:24:06.769 --> 00:24:13.710 But really its function is probably something that's only been recently understood in the context of phase separation.

223 00:24:13.710 --> 00:24:20.373 Yeah, I'm happy that we've had a chance to talk about phase separation at such a hot topic in cell biology right now,

224 00:24:20.373 --> 00:24:26.696 it feels like every cell biology seminar that you go to somebody says face separation at some point or another.

225 00:24:26.696 --> 00:24:35.788 I think that that's true at the same time I think there's also starting to be a little bit of a backlash where people are seeing phase separation everywhere and

226 00:24:35.788 --> 00:24:45.500 I will just make the point that most of the evidence for face separation or at least most of the biochemical understanding for phase separation comes from in vitro studies.

227 00:24:45.500 --> 00:24:53.039 And there are still a major questions about not just whether it occurs in cells because I think that there's good evidence for that,

228 00:24:53.039 --> 00:24:55.534 but really what the functional importances,

229 00:24:55.534 --> 00:25:05.342 so that means what we need in cell biology are tools that can dissect the phase separation behavior from the other functions of the structured domains of those proteins and

230 00:25:05.342 --> 00:25:12.654 a lot of that work is yet to be done and so I think there's a real need in the future for a kind of dissecting the role of phase

231 00:25:12.654 --> 00:25:17.869 separation from with regards to the function of the processes in which it's been implicated.

232 00:25:17.869 --> 00:25:22.586 I think there's also room for other phase changes right so there's this idea.

233 00:25:22.586 --> 00:25:24.765 Liquid liquid like fairies changes,

234 00:25:24.765 --> 00:25:27.001 which which Megan just talked about,

235 00:25:27.001 --> 00:25:31.115 but there's also evidence that complexes of proteins can form gels.

236 00:25:31.115 --> 00:25:37.526 For example, actually one could argue that some of the initial data supporting the concept that proteins,

237 00:25:37.526 --> 00:25:45.811 particularly intrinsically disordered proteins, which many of these phase separated demands are considered the constituents are in fact,

238 00:25:45.811 --> 00:25:50.650 these intrinsically disordered proteins that conform multi valent interactions,

239 00:25:50.650 --> 00:26:02.305 which allows them to. They separate was discovered earlier than that and actually in the context of the nuclear pore and this is work done by colleagues dirt.

240 00:26:02.305 --> 00:26:06.473 Garlic and Germany, who suggested almost 20 years ago.

241 00:26:06.473 --> 00:26:14.736 The idea actually that the nuclear pore which is this conduit that controls all molecular traffic is itself.

242 00:26:14.736 --> 00:26:19.739 The reason why it can be selective and to what can go through it,

243 00:26:19.739 --> 00:26:24.589 which is key to write establishing this nuclear insider plasmic.

244 00:26:24.589 --> 00:26:29.446 I'm barrier. Is actually mediated through phase property?

245 00:26:29.446 --> 00:26:36.000 Where these these these nuclear pore proteins form essentially a gel and they form a gel,

246 00:26:36.000 --> 00:26:49.105 which is capable actually at least in a test tube re capitulating many of the fundamental aspects of nuclear transport and that means that it's selected for some molecules whereas

247 00:26:49.105 --> 00:26:56.460 a excludes others, and this is was really pioneering work and what's interesting is that so it's not?

248 00:26:56.460 --> 00:27:00.188 Are liquid? On the other hand,

249 00:27:00.188 --> 00:27:11.634 a lot of these phase separated demands that Megan brought up do actually change their properties over a native age actually and so they can move from a liquid state

250 00:27:11.634 --> 00:27:17.046 to a gel like State to even more solid state and this is thought to be often.

251 00:27:17.046 --> 00:27:23.636 Continuum also related to function in ways that maybe also pathological so in some some cases.

252 00:27:23.636 --> 00:27:33.579 These domains actually essentially can never be disassembled may esentially become stationary and obviously the dynamics are very critical.

253 00:27:33.579 --> 00:27:43.510 For their function and so I think one of the interesting things that we're going to have to address in the future is sort of how these chromatin domains if

254 00:27:43.510 --> 00:27:54.016 they do move to these sort of solid like states are there mechanisms to release them from that and other ways to potentially clear these aggregates if you will from

255 00:27:54.016 --> 00:27:59.873 the nucleus? Which is another interesting area of nuclear biology that we're interested in.

