AbGradCon 2018

1
00:00:00,260 --> 00:00:12,270
[Music]

2
00:00:17,470 --> 00:00:15,039
and I work with dr. Sudha WrestleManias

3
00:00:20,320 --> 00:00:17,480
graduate student and today I'm going to

4
00:00:22,870 --> 00:00:20,330
talk to you about why it is important to

5
00:00:24,790 --> 00:00:22,880
consider the prebiotic complexity when

6
00:00:27,880 --> 00:00:24,800
you are studying the relevant 3 biotic

7
00:00:29,980 --> 00:00:27,890
reactions so I was going to start with

8
00:00:31,900 --> 00:00:29,990
the concept of R in the world but there

9
00:00:34,150 --> 00:00:31,910
it has already done a very good job so

10
00:00:35,920 --> 00:00:34,160
I'll just give this slide and we'll go

11
00:00:40,060 --> 00:00:35,930
to the steps that are actually involved

12
00:00:45,580 --> 00:00:40,070
you really are in a world so you have

13
00:00:47,830 --> 00:00:45,590

this yeah you have so how do you get to

14
00:00:50,049 --> 00:00:47,840
a functional RNA from ER you know a bag

15
00:00:52,330 --> 00:00:50,059
of just random chemicals so you have

16
00:00:55,180 --> 00:00:52,340
some chemicals and then suppose if you

17
00:00:57,430 --> 00:00:55,190
form ribonucleotides and then they get

18
00:00:59,680 --> 00:00:57,440
together to form this RNA some of them

19
00:01:02,259 --> 00:00:59,690
which will be functional so the next

20
00:01:04,750 --> 00:01:02,269
step is once you have this functional

21
00:01:07,570 --> 00:01:04,760
molecule you also need to you know copy

22
00:01:09,820 --> 00:01:07,580
this information very accurately so that

23
00:01:11,890 --> 00:01:09,830
you retain the function so you go on

24
00:01:14,080 --> 00:01:11,900
copying these reactions and then you do

25
00:01:16,210 --> 00:01:14,090
undergo natural selection and in

26
00:01:18,490 --> 00:01:16,220
somewhere in here these functional

27
00:01:20,530 --> 00:01:18,500
molecules get encapsulated to give you a

28
00:01:24,670 --> 00:01:20,540
structure something similar to a

29
00:01:27,280 --> 00:01:24,680
protocell ok so out of in the scheme and

30
00:01:30,580 --> 00:01:27,290
I know there are other views about you

31
00:01:32,800 --> 00:01:30,590
know how I never could not have evolved

32
00:01:34,359 --> 00:01:32,810
de novo like there are P RNA boys and so

33
00:01:36,789 --> 00:01:34,369
on and so forth but I'm going to stick

34
00:01:39,039 --> 00:01:36,799
to our inner world and where I work is

35
00:01:42,010 --> 00:01:39,049
right here where I study the RNA

36
00:01:43,960 --> 00:01:42,020
replication so I study how accurately

37
00:01:47,139 --> 00:01:43,970
the RNA replicates in absence of any

38
00:01:49,480 --> 00:01:47,149

enzymes so a lot of prebiotic chemists

39
00:01:51,789 --> 00:01:49,490
what they do is they pick and choose

40
00:01:53,649 --> 00:01:51,799
their favorite reactions and consider

41
00:01:56,350 --> 00:01:53,659
only the molecules which they are

42
00:01:58,330 --> 00:01:56,360
studying so whoever is studying is

43
00:02:00,609 --> 00:01:58,340
replication they only consider RNA and

44
00:02:03,069 --> 00:02:00,619
similar is for amino acids and lipids

45
00:02:05,050 --> 00:02:03,079
and so on and so forth but if you

46
00:02:06,760 --> 00:02:05,060
remember the graph or the HPLC profile

47
00:02:08,490 --> 00:02:06,770
that Becky showed us this morning from

48
00:02:10,870 --> 00:02:08,500
for most reactions you have like

49
00:02:12,490 --> 00:02:10,880
hundreds of Peaks there and the ribose

50
00:02:14,980 --> 00:02:12,500
is the only tiny fraction of the for

51
00:02:16,390 --> 00:02:14,990
most reaction that you get and similar

52
00:02:18,040 --> 00:02:16,400
