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Lab Members (Present)

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- Fiore Guessan
- Adam Hallam

Dr. Ramanarayan Krishnamurthy
Scripps Research Institute
San Diego, CA

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1
00:00:10,920 --> 00:00:08,850
hi everyone I'm Raghav and from

2
00:00:13,709 --> 00:00:10,930
University of Missouri and I am

3
00:00:17,370 --> 00:00:13,719
interested in understanding rnase role

4
00:00:20,460 --> 00:00:17,380
in the origins of life and I'm I am

5
00:00:22,499 --> 00:00:20,470
basically concerned with how RNA

6
00:00:26,819 --> 00:00:22,509
catalyzes biologically relevant

7
00:00:28,979 --> 00:00:26,829
reactions I'm Thank You Kristen for the

8
00:00:30,960 --> 00:00:28,989
RNA world hypothesis intro that was

9
00:00:33,210 --> 00:00:30,970
really good so basically the RNA world

10
00:00:35,729 --> 00:00:33,220
hypothesis says that like during the

11
00:00:38,490 --> 00:00:35,739
early origins and evolution of life it

12
00:00:40,890 --> 00:00:38,500
was the RNA that was the workhorse both

13
00:00:43,710 --> 00:00:40,900

acting both as a catalyst and also as a

14
00:00:45,720 --> 00:00:43,720
genetic information carrier which is of

15
00:00:48,450 --> 00:00:45,730
course now done by the DNA and the

16
00:00:50,580 --> 00:00:48,460
protein although originally it was put

17
00:00:52,800 --> 00:00:50,590
forward to circumvent the chicken or egg

18
00:00:54,870 --> 00:00:52,810
paradox whether DNA came first or the

19
00:00:57,840 --> 00:00:54,880
army came first well recently we've

20
00:00:59,910 --> 00:00:57,850
actually found many catalytic rnase by

21
00:01:03,180 --> 00:00:59,920
in vitro evolution and it has really

22
00:01:06,090 --> 00:01:03,190
strengthened this hypothesis so I'm

23
00:01:08,789 --> 00:01:06,100
interested in phosphoryl transfer and

24
00:01:12,690 --> 00:01:08,799
and how RNA catalyzes phosphoryl

25
00:01:19,169 --> 00:01:12,700
transfer for example well in modern

26
00:01:23,940 --> 00:01:19,179
biology what we have ok so in modern

27
00:01:25,950 --> 00:01:23,950
biology we know that all these all the

28
00:01:30,749 --> 00:01:25,960
phosphorylation reactions are actually

29
00:01:32,399 --> 00:01:30,759
catalyzed by protein kinases and in case

30
00:01:34,709 --> 00:01:32,409
of the RNA well you do you think that

31
00:01:37,279 --> 00:01:34,719
there would be kinase ribozymes or RNA

32
00:01:41,580 --> 00:01:37,289
enzymes that can catalyze this reaction

33
00:01:44,129 --> 00:01:41,590
so how do you find catalytic RNA is that

34
00:01:46,109 --> 00:01:44,139
that have this activity to transfer

35
00:01:48,300 --> 00:01:46,119
phosphate group so we are actually

36
00:01:50,160 --> 00:01:48,310
selecting for our enzymes that can

37
00:01:53,519 --> 00:01:50,170
phosphorylate themselves so they take

38
00:01:55,739 --> 00:01:53,529

the phosphate off of a phosphoryl donor

39
00:01:59,999 --> 00:01:55,749
and then transfer onto themselves so

40
00:02:02,639 --> 00:02:00,009
that's what they would look like and the

41
00:02:04,349 --> 00:02:02,649
way you can find these active rnase well

42
00:02:06,620 --> 00:02:04,359
well the way you can partition the

43
00:02:09,059 --> 00:02:06,630
active rnase from the inactive rnase is

44
00:02:11,339 --> 00:02:09,069
so if you notice carefully there the

45
00:02:13,920 --> 00:02:11,349
phosphoryl donor actually has a sulfur

46
00:02:17,220 --> 00:02:13,930
instead of an oxygen on the gamma

47
00:02:19,470 --> 00:02:17,230
position of the triphosphate so any RNA

48
00:02:19,980 --> 00:02:19,480
that is active actually gets a sulfur

49
00:02:22,890 --> 00:02:19,990
along

50
00:02:25,620 --> 00:02:22,900
with the phosphate so if you separate

51
00:02:27,000 --> 00:02:25,630
these rnase out on a polyacrylamide gel

52
00:02:29,610 --> 00:02:27,010
where one of the layer in the middle

53
00:02:31,800 --> 00:02:29,620
contains mercury the sulfur interacts

54
00:02:34,110 --> 00:02:31,810
with the mercury and then all the rnase

55
00:02:37,110 --> 00:02:34,120
that have the sulfur get trapped in the

56
00:02:40,290 --> 00:02:37,120
mercury layer so if the RNA is active

57
00:02:42,900 --> 00:02:40,300
and if it is the activity of a guyanese

58
00:02:44,550 --> 00:02:42,910
ribozyme then it gets the sulfur along

59
00:02:46,080 --> 00:02:44,560
