Krishna Sapkota

Identity & Metabolism of CoA Linked RNA

1
00:00:00,240 --> 00:00:10,949
[Music]

2
00:00:17,490 --> 00:00:14,739
this afternoon I'll be talking about are

3
00:00:19,839 --> 00:00:17,500
any species that are relevant to the

4
00:00:23,710 --> 00:00:19,849
origin of life or are any wool are any

5
00:00:25,599 --> 00:00:23,720
wool tunic if you are not familiar with

6
00:00:27,759 --> 00:00:25,609
irony there are three biopolymers in

7
00:00:29,800 --> 00:00:27,769
current world let's the three major

8
00:00:31,989 --> 00:00:29,810
biopolymers one is DNA that to

9
00:00:33,640 --> 00:00:31,999
information stories and there is RNA

10
00:00:36,310 --> 00:00:33,650
that is kind of reached between DNA and

11
00:00:37,930 --> 00:00:36,320
protein linking the information or

12
00:00:40,150 --> 00:00:37,940
carrying the information coded in DNA

13
00:00:41,860 --> 00:00:40,160

into the protein and proteins are the

14
00:00:44,410 --> 00:00:41,870
functional molecules that to all

15
00:00:47,380 --> 00:00:44,420
structure looking catalysis and binding

16
00:00:51,010 --> 00:00:47,390
and so on and so forth I'll be talking

17
00:00:53,350 --> 00:00:51,020
about RNA hr1 one special class of RNA

18
00:00:58,360 --> 00:00:53,360
that may be related it is related to the

19
00:01:00,550 --> 00:00:58,370
origin of life so everyone in the room

20
00:01:04,110 --> 00:01:00,560
is interested how life originated in the

21
00:01:06,220 --> 00:01:04,120
planet or something similar to the

22
00:01:08,020 --> 00:01:06,230
prebiotic all may be happening somewhere

23
00:01:13,180 --> 00:01:08,030
around the universe so we have a very

24
00:01:16,210 --> 00:01:13,190
nice talk about these small molecules

25
00:01:19,720 --> 00:01:16,220
which are prebiotic chemicals in the

26
00:01:21,700 --> 00:01:19,730
previous lessons one widely accepted

27
00:01:24,399 --> 00:01:21,710
hypothesis about what is enough life in

28
00:01:28,030 --> 00:01:24,409
our theories RNA world hypothesis which

29
00:01:31,560 --> 00:01:28,040
tells RNA is the first molecule first

30
00:01:33,700 --> 00:01:31,570
biomolecule appeared which sort in both

31
00:01:37,090 --> 00:01:33,710
information stories and functional

32
00:01:39,219 --> 00:01:37,100
capacity so in the prebiotic world is

33
00:01:41,170 --> 00:01:39,229
small organic chemicals organic

34
00:01:43,990 --> 00:01:41,180
molecules appeared somehow and then

35
00:01:47,469 --> 00:01:44,000
these condense together form bigger

36
00:01:50,770 --> 00:01:47,479
molecules which appear similar to or

37
00:01:53,289 --> 00:01:50,780
exactly similar same to like for RNA

38
00:01:56,740 --> 00:01:53,299

monomers that we see today a dilution

39
00:02:00,370 --> 00:01:56,750
guanine cytosine and uracil these for

40
00:02:02,980 --> 00:02:00,380
RNA monomers polymerized in a phosphate

41
00:02:05,980 --> 00:02:02,990
phosphate backbone giving us primitive

42
00:02:08,979 --> 00:02:05,990
RNA oligomers these oligomers

43
00:02:09,459 --> 00:02:08,989
learned to like it with other RNA

44
00:02:12,160 --> 00:02:09,469
oligomers

45
00:02:14,059 --> 00:02:12,170
making themself a bigger and they learn

46
00:02:16,630 --> 00:02:14,069
to call

47
00:02:19,130 --> 00:02:16,640
themselves in a fateful way and then

48
00:02:23,059 --> 00:02:19,140
learn to change the sequences around

49
00:02:26,780 --> 00:02:23,069
muted like causing mutations and the

50
00:02:28,580 --> 00:02:26,790
more favorable mutations were selected

51
00:02:32,000 --> 00:02:28,590
or successful mutations were selected

52
00:02:34,009 --> 00:02:32,010
and the same biopolymers are folded in a

53
00:02:36,289 --> 00:02:34,019
special way creating a functional

54
00:02:38,420 --> 00:02:36,299