is the case with Fischer trough

53
00:02:18,620 --> 00:02:18,050
synthesis like you have thousands of

54
00:02:20,270 --> 00:02:18,630
products so

55
00:02:23,090 --> 00:02:20,280
you consider any prebiotic reactions

56
00:02:25,460 --> 00:02:23,100
there is no single product so the

57
00:02:27,170 --> 00:02:25,470
situation might be something similar to

58
00:02:29,570 --> 00:02:27,180
this like if I want to study lipids

59
00:02:31,670 --> 00:02:29,580
there are also other molecules which are

60
00:02:33,830 --> 00:02:31,680
there in this mixture we just ignore

61
00:02:36,590 --> 00:02:33,840
them but probably we shouldn't ignore

62
00:02:39,650 --> 00:02:36,600
them because they might have effects on

63
00:02:42,410 --> 00:02:39,660

our favorite reactions so for example if

64
00:02:44,600 --> 00:02:42,420
you have a molecule which is this tiny

65
00:02:47,060 --> 00:02:44,610
it could probably survive in a pool of

66
00:02:49,250 --> 00:02:47,070
large giant molecules I mean that might

67
00:02:52,790 --> 00:02:49,260
not affect it but if you have a molecule

68
00:02:55,400 --> 00:02:52,800
of similar size the it starts with the

69
00:02:57,530 --> 00:02:55,410
diffusion of this molecule gets affected

70
00:02:59,600 --> 00:02:57,540
because of just because of you have a

71
00:03:01,070 --> 00:02:59,610
lot of molecules in there and there will

72
00:03:04,400 --> 00:03:01,080
be another effects and so on and so

73
00:03:07,100 --> 00:03:04,410
forth so what we decided is okay let us

74
00:03:09,800 --> 00:03:07,110
add some background molecules to this

75
00:03:12,650 --> 00:03:09,810
RNA replication reactions and see what

76
00:03:14,420 --> 00:03:12,660
what what's the effect so because RNA

77
00:03:17,570 --> 00:03:14,430
replication is my favorite reaction I

78
00:03:19,070 --> 00:03:17,580
selected RNA template and primer so we

79
00:03:20,480 --> 00:03:19,080
have this template and we have four

80
00:03:22,910 --> 00:03:20,490
versions of template because we have

81
00:03:25,880 --> 00:03:22,920
four bases and then there is this primer

82
00:03:27,560 --> 00:03:25,890
and so what I do is I add one one over

83
00:03:30,590 --> 00:03:27,570
at a time and study the rate of the

84
00:03:33,230 --> 00:03:30,600
reaction so the selected monomer is is

85
00:03:34,820 --> 00:03:33,240
this which is a modification at the

86
00:03:36,590 --> 00:03:34,830
phosphate end of the monomer just to

87
00:03:37,670 --> 00:03:36,600
enhance the rate of the reaction so that

88
00:03:40,160 --> 00:03:37,680

I can study it on the laboratory

89
00:03:42,200 --> 00:03:40,170
timescale and the course I'll do is that

90
00:03:44,150 --> 00:03:42,210
we selected our two the first one is a

91
00:03:46,220 --> 00:03:44,160
lipid molecule and why lipid molecule

92
00:03:47,780 --> 00:03:46,230
because it has implications in forming

93
00:03:50,330 --> 00:03:47,790
this protists cellular membranes or

94
00:03:52,610 --> 00:03:50,340
boundary conditions and the second one

95
00:03:55,490 --> 00:03:52,620
is peg because a lot of biochemical

96
00:03:57,260 --> 00:03:55,500
studies also include peg because it

97
00:03:58,580 --> 00:03:57,270
makes molecular crowding and so on and

98
00:03:59,800 --> 00:03:58,590
so forth so we selected these two

99
00:04:03,070 --> 00:03:59,810
molecules okay

100
00:04:05,660 --> 00:04:03,080
so how do we study this reaction so we

101
00:04:07,640 --> 00:04:05,670
analyze it using gel electrophoresis and

102
00:04:09,710 --> 00:04:07,650
then we get the rate of the reaction so

103
00:04:11,750 --> 00:04:09,720
you have for templating basis for

104
