AbGradCon 2018

1
00:00:00,260 --> 00:00:11,789
[Music]

2
00:00:17,560 --> 00:00:14,980
after everyone I area from just

3
00:00:19,750 --> 00:00:17,570
University in Prague Czech Republic and

4
00:00:21,970 --> 00:00:19,760
today I'm going to introduce to my

5
00:00:24,070 --> 00:00:21,980
project test of genetic code evolution

6
00:00:26,230 --> 00:00:24,080
hypothesis all the best evolution of the

7
00:00:29,859 --> 00:00:26,240
RNA binding domain of the ribosomal

8
00:00:32,520 --> 00:00:29,869
protein at 11:00 okay first of all I

9
00:00:34,979 --> 00:00:32,530
introduced like how was a ball with a

10
00:00:38,500 --> 00:00:34,989
pathetically was evolved with the

11
00:00:41,799 --> 00:00:38,510
genetic code different theory assumes

12
00:00:44,770 --> 00:00:41,809
that the original genetic code coding

13
00:00:47,829 --> 00:00:44,780

only for a small set of amino acid a

14
00:00:49,979 --> 00:00:47,839
dismal set of amino acid were introduced

15
00:00:52,989 --> 00:00:49,989
like in the answer to prebiotic protein

16
00:00:55,509 --> 00:00:52,999
but how the scientist like the side

17
00:00:58,149 --> 00:00:55,519
which an amino acid I've introduced are

18
00:01:01,000 --> 00:00:58,159
like early one and which other one could

19
00:01:02,289 --> 00:01:01,010
be like late amino acid particularly

20
00:01:05,890 --> 00:01:02,299
this guy Hicks

21
00:01:07,870 --> 00:01:05,900
Audrey's like summarize all the

22
00:01:11,170 --> 00:01:07,880
different theories and also all the

23
00:01:14,020 --> 00:01:11,180
different evidence about the evidence

24
00:01:16,240 --> 00:01:14,030
abundancy of the different amino acid in

25
00:01:18,130 --> 00:01:16,250
for instance in the Miller experiment

26
00:01:21,279 --> 00:01:18,140
Mathieu right or in the presence of

27
00:01:24,160 --> 00:01:21,289
hydrothermal vents so it just classify

28
00:01:27,099 --> 00:01:24,170
all the amino acid amazing of the de

29
00:01:29,590 --> 00:01:27,109
árbol dance and wonders in this in this

30
00:01:33,639 --> 00:01:29,600
environment and here in the table we can

31
00:01:36,490 --> 00:01:33,649
see that in a table we can see we can

32
00:01:38,289 --> 00:01:36,500
split the the chart into part the first

33
00:01:40,510 --> 00:01:38,299
part are represented like the early

34
00:01:44,050 --> 00:01:40,520
amino acid in see the bottom part of the

35
00:01:45,429 --> 00:01:44,060
chart is like the late immunity because

36
00:01:47,740 --> 00:01:45,439
we can also find like some evidence

37
00:01:49,569 --> 00:01:47,750
indirectly in the genetic code in fact

38
00:01:51,279 --> 00:01:49,579

we will see that the in green is

39
00:01:54,489 --> 00:01:51,289
represented like the early immunity the

40
00:01:57,249 --> 00:01:54,499
represent more than 60 percentage of the

41
00:01:59,550 --> 00:01:57,259
coding the code on that coding for amino

42
00:02:02,709 --> 00:01:59,560
acid respect to the late amino acid but

43
00:02:06,160 --> 00:02:02,719
for the moment are just like sampling

44
00:02:08,999 --> 00:02:06,170
just theory so no one and as demonstrate

45
00:02:13,990 --> 00:02:09,009
experimental II this different theories

46
00:02:15,910 --> 00:02:14,000
so my project is to verify that it's

47
00:02:18,940 --> 00:02:15,920
possible that a protein composite of

48
00:02:19,390 --> 00:02:18,950
only early amino acid amino is it is

49
00:02:23,860 --> 00:02:19,400
still

50
00:02:26,380 --> 00:02:23,870
Lentini function or extraction and so

51
00:02:28,270 --> 00:02:26,390
how to do this feel more or less is the

52
00:02:30,039 --> 00:02:28,280
flowchart of my project so the first

53
00:02:33,160 --> 00:02:30,049
step is like the selection of a target

54
00:02:34,990 --> 00:02:33,170
protein like a modern protein with some

55
00:02:37,420 --> 00:02:35,000
faction function could be like an enzyme

56
00:02:40,270 --> 00:02:37,430
or like a binding protein after that

57
00:02:43,420 --> 00:02:40,280
generate the library compose it only of

58
00:02:45,940 --> 00:02:43,430
early amino acid so substitute all the

59
00:02:47,170 --> 00:02:45,950
late amino acid with the early one and

60
00:02:49,539 --> 00:02:47,180
the next step will be the

61
00:02:51,009 --> 00:02:49,549
