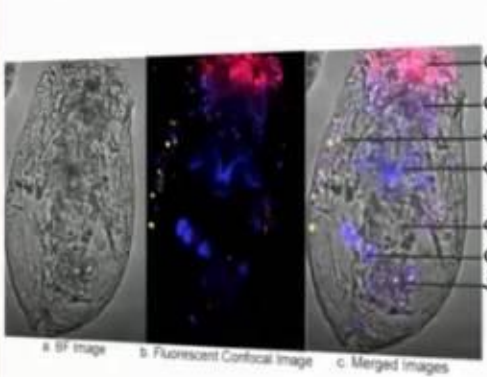
Microscopy Images from *B. manjavacas* Immunohistochemistry



B.L. Jones, et al. Stress Granules form in Brachionus manjavacas (Rotifera) in response to a variety of stresso
In Review: Comparative Biochemistry and Physiology. Part A. 2013.

1
00:00:10,670 --> 00:00:08,210
hi my name is Brandi Jones I'm a

2
00:00:12,980 --> 00:00:10,680
postdoctoral fellow with the NAI Center

3
00:00:14,930 --> 00:00:12,990
for a ribosomal of origins and evolution

4
00:00:17,029 --> 00:00:14,940
at Georgia Tech and today I want to talk

5
00:00:18,439 --> 00:00:17,039
to you about a special group of

6
00:00:20,840 --> 00:00:18,449
extremists Al's they were kind of

7
00:00:23,090 --> 00:00:20,850
skipped during the morning session but

8
00:00:25,340 --> 00:00:23,100
they're rotifers they are eukaryotes

9
00:00:28,460 --> 00:00:25,350
they're microscopic animals and they

10
00:00:31,670 --> 00:00:28,470
live in very diverse habitats in my work

11
00:00:33,049 --> 00:00:31,680
focuses on the molecular biology of how

12
00:00:35,810 --> 00:00:33,059
they're able to survive in those

13
00:00:37,459 --> 00:00:35,820

habitats and we know a lot is known

14
00:00:39,500 --> 00:00:37,469
about prokaryotes the very little is

15
00:00:41,770 --> 00:00:39,510
known about eukaryotes extreme of house

16
00:00:44,329 --> 00:00:41,780
so that's what my work practices are

17
00:00:46,779 --> 00:00:44,339
just some examples of where you can find

18
00:00:50,000 --> 00:00:46,789
rotifers and they live in alkaline lakes

19
00:00:52,759 --> 00:00:50,010
acidic blakes you can find them in hyper

20
00:00:55,369 --> 00:00:52,769
saline habitats as well as i know we've

21
00:00:59,750 --> 00:00:55,379
heard a lot about dr ali and target hell

22
00:01:05,060 --> 00:00:59,760
you can find them there as well they

23
00:01:07,310 --> 00:01:05,070
also are able to adapt to their habit

24
00:01:09,289 --> 00:01:07,320
the habitats in which they live where if

25
00:01:11,920 --> 00:01:09,299
you get extreme desiccation they can go

26
00:01:14,930 --> 00:01:11,930
from a very active living form to a

27
00:01:17,330 --> 00:01:14,940
desiccated dormant form that upon

28
00:01:20,330 --> 00:01:17,340
rehydration years later will become

29
00:01:23,510 --> 00:01:20,340
active adult and able to let live

30
00:01:28,610 --> 00:01:23,520
metabolize and just to give you an idea

31
00:01:30,650 --> 00:01:28,620
the adult can go from active swimming to

32
00:01:33,260 --> 00:01:30,660
that desiccated form within 30 minutes

33
00:01:36,050 --> 00:01:33,270
and upon rehydration you get the active

34
00:01:38,570 --> 00:01:36,060
swimming adult form within the same time

35
00:01:40,870 --> 00:01:38,580
period and so my question is how do the

36
00:01:43,100 --> 00:01:40,880
ribosomes are they able to survive and

37
00:01:47,690 --> 00:01:43,110
become active when that within that

38
00:01:49,490 --> 00:01:47,700

short of time period the model organism

39
00:01:52,550 --> 00:01:49,500
for my study is bright be honest Maha

40
00:01:54,670 --> 00:01:52,560
vacas is a rotor for a microscopic

41
00:01:57,830 --> 00:01:54,680
aquatic organism is able to withstand

42
00:02:01,430 --> 00:01:57,840
extreme heat desiccation UV irradiation

43
00:02:06,770 --> 00:02:01,440
and this here is a female carrying a

44
00:02:09,770 --> 00:02:06,780
sexual eggs they undergo both asexual

45
00:02:12,140 --> 00:02:09,780
and sexual life cycles usually it's a

46
00:02:13,990 --> 00:02:12,150
sexual where they develop the asexual

47
00:02:17,330 --> 00:02:14,000
