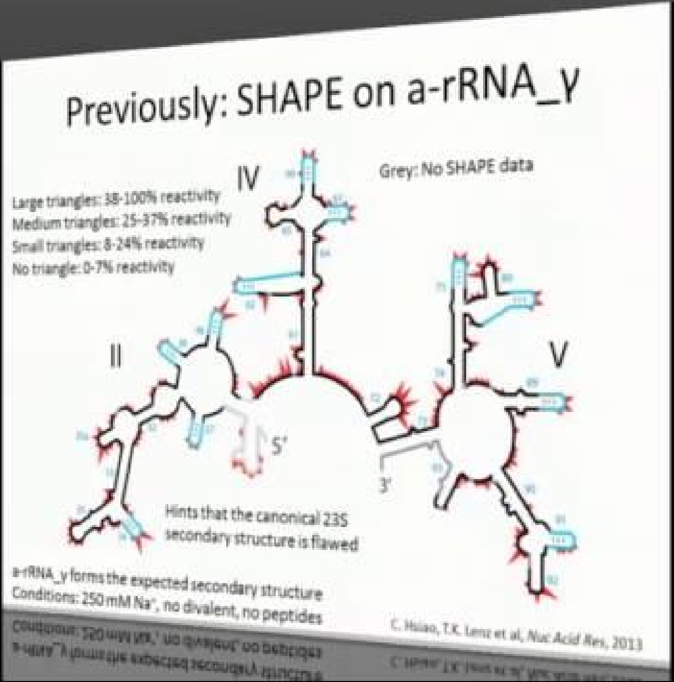

Previously: SHAPE on a-rRNA_γ

Grey: No SHAPE data

Large triangles: 38-100% reactivity
Medium triangles: 25-37% reactivity
Small triangles: 8-24% reactivity
No triangle: 0-7% reactivity

Hints that the canonical 23S secondary structure is flawed

a-rRNA_γ forms the expected secondary structure
Conditions: 250 mM Na^+, no divalent, no peptides

