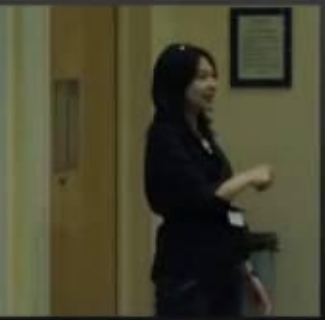

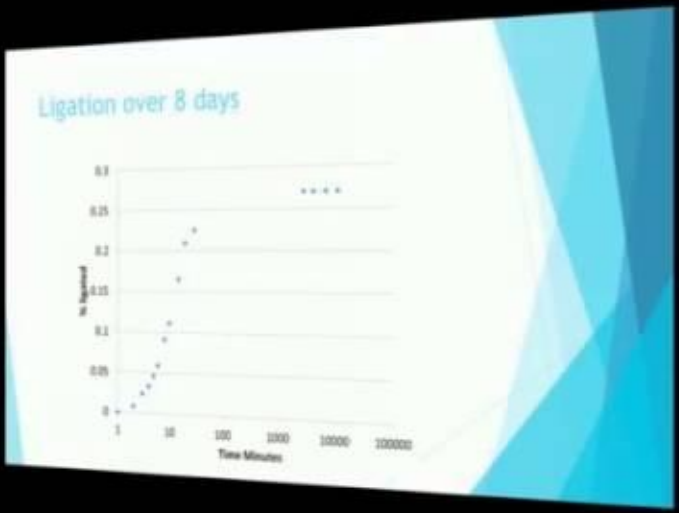
Ligation over 8 days
% ligated
0.3
0.25
0.2
0.15
0.1
0.05
0
1
10
100
1000
10000
100000
Time Minutes