eggs but if you get some type of

48
00:02:19,670 --> 00:02:17,340
environmental key or signal usually it's

49
00:02:21,830 --> 00:02:19,680
an environmental stress they it

50
00:02:23,509 --> 00:02:21,840
tourism to go into a sexual life cycle

51
00:02:26,270 --> 00:02:23,519
where they produce these resting eggs

52
00:02:28,339 --> 00:02:26,280
that can remain dormant for hundreds of

53
00:02:30,470 --> 00:02:28,349
years they can become completely

54
00:02:34,089 --> 00:02:30,480
desiccated you can expose them to

55
00:02:36,349 --> 00:02:34,099
extreme heat UV radiation but upon

56
00:02:40,670 --> 00:02:36,359
rehydration they're able to continue

57
00:02:42,050 --> 00:02:40,680
developing until normal adults and this

58
00:02:45,170 --> 00:02:42,060
is just an experiment I did with the

59
00:02:47,990 --> 00:02:45,180
active rotifers adult rotifers from

60
00:02:49,699 --> 00:02:48,000
three different species and then those

61
00:02:53,659 --> 00:02:49,709
desiccated forms that was just telling

62
00:02:56,360 --> 00:02:53,669
you about if you incubate and this is a

63
00:02:58,460 --> 00:02:56,370

survival ship curve and it's on the

64
00:03:01,460 --> 00:02:58,470
y-axis is the proportion surviving on

65
00:03:02,929 --> 00:03:01,470
the exes the temperature exposed to so

66
00:03:05,449 --> 00:03:02,939
the adults when exposed to temperatures

67
00:03:07,970 --> 00:03:05,459
higher than 40 degrees they die but

68
00:03:09,740 --> 00:03:07,980
those are dormant cysts you can expose

69
00:03:12,470 --> 00:03:09,750
them to temperature is greater than 100

70
00:03:16,399 --> 00:03:12,480
degrees and then upon rehydration they

71
00:03:18,220 --> 00:03:16,409
go on metabolizing so they're very um

72
00:03:20,959 --> 00:03:18,230
they can withstand extreme temperatures

73
00:03:23,300 --> 00:03:20,969
so my goal is to gather insight on how

74
00:03:27,530 --> 00:03:23,310
the ribosomes are able to withstand

75
00:03:29,809 --> 00:03:27,540
those environmental stresses how once

76
00:03:31,399 --> 00:03:29,819
you remove those organisms from the

77
00:03:34,789 --> 00:03:31,409
environmental stress the ribosomes are

78
00:03:42,020 --> 00:03:34,799
able to continue translating and form

79
00:03:44,300 --> 00:03:42,030
proteins so what I know from other

80
00:03:46,420 --> 00:03:44,310
eukaryotes is that usually when you have

81
00:03:49,689 --> 00:03:46,430
some type of environmental stress

82
00:03:52,909 --> 00:03:49,699
translation of housekeeping genes in

83
00:03:54,949 --> 00:03:52,919
normal genes they it stops the ribosome

84
00:03:56,509 --> 00:03:54,959
will fall apart you'll get the small

85
00:03:59,449 --> 00:03:56,519
subunit falls apart from the large

86
00:04:03,080 --> 00:03:59,459
subunit and the small subunit associates

87
00:04:05,240 --> 00:04:03,090
with proteins known to initiate

88
00:04:07,399 --> 00:04:05,250

translation as well as mrnas that are

89
00:04:12,439 --> 00:04:07,409
not even translated and they move into

90
00:04:16,129 --> 00:04:12,449
these stress granules and here's just an

91
00:04:18,949 --> 00:04:16,139
example from a human cell line here

92
00:04:20,960 --> 00:04:18,959
we're looking at and we're just we type

93
00:04:24,110 --> 00:04:20,970
of proteins fluoresce with fluorescent

94
00:04:26,839 --> 00:04:24,120
markers this is from a paper from 2009

95
00:04:29,769 --> 00:04:26,849
you have the large subunit and green the

96
00:04:31,839 --> 00:04:29,779
small subunit in red and then you have a

97
00:04:33,020 --> 00:04:31,849
initiation factor shown here in blue

98