with the phosphate and gets trapped in

60
00:02:47,730 --> 00:02:46,090
the mercury later so you can you can

61
00:02:49,830 --> 00:02:47,740
basically cut out this band that is

62
00:02:52,530 --> 00:02:49,840
trapped in the mercury layer reverse

63
00:02:54,390 --> 00:02:52,540

transcribe it and then pcr-amplified do

64
00:02:57,990 --> 00:02:54,400
this process over and over again until

65
00:03:01,080 --> 00:02:58,000
you finally enrich your pool with active

66
00:03:03,330 --> 00:03:01,090
RNA so essentially you start out with 10

67
00:03:06,660 --> 00:03:03,340
to the 14th different sequences and you

68
00:03:10,500 --> 00:03:06,670
end up with a fraction of them that have

69
00:03:12,990 --> 00:03:10,510
the desired activity so this brings to

70
00:03:16,190 --> 00:03:13,000
part 1 of my talk which has to do with

71
00:03:19,170 --> 00:03:16,200
understanding how this ribozyme

72
00:03:21,030 --> 00:03:19,180
catalyzes phosphoryl transfer how ripe

73
00:03:25,620 --> 00:03:21,040
is not what kind of products can

74
00:03:27,480 --> 00:03:25,630
ribozymes form off of this reaction so

75
00:03:31,560 --> 00:03:27,490
this ribozyme was actually selected in

76
00:03:34,140 --> 00:03:31,570
my lab as so the GTP gamma s is the

77
00:03:37,260 --> 00:03:34,150
phosphate donor it uses magnesium and

78
00:03:42,330 --> 00:03:37,270
copper and phosphorylates at this second

79
00:03:44,550 --> 00:03:42,340
guanosine position and so that this is

80
00:03:46,920 --> 00:03:44,560
basically what a polyacrylamide gel

81
00:03:49,560 --> 00:03:46,930
would look like as the time increases

82
00:03:52,800 --> 00:03:49,570
you see more and more RNA getting

83
00:03:57,210 --> 00:03:52,810
phosphorylated and and gets trapped at

84
00:03:58,950 --> 00:03:57,220
the mercury layer okay so one of the

85
00:04:01,860 --> 00:03:58,960
first things I wanted to understand was

86
00:04:06,870 --> 00:04:01,870
how does the two prime hydroxyl in an

87
00:04:08,880 --> 00:04:06,880
RNA in this particular ribozyme play

88
00:04:13,290 --> 00:04:08,890

what can rule the two prime hydroxyl

89
00:04:15,150 --> 00:04:13,300
plays in this RNA structure so we know

90
00:04:16,920 --> 00:04:15,160
that two prime hydroxyl is involved in

91
00:04:19,070 --> 00:04:16,930
different things like a lot of different

92
00:04:23,550 --> 00:04:19,080
things for example here it's shown

93
00:04:26,580 --> 00:04:23,560
coordinating a metal ion and it is also

94
00:04:28,950 --> 00:04:26,590
involved in hydrogen bond as a hydrogen

95
00:04:32,850 --> 00:04:28,960
bond donor or hydrogen bond acceptor so

96
00:04:33,700 --> 00:04:32,860
so so it is very important to form the

97
00:04:37,470 --> 00:04:33,710
RNA straw

98
00:04:45,460 --> 00:04:37,480
the active RNA structures and ultimately

99
00:04:47,380 --> 00:04:45,470
give RNA the catalytic activity so to

100
00:04:49,780 --> 00:04:47,390
figure out whether the stew prime

101
00:04:52,570 --> 00:04:49,790
hydroxyls in this RNA is important what

102
00:04:55,630 --> 00:04:52,580
kind of two prime moriches are important

103
00:04:58,810 --> 00:04:55,640
in this ribozyme what we did was we we

104
00:05:00,730 --> 00:04:58,820
try to substitute 2 prime we try to

105
00:05:03,040 --> 00:05:00,740
substitute the nucleotides with two

106
00:05:05,440 --> 00:05:03,050
prime fluoro nucleotides so you

107
00:05:08,110 --> 00:05:05,450
basically transcribe this ribozyme with

108
00:05:10,630 --> 00:05:08,120
either ribonucleotides that have to

109
00:05:12,820 --> 00:05:10,640
prime hydroxyl or ribonucleotides that

110
00:05:14,710 --> 00:05:12,830
have to prime fluorine so we we either

111
00:05:17,710 --> 00:05:14,720
made the RNA with all two prime hydroxyl

112
00:05:19,660 --> 00:05:17,720
or substituted all geez with two prime

113
00:05:23,680 --> 00:05:19,670

fluoro or all a's with two prime photo

114
00:05:25,780 --> 00:05:23,690
all C's or all use one at a time so

115
00:05:29,260 --> 00:05:25,790
again the same idea to prime hydroxyl

116
00:05:30,930 --> 00:05:29,270
the RNA without the phosphate donor the

117
00:05:33,190 --> 00:05:30,940
RNA of course doesn't get phosphorylated

118
00:05:35,410 --> 00:05:33,200
once you have the phosphoryl donor it

119
00:05:38,740 --> 00:05:35,420
gets phosphorylated so get stuck at the

120
00:05:42,250 --> 00:05:38,750
mercury layer and we were really