256 00:27:59.873 --> 00:28:04.099 We're interested in essentially how you are able to recognize.

257 00:28:04.099 --> 00:28:07.478 Clear damage from within this nuclear compartment,

258 00:28:07.478 --> 00:28:13.439 which is generally thought to be segregated from some of the major decorative organelles.

259 00:28:13.439 --> 00:28:15.890 For example, process called Atapa G,

260 00:28:15.890 --> 00:28:26.090 which is essentially a process where the cell can eat large chunks of the site is or even eat portions of organelles and in order to sort of clear damage

261 00:28:26.090 --> 00:28:31.721 and clear stress from the cell and this is also accumulates with age in the nucleus.

262 00:28:31.721 --> 00:28:35.180 But how you actually access the nucleus by this.

263 00:28:35.180 --> 00:28:42.690 Dark machinery is actually a very enigmatic in some despite evidence that it probably happens if that makes sense.

264 00:28:42.690 --> 00:28:49.410 What do you think the next big thing is that we're going to learn about the nucleus?

265 00:28:49.410 --> 00:28:54.105 Like what are you anticipating is going to come out next?

266 00:28:54.105 --> 00:29:07.670 I think one of the major areas that was really unanticipated Anas come from numerous fronts over the past probably only 5 years is the recognition that.

267 00:29:07.670 --> 00:29:11.339 Segregating the we can think of as the host genome.

268 00:29:11.339 --> 00:29:23.269 The genome of the cell inside the nucleus is really a critical aspect of ensuring that the innate immune system is able to function properly so the innate immune system

269 00:29:23.269 --> 00:29:26.445 as surveillance mechanisms in the cytoplasm,

270 00:29:26.445 --> 00:29:36.185 which are looking for RNA and DNA because that is a sign that the cell is infected with a bacteria or with a virus and that leads to an 8

271 00:29:36.185 --> 00:29:43.019 immune signaling which can lead to inflammation can bring in the adaptive immune system etc.

272 00:29:43.019 --> 00:29:53.019 Those mechanisms when you think about it really rely on the fact that the DNA is housed in the nucleus so that it's not surveilled by those receptors that are

273 00:29:53.019 --> 00:29:55.787 out there looking for nucleic acids and so,

274 00:29:55.787 --> 00:29:58.365 if we come back to some of the concepts.

275 00:29:58.365 --> 00:30:00.692 We already talked about for example,

276 00:30:00.692 --> 00:30:10.567 that you can have these ruptures of the nucleus that expose the DNA to the cytoplasm if you have defects in these nuclear pore complex is so that you're not

277 00:30:10.567 --> 00:30:14.039 able to maintain the barrier of the nucleus properly.

278 00:30:14.039 --> 00:30:27.304 This can lead to the exposure of the genomic DNA to this machinery and this is something that I think we didn't really quite anticipate how important nuclear compartmentalization is

279 00:30:27.304 --> 00:30:33.644 to prevent inflammation. So this you can think of this in the context of autoimmunity.

280 00:30:33.644 --> 00:30:40.642 For example, you're going to get an autoimmune and inflammatory reaction if these systems fail.

281 00:30:40.642 --> 00:30:44.140 The other context of this that was probably not.

282 00:30:44.140 --> 00:30:48.932 Also, none anticipated anticipated comes from cancer biology.

283 00:30:48.932 --> 00:30:57.050 So it may well be that these kind of classic changes on nuclear architecture that are known to manifest,

284 00:30:57.050 --> 00:31:05.013 particularly metastatic cancer cells so all cancer is diagnosed and staged by looking at nuclear size.

285 00:31:05.013 --> 00:31:14.910 Nuclear appearance and the kind of appearance of chromatin and nuclear bodies like the nucleolus and we really don't understand.

286 00:31:14.910 --> 00:31:24.013 Why that's such a good diagnostic which is kind of very frustrating for people who have been studying nuclear architecture for their entire careers.

