
AbSciCon
2019

1
00:00:00,790 --> 00:00:07,320
[Music]

2
00:00:12,180 --> 00:00:09,500
[Applause]

3
00:00:15,090 --> 00:00:12,190
absolutely fantastic system and it

4
00:00:18,359 --> 00:00:15,100
really lets us get some clues about

5
00:00:19,530 --> 00:00:18,369
evolution before Luca but I'd just like

6
00:00:22,080 --> 00:00:19,540
to let you know it's not the only game

7
00:00:24,420 --> 00:00:22,090
in town there are there RNAs that we can

8
00:00:26,370 --> 00:00:24,430
look at that were present in Luca not

9
00:00:29,279 --> 00:00:26,380
very many but one of them is is a

10
00:00:31,350 --> 00:00:29,289
nucleus and for those of you who know a

11
00:00:32,970 --> 00:00:31,360
lot about RNA you priority guess that

12
00:00:36,390 --> 00:00:32,980
I'm talking about ribonuclease P

13
00:00:38,400 --> 00:00:36,400

ribonuclease P is a nearly universal

14
00:00:40,080 --> 00:00:38,410
ribozyme as say there are a few

15
00:00:41,310 --> 00:00:40,090
organisms scattered here and there

16
00:00:43,740 --> 00:00:41,320
around the tree of life that have

17
00:00:45,840 --> 00:00:43,750
dispensed with the RNA component of RNA

18
00:00:50,400 --> 00:00:45,850
SP but by and large pretty much

19
00:00:53,490 --> 00:00:50,410
everything uses an rnase p RNA to

20
00:00:56,970 --> 00:00:53,500
process transfer RNAs so the main thing

21
00:00:58,470 --> 00:00:56,980
RNA HP does is it takes a pre T RNA cuts

22
00:01:00,780 --> 00:00:58,480
off sequence from the five prime end and

23
00:01:02,610 --> 00:01:00,790
gives you a maturity RNA they can go on

24
00:01:05,670 --> 00:01:02,620
and do its job with the ribosome and

25
00:01:09,859 --> 00:01:05,680
make proteins okay alright so that's

26
00:01:12,090 --> 00:01:09,869
what it does as I said it is present in

27
00:01:13,560 --> 00:01:12,100
all three domains of life here are

28
00:01:17,100 --> 00:01:13,570
examples from bacteria archaea and

29
00:01:19,740 --> 00:01:17,110
eukaryotes and as you can see they all

30
00:01:21,030 --> 00:01:19,750
have they have different secondary

31
00:01:23,100 --> 00:01:21,040
structures but there are certain

32
00:01:24,929 --> 00:01:23,110
elements that are shared between all

33
00:01:26,640 --> 00:01:24,939
three of them and so they have certain

34
00:01:28,020 --> 00:01:26,650
sequence elements in common they have

35
00:01:30,780 --> 00:01:28,030
certain structural elements in common

36
00:01:33,149 --> 00:01:30,790
and they're they're widely dispersed

37
00:01:36,690 --> 00:01:33,159
across all three domains so it's quite

38
00:01:39,030 --> 00:01:36,700

clear that this was present in Luca and

39
00:01:40,770 --> 00:01:39,040
in fact one other interest thinking

40
00:01:43,440 --> 00:01:40,780
about RNA SP is that it's a ribozyme

41
00:01:47,730 --> 00:01:43,450
which there are you know not that many

42
00:01:50,340 --> 00:01:47,740
of and it's it's true in biology RNA SP

43
00:01:53,100 --> 00:01:50,350
has always has at least one protein

44
00:01:55,520 --> 00:01:53,110
component but it's not necessary for

45
00:01:57,630 --> 00:01:55,530
catalysis so this is a ribozyme and

46
00:01:59,940 --> 00:01:57,640
because it doesn't mean it's protein and

47
00:02:02,999 --> 00:01:59,950
it's universally conserved and it was in

48
00:02:04,649 --> 00:02:03,009
Luke I like to point out that I wouldn't

49
00:02:06,810 --> 00:02:04,659
say that RNA SP is older than ribosome

50
00:02:09,180 --> 00:02:06,820
but it is something that could be older

51
00:02:11,460 --> 00:02:09,190
than the ribosome and it's not possibly

52
00:02:13,229 --> 00:02:11,470
the only other system where we could

53
00:02:15,210 --> 00:02:13,239
have a to follow a continuous

54
00:02:19,470 --> 00:02:15,220