121
00:05:44,680 --> 00:05:42,260
surprised that when you replace all

122
00:05:48,220 --> 00:05:44,690
these ribonucleotides with two prime

123
00:05:51,250 --> 00:05:48,230
floros they still get phosphorylated and

124
00:05:55,110 --> 00:05:51,260
the ribozyme is still active so we

125
00:05:57,490 --> 00:05:55,120
wanted to know more about about this

126
00:05:59,590 --> 00:05:57,500
this ribozyme because of what this tells

127
00:06:02,530 --> 00:05:59,600
us is that actually no two prime

128
00:06:04,570 --> 00:06:02,540
hydroxyl is important for this or no

129
00:06:07,240 --> 00:06:04,580
particular two prime hydroxyl is

130
00:06:09,730 --> 00:06:07,250
important for the activity because I

131
00:06:11,830 --> 00:06:09,740
also tried making the ribozyme as a

132
00:06:13,960 --> 00:06:11,840
complete deoxyribose I meant that is

133
00:06:15,880 --> 00:06:13,970
dead so of course like if you start

134
00:06:19,060 --> 00:06:15,890
replacing multiple to prime hydroxyls

135
00:06:25,810 --> 00:06:19,070
from multiple nucleotides you lose the

136
00:06:28,660 --> 00:06:25,820
activity so initially what we had found

137
00:06:32,200 --> 00:06:28,670
was that for the ribozyme that with the

138
00:06:34,840 --> 00:06:32,210

two prime hydroxyl the rate of the

139
00:06:37,120 --> 00:06:34,850
reaction increases as you increase the

140
00:06:39,370 --> 00:06:37,130
pH and one of the one obvious

141
00:06:41,500 --> 00:06:39,380
explanation to this would be as you

142
00:06:43,990 --> 00:06:41,510
increase the pH the two prime hydroxyl

143
00:06:47,290 --> 00:06:44,000
gets deprotonated and that somehow

144
00:06:49,589 --> 00:06:47,300
increases the rate of the reaction

145
00:06:52,779 --> 00:06:49,599
now that I had all the two prime 40

146
00:06:54,520 --> 00:06:52,789
ribozymes I I tested all these ribozymes

147
00:06:56,740 --> 00:06:54,530
with the two prime for in positions at

148
00:07:00,189 --> 00:06:56,750
different PHS as well and they all

149
00:07:03,129 --> 00:07:00,199
showed similar behavior and they all

150
00:07:05,439 --> 00:07:03,139
were being stimulated at higher pH as

151
00:07:07,270 --> 00:07:05,449
well now what this tells us is that it's

152
00:07:09,369 --> 00:07:07,280
not the two prime hydroxyl that is being

153
00:07:12,790 --> 00:07:09,379
deprotonated but there is something else

154
00:07:15,100 --> 00:07:12,800
going on and and and we would be really

155
00:07:17,200 --> 00:07:15,110
intrigued by this data because all this

156
00:07:19,450 --> 00:07:17,210
time we also thought that it was the two

157
00:07:21,760 --> 00:07:19,460
prime hydroxyl that was getting

158
00:07:24,490 --> 00:07:21,770
phosphorylated but it might not be the

159
00:07:26,740 --> 00:07:24,500
case anymore so to answer that the

160
00:07:30,249 --> 00:07:26,750
questions what we started to do was so

161
00:07:32,740 --> 00:07:30,259
this is the full ribozyme what I did was

162
00:07:34,719 --> 00:07:32,750
I basically designed a trans-acting

163
00:07:36,459 --> 00:07:34,729

version of the ribozyme where the

164
00:07:38,589 --> 00:07:36,469
phosphor the strength the part that gets

165
00:07:40,629 --> 00:07:38,599
phosphorylated is actually separated

166
00:07:42,459 --> 00:07:40,639
from the catalytic strand so you bring

167
00:07:44,499 --> 00:07:42,469
these two molecules together the

168
00:07:46,930 --> 00:07:44,509
catalytic strand base pairs with the

169
00:07:49,420 --> 00:07:46,940
substrate strand and phosphorylates at

170
00:07:52,420 --> 00:07:49,430
this guanosine position now what what

171
00:07:54,490 --> 00:07:52,430
this the design enabled us to do was we

172
00:07:56,830 --> 00:07:54,500
could actually test different substrates

173
00:07:59,860 --> 00:07:56,840
now and that's basically what I started

174
00:08:02,709 --> 00:07:59,870
out doing first of all I I used on all

175
00:08:04,420 --> 00:08:02,719
RNA backbone as a substrate and all DNA

176
00:08:08,939 --> 00:08:04,430
as the backbone as the substrate and

177
00:08:12,909 --> 00:08:08,949
then a DNA RNA hybrid substrate as well

178
00:08:14,200 --> 00:08:12,919
when to our surprise everything all of

179
00:08:16,390 --> 00:08:14,210
these three substrates got

180
00:08:18,969 --> 00:08:16,400
phosphorylated now what is really

181
00:08:22,379 --> 00:08:18,979
intriguing was that for the second

182