characterization of the mutant so

62
00:02:53,470 --> 00:02:51,019
understand if the Alpha recently the

63
00:02:56,940 --> 00:02:53,480

mutant maintain the structure maintain

64
00:03:00,339 --> 00:02:56,950
the function or both or nothing

65
00:03:03,580 --> 00:03:00,349
okay so the first step was the target

66
00:03:05,770 --> 00:03:03,590
was the selection of the target to do

67
00:03:08,050 --> 00:03:05,780
this we have to follow some strictly

68
00:03:11,170 --> 00:03:08,060
criteria the first one the protein the

69
00:03:13,420 --> 00:03:11,180
target should be small so between 50 to

70
00:03:16,119 --> 00:03:13,430
100 amino acid this is more related to

71
00:03:17,649 --> 00:03:16,129
the most practical part because of

72
00:03:19,690 --> 00:03:17,659
course if we had like a small protein

73
00:03:22,539 --> 00:03:19,700
more than 100 no is it we will generate

74
00:03:25,509 --> 00:03:22,549
a library of like more than 10 to the

75
00:03:28,180 --> 00:03:25,519
power of 3040 mutants that it's

76
00:03:31,390 --> 00:03:28,190
impossible to use like in practical way

77
00:03:33,490 --> 00:03:31,400
to manage this kind of library second

78
00:03:36,339 --> 00:03:33,500
step the protein should be like conserve

79
00:03:38,020 --> 00:03:36,349
it and also this step this criteria is

80
00:03:40,930 --> 00:03:38,030
really important in order to reduce the

81
00:03:43,960 --> 00:03:40,940
size of library because we'd like we can

82
00:03:45,940 --> 00:03:43,970
use like multiple sequence alignment or

83
00:03:49,119 --> 00:03:45,950
like a structural element in order to

84
00:03:51,789 --> 00:03:49,129
reduce the size of the library search

85
00:03:54,009 --> 00:03:51,799
pepper criteria is that this target

86
00:03:56,199 --> 00:03:54,019
should be maintained like the should

87
00:03:58,599 --> 00:03:56,209
have like the early amino acid in the

88
00:04:00,670 --> 00:03:58,609

most critical position for the function

89
00:04:02,110 --> 00:04:00,680
or for the structure this is quite

90
00:04:03,670 --> 00:04:02,120
obvious because for instance if we

91
00:04:07,150 --> 00:04:03,680
selected I don't know like an enzyme

92
00:04:08,800 --> 00:04:07,160
with the arginine in the one arginine or

93
00:04:11,199 --> 00:04:08,810
lysine in the active site if we

94
00:04:13,379 --> 00:04:11,209
substitute the arginine lysis is really

95
00:04:16,870 --> 00:04:13,389
probable that the protein will lose the

96
00:04:19,330 --> 00:04:16,880
the activity so we need to find also

97
00:04:21,819 --> 00:04:19,340
someone that maintain the amino acid in

98
00:04:24,120 --> 00:04:21,829
the critical position after that we need

99
00:04:26,379 --> 00:04:24,130
the target is well characterized and

100
00:04:28,540 --> 00:04:26,389
with the possibility to have like a

101
00:04:33,220 --> 00:04:28,550
selection method so to select the mutant

102
00:04:35,410 --> 00:04:33,230
so to perform very practically and

103
00:04:37,960 --> 00:04:35,420
the best target it was chosen was like

104
00:04:41,110 --> 00:04:37,970
the RNA binding domain of the ribosome

105
00:04:43,030 --> 00:04:41,120
betrothal at 11:00 this this domain is

106
00:04:46,900 --> 00:04:43,040
composed of seven three smaller domain

107
00:04:49,480 --> 00:04:46,910
76 amino acid so correspond to over the

108
00:04:53,170 --> 00:04:49,490
first criteria is like its function is

109
00:04:58,060 --> 00:04:53,180
to bind the specific sequence of RNA

110
00:04:59,740 --> 00:04:58,070
from the ribosomal RNA it's a

111
00:05:01,810 --> 00:04:59,750
conservative course it's a protein of

112
00:05:04,660 --> 00:05:01,820
the ribosome so is strictly conserving

113
00:05:07,270 --> 00:05:04,670

in most of species and in most of the

114
00:05:10,090 --> 00:05:07,280
species and in particularly the amino

115
00:05:13,020 --> 00:05:10,100
acid it arguing involved within the

116
00:05:16,030 --> 00:05:13,030
interaction with the DNA are mostly

117
00:05:17,710 --> 00:05:16,040
eliminated except for a recent lysine

118
00:05:20,020 --> 00:05:17,720
arginine but most of them are like a

119
00:05:24,700 --> 00:05:20,030
glycine Sarah so it's really good

120
00:05:27,730 --> 00:05:24,710
candidate for the but also if it's small