molecule and somehow this informational

55
00:02:40,490 --> 00:02:38,430
molecule and this functional molecule in

56
00:02:43,569 --> 00:02:40,500
turn are enclosed in our lipid vesicles

57
00:02:47,690 --> 00:02:43,579
creating a first system that is able to

58
00:02:49,789 --> 00:02:47,700
store information or that is able to

59
00:02:52,039 --> 00:02:49,799
support itself and store the information

60
00:02:55,129 --> 00:02:52,049
which we sometimes called a rebel cell

61
00:02:59,539 --> 00:02:55,139
arrival site and then from this point on

62
00:03:01,129 --> 00:02:59,549
the Darwinian evolution started so once

63
00:03:04,129 --> 00:03:01,139

the Darwinian evolutionist I drew the

64
00:03:06,559 --> 00:03:04,139
the system start just getting started

65
00:03:09,949 --> 00:03:06,569
getting more complex and complex so this

66
00:03:11,809 --> 00:03:09,959
is a rival site and the complexity in it

67
00:03:15,619 --> 00:03:11,819
gets increased with the evolution and

68
00:03:18,439 --> 00:03:15,629
with increasing complexity RNA itself

69
00:03:21,439 --> 00:03:18,449
cannot do could not do all the reactions

70
00:03:23,599 --> 00:03:21,449
needed so it needs it needed the help of

71
00:03:26,390 --> 00:03:23,609
other molecules that has the required

72
00:03:28,909 --> 00:03:26,400
functional groups for example 4s I'll

73
00:03:30,920 --> 00:03:28,919
transfer reactions simply carbon

74
00:03:33,530 --> 00:03:30,930
transfer reactions it needed our salt

75
00:03:36,890 --> 00:03:33,540
for functional groups or tile and the

76
00:03:40,909 --> 00:03:36,900
coenzyme a appeared in electron transfer

77
00:03:43,729 --> 00:03:40,919
reactions and oxidation reduction

78
00:03:45,619 --> 00:03:43,739
reactions it needed nad and 8 years and

79
00:03:49,129 --> 00:03:45,629
slave in Co enzymes and all these

80
00:03:51,199 --> 00:03:49,139
coenzymes appeared all common also there

81
00:03:53,780 --> 00:03:51,209
is one common thing about these

82
00:03:56,439 --> 00:03:53,790
coenzymes which is they all have this

83
00:03:59,379 --> 00:03:56,449
adenosine part attached to it which is

84
00:04:01,719 --> 00:03:59,389
which which tells us that these are

85
00:04:08,080 --> 00:04:01,729
coenzymes could be of molecular fossils

86
00:04:13,509 --> 00:04:09,910
I will be talking basically about

87
00:04:15,339 --> 00:04:13,519
coenzyme a suppose I made involves in s

88
00:04:17,740 --> 00:04:15,349

and transfer reactions because it has

89
00:04:22,090 --> 00:04:17,750
this sulfur or tile functional group at

90
00:04:24,730 --> 00:04:22,100
the end someone is talking about kick

91
00:04:25,930 --> 00:04:24,740
that database at least get some less so

92
00:04:28,030 --> 00:04:25,940
you got kicked it away certainly and if

93
00:04:29,439 --> 00:04:28,040
you go to the kick database around ten

94
00:04:31,629 --> 00:04:29,449
percent of things I am stunning in our

95
00:04:33,969 --> 00:04:31,639
show right now need this molecule for

96
00:04:35,800 --> 00:04:33,979
the function so this is an important

97
00:04:40,000 --> 00:04:35,810
molecule and if this molecule is

98
00:04:44,980 --> 00:04:40,010
believed to exist since the sins are any

99
00:04:46,120 --> 00:04:44,990
water since it has higher functional

100
00:04:51,730 --> 00:04:46,130
group there it can form a Thai

101
00:04:54,610 --> 00:04:51,740
restaurant which is thought to serve as

102
00:04:57,010 --> 00:04:54,620
a free energy source during the during

103
00:05:01,450 --> 00:04:57,020
the evolution during the reaction in

104
00:05:03,790 --> 00:05:01,460
evoking evolution so the question is how

105
00:05:05,770 --> 00:05:03,800
this coenzyme a molecule formed for the

106
00:05:09,790 --> 00:05:05,780