00:08:24,519 --> 00:08:22,389
guanosine the only like the only obvious

183
00:08:27,760 --> 00:08:24,529
phosphorylation site is the two prime

184
00:08:30,760 --> 00:08:27,770
hydroxyl and in a DNA we know from

185
00:08:34,209 --> 00:08:30,770
biochem or biology 101 that there is

186
00:08:37,029 --> 00:08:34,219
absolutely no two prime hydroxyl so this

187
00:08:40,000 --> 00:08:37,039
is actually first observation of direct

188
00:08:43,990 --> 00:08:40,010

nucleobase phosphorylation by a kinase

189
00:08:46,120 --> 00:08:44,000
ribozyme now why this is important is

190
00:08:49,360 --> 00:08:46,130
because once you start modifying

191
00:08:52,300 --> 00:08:49,370
nucleotides and early prebiotic earth

192
00:08:55,240 --> 00:08:52,310
would have more nucleotides to sample

193
00:08:57,699 --> 00:08:55,250
from it would have that's basically a

194
00:08:59,980 --> 00:08:57,709
way of generating different kinds of

195
00:09:01,180 --> 00:08:59,990
nucleotides so that nature can sample to

196
00:09:05,230 --> 00:09:01,190
see which one

197
00:09:06,700 --> 00:09:05,240
is optimum for life to use so if you

198
00:09:09,760 --> 00:09:06,710
actually go back to the structure of

199
00:09:11,770 --> 00:09:09,770
this complex everything from the

200
00:09:13,180 --> 00:09:11,780
substrate is actually base paired with

201
00:09:16,150 --> 00:09:13,190
the ribozyme so that's actually pretty

202
00:09:17,950 --> 00:09:16,160
boring it's just the DNA RNA helix

203
00:09:20,200 --> 00:09:17,960
there's not much going on but if you

204
00:09:23,800 --> 00:09:20,210
look at the business end here this GGA

205
00:09:26,020 --> 00:09:23,810
is this phi prime GG and a there it's

206
00:09:28,840 --> 00:09:26,030
free and it is free to do stuff it can

207
00:09:32,410 --> 00:09:28,850
actually interact with other parts of

208
00:09:35,170 --> 00:09:32,420
the ribozyme so i started out testing

209
00:09:37,810 --> 00:09:35,180
whether you can change this GG na so

210
00:09:41,830 --> 00:09:37,820
that's GGA to other nucleotides or not

211
00:09:44,470 --> 00:09:41,840
so if you mutated this G to a C or tea

212
00:09:46,390 --> 00:09:44,480
or a the ribozyme is dead so these are

213
00:09:49,060 --> 00:09:46,400

very soft mutations where you're only

214
00:09:51,160 --> 00:09:49,070
changing some atoms so in this case

215
00:09:54,220 --> 00:09:51,170
you're going from g2 and in a scene

216
00:09:58,210 --> 00:09:54,230
where you're only taking this amine out

217
00:10:00,910 --> 00:09:58,220
and for this g1 and g2 pretty much any

218
00:10:03,850 --> 00:10:00,920
soft mutation that you do the ribozyme

219
00:10:07,930 --> 00:10:03,860
is dead like it absolutely is very

220
00:10:09,220 --> 00:10:07,940
specific to this GG a sequence now this

221
00:10:11,320 --> 00:10:09,230
will this will be very important we'll

222
00:10:15,640 --> 00:10:11,330
come back to this again for the second

223
00:10:18,460 --> 00:10:15,650
part of my talk we basically then wanted

224
00:10:22,600 --> 00:10:18,470
to understand what kind of product is

225
00:10:25,180 --> 00:10:22,610
this ribozyme forming so do you answer

226
00:10:27,850 --> 00:10:25,190
that I basically took the substrate did

227
00:10:30,160 --> 00:10:27,860
not react with the ribozyme but added a

228
00:10:33,970 --> 00:10:30,170
phosphate on the very five prime end of

229
00:10:36,760 --> 00:10:33,980
the ribose sugar take this substrate

230
00:10:39,700 --> 00:10:36,770
incubated it at different ph is from

231
00:10:42,010 --> 00:10:39,710
nine to four point five and you can see

232
00:10:43,900 --> 00:10:42,020
that the Firefall now the product that

233
00:10:47,890 --> 00:10:43,910
has the phosphate the phosphate is still

234
00:10:50,350 --> 00:10:47,900
stable at all this pH range next what I

235
00:10:52,990 --> 00:10:50,360
did was I took the same substrate now

236
00:10:54,970 --> 00:10:53,000
this time added the phosphate using the

237
00:10:57,190 --> 00:10:54,980
ribozyme and and we know that the

238
00:10:59,050 --> 00:10:57,200

phosphate is on a nuclear base it's not

239
00:11:00,910 --> 00:10:59,060
on the two prime hydroxyl but we don't

240
00:11:03,880 --> 00:11:00,920
know what Adam the phosphate is attached

241
00:11:05,770 --> 00:11:03,890
to now you take this product formed by

242
00:11:07,990 --> 00:11:05,780
the ribosome and do the same experiment