00:04:13,850 --> 00:04:11,760
nucleotides that come in so you have 16

105
00:04:15,860 --> 00:04:13,860
reactions and I studied them in four

106
00:04:18,320 --> 00:04:15,870
different conditions so 64 different

107
00:04:20,660 --> 00:04:18,330
reactions so let me first show you the

108
00:04:22,370 --> 00:04:20,670
results from match traditional reaction

109
00:04:25,250 --> 00:04:22,380
so by match tradition what I mean is

110
00:04:26,930 --> 00:04:25,260
when you try to add a across or new

111
00:04:28,310 --> 00:04:26,940
templating base that's a mass reduction

112
00:04:29,990 --> 00:04:28,320
reaction so there are four nice

113
00:04:32,600 --> 00:04:30,000

traditions and there are twelve

114
00:04:36,080 --> 00:04:32,610
mismatched traditions so what happens

115
00:04:38,689 --> 00:04:36,090
masterda station reaction so nothing

116
00:04:40,369 --> 00:04:38,699
happens to these two reactions so on

117
00:04:42,439 --> 00:04:40,379
x-axis you have the incoming nucleotide

118
00:04:44,899 --> 00:04:42,449
so when the incoming nucleotide is a

119
00:04:47,149 --> 00:04:44,909
pyrimidine which is C or you nothing

120
00:04:49,159 --> 00:04:47,159
really happens but then you have an

121
00:04:51,230 --> 00:04:49,169
incoming nucleotide versus G and a which

122
00:04:53,119 --> 00:04:51,240
is actually a purine the rate of the

123
00:04:55,070 --> 00:04:53,129
reaction goes down if you have these

124
00:04:58,279 --> 00:04:55,080
background molecules in the picture okay

125
00:05:00,860 --> 00:04:58,289
so this might be a more a bit more clear

126
00:05:02,360 --> 00:05:00,870
in the next slide which is this so an

127
00:05:04,730 --> 00:05:02,370
x-axis what I have done is I've plotted

128
00:05:06,290 --> 00:05:04,740
the rate difference between a control

129
00:05:07,730 --> 00:05:06,300
reaction where you do not have any

130
00:05:09,469 --> 00:05:07,740
background molecules the reaction is

131
00:05:11,600 --> 00:05:09,479
happening in buffered condition just the

132
00:05:13,040 --> 00:05:11,610
buffer and the second where I have the

133
00:05:15,439 --> 00:05:13,050
background molecules so that's on the

134
00:05:17,899 --> 00:05:15,449
x-axis and I am normalized that on the

135
00:05:19,519 --> 00:05:17,909
y-axis because rate of all different

136
00:05:21,769 --> 00:05:19,529
reactions is different like master

137
00:05:23,990 --> 00:05:21,779
additions take place at a faster rate so

138
00:05:25,670 --> 00:05:24,000

these two points they stand out

139
00:05:27,200 --> 00:05:25,680
everything else is clustered near zero

140
00:05:29,029 --> 00:05:27,210
zero but these two points stand out

141
00:05:34,279 --> 00:05:29,039
where you have addition of a purine

142
00:05:35,929 --> 00:05:34,289
across a pyrimidine so yes so I mean how

143
00:05:38,149 --> 00:05:35,939
does it matter right only two out of 16

144
00:05:40,670 --> 00:05:38,159
reactions are getting affected so what's

145
00:05:42,019 --> 00:05:40,680
the big deal but just now I told you

146
00:05:43,730 --> 00:05:42,029
that these are the master addition

147
00:05:46,040 --> 00:05:43,740
reactions so these are two of the four

148
00:05:49,279 --> 00:05:46,050
fastest reactions in those 16 reactions

149
00:05:50,659 --> 00:05:49,289
so what they do is they affect the

150
00:05:52,219 --> 00:05:50,669
number of times you are getting a

151
00:05:53,450 --> 00:05:52,229
correct addition to your primer so

152
00:05:56,360 --> 00:05:53,460
number of times you are getting a

153
00:05:58,269 --> 00:05:56,370
correct replication so that's depicted

154
00:06:00,740 --> 00:05:58,279
here so if you consider this graph