121
00:05:30,910 --> 00:05:27,740
protein still the library will be huge

122
00:05:33,520 --> 00:05:30,920
like we can talk like ten to the power

123
00:05:35,860 --> 00:05:33,530
of 30 variant if we won't substitute all

124
00:05:38,470 --> 00:05:35,870
the late amino acid with the early one

125
00:05:40,930 --> 00:05:38,480
so in order to reduce this one we have

126
00:05:43,170 --> 00:05:40,940
perform like different multiple sequence

127
00:05:46,450 --> 00:05:43,180
alignment or also structural analysis

128
00:05:48,850 --> 00:05:46,460
for instance we can we can reduce the

129
00:05:51,010 --> 00:05:48,860
size of library for instance is in IBD

130
00:05:53,410 --> 00:05:51,020
for in our target protein for this

131
00:05:55,560 --> 00:05:53,420
innocence pot could be like an arginine

132
00:05:58,630 --> 00:05:55,570
but we can find that in a really

133
00:06:02,050 --> 00:05:58,640
collagen product protein that could be

134
00:06:03,970 --> 00:06:02,060
like a Spartacus II that and these

135
00:06:05,950 --> 00:06:03,980
mutations to maintain the function so we

136
00:06:08,170 --> 00:06:05,960
can reduce the size of library in this

137
00:06:10,510 --> 00:06:08,180
way just with a multiple sequence

138
00:06:12,340 --> 00:06:10,520

alignment all for instance depend on the

139
00:06:13,660 --> 00:06:12,350
position of the late amino acid for

140
00:06:16,030 --> 00:06:13,670
instance if a late amino acid the

141
00:06:18,610 --> 00:06:16,040
present is like an alpha helix we can

142
00:06:20,950 --> 00:06:18,620
for instance avoid like the presence of

143
00:06:23,050 --> 00:06:20,960
a protein of prolene or other kind of

144
00:06:23,740 --> 00:06:23,060
immunity at the end of this analysis we

145
00:06:26,920 --> 00:06:23,750
are fine

146
00:06:29,140 --> 00:06:26,930
the we are reaching like the size of 10

147
00:06:32,260 --> 00:06:29,150
to the power of 10 that is still huge

148
00:06:34,780 --> 00:06:32,270
number but with the the mRNA display

149
00:06:38,290 --> 00:06:34,790
technique it's still possible to express

150
00:06:40,060 --> 00:06:38,300
this kind of library this technique is

151
00:06:41,620 --> 00:06:40,070
really powerful because respect to the

152
00:06:43,060 --> 00:06:41,630
other one for instance like the page

153
00:06:45,880 --> 00:06:43,070
display or the technique the technique

154
00:06:47,150 --> 00:06:45,890
that you can express a library maximum

155
00:06:49,040 --> 00:06:47,160
10 to the power of nine

156
00:06:53,480 --> 00:06:49,050
clone in these cases we can express

157
00:06:54,890 --> 00:06:53,490
express a 10 to the power 3 so 3

158
00:06:58,520 --> 00:06:54,900
magnitude more respect of the other

159
00:07:00,170 --> 00:06:58,530
technique this technique use it

160
00:07:02,420 --> 00:07:00,180
practically connect together the

161
00:07:05,210 --> 00:07:02,430
genotype to the phenotype so we have

162
00:07:06,080 --> 00:07:05,220
like the RNA the protein linked to its

163
00:07:07,910 --> 00:07:06,090

mRNA

164
00:07:11,060 --> 00:07:07,920
how it's possible thanks to the

165
00:07:12,590 --> 00:07:11,070
promising and these molecules so during

166
00:07:14,480 --> 00:07:12,600
the translation the ribosome when

167
00:07:17,060 --> 00:07:14,490
arrived to the three terminal interact

168
00:07:20,180 --> 00:07:17,070
with the promising they enter inside the

169
00:07:22,460 --> 00:07:20,190
ribosome a link the protein and in same

170
00:07:26,450 --> 00:07:22,470
time disassembly the ribosome so we have

171
00:07:28,820 --> 00:07:26,460
like the RNA linkage to the protein but

172
00:07:30,860 --> 00:07:28,830
before to perform the before to perform

173
00:07:33,950 --> 00:07:30,870
the mRNA display technique we have to

174
00:07:35,750 --> 00:07:33,960
optimize the selection method before the

175
00:07:40,400 --> 00:07:35,760
protein and we have optimized with the

176
00:07:41,930 --> 00:07:40,410
with the wall type so we use it like a

177
00:07:43,850 --> 00:07:41,940
cell free expression system so it's

178
00:07:46,510 --> 00:07:43,860
meaning that we didn't use the cell but

179
00:07:49,730 --> 00:07:46,520
just the extract of ribosome and all the

180
00:07:52,100 --> 00:07:49,740