243
00:11:09,970 --> 00:11:08,000
incubated at different pages the

244
00:11:13,450 --> 00:11:09,980
phosphate actually starts to come off so

245
00:11:14,889 --> 00:11:13,460
chemically speaking the ribozyme forms a

246
00:11:18,129 --> 00:11:14,899
different product they

247
00:11:21,369 --> 00:11:18,139
and what you have if it was just a

248
00:11:23,049 --> 00:11:21,379
ribose phosphorylation and then I

249
00:11:24,699 --> 00:11:23,059
started digging out literature to see

250
00:11:26,499 --> 00:11:24,709
like what people have done this is like

251
00:11:28,600 --> 00:11:26,509
I don't know I don't even know when this

252
00:11:30,460 --> 00:11:28,610
paper was probably uh give us from 2004

253
00:11:33,519 --> 00:11:30,470
but what what this paper essentially

254
00:11:37,359 --> 00:11:33,529
said was that in acidic conditions the

255
00:11:40,109 --> 00:11:37,369
pn bond they break faster than p 0 bond

256
00:11:42,730 --> 00:11:40,119
so what this suggests is that the

257
00:11:45,340 --> 00:11:42,740
phosphate might actually be linked in

258
00:11:46,989 --> 00:11:45,350
one of these two atoms so we still don't

259
00:11:50,230 --> 00:11:46,999
know where the phosphate is linked but

260
00:11:53,069 --> 00:11:50,240
it is pretty interesting that we are

261
00:11:57,189 --> 00:11:53,079
starting to see this novel targets for

262
00:11:59,679 --> 00:11:57,199
RNA catalyzed phosphorylation so that's

263
00:12:02,439 --> 00:11:59,689

the sort of summary of the first part of

264
00:12:04,329 --> 00:12:02,449
my talk I guess is that this ribozyme

265
00:12:08,619 --> 00:12:04,339
phosphorylates the nuclear base directly

266
00:12:10,090 --> 00:12:08,629
and the phosphoryl acceptor is likely an

267
00:12:12,910 --> 00:12:10,100
end we don't know if whether it's an end

268
00:12:14,919 --> 00:12:12,920
to or an n1 and obviously this is the

269
00:12:17,710 --> 00:12:14,929
first evidence of direct modification by

270
00:12:20,110 --> 00:12:17,720
of nuclear base by an re enzyme and as i

271
00:12:23,230 --> 00:12:20,120
said earlier nucleobase modification

272
00:12:25,299 --> 00:12:23,240
would have been a nice way to explore

273
00:12:30,220 --> 00:12:25,309
the chemical space four nucleotides in

274
00:12:33,129 --> 00:12:30,230
an early RNA work so this brings to sort

275
00:12:37,150 --> 00:12:33,139
of the second part of my talk and and

276
00:12:39,759 --> 00:12:37,160
and so you have all these ribosomes that

277
00:12:42,699 --> 00:12:39,769
people select and and they they say oh

278
00:12:44,710 --> 00:12:42,709
we found this ribozyme but they just end

279
00:12:47,379 --> 00:12:44,720
it at bat what I what are you going to

280
00:12:51,100 --> 00:12:47,389
do with it so I was looking for ideas to

281
00:12:54,790 --> 00:12:51,110
use this ribozyme to regulate other RNAs

282
00:12:59,199 --> 00:12:54,800
that are of biological or Astra

283
00:13:01,090 --> 00:12:59,209
biological significance I guess so if

284
00:13:03,639 --> 00:13:01,100
you look at the ribozyme structure with

285
00:13:05,350 --> 00:13:03,649
a substrate everything else as I pointed

286
00:13:07,239 --> 00:13:05,360
out earlier is base paired with a

287
00:13:09,669 --> 00:13:07,249
substrate which is pretty boring like in

288
00:13:12,220 --> 00:13:09,679

principle you should be able to change

289
00:13:14,650 --> 00:13:12,230
all these base pairs and the ribozyme

290
00:13:21,850 --> 00:13:14,660
should still be active as long as this

291
00:13:26,559 --> 00:13:21,860
part is kept constant and so that's

292
00:13:28,720 --> 00:13:26,569
basically what i was studying so one of

293
00:13:31,090 --> 00:13:28,730
the functional RNA is that i wanted

294
00:13:33,460 --> 00:13:31,100
target using this ribozyme was the ATP

295
00:13:37,240 --> 00:13:33,470
aptima so this RNA molecule actually

296
00:13:40,210 --> 00:13:37,250
binds to ATP and it was discovered by

297
00:13:43,120 --> 00:13:40,220
Jack szostak in and I think it was early

298
00:13:46,120 --> 00:13:43,130
90s but if you if you look at the

299
00:13:48,519 --> 00:13:46,130
sequence the sequence of the ATP aptima

300
00:13:50,470 --> 00:13:48,529
already has a GGA and and that's why I

301
00:13:53,980 --> 00:13:50,480
wanted you guys to keep in mind about

302
00:13:55,990 --> 00:13:53,990
that five prime GGA terminal because the

303