first time and to answer that question

107
00:05:12,520 --> 00:05:09,800
we have selected from thousands of

108
00:05:14,350 --> 00:05:12,530
trillions of RNA library some RNA

109
00:05:17,050 --> 00:05:14,360
sequences that can catalyze the

110
00:05:19,450 --> 00:05:17,060
synthesis of this molecule itself so RNA

111
00:05:23,680 --> 00:05:19,460
can catalyze the synthesis of coenzyme a

112
00:05:28,830 --> 00:05:23,690
which we call them coenzyme a synthetic

113
00:05:33,779 --> 00:05:28,840

those ribozymes so this tells us that

114
00:05:38,469 --> 00:05:33,789
RNA itself may have synthesized the

115
00:05:40,060 --> 00:05:38,479
required functional groups that it that

116
00:05:44,170 --> 00:05:40,070
the right side needed in the RNA world

117
00:05:46,480 --> 00:05:44,180
so this thing us there could be that

118
00:05:54,100 --> 00:05:46,490
could have been a complex metabolic

119
00:05:56,529 --> 00:05:54,110
system composed just of RNA so the

120
00:05:59,680 --> 00:05:56,539
question is the question now is so har

121
00:06:01,510 --> 00:05:59,690
na can catalyze the formation of

122
00:06:03,940 --> 00:06:01,520
coenzyme a in the formation of the

123
00:06:05,740 --> 00:06:03,950
thyristors are these molecules are these

124
00:06:09,490 --> 00:06:05,750
queens I'm a link to her molecules

125
00:06:12,310 --> 00:06:09,500
existing current biology if they do they

126
00:06:15,100 --> 00:06:12,320
could give us a clue about how all these

127
00:06:17,860 --> 00:06:15,110
things happened in the periodic world

128
00:06:20,260 --> 00:06:17,870
because these selections these molecules

129
00:06:21,540 --> 00:06:20,270
are from are selected from artificially

130
00:06:25,110 --> 00:06:21,550
created random

131
00:06:29,820 --> 00:06:25,120
library containing 10 power 14 to 10

132
00:06:33,420 --> 00:06:29,830
power 15 unique sequences so the

133
00:06:35,400 --> 00:06:33,430
question is to query linked to RNA exist

134
00:06:37,680 --> 00:06:35,410
in current biology and the till balloon

135
00:06:40,470 --> 00:06:37,690
from Harvard insert this question and

136
00:06:43,500 --> 00:06:40,480
the answer is yes there are we linked

137
00:06:48,120 --> 00:06:43,510
RNA species and we Tyra Stirling tyranny

138
00:06:50,490 --> 00:06:48,130

species existing in the RNA pool of

139
00:06:51,660 --> 00:06:50,500
current bacterial cell at least in

140
00:06:53,940 --> 00:06:51,670
bacterial cell we know we know that

141
00:06:54,270 --> 00:06:53,950
maybe in the eukaryotic also we don't

142
00:07:02,400 --> 00:06:54,280
know

143
00:07:05,240 --> 00:07:02,410
the sequence is down here and what they

144
00:07:07,950 --> 00:07:05,250
are doing right now if we are functional

145
00:07:09,660 --> 00:07:07,960
since we don't know the sequence we

146
00:07:13,620 --> 00:07:09,670
don't know how they evolved and what

147
00:07:16,110 --> 00:07:13,630
they what is the role in the cell so the

148
00:07:17,820 --> 00:07:16,120
goal of my research is to capture these

149
00:07:20,550 --> 00:07:17,830
RNA sequences based on this tile

150
00:07:22,920 --> 00:07:20,560
functional group sequence them and then

151
00:07:25,040 --> 00:07:22,930
find out how they evolved and what they

152
00:07:27,840 --> 00:07:25,050
are doing there so the first question is

153
00:07:30,390 --> 00:07:27,850
the first of all is capture them and

154
00:07:33,720 --> 00:07:30,400
find out what are the sequence by doing

155
00:07:38,130 --> 00:07:33,730
sequencing and the second goal of the

156
00:07:40,650 --> 00:07:38,140
project is find out how these RNA

157
00:07:42,480 --> 00:07:40,660
species are being made in the cell what

158
00:07:45,270 --> 00:07:42,490
is the mechanism there could be two

159
00:07:48,060 --> 00:07:45,280