C. Hsiao, T.K. Lenz et al, *Nuc Acid Res*, 2013

1
00:00:14,760 --> 00:00:09,959
for Speaker of this session is me I am

2
00:00:16,859 --> 00:00:14,770
me I am from Georgia Tech I work kind of

3
00:00:18,870 --> 00:00:16,869
co advised between Nick head and Lauren

4
00:00:22,019 --> 00:00:18,880
Williams we're really lucky Georgia Tech

5
00:00:25,019 --> 00:00:22,029
to have a lot of funding for origins of

6
00:00:26,880 --> 00:00:25,029
life research so quick plug so I'm

7
00:00:29,040 --> 00:00:26,890
working in the center for ribosomal

8
00:00:30,870 --> 00:00:29,050
orange enough evolution so that's why

9
00:00:32,970 --> 00:00:30,880
today I'm going to be talking about what

10
00:00:34,800 --> 00:00:32,980
I'm what's probably going to drive some

11
00:00:38,939 --> 00:00:34,810
of the rock people in here crazy which

12
00:00:41,430 --> 00:00:38,949
is molecular paleontology so in this

13
00:00:42,810 --> 00:00:41,440

case we're kind of working on a top-down

14
00:00:46,080 --> 00:00:42,820
approach where we're trying to use

15
00:00:48,119 --> 00:00:46,090
what's left in modern biology and drill

16
00:00:49,560 --> 00:00:48,129
back as far as we can into evolutionary

17
00:00:52,470 --> 00:00:49,570
history with that and it turns out

18
00:00:55,319 --> 00:00:52,480
luckily we can drill back pretty far so

19
00:00:56,849 --> 00:00:55,329
you guys are already generally familiar

20
00:00:59,880 --> 00:00:56,859
with the ribosome you about to become

21
00:01:01,319 --> 00:00:59,890
intimately familiar with the ribosome so

22
00:01:05,009 --> 00:01:01,329
you guys have already seen the central

23
00:01:07,050 --> 00:01:05,019
dogma this is where the ribosome fits in

24
00:01:10,080 --> 00:01:07,060
here so the ribosome is what turns RNA

25
00:01:13,310 --> 00:01:10,090
into protein it is in terms of

26
00:01:17,969 --> 00:01:13,320
biomolecules huge it's one mega dalton

27
00:01:19,920 --> 00:01:17,979
1000 1,000,000 Dalton's it is as I

28
00:01:22,080 --> 00:01:19,930
already mentioned earlier probably well

29
00:01:23,999 --> 00:01:22,090
established maybe not in its full modern

30
00:01:25,139 --> 00:01:24,009
form but in a pretty advanced forum at

31
00:01:29,370 --> 00:01:25,149
the time of the last Universal common

32
00:01:31,889 --> 00:01:29,380
ancestor this is generally how it works

33
00:01:35,010 --> 00:01:31,899
in a sort of schematic way where it

34
00:01:38,100 --> 00:01:35,020
reads the messenger RNA and these tRNA

35
00:01:41,609 --> 00:01:38,110
serve as adapter molecules that turn

36
00:01:46,380 --> 00:01:41,619
that code into the specific amino acid

37
00:01:50,700 --> 00:01:46,390
that that code codes for and then you

38
00:01:53,279 --> 00:01:50,710

make a protein note of that this is what

39
00:01:55,230 --> 00:01:53,289
it looks like in more depth so here we

40
00:01:58,080 --> 00:01:55,240
have separated out the small subunit

41
00:02:02,179 --> 00:01:58,090
which is responsible for binding the

42
00:02:05,310 --> 00:02:02,189
messenger RNA and it is comprised of

43
00:02:08,460 --> 00:02:05,320
what's called the 16s ribosomal RNA and

44
00:02:09,840 --> 00:02:08,470
about 20 ribosomal proteins but I don't

45
00:02:11,100 --> 00:02:09,850
care about that this morning I'm going

46
00:02:13,949 --> 00:02:11,110
to talk to you solely about the large

47
00:02:15,990 --> 00:02:13,959
subunit which is responsible for the

48
00:02:17,670 --> 00:02:16,000
peptide bond formation so it's pretty

49
00:02:19,190 --> 00:02:17,680
widely accepted that the small subunit

50
00:02:21,380 --> 00:02:19,200
came later

51
00:02:22,850 --> 00:02:21,390
Lucien so we're trying to go back as far

52
00:02:25,460 --> 00:02:22,860
as we can so we're just going to look at

53
00:02:29,479 --> 00:02:25,470
the large subunit so it's where the

54
00:02:33,800 --> 00:02:29,489
actual chemistry happens so the 23s and

55
00:02:36,140 --> 00:02:33,810
5s ribosomal RNAs are the RNA components

56
00:02:37,699 --> 00:02:36,150
of the large subunit again I'm not going

57
00:02:38,960 --> 00:02:37,709
to talk about the 5s because it's much