00:13:59,410 --> 00:13:56,000
ribozyme actually absolutely needs it

304
00:14:01,900 --> 00:13:59,420
and turns out that the second G that the

305
00:14:06,600 --> 00:14:01,910
ribozyme would modify it actually makes

306
00:14:09,490 --> 00:14:06,610
a direct contact with the ligand a MP

307
00:14:11,350 --> 00:14:09,500
acting as a hydrogen bond donor so we

308
00:14:13,810 --> 00:14:11,360
wanted to see if whether we could design

309
00:14:16,780 --> 00:14:13,820
a ribozyme that would phosphorylate ATP

310
00:14:20,230 --> 00:14:16,790
a primer and and and what would happen

311
00:14:22,720 --> 00:14:20,240
to the ATP after function so this is I

312
00:14:25,090 --> 00:14:22,730
guess the diagram kind of messed I'm

313
00:14:27,040 --> 00:14:25,100

sorry about that but but you can't

314
00:14:29,110 --> 00:14:27,050
basically say anything but anyway we

315
00:14:31,720 --> 00:14:29,120
designed the ribozyme and turns out that

316
00:14:34,960 --> 00:14:31,730
you can phosphorylate the ATP a primer

317
00:14:37,090 --> 00:14:34,970
and as you can see it the phosphorylated

318
00:14:40,750 --> 00:14:37,100
ATP aptima again is retain in that

319
00:14:43,780 --> 00:14:40,760
mercury layer so the next thing I want

320
00:14:46,090 --> 00:14:43,790
to study was what happens when this ATP

321
00:14:49,059 --> 00:14:46,100
a primer is phosphorylated is it still

322
00:14:53,889 --> 00:14:49,069
functional and and the way you can test

323
00:14:59,379 --> 00:14:53,899
that is you have a column with a gross

324
00:15:03,579 --> 00:14:59,389
that has ATP embedded in it and what you

325
00:15:06,400 --> 00:15:03,589
do is you add ATP epimer the the RNAs

326
00:15:08,379 --> 00:15:06,410
that bind to ATP bind to the column and

327
00:15:12,160 --> 00:15:08,389
the unbound RNA is basically flow

328
00:15:16,689 --> 00:15:12,170
through and you wash multiple times so

329
00:15:22,480 --> 00:15:16,699
all the unbound rnase is is alluded out

330
00:15:24,040 --> 00:15:22,490
and then you add a free ATP molecules so

331
00:15:27,189 --> 00:15:24,050
that the RNAs that are bound to the

332
00:15:30,069 --> 00:15:27,199
agarose now grab onto the free ATP

333
00:15:32,740 --> 00:15:30,079
molecules and then they allude out so

334
00:15:34,900 --> 00:15:32,750
this is basically what I did ATP aptima

335
00:15:36,970 --> 00:15:34,910
without the phosphate you add to the

336
00:15:41,439 --> 00:15:36,980
column then you start washing they come

337
00:15:42,249 --> 00:15:41,449
off and then well they come off and then

338
00:15:44,109 --> 00:15:42,259

the

339
00:15:45,969 --> 00:15:44,119
certain amount of ATP a primer that is

340
00:15:48,879 --> 00:15:45,979
still bound to the column and then when

341
00:15:51,729 --> 00:15:48,889
you wash it with ATP containing buffer

342
00:15:53,559 --> 00:15:51,739
then the eye primers bind to the

343
00:15:56,619 --> 00:15:53,569
free-floating ATP and then they allude

344
00:15:59,259 --> 00:15:56,629
out as well the story is different when

345
00:16:01,539 --> 00:15:59,269
the ATP a primer is phosphorylated the

346
00:16:03,489 --> 00:16:01,549
abbé de mer can no longer bind to the

347
00:16:07,269 --> 00:16:03,499
column so this could be a very

348
00:16:10,629 --> 00:16:07,279
interesting way of regulating functional

349
00:16:12,639 --> 00:16:10,639
rnase in an RNA world because because if

350
00:16:14,319 --> 00:16:12,649
there is an a functional area if there

351
00:16:17,019 --> 00:16:14,329
wasn't functional RNA molecule that

352
00:16:19,269 --> 00:16:17,029
bound to ntps and if there were

353
00:16:21,789 --> 00:16:19,279
ribozymes that started phosphorylating

354
00:16:24,279 --> 00:16:21,799
these these functional domains of these

355
00:16:27,969 --> 00:16:24,289
epimers then the ribozyme of the app

356
00:16:30,699 --> 00:16:27,979
members would no longer be functional so

357
00:16:32,439 --> 00:16:30,709
this brings to then I started looking at

358
00:16:34,419 --> 00:16:32,449
whether okay you can you can

359
00:16:38,199 --> 00:16:34,429
phosphorylate ATP a primers can you

360
00:16:41,229 --> 00:16:38,209
phosphorylate longer RNA chains and and

361
00:16:43,359 --> 00:16:41,239
this is sort of like a background of why

362
00:16:45,549 --> 00:16:43,369
we want to do that so this is just a

363
00:16:48,279 --> 00:16:45,559

simple primary extension a say that I'm