translation I keep my fist timer for

181
00:07:54,680 --> 00:07:52,110
this patient so we will Express where

182
00:07:56,270 --> 00:07:54,690
the the protein were expressing was

183
00:07:59,540 --> 00:07:56,280
pricey and after that were incubated

184
00:08:02,810 --> 00:07:59,550
with the target RNA it was functional ID

185
00:08:05,920 --> 00:08:02,820
with a biotin molecules after that for

186
00:08:09,200 --> 00:08:05,930
the selection we can was like the

187
00:08:10,940 --> 00:08:09,210
complex protein and target so when

188
00:08:13,580 --> 00:08:10,950

incubated with the strategy bits so it's

189
00:08:15,650 --> 00:08:13,590
possible to select the the complex and

190
00:08:17,840 --> 00:08:15,660
here we can see like the reservoir like

191
00:08:19,610 --> 00:08:17,850
job well like in the first Lane we can

192
00:08:24,020 --> 00:08:19,620
see like the fluid row so all the

193
00:08:25,700 --> 00:08:24,030
protein that doesn't bind the tRNA and

194
00:08:29,090 --> 00:08:25,710
it's empty so it's meaning that all

195
00:08:32,180 --> 00:08:29,100
protein interact with the RNA with the

196
00:08:34,700 --> 00:08:32,190
target RNA in the washing step so it's a

197
00:08:36,529 --> 00:08:34,710
nothing so it's meaning that no we can

198
00:08:39,409 --> 00:08:36,539
bind there so all the protein binded an

199
00:08:41,810 --> 00:08:39,419
illusion you can see all our product it

200
00:08:44,360 --> 00:08:41,820
means that this protein this selection

201
00:08:48,650 --> 00:08:44,370
method is at least for the what type is

202
00:08:50,330 --> 00:08:48,660
tiwa be able so we have told you we have

203
00:08:54,310 --> 00:08:50,340
introduced with this selection method

204
00:08:57,200 --> 00:08:54,320
it's inside the Emily display technique

205
00:08:59,240 --> 00:08:57,210
here is like how to explain just let the

206
00:09:01,319 --> 00:08:59,250
floor show the the flowchart of the of

207
00:09:04,170 --> 00:09:01,329
this technique

208
00:09:07,490 --> 00:09:04,180
start with the DNA so we have like the

209
00:09:11,069 --> 00:09:07,500
DNA library after that we transcribe the

210
00:09:14,220 --> 00:09:11,079
DNA library RNA after that there will be

211
00:09:18,660 --> 00:09:14,230
the step where the RNA will be linked it

212
00:09:20,460 --> 00:09:18,670
to the promise in molecules and in the

213
00:09:23,009 --> 00:09:20,470

linker there will be like a fluorescent

214
00:09:24,600 --> 00:09:23,019
probe that will be useful for the next

215
00:09:26,759 --> 00:09:24,610
step just to follow like the different

216
00:09:29,970 --> 00:09:26,769
paper in the presence of not of the

217
00:09:32,309 --> 00:09:29,980
protein after that after that we have

218
00:09:34,439 --> 00:09:32,319
this construct like RNA plus promising

219
00:09:36,240 --> 00:09:34,449
there will be the expression so the

220
00:09:38,639 --> 00:09:36,250
junction between like the protein and

221
00:09:41,309 --> 00:09:38,649
the hair na and after that we have like

222
00:09:45,120 --> 00:09:41,319
so this library composite of like 10 to

223
00:09:49,350 --> 00:09:45,130
the power to the 10 to power 10 variant

224
00:09:52,199 --> 00:09:49,360
that will be selected toward the target

225
00:09:55,019 --> 00:09:52,209
RNA after that there will be like the

226
00:09:57,509 --> 00:09:55,029
aleutian and this type of reverse

227
00:09:59,699 --> 00:09:57,519
transcription because here we have DNA

228
00:10:02,999 --> 00:09:59,709
in this step we need the DNA for the

229
00:10:05,460 --> 00:10:03,009
sequencing or a new round of all to

230
00:10:06,840 --> 00:10:05,470
perform a new round of selection so

231
00:10:10,949 --> 00:10:06,850
there will be like just read less

232
00:10:12,360 --> 00:10:10,959
transcription so from DNA RNA DNA it's

233
00:10:14,249 --> 00:10:12,370
important also the negative control

234
00:10:16,170 --> 00:10:14,259
because I don't know it's someone worked

235
00:10:19,650 --> 00:10:16,180
with the RNA but the RNA is really

236
00:10:22,499 --> 00:10:19,660
sticky so we have to subtract the noise

237
00:10:24,120 --> 00:10:22,509
from the from the of the project so more

238
00:10:26,179 --> 00:10:24,130

or less for the negative control is the

239