155
00:06:03,200 --> 00:06:00,750
anyways the addition the frequency of

156
00:06:05,089 --> 00:06:03,210
addition across a and u is not that

157
00:06:07,159 --> 00:06:05,099
accurate and it's already known in the

158
00:06:09,290 --> 00:06:07,169
literature what happens if you start

159
00:06:12,469 --> 00:06:09,300
adding these core solutes this happens

160
00:06:15,320 --> 00:06:12,479
so the frequency of addition of G across

161
00:06:17,480 --> 00:06:15,330
U which is a bobble pair it considered

162
00:06:20,329 --> 00:06:17,490
considerably increases when you have

163
00:06:23,179 --> 00:06:20,339

this core solutes in the background you

164
00:06:24,709 --> 00:06:23,189
know so if you convert these frequencies

165
00:06:26,480 --> 00:06:24,719
to mutation rates

166
00:06:28,189 --> 00:06:26,490
you obviously have higher mutation rates

167
00:06:31,040 --> 00:06:28,199
when you have these background molecules

168
00:06:34,459 --> 00:06:31,050
so and just remember we have added just

169
00:06:36,499 --> 00:06:34,469
tuned and not a lot of them so the the

170
00:06:38,659 --> 00:06:36,509
effect is really drastic if you have a

171
00:06:42,829 --> 00:06:38,669
you templating base like it goes from

172
00:06:44,839 --> 00:06:42,839
31% to 51% of error that's occurring so

173
00:06:45,710 --> 00:06:44,849
if you have a functional sequence which

174
00:06:47,990 --> 00:06:45,720
is like really

175
00:06:50,090 --> 00:06:48,000
Niraj you will and in the scenario if

176
00:06:51,320 --> 00:06:50,100
it's replicating it will quickly lose

177
00:06:54,770 --> 00:06:51,330
this function because it's not

178
00:06:56,450 --> 00:06:54,780
replicating correctly so that's so the

179
00:06:59,450 --> 00:06:56,460
main point in this talk that I want to

180
00:07:01,160 --> 00:06:59,460
get across here is we need to consider

181
00:07:03,050 --> 00:07:01,170
the presence of these background

182
00:07:04,670 --> 00:07:03,060
molecules on any of the previous 3

183
00:07:07,040 --> 00:07:04,680
biotic reaction that we have considered

184
00:07:09,050 --> 00:07:07,050
because I just added to background

185
00:07:12,170 --> 00:07:09,060
molecules and it's actually already is

186
00:07:15,200 --> 00:07:12,180
reducing the accuracy of the replication

187
00:07:17,150 --> 00:07:15,210
and so it will affect the the way the

188
00:07:21,560 --> 00:07:17,160

functional sequences are evolving and

189
00:07:23,660 --> 00:07:21,570
everything ok and right now is a good

190
00:07:25,340 --> 00:07:23,670
time to do it because we also have very

191
00:07:27,650 --> 00:07:25,350
good analytical techniques and

192
00:07:30,230 --> 00:07:27,660
everything so what we want to do next

193
00:07:31,610 --> 00:07:30,240
from here is we want to understand why

194
00:07:34,730 --> 00:07:31,620
this is happening so they could make two

195
00:07:36,980 --> 00:07:34,740
reasons why this is happening there is a

196
00:07:38,390 --> 00:07:36,990
reason why there's RNA and lipid

197
00:07:41,060 --> 00:07:38,400
interaction so we are trying to

198
00:07:43,460 --> 00:07:41,070
understand that interaction using some

199
00:07:45,650 --> 00:07:43,470
microscopy and probably I'll have the

200
00:07:48,110 --> 00:07:45,660
results pretty soon but I'm going to

201
00:07:50,540 --> 00:07:48,120
share with you the data for enemas so

202
00:07:52,640 --> 00:07:50,550
with NMR what we want to do is we want

203
00:07:54,590 --> 00:07:52,650
to understand the nucleotide stacking so

204
00:07:57,950 --> 00:07:54,600
the hypothesis is because we have this

205
00:08:00,260 --> 00:07:57,960
all molecular crowding agents the