364
00:16:50,109 --> 00:16:48,289
showing here you have a template you

365
00:16:51,939 --> 00:16:50,119
have a primer the primer binds to the

366
00:16:54,429 --> 00:16:51,949
template and then you can add of DNA

367
00:16:58,299 --> 00:16:54,439
polymerase to extend it whereas if you

368
00:17:01,059 --> 00:16:58,309
have a phosphorylated template you add

369
00:17:02,769 --> 00:17:01,069
the primer anneal it extend it but the

370
00:17:05,860 --> 00:17:02,779
extension stops right where the

371
00:17:09,069 --> 00:17:05,870
phosphate is and this is basically what

372
00:17:11,470 --> 00:17:09,079
this just shows primer extended primer

373
00:17:13,569 --> 00:17:11,480
once you add the phosphate it stops

374
00:17:16,179 --> 00:17:13,579
right where the phosphate is you can

375
00:17:17,860 --> 00:17:16,189
make the template longer again you see

376
00:17:21,059 --> 00:17:17,870
extension but as soon as you add the

377
00:17:25,689 --> 00:17:21,069
phosphate it stops right there as well

378
00:17:27,669 --> 00:17:25,699
now what I was trying to get out was you

379
00:17:31,740 --> 00:17:27,679
have em RNA sequences that might also

380
00:17:34,869 --> 00:17:31,750
have GGA in them so what happens if you

381
00:17:36,909 --> 00:17:34,879
phosphorylated a G and and while the

382
00:17:39,430 --> 00:17:36,919
ribosome is trying to scan through this

383
00:17:43,649 --> 00:17:39,440
mRNA sequence what does it do when it

384
00:17:46,869 --> 00:17:43,659
encounters a modified nucleotide and and

385
00:17:49,360 --> 00:17:46,879
we know that the codon anticodon

386
00:17:51,519 --> 00:17:49,370
interactions are primarily governed by

387
00:17:53,799 --> 00:17:51,529
the Watson and Crick base pairing and

388
00:17:54,570 --> 00:17:53,809

when you're phosphorylating nuclear base

389
00:17:56,279 --> 00:17:54,580
and g

390
00:17:58,080 --> 00:17:56,289
you're essentially messing up with that

391
00:18:00,269 --> 00:17:58,090
watson-crick base pairing and and I

392
00:18:03,060 --> 00:18:00,279
think this might be a bird this might be

393
00:18:06,360 --> 00:18:03,070
a really good way to turn off genes as

394
00:18:09,389 --> 00:18:06,370
well so do you answer that question we

395
00:18:12,690 --> 00:18:09,399
started we started generating new

396
00:18:17,070 --> 00:18:12,700
ribozymes that would target sequences of

397
00:18:19,230 --> 00:18:17,080
a longer RNA chain so we this is an EG

398
00:18:21,870 --> 00:18:19,240
enhanced green fluorescent protein mRNA

399
00:18:25,769 --> 00:18:21,880
sequence and it turns out that it has 11

400
00:18:29,250 --> 00:18:25,779
sites that have a GGA sequence in them

401
00:18:30,990 --> 00:18:29,260
and i think everything has shifted so

402
00:18:33,509 --> 00:18:31,000
you can't really see there's one there's

403
00:18:36,269 --> 00:18:33,519
one there's one but but there are 11

404
00:18:38,370 --> 00:18:36,279
sites that have GGA in it so we

405
00:18:40,680 --> 00:18:38,380
basically generated ribozymes to target

406
00:18:42,629 --> 00:18:40,690
each of these 11 sites and turns out we

407
00:18:45,210 --> 00:18:42,639
can actually phosphorylate multiple

408
00:18:46,649 --> 00:18:45,220
sites in g enhance green fluorescent

409
00:18:47,879 --> 00:18:46,659
protein so what we're going to do next

410
00:18:50,220 --> 00:18:47,889
is we're going to take this

411
00:18:52,799 --> 00:18:50,230
phosphorylated mrna and do an in vitro

412
00:18:54,960 --> 00:18:52,809
translation experiment to see whether

413
00:18:58,220 --> 00:18:54,970

this phosphorylated mrnas are

414
00:19:01,830 --> 00:18:58,230
translatable or not if they are well if

415
00:19:04,710 --> 00:19:01,840
what we hope is that the translation is

416
00:19:07,860 --> 00:19:04,720
shut off after phosphorylation and this

417
00:19:10,730 --> 00:19:07,870
would mean that phosphorylation by

418
00:19:13,110 --> 00:19:10,740
ribosomes could be a new avenue for

419
00:19:14,909 --> 00:19:13,120
artificial genetic regulation and

420
00:19:17,490 --> 00:19:14,919
possibly could be used in synthetic

421
00:19:20,700 --> 00:19:17,500
biology as well we're also working on to

422
00:19:22,680 --> 00:19:20,710
reverse the phosphor alysha the first

423
00:19:25,649 --> 00:19:22,690
part of my talk I showed that at low PH

424
00:19:27,629 --> 00:19:25,659
you start losing the phosphate so we're

425