00:10:28,439 --> 00:10:26,189
same step is the same process a

240
00:10:29,670 --> 00:10:28,449
parameter the only difference that to

241
00:10:32,910 --> 00:10:29,680
the approach the library will be

242
00:10:35,670 --> 00:10:32,920
incubated not with the the target RNA

243
00:10:37,439 --> 00:10:35,680
but just with the bits so in this way we

244
00:10:40,259 --> 00:10:37,449
can select it that the project that bind

245
00:10:44,100 --> 00:10:40,269
us specifically the the surface or the

246
00:10:48,360 --> 00:10:44,110
streptavidin or like the complex and

247
00:10:50,429 --> 00:10:48,370
after that is the same okay here we can

248
00:10:52,110 --> 00:10:50,439
see step by step because the mmm display

249
00:10:54,090 --> 00:10:52,120
respect to other technique is not

250
00:10:56,639 --> 00:10:54,100
something like a commercial a commercial

251
00:10:59,490 --> 00:10:56,649
key we need to perform and to optimize

252
00:11:02,939 --> 00:10:59,500
every single step here is the first step

253
00:11:05,340 --> 00:11:02,949
so the link the legation between the RNA

254
00:11:06,840 --> 00:11:05,350
and pure marking the promise is actually

255
00:11:09,540 --> 00:11:06,850
contain a fluorescence molecule so we

256
00:11:11,879 --> 00:11:09,550
can visualize in in fluorescence and we

257
00:11:14,130 --> 00:11:11,889
can see like the free pure attack so and

258
00:11:18,180 --> 00:11:14,140
in the the top of the part like the

259
00:11:20,040 --> 00:11:18,190
congregated MRNA to the to the to the

260
00:11:22,500 --> 00:11:20,050
porta marking on the other side we can

261
00:11:24,210 --> 00:11:22,510
see the visualization with like the

262
00:11:26,310 --> 00:11:24,220
common would be visualization with the

263
00:11:28,650 --> 00:11:26,320

generate and we can see that the the

264
00:11:30,990 --> 00:11:28,660
uilt it's more than eighty percent its

265
00:11:34,519 --> 00:11:31,000
meaning that still some RNA does bind

266
00:11:38,370 --> 00:11:34,529
the demise it but the process feel okay

267
00:11:40,829 --> 00:11:38,380
after that the the error name will be as

268
00:11:43,560 --> 00:11:40,839
price it will be translated and here we

269
00:11:46,500 --> 00:11:43,570
can see the difference between before

270
00:11:49,199 --> 00:11:46,510
and after so all the mRNA and after the

271
00:11:52,079 --> 00:11:49,209
translation so we can see a shift data

272
00:11:55,819 --> 00:11:52,089
meaning that there is like protein plus

273
00:11:59,699 --> 00:11:55,829
mrna so very we there is like expression

274
00:12:01,920 --> 00:11:59,709
and after that the selection so here is

275
00:12:03,900 --> 00:12:01,930
represented like the Pluto so all the

276
00:12:08,100 --> 00:12:03,910
library all the variant mutant that are

277
00:12:10,800 --> 00:12:08,110
not able to bind the target RNA so loose

278
00:12:13,860 --> 00:12:10,810
lost the function and it's the most

279
00:12:16,199 --> 00:12:13,870
abundant of course because no holding or

280
00:12:17,940 --> 00:12:16,209
like protein are not able to bind we

281
00:12:20,490 --> 00:12:17,950
worship that so just to remove like the

282
00:12:22,769 --> 00:12:20,500
wicked binder and the Lewisham so just

283
00:12:25,530 --> 00:12:22,779
this one its meaning that some protein

284
00:12:28,199 --> 00:12:25,540
maintain the function so composite of

285
00:12:29,610 --> 00:12:28,209
all early immune system maintain the

286
00:12:32,550 --> 00:12:29,620
function to interact with the target

287
00:12:34,560 --> 00:12:32,560
renege after that there will be the

288
00:12:36,540 --> 00:12:34,570

reverse transcription after the reverse

289
00:12:38,220 --> 00:12:36,550
at ratio this is like the visualization

290
00:12:40,110 --> 00:12:38,230
with the best prescription show after

291
00:12:42,780 --> 00:12:40,120
the aleutian will be retro transcribe

292
00:12:44,160 --> 00:12:42,790
and we are completed like the negative

293
00:12:48,000 --> 00:12:44,170
control the same drop from the negative

294
00:12:49,829 --> 00:12:48,010
control so without tRNA target and the

295
00:12:51,300 --> 00:12:49,839
library we can see at the beginning

296
00:12:53,490 --> 00:12:51,310
right round by round because was

297
00:12:56,670 --> 00:12:53,500