58
00:02:42,410 --> 00:02:38,970
later we're going to talk solely about

59
00:02:43,729 --> 00:02:42,420
the 23s RNA and a subset of these 30

60
00:02:47,839 --> 00:02:43,739
proteins so when you're looking at this

61
00:02:49,220 --> 00:02:47,849
this here's the 5s RNA I will generally

62
00:02:51,020 --> 00:02:49,230
tell you today that if something looks

63
00:02:53,839 --> 00:02:51,030

like it's on the exterior it's probably

64
00:02:55,220 --> 00:02:53,849
newer so the 5s RNA can see is right out

65
00:02:58,009 --> 00:02:55,230
of here on the outside probably a lot

66
00:03:00,289 --> 00:02:58,019
newer addition to the ribosome and then

67
00:03:02,839 --> 00:03:00,299
these beige things here that look like

68
00:03:05,210 --> 00:03:02,849
hela sees those are the right the 23s

69
00:03:06,830 --> 00:03:05,220
and the 16s ribosomal RNAs these purple

70
00:03:08,120 --> 00:03:06,840
guys that are sort of stuck in all over

71
00:03:10,550 --> 00:03:08,130
the surface those are the ribosomal

72
00:03:12,550 --> 00:03:10,560
proteins so this in particular is a

73
00:03:18,319 --> 00:03:12,560
bacterial ribosome you care what

74
00:03:21,770 --> 00:03:18,329
ribosomes are even bigger so what we

75
00:03:24,650 --> 00:03:21,780
wanted to do was try and resurrect an

76
00:03:26,270 --> 00:03:24,660
ancestral version of the ribosome so

77
00:03:28,640 --> 00:03:26,280
what we call it was the ancestral

78
00:03:31,550 --> 00:03:28,650
peptidyl transferase center a ptc for

79
00:03:33,050 --> 00:03:31,560
short and basically because that's what

80
00:03:34,640 --> 00:03:33,060
we wanted to do we want it to be able to

81
00:03:37,520 --> 00:03:34,650
make peptide bonds to do the peptidyl

82
00:03:39,770 --> 00:03:37,530
transferase reaction but in a much more

83
00:03:42,470 --> 00:03:39,780
simplified way than we have today so

84
00:03:45,289 --> 00:03:42,480
included in our model are the ancestral

85
00:03:47,720 --> 00:03:45,299
ribosomal RNA it's a very stripped down

86
00:03:49,970 --> 00:03:47,730
version of the 23s RNA which i'll show

87
00:03:52,610 --> 00:03:49,980
you on the next slide and it's stitched

88
00:03:54,140 --> 00:03:52,620

together with these tetra loops which

89
00:03:55,340 --> 00:03:54,150
all you need to know about those is that

90
00:03:57,890 --> 00:03:55,350
they're very stable we know that they

91
00:03:59,569 --> 00:03:57,900
fold in a very predictable way so

92
00:04:02,890 --> 00:03:59,579
they're kind of anywhere that we made a

93
00:04:04,879 --> 00:04:02,900
cut in the RNA we put those in and five

94
00:04:06,920 --> 00:04:04,889
ribosomal peptides that are highly

95
00:04:09,110 --> 00:04:06,930
conserved and penetrate very deeply down

96
00:04:12,170 --> 00:04:09,120
into the core of the ribosome those are

97
00:04:14,629 --> 00:04:12,180
derived from ribosomal proteins large

98
00:04:17,300 --> 00:04:14,639
subunit ribosome proteins l2 l3 l4 l5

99
00:04:18,920 --> 00:04:17,310
teen and l 22 all you really need to

100
00:04:20,449 --> 00:04:18,930
know about those is that there's a bunch

101
00:04:24,500 --> 00:04:20,459
of them this is just a few of them and

102
00:04:26,180 --> 00:04:24,510
that the L stands for large subunit so

103
00:04:29,330 --> 00:04:26,190
in this case in the background here you

104
00:04:30,830 --> 00:04:29,340
can see the entire 23s ribosomal RNA for

105
00:04:32,450 --> 00:04:30,840
comparison so you can see how much we

106
00:04:34,189 --> 00:04:32,460
really have stripped away

107
00:04:39,170 --> 00:04:34,199
in this really it's about eighty percent

108
00:04:40,670 --> 00:04:39,180
of the RNA and so these are the co

109
00:04:42,020 --> 00:04:40,680
crystallized logins from this crystal

110
00:04:43,640 --> 00:04:42,030
structure that just give you an idea of

111
00:04:46,339 --> 00:04:43,650
where in this beast the actual chemistry

112
00:04:50,480 --> 00:04:46,349
happens so it's right down in here right

113
00:04:53,390 --> 00:04:50,490

in the center so this is what the

114
00:04:54,920 --> 00:04:53,400
secondary structure of the 23s ribosomal

115