evolutionary track from before the

55
00:02:22,080 --> 00:02:19,480
ribosome so now okay so it's pretty cool

56
00:02:22,480 --> 00:02:22,090
we should learn more about it so they're

57
00:02:25,150 --> 00:02:22,490
a bunch of

58
00:02:27,220 --> 00:02:25,160
and last session about the accretion

59
00:02:29,800 --> 00:02:27,230
model so I'm going to tune in details

60
00:02:32,310 --> 00:02:29,810
but some model developed by my largely

61
00:02:36,370 --> 00:02:32,320
by my collaborators at Georgia Tech and

62
00:02:38,860 --> 00:02:36,380
it explains ribosome evolution and I

63
00:02:41,620 --> 00:02:38,870

just there are sort of three key

64
00:02:43,240 --> 00:02:41,630
features as far as I can tell from from

65
00:02:45,670 --> 00:02:43,250
you know talking to them and reading

66
00:02:47,460 --> 00:02:45,680
their papers and that is that one of the

67
00:02:51,460 --> 00:02:47,470
key features of this model is that

68
00:02:53,920 --> 00:02:51,470
structural RNA is if you look at an RNA

69
00:02:57,340 --> 00:02:53,930
structure you can see a record of where

70
00:02:58,540 --> 00:02:57,350
elements were inserted into it that if

71
00:03:00,310 --> 00:02:58,550
you look at a three-dimensional

72
00:03:02,080 --> 00:03:00,320
structure you can also get information

73
00:03:06,550 --> 00:03:02,090
about the order in which elements were

74
00:03:09,730 --> 00:03:06,560
added and that these RNAs generally grow

75
00:03:13,630 --> 00:03:09,740
from a common core and so that's the

76
00:03:17,020 --> 00:03:13,640
model with the ribosome so why not apply

77
00:03:18,310 --> 00:03:17,030
to RNA SP the this model relies on

78
00:03:20,380 --> 00:03:18,320
three-dimensional structures from

79
00:03:22,600 --> 00:03:20,390
different domains of life and now for

80
00:03:25,480 --> 00:03:22,610
RNA SP as of about a week ago we have

81
00:03:28,870 --> 00:03:25,490
bacterial eukaryote and this just came

82
00:03:31,600 --> 00:03:28,880
out our kale rnase p structure and so we

83
00:03:32,860 --> 00:03:31,610
have a high-resolution three-dimensional

84
00:03:35,950 --> 00:03:32,870
structural information for all three

85
00:03:37,330 --> 00:03:35,960
domains of life and so I just want to go

86
00:03:39,220 --> 00:03:37,340
through a couple of examples of some of

87
00:03:42,070 --> 00:03:39,230
the the features the accretion model as

88
00:03:44,050 --> 00:03:42,080

they show up in RNA speak so this is P

89
00:03:47,140 --> 00:03:44,060
18 and it's one of the one of the HeLa

90
00:03:49,780 --> 00:03:47,150
C's in RNA SP from from bacterial

91
00:03:51,430 --> 00:03:49,790
structure and what I'm showing is what

92
00:03:53,860 --> 00:03:51,440
we consider an insertion site that is a

93
00:03:56,740 --> 00:03:53,870
site where it looks like you could have

94
00:03:58,750 --> 00:03:56,750
put an element into the RNA structure

95
00:04:00,070 --> 00:03:58,760
without disrupting the rest of the

96
00:04:02,230 --> 00:04:00,080
structure and that is to say this this

97
00:04:03,910 --> 00:04:02,240
part that's shown in cyan we think was

98
00:04:06,940 --> 00:04:03,920
dropped into the part that's shown in

99
00:04:08,410 --> 00:04:06,950
blue and and you might think okay yeah

100
00:04:10,540 --> 00:04:08,420
well the backbone it just it comes

101
00:04:11,740 --> 00:04:10,550
together just because the backbone is

102
00:04:13,390 --> 00:04:11,750
coming close together doesn't mean that

103
00:04:15,190 --> 00:04:13,400
it used to be connected but if we

104
00:04:18,130 --> 00:04:15,200
overlay it with the RK lar an ASP

105
00:04:19,810 --> 00:04:18,140
structure then we see that you know you

106
00:04:22,120 --> 00:04:19,820
basically have the same structure - this

