
AbSciCon
2019

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

2
00:00:11,990 --> 00:00:09,340
[Applause]

3
00:00:14,060 --> 00:00:12,000
good afternoon everybody

4
00:00:16,670 --> 00:00:14,070
this is going to be a kind of a tale of

5
00:00:20,870 --> 00:00:16,680
a set of reactions based on this DCC

6
00:00:24,189 --> 00:00:20,880
system okay so a little bit of a

7
00:00:26,870 --> 00:00:24,199
preamble or a prologue to the story

8
00:00:29,529 --> 00:00:26,880
there are various kinds of hypothesis as

9
00:00:32,439 --> 00:00:29,539
to how prebiotic life could have emerged

10
00:00:35,570 --> 00:00:32,449
into the last Universal common ancestor

11
00:00:37,759 --> 00:00:35,580
but assuming that there was a

12
00:00:39,140 --> 00:00:37,769
hypothetical RNA world the question

13
00:00:40,910 --> 00:00:39,150

remains as to how the our own

14
00:00:44,090 --> 00:00:40,920
hypothetical on a wall got to the Lucas

15
00:00:46,610 --> 00:00:44,100
stage if we look at life from a top-down

16
00:00:50,240 --> 00:00:46,620
approach one eventually goes down to the

17
00:00:52,460 --> 00:00:50,250
ribosome which I strongly believe based

18
00:00:55,490 --> 00:00:52,470
on the evidence it's the largest white

19
00:00:57,860 --> 00:00:55,500
elephant in the room of prebiotic life

20
00:00:59,240 --> 00:00:57,870
and also the Luca if you look at the

21
00:01:01,670 --> 00:00:59,250
ribosome you go deep down to the

22
00:01:03,979 --> 00:01:01,680
ribosome you end up with the PTC but

23
00:01:06,020 --> 00:01:03,989
it's almost like at the end of the

24
00:01:07,850 --> 00:01:06,030
ladder when you look down what do you

25
00:01:09,469 --> 00:01:07,860
see what do you see is a prebiotic wall

26
00:01:11,539 --> 00:01:09,479
assuming that there was a hypothetical

27
00:01:12,920 --> 00:01:11,549
on a wall how did we get from the

28
00:01:19,010 --> 00:01:12,930
hypothetical on a wall to the locust

29
00:01:21,620 --> 00:01:19,020
stage so the the current hypothesis is

30
00:01:23,810 --> 00:01:21,630
that the hypothetical in a world was

31
00:01:26,029 --> 00:01:23,820
probably terminated by a proto Ramazan

32
00:01:28,429 --> 00:01:26,039
wall an ancient peptide making machine

33
00:01:30,520 --> 00:01:28,439
however there's a small problem the

34
00:01:32,749 --> 00:01:30,530
absence of a demonstrable replicase

35
00:01:36,639 --> 00:01:32,759
therefore the absence of this replicase

36
00:01:40,489 --> 00:01:36,649
we have heard a lot about the dry cycles

37
00:01:43,160 --> 00:01:40,499
in a similar fashion we have this

38
00:01:45,080 --> 00:01:43,170

alternate system which was developed in

39
00:01:46,730 --> 00:01:45,090
a lab it's called the dynamic

40
00:01:48,560 --> 00:01:46,740
combinatorial chemistry system which

41
00:01:51,410 --> 00:01:48,570
uses cycles of synthesis and degradation

42
00:01:54,020 --> 00:01:51,420
degradation not much different for the

43
00:01:59,179 --> 00:01:54,030
wet/dry cycles there's one big caveat

44
00:02:01,190 --> 00:01:59,189
though the the enzymes that we use are

45
00:02:04,639 --> 00:02:01,200
not particularly very prebiotic not at

46
00:02:07,039 --> 00:02:04,649
all however that's not the point whether

47
00:02:10,639 --> 00:02:07,049
we use enzymes or whether it was any the

48
00:02:12,710 --> 00:02:10,649
system of degradation and ligation the

49
00:02:14,420 --> 00:02:12,720
RNA sequence accretion pathways are

50
00:02:17,570 --> 00:02:14,430
likely to have been along similar lines

51
00:02:21,020 --> 00:02:17,580
that's the central idea now what did we

52
00:02:22,100 --> 00:02:21,030
do we use two enzymes one of them is the

53
00:02:26,480 --> 00:02:22,110
nucleus

54
00:02:28,970 --> 00:02:26,490
nucleic acids while having absolutely

55
00:02:31,490 --> 00:02:28,980
zero proteolytic activity and when it

56
00:02:33,020 --> 00:02:31,500
does the degradation it creates a five

57
00:02:35,840 --> 00:02:33,030
prime phosphate N and three prime

58
00:02:38,930 --> 00:02:35,850
evasion in the degraded product which is

59
00:02:41,570 --> 00:02:38,940
perfect as a substrate for the following

60
00:02:44,480 --> 00:02:41,580
enzyme which is a t4 RNA ligase one

61
00:02:49,280 --> 00:02:44,490
which catalyzes the ligation of these

62
00:02:52,340 --> 00:02:49,290
two products if the help of ATP so what

63
00:02:54,410 --> 00:02:52,350

do we do we put them together in a

64
00:02:58,040 --> 00:02:54,420
buffer system which we establish along

65
00:03:00,590 --> 00:02:58,050
with an RNA ala go and while one enzyme

66
00:03:02,660 --> 00:03:00,600
cuts the other our game enzyme puts it

67
00:03:05,900 --> 00:03:02,670
puts it together and this cycle goes on

68
00:03:07,970 --> 00:03:05,910
or a period of time in day and the going

69
00:03:11,150 --> 00:03:07,980
assumption is that this will help in

70
00:03:13,520 --> 00:03:11,160
increase in buildup of the RNA size if

71
00:03:15,110 --> 00:03:13,530
not complexity I'm I'm very nervous

72
00:03:18,260 --> 00:03:15,120
about using the word complexity after

73
00:03:20,930 --> 00:03:18,270
today's morning session yeah so this is

74
00:03:22,940 --> 00:03:20,940
exactly what happens shuffling of cards

75
00:03:24,800 --> 00:03:22,950
but with one small difference

76
00:03:26,330 --> 00:03:24,810
assuming that instead of just one deck

77
00:03:29,060 --> 00:03:26,340
of cards there are several decks of

78
00:03:31,250 --> 00:03:29,070
cards each deck of cards refers to one

79
00:03:32,690 --> 00:03:31,260
oligo in the entire mix so every time

80
00:03:35,000 --> 00:03:32,700
you shuffle them there is a new

81
00:03:37,100 --> 00:03:35,010
combination and hopefully build up of

82
00:03:41,030 --> 00:03:37,110
size not complexity we do not know yet

83
00:03:43,430 --> 00:03:41,040
okay so what we did we chose the seed

84
00:03:45,949 --> 00:03:43,440
Allah go which is a reasonably humble

85
00:03:49,430 --> 00:03:45,959
oligo it has no secondary structure so

86
00:03:53,080 --> 00:03:49,440
convenient and it has just one small

87
00:03:55,970 --> 00:03:53,090
little repeat of a followed by three C's

88
00:03:57,800 --> 00:03:55,980

but absolutely no secondary structure so

89
00:03:59,479 --> 00:03:57,810
you take the seed Allah go combine it

90
00:04:02,210 --> 00:03:59,489
with the benzene is and ligase along

91
00:04:04,670 --> 00:04:02,220
with ATP run it for 180 minutes take

92
00:04:06,979 --> 00:04:04,680
wood samples every 30 minutes sequence

93
00:04:09,199 --> 00:04:06,989
the same and also run gels of each of

94
00:04:12,380 --> 00:04:09,209
these samples together and what do we

95
00:04:15,199 --> 00:04:12,390
see when we run the gel we immediately

96
00:04:17,390 --> 00:04:15,209
see a forty mer which we suspect to be

97
00:04:19,699 --> 00:04:17,400
the only cue ligated with itself and we

98
00:04:24,950 --> 00:04:19,709
also see bands that gradually increase

99
00:04:26,870 --> 00:04:24,960
in intensity but past the 120 minute

100
00:04:29,930 --> 00:04:26,880
point they start decreasing because the

101
00:04:33,800 --> 00:04:29,940
ATP gets used up okay when we sequence

102
00:04:35,070 --> 00:04:33,810
these reaction pools we make DNA

103
00:04:36,570 --> 00:04:35,080
libraries

104
00:04:38,610 --> 00:04:36,580
which had its own set of problems are

105
00:04:42,059 --> 00:04:38,620
not to be talking about them but when

106
00:04:47,459 --> 00:04:42,069
you look at the sequences the blue bar

107
00:04:50,279 --> 00:04:47,469
here is the seed oligo which decreases

108
00:04:52,559 --> 00:04:50,289
over time which I which one would think

109
00:04:54,480 --> 00:04:52,569
is intuitively expected but what is

110
00:04:56,820 --> 00:04:54,490
interesting is the increase in the

111
00:04:59,309 --> 00:04:56,830
number of unique sequences which is

112
00:05:00,749 --> 00:04:59,319
unique to each time point at 30 minute

113
00:05:04,170 --> 00:05:00,759

time point there is a set of unique

114
00:05:06,149 --> 00:05:04,180
sequences that are a comparative

115
00:05:07,290 --> 00:05:06,159
compared to the seed or liqueur here at

116
00:05:08,459 --> 00:05:07,300
this level and then they keep on

117
00:05:11,249 --> 00:05:08,469
increasing in percentage

118
00:05:13,110 --> 00:05:11,259
this shows the percentage and at the 120

119
00:05:16,290 --> 00:05:13,120
and 150 minute points they are almost

120
00:05:19,709 --> 00:05:16,300
more than 85% of the total RNA pol but

121
00:05:21,930 --> 00:05:19,719
as we saw on the gel past the 120 150

122
00:05:24,600 --> 00:05:21,940
minute point 80 baguettes used up so the

123
00:05:26,100 --> 00:05:24,610
total oral oral RNA pool starts getting

124
00:05:27,899 --> 00:05:26,110
degraded because the benzene here takes

125
00:05:32,850 --> 00:05:27,909
all it's almost like a catch me if you

126
00:05:34,379 --> 00:05:32,860
can experiment so what did we learned

127
00:05:37,559 --> 00:05:34,389
from this particular explained is that

128
00:05:40,980 --> 00:05:37,569
the DCC system definitely produces

129
00:05:42,899 --> 00:05:40,990
products larger than the scene and point

130
00:05:45,209 --> 00:05:42,909
mutations are very very rare in this

131
00:05:47,040 --> 00:05:45,219
system and most of the changes involve

132
00:05:51,300 --> 00:05:47,050
insertion and deletion of small blocks

133
00:05:53,219 --> 00:05:51,310
akin to recombination events however if

134
00:05:56,189 --> 00:05:53,229
only we could manage to figure out a way

135
00:05:58,769 --> 00:05:56,199
to keep on adding ATP we'll have more

136
00:06:03,180 --> 00:05:58,779
room for extra RNA sequence space

137
00:06:05,820 --> 00:06:03,190
exploration past one 180 minutes okay

138
00:06:07,740 --> 00:06:05,830

now it is not realistic that in the

139
00:06:10,200 --> 00:06:07,750
ancient are on a wall if there was one

140
00:06:11,670 --> 00:06:10,210
that they'll just be 1 L ago what if

141
00:06:14,820 --> 00:06:11,680
there are multiple early goes so we use

142
00:06:18,269 --> 00:06:14,830
multiple seeds what we what we chose was

143
00:06:21,329 --> 00:06:18,279
this mini helix alanine our tRNA which

144
00:06:24,540 --> 00:06:21,339
has we just split it into four different

145
00:06:27,269 --> 00:06:24,550
parts and put them all together hoping

146
00:06:31,079 --> 00:06:27,279
to see what happens and what happened

147
00:06:32,820 --> 00:06:31,089
after 15 minutes distinctly this

148
00:06:34,350 --> 00:06:32,830
particular section with the CCA end

149
00:06:36,899 --> 00:06:34,360
completely disappeared you cannot see

150
00:06:43,740 --> 00:06:36,909
rain right here but after 15 minutes you

151
00:06:45,530 --> 00:06:43,750
still see the other other 3 on top but

152
00:06:48,500 --> 00:06:45,540
after 180 minutes

153
00:06:51,140 --> 00:06:48,510
you find that this three these three

154
00:06:55,010 --> 00:06:51,150
have been ligated together - the fourth

155
00:06:57,770 --> 00:06:55,020
part and there this one was nowhere in

156
00:06:59,870 --> 00:06:57,780
the picture in the initial RNA pool they

157
00:07:04,520 --> 00:06:59,880
have not occupied the amongst the first

158
00:07:08,120 --> 00:07:04,530
top ten now the original seed from which

159
00:07:10,820 --> 00:07:08,130
the four roll egos were derived had just

160
00:07:12,650 --> 00:07:10,830
one copy number that kind of reverses

161
00:07:14,870 --> 00:07:12,660
back to the stiffened golden paradox as

162
00:07:17,930 --> 00:07:14,880
I call it if you had to start life with

163
00:07:19,850 --> 00:07:17,940

pieces of the ribosomal RNA with

164
00:07:22,130 --> 00:07:19,860
something like this or even wet/dry

165
00:07:24,110 --> 00:07:22,140
cycles it's highly unlikely we will get

166
00:07:26,740 --> 00:07:24,120
something like the pre PTC so that's a

167
00:07:30,530 --> 00:07:26,750
huge challenge we have to keep in mind

168
00:07:32,030 --> 00:07:30,540
okay now it is also a reasonably fair

169
00:07:34,160 --> 00:07:32,040
assumption based on all the talks we

170
00:07:35,780 --> 00:07:34,170
have listened to that the RNA world was

171
00:07:38,420 --> 00:07:35,790
not just an RNA wall it must have had

172
00:07:40,040 --> 00:07:38,430
inputs of peptides or amino acids so how

173
00:07:42,260 --> 00:07:40,050
do the presence of peptides or amino

174
00:07:44,840 --> 00:07:42,270
acids could have impacted this sequence

175
00:07:47,720 --> 00:07:44,850
space exploration to do that we chose

176
00:07:49,460 --> 00:07:47,730
two peptides one a peptide of ancient

177
00:07:51,530 --> 00:07:49,470
origin which is highly conserved

178
00:07:55,010 --> 00:07:51,540
extension of the ribosomal protein l2

179
00:07:56,930 --> 00:07:55,020
and as a control we choose a second

180
00:07:59,210 --> 00:07:56,940
peptide which is of much more recent

181
00:08:00,530 --> 00:07:59,220
origin which is FM LP which plays an

182
00:08:03,500 --> 00:08:00,540
important role in the human immune

183
00:08:05,270 --> 00:08:03,510
system it's a short peptide and we did

184
00:08:07,760 --> 00:08:05,280
the same by putting each of these

185
00:08:09,350 --> 00:08:07,770
separately into this DCC system with the

186
00:08:15,530 --> 00:08:09,360
two enzymes along along with the oligo

187
00:08:17,660 --> 00:08:15,540
what do we see we see that while l2

188
00:08:19,790 --> 00:08:17,670

inhibits formation of the larger product

189
00:08:22,460 --> 00:08:19,800
FML b appears to favor the ligated

190
00:08:25,820 --> 00:08:22,470
product we do not know why but this is a

191
00:08:27,830 --> 00:08:25,830
very good sample to just show how the

192
00:08:30,050 --> 00:08:27,840
presence of peptides or amino acids

193
00:08:34,100 --> 00:08:30,060
could impact sequence space exploration

194
00:08:37,130 --> 00:08:34,110
when they went without them now looking

195
00:08:40,580 --> 00:08:37,140
ahead we are thinking of using the DC

196
00:08:44,060 --> 00:08:40,590
system we with rnas as well as prebiotic

197
00:08:46,100 --> 00:08:44,070
little-o and amino acids these are amino

198
00:08:48,260 --> 00:08:46,110
acids which have been catalogued very

199
00:08:50,450 --> 00:08:48,270
well by Paul Higgs and as well as

200
00:08:52,190 --> 00:08:50,460
attentions group and I'm still issuing

201
00:08:54,380 --> 00:08:52,200
using combination of these amino acids

202
00:08:56,330 --> 00:08:54,390
and short peptides again I have to make

203
00:08:58,220 --> 00:08:56,340
this caveat that's P P odd prebiotic

204
00:08:59,360 --> 00:08:58,230
systems did not have these enzymes we

205
00:09:01,010 --> 00:08:59,370
are only interested

206
00:09:05,320 --> 00:09:01,020
the dynamics of the RNA sequence space

207
00:09:10,130 --> 00:09:05,330
exploration right now here is their blog

208
00:09:11,750 --> 00:09:10,140
it's a seminal paper from Col who's in

209
00:09:13,550 --> 00:09:11,760
an atom to determine what sorts of

210
00:09:15,320 --> 00:09:13,560
prebiotic interactions between poly

211
00:09:18,020 --> 00:09:15,330
nucleotides and poly mean acids or their

212
00:09:19,640 --> 00:09:18,030
derivatives of possible while doing so

213
00:09:21,140 --> 00:09:19,650

we hope that this knowledge will in turn

214
00:09:23,270 --> 00:09:21,150
lead to the development of a concept of

215
00:09:25,430 --> 00:09:23,280
what a workable primitive form of

216
00:09:28,220 --> 00:09:25,440
translation could have been like so

217
00:09:30,530 --> 00:09:28,230
towards this visionary goal of the late

218
00:09:33,590 --> 00:09:30,540
Carlos I think it is important for us to

219
00:09:36,080 --> 00:09:33,600
trace the roots of the ribosome through

220
00:09:44,930 --> 00:09:36,090
various systems including the DCC that's

221
00:09:47,990 --> 00:09:44,940
all at thank you are there any questions

222
00:09:49,970 --> 00:09:48,000
out in the audience if anyone's

223
00:09:52,340 --> 00:09:49,980
gathering up the courage for actually

224
00:09:54,680 --> 00:09:52,350
have a question these sequences that you

225
00:09:57,230 --> 00:09:54,690
do see that are showing up are they

226
00:09:59,000 --> 00:09:57,240
correlated with any particular property

227
00:10:00,650 --> 00:09:59,010
that causes them to show up because it

228
00:10:02,870 --> 00:10:00,660
seems like in terms of sequence base

229
00:10:04,340 --> 00:10:02,880
there are so many possibilities so is

230
00:10:06,080 --> 00:10:04,350
there any commonality for what keeps

231
00:10:07,610 --> 00:10:06,090
showing up you Moore's life that they

232
00:10:08,960 --> 00:10:07,620
removed because I might be running out

233
00:10:12,410 --> 00:10:08,970
of time which is a very good habit of

234
00:10:15,260 --> 00:10:12,420
mine so benzene is the enzyme is not

235
00:10:18,410 --> 00:10:15,270
particularly agnostic it's very we found

236
00:10:23,540 --> 00:10:18,420
that from the from the statistics it

237
00:10:24,830 --> 00:10:23,550
cuts at anywhere after or before a C so

238
00:10:28,070 --> 00:10:24,840

that could be the reason why they are

239
00:10:30,170 --> 00:10:28,080
appearing however if we also tried a

240
00:10:32,470 --> 00:10:30,180
different system by using alkaline

241
00:10:34,880 --> 00:10:32,480
hydrolysis combined with another laggies

242
00:10:38,000 --> 00:10:34,890
which combines three prime phosphate

243
00:10:40,460 --> 00:10:38,010
with F with Phi prime OS but that enzyme

244
00:10:42,140 --> 00:10:40,470
number one it forms cyclical products it

245
00:10:46,640 --> 00:10:42,150
doesn't push the reaction forward number

246
00:10:50,110 --> 00:10:46,650
two it's ridiculously expensive all

247
00:10:52,280 --> 00:10:50,120
right dr. Thirumalai thank you very much