00:19:30,060 --> 00:19:27,639
also trying to explore what happens once

426
00:19:32,190 --> 00:19:30,070
the phosphate is lost can you regain the

427
00:19:35,840 --> 00:19:32,200
active functional activity that you

428
00:19:38,070 --> 00:19:35,850
initially had as well so with that well

429
00:19:41,850 --> 00:19:38,080
this is a summary of the second part

430
00:19:44,789 --> 00:19:41,860
well functional rnase can be targeted

431
00:19:46,799 --> 00:19:44,799
for ribozyme mediated regulation of

432
00:19:50,850 --> 00:19:46,809
other functional RNA molecules so you're

433
00:19:52,860 --> 00:19:50,860
you're basically so this is literally

434
00:19:55,830 --> 00:19:52,870
making like an RNA world because you are

435
00:19:59,659 --> 00:19:55,840
controlling activity of one functional

436
00:20:03,360 --> 00:19:59,669
RNA molecules with another ribozyme and

437
00:20:06,000 --> 00:20:03,370
mRNA molecules mrna phosphorylation may

438
00:20:08,130 --> 00:20:06,010

potentially be useful for artificially

439
00:20:11,100 --> 00:20:08,140
regulating gene expression

440
00:20:14,310 --> 00:20:11,110
and this basically means that metabolic

441
00:20:16,980 --> 00:20:14,320
networks in an RNA world could be

442
00:20:18,930 --> 00:20:16,990
mediated by ribosomes and with that I

443
00:20:21,810 --> 00:20:18,940
think that's it yeah I'd like to thank

444
00:20:30,100 --> 00:20:21,820
my lab and of course of you all for your

445
00:20:42,109 --> 00:20:39,680
any questions thanks for that it was an

446
00:20:44,659 --> 00:20:42,119
interesting talk now you mentioned a

447
00:20:46,460 --> 00:20:44,669
little bit before the relationship

448
00:20:48,830 --> 00:20:46,470
between that proteins and and sort of

449
00:20:51,019 --> 00:20:48,840
RNA enzymes and when I think of a

450
00:20:54,200 --> 00:20:51,029
protein you know the active site usually

451
00:20:56,960 --> 00:20:54,210
has a metal there right right so how

452
00:20:59,570 --> 00:20:56,970
does this work with RNA enzymes beat DJ

453
00:21:02,810 --> 00:20:59,580
is it so you incorporate a metal at some

454
00:21:05,539 --> 00:21:02,820
point well some are enzymes they utilize

455
00:21:07,039 --> 00:21:05,549
may require metals absolutely but there

456
00:21:09,859 --> 00:21:07,049
have been some that are metal

457
00:21:11,930 --> 00:21:09,869
independent as well this one this one

458
00:21:14,419 --> 00:21:11,940
particular actually uses magnesium and

459
00:21:16,129 --> 00:21:14,429
and copper for some reason but yeah just

460
00:21:17,749 --> 00:21:16,139
a quick follow-up do you know how those

461
00:21:20,060 --> 00:21:17,759
metals are incorporated I mean today

462
00:21:21,889 --> 00:21:20,070
mostly are they kind of dynamic like do

463
00:21:23,869 --> 00:21:21,899

they come in and go out or are they

464
00:21:25,310 --> 00:21:23,879
fixed like when you have this kind of

465
00:21:27,830 --> 00:21:25,320
structure that's almost like a protein

466
00:21:29,419 --> 00:21:27,840
it's pretty much fixed just like

467
00:21:31,669 --> 00:21:29,429
proteins there's specific metal binding

468
00:21:33,169 --> 00:21:31,679
sites metal ion binding sites and and

469
00:21:35,180 --> 00:21:33,179
these metal ions usually interact with

470
00:21:36,710 --> 00:21:35,190
the two prime hydroxyls as well and so

471
00:21:44,509 --> 00:21:36,720
it's really important for the RNA

472
00:21:47,210 --> 00:21:44,519
folding Thanks a quick question about

473
00:21:50,979 --> 00:21:47,220
your first part of your talk so you are

474
00:21:55,070 --> 00:21:50,989
showing the the function of your

475
00:21:57,919 --> 00:21:55,080
ribozyme against pH right I noticed the

476
00:21:59,869 --> 00:21:57,929
the Ranger you did was a little bit

477
00:22:02,180 --> 00:21:59,879
tight did you try pushing it very far

478
00:22:04,190 --> 00:22:02,190
out of here actually yes so that work

479
00:22:06,229 --> 00:22:04,200
had already been done before and I was

480
00:22:07,999 --> 00:22:06,239
basically only testing it at a small

481
00:22:10,340 --> 00:22:08,009
range and and yes it is evident in our

482
00:22:13,220 --> 00:22:10,350
larger range as well I think we have

483
00:22:15,229 --> 00:22:13,230
gone from five to eight point five or

484
00:22:18,019 --> 00:22:15,239
something like that Thank you Thank

485
00:22:24,229 --> 00:22:18,029
other questions maybe some from say