107
00:04:24,370 --> 00:04:22,130
p 18 and of course the other thing about

108
00:04:25,750 --> 00:04:24,380
that it there are other reasons we kind

109
00:04:28,090 --> 00:04:25,760
of know this element is probably a later

110
00:04:30,490 --> 00:04:28,100
edition it's not in eukaryotes or

111
00:04:32,510 --> 00:04:30,500
archaea and it's not even known all in

112
00:04:34,129 --> 00:04:32,520
all bacteria so

113
00:04:35,839 --> 00:04:34,139

but at any rate we can see some of those

114
00:04:37,820 --> 00:04:35,849
features in RNA SP like we see in the

115
00:04:39,529 --> 00:04:37,830
ribosome another thing that we've seen

116
00:04:41,210 --> 00:04:39,539
the ribosome a lot of are these a minor

117
00:04:43,839 --> 00:04:41,220
interactions they're also present in RNA

118
00:04:46,309 --> 00:04:43,849
SP and so what's going on there is that

119
00:04:47,839 --> 00:04:46,319
if you look you can see in blue we have

120
00:04:49,610 --> 00:04:47,849
a double-stranded RNA that

121
00:04:51,260 --> 00:04:49,620
double-stranded RNA can form on its own

122
00:04:53,629 --> 00:04:51,270
it doesn't need the part that's shown in

123
00:04:54,860 --> 00:04:53,639
cyan and in green okay the part that's

124
00:04:56,450 --> 00:04:54,870
shown in green these are a couple of

125
00:04:57,320 --> 00:04:56,460
Dennis teens that are looped out and

126
00:04:58,790 --> 00:04:57,330
they're interacting with the minor

127
00:05:00,920 --> 00:04:58,800
groove of the double-stranded RNA that

128
00:05:02,900 --> 00:05:00,930
structure will not form if the

129
00:05:05,570 --> 00:05:02,910
double-stranded RNA is not there and so

130
00:05:06,890 --> 00:05:05,580
the thinking is that the independent

131
00:05:08,839 --> 00:05:06,900
structure is older than the dependent

132
00:05:10,820 --> 00:05:08,849
structure okay so we have those two

133
00:05:12,920 --> 00:05:10,830
elements we can find insertion sites and

134
00:05:15,770 --> 00:05:12,930
we can you know establish chronology

135
00:05:17,420 --> 00:05:15,780
based on three-dimensional structure so

136
00:05:20,719 --> 00:05:17,430
then if we go on from there and we kind

137
00:05:24,170 --> 00:05:20,729
of build up the structure from a single

138
00:05:26,360 --> 00:05:24,180

core we can kind of have a model of how

139
00:05:27,800 --> 00:05:26,370
our NASPE vault and so what I'm showing

140
00:05:35,210 --> 00:05:27,810
here is RNAs P substrate

141
00:05:37,370 --> 00:05:35,220
it's a tRNA alright so basically this

142
00:05:38,629 --> 00:05:37,380
part right here kind of shown in gray is

143
00:05:39,980 --> 00:05:38,639
the piece that's going to get cut off

144
00:05:41,529 --> 00:05:39,990
there are a couple of metals at the

145
00:05:44,300 --> 00:05:41,539
active site where the cleavage happens

146
00:05:47,810 --> 00:05:44,310
and and we'll just kind of walk through

147
00:05:49,430 --> 00:05:47,820
the evolution of RNA SP so we start to

148
00:05:52,219 --> 00:05:49,440
have a you know different chunks get

149
00:05:55,310 --> 00:05:52,229
added on after the first four pieces are

150
00:05:57,080 --> 00:05:55,320
added we have basically a step we

151
00:05:59,749 --> 00:05:57,090
basically have everything you you you

152
00:06:04,070 --> 00:05:59,759
should need for catalysis and then as

153
00:06:06,379 --> 00:06:04,080
you continue on in the model with you

154
00:06:08,510 --> 00:06:06,389
know through time you add on additional

155
00:06:10,580 --> 00:06:08,520
elements that recognize larger and

156
00:06:12,050 --> 00:06:10,590
larger portions of the tRNA and also

157
00:06:15,200 --> 00:06:12,060
help to stabilize some of the structure

158
00:06:16,700 --> 00:06:15,210
and then we can go beyond Luca with this

159
00:06:18,649 --> 00:06:16,710
particular structure there are a couple

160
00:06:21,350 --> 00:06:18,659
of additional elements that are you know

161
00:06:24,140 --> 00:06:21,360
specific to this lineage okay

162
00:06:29,779 --> 00:06:24,150
so that's the fun movie here's a couple

163
00:06:31,490 --> 00:06:29,789

of the views how am I on time okay okay

164
00:06:34,279 --> 00:06:31,500
oh great please I'm okay so here's a

165
00:06:36,070 --> 00:06:34,289
couple of different views of the of the

166
00:06:38,270 --> 00:06:36,080
model and broken up in different pieces

167
00:06:39,080 --> 00:06:38,280
so what do we do with this now that we

168
00:06:40,519 --> 00:06:39,090
have this model well there a couple

169
00:06:42,140 --> 00:06:40,529
things we can do and one of the first

170
00:06:44,480 --> 00:06:42,150
things I wanted to do is sort of track

171
00:06:46,320 --> 00:06:44,490
how does the interface between RNA SP

172
00:06:49,619 --> 00:06:46,330
and its substrate change over time

173
00:06:51,899 --> 00:06:49,629
so we start here on the left yes

174
00:06:53,339 --> 00:06:51,909
very early on and so some of the first

175
00:06:54,839 --> 00:06:53,349
things that they are contacted over the

176
00:06:57,360 --> 00:06:54,849
cleavage site that's not surprising and

177
00:06:59,010 --> 00:06:57,370
then there's also part of the the tRNA

178
00:07:01,350 --> 00:06:59,020
that's one helical turn away from the

179
00:07:04,290 --> 00:07:01,360
cleavage site and then as we go forward

180
00:07:05,790 --> 00:07:04,300
in history we start to contact more of

181
00:07:09,510 --> 00:07:05,800
the leader sequence the part that we cut

182
00:07:11,999 --> 00:07:09,520
off and then even more of the acceptor

183
00:07:13,589 --> 00:07:12,009
stem then the T stem T loop and then the

184
00:07:14,670 --> 00:07:13,599
last thing that gets contacted is the D

185
00:07:16,350 --> 00:07:14,680
loop and what's kind of interesting

186
00:07:17,790 --> 00:07:16,360
there is that it's not really until you

187
00:07:19,920 --> 00:07:17,800
get to Luca that you start interacting

188
00:07:23,459 --> 00:07:19,930

with parts of the tRNA they're sort of

189
00:07:25,559 --> 00:07:23,469
unique to tRNA I mean really I mean so

190
00:07:27,450 --> 00:07:25,569
so obviously the acceptor stem is at the

191
00:07:30,270 --> 00:07:27,460
ends is unique it has a CCA tail and all

192
00:07:32,850 --> 00:07:30,280
that but it's essentially interacting

193
00:07:35,760 --> 00:07:32,860
with what you might call mini helix up

194
00:07:38,129 --> 00:07:35,770
until Luca potentially so that suggests

195
00:07:42,270 --> 00:07:38,139
that you know maybe the full tier and I

196
00:07:45,209 --> 00:07:42,280
didn't come till later okay so and just

197
00:07:47,330 --> 00:07:45,219
to I don't really have well this is my

198
00:07:49,830 --> 00:07:47,340
last slide and and I'll just say that

199
00:07:52,080 --> 00:07:49,840
we're kind of right in the thick of it

200
00:07:53,879 --> 00:07:52,090
and like figuring out this model and and

201
00:07:55,320 --> 00:07:53,889
what it all means and but one of the

202
00:07:56,820 --> 00:07:55,330
things that occurred to me is that by

203
00:07:59,430 --> 00:07:56,830
tracking the way this interacts with

204
00:08:01,200 --> 00:07:59,440
tRNA we might be able to synchronize the

205
00:08:03,149 --> 00:08:01,210
evolution of RNA SP with the evolution

206
00:08:05,100 --> 00:08:03,159
of the ribosome with the idea that you

207
00:08:08,279 --> 00:08:05,110
know some of the same when that the

208
00:08:10,080 --> 00:08:08,289
ribosome and RNA SP might be contacting

209
00:08:13,740 --> 00:08:10,090