performing like 60-pound with times

298
00:12:59,130 --> 00:12:56,680
there was like a noise but after several

299
00:13:00,900 --> 00:12:59,140
time we start to decrease sensible the

300
00:13:03,360 --> 00:13:00,910
noise but still there so it's meaning

301
00:13:05,910 --> 00:13:03,370
that we there is like a subpopulation or

302
00:13:09,540 --> 00:13:05,920
protein that does not bind at the target

303
00:13:11,160 --> 00:13:09,550
RNA but only the bits but to understand

304
00:13:14,819 --> 00:13:11,170
which which was like this kind of

305
00:13:17,850 --> 00:13:14,829
sequence we have we have a sequencing

306
00:13:20,280 --> 00:13:17,860
the library at the negative control hey

307
00:13:21,630 --> 00:13:20,290
add the result of the from the neck the

308
00:13:25,980 --> 00:13:21,640
sequencing from the next-gen sequencing

309
00:13:28,199 --> 00:13:25,990
and ok of course the best binder was the

310
00:13:30,239 --> 00:13:28,209
water i don't know was how is possible

311
00:13:33,929 --> 00:13:30,249
there was like a contamination from the

312
00:13:36,720 --> 00:13:33,939
first round so the best bandit of course

313
00:13:39,059 --> 00:13:36,730

is the water but it at least its meaning

314
00:13:41,369 --> 00:13:39,069
that the method its raw booze I mean

315
00:13:44,069 --> 00:13:41,379
because the put post our maker is to

316
00:13:45,509 --> 00:13:44,079
select the best binder and the worst

317
00:13:49,280 --> 00:13:45,519
part of course is the best like that

318
00:13:51,389 --> 00:13:49,290
but here you see if we can find the

319
00:13:54,319 --> 00:13:51,399
contamination the noise you can see that

320
00:13:57,179 --> 00:13:54,329
around my round this this sequence is

321
00:13:59,609 --> 00:13:57,189
equal present about 19 the same

322
00:14:02,160 --> 00:13:59,619
abundance in the negative control and in

323
00:14:04,590 --> 00:14:02,170
the library so it mean that stick to the

324
00:14:06,809 --> 00:14:04,600
beat but we have found other three

325
00:14:09,569 --> 00:14:06,819
sequence especially the sequence number

326
00:14:12,540 --> 00:14:09,579
nine that is a really really good ratio

327
00:14:15,210 --> 00:14:12,550
between library so the samples with the

328
00:14:18,090 --> 00:14:15,220
RNA and the control negative control

329
00:14:19,919 --> 00:14:18,100
that mean that this this sequence this

330
00:14:22,319 --> 00:14:19,929
protein does not have the tendencies to

331
00:14:24,569 --> 00:14:22,329
bind the naked intruder to the beat so

332
00:14:26,819 --> 00:14:24,579
it's really probable that this protein

333
00:14:32,509 --> 00:14:26,829
is still buying all the mounting the

334
00:14:38,340 --> 00:14:36,269
here we have like Alana align the there

335
00:14:40,350 --> 00:14:38,350
was like the alignment of the different

336
00:14:42,509 --> 00:14:40,360
the selected protein respect to the work

337
00:14:46,530 --> 00:14:42,519
type and we can find that the Sam

338
00:14:48,809 --> 00:14:46,540

mutation welcome on in the different

339
00:14:51,259 --> 00:14:48,819
clue so for instance in some cases

340
00:14:54,359 --> 00:14:51,269
really interesting for instance like in

341
00:14:56,609 --> 00:14:54,369
there was like a substitution presented

342
00:15:00,269 --> 00:14:56,619
in the late 14th like the adenine

343
00:15:02,160 --> 00:15:00,279
welcome well substitute by aspartic acid

344
00:15:08,400 --> 00:15:02,170
so there was a completely a change of

345
00:15:10,199 --> 00:15:08,410
charge in an abandoned furthermore they

346
00:15:11,210 --> 00:15:10,209
were the during the reverse

347
00:15:14,280 --> 00:15:11,220
transcription

348
00:15:17,730 --> 00:15:14,290
there were also were inserted other

349
00:15:21,629 --> 00:15:17,740
mutation we can see that for instance in

350
00:15:24,600 --> 00:15:21,639
the the red arrow there should be like

351
00:15:26,460 --> 00:15:24,610
the well substituted like later early

352
00:15:27,989 --> 00:15:26,470
amino acid with other elimination this

353
00:15:30,210 --> 00:15:27,999
is spontaneous process during the

354
00:15:33,019 --> 00:15:30,220
reverse transition prescription but this

355
00:15:36,269 --> 00:15:33,029
mutation will well still maintain it and