possible pathways that these are any

160
00:07:51,660 --> 00:07:48,070
species can some can be formed when it's

161
00:07:55,440 --> 00:07:51,670
co-transcriptional which is since this

162
00:07:58,800 --> 00:07:55,450
part is Eddy notion and which kind of

163
00:08:01,350 --> 00:07:58,810

which is exactly same is ATP this part

164
00:08:03,030 --> 00:08:01,360
can initiate the transcription by

165
00:08:05,670 --> 00:08:03,040
competing with ATP under certain

166
00:08:08,400 --> 00:08:05,680
promoters so this is a cool

167
00:08:10,790 --> 00:08:08,410
transcriptional mode of formation which

168
00:08:15,210 --> 00:08:10,800
could be happening and there is another

169
00:08:17,580 --> 00:08:15,220
possible pathway which is there may be

170
00:08:20,520 --> 00:08:17,590
some enzymes linking this first poop

171
00:08:22,050 --> 00:08:20,530
endeth in part to ATP initiated RNA

172
00:08:24,150 --> 00:08:22,060
forming these colonies

173
00:08:26,190 --> 00:08:24,160
so we don't know what whether the

174
00:08:27,900 --> 00:08:26,200
colonies are being made by

175
00:08:32,259 --> 00:08:27,910
co-transcriptional mode or whose

176
00:08:37,209 --> 00:08:34,929
so the goal is to capture the cuellar

177
00:08:39,040 --> 00:08:37,219
and sequences and cuellar any species

178
00:08:41,109 --> 00:08:39,050
and sequence them and figure out how

179
00:08:43,269 --> 00:08:41,119
they are being made so to capture them

180
00:08:45,910 --> 00:08:43,279
we have to develop a RNA capture

181
00:08:47,829 --> 00:08:45,920
protocol or method and to develop our

182
00:08:50,379 --> 00:08:47,839
method we need our eat we need an easy

183
00:08:53,439 --> 00:08:50,389
access to lab synthesize quailing

184
00:08:55,179 --> 00:08:53,449
colonies so we are our lab have has

185
00:08:58,350 --> 00:08:55,189
previously developed a technique to

186
00:09:00,999 --> 00:08:58,360
label coenzyme a on the RNA phi prime in

187
00:09:04,150 --> 00:09:01,009
since coenzyme a has that any notion

188
00:09:05,939 --> 00:09:04,160

part on it it can compete with RNA under

189
00:09:09,340 --> 00:09:05,949
all right promoter under t7 promoter

190
00:09:11,169 --> 00:09:09,350
with t7 RNA polymerase in the some RNA

191
00:09:16,509 --> 00:09:11,179
transcript will automatically be labeled

192
00:09:18,189 --> 00:09:16,519
with coenzyme a but we need a default

193
00:09:20,199 --> 00:09:18,199
photo enzyme a to initiate the

194
00:09:23,259 --> 00:09:20,209
transcripts on there and this differs

195
00:09:27,160 --> 00:09:23,269
for coenzyme a itself is a problem it it

196
00:09:29,109 --> 00:09:27,170
is extremely expensive molecule well not

197
00:09:32,650 --> 00:09:29,119
let's not say extremely but it is

198
00:09:36,999 --> 00:09:32,660
expensive extremely the kind of TOEFL so

199
00:09:39,309 --> 00:09:37,009
and then we started with developing our

200
00:09:42,730 --> 00:09:39,319
one method for the to make this d4 spoke

201
00:09:45,939 --> 00:09:42,740
we we started we we use the enzymatic

202
00:09:49,299 --> 00:09:45,949
strategy nature has two different

203
00:09:52,059 --> 00:09:49,309
pathways to dedicated for the synthesis

204
00:09:53,919 --> 00:09:52,069
of coenzyme a so we use a pathway called

205
00:09:57,609 --> 00:09:53,929
coenzyme a salvage pathway which has

206
00:09:59,889 --> 00:09:57,619
three total enzymes while cloning these

207
00:10:02,470 --> 00:09:59,899
enzymes we came up with a new cloning

208
00:10:06,549 --> 00:10:02,480
strategy which is shown in this picture

209
00:10:09,329 --> 00:10:06,559
which is based on just a PCR read

210
00:10:11,319 --> 00:10:09,339
we don't need restriction digestion and

211
00:10:12,850 --> 00:10:11,329
ligation steps maybe molecular