parts of the tRNA at the same time and

210
00:08:16,080 --> 00:08:13,750
so like I said before RNA SP we don't

211
00:08:18,180 --> 00:08:16,090
contact the D loop until very late and

212
00:08:19,559 --> 00:08:18,190
and the ribosome doesn't start to really

213
00:08:21,719 --> 00:08:19,569

interact with the D loop either until

214
00:08:24,029 --> 00:08:21,729
fairly late so that something along

215
00:08:25,920 --> 00:08:24,039
there we've got a lot of work to do to

216
00:08:27,029 --> 00:08:25,930
really flesh out this model and make

217
00:08:30,450 --> 00:08:27,039
sure it's well synchronized with the

218
00:08:31,409 --> 00:08:30,460
ribosome I this is definitely one of

219
00:08:32,880 --> 00:08:31,419
those projects where you know I'm

220
00:08:35,909 --> 00:08:32,890
talking about it as I'm thinking about

221
00:08:37,500 --> 00:08:35,919
it and so we don't have a nicely tied up

222
00:08:40,290 --> 00:08:37,510
package for what it all means but I

223
00:08:41,760 --> 00:08:40,300
think this is a very important system it

224
00:08:43,889 --> 00:08:41,770
has a lot of potential I haven't heard a

225
00:08:46,160 --> 00:08:43,899
lot of talk about it at origin of life

226
00:08:48,540 --> 00:08:46,170
or astrobiology conferences in a while

227
00:08:49,889 --> 00:08:48,550
you know and so I think we need to

228
00:08:52,110 --> 00:08:49,899
revisit it especially now that we have

229
00:08:53,009 --> 00:08:52,120
all the structural information and with

230
00:08:56,040 --> 00:08:53,019
that I just like to thank my

231
00:08:56,860 --> 00:08:56,050
collaborators at Georgia Tech especially

232
00:08:58,360 --> 00:08:56,870
Anton who

233
00:09:00,130 --> 00:08:58,370
I think it was last night or night

234
00:09:04,090 --> 00:09:00,140
before we spent several hours looking at

235
00:09:06,700 --> 00:09:04,100
structures having a lot of fun and this

236
00:09:09,790 --> 00:09:06,710
work was was done I'm at NASA there at

237
00:09:11,710 --> 00:09:09,800
Georgia Tech and I think you for your

238
00:09:23,920 --> 00:09:11,720

attention and I hope we have a little

239
00:09:26,650 --> 00:09:23,930
time for questions is the protein

240
00:09:30,340 --> 00:09:26,660
ancient or does it what can you say

241
00:09:32,890 --> 00:09:30,350
about the protein well you can you can

242
00:09:36,490 --> 00:09:32,900
say that it's it's totally dispensable

243
00:09:37,480 --> 00:09:36,500
it looks pretty it's I mean it's really

244
00:09:39,160 --> 00:09:37,490
supposed to care it's got it's a it's

245
00:09:41,760 --> 00:09:39,170
mixed alpha-helix you some beta sheets I

246
00:09:46,690 --> 00:09:41,770
mean doesn't really match what some of

247
00:09:49,140 --> 00:09:46,700
you know the accretion models or the

248
00:09:52,960 --> 00:09:49,150
Williams Labs model on early proteins

249
00:09:55,420 --> 00:09:52,970
and it it seems to be a lot of what's

250
00:09:55,990 --> 00:09:55,430
involved is is recognizing the the Phi

251
00:09:58,930 --> 00:09:56,000
Prime leader

252
00:10:00,280 --> 00:09:58,940
more than it does help catalysis but

253
00:10:02,050 --> 00:10:00,290
it's primarily sort of a recognition

254
00:10:03,460 --> 00:10:02,060
thing and and I should point out that

255
00:10:05,050 --> 00:10:03,470
the other artis so that's the bacterial

256
00:10:07,390 --> 00:10:05,060
RTSP just has this one protein that's

257
00:10:09,430 --> 00:10:07,400
shown in brown here over the left but

258
00:10:12,190 --> 00:10:09,440
our kale and eukaryotic Arnie's peas

259
00:10:15,340 --> 00:10:12,200
have several more proteins eukaryotes

260
00:10:17,890 --> 00:10:15,350
have up to ten or so and and an half a

261
00:10:19,180 --> 00:10:17,900
dozen for archaea so and you can and