206
00:08:03,650 --> 00:08:00,270
nucleotide stacking is getting enhanced

207
00:08:05,450 --> 00:08:03,660
in these situations because let me

208
00:08:06,830 --> 00:08:05,460
remind you that only purine reactions

209
00:08:08,630 --> 00:08:06,840
are getting affected so purines are

210
00:08:11,690 --> 00:08:08,640
known to stack better so if that

211
00:08:13,730 --> 00:08:11,700
stacking the that stacking increases in

212
00:08:16,040 --> 00:08:13,740
presence of background molecules the

213
00:08:18,140 --> 00:08:16,050

purines won't be available for addition

214
00:08:21,260 --> 00:08:18,150
to the RNA primer so that's what we are

215
00:08:24,530 --> 00:08:21,270
studying using p1 NMR data right now so

216
00:08:26,510 --> 00:08:24,540
these and I'm just standardizing these

217
00:08:29,570 --> 00:08:26,520
with help of Herschel here in my

218
00:08:31,730 --> 00:08:29,580
Institute so this is an even relaxation

219
00:08:34,190 --> 00:08:31,740
data for a MP and which is a purine and

220
00:08:36,230 --> 00:08:34,200
this is Steven relaxation data for UMP

221
00:08:38,180 --> 00:08:36,240
which is a pyrimidine so if you see as

222
00:08:40,339 --> 00:08:38,190
you go on increasing the concentration

223
00:08:42,170 --> 00:08:40,349
the relaxation time decreases which

224
00:08:46,370 --> 00:08:42,180
means that the molecular size is

225
00:08:49,490 --> 00:08:46,380
increasing so a MP is stacking better

226
00:08:51,800 --> 00:08:49,500
than UMP and this is a standardization

227
00:08:55,100 --> 00:08:51,810
and we going to you know extend this

228
00:08:57,110 --> 00:08:55,110
tool when we add peg or when the egg the

229
00:08:58,769 --> 00:08:57,120
LPC vesicle is what happens and any

230
00:09:01,850 --> 00:08:58,779
suggestions from you guys

231
00:09:05,310 --> 00:09:01,860
welcome because yeah this is just fresh

232
00:09:07,769 --> 00:09:05,320
so yes with that I think these are my

233
00:09:10,290 --> 00:09:07,779
acknowledgments I thank my professor and

234
00:09:19,259 --> 00:09:10,300
lab members and these are our funding

235
00:09:33,269 --> 00:09:19,269
sources and thank you all right do we

236
00:09:39,809 --> 00:09:36,900
I am is there a reason specifically why

237
00:09:44,989 --> 00:09:39,819
you use the non-traditional 3-prime

238
00:09:48,269 --> 00:09:44,999

terminated primer a non hydroxyl yeah so

239
00:09:51,389 --> 00:09:48,279
the reason that I've used it is because

240
00:09:53,790 --> 00:09:51,399
if you try and add a phosphate to a H

241
00:09:56,460 --> 00:09:53,800
that will not happen at room temperature

242
00:09:59,579 --> 00:09:56,470
so that's why because a mine is a better

243
00:10:00,989 --> 00:09:59,589
nucleophile than hydroxyl group so

244
00:10:02,610 --> 00:10:00,999
that's why we have modified the primer

245
00:10:05,340 --> 00:10:02,620
so that I actually can study the

246
00:10:09,449 --> 00:10:05,350
reactions in that skin like in 24 hours

247
00:10:11,400 --> 00:10:09,459
or so and this is a very and a lot of

248
00:10:20,720 --> 00:10:11,410
non enzymatic replication studies they

249
00:10:26,249 --> 00:10:23,610
guess I was just wondering um what would

250
00:10:28,079 --> 00:10:26,259
happen cuz I mean obviously the question

251
00:10:29,759 --> 00:10:28,089
is always what if you try other sort of

252
00:10:32,069 --> 00:10:29,769
CO solutes and things like that and you

253
00:10:34,710 --> 00:10:32,079
know they're limited limited on time and

254
00:10:36,480 --> 00:10:34,720
effort everything but what do you think

255
00:10:38,879 --> 00:10:36,490
if you you know we're including

256
00:10:40,439 --> 00:10:38,889
