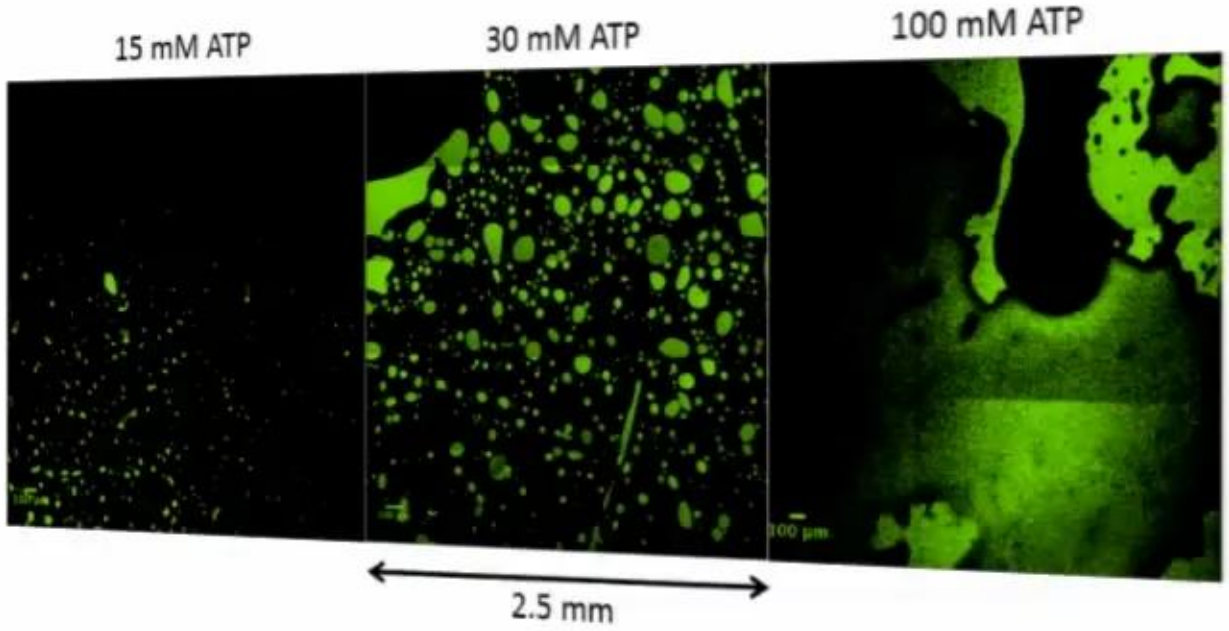
Complex Coacervates



Jia, et al. *Orig. Life Evol. Biosph.* **2014**

1
00:00:13,140 --> 00:00:11,250
alright hi everyone um yeah so first of

2
00:00:15,800 --> 00:00:13,150
all thanks for having me um first time

3
00:00:19,920 --> 00:00:15,810
speaker so it's been going well so far

4
00:00:22,410 --> 00:00:19,930
and i work for the Shostak lab at

5
00:00:24,690 --> 00:00:22,420
harvard and we're interested in a

6
00:00:27,090 --> 00:00:24,700
probiotic RNA replication so I'm going

7
00:00:30,480 --> 00:00:27,100
to talk a little bit about non-enzymatic

8
00:00:34,819 --> 00:00:30,490
RNA replication and then how tides can

9
00:00:42,119 --> 00:00:39,239
yeah is this good great alright so we

10
00:00:46,290 --> 00:00:42,129
got a pretty nice introduction today to

11
00:00:48,630 --> 00:00:46,300
the RNA world by from the warm-up talk

12
00:00:51,989 --> 00:00:48,640
and so just briefly presidents

13
00:00:54,059 --> 00:00:51,999

present-day cells we believe that you

14
00:00:56,869 --> 00:00:54,069
know center dot central dogma holds you

15
00:00:59,849 --> 00:00:56,879
go from DNA to RNA to protein but

16
00:01:02,819 --> 00:00:59,859
previously you know in the evolution of

17
00:01:06,330 --> 00:01:02,829
life it's possible that RNA was the

18
00:01:09,690 --> 00:01:06,340
predominant molecule inside sales and

19
00:01:11,310 --> 00:01:09,700
because they store stores and passes

20
00:01:13,109 --> 00:01:11,320
down genetic information and it can

21
00:01:17,359 --> 00:01:13,119
catalyze chemical reactions and to that

22
00:01:20,700 --> 00:01:17,369
end multiple attempts have been

23
00:01:23,069 --> 00:01:20,710
undergone to kind of show whether

24
00:01:25,350 --> 00:01:23,079
prebiotic RNA synthesis and replication

25
00:01:30,649 --> 00:01:25,360
is possible and efficient and also

26
00:01:33,319 --> 00:01:30,659
research with with ribozymes

27
00:01:37,050 --> 00:01:33,329
specifically I'm really interested in

28
00:01:39,300 --> 00:01:37,060
non-enzymatic non ribose I matic RNA

29
00:01:41,910 --> 00:01:39,310
replication and you can imagine at some

30
00:01:43,830 --> 00:01:41,920
point it's probably likely that if you

31
00:01:46,380 --> 00:01:43,840
believe in the RNA world hypothesis

32
00:01:48,749 --> 00:01:46,390
first of all that um an RNA dependent

33
00:01:51,770 --> 00:01:48,759
RNA polymerase should have been

34
00:01:54,870 --> 00:01:51,780
necessary to efficiently replicate life

35
00:01:57,899 --> 00:01:54,880
so if we kind of go to this simplistic

36
00:01:59,819 --> 00:01:57,909
model here um you have a template and a

37
00:02:05,609 --> 00:01:59,829
primer templates and black primers in

38
00:02:07,139 --> 00:02:05,619

red and to get replication going you can

39
00:02:10,109 --> 00:02:07,149
extend the primer you form the

40
00:02:11,550 --> 00:02:10,119
full-length compliment you kind of break

41
00:02:15,720 --> 00:02:11,560
that up and then you repeat the process

42
00:02:18,710 --> 00:02:15,730
and this is what essentially PCR does on

43
00:02:20,840 --> 00:02:18,720
the larger and more exponential scale

44
00:02:23,900 --> 00:02:20,850