262
00:10:20,950 --> 00:10:19,190
what's kind of interesting is in some

263
00:10:22,990 --> 00:10:20,960

cases you can kind of see deletions that

264
00:10:24,340 --> 00:10:23,000
happen in eukaryotes archaea where you

265
00:10:27,720 --> 00:10:24,350
kind of have a protein that seems to be

266
00:10:30,790 --> 00:10:27,730
coming and and and compensating for or

267
00:10:34,180 --> 00:10:30,800
you know taking the place of an RNA

268
00:10:35,550 --> 00:10:34,190
element anyway cool stuff but I was

269
00:10:38,590 --> 00:10:35,560
taken when he showed the three

270
00:10:39,250 --> 00:10:38,600
ribonuclease Peas two of them one from

271
00:10:42,280 --> 00:10:39,260
Ithaca

272
00:10:46,720 --> 00:10:42,290
called a caucus you know CIN and the

273
00:10:48,820 --> 00:10:46,730
other are thermophilic are is there any

274
00:10:52,000 --> 00:10:48,830
implication from what you're doing that

275
00:10:55,570 --> 00:10:52,010
perhaps the first ribonuclease P was

276
00:10:57,730 --> 00:10:55,580
high-temperature and that whether or not

277
00:11:00,250 --> 00:10:57,740
that might actually fit with some of the

278
00:11:02,500 --> 00:11:00,260
models that indicate that Luca was also

279
00:11:04,270 --> 00:11:02,510
a high-temperature growing well I'll

280
00:11:06,340 --> 00:11:04,280
just say that the examples I showed

281
00:11:07,180 --> 00:11:06,350
where the example were the examples for

282
00:11:08,350 --> 00:11:07,190
which we have three-dimensional

283
00:11:09,470 --> 00:11:08,360
structures and so it really just speaks

284
00:11:11,480 --> 00:11:09,480
the fact that the thermo file

285
00:11:14,600 --> 00:11:11,490
are easier to get structural information

286
00:11:17,600 --> 00:11:14,610
on some of the the RNA is associated

287
00:11:21,530 --> 00:11:17,610
thermophiles tend to be yeah but you

288
00:11:23,750 --> 00:11:21,540

don't have a model that indicates what

289
00:11:27,800 --> 00:11:23,760
the first ribonuclease p would have

290
00:11:29,509 --> 00:11:27,810
looked like well we do I mean as the the

291
00:11:31,129 --> 00:11:29,519
movie we kind of march through and we

292
00:11:32,840 --> 00:11:31,139
haven't done I mean what what's nice

293
00:11:35,540 --> 00:11:32,850
about the system is its fairly small and

294
00:11:36,710 --> 00:11:35,550
we can go and characterize the activity

295
00:11:39,079 --> 00:11:36,720
of some of these contracts so some of

296
00:11:41,360 --> 00:11:39,089
those all those different steps we plan

297
00:11:42,740 --> 00:11:41,370
to make in the lab and they won't be

298
00:11:45,410 --> 00:11:42,750
that difficult to make it's not a very

299
00:11:47,990 --> 00:11:45,420
large RNA and it we know that a lot of

300
00:11:49,400 --> 00:11:48,000
truncated forms of RNA SP are cadelec

301
00:11:50,780 --> 00:11:49,410
the active you can cut off tremendous

302
00:11:52,790 --> 00:11:50,790
amounts of RNA SP and still have

303
00:11:54,860 --> 00:11:52,800
activity so we're very optimistic that

304
00:11:57,050 --> 00:11:54,870
as we build constructs that correspond

305
00:11:58,639 --> 00:11:57,060
to the different steps in the secretion

306
00:12:00,019 --> 00:11:58,649
model that we'll be able to test them

307
00:12:01,819 --> 00:12:00,029
and characterize you know what

308
00:12:04,400 --> 00:12:01,829
temperature do they work best at what pH

309
00:12:08,240 --> 00:12:04,410
what ions how they respond to iron you

310
00:12:09,860 --> 00:12:08,250
know things like that I was really taken

311
00:12:12,949 --> 00:12:09,870
by the fact that do you have

312
00:12:17,509 --> 00:12:12,959
ribonuclease be functional above 90

313
00:12:25,880 --> 00:12:17,519

degrees and very interested in how that