356
00:15:38,669 --> 00:15:36,279
still maintain at least years like this

357
00:15:40,559 --> 00:15:38,679
is previous just homology modeling so we

358
00:15:42,269 --> 00:15:40,569
can see that at least it's the first

359
00:15:44,760 --> 00:15:42,279
analysis seems the

360
00:15:47,970 --> 00:15:44,770
the the the mutant maintain the function

361
00:15:49,560 --> 00:15:47,980
it seems that Monty also the the

362
00:15:51,360 --> 00:15:49,570
structure but for the moment is a

363
00:15:54,840 --> 00:15:51,370

previous data is just Amala G modeling

364
00:15:57,060 --> 00:15:54,850
so okay in conclusion the future work

365
00:16:00,150 --> 00:15:57,070
will be like of course the expression of

366
00:16:02,940 --> 00:16:00,160
the single protein characterization just

367
00:16:05,760 --> 00:16:02,950
to understand the binding constant with

368
00:16:08,850 --> 00:16:05,770
the target RNA and of course the the

369
00:16:11,160 --> 00:16:08,860
crystal structure on with the RNA so in

370
00:16:14,269 --> 00:16:11,170
conclusion we have identified a robust

371
00:16:17,610 --> 00:16:14,279
target for the reverse evolution project

372
00:16:20,430 --> 00:16:17,620
was customized the amenities the mRNA

373
00:16:22,610 --> 00:16:20,440
display technique for the best best

374
00:16:26,040 --> 00:16:22,620
mutant selection and it was identified

375
00:16:29,370 --> 00:16:26,050
three different protein able to bind the

376
00:16:33,120 --> 00:16:29,380
RNA also in presence of only early amino

377
00:16:35,670 --> 00:16:33,130
acid i'd like to thanks of my group from

378
00:16:38,640 --> 00:16:35,680
Chester University and particularly

379
00:16:40,550 --> 00:16:38,650
professor cosmic evolution from the SE

380
00:16:58,710 --> 00:16:40,560
in Japan for the collaboration and

381
00:17:01,500 --> 00:16:58,720
thanks to you questions very so you

382
00:17:03,180 --> 00:17:01,510
identified as a candidate this protein

383
00:17:05,309 --> 00:17:03,190
because it had a bunch of the early

384
00:17:09,059 --> 00:17:05,319
amino acids that important positions are

385
00:17:10,640 --> 00:17:09,069
there classes of protein that have much

386
00:17:13,919 --> 00:17:10,650
that have a larger tendency to have

387
00:17:16,380 --> 00:17:13,929
important sites Phoebe's early ones

388
00:17:18,390 --> 00:17:16,390

versus later ones no no they are protein

389
00:17:21,030 --> 00:17:18,400
that contain only early amino acid and

390
00:17:23,280 --> 00:17:21,040
we can cluster in some in some part but

391
00:17:25,380 --> 00:17:23,290
all the sequencer that we have selected

392
00:17:26,669 --> 00:17:25,390
contain only early memories know what I

393
00:17:29,700 --> 00:17:26,679
mean what I mean is when you look when

394
00:17:32,040 --> 00:17:29,710
you're looking at existent proteins I

395
00:17:33,380 --> 00:17:32,050
mean like like so you're saying there

396
00:17:35,880 --> 00:17:33,390
are ones that have there are existing

397
00:17:39,450 --> 00:17:35,890
proteins that you can grab say that only

398
00:17:41,669 --> 00:17:39,460
have in the pretty well it was plausible

399
00:17:44,100 --> 00:17:41,679
that was some protein like that right

400
00:17:46,320 --> 00:17:44,110
but no evidence I mean it was just

401
00:17:48,900 --> 00:17:46,330
basing on the like a simple Inga like on

402
00:17:51,030 --> 00:17:48,910
the mater I tend on the prebiotic food

403
00:17:52,960 --> 00:17:51,040
but no protein was fine that contains

404
00:17:54,669 --> 00:17:52,970
only eliminating

405
00:17:56,980 --> 00:17:54,679
there are if there are modern protein

406
00:17:58,840 --> 00:17:56,990
classes that are more biased towards

407
00:18:00,850 --> 00:17:58,850
early amino acids than later today I

408
00:18:03,250 --> 00:18:00,860
mean if there are functions in the cell

409
00:18:20,600 --> 00:18:03,260
that seems that way what do I mean no

410
00:18:25,159 --> 00:18:23,000
thank you for your presentation I

411
00:18:27,680 --> 00:18:25,169
realize that you already have a lot of

412
00:18:29,720 --> 00:18:27,690
possible combinations in your essay when

413
00:18:32,029 --> 00:18:29,730

you start but I was wondering if you

414
00:18:34,190 --> 00:18:32,039
ever considered adding prebiotic