00:04:58,370 --> 00:04:54,930
RNA looks like secondary structure

116
00:05:00,379 --> 00:04:58,380
rather than this here which is a 3d

117
00:05:03,230 --> 00:05:00,389
structure is basically just laid flat

118
00:05:06,529 --> 00:05:03,240
for simplicity's sake so anywhere that

119
00:05:07,730 --> 00:05:06,539
there's a sort of two lines close to

120
00:05:09,439 --> 00:05:07,740
each other that means that there's base

121
00:05:12,529 --> 00:05:09,449
pairing going in between going between

122
00:05:14,990 --> 00:05:12,539
those two strands of the RNA so in the

123
00:05:18,200 --> 00:05:15,000
dash line here in the back that's the

124
00:05:21,110 --> 00:05:18,210
entire 23s ribosomal RNA in the black is

125
00:05:22,610 --> 00:05:21,120
in the sort of glue is what we kept so

126
00:05:24,499 --> 00:05:22,620
you can see again we'd stripped a lot of

127
00:05:27,110 --> 00:05:24,509
this away we made actually made this

128
00:05:29,330 --> 00:05:27,120
part in the lab which is it represents

129
00:05:33,770 --> 00:05:29,340
about twenty percent of a bacterial

130
00:05:35,629 --> 00:05:33,780
large subunit RNA and made it with some

131
00:05:37,270 --> 00:05:35,639
nice pcr techniques that I wish I had

132
00:05:40,790 --> 00:05:37,280
the time tell you about but I don't and

133
00:05:42,560 --> 00:05:40,800
these ribosomes peptides which I can

134
00:05:44,420 --> 00:05:42,570
tell you about more in a couple slides

135
00:05:47,570 --> 00:05:44,430
but first we need to tell you about my

136
00:05:50,510 --> 00:05:47,580
methods so a main method that I use is

137
00:05:51,589 --> 00:05:50,520
called shape and that stands actually

138
00:05:53,540 --> 00:05:51,599

Jessica showed some of this yesterday

139
00:05:55,520 --> 00:05:53,550
but it stands for selectively two prime

140
00:05:57,589 --> 00:05:55,530
hydroxyl isolation analyzed by primer

141
00:06:01,520 --> 00:05:57,599
extension astrobiologist and their

142
00:06:03,529 --> 00:06:01,530
acronyms so the way that it works in a

143
00:06:06,050 --> 00:06:03,539
very basic way is you have a chemical

144
00:06:09,140 --> 00:06:06,060
that selectively modifies your RNA at

145
00:06:11,120 --> 00:06:09,150
positions where it is flexible ie not

146
00:06:14,540 --> 00:06:11,130
base paired so what you get in the end

147
00:06:18,110 --> 00:06:14,550
is a readout of the places in your RNA

148
00:06:22,040 --> 00:06:18,120
that are that are not based paired and

149
00:06:26,180 --> 00:06:22,050
then from there we do reverse

150
00:06:29,060 --> 00:06:26,190
transcription into you just using an

151
00:06:31,730 --> 00:06:29,070
enzyme we hijack biology basically to

152
00:06:33,290 --> 00:06:31,740
make the complementary DNA that stops

153
00:06:35,810 --> 00:06:33,300
when it hits one of these modifications

154
00:06:38,839 --> 00:06:35,820
so what we're left with is a library of

155
00:06:41,089 --> 00:06:38,849
pieces of DNA that represent the places

156
00:06:43,579 --> 00:06:41,099
that are flexible in the corresponding

157
00:06:45,500 --> 00:06:43,589
RNA we run that on capillary

158
00:06:47,150 --> 00:06:45,510
electrophoresis which if you know any

159
00:06:50,690 --> 00:06:47,160
with gel electrophoresis is we act the

160
00:06:53,420 --> 00:06:50,700
same but its run in a capillary and from

161
00:06:55,370 --> 00:06:53,430
there we can process that data should we

162
00:06:57,410 --> 00:06:55,380
use in-house MATLAB script so we've

163
00:06:59,300 --> 00:06:57,420

developed to get a single nucleotide

164
00:07:04,700 --> 00:06:59,310
readout of the flexibility of that piece

165
00:07:07,460 --> 00:07:04,710
of RNA so we did that on this ancestral

166
00:07:09,920 --> 00:07:07,470
piece of ribosomal RNA because we wanted

167
00:07:11,180 --> 00:07:09,930
to see basically if regardless of the

168
00:07:13,250 --> 00:07:11,190
fact that we stripped away about eighty

169
00:07:17,120 --> 00:07:13,260
percent of the RNA if it still folds in

170
00:07:19,970 --> 00:07:17,130
a similar way and it does what you can

171
00:07:21,710 --> 00:07:19,980