1
00:00:13,129 --> 00:00:10,670
okay so I'll start out with presenting

2
00:00:16,280 --> 00:00:13,139
my goal is to find a small efficient

3
00:00:17,870 --> 00:00:16,290
ligating right design and I hope I know

4
00:00:20,510 --> 00:00:17,880
you guys know what a ribozyme is it's an

5
00:00:23,720 --> 00:00:20,520
RNA that has catalytic function and why

6
00:00:25,519 --> 00:00:23,730
ligation well um you're going to talk

7
00:00:28,220 --> 00:00:25,529
about origin of life studies you want to

8
00:00:30,769 --> 00:00:28,230
try to generate more complex products so

9
00:00:32,569 --> 00:00:30,779
your product should be more complex than

10
00:00:34,280 --> 00:00:32,579
your starting material and that's what

11
00:00:35,810 --> 00:00:34,290
ligation is ligation is taking two

12
00:00:37,910 --> 00:00:35,820
pieces of RNA you're putting them

13
00:00:40,490 --> 00:00:37,920

together you're like a ting them into

14
00:00:42,980 --> 00:00:40,500
one product a more complex product so I

15
00:00:45,050 --> 00:00:42,990
want to increase ligation in a ribozyme

16
00:00:48,470 --> 00:00:45,060
and I want to try using different

17
00:00:50,710 --> 00:00:48,480
conditions particularly by freezing and

18
00:00:52,580 --> 00:00:50,720
then I want to increase that yield by

19
00:00:56,030 --> 00:00:52,590
introducing thermal cycling or

20
00:00:57,560 --> 00:00:56,040
freeze-thaw cycles so I won't go very

21
00:00:59,450 --> 00:00:57,570
much in the background for the RNA world

22
00:01:02,569 --> 00:00:59,460
I I'm gonna see I'm assuming that

23
00:01:06,460 --> 00:01:02,579
everyone know this by now um that this

24
00:01:08,990 --> 00:01:06,470
idea started around the late 1960s and

25
00:01:11,179 --> 00:01:09,000
it suggested that RNA is the first

26
00:01:13,099 --> 00:01:11,189
biomolecule as opposed to DNA or protein

27
00:01:16,160 --> 00:01:13,109
and it's because RNA can store

28
00:01:18,260 --> 00:01:16,170
information and it also has an enzymatic

29
00:01:20,809 --> 00:01:18,270
function but the mage one of the major

30
00:01:22,849 --> 00:01:20,819
problems for RNA is that it's fragile

31
00:01:24,649 --> 00:01:22,859
and if you ask anybody who worked with

32
00:01:26,419 --> 00:01:24,659
RNA they'll tell you it's prone to

33
00:01:28,730 --> 00:01:26,429
degradation so if you heat it it'll

34
00:01:30,940 --> 00:01:28,740
probably degrade and pray if you have

35
00:01:34,459 --> 00:01:30,950
something like magnesium in your sample

36
00:01:37,129 --> 00:01:34,469
um so one solution to that is lowering

37
00:01:39,649 --> 00:01:37,139
your incubation temperature and when you

38
00:01:41,840 --> 00:01:39,659

I guess when a lot of people think about

39
00:01:43,669 --> 00:01:41,850
freezing or ice they think about all the

40
00:01:45,980 --> 00:01:43,679
water molecules and all the solute sin

41
00:01:48,349 --> 00:01:45,990
solid phase well that's true if you have

42
00:01:49,940 --> 00:01:48,359
if you go below this eutectic

43
00:01:52,539 --> 00:01:49,950
temperature on this phase diagram here

44
00:01:54,859 --> 00:01:52,549
what we're interested in is this

45
00:01:57,859 --> 00:01:54,869
triangle area here where the majority of

46
00:02:00,859 --> 00:01:57,869
the water molecule isn't is in an ice

47
00:02:03,949 --> 00:02:00,869
crystal but the remaining liquid water

48
00:02:06,410 --> 00:02:03,959
is mixed with your solute and that makes

49
00:02:08,359 --> 00:02:06,420
up your eutectic composition and this

50
00:02:12,650 --> 00:02:08,369
eutectic composition exists in channels

51
00:02:15,080 --> 00:02:12,660
very small micro meter in width grooves

52
00:02:17,870 --> 00:02:15,090
and it's shown right here so very small

53
00:02:18,559 --> 00:02:17,880
tunnels a nice and this provides a

54
00:02:21,440 --> 00:02:18,569
protective

55
00:02:23,809 --> 00:02:21,450
for your RNA it concentrates your solute

56
00:02:25,369 --> 00:02:23,819
and for your RNA you could also reach

57
00:02:27,369 --> 00:02:25,379
alternative stable conformation that

58
00:02:29,569 --> 00:02:27,379
wouldn't be possible if you tried

59
00:02:32,569 --> 00:02:29,579
incubating at higher temperatures and

60
00:02:35,780 --> 00:02:32,579
and above all your RNA can still retain

61
00:02:39,920 --> 00:02:35,790
some enzymatic function this is

62
00:02:41,899 --> 00:02:39,930
demonstrated by by the r18 polymerase

63
00:02:45,020 --> 00:02:41,909

this this was this is from a paper in

64
00:02:48,009 --> 00:02:45,030
2010 the r18 polymerase is an artificial

65
00:02:51,800 --> 00:02:48,019
ribozyme it's lab-created and it's

66
00:02:55,580 --> 00:02:51,810
outlined in black right here and it

67
00:02:58,129 --> 00:02:55,590
binds to this template in green and it

68
00:03:00,740 --> 00:02:58,139
adds nucleotides one by one to this

69
00:03:02,300 --> 00:03:00,750
primer strand right here so when people

70
00:03:03,979 --> 00:03:02,310
think about polymerase they think about

71
00:03:06,619 --> 00:03:03,989
a polymerase that's made out of protein

72
00:03:08,509 --> 00:03:06,629
and a common one is this t7 RNA

73
00:03:11,809 --> 00:03:08,519
polymerase right here from bacteriophage

74
00:03:14,990 --> 00:03:11,819
and it does wonderfully at 37 degrees so

75
00:03:17,000 --> 00:03:15,000
the higher you see these bands up here

76
00:03:19,759 --> 00:03:17,010
the higher you go the more the longer

77
00:03:21,259 --> 00:03:19,769
the projects are but at negative 7 if

78
00:03:25,749 --> 00:03:21,269
you freeze it you don't see any

79
00:03:29,839 --> 00:03:25,759
polymerization so how does the r18 fair

80
00:03:31,039 --> 00:03:29,849
at 37 it's not shown here at 37 it

81
00:03:33,920 --> 00:03:31,049
doesn't do so well because the

82
00:03:36,080 --> 00:03:33,930
polymerase degrades but if you start at