00:04:35,560 --> 00:04:33,030
apply

99
00:04:38,660 --> 00:04:35,570
of stress with arsenite you get

100
00:04:41,810 --> 00:04:38,670
co-localization of the large the small

101
00:04:44,150 --> 00:04:41,820
subunit as well as the initiation factor

102
00:04:46,610 --> 00:04:44,160
but the large subunit doesn't colocalize

103
00:04:49,160 --> 00:04:46,620
with them and this right here is what we

104
00:04:51,860 --> 00:04:49,170
call a stress granule you see it here

105
00:04:53,720 --> 00:04:51,870
and shown over here is the same thing

106
00:04:56,690 --> 00:04:53,730
these three proteins or three initiation

107
00:04:58,580 --> 00:04:56,700
factors upon I'm stress you get

108
00:05:04,460 --> 00:04:58,590
co-localization of all three proteins

109
00:05:06,770 --> 00:05:04,470
and stress granule production so my

110
00:05:09,200 --> 00:05:06,780
hypothesis is that these stress granules

111
00:05:11,360 --> 00:05:09,210
form and the rotor first or in periods

112
00:05:13,490 --> 00:05:11,370
of environmental stress and they protect

113
00:05:15,350 --> 00:05:13,500

the ribosome during those periods and

114
00:05:17,810 --> 00:05:15,360
then upon removal of the environmental

115
00:05:20,810 --> 00:05:17,820
stress you get reassociation of the

116
00:05:25,400 --> 00:05:20,820
ribosome and the organism are able to go

117
00:05:27,230 --> 00:05:25,410
on metabolizing so what I know from

118
00:05:29,870 --> 00:05:27,240
previous studies done with both use the

119
00:05:31,760 --> 00:05:29,880
mammalian cells is that unstressed cells

120
00:05:35,120 --> 00:05:31,770
do not have stress granules they're not

121
00:05:37,220 --> 00:05:35,130
present he upon heat stress you get

122
00:05:40,730 --> 00:05:37,230
stress granule production as well as

123
00:05:43,610 --> 00:05:40,740
starvation hyperosmolarity and then if

124
00:05:46,210 --> 00:05:43,620
you incubate them in the presence of an

125
00:05:49,660 --> 00:05:46,220
antibiotic called cycloheximide it

126
00:05:52,040 --> 00:05:49,670
inhibits stress final formation but the

127
00:05:55,070 --> 00:05:52,050
antibiotic promote stress for Niall

128
00:05:59,450 --> 00:05:55,080
formation so I decided to test all these

129
00:06:01,460 --> 00:05:59,460
conditions with my rotifers and the

130
00:06:04,640 --> 00:06:01,470
different environmental stresses i use

131
00:06:08,090 --> 00:06:04,650
whereas modoch stress you normally grow

132
00:06:12,470 --> 00:06:08,100
the Rutherfords in fifth artificial

133
00:06:15,980 --> 00:06:12,480
seawater containing 15 ppt salt I buried

134
00:06:18,770 --> 00:06:15,990
it up to 45 p bt i deprived them of

135
00:06:21,230 --> 00:06:18,780
nutrients for several days and then I

136
00:06:22,970 --> 00:06:21,240
heat shock them and I did all of those

137
00:06:24,680 --> 00:06:22,980
things and then I followed the movement

138
00:06:28,970 --> 00:06:24,690

of three proteins known to be associated

139
00:06:30,830 --> 00:06:28,980
with stress granules the three proteins

140
00:06:34,850 --> 00:06:30,840
are TI a one is that an rna-binding

141
00:06:37,510 --> 00:06:34,860
protein is involved in mrna splicing and

142
00:06:40,670 --> 00:06:37,520
then to eukaryotic initiation factors

143
00:06:42,650 --> 00:06:40,680
eif4e and ya s-3b all three of these

144
00:06:44,900 --> 00:06:42,660
proteins are known to co-localize the

145
00:06:45,690 --> 00:06:44,910
stress granules upon stress

146
00:06:50,580 --> 00:06:45,700
environmental

147
00:06:52,050 --> 00:06:50,590
stress in yeast and among yourselves so

148
00:06:55,050 --> 00:06:52,060
just so you can follow the rest of my

149
00:06:57,180 --> 00:06:55,060
experiment to follow the proteins i use

150
00:07:00,120 --> 00:06:57,190
anna antibodies and they were

151
00:07:01,950 --> 00:07:00,130
fluorescently tagged so here TI a one

152
00:07:06,930 --> 00:07:01,960
will always appear red in my pictures

153
00:07:10,200 --> 00:07:06,940
eif 3b will appear magenta eif4e will be

154
00:07:13,160 --> 00:07:10,210
green and then the DNA and some pictures

155
00:07:16,290 --> 00:07:13,170
I sing with that be in an appears blue

156
00:07:19,080 --> 00:07:16,300
and this is just an example of the

157
00:07:20,880 --> 00:07:19,090
microscopy pictures you'll see here's a

158
00:07:24,870 --> 00:07:20,890
bright field image showing just the

159
00:07:28,050 --> 00:07:24,880
rotifer here's a confocal image you see

160
00:07:30,690 --> 00:07:28,060
the nuclei and some of the red and

161
00:07:32,400 --> 00:07:30,700
magenta and some of the green and those

162
00:07:34,740 --> 00:07:32,410
are the three proteins and wherever

163
00:07:37,470 --> 00:07:34,750

those proteins co-localize you'll see a

164
00:07:39,720 --> 00:07:37,480
yellow or orange color shown here you're

165
00:07:41,790 --> 00:07:39,730
here and then I have them labeled so you

166
00:07:47,400 --> 00:07:41,800
can see the organs and which they

167
00:07:51,390 --> 00:07:47,410
colocalize so in my first experiment I

168
00:07:56,400 --> 00:07:51,400
deprived them of nutrients for 48 hours

169
00:07:58,740 --> 00:07:56,410
and just so you can see each column here

170
00:08:02,850 --> 00:07:58,750
is the individual protein that I

171
00:08:05,550 --> 00:08:02,860
followed so y is 3 be as you continue

172
00:08:11,100 --> 00:08:05,560
with starvation you see aggregation of

173
00:08:13,950 --> 00:08:11,110
eif 3b as well as eif4e and CIA one they

174
00:08:15,720 --> 00:08:13,960
each begin to aggregate amongst

175
00:08:17,810 --> 00:08:15,730
themselves but then i'm showing you a

176
00:08:21,030 --> 00:08:17,820
merged image and you can see that

177
00:08:23,070 --> 00:08:21,040
they're colocalize into the same

178
00:08:26,430 --> 00:08:23,080
location and just in case you can't see

179
00:08:28,530 --> 00:08:26,440
what i see i use a software that labels

180
00:08:31,290 --> 00:08:28,540
white and where those three proteins

181
00:08:33,540 --> 00:08:31,300
co-localize so you see as you increase

182
00:08:35,790 --> 00:08:33,550
the time period of starvation you're

183
00:08:37,620 --> 00:08:35,800
going to increase of colocalization of

184
00:08:39,690 --> 00:08:37,630
the three proteins within the major

185
00:08:42,270 --> 00:08:39,700
organ systems which is indicative of

186
00:08:45,210 --> 00:08:42,280
stress granule formation and here I've

187
00:08:47,520 --> 00:08:45,220
just zoomed in and you see the yellow

188
00:08:50,610 --> 00:08:47,530

and orange here I've also done some

189
00:08:57,650 --> 00:08:50,620
statistical analysis using a matters

190
00:09:00,680 --> 00:08:59,090
the coefficient when you get a zero it

191
00:09:02,510 --> 00:09:00,690
means there's no colocalization of the

192
00:09:04,880 --> 00:09:02,520
proteins and a one means perfect

193
00:09:09,050 --> 00:09:04,890
colocalization and you can see it goes

194
00:09:14,270 --> 00:09:09,060
from point 344 at 24 hours and it peaks

195
00:09:21,320 --> 00:09:14,280
at point seven on at 48 hours does that

196
00:09:23,660 --> 00:09:21,330
mean five left or left okay and then my

197
00:09:27,680 --> 00:09:23,670
next experiment is osmotic shop where I

198
00:09:31,490 --> 00:09:27,690
buried it from the 15 ppt asw to the 35

199
00:09:34,820 --> 00:09:31,500
on ppt so the 15 ppt is the control and

200
00:09:37,760 --> 00:09:34,830
as you see as we increase the osmolarity

201
00:09:39,800 --> 00:09:37,770
you get an increase in aggregation of

202
00:09:41,600 --> 00:09:39,810
each of the individual proteins it's not

203
00:09:43,880 --> 00:09:41,610
as dramatic as we saw before in the

204
00:09:47,450 --> 00:09:43,890
nutrient deprivation but you do see

205
00:09:51,260 --> 00:09:47,460
co-localization and it goes from point

206
00:09:54,140 --> 00:09:51,270
three seven in the control 2.79 so

207
00:09:59,510 --> 00:09:54,150
that's indicative of colocalization is

208
00:10:02,000 --> 00:09:59,520
stressful any old formation I under heat

209
00:10:04,480 --> 00:10:02,010
stress I buried the time that we heat

210
00:10:06,830 --> 00:10:04,490
shock them from zero to 20 minutes and

211
00:10:09,110 --> 00:10:06,840
you see the same pattern you see an

212
00:10:11,510 --> 00:10:09,120
increase in aggregation of each of the

213
00:10:14,930 --> 00:10:11,520

individual proteins and an increase in

214
00:10:19,640 --> 00:10:14,940
colocalization and the major organ organ

215
00:10:22,160 --> 00:10:19,650
systems which is like the other is

216
00:10:26,090 --> 00:10:22,170
indicative of coagulation and stress

217
00:10:28,310 --> 00:10:26,100
granule formation so here I wanted to

218
00:10:31,370 --> 00:10:28,320
show you that the stress final formation

219
00:10:33,260 --> 00:10:31,380
is dynamic you get stress granules form

220
00:10:35,780 --> 00:10:33,270
but when you remove the stress those

221
00:10:38,090 --> 00:10:35,790
stressful angles will go away so here at

222
00:10:40,220 --> 00:10:38,100
unstressed rotifers you don't see any of

223
00:10:43,250 --> 00:10:40,230
those allegations and co-localization

224
00:10:46,460 --> 00:10:43,260
you see it with 30 minutes of heat

225
00:10:48,110 --> 00:10:46,470
stress and then I let them recover for

226
00:10:51,680 --> 00:10:48,120
three hours so I put them back at their

227
00:10:53,870 --> 00:10:51,690
normal temperature to grow and that was

228
00:10:55,670 --> 00:10:53,880
room temperature and you see a decrease

229
00:10:58,610 --> 00:10:55,680
in the amount of stress my angles formed

230
00:11:03,460 --> 00:10:58,620
so these stress granules are dynamic

231
00:11:04,630 --> 00:11:03,470
they form and then they dissipate and

232
00:11:06,640 --> 00:11:04,640
here

233
00:11:09,010 --> 00:11:06,650
antibody puromycin that's known to

234
00:11:11,920 --> 00:11:09,020
promote stress for information and other

235
00:11:14,050 --> 00:11:11,930
organisms we see that when you incubate

236
00:11:16,330 --> 00:11:14,060
them with the pur- inand the heat stress

237
00:11:20,160 --> 00:11:16,340
you get on the dramatic amount of stress

238
00:11:22,420 --> 00:11:20,170

granules forming and then the other

239
00:11:24,880 --> 00:11:22,430
antibiotic cyclohexyl might that's known

240
00:11:26,350 --> 00:11:24,890
to prevent stress triangle formation I'm

241
00:11:31,170 --> 00:11:26,360
just the same here you don't see any

242
00:11:34,150 --> 00:11:31,180
colocalization of those proteins and

243
00:11:37,150 --> 00:11:34,160
here it's a deviation from my other

244
00:11:41,100 --> 00:11:37,160
pictures the green here is actually the

245
00:11:46,510 --> 00:11:41,110
large ribosomal subunit I labeled it I

246
00:11:48,370 --> 00:11:46,520
labeled puromycin with the green

247
00:11:51,520 --> 00:11:48,380
fluorescent well here is 50 which is