212
00:10:14,819 --> 00:10:12,860
biologists understand understand this

213
00:10:17,910 --> 00:10:14,829

better

214
00:10:20,710 --> 00:10:17,920
we came up with a new pruning technique

215
00:10:22,900 --> 00:10:20,720
which is very simple and efficient and

216
00:10:27,699 --> 00:10:22,910
able to publish this cloning method in

217
00:10:29,919 --> 00:10:27,709
plus one two years ago if we if someone

218
00:10:33,549 --> 00:10:29,929
in the room is facing a difficult time

219
00:10:35,109 --> 00:10:33,559
in like crowning using a restriction

220
00:10:37,840 --> 00:10:35,119
dilation in like some method we can

221
00:10:39,609 --> 00:10:37,850
maybe we can talk about the new method

222
00:10:41,189 --> 00:10:39,619
which does not need digestion and

223
00:10:44,829 --> 00:10:41,199
ligation

224
00:10:46,150 --> 00:10:44,839
using the new method we call two enzymes

225
00:10:49,240 --> 00:10:46,160
of the coil salad

226
00:10:53,069 --> 00:10:49,250
into a plasmid we created using this

227
00:10:55,300 --> 00:10:53,079
plasmid we made two proteins which are

228
00:10:59,230 --> 00:10:55,310
able to make this device work away from

229
00:11:02,379 --> 00:10:59,240
the commercially available Penta teen

230
00:11:04,509 --> 00:11:02,389
this molecule and then this carried out

231
00:11:07,300 --> 00:11:04,519
this enzymatic synthesis we had some

232
00:11:09,970 --> 00:11:07,310
purified distance between Jamie and we

233
00:11:13,269 --> 00:11:09,980
were able to publish this this part in

234
00:11:15,850 --> 00:11:13,279
by organic chemistry last year so now we

235
00:11:17,949 --> 00:11:15,860
have a method to make why are any in the

236
00:11:19,840 --> 00:11:17,959
lab and we have an easy access to defer

237
00:11:23,590 --> 00:11:19,850
spoke way we can go ahead and work on

238
00:11:26,619 --> 00:11:23,600

cuellar and it captured protocol so I

239
00:11:31,900 --> 00:11:26,629
started we are any capture protocol with

240
00:11:35,199 --> 00:11:31,910
the lab made a RNA and then I was

241
00:11:38,400 --> 00:11:35,209
playing with chemistry on this tile so

242
00:11:41,889 --> 00:11:38,410
the idea is you have a RNA pool that has

243
00:11:44,290 --> 00:11:41,899
where as summit RNA sequences are

244
00:11:48,340 --> 00:11:44,300
labeled with coenzyme a and most of them

245
00:11:50,980 --> 00:11:48,350
are not so we prepared three types of

246
00:11:54,100 --> 00:11:50,990
solid beets which can specifically react

247
00:11:56,350 --> 00:11:54,110
with this tile so these are beets and

248
00:11:57,370 --> 00:11:56,360
under in the base this is a Malamute

249
00:12:02,769 --> 00:11:57,380
functional group

250
00:12:04,840 --> 00:12:02,779
with this tile and links this tile and

251
00:12:08,740 --> 00:12:04,850
whatever link to this tile to the beets

252
00:12:10,629 --> 00:12:08,750
so that we can specifically capture the

253
00:12:12,970 --> 00:12:10,639
tile containing molecules on the on the

254
00:12:16,420 --> 00:12:12,980
beets and another one another beets with

255
00:12:18,460 --> 00:12:16,430
I work with is iodoform generalized

256
00:12:21,850 --> 00:12:18,470
beets this iro also reacts with this

257
00:12:23,530 --> 00:12:21,860
tile and then linking the tile

258
00:12:26,110 --> 00:12:23,540
containing molecule permanently or

259
00:12:28,660 --> 00:12:26,120
covalently with the beads so that we can

260
00:12:30,160 --> 00:12:28,670
capture them or purify them by some

261
00:12:32,499 --> 00:12:30,170
chromatograph you check some

262
00:12:34,299 --> 00:12:32,509
chromatographic techniques later and the

263
00:12:35,199 --> 00:12:34,309

third type of beets I work with is tile

264
00:12:37,389 --> 00:12:35,209
containing beets

265
00:12:38,949 --> 00:12:37,399
this these tiles can make disulfide

266
00:12:42,220 --> 00:12:38,959
bonds with us with another tile

267
00:12:44,199 --> 00:12:42,230
containing molecules so that we can use

268
00:12:47,410 --> 00:12:44,209
them to capture these tile containing

269
00:12:50,829 --> 00:12:47,420
molecules specifically so I prepare

270
00:12:54,280 --> 00:12:50,839
these beads and then incubated days

271
00:12:56,970 --> 00:12:54,290
beats with a RNAi poop and the

272
00:12:59,890 --> 00:12:56,980
expectation is is this so if it did

273
00:13:02,080 --> 00:12:59,900
malee made will react with tile i

274
00:13:07,120 --> 00:13:02,090
also react with tile and that this tile

275
00:13:09,250 --> 00:13:07,130
column will give me a disulfide and then

276
00:13:11,440 --> 00:13:09,260
once this is captured once this RNA is

277
00:13:14,770 --> 00:13:11,450
captured we have to release it so that

278
00:13:16,930 --> 00:13:14,780
we can sequence it and the release for

279
00:13:19,510 --> 00:13:16,940
the release I used three different

280
00:13:21,850 --> 00:13:19,520
protocols one being cleaving a disulfide

281
00:13:28,060 --> 00:13:21,860
there in releasing the RNA part this is

282
00:13:31,300 --> 00:13:28,070
done by DT T releasing a RNA and in the

283
00:13:33,610 --> 00:13:31,310
Kyoto beats we used some some group

284
00:13:35,530 --> 00:13:33,620
called photo cleavable group which can

285
00:13:37,720 --> 00:13:35,540
be cleaved off by signing certain

286
00:13:39,940 --> 00:13:37,730
wavelength of UV light and in these type

287
00:13:42,760 --> 00:13:39,950
of beats I use light to cleave off the

288
00:13:45,010 --> 00:13:42,770

RNA sequences from the beats and in

289
00:13:47,530 --> 00:13:45,020
another type of column which is melamed

290
00:13:49,420 --> 00:13:47,540
functionalized columns I used our

291
00:13:52,720 --> 00:13:49,430
special files of enzyme called the new

292
00:13:55,330 --> 00:13:52,730
dekes hydrolysis to release this RNA

293
00:13:57,190 --> 00:13:55,340
sequences captured there so new tricks

294
00:13:59,910 --> 00:13:57,200
enzyme is a special class of hydrolysis

295
00:14:02,680 --> 00:13:59,920
that cleaves right there so this is a

296
00:14:05,200 --> 00:14:02,690
pen dip in part of coenzyme a and this

297
00:14:07,720 --> 00:14:05,210
is the RNA part so once knew once new

298
00:14:10,540 --> 00:14:07,730
dekes reacts on these are any species or

299
00:14:12,940 --> 00:14:10,550
whatever it capture there it it cuts off

300
00:14:15,370 --> 00:14:12,950
it cuts right there in between these two

301
00:14:21,040 --> 00:14:15,380
phosphates and releases whatever is

302
00:14:25,030 --> 00:14:21,050
there in the RNA part so after the

303
00:14:26,740 --> 00:14:25,040
releasing we ligated our adapter

304
00:14:28,660 --> 00:14:26,750
molecule on both five Prime and three

305
00:14:31,840 --> 00:14:28,670
prime end of the RNA so that we can

306
00:14:33,730 --> 00:14:31,850
sequence them after ligation we did

307
00:14:35,590 --> 00:14:33,740
reverse transcription get back to TNA

308
00:14:38,800 --> 00:14:35,600
and then did pshare to amplify the

309
00:14:41,020 --> 00:14:38,810
signal and did next-gen sequencing so so

310
00:14:42,670 --> 00:14:41,030
far so good winning we developed our

311
00:14:44,950 --> 00:14:42,680
method we got the RNA from total

312
00:14:47,230 --> 00:14:44,960
bacterial pool and we send around 20

313
00:14:49,480 --> 00:14:47,240

clones for the sequencing but when we

314
00:14:52,360 --> 00:14:49,490
saw the sequencing result I was very sad

315
00:14:54,370 --> 00:14:52,370
because I was expecting to get this coil

316
00:14:58,210 --> 00:14:54,380
entirely from this protocol but what I

317
00:15:01,060 --> 00:14:58,220