pcrs polymerase chain reaction can

45
00:02:28,190 --> 00:02:23,910
amplify DNA expect exponentially it's

46
00:02:32,810 --> 00:02:28,200
used in labs everywhere and just a kind

47
00:02:35,170 --> 00:02:32,820
of as a comparison so modern pcr and

48
00:02:38,240 --> 00:02:35,180
prebiotic what we believe could be

49
00:02:41,060 --> 00:02:38,250
prebiotic replication you don't have

50
00:02:42,470 --> 00:02:41,070
such a huge axis of primer requires more

51
00:02:44,810 --> 00:02:42,480
magnesium because the rate of reaction

52
00:02:48,520 --> 00:02:44,820
is slower we assume there's no

53
00:02:51,230 --> 00:02:48,530
polymerase enzyme or ribozyme and

54
00:02:52,430 --> 00:02:51,240
sometimes it's required that the amount

55
00:02:54,410 --> 00:02:52,440
of nucleotides that you use are

56
00:02:58,820 --> 00:02:54,420
activated kind of lower the energy

57
00:03:02,390 --> 00:02:58,830
barrier for this reaction and so if you

58
00:03:04,970 --> 00:03:02,400
go into a prebiotic kind of model once

59
00:03:08,060 --> 00:03:04,980
you get to this you know separated

60
00:03:10,340 --> 00:03:08,070
replicated strand this guy here if you

61
00:03:13,330 --> 00:03:10,350
want to kind of effect a second round of

62
00:03:17,449 --> 00:03:13,340
replication yes in primer but due to

63
00:03:19,940 --> 00:03:17,459

thermodynamic and kinetic issues what

64
00:03:21,980 --> 00:03:19,950
happens is the fully formed duplex RIA

65
00:03:23,780 --> 00:03:21,990
needles and that's because the full

66
00:03:25,910 --> 00:03:23,790
fully formed OOP flex full length is

67
00:03:31,340 --> 00:03:25,920
much more thermodynamically stable than

68
00:03:33,229 --> 00:03:31,350
the primer template complex and also you

69
00:03:34,759 --> 00:03:33,239
can undergo strand displacement and the

70
00:03:37,100 --> 00:03:34,769
longer strand will displace the shorter

71
00:03:39,140 --> 00:03:37,110
strands so if you get end up in kind of

72
00:03:42,380 --> 00:03:39,150
this regime you can't really replicate

73
00:03:44,509 --> 00:03:42,390
any more stops you're done cells dead or

74
00:03:48,160 --> 00:03:44,519
I mean it's not dead but can't grow you

75
00:03:51,770 --> 00:03:48,170
can't evolve and so all right my

76
00:03:54,380 --> 00:03:51,780
question then is like how can we inhibit

77
00:03:57,050 --> 00:03:54,390
this process how can we hit inhibit the

78
00:03:58,759 --> 00:03:57,060
rate of strain renewing and you know

79
00:04:00,050 --> 00:03:58,769
there's some things you can do in the

80
00:04:02,720 --> 00:04:00,060
only the second order process you can

81
00:04:05,210 --> 00:04:02,730
dilute it everything slows down but then

82
00:04:07,220 --> 00:04:05,220
you kind of it's pointless because all

83
00:04:10,039 --> 00:04:07,230
the reactions go slowed and might not be

84
00:04:11,420 --> 00:04:10,049
able to do anything you can in a

85
00:04:13,340 --> 00:04:11,430
laboratory setting increase the primer

86
00:04:15,830 --> 00:04:13,350
concentration but again in a prebiotic

87
00:04:19,099 --> 00:04:15,840
setting that might not have been totally

88
00:04:21,349 --> 00:04:19,109

plausible some people have tried to use

89
00:04:23,210 --> 00:04:21,359
complementary all goo nucleotide

90
00:04:26,210 --> 00:04:23,220
fragments of small fragments that bind

91
00:04:28,969 --> 00:04:26,220
to both strands that can physically

92
00:04:31,790 --> 00:04:28,979
block annealing but the fragments

93
00:04:32,960 --> 00:04:31,800
themselves if you like have them at high

94
00:04:36,439 --> 00:04:32,970
concentration they'll kind of self

95
00:04:38,659 --> 00:04:36,449
inhibit kind of be really strange and

96
00:04:40,850 --> 00:04:38,669
not really worked very well and we're

97
00:04:43,879 --> 00:04:40,860
currently also working on you know using

98
00:04:47,659 --> 00:04:43,889
some viscous environments that can also

99
00:04:51,320 --> 00:04:47,669
possibly you know physically slow down

100
00:04:54,409 --> 00:04:51,330
inhaling and so earlier in the first

101
00:04:56,689 --> 00:04:54,419
talk Nick was it he mentioned that there

102
00:05:00,649 --> 00:04:56,699
are a lot of RNA protein interactions

103
00:05:03,559 --> 00:05:00,659
that arm involve cationic peptides and

104
00:05:08,270 --> 00:05:03,569
cationic residues and it's been shown

105
00:05:09,980 --> 00:05:08,280
that ATP can interact with simple

106
00:05:11,899 --> 00:05:09,990
polypeptides made of lysine and arginine

107
00:05:13,520 --> 00:05:11,909
and they form these coacervate

108
00:05:16,550 --> 00:05:13,530
structures which is basically a second

109
00:05:20,719 --> 00:05:16,560
phase and it kind of separate and they

110
00:05:22,730 --> 00:05:20,729
can interact with RNA molecules RNA will

111
00:05:25,339 --> 00:05:22,740
preferentially separate into one of the

112
00:05:28,339 --> 00:05:25,349
two strands so we're like okay well it's

113
00:05:31,939 --> 00:05:28,349

possible that you know is it possible we

114
00:05:34,180 --> 00:05:31,949
can get some type of you know RNA

115
00:05:37,159 --> 00:05:34,190
binding event to these small

116
00:05:40,700 --> 00:05:37,169
polypeptides and kind of slow down the

117
00:05:44,689 --> 00:05:40,710
rate of annealing so basic polypeptides

118
00:05:47,059 --> 00:05:44,699
we studied specifically arginine but you

119
00:05:51,080 --> 00:05:47,069
know lysine could also work there also

120
00:05:52,610 --> 00:05:51,090
other issues with lysine I'm not going

121
00:05:54,649 --> 00:05:52,620
to talk about this in detail with a nice

122
00:05:57,620 --> 00:05:54,659
talk yesterday that kind of outline

123
00:06:01,040 --> 00:05:57,630
where all the plausible points of entry

124
00:06:04,010 --> 00:06:01,050
that peptides could have had in in the

125
00:06:06,110 --> 00:06:04,020
prebiotic earth you know all these

126
00:06:08,719 --> 00:06:06,120
different guys we can talk about this

127
00:06:11,689 --> 00:06:08,729
later if you're interested and most

128
00:06:14,029 --> 00:06:11,699
recently so some of you might know

129
00:06:15,890 --> 00:06:14,039
arginine is like pretty low in abundance

130
00:06:19,790 --> 00:06:15,900
in a lot of these experiments and in

131
00:06:22,129 --> 00:06:19,800
meteorites dust grains and reap most

132
00:06:24,399 --> 00:06:22,139
recently it's been shown that there is a

133
00:06:28,219 --> 00:06:24,409
prebiotic we plausible synthesis of

134
00:06:30,469 --> 00:06:28,229
arginine in cyanide rich environment

135
00:06:33,200 --> 00:06:30,479
that's reducing and contains a lot of

136
00:06:36,529 --> 00:06:33,210
hydrogen sulfide and this is work done

137
00:06:39,860 --> 00:06:36,539
by the Sutherland group in the UK and so

138
00:06:42,879 --> 00:06:39,870

here we go all right so can we use these

139
00:06:45,550 --> 00:06:42,889
peptides in a way to push the

140
00:06:48,520 --> 00:06:45,560
action in this way so that you can

141
00:06:53,050 --> 00:06:48,530
affect multiple rounds of polymerization

142
00:06:55,659 --> 00:06:53,060
and so we initially did this by testing

143
00:06:57,600 --> 00:06:55,669
the first rate of strand annealing in

144
00:07:03,550 --> 00:06:57,610
the presence and absence of peptides and

145
00:07:06,429 --> 00:07:03,560
we use the nucleic acid analog here's a

146
00:07:10,839 --> 00:07:06,439
to me no purine it's an editing analog

147
00:07:13,839 --> 00:07:10,849
and it you know bonds just as well to

148
00:07:18,100 --> 00:07:13,849
uracil and what's nice about this is

149
00:07:20,499 --> 00:07:18,110
it's fluorescent and it the fluorescence

150
00:07:21,999 --> 00:07:20,509
quenches when it's in a more ordered

151
00:07:24,640 --> 00:07:22,009
state so it's when when it's more

152
00:07:26,769 --> 00:07:24,650
stacked and when it's been a duplex the

153
00:07:29,320 --> 00:07:26,779
fluorescence will decrease and we can

154
00:07:32,649 --> 00:07:29,330
use this as a direct measure of the

155
00:07:36,929 --> 00:07:32,659
fraction of RNA and one or the other for

156
00:07:40,679 --> 00:07:36,939
in an assay and we use a stop folks

157
00:07:44,760 --> 00:07:40,689
spectrometer which you basically can

158
00:07:49,059 --> 00:07:44,770
inter inject both strands separately and

159
00:07:54,070 --> 00:07:49,069
measure kinetics immediately that's very

160
00:07:57,640 --> 00:07:54,080
unfortunate yeah alright well basically

161
00:08:00,820 --> 00:07:57,650
what this what this figure shows is that

162
00:08:02,589 --> 00:08:00,830
the y-axis is the fraction of RNA in a

163
00:08:05,529 --> 00:08:02,599

single-stranded state and the x axis is

164
00:08:08,980 --> 00:08:05,539
time and this is the annealing where the

165
00:08:11,260 --> 00:08:08,990
annealing of 2 15 more rnase in the

166
00:08:16,089 --> 00:08:11,270
presence of different peptides and the

167
00:08:19,119 --> 00:08:16,099
black is with no peptide and yellow is

168
00:08:21,939 --> 00:08:19,129
with a arginine 5m ER and these guys up

169
00:08:25,389 --> 00:08:21,949
here or longer our genes and we can see

170
00:08:28,689 --> 00:08:25,399
that the first curve here the black

171
00:08:31,179 --> 00:08:28,699
curve that without peptides the

172
00:08:33,610 --> 00:08:31,189
transition from single-stranded to

173
00:08:37,029 --> 00:08:33,620
double-stranded is very fast but once

174
00:08:39,250 --> 00:08:37,039
you add peptides in its starts to slow

175
00:08:41,909 --> 00:08:39,260
down is this like is it okay if I do it

176
00:08:44,199 --> 00:08:41,919
this way you guys all yeah all right

177
00:08:49,889 --> 00:08:44,209
because all the figures now are going to

178
00:08:54,519 --> 00:08:49,899
be like really crabby okay it's too bad

179
00:08:56,230 --> 00:08:54,529
so we by increasing the peptide

180
00:08:58,000 --> 00:08:56,240
concentration

181
00:09:00,760 --> 00:08:58,010
each of these colors is a different

182
00:09:04,210 --> 00:09:00,770
length of peptide and this is the

183
00:09:06,130 --> 00:09:04,220
annealing half-life of an annealing

184
00:09:10,210 --> 00:09:06,140
reaction and so by increasing the

185
00:09:12,550 --> 00:09:10,220
peptide concentration the time that it

186
00:09:16,060 --> 00:09:12,560
takes to neil increases so the nailing

187
00:09:19,600 --> 00:09:16,070
rate is inhibited somewhat and when you

188
00:09:21,940 --> 00:09:19,610

go from a 5m ER to a 7 to a 9 mer to

189
00:09:25,480 --> 00:09:21,950
attend mer the longer the arginine

190
00:09:27,699 --> 00:09:25,490
peptide that you're using the greater

191
00:09:31,440 --> 00:09:27,709
the inhibition of the annealing effect

192
00:09:34,870 --> 00:09:31,450
and so larger longer peptides a new

193
00:09:36,880 --> 00:09:34,880
cause a greater slowing of really you

194
00:09:40,090 --> 00:09:36,890
can increase the length of the RNA and

195
00:09:44,199 --> 00:09:40,100
the longer the RNA in the presence of

196
00:09:46,660 --> 00:09:44,209
peptide the longer takes 42 RNA to nail

197
00:09:49,240 --> 00:09:46,670
and this is great because of course the

198
00:09:51,190 --> 00:09:49,250
primer is much shorter than a replicated

199
00:09:55,150 --> 00:09:51,200
strand so we want in the presence of

200
00:09:58,389 --> 00:09:55,160
peptides to see that the primer a nails

201
00:09:59,829 --> 00:09:58,399
faster than this replicated strand

202
00:10:04,660 --> 00:09:59,839
otherwise this whole system is

203
00:10:07,810 --> 00:10:04,670
meaningless unfortunately we came across

204
00:10:09,670 --> 00:10:07,820
couple problems the traditional

205
00:10:13,360 --> 00:10:09,680
non-enzymatic replication machinery

206
00:10:15,670 --> 00:10:13,370
requires magnesium and magnesium at

207
00:10:18,360 --> 00:10:15,680
increasing concentrations decreases the

208
00:10:21,699 --> 00:10:18,370
annealing time of RNA so it kind of

209
00:10:23,769 --> 00:10:21,709
disrupts the peptide RNA interaction and

210
00:10:26,740 --> 00:10:23,779
you'll go back to a much faster in

211
00:10:31,240 --> 00:10:26,750
Newton time same with our activated

212
00:10:32,680 --> 00:10:31,250
monomer we use a modified G monomer if

213
00:10:35,590 --> 00:10:32,690

you're interested we can talk about this

214
00:10:38,819 --> 00:10:35,600
later and that also because it's charged

215
00:10:42,100 --> 00:10:38,829
it disrupts this interaction and

216
00:10:44,880 --> 00:10:42,110
decrease the effectiveness of the

217
00:10:49,000 --> 00:10:44,890
peptide in slowing the India trade so

218
00:10:52,030 --> 00:10:49,010
what do we do now so we took the we use

219
00:10:55,600 --> 00:10:52,040
this system here and it's a system where

220
00:10:59,410 --> 00:10:55,610
we can add up to 4G bases on to a

221
00:11:01,740 --> 00:10:59,420
fluorescently labeled primer we incubate

222
00:11:04,449 --> 00:11:01,750
this for many hours without enzymes

223
00:11:07,290 --> 00:11:04,459
anything and you can actually extend you

224
00:11:11,880 --> 00:11:07,300
can see extension over many hours

225
00:11:13,920 --> 00:11:11,890
so I think that's pretty cool and the

226
00:11:16,769 --> 00:11:13,930
rate of extension with and without

227
00:11:18,810 --> 00:11:16,779
peptides you know differs by about

228
00:11:22,320 --> 00:11:18,820
thirty percent but when you have peptide

229
00:11:23,759 --> 00:11:22,330
the annealing rate decreases or the

230
00:11:25,980 --> 00:11:23,769
annealing rate decreases by over three

231
00:11:28,680 --> 00:11:25,990
orders of magnitude so by sacrificing

232
00:11:31,430 --> 00:11:28,690
thirty percent in the right of the

233
00:11:35,069 --> 00:11:31,440
replication you can affect such a huge

234
00:11:37,500 --> 00:11:35,079
change in the annealing rate and so the

235
00:11:39,810 --> 00:11:37,510
kind of big experiment we did here is we

236
00:11:43,170 --> 00:11:39,820
started with a reverse complement and a

237
00:11:45,960 --> 00:11:43,180
template premio added peptide added

238
00:11:47,940 --> 00:11:45,970

primer heated it up cooled it down

239
00:11:52,980 --> 00:11:47,950
immediately due to some thermodynamic

240
00:11:54,360 --> 00:11:52,990
and kinetic considerations we have to

241
00:11:56,460 --> 00:11:54,370
cool down immediately otherwise the

242
00:11:59,610 --> 00:11:56,470
reaction doesn't work and we can see

243
00:12:00,990 --> 00:11:59,620
here that the third line is in the

244
00:12:02,400 --> 00:12:01,000
presence of reverse complement but

245
00:12:04,380 --> 00:12:02,410
without peptide and you can see that

246
00:12:09,870 --> 00:12:04,390
there's no primer extension there's no

247
00:12:12,930 --> 00:12:09,880
replication because presumably the the

248
00:12:15,810 --> 00:12:12,940
strand the blue strand is renewing

249
00:12:17,280 --> 00:12:15,820
faster but in the fourth line here you

250
00:12:21,660 --> 00:12:17,290
can see that in the presence of peptide

251
00:12:24,660 --> 00:12:21,670
we do get some form of primer extension

252
00:12:25,920 --> 00:12:24,670
we do get replication and I think this

253
00:12:27,750 --> 00:12:25,930
is one of the first times it's been

254
00:12:31,740 --> 00:12:27,760
shown that you can get multiple rounds

255
00:12:35,190 --> 00:12:31,750
of a non-enzymatic primer extension and

256
00:12:37,130 --> 00:12:35,200
so yeah we we hope to optimize this

257
00:12:39,480 --> 00:12:37,140
procedure to kind of push the

258
00:12:42,030 --> 00:12:39,490
equilibrium even further into this

259
00:12:44,490 --> 00:12:42,040
direction hopefully the ultimate goal is

260
00:12:47,100 --> 00:12:44,500
to kind of create this protocell that's

261
00:12:54,009 --> 00:12:47,110
self sufficient so thank you every one

262
00:12:58,479 --> 00:12:56,319
sorry about the figures I should have

263
00:13:01,090 --> 00:12:58,489

checked it I'm going to use my privilege

264
00:13:03,309 --> 00:13:01,100