different calls to Co solutes or what

257
00:10:42,299 --> 00:10:40,449
would be sort of the the next one you

258
00:10:44,340 --> 00:10:42,309
would want to want to try that you think

259
00:10:46,049 --> 00:10:44,350
would have an interesting effect okay so

260
00:10:47,819 --> 00:10:46,059
the next one that we actually wanted to

261
00:10:50,069 --> 00:10:47,829
try and that we are trying right now is

262
00:10:51,869 --> 00:10:50,079
replacing the lipid with fatty acids

263
00:10:55,499 --> 00:10:51,879

because that's more like prebiotic

264
00:10:57,840 --> 00:10:55,509
really relevant and then we read I mean

265
00:11:00,540 --> 00:10:57,850
we might wanna go to dipeptides or try

266
00:11:02,519 --> 00:11:00,550
peptides which actually bind to RNA by

267
00:11:04,850 --> 00:11:02,529
electrostatic interactions and see

268
00:11:13,690 --> 00:11:04,860
whether they have any effect or not

269
00:11:19,910 --> 00:11:17,450
so you are noting that the extra solutes

270
00:11:22,550 --> 00:11:19,920
do have the higher rate of mutation but

271
00:11:26,900 --> 00:11:22,560
could that loss of fidelity actually be

272
00:11:30,920 --> 00:11:26,910
beneficial towards increased great

273
00:11:34,040 --> 00:11:30,930
presentation evolution of life so there

274
00:11:36,410 --> 00:11:34,050
are two scenarios here if you are

275
00:11:38,780 --> 00:11:36,420
already upon I mean if if it is already

276
00:11:40,880 --> 00:11:38,790
functional sequence it is not beneficial

277
00:11:43,160 --> 00:11:40,890
so the if it is a functional sequence it

278
00:11:45,050 --> 00:11:43,170
wants to get into capsulated probably

279
00:11:47,060 --> 00:11:45,060
maybe because then it will be away from

280
00:11:48,860 --> 00:11:47,070
all these Co solutes because if the

281
00:11:50,060 --> 00:11:48,870
mutation rate is high the functional

282
00:11:52,310 --> 00:11:50,070
sequence will not get replicated

283
00:11:53,990 --> 00:11:52,320
correctly and because of the loss of

284
00:11:57,410 --> 00:11:54,000
information you might lose the function

285
00:11:59,360 --> 00:11:57,420
but again it might be helpful in sort of

286
00:12:01,460 --> 00:11:59,370
another scenario when you want to get to

287
00:12:04,940 --> 00:12:01,470
the in functional sequence like you can

288
00:12:07,790 --> 00:12:04,950

probably explore a lot of sequence space

289
00:12:18,260 --> 00:12:07,800
so yeah it's it's like it might be

290
00:12:19,760 --> 00:12:18,270
helpful in one scenario versus when you

291
00:12:21,800 --> 00:12:19,770
were talking about the t1 relaxation

292
00:12:23,210 --> 00:12:21,810
times I think it was in the last slide

293
00:12:26,150 --> 00:12:23,220
do you think it's possible that the

294
00:12:28,070 --> 00:12:26,160
period nucleotides are aggregating at

295
00:12:29,420 --> 00:12:28,080
the higher concentrations and maybe

296
00:12:32,780 --> 00:12:29,430
that's why you're saying it like an

297
00:12:34,190 --> 00:12:32,790
apparent decrease yeah so that that's

298
00:12:36,590 --> 00:12:34,200
what we are trying to study actually

299
00:12:37,790 --> 00:12:36,600
whether they are aggregating or they are

300
00:12:39,350 --> 00:12:37,800
forming some sort of secondary

301
00:12:42,260 --> 00:12:39,360
structures or whatever higher higher

302
00:12:45,199 --> 00:12:42,270
order structures so that's what we want

303
00:12:48,680 --> 00:12:45,209
to try using t1 relaxation data and we

304
00:12:50,150 --> 00:12:48,690
are seeing that MP does show decrease in

305
00:12:51,860 --> 00:12:50,160
T and relaxation

306
00:12:54,230 --> 00:12:51,870
maybe it's getting aggregated or