83
00:03:38,149 --> 00:03:36,090
a slightly lower temperature than a room

84
00:03:41,110 --> 00:03:38,159
temperature it does compare ibly well as

85
00:03:48,110 --> 00:03:41,120
the protein polymerase but the major

86
00:03:51,050 --> 00:03:48,120
difference is at negative 7 so here the

87
00:03:53,749 --> 00:03:51,060
r18 can still catalyze polymerization at

88
00:03:57,860 --> 00:03:53,759

negative 7 below freezing where the

89
00:03:59,629 --> 00:03:57,870
protein equivalent you see no product at

90
00:04:02,689 --> 00:03:59,639
all so this is something that RNA can do

91
00:04:04,939 --> 00:04:02,699
that the protein can't but this plum

92
00:04:07,879 --> 00:04:04,949
race is a little too long I don't it's a

93
00:04:12,110 --> 00:04:07,889
little too big for what I want to what I

94
00:04:13,280 --> 00:04:12,120
want to work with so this this is what

95
00:04:16,610 --> 00:04:13,290
I'm actually working with this is the

96
00:04:19,810 --> 00:04:16,620
hammerhead ribozyme and this was a first

97
00:04:22,640 --> 00:04:19,820
found in the mid-1980s in plant varese

98
00:04:24,969 --> 00:04:22,650
this specific sequence though is from

99
00:04:28,070 --> 00:04:24,979
justice omen which is a parasitic worm

100
00:04:30,980 --> 00:04:28,080
so this fri design is traditionally

101
00:04:32,030 --> 00:04:30,990
known to cleave and not like it even

102
00:04:36,500 --> 00:04:32,040
though they're both reverse

103
00:04:38,570 --> 00:04:36,510
reactions so in a cleavage reaction you

104
00:04:41,630 --> 00:04:38,580
would have the enzyme which I outlined

105
00:04:43,700 --> 00:04:41,640
in red here bind to the substrate in

106
00:04:45,740 --> 00:04:43,710
blue and it would cleave it or cut into

107
00:04:48,470 --> 00:04:45,750
two pieces generating two fragments of

108
00:04:51,290 --> 00:04:48,480
this p1 fragment down here and this P to

109
00:04:53,030 --> 00:04:51,300
fragment up there in a ligation reaction

110
00:04:56,090 --> 00:04:53,040
the opposite would happen where the

111
00:04:57,650 --> 00:04:56,100
ribozyme binds to p1 fragment and the p2

112
00:05:01,130 --> 00:04:57,660
fragment and it would like it it back

113
00:05:02,900 --> 00:05:01,140

together back to one strand efficient

114
00:05:04,960 --> 00:05:02,910
ligation with this sequence wasn't

115
00:05:08,960 --> 00:05:04,970
demonstrated until two thousand seven

116
00:05:11,570 --> 00:05:08,970
here the p1 fragment is labeled and if

117
00:05:13,760 --> 00:05:11,580
it's like ated to p2 then you should see

118
00:05:16,640 --> 00:05:13,770
this band shift up here and this was

119
00:05:18,590 --> 00:05:16,650
done at room temperature but it was only

120
00:05:20,750 --> 00:05:18,600
possible if you added millimolar amounts

121
00:05:24,110 --> 00:05:20,760
of magnesium and this is a fast reaction

122
00:05:27,200 --> 00:05:24,120
so here 18 seconds you're done and their

123
00:05:29,500 --> 00:05:27,210
maximum yield 23% I thought this was a

124
00:05:33,020 --> 00:05:29,510
great ribozyme to start out with I

125
00:05:34,630 --> 00:05:33,030
started out with magnesium repeating

126
00:05:37,580 --> 00:05:34,640
their experiments but I also tried

127
00:05:39,200 --> 00:05:37,590
calcium and iron and in short they

128
00:05:40,400 --> 00:05:39,210
worked but that's not the focus of this

129
00:05:45,200 --> 00:05:40,410
talk although you're welcome to ask me

130
00:05:47,300 --> 00:05:45,210
about it iron people so what I'm focused

131
00:05:49,610 --> 00:05:47,310
on is ligation through freezing and if

132
00:05:52,430 --> 00:05:49,620
you do it this way you don't have to

133
00:05:55,790 --> 00:05:52,440
have divalent cations in your sample um

134
00:05:58,070 --> 00:05:55,800
this this is just a shorter example of

135
00:06:02,180 --> 00:05:58,080
the different temperatures I used and I

136
00:06:05,600 --> 00:06:02,190
think that C is supposed to go there but

137
00:06:07,700 --> 00:06:05,610
temperature alone isn't enough so as

138
00:06:08,900 --> 00:06:07,710

demonstrated here these two lanes right

139
00:06:11,240 --> 00:06:08,910
here they're incubated at the same

140
00:06:13,490 --> 00:06:11,250
temperature negative 10 but one was

141
00:06:16,220 --> 00:06:13,500
frozen before incubation and one remain

142
00:06:17,270 --> 00:06:16,230
liquid the entire time so even though

143
00:06:18,620 --> 00:06:17,280
it's the same rank you Batian

144
00:06:21,380 --> 00:06:18,630
temperature you still have very

145
00:06:23,720 --> 00:06:21,390
different results this demonstrates that

146
00:06:27,590 --> 00:06:23,730
you need freezing or the concentrating

147
00:06:30,020 --> 00:06:27,600
effect to get ligation so next we looked

148
00:06:32,600 --> 00:06:30,030
at how fast this reaction is and while

149
00:06:35,720 --> 00:06:32,610
it's not that 18 seconds from that you

150
00:06:39,140 --> 00:06:35,730
get with magnesium we still get a higher

151
00:06:42,230 --> 00:06:39,150
final yield and based on this the

152
00:06:44,450 --> 00:06:42,240
majority ligations done in an hour so we

153
00:06:46,490 --> 00:06:44,460
decided to use one hour in our thermal

154
00:06:50,000 --> 00:06:46,500
cycling experiments so thermal cycling

155
00:06:54,260 --> 00:06:50,010
is freestyle cycling and it's supposed

156
00:06:56,450 --> 00:06:54,270
to mimic day-night cycles so each of our

157
00:06:58,280 --> 00:06:56,460
cycles is there's a two-minute heat step

158
00:07:01,159 --> 00:06:58,290
and then there's an hour long incubation

159
00:07:03,080 --> 00:07:01,169
free step so that's one cycle here and

160
00:07:06,640 --> 00:07:03,090
then two cycles you have to heat steps

161
00:07:09,230 --> 00:07:06,650
and then two-hour-long free steps and

162
00:07:15,020 --> 00:07:09,240
that wasn't supposