
AbSciCon
2019

1
00:00:00,790 --> 00:00:07,320
[Music]

2
00:00:11,190 --> 00:00:08,990
[Applause]

3
00:00:13,140 --> 00:00:11,200
so we've been interested in complex

4
00:00:15,990 --> 00:00:13,150
class rates as model prebiotic

5
00:00:18,359 --> 00:00:16,000
compartments because they are very easy

6
00:00:21,150 --> 00:00:18,369
to assemble and they can accumulate

7
00:00:23,519 --> 00:00:21,160
different biomolecules that are very

8
00:00:26,009 --> 00:00:23,529
relevant to provide a chemistry so a

9
00:00:28,439 --> 00:00:26,019
little bit of background on how these

10
00:00:32,660 --> 00:00:28,449
guys form is through assembly of

11
00:00:35,310 --> 00:00:32,670
polyelectrolytes so I'm showing here

12
00:00:37,290 --> 00:00:35,320
poly anion and poly cation they can

13
00:00:39,810 --> 00:00:37,300

assemble together to form these dense

14
00:00:41,369 --> 00:00:39,820
phase and dilute phase so the dense

15
00:00:44,069 --> 00:00:41,379
phase actually contains a lot of all the

16
00:00:46,619 --> 00:00:44,079
electrolytes and and charged molecules

17
00:00:49,139 --> 00:00:46,629
since and since RNA is highly charged

18
00:00:52,770 --> 00:00:49,149
they can also be expected to accumulate

19
00:00:55,259 --> 00:00:52,780
within these droplets so before I joined

20
00:00:57,119 --> 00:00:55,269
Phil's lab graduates in the lab Erica

21
00:01:00,139 --> 00:00:57,129
Frankel she had done a lot of nice work

22
00:01:03,540 --> 00:01:00,149
with the system of poly allele amine

23
00:01:05,820 --> 00:01:03,550
hydrochloride and ATP where obviously

24
00:01:08,220 --> 00:01:05,830
the poly a lil amine is the poly cation

25
00:01:09,600 --> 00:01:08,230
on the ATP is the poly anion and when

26
00:01:12,560 --> 00:01:09,610
you add these things together you form

27
00:01:14,490 --> 00:01:12,570
these really nice droplets that

28
00:01:16,649 --> 00:01:14,500
accumulate I'm not gonna go into too

29
00:01:18,330 --> 00:01:16,659
much detail but what she showed was you

30
00:01:22,319 --> 00:01:18,340
could really accumulate magnesium

31
00:01:25,170 --> 00:01:22,329
nucleotide and RNA for example when she

32
00:01:27,719 --> 00:01:25,180
used ADP and poly ethyl amine to form

33
00:01:30,620 --> 00:01:27,729
these Kumasi rates and only added about

34
00:01:32,700 --> 00:01:30,630
5 milli molar magnesium bulk the

35
00:01:35,010 --> 00:01:32,710
concentration of magnesium in the

36
00:01:36,780 --> 00:01:35,020
coacervate swear upwards of molar so

37
00:01:38,819 --> 00:01:36,790
that's tremendous enhancements of

38
00:01:41,130 --> 00:01:38,829

magnesium levels which might be very

39
00:01:43,200 --> 00:01:41,140
relevant for chemistry's like non

40
00:01:45,719 --> 00:01:43,210
enzymatic RNA polymerize ation or even

41
00:01:51,719 --> 00:01:45,729
ribozyme catalyze RNA polymerize ation

42
00:01:55,649 --> 00:01:51,729
as well and obviously what she also

43
00:01:57,569 --> 00:01:55,659
found was that RNA different rnase were

44
00:02:01,380 --> 00:01:57,579
also highly enriched in these Kumasi

45
00:02:03,899 --> 00:02:01,390
rates so what we started to do was we

46
00:02:05,730 --> 00:02:03,909
started exploring different poly cations

47
00:02:07,620 --> 00:02:05,740
and poly anions to form these class

48
00:02:09,450 --> 00:02:07,630
rates and ask whether they can

49
00:02:12,450 --> 00:02:09,460
accumulate rnase and whether they can

50
00:02:14,780 --> 00:02:12,460
participate in chemistry's that we care

51
00:02:18,119 --> 00:02:14,790
about in terms of RNA world hypothesis

52
00:02:21,100 --> 00:02:18,129
so you can see that all the go lysine

53
00:02:23,620 --> 00:02:21,110
poly dialy o dimethyl

54
00:02:25,390 --> 00:02:23,630
for IP DAC all the arginine they all

55
00:02:28,510 --> 00:02:25,400
formed these nice class roommates when

56
00:02:30,760 --> 00:02:28,520
we add all ego RNA to the solution and

57
00:02:34,870 --> 00:02:30,770
what I didn't tell you was that we

58
00:02:37,660 --> 00:02:34,880
actually added a size five or site we

59
00:02:39,900 --> 00:02:37,670
labeled RNA in these class arrays and

60
00:02:42,550 --> 00:02:39,910
you can see that the RNA really

61
00:02:44,410 --> 00:02:42,560
accumulates in these droplets and we're

62
00:02:46,120 --> 00:02:44,420
going to use the same RNA for template

63
00:02:50,140 --> 00:02:46,130

directed RNA polymerize ation that i'm

64
00:02:52,090 --> 00:02:50,150
gonna talk to you guys here so again

65
00:02:55,660 --> 00:02:52,100
these are the different classifications

66
00:02:58,720 --> 00:02:55,670
that we're using we're using poly a RNA

67
00:03:00,720 --> 00:02:58,730
to form the to have the poly anionic

68
00:03:04,690 --> 00:03:00,730
part and we're using pollyali Lamine

69
00:03:06,940 --> 00:03:04,700
PTAC illegal I seen illegal arginine as

70
00:03:09,520 --> 00:03:06,950
the poly cationic part and what we do is

71
00:03:11,500 --> 00:03:09,530
we add this RNA primer template complex

72
00:03:14,770 --> 00:03:11,510
and you can see that the template has a

73
00:03:17,140 --> 00:03:14,780
stretch of C's where this activated

74
00:03:19,870 --> 00:03:17,150
nucleotide that is aji can come in and

75
00:03:21,790 --> 00:03:19,880
the primer gets extended and what we do

76
00:03:23,590 --> 00:03:21,800
is that we add everything together and

77
00:03:25,210 --> 00:03:23,600
then we separate the two phases so that

78
00:03:27,280 --> 00:03:25,220
the coacervate phase is separate from

79
00:03:29,729 --> 00:03:27,290
the dilute phase and we monitor the

80
00:03:33,790 --> 00:03:29,739
reaction in these two phases separately

81
00:03:36,009 --> 00:03:33,800
so what happens when you don't have any

82
00:03:38,500 --> 00:03:36,019
class rates of course you see that the

83
00:03:39,850 --> 00:03:38,510
RNA starts to the primer starts to get

84
00:03:42,640 --> 00:03:39,860
longer because this monomer gets

85
00:03:45,370 --> 00:03:42,650
incorporated and what was really

86
00:03:46,900 --> 00:03:45,380
interesting was that in the case of the

87
00:03:49,060 --> 00:03:46,910
dilute phase which is the supernatant

88
00:03:51,550 --> 00:03:49,070

phase you don't really see that much RNA

89
00:03:53,080 --> 00:03:51,560
which is expected because a lot of the

90
00:03:55,600 --> 00:03:53,090
RNA ends up in the condensed phase

91
00:03:58,390 --> 00:03:55,610
inside the coacervate but while striking

92
00:04:00,220 --> 00:03:58,400
was that in the condensed phase only the

93
00:04:02,320 --> 00:04:00,230
B tack only the class of rates that

94
00:04:04,960 --> 00:04:02,330
formed that were formed from the B DAC

95
00:04:07,120 --> 00:04:04,970
and all ego RNA they supported the non

96
00:04:09,430 --> 00:04:07,130
enzymatic polymerization and you can see

97
00:04:12,009 --> 00:04:09,440
that all the ones that don't support non

98
00:04:15,220 --> 00:04:12,019
enzymatic polymerization with PAH oligo

99
00:04:16,750 --> 00:04:15,230
arginine and all ego lysine they can not

100
00:04:18,219 --> 00:04:16,760
only engage in short charged

101
00:04:20,140 --> 00:04:18,229
interactions but they also have this

102
00:04:22,420 --> 00:04:20,150
ability to hydrogen bonds so we think

103
00:04:24,700 --> 00:04:22,430
that this super high concentration of

104
00:04:26,230 --> 00:04:24,710
hydrogen bond donors acceptors and and

105
00:04:30,040 --> 00:04:26,240
charged groups within the co acid rates

106
00:04:32,200 --> 00:04:30,050
they might be somehow inhibiting this

107
00:04:33,879 --> 00:04:32,210
reaction potentially miss folding or

108
00:04:39,820 --> 00:04:33,889
unfolding the primer temper

109
00:04:41,080 --> 00:04:39,830
complex since we knew that some of the

110
00:04:43,420 --> 00:04:41,090
crafts are eight other crafts of eight

111
00:04:44,920 --> 00:04:43,430
systems in increase the magnesium

112
00:04:46,149 --> 00:04:44,930
concentrations within the condensed

113
00:04:48,070 --> 00:04:46,159

phase we thought it might be interesting

114
00:04:51,040 --> 00:04:48,080
to look at non enzymatic polymerization

115
00:04:52,899 --> 00:04:51,050
in different magnesium concentrations in

116
00:04:54,519 --> 00:04:52,909
the presence of colossal rates so you

117
00:04:56,260 --> 00:04:54,529
can see that without any class of rates

118
00:04:59,170 --> 00:04:56,270
in buffer as you decrease the magnesium

119
00:05:01,950 --> 00:04:59,180
level you do see you do take a hit in

120
00:05:05,739 --> 00:05:01,960
how long the RNA is formed by this

121
00:05:07,629 --> 00:05:05,749
reaction and what was really interesting

122
00:05:09,610 --> 00:05:07,639
was when we did the same thing but in

123
00:05:12,519 --> 00:05:09,620
the presence of P deck and oligo RNA

124
00:05:14,559 --> 00:05:12,529
Kassovitz you deuce take a little bit of

125
00:05:16,450 --> 00:05:14,569
hit in the beginning but then even when

126
00:05:18,429 --> 00:05:16,460
we didn't add any magnesium you stills

127
00:05:20,939 --> 00:05:18,439
formed like decent amount of plus one

128
00:05:23,439 --> 00:05:20,949
and you even see some amount of plus two

129
00:05:26,170 --> 00:05:23,449
of band form from non enzymatic

130
00:05:29,050 --> 00:05:26,180
polymerization and and that's quantified

131
00:05:31,809 --> 00:05:29,060
here and so to really get into more

132
00:05:34,570 --> 00:05:31,819
detail of what was going on we decided

133
00:05:37,600 --> 00:05:34,580
to add an excess of EDTA to the reaction

134
00:05:39,399 --> 00:05:37,610
so when we added ATT a very little

135
00:05:40,779 --> 00:05:39,409
background reaction that was that was

136
00:05:42,999 --> 00:05:40,789
happening even in the absence of

137
00:05:44,800 --> 00:05:43,009
magnesium that goes away as well as

138
00:05:47,769 --> 00:05:44,810

expected because there might be some

139
00:05:49,570 --> 00:05:47,779
background dye valence that the ETA is

140
00:05:52,209 --> 00:05:49,580
chelating and what was really surprising

141
00:05:53,889 --> 00:05:52,219
was that when we added the PTAC which is

142
00:05:55,839 --> 00:05:53,899
the cationic polymers that we were using

143
00:05:59,139 --> 00:05:55,849
to form the coacervate we actually

144
00:06:01,389 --> 00:05:59,149
rescue the the the the reaction back so

145
00:06:03,639 --> 00:06:01,399
what this was telling us was that not

146
00:06:05,320 --> 00:06:03,649
only that Kosar rates can act as this

147
00:06:07,389 --> 00:06:05,330
passive compartments that are just

148
00:06:10,149 --> 00:06:07,399
taking up RNA molecules but they might

149
00:06:11,559 --> 00:06:10,159
be actually engaging in in the chemistry

150
00:06:14,589 --> 00:06:11,569
as well so that was really interesting

151
00:06:18,369 --> 00:06:14,599
so having looked at non enzymatic

152
00:06:20,980 --> 00:06:18,379
polymerization we decided to now you

153
00:06:24,219 --> 00:06:20,990
know go study a little more complex

154
00:06:26,800 --> 00:06:24,229
system and the the sequel to this you've

155
00:06:29,499 --> 00:06:26,810
already heard yesterday from phil where

156
00:06:31,659 --> 00:06:29,509
we wanted to look at ribozyme catalysis

157
00:06:33,790 --> 00:06:31,669
within within complex krasin rate system

158
00:06:36,939 --> 00:06:33,800
and the model we use is the hammerhead

159
00:06:39,879 --> 00:06:36,949
ribozyme which is shown in shown here so

160
00:06:42,009 --> 00:06:39,889
the black strand is the enzyme strand

161
00:06:44,529 --> 00:06:42,019
which base pairs with a substrate strand

162
00:06:46,389 --> 00:06:44,539
which is shown in green and then cuts

163
00:06:47,570 --> 00:06:46,399

the substrate right where this red

164
00:06:51,560 --> 00:06:47,580
triangle is

165
00:06:54,500 --> 00:06:51,570
and so the idea was you know if in an

166
00:06:55,880 --> 00:06:54,510
early Earth the the enzymes were

167
00:06:58,220 --> 00:06:55,890
probably not very abundant they're

168
00:07:00,290 --> 00:06:58,230
probably very scarce and so how do you

169
00:07:01,760 --> 00:07:00,300
and further insult for this particular

170
00:07:04,070 --> 00:07:01,770
reaction to happen of course the enzyme

171
00:07:06,380 --> 00:07:04,080
needs to find the substrate so if you

172
00:07:08,780 --> 00:07:06,390
are and by the way this is just a native

173
00:07:11,660 --> 00:07:08,790
gel that shows the association between

174
00:07:13,880 --> 00:07:11,670
the enzyme and the substrate so if you

175
00:07:16,370 --> 00:07:13,890
have a lot of enzyme present then you

176
00:07:18,440 --> 00:07:16,380
start to associate with the substrate so

177
00:07:21,620 --> 00:07:18,450
if the molecule becomes larger so it

178
00:07:23,300 --> 00:07:21,630
moves up but if you don't have enough

179
00:07:24,950 --> 00:07:23,310
enzyme you're below the dissociation

180
00:07:27,980 --> 00:07:24,960
constant then you don't see much

181
00:07:29,750 --> 00:07:27,990
association and and and so the substrate

182
00:07:32,060 --> 00:07:29,760
is not really interacting with the

183
00:07:34,070 --> 00:07:32,070
enzyme and presumably when the

184
00:07:36,410 --> 00:07:34,080
functional molecules were very scarce in

185
00:07:38,360 --> 00:07:36,420
the early Earth you are probably but we

186
00:07:40,850 --> 00:07:38,370
were probably dealing with this sort of

187
00:07:43,040 --> 00:07:40,860
regime SOCAN complex Craster rates

188
00:07:45,020 --> 00:07:43,050

activate these kind of chemistry's when

189
00:07:47,780 --> 00:07:45,030
you have a very limited functional RNA

190
00:07:50,330 --> 00:07:47,790
molecules so let's see what happens so

191
00:07:52,640 --> 00:07:50,340
again we tested these sort of ribozyme

192
00:07:54,620 --> 00:07:52,650
reactions with PTAC and an oligarchic

193
00:07:56,720 --> 00:07:54,630
acid casa rates so the way we do these

194
00:07:58,460 --> 00:07:56,730
experiments is we first add the poly

195
00:08:00,890 --> 00:07:58,470
cation and the pollyanna and to form the

196
00:08:03,470 --> 00:08:00,900
coacervate and then we add the ribozyme

197
00:08:06,770 --> 00:08:03,480
and the substrate separately and and and

198
00:08:10,520 --> 00:08:06,780
and monitor the reaction so to our

199
00:08:12,080 --> 00:08:10,530
surprise indeed well in case a buffer

200
00:08:14,090 --> 00:08:12,090
where we don't have any co acid rates

201
00:08:17,510 --> 00:08:14,100
you don't see a lot of reaction which is

202
00:08:19,610 --> 00:08:17,520
the cleavage of the substrate because of

203
00:08:21,650 --> 00:08:19,620
course since there's only 5 nano mol or

204
00:08:23,390 --> 00:08:21,660
enzyme and the KD i think you at 1

205
00:08:25,550 --> 00:08:23,400
millimolar we estimated it to be about

206
00:08:27,260 --> 00:08:25,560
250 nanomolar so there's not a lot of

207
00:08:28,910 --> 00:08:27,270
association between the ribozyme and the

208
00:08:31,280 --> 00:08:28,920
substrate so you don't see a lot of

209
00:08:33,290 --> 00:08:31,290
reaction whereas when we have the p deck

210
00:08:35,660 --> 00:08:33,300
and the d-10 class of rates you you see

211
00:08:38,030 --> 00:08:35,670
robust enhancement of the ribozyme

212
00:08:39,680 --> 00:08:38,040
chemistry so not only about what we

213
00:08:41,240 --> 00:08:39,690

think is going on is that the cursor

214
00:08:43,190 --> 00:08:41,250
rates are bringing these RNA molecules

215
00:08:45,410 --> 00:08:43,200
together and promoting RNA RNA

216
00:08:48,530 --> 00:08:45,420
interactions that are not possible in

217
00:08:51,440 --> 00:08:48,540
dilute solutions we tested several

218
00:08:54,770 --> 00:08:51,450
different class rate compositions for

219
00:08:58,060 --> 00:08:54,780
example PTAC and illegal aspartic acid

220
00:09:00,230 --> 00:08:58,070
obviously they activate the ribozyme

221
00:09:01,220 --> 00:09:00,240
illegal lysine and all the grass partic

222
00:09:03,260 --> 00:09:01,230
acid they

223
00:09:06,050 --> 00:09:03,270
these classmates also tended to activate

224
00:09:08,060 --> 00:09:06,060
a ribozyme a legal arginine on the other

225
00:09:10,100 --> 00:09:08,070
hand we saw inhibition of the reaction

226
00:09:11,660 --> 00:09:10,110
and we do the same and I think based on

227
00:09:13,730 --> 00:09:11,670
Irene's work

228
00:09:15,710 --> 00:09:13,740
we know that arginine is highly

229
00:09:17,900 --> 00:09:15,720
interactive with RNA and then I think

230
00:09:20,270 --> 00:09:17,910
the Aligarh journey is probably

231
00:09:21,530 --> 00:09:20,280
misfolding the ribozyme when it gets

232
00:09:26,120 --> 00:09:21,540
inside the complex

233
00:09:28,460 --> 00:09:26,130
Craster rates and finally what we

234
00:09:30,470 --> 00:09:28,470
decided to do was we decided to test

235
00:09:33,170 --> 00:09:30,480
whether this sort of system is

236
00:09:35,780 --> 00:09:33,180
widespread can other enzymes also be

237
00:09:38,980 --> 00:09:35,790
activated using these this mechanism and

238
00:09:42,500 --> 00:09:38,990

so this is the 10:23 DNA's I'm which was

239
00:09:44,540 --> 00:09:42,510
which is from Gerry Joyce's lab and it

240
00:09:46,580 --> 00:09:44,550
also catalyzed a similar chemistry the

241
00:09:49,280 --> 00:09:46,590
DNA strand base pairs with a substrate

242
00:09:51,530 --> 00:09:49,290
which is shown in blue and cuts where

243
00:09:54,050 --> 00:09:51,540
the red triangle is and here you can see

244
00:09:56,060 --> 00:09:54,060
that with high ends the DNA is I'm

245
00:09:58,100 --> 00:09:56,070
concentration you can see nice cleavage

246
00:10:00,050 --> 00:09:58,110
of the substrate but when you reduce the

247
00:10:02,000 --> 00:10:00,060
DNA's I'm to five nano molar you don't

248
00:10:04,400 --> 00:10:02,010
see a lot of chemistry being happening

249
00:10:05,990 --> 00:10:04,410
and when we do the same reaction now in

250
00:10:08,210 --> 00:10:06,000
the presence of complex class of eight

251
00:10:10,880 --> 00:10:08,220
so you can see that the DNA's I'm also

252
00:10:12,920 --> 00:10:10,890
gets turned on and we've done this with

253
00:10:14,630 --> 00:10:12,930
also hairpin ribozyme which I didn't

254
00:10:16,750 --> 00:10:14,640
have time to talk about in that case the

255
00:10:19,220 --> 00:10:16,760
enzyme actually gets activated by

256
00:10:20,660 --> 00:10:19,230
concentration of sperm in which is not

257
00:10:23,740 --> 00:10:20,670
really in our neighbor a small molecule

258
00:10:26,030 --> 00:10:23,750
so there there there multiple ways that

259
00:10:30,110 --> 00:10:26,040
coacervate seem to be enhancing

260
00:10:32,150 --> 00:10:30,120
different nucleic acid enzymes and

261
00:10:35,090 --> 00:10:32,160
that's just the quantification from

262
00:10:37,370 --> 00:10:35,100
different magnesium levels so in summary

263
00:10:39,560 --> 00:10:37,380

what I want to talk about is that you

264
00:10:41,690 --> 00:10:39,570
know we've shown that specific complex

265
00:10:44,570 --> 00:10:41,700
class rates can not only partition RNA

266
00:10:46,400 --> 00:10:44,580
molecules but they can also enhance

267
00:10:49,300 --> 00:10:46,410
template directed polymerization under

268
00:10:51,740 --> 00:10:49,310
limiting magnesium concentrations and

269
00:10:55,250 --> 00:10:51,750
concentration of RNA and other Co

270
00:10:58,040 --> 00:10:55,260
solutes can lead to enhancements of RNA

271
00:10:59,780 --> 00:10:58,050
catalysis and finally we we've shown

272
00:11:02,060 --> 00:10:59,790
that complex crossbred mediated

273
00:11:05,060 --> 00:11:02,070
enhancements seem to be general as in

274
00:11:07,100 --> 00:11:05,070
different different composition of cras

275
00:11:09,440 --> 00:11:07,110
rates can activate different nucleic

276
00:11:11,750 --> 00:11:09,450
acid enzymes so we think that this might

277
00:11:14,569 --> 00:11:11,760
be a very useful thing to study for

278
00:11:17,179 --> 00:11:14,579
diverse nucleic acid enzymes and

279
00:11:19,549 --> 00:11:17,189
with that I would like to thank Phil's

280
00:11:21,350 --> 00:11:19,559
lab and and Chris's lab who's been like

281
00:11:23,150 --> 00:11:21,360
really instrumental in and we have had

282
00:11:24,769 --> 00:11:23,160
an awesome collaboration in all those

283
00:11:26,989 --> 00:11:24,779
liquid liquid phase separated systems

284
00:11:28,669 --> 00:11:26,999
and we're starting to look at liquid

285
00:11:31,579 --> 00:11:28,679
liquid phase separation in cells with

286
00:11:33,410 --> 00:11:31,589
Geraldine City at Johns Hopkins we're

287
00:11:35,239 --> 00:11:33,420
starting to do some ribozyme catalyzed

288
00:11:38,710 --> 00:11:35,249

RNA polymerize ation with Gerry Joyce

289
00:11:41,299 --> 00:11:38,720
and we're also looking nan non-enzymatic

290
00:11:42,590 --> 00:11:41,309
oligonucleotide phosphorylation with Ron

291
00:11:44,720 --> 00:11:42,600
Krishnamurthy lab so a lot of cool