287 00:31:24.013 --> 00:31:27.130 So I think that's a major big unanswered question.

288 00:31:27.130 --> 00:31:30.429 But to come back to the kind of New Horizons of that.

289 00:31:30.429 --> 00:31:34.890 One idea is that whatever is a driver of those structural abnormalities.

290 00:31:34.890 --> 00:31:45.520 These kind of nuclear ruptures can lead to the engagement of the innate immune system and that what you normally think about the contents of infection could be really useful

291 00:31:45.520 --> 00:31:49.920 as a way that multi cellular organisms are able to identify these cells.

292 00:31:49.920 --> 00:31:52.820 That have manifested with this kind of damage,

293 00:31:52.820 --> 00:31:57.820 and to remove them so just like I saw a Organism wants to remove infected cells.

294 00:31:57.820 --> 00:32:08.685 It also probably wants to remove cells that have undergone this kind of catastrophic damage leading to losses of genome integrity and that it might surveil that by looking for

295 00:32:08.685 --> 00:32:11.789 these defects in the nuclear barrier.

296 00:32:11.789 --> 00:32:16.530 At the same time, it's also likely that a lot of our cancer therapies.

297 00:32:16.530 --> 00:32:28.351 When we irradiate cells that can lead to failures of mitosis where we don't re establish the nuclear Berryer when cells exit the mitotic mitosis when they would have segregated

298 00:32:28.351 --> 00:32:34.093 their chromosomes and this probably also leads to surveillance by the same machinery.

299 00:32:34.093 --> 00:32:42.240 Zan so there's a new recognition that molecules involved in an AI mean sensing these are molecules like see gas and sting.

300 00:32:42.240 --> 00:32:53.471 Which the immunologists have been studying for a long time are likely very important for cells for organisms to call bad cells,

301 00:32:53.471 --> 00:33:09.002 but also for therapies to work to allow to allow a patient to respond to radiation by actually killing tumor cells that those processes are actually dependent on this assessing

302 00:33:09.002 --> 00:33:12.250 the integrity of the nuclear barrier.

303 00:33:12.250 --> 00:33:14.991 Is probably something that was happening all along?

304 00:33:14.991 --> 00:33:21.426 When we've developed therapies but we didn't know that that was the mechanism and so understanding that mechanism better,

305 00:33:21.426 --> 00:33:26.912 so that we can actually leverage it more effectively and cancer therapy and particularly immunotherapy,

306 00:33:26.912 --> 00:33:28.336 which is a really rapidly.

307 00:33:28.336 --> 00:33:34.980 Expanding aspect of cancer. Therapies is something that really may come back to this fundamental cell biology of the nucleus,

308 00:33:34.980 --> 00:33:35.930 which is exciting.

309 00:33:35.930 --> 00:33:38.250

310 00:33:38.250 --> 00:33:46.564 So it seems like soft matter physics and just this phase separation question is becoming or is showing to be ineligible to cell biology?

311 00:33:46.564 --> 00:33:57.380 Can you talk about maybe some of the challenges that you faced being primarily biologists and moving into a field now that is historically been dominated by physicists.

312 00:33:57.380 --> 00:34:03.619 I I mean, I think that it was interesting is a lot of the early discoveries here.

313 00:34:03.619 --> 00:34:11.230 We're sort of made by physicists working in biology fields and I think what's really most exciting.

314 00:34:11.230 --> 00:34:13.893 I think about modern cell biology?

315 00:34:13.893 --> 00:34:17.545 Is actually how multidisciplinary it really is.

316 00:34:17.545 --> 00:34:20.362 And so physicists bio fermentations.

317 00:34:20.362 --> 00:34:24.318 Computational computer scientists. You know 'cause.

318 00:34:24.318 --> 00:34:27.438 We have to deal with huge data analysis.

319 00:34:27.438 --> 00:34:29.769 Now, if large datasets from.

320 00:34:29.769 --> 00:34:41.650 Really sophisticated electron microscopy from really sophisticated high throughput screening in these sort of things that machine learning is really a big part of what's coming in in cell biology?

321 00:34:41.650 --> 00:34:52.567 So I think that one of the most exciting features is actually how multidisciplinary cell biology has become Megan can comment probably more about that since she works directly with

322 00:34:52.567 --> 00:34:55.597 physicists. Yeah, I mean for me as I said,

323 00:34:55.597 --> 00:34:58.456 I started as a biophysicist really by training,

324 00:34:58.456 --> 00:35:03.460 so it's to me. It's a fantastic development of cell that cell biology really needs.

325 00:35:03.460 --> 00:35:08.166 People who are used to thinking about those aspects of problems so physicists.

326 00:35:08.166 --> 00:35:19.367 You're absolutely right soft matter physicists are actually that it's a group of soft matter physicists from which this original concept of phase separation and cell biology arose from so

327 00:35:19.367 --> 00:35:26.119 that you're exactly right that is a really important lens through which to see these aspects of cell biology.

328 00:35:26.119 --> 00:35:29.346 Uh what island and we have been in my own work,

329 00:35:29.346 --> 00:35:35.059 actually some of the most impactful concepts are coming from soft matter physicists?

330 00:35:35.059 --> 00:35:42.789 Who are studying phase separation and non biological systems and that's that's my very impactful for us.

331 00:35:42.789 --> 00:35:54.126 I think there are some challenges so one of the challenges is that soft matter physicists are used to thinking or have classically described these problems from equilibrium models,

332 00:35:54.126 --> 00:35:59.137 which makes a lot of sense when you're working on inert non biological systems,

333 00:35:59.137 --> 00:36:01.456 but living cells are absolutely not.

334 00:36:01.456 --> 00:36:07.782 At equilibrium so we really need to work with physicists and we there are individuals in this field,

335 00:36:07.782 --> 00:36:14.860 that are are making steps towards this to start to be sure that our theory that we're applying to these problems.

336 00:36:14.860 --> 00:36:19.076 Is actually well suited to the complexities of the biology?

337 00:36:19.076 --> 00:36:26.315 While not making it so complex that you can't try to use first principles to define and understand it,

338 00:36:26.315 --> 00:36:27.650 so in my own work,

339 00:36:27.650 --> 00:36:33.271 I work. I've worked for 8 years with a physicist colleagues who's here at Yale.

340 00:36:33.271 --> 00:36:44.585 Dr Simon Mochary, an that has been critical to all of the work that we've done on nuclear mechanics and work that we're doing on chromatin organization and so I

341 00:36:44.585 --> 00:36:48.239 think that this is going to be absolutely essential.

342 00:36:48.239 --> 00:36:53.224 And what I've seen at least is that it can be extremely successful.

343 00:36:53.224 --> 00:37:05.173 If you just have people who are really driven and interested to work with others across disciplines and also you need a few people who can bridge the languages of

344 00:37:05.173 --> 00:37:16.019 these different fields. But it's been the absolutely most rewarding part of for me doing science at Yale has been through these interactions.

345 00:37:16.019 --> 00:37:21.492 With physicists and also more recently with engineers so we also work with doctor.

346 00:37:21.492 --> 00:37:25.184 Corey o'hearn with him. We do increasingly simulations,

347 00:37:25.184 --> 00:37:31.184 which isn't also I'm really another impactful approach in Cell Biology Doctor Tom Pollard.

348 00:37:31.184 --> 00:37:42.590 One of our esteemed faculty here would be the 1st to say that you don't really understand something until you can derive a mathematical model that can explain the behaviors

349 00:37:42.590 --> 00:37:46.480 that we observe in living cells and I think that that is a.

350 00:37:46.480 --> 00:37:50.931 A good goal to have it is certainly one that we have in our science.

351 00:37:50.931 --> 00:37:53.512 So our last question is to each of you?

352 00:37:53.512 --> 00:37:57.110 What's your favorite fun fact about the nucleus?

353 00:37:57.110 --> 00:38:00.621 We just talked about this on the way and I mean,

354 00:38:00.621 --> 00:38:02.914 I think the classic fact right,

355 00:38:02.914 --> 00:38:09.577 which I think is a fun fact is that you have essentially 2 meters of DNA in each cell right?

356 00:38:09.577 --> 00:38:16.420 That is somehow compacted into a tiny volume nucleus is sort of 6 microns in diameter.

357 00:38:16.420 --> 00:38:21.954 Uhm I think other fun facts would be that I think we talked a bit about nuclear shape.

358 00:38:21.954 --> 00:38:28.568 I mean, I think there is this conceptual ization in every textbook that you guys have that the nucleus?

359 00:38:28.568 --> 00:38:34.483 Is this sort of round ball and it turns out there's actually a plethora of different shapes,

360 00:38:34.483 --> 00:38:44.851 depending on cell type, so a lot of nuclear actually more like squash pancakes and all nuclear actually like sort of beads on a string that really multi lobed and

361 00:38:44.851 --> 00:38:56.204 really elaborately. They have very different morphologies and the idea is that those morphologies reflect the function of those cells and I think one thing we haven't talked about is

362 00:38:56.204 --> 00:38:58.320 is how all the cells in our body,

363 00:38:58.320 --> 00:39:00.063 have the same genome right.

364 00:39:00.063 --> 00:39:10.518 And yet they do very different things where tissues that very unique functions and I think this is one of the fundamental questions is how does nuclear shape relate to

365 00:39:10.518 --> 00:39:15.739 those unique functions? The other half.

366 00:39:15.739 --> 00:39:29.063 So fun. The other fun fact in the context of this issue that you put together on organelles is that as we've already discussed the classic definition of organelles is

367 00:39:29.063 --> 00:39:31.807 that their membrane bound compartments,

368 00:39:31.807 --> 00:39:37.230 but as we've already described the nuclear envelope is a membrane compartment.

369 00:39:37.230 --> 00:39:43.681 That's full of holes those holes are filled by these nuclear pore complex is but nonetheless.

370 00:39:43.681 --> 00:39:50.070 I think it's actually very unique in that it is really not just an intact membrane sheet.

371 00:39:50.070 --> 00:39:54.302 Ross, which things can only be pumped by channels for example,

372 00:39:54.302 --> 00:39:56.385 are imported through you know,

373 00:39:56.385 --> 00:40:00.483 small channels where unfolded proteins can be trans located?

374 00:40:00.483 --> 00:40:05.119 In fact, it has these 50 nanometer diameter holes all throughout it,

375 00:40:05.119 --> 00:40:10.829 which is really big right and so while we while it is one of the classic organelles.

376 00:40:10.829 --> 00:40:20.139 There really is a whole host of biology that we have to understand and that it has to have to actually maintain that compartmentalization.

377 00:40:20.139 --> 00:40:31.045 And and that's really kind of unique is just to keep in mind that it's not actually an intact membrane and how cells enough to be really careful to actually

378 00:40:31.045 --> 00:40:34.650 maintain its specific identity.

379 00:40:34.650 --> 00:40:42.054 Awesome so thanks so much to Doctor King and Doctor Lusk for joining us on this episode of the YJBM podcast.

380 00:40:42.054 --> 00:40:49.188 Like many scientists today. They're on Twitter so if you would like to follow them for more nucleus fun.

381 00:40:49.188 --> 00:40:51.634 You can follow them atleast King L.

382 00:40:51.634 --> 00:40:54.418 That's LUSKINGL and at Peel ask for you.

383 00:40:54.418 --> 00:40:57.869 That's PLUSK the number 4 and the letter U?

384 00:40:57.869 --> 00:41:01.896 There are many people behind this podcast that you never get a chance to hear.

385 00:41:01.896 --> 00:41:03.425 Thank you to the Yale School,

386 00:41:03.425 --> 00:41:06.074 Medicine for being our home for YJBM an the podcast.

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390 00:41:19.326 --> 00:41:26.307 Amelia Hartworth, and John Venturafinally thanks to you for tuning into this episode of the Yale Journal of Biology and medicine podcasts.

391 00:41:26.307 --> 00:41:28.498 We love to hear your feedback in question,

392 00:41:28.498 --> 00:41:31.199 so feel free to tell us your thoughts by emailing us.

393 00:41:31.199 --> 00:41:39.570 yjbm@yale.edu if you enjoyed our podcast we share it on SoundCloud or Apple podcasts?

394 00:41:39.570 --> 00:41:40.284 Thanks.