as the first question so it looks like

265
00:13:04,720 --> 00:13:03,319
that was almost an exponential increases

266
00:13:07,179 --> 00:13:04,730
you were increasing the length of the

267
00:13:09,220 --> 00:13:07,189
peptide right have you gone beyond 10 is

268
00:13:13,210 --> 00:13:09,230
that does it turn over at some point and

269
00:13:16,269 --> 00:13:13,220
stop being as effective or um we haven't

270
00:13:18,699 --> 00:13:16,279
gone above 10 and the rationale there is

271
00:13:20,979 --> 00:13:18,709
just the shorter the peptide the easier

272
00:13:24,299 --> 00:13:20,989
it would have been ache I would guess

273
00:13:26,590 --> 00:13:24,309
that you could you would just keep going

274
00:13:30,579 --> 00:13:26,600
one of the things we tested was a

275
00:13:33,729 --> 00:13:30,589
cationic dextran polymer which is like a

276
00:13:36,999 --> 00:13:33,739
I think of polysaccharide and we see is

277
00:13:39,129 --> 00:13:37,009
the kneeling is barely visible it

278
00:13:43,119 --> 00:13:39,139
basically like completely stops it so

279
00:13:55,449 --> 00:13:43,129
cool other poly cations could also have

280
00:13:58,449 --> 00:13:55,459
the same effect any other questions so

281
00:14:00,160 --> 00:13:58,459
obviously this is sort of you started

282
00:14:02,470 --> 00:14:00,170
with the assumption of like an RNA world

283
00:14:05,859 --> 00:14:02,480
type scenario but then you've invoked

284
00:14:07,780 --> 00:14:05,869
peptides to sort of keep the RNA world

285
00:14:09,460 --> 00:14:07,790
going right and so this is something

286
00:14:11,919 --> 00:14:09,470
that seems to be coming up all over the

287
00:14:14,829 --> 00:14:11,929
place is that the RNA world was clearly

288
00:14:16,600 --> 00:14:14,839

not just an RNA world so I mean do you

289
00:14:19,869 --> 00:14:16,610
have anything that you think do you

290
00:14:21,970 --> 00:14:19,879
think RNA was playing specific roles or

291
00:14:24,340 --> 00:14:21,980
most roles or do you think that there

292
00:14:25,689 --> 00:14:24,350
was just sort of a lot of RNA but there

293
00:14:29,549 --> 00:14:25,699
was also a lot of other stuff going on

294
00:14:33,069 --> 00:14:29,559
yeah so I guess it's very possible that

295
00:14:36,819 --> 00:14:33,079
the peptides and RNA emerge kind of

296
00:14:38,980 --> 00:14:36,829
independently um weather at what point

297
00:14:41,710 --> 00:14:38,990
they kind of became interdependent I'm

298
00:14:45,100 --> 00:14:41,720
not so sure but I guess that would also

299
00:14:46,989 --> 00:14:45,110
depend on like in my opinion the length

300
00:14:49,900 --> 00:14:46,999
of both of the polymers that are made

301
00:14:52,679 --> 00:14:49,910
obviously the longer the polymer is the

302
00:14:56,169 --> 00:14:52,689
more like it's multivalent the more

303
00:14:57,909 --> 00:14:56,179
possibility for interaction so you know

304
00:15:01,150 --> 00:14:57,919
it's hard to say it'd be interesting to

305
00:15:03,460 --> 00:15:01,160
see you know some some more people work

306
00:15:05,920 --> 00:15:03,470
on this and I know a lot of people are

307
00:15:10,980 --> 00:15:05,930
kind of leaning towards a pet