248
00:11:53,320 --> 00:11:51,530
green and then I incubated it with the

249
00:11:54,850 --> 00:11:53,330
rotor first after they have been heat

250
00:11:57,100 --> 00:11:54,860
stress though it's going to bind to the

251
00:12:00,190 --> 00:11:57,110
large ribosomal subunit which is known

252
00:12:01,870 --> 00:12:00,200
in other organisms not to move into

253
00:12:04,840 --> 00:12:01,880
stress granules and usually on the

254
00:12:07,620 --> 00:12:04,850
periphery and you see aggregation with

255
00:12:09,850 --> 00:12:07,630
heat stress so these are the other two

256
00:12:11,590 --> 00:12:09,860
proteins that you saw before you see

257
00:12:13,780 --> 00:12:11,600
that there are co-localized like they

258
00:12:14,950 --> 00:12:13,790
did before the red and magenta but the

259
00:12:18,490 --> 00:12:14,960
green which is the large ribosomal

260
00:12:25,030 --> 00:12:18,500
subunit is not co-localize arrest

261
00:12:27,130 --> 00:12:25,040
renewals so just in conclusion what I've

262
00:12:28,540 --> 00:12:27,140
seen in rotor first is consistent with

263
00:12:31,450 --> 00:12:28,550

what we see in both yeast in mammalian

264
00:12:33,610 --> 00:12:31,460
cells that an unstressed cells you do

265
00:12:36,280 --> 00:12:33,620
not get stressful annual formation but

266
00:12:38,170 --> 00:12:36,290
in other environmental stresses that we

267
00:12:39,760 --> 00:12:38,180
test it we got stress granule formation

268
00:12:42,300 --> 00:12:39,770
just as you see in yeast and mammalian

269
00:12:44,680 --> 00:12:42,310
cells the antibiotic purim I

270
00:12:47,410 --> 00:12:44,690
cycloheximide prevented stress mariel

271
00:12:51,820 --> 00:12:47,420
formation and pura myosin promoted it

272
00:12:53,920 --> 00:12:51,830
and the components of the stress

273
00:12:57,280 --> 00:12:53,930
granules and rotor first were consistent

274
00:13:00,160 --> 00:12:57,290
with what we see in other organisms so

275
00:13:04,000 --> 00:13:00,170
just what I can conclude from this study

276
00:13:05,770 --> 00:13:04,010
is that the stress granules are most

277
00:13:07,930 --> 00:13:05,780
probably of an ancient origin because

278
00:13:12,150 --> 00:13:07,940
you see them early in the evolution of

279
00:13:15,460 --> 00:13:12,160
animals rotifers as well as in mammals

280
00:13:17,079 --> 00:13:15,470
and I want to thank on the labs I work

281
00:13:18,819 --> 00:13:17,089
with the snow lab

282
00:13:21,129 --> 00:13:18,829
at the school of biology at Georgia Tech

283
00:13:23,590 --> 00:13:21,139
the Williams lab in school chemistry at

284
00:13:27,160 --> 00:13:23,600
Georgia Tech and the Dunham lab at Emory

285
00:13:28,749 --> 00:13:27,170
as well as NASA and my Center on the

286
00:13:35,110 --> 00:13:28,759
Center for ribosomal origins and

287
00:13:42,369 --> 00:13:35,120
evolution thank you do we have any

288
00:13:43,960 --> 00:13:42,379

questions for brandy hi sorry if I miss

289
00:13:45,939 --> 00:13:43,970
this but is there anything special about

290
00:13:47,670 --> 00:13:45,949
the stress granules of the rotifers that

291
00:13:51,040 --> 00:13:47,680
makes them better able to withstand

292
00:13:54,340 --> 00:13:51,050
straw so that would be what I want to

293
00:13:55,989 --> 00:13:54,350
determine later on we don't know now is

294
00:13:58,660 --> 00:13:55,999
the first time they've ever been seen in

295
00:14:00,429 --> 00:13:58,670
rotifers but I would like to see if the

296
00:14:02,769 --> 00:14:00,439
stress granules swarm quicker and

297
00:14:04,150 --> 00:14:02,779