see here is that anywhere that there's

172
00:07:24,320 --> 00:07:21,720
these triangles that means that it is

173
00:07:25,520 --> 00:07:24,330
flexible and those triangles line up

174
00:07:27,290 --> 00:07:25,530
really well with the single-stranded

175
00:07:30,920 --> 00:07:27,300
regions which we expect to be flexible

176
00:07:33,500 --> 00:07:30,930
so we were very happy about that and in

177
00:07:35,720 --> 00:07:33,510
this case I want to highlight that this

178
00:07:38,660 --> 00:07:35,730
was done just with the RNA on its own

179
00:07:40,340 --> 00:07:38,670
and some monovalent cations which just

180
00:07:42,890 --> 00:07:40,350
help to neutralize the backbone so that

181
00:07:44,900 --> 00:07:42,900
you get secondary structure formation

182
00:07:48,110 --> 00:07:44,910
you get the Gila sees actually able to

183
00:07:50,660 --> 00:07:48,120
form so no divalent cations or no none

184
00:07:52,670 --> 00:07:50,670
of the ribosome peptides we did have

185
00:07:54,650 --> 00:07:52,680
some hints early on that certain parts

186
00:07:56,540 --> 00:07:54,660
of the secondary structure that we were

187
00:07:58,820 --> 00:07:56,550
working with were not great this is

188
00:08:01,160 --> 00:07:58,830

going to be a little bit of an aside but

189
00:08:03,500 --> 00:08:01,170
for instance this region here I would

190
00:08:05,030 --> 00:08:03,510
have expected these nucleotides to be

191
00:08:06,950 --> 00:08:05,040
reactive but they were not but when I

192
00:08:08,990 --> 00:08:06,960
went back and looked at the actual 3d

193
00:08:13,070 --> 00:08:09,000
crystal structure it looked a little bit

194
00:08:16,010 --> 00:08:13,080
more like this where my data lines up a

195
00:08:20,930 --> 00:08:16,020
lot better and I was happy again so we

196
00:08:22,580 --> 00:08:20,940
had hints early on the 23s secondary

197
00:08:25,370 --> 00:08:22,590
structure which we derived this

198
00:08:27,920 --> 00:08:25,380
representation from was not perfect and

199
00:08:30,620 --> 00:08:27,930
so some guys in our lab decided to take

200
00:08:34,219 --> 00:08:30,630
that and run with it and rewrite the

201
00:08:35,390 --> 00:08:34,229
ribosome bible essentially so that big

202
00:08:39,260 --> 00:08:35,400
secondary structure i showed you the

203
00:08:41,089 --> 00:08:39,270
beginning is largely the way that it

204
00:08:44,180 --> 00:08:41,099
looks because of historical purposes and

205
00:08:47,000 --> 00:08:44,190
as young scientists we all know how much

206
00:08:49,670 --> 00:08:47,010
we hate that sort of stuff so in this

207
00:08:51,950 --> 00:08:49,680
case they basically redrew the map so

208
00:08:53,690 --> 00:08:51,960
that it looked so that it didn't have a

209
00:08:55,400 --> 00:08:53,700
gap between the two halves for starters

210
00:08:57,290 --> 00:08:55,410
and so that it represented more

211
00:08:58,880 --> 00:08:57,300
accurately the base pairing that's seen

212
00:08:59,480 --> 00:08:58,890
in the crystal structure so in

213
00:09:02,360 --> 00:08:59,490

particular

214
00:09:04,519 --> 00:09:02,370
it's all just brought together and this

215
00:09:06,019 --> 00:09:04,529
domain here which we call domain 0

216
00:09:10,340 --> 00:09:06,029
because they'd already named the others

217
00:09:14,510 --> 00:09:10,350
1 through 6 little facetious maybe but

218
00:09:16,250 --> 00:09:14,520
so is really makes up the core of the

219
00:09:20,210 --> 00:09:16,260
ribosome so what you're seeing here in

220
00:09:24,010 --> 00:09:20,220
blue is the ancestral ribosomal RNA just

221
00:09:30,260 --> 00:09:27,650
so going back to the ribosomal protein /

222
00:09:32,420 --> 00:09:30,270
peptides this is what the ribosomal

223
00:09:34,100 --> 00:09:32,430
proteins in general look like they have

224
00:09:37,220 --> 00:09:34,110
these big globular domains and these

225
00:09:38,780 --> 00:09:37,230
long fingers that have sort of really no

226
00:09:43,190 --> 00:09:38,790
structure that penetrate down deeply

227
00:09:45,470 --> 00:09:43,200
into the ribosome they vary in size

228
00:09:48,190 --> 00:09:45,480