got was some key RNA sequences so we

318
00:15:03,070 --> 00:15:01,070
know the tRNA sequences are heavily

319
00:15:05,470 --> 00:15:03,080
modified the tRNAs are heavily wouldíve

320
00:15:07,600 --> 00:15:05,480
modified with tile this is this is an

321
00:15:09,580 --> 00:15:07,610
example this is a type 4 high reading

322
00:15:13,410 --> 00:15:09,590
there are other multiple modified you

323
00:15:16,300 --> 00:15:13,420
readings with tiles so this protocol

324
00:15:20,440 --> 00:15:16,310
captured these tire readings on tRNAs

325
00:15:23,950 --> 00:15:20,450
not the coy RNA so since Cuellar and A's

326
00:15:26,680 --> 00:15:23,960
are very less like their abundance is

327
00:15:28,200 --> 00:15:26,690
very less compared to the tRNA we simply

328
00:15:30,820 --> 00:15:28,210
missed Co irony

329
00:15:33,400 --> 00:15:30,830
since this protocol failed I moved on

330
00:15:37,480 --> 00:15:33,410
and then developed other two strategies

331
00:15:41,170 --> 00:15:37,490
to capture these coy RNA species so one

332
00:15:43,660 --> 00:15:41,180
is based on the enzymes which we call

333
00:15:45,280 --> 00:15:43,670
the enzymatic strategy and another one

334
00:15:48,820 --> 00:15:45,290
is chemical strategy based on the

335
00:15:51,160 --> 00:15:48,830
chemistry so for the enzymatic strategy

336
00:15:53,470 --> 00:15:51,170
I'm looking for any specific enzyme

337
00:15:55,770 --> 00:15:53,480
called acid core I like it in the class

338
00:16:00,190 --> 00:15:55,780

of a sheet coral I guess that can

339
00:16:03,100 --> 00:16:00,200
catalyze the the linkage or covalent

340
00:16:05,320 --> 00:16:03,110
bond formation between some fatty acid

341
00:16:08,050 --> 00:16:05,330
modified with biotin on one end and

342
00:16:09,760 --> 00:16:08,060
coenzyme ellington asa so these enzymes

343
00:16:13,780 --> 00:16:09,770
this is a huge class of enzymes that

344
00:16:16,330 --> 00:16:13,790
catalyzes a reaction of coenzyme a and

345
00:16:19,240 --> 00:16:16,340
free fatty acids but there are no exam

346
00:16:21,640 --> 00:16:19,250
in nature that can work on modified

347
00:16:23,890 --> 00:16:21,650
coenzyme a let's say boiling - RNA and

348
00:16:26,410 --> 00:16:23,900
modified fatty acid let's say biotin

349
00:16:29,130 --> 00:16:26,420
fatty acids so using structure guided

350
00:16:34,170 --> 00:16:29,140
engineering and some sub mutation

351
00:16:36,610 --> 00:16:34,180
mutation are guided by structure

352
00:16:39,550 --> 00:16:36,620
proteins are three dimensional strict

353
00:16:42,820 --> 00:16:39,560
structure we are creating we're trying

354
00:16:45,610 --> 00:16:42,830
to create our new enzyme that can accept

355
00:16:47,950 --> 00:16:45,620
modified coenzyme a sorry modified fatty

356
00:16:51,940 --> 00:16:47,960
acid and modified coenzyme a is a

357
00:16:54,430 --> 00:16:51,950
substrate and covalently links the

358
00:16:56,860 --> 00:16:54,440
carboxylic acid and the tile part

359
00:16:58,900 --> 00:16:56,870
creating a thioester bond once it is

360
00:17:01,570 --> 00:16:58,910
once the molecule is enzymatically

361
00:17:03,580 --> 00:17:01,580
labeled with biotin we can capture this

362
00:17:07,410 --> 00:17:03,590
biotin molecules on the step traveling

363
00:17:09,810 --> 00:17:07,420

beads and purified purified and then

364
00:17:12,640 --> 00:17:09,820
next starting is user

365
00:17:14,710 --> 00:17:12,650
pending in Alex which is we are trying

366
00:17:19,120 --> 00:17:14,720
to modify the panteth in part with

367
00:17:20,770 --> 00:17:19,130
biotin and some linkers some some label

368
00:17:23,079 --> 00:17:20,780
layer so that these molecules will be

369
00:17:25,270 --> 00:17:23,089
sent to the bacterial cell bacteria will

370
00:17:26,549 --> 00:17:25,280
level their query RNA with the with the

371
00:17:28,409 --> 00:17:26,559
new pending

372
00:17:30,090 --> 00:17:28,419
since Pete and Alex are modified with

373
00:17:31,669 --> 00:17:30,100
biotene we can capture them money step

374
00:17:36,509 --> 00:17:31,679
evident Peaks so these are two

375
00:17:39,330 --> 00:17:36,519
strategies and I'm working - working to

376
00:17:42,049 --> 00:17:39,340
ask them trying to get the enzyme like I

377
00:17:47,039 --> 00:17:42,059
purified multiple enzymes from bacteria

378
00:17:49,379 --> 00:17:47,049
from e.coli trying to like expect

379
00:17:52,289 --> 00:17:49,389
expecting the enzymes when modified we

380
00:17:54,419 --> 00:17:52,299
can do this reaction so that enzyme

381
00:17:56,820 --> 00:17:54,429
reaction will be specific and then I'm

382
00:17:59,460 --> 00:17:56,830
modifying the pendant in part with by

383
00:18:02,009 --> 00:17:59,470
putting some putting a biotin and

384
00:18:04,649 --> 00:18:02,019
multiple lights and residues on it so it

385
00:18:07,200 --> 00:18:04,659
has a pendant in part that will be used

386
00:18:08,909 --> 00:18:07,210
to make way RNA by the cell and it has

387
00:18:10,430 --> 00:18:08,919
multiple license residues that will help

388
00:18:13,499 --> 00:18:10,440

this molecule to get inside the

389
00:18:15,450 --> 00:18:13,509
bacterial cell membrane I'm in two key

390
00:18:19,049 --> 00:18:15,460
to get across the cell membrane and then

391
00:18:21,479 --> 00:18:19,059
I'm doing the transfer D study of

392
00:18:24,570 --> 00:18:21,489
studies of these molecules and luckily

393
00:18:26,669 --> 00:18:24,580
these molecules are being acted like are

394
00:18:28,349 --> 00:18:26,679
accepted by the enzymes of COI synthetic

395
00:18:30,690 --> 00:18:28,359
pathway so there is a good chance that

396
00:18:34,710 --> 00:18:30,700
these molecules will end up in the RNA

397
00:18:36,409 --> 00:18:34,720
of the bacteria and then I'm also

398
00:18:40,169 --> 00:18:36,419
working with Cuellar and a metabolism

399
00:18:42,389 --> 00:18:40,179
trying to answer how these core RNAs are

400
00:18:46,169 --> 00:18:42,399
being turned up like how the toner what

401
00:18:48,779 --> 00:18:46,179
happens I'm not talking a lot about this

402
00:18:52,769 --> 00:18:48,789
area since the time is up and then do it

403
00:18:54,779 --> 00:18:52,779
this hopefully I convinced you these

404
00:18:57,690 --> 00:18:54,789
qualities are very important to

405
00:19:01,080 --> 00:18:57,700
understand how RNA the world started and

406
00:19:03,899 --> 00:19:01,090
they can give us a good clue on how our

407
00:19:09,660 --> 00:19:03,909
and will start it and I'm doing working

408
00:19:13,950 --> 00:19:11,269
[Applause]

409
00:19:17,789 --> 00:19:13,960
your question yep

410
00:19:18,450 --> 00:19:17,799
I know you yeah okay so um quick

411
00:19:20,999 --> 00:19:18,460
question

412
00:19:23,129 --> 00:19:21,009
you want to use the biotinylated Panther

413
00:19:24,749 --> 00:19:23,139

theme as a way to basically labeling

414
00:19:26,249 --> 00:19:24,759
darnay's yeah but are you concerned at

415
00:19:27,599 --> 00:19:26,259
all that if there is some kind of

416
00:19:29,970 --> 00:19:27,609
post-transcriptional modification

417
00:19:31,859 --> 00:19:29,980
happening that those enzymes would not

418
00:19:33,180 --> 00:19:31,869
be able to add the modified panda thing

419
00:19:36,060 --> 00:19:33,190
and therefore you're not actually saying

420
00:19:41,140 --> 00:19:36,070
- yep Bejo yeah cuz you might not see a

421
00:19:49,460 --> 00:19:47,450
yeah all right one more quick one all

422
00:19:51,110 --> 00:19:49,470
right if not we're gonna move on in the