rotifers if you if they're more dynamic

298
00:14:10,720 --> 00:14:04,160
i'm not sure but that's something I

299
00:14:12,939 --> 00:14:10,730
definitely want to look into I could you

300
00:14:17,799 --> 00:14:12,949
go back to your slide with the the heat

301
00:14:20,290 --> 00:14:17,809
stress over time early earlier yeah that

302
00:14:24,280 --> 00:14:20,300
one the very bottom with the T 1 a 1

303
00:14:26,829 --> 00:14:24,290
where it's not showing any sort of and

304
00:14:28,840 --> 00:14:26,839
any red dump yes what's going on there I

305
00:14:31,509 --> 00:14:28,850
mean why is it just disappeared do you

306
00:14:34,949 --> 00:14:31,519
think it has a lot of it I don't know if

307
00:14:38,499 --> 00:14:34,959
you can see it yeah it has a lot of it

308
00:14:40,269 --> 00:14:38,509
okay because all right maybe I've just

309
00:14:42,879 --> 00:14:40,279
read blind for the bottom there but I

310
00:14:46,269 --> 00:14:42,889
doesn't see anything yeah it's there I

311
00:14:49,299 --> 00:14:46,279
do think I agree that there is more here

312
00:14:53,429 --> 00:14:49,309
than there but it's still a lot I'm

313
00:14:55,809 --> 00:14:53,439

present but I think there's a limit to

314
00:14:57,549 --> 00:14:55,819
the stress granules form they're

315
00:15:00,369 --> 00:14:57,559
supposed to be dynamic and I really

316
00:15:02,139 --> 00:15:00,379
didn't give the animals and opportunity

317
00:15:04,540 --> 00:15:02,149
to it just like they would normally do I

318
00:15:08,290 --> 00:15:04,550
just take them and move them from their

319
00:15:10,360 --> 00:15:08,300
normal growth temperature to this heat

320
00:15:12,579 --> 00:15:10,370
shock and normally they would have time

321
00:15:14,829 --> 00:15:12,589
to acclimate to it so I think probably

322
00:15:19,079 --> 00:15:14,839
20 minutes was pushing the limits for

323
00:15:21,549 --> 00:15:19,089
them and so they start to kind of guy

324
00:15:23,829 --> 00:15:21,559
let's do you use pure mind sending what

325
00:15:27,429 --> 00:15:23,839
was the other cycloheximide do you know

326
00:15:29,600 --> 00:15:27,439
how that works how that antibiotic and

327
00:15:32,329 --> 00:15:29,610
so cyclohexyl might prevent

328
00:15:35,509 --> 00:15:32,339
elongation and Pyramus and prevent

329
00:15:39,620 --> 00:15:35,519
initiation of I'm translation and the

330
00:15:41,900 --> 00:15:39,630
prevent initiation it promotes a signal

331
00:15:44,360 --> 00:15:41,910
a signaling cascade that promotes these

332
00:15:45,769 --> 00:15:44,370
stress modules formation and the cycle

333
00:15:48,889 --> 00:15:45,779
hexa might because you're stopping it at

334
00:15:51,019 --> 00:15:48,899
a different time point it doesn't start

335
00:15:53,000 --> 00:15:51,029
that cascade by segment with cascading

336
00:15:54,860 --> 00:15:53,010
the same one would it be worth looking

337
00:15:58,400 --> 00:15:54,870
at other antibiotics to give you clues

338
00:15:59,990 --> 00:15:58,410

about Eric um they have been like with

339
00:16:02,030 --> 00:16:00,000
the east of mammalian cells there are

340
00:16:04,009 --> 00:16:02,040
there a number of different antibiotics

341
00:16:06,400 --> 00:16:04,019
that they have looked at and that just

342
00:16:08,920 --> 00:16:06,410
grabbed two from the list but if you are

343
00:16:11,810 --> 00:16:08,930
the antibiotics that prevent an

344
00:16:14,660 --> 00:16:11,820
initiation of translation all promote

345
00:16:16,880 --> 00:16:14,670
stress granule formation because you end

346
00:16:21,530 --> 00:16:16,890
up getting on phosphorylation of one of