depending on how far they penetrate and

229
00:09:52,400 --> 00:09:48,200
how much of it actually penetrates from

230
00:09:54,170 --> 00:09:52,410
18 amino acids to 45 this is what they

231
00:09:57,550 --> 00:09:54,180
look like if we look at the crystal

232
00:10:01,280 --> 00:09:57,560
structure of the in social ribosomal RNA

233
00:10:04,069 --> 00:10:01,290
with these individual peptides so you

234
00:10:05,480 --> 00:10:04,079
can see that they're kind of peppered

235
00:10:07,010 --> 00:10:05,490
around in some cases they get pretty

236
00:10:08,930 --> 00:10:07,020
close to the very middle which is where

237
00:10:10,480 --> 00:10:08,940
the chemistry happens but for the most

238
00:10:13,040 --> 00:10:10,490

part what these are here for is to

239
00:10:15,160 --> 00:10:13,050
stabilize the background the backbone so

240
00:10:17,540 --> 00:10:15,170
that you can get these negative charged

241
00:10:20,060 --> 00:10:17,550
are pieces of RNA coming closely

242
00:10:22,630 --> 00:10:20,070
together so previously we've been able

243
00:10:26,199 --> 00:10:22,640
to show that four of those five

244
00:10:29,660 --> 00:10:26,209
ribosomal peptides do actually bind our

245
00:10:31,340 --> 00:10:29,670
ribosomal RNA I don't have time to go

246
00:10:33,470 --> 00:10:31,350
into this but if you'd like to discuss

247
00:10:35,180 --> 00:10:33,480
it later I'd be happy to is this is a

248
00:10:41,090 --> 00:10:35,190
bunch of experiments that I didn't do

249
00:10:43,910 --> 00:10:41,100
but the only one that didn't was l2 but

250
00:10:45,949 --> 00:10:43,920
we didn't know if it was binding in the

251
00:10:47,720 --> 00:10:45,959
exact place that we wanted it to so

252
00:10:49,550 --> 00:10:47,730
that's where my research came in and I

253
00:10:51,230 --> 00:10:49,560
went through and used the crystal

254
00:10:53,569 --> 00:10:51,240
structure to say hey where's where these

255
00:10:55,970 --> 00:10:53,579
things actually are where do we want

256
00:10:57,260 --> 00:10:55,980
them to bind and in the secondary

257
00:10:59,690 --> 00:10:57,270
structure it looks something like this

258
00:11:01,400 --> 00:10:59,700
just in the different colors are the

259
00:11:03,590 --> 00:11:01,410
different locations that we might expect

260
00:11:07,760 --> 00:11:03,600
to see contacts if they're binding at

261
00:11:11,090 --> 00:11:07,770
the expected places this is what my raw

262
00:11:13,369 --> 00:11:11,100
shape data looks at looks like I'm not

263
00:11:16,219 --> 00:11:13,379

going to try and help you guys in

264
00:11:17,929 --> 00:11:16,229
too much except to know that anywhere so

265
00:11:19,279 --> 00:11:17,939
that any location with an error here

266
00:11:22,639 --> 00:11:19,289
that you kind of see a peak that

267
00:11:24,979 --> 00:11:22,649
increases or decreases so there's some

268
00:11:26,989 --> 00:11:24,989
that that appear as we increase the

269
00:11:30,139 --> 00:11:26,999
amount of these peptides in this case

270
00:11:32,029 --> 00:11:30,149
it's the l4 peptide that means that

271
00:11:35,689 --> 00:11:32,039
there is a change in the structure at

272
00:11:37,249 --> 00:11:35,699
that location you know it's a local in a

273
00:11:39,259 --> 00:11:37,259
lot of cases just a single nucleotide

274
00:11:41,179 --> 00:11:39,269
that's maybe becoming less flexible so

275
00:11:45,109 --> 00:11:41,189
maybe more lockdown or becoming more

276
00:11:47,869 --> 00:11:45,119
flexible and floppy when so far I've

277
00:11:50,299 --> 00:11:47,879
done l4 and I'll 22 peptides and when I

278
00:11:51,769 --> 00:11:50,309
compare where those actual changes

279
00:11:53,539 --> 00:11:51,779
happen this is in a very rough way I

280
00:11:56,109 --> 00:11:53,549
haven't fully processes data yet because

281
00:11:58,460 --> 00:11:56,119
it's kind of a nightmare to process but

282
00:12:00,559 --> 00:11:58,470
if I sort of do a really rough

283
00:12:04,639 --> 00:12:00,569
estimation these are the positions that

284
00:12:08,149 --> 00:12:04,649
I see some of the biggest changes at and

285
00:12:10,219 --> 00:12:08,159