415
00:18:37,009 --> 00:18:34,200
irrelevant amino acids that we do not

416
00:18:40,940 --> 00:18:37,019
find in proteins nowadays for example or

417
00:18:42,529 --> 00:18:40,950
anything could sort solve the problem

418
00:18:45,200 --> 00:18:42,539
that right now you're not able to use

419
00:18:47,659 --> 00:18:45,210
any positively charged amino acids yeah

420
00:18:49,730 --> 00:18:47,669
in this is the first step so we have

421
00:18:51,889 --> 00:18:49,740
selected only the early amino acid

422
00:18:53,930 --> 00:18:51,899
present in the genetic code but of

423
00:18:56,269 --> 00:18:53,940
course the most Theory provide like

424
00:18:58,399 --> 00:18:56,279
evidence that was like composite the

425
00:19:01,279 --> 00:18:58,409
early amino acid of like conventional

426
00:19:03,379 --> 00:19:01,289
it's like the modern one so like the

427
00:19:05,750 --> 00:19:03,389
present is but also non-conventional one

428
00:19:07,549 --> 00:19:05,760
forest and one of the cases like the old

429
00:19:10,159 --> 00:19:07,559
meeting in fact if we notice the the

430
00:19:12,049 --> 00:19:10,169
table was not positive charged amino

431
00:19:14,269 --> 00:19:12,059
acids quite weird so it's possible that

432
00:19:15,889 --> 00:19:14,279
with the ornithine we replace it after

433
00:19:18,019 --> 00:19:15,899
that one so but there will be the next

434
00:19:20,090 --> 00:19:18,029
step of the project so first up like

435
00:19:21,740 --> 00:19:20,100
this one and after that we see two

436
00:19:23,870 --> 00:19:21,750
looking for the non-conventional but

437
00:19:25,570 --> 00:19:23,880
this is a quite difficult because you

438
00:19:27,620 --> 00:19:25,580

need to integrate in the like

439
00:19:29,470 --> 00:19:27,630
translation system the possibility to

440
00:19:32,690 --> 00:19:29,480
insert non-conventional amino acid is

441
00:19:35,169 --> 00:19:32,700
it's not so easy to modify the tRNA

442
00:19:37,750 --> 00:19:35,179
synthetase G and blah blah blah

443
00:19:40,940 --> 00:19:37,760
thank you very much for your talk I

444
00:19:43,549 --> 00:19:40,950
assume that the positive charges in the

445
00:19:47,090 --> 00:19:43,559
proteins are interacting with a negative

446
00:19:50,750 --> 00:19:47,100
charge of the phosphate in the renamed

447
00:19:52,639 --> 00:19:50,760
and but in your new proteins several of

448
00:19:56,000 --> 00:19:52,649
those positive charges are genes and

449
00:19:57,100 --> 00:19:56,010
license are now negative like in

450
00:19:59,990 --> 00:19:57,110
something

451
00:20:03,379 --> 00:20:00,000
aspartic acid do you have any idea or

452
00:20:05,649 --> 00:20:03,389
any model of how now these negative

453
00:20:10,430 --> 00:20:05,659
amino acids are interacting with with

454
00:20:12,409 --> 00:20:10,440
okay thank you in the most the only

455
00:20:14,629 --> 00:20:12,419
parts that interact with the DNA is

456
00:20:24,289 --> 00:20:14,639
particularly these alpha Alex and this

457
00:20:26,419 --> 00:20:24,299
loop and mostly and and this alpha helix

458
00:20:29,210 --> 00:20:26,429
and this loop is covered in this part so

459
00:20:32,120 --> 00:20:29,220
this remain is remaining it practically

460
00:20:34,310 --> 00:20:32,130
the same and probably these when it's

461
00:20:36,140 --> 00:20:34,320
like for this this charge

462
00:20:38,420 --> 00:20:36,150
on the other part of the Alfa Alex so

463
00:20:42,080 --> 00:20:38,430

it's not interact directly with the tRNA

464
00:20:46,010 --> 00:20:42,090
so probably is not important for that

465
00:20:47,660 --> 00:20:46,020
binding and also it's also possible that

466
00:20:50,690 --> 00:20:47,670
I don't know maybe some positive charge

467
00:20:54,440 --> 00:20:50,700
like the KT or cation can can function

468
00:20:56,870 --> 00:20:54,450
like like a bridge so it's also possible

469
00:20:58,400 --> 00:20:56,880
but I know this is only a monetary model

470
00:21:01,100 --> 00:20:58,410
in fact next step would be to have the

471
00:21:03,890 --> 00:21:01,110
crystal with the target RNA it wanted us

472
00:21:08,630 --> 00:21:03,900
to know how it's like fit so future warp

473
00:21:09,480 --> 00:21:08,640
I hope that will be soon okay let's take