so for the l4 in particular let's say

286
00:12:12,589 --> 00:12:10,229
this is one of the places that i do see

287
00:12:14,960 --> 00:12:12,599
a change and that is one of the places

288
00:12:16,579 --> 00:12:14,970

that i expect to sorry down here that i

289
00:12:18,919 --> 00:12:16,589
expect to see a change so very close

290
00:12:22,309 --> 00:12:18,929
which makes me very happy and again

291
00:12:25,129 --> 00:12:22,319
right here there's some not too far away

292
00:12:27,049 --> 00:12:25,139
that i expected to have a change in in

293
00:12:30,019 --> 00:12:27,059
their structures so what i can say right

294
00:12:33,379 --> 00:12:30,029
now is that both the l4 and l5 tides do

295
00:12:36,199 --> 00:12:33,389
induce modest changes in the second in

296
00:12:38,090 --> 00:12:36,209
the structure and in some cases at

297
00:12:41,449 --> 00:12:38,100
locations near where we expected them to

298
00:12:44,479 --> 00:12:41,459
and l3 alone I didn't show because it's

299
00:12:46,189 --> 00:12:44,489
boring it doesn't seem to bind at least

300
00:12:48,319 --> 00:12:46,199
in a specific way so it may still bind

301
00:12:50,419 --> 00:12:48,329
but just not the exact way that we

302
00:12:51,679 --> 00:12:50,429
intended it may be that it's dependent

303
00:12:54,229 --> 00:12:51,689
on having one of those other peptides

304
00:12:56,119 --> 00:12:54,239
there first so that you know you need to

305
00:12:58,909 --> 00:12:56,129
have one so that the other one can kind

306
00:13:01,129 --> 00:12:58,919
of cooperatively come in and in bind and

307
00:13:03,019 --> 00:13:01,139
in the future we're going to start doing

308
00:13:05,989 --> 00:13:03,029
these in combinations so that we can get

309
00:13:07,579 --> 00:13:05,999
an idea if there's an actual order of

310
00:13:08,989 --> 00:13:07,589
assembly and that sort of thing and also

311
00:13:11,779 --> 00:13:08,999
start adding back in stuff like

312
00:13:15,579 --> 00:13:11,789
magnesium which is also very important

313
00:13:18,139 --> 00:13:15,589

in forming RNA large RNA structures and

314
00:13:19,689 --> 00:13:18,149
that's everything I had these are all

315
00:13:22,849 --> 00:13:19,699
the people that helped with my work and

316
00:13:25,129 --> 00:13:22,859
once again thank NASA for funding us and

317
00:13:28,879 --> 00:13:25,139
for finding this conference in a large

318
00:13:30,349 --> 00:13:28,889
part and also the Center for ribosomal

319
00:13:43,639 --> 00:13:30,359
origins and evolution I will happily

320
00:13:50,349 --> 00:13:43,649
take any questions Bradley I see you

321
00:13:53,960 --> 00:13:50,359
have a question it's better be good oh

322
00:13:56,840 --> 00:13:53,970
it's awesome all right so you mentioned

323
00:13:59,389 --> 00:13:56,850
your future studies doing magnesium

324
00:14:02,269 --> 00:13:59,399
adding it there yeah you see where I'm

325
00:14:03,769 --> 00:14:02,279
going any temptation to try it with iron

326
00:14:07,069 --> 00:14:03,779
since I know your labs have the

327
00:14:09,319 --> 00:14:07,079
capability yes yeah that's definitely a

328
00:14:13,299 --> 00:14:09,329
down the road sort of thing too yeah

329
00:14:16,429 --> 00:14:13,309
because because iron has been shown to

330
00:14:18,710 --> 00:14:16,439
really mimic magnesium in terms of the

331
00:14:21,019 --> 00:14:18,720
way that it interacts with RNA so yes

332
00:14:22,699 --> 00:14:21,029
for certain I would like to do that part

333
00:14:25,099 --> 00:14:22,709
of it is just instrumentation issues we

334
00:14:26,689 --> 00:14:25,109
are in the process of getting an

335
00:14:30,019 --> 00:14:26,699
anaerobic chamber that will allow us to

336
00:14:32,359 --> 00:14:30,029
do it but we've got it from somebody

337
00:14:35,479 --> 00:14:32,369
else who cut it in half and tried to put

338
00:14:36,649 --> 00:14:35,489

it back together and so it leaks so

339
00:14:38,149 --> 00:14:36,659
we're getting the manufacturers to fix

340
00:14:39,649 --> 00:14:38,159
it but once we have that we should be

341
00:14:41,629 --> 00:14:39,659
able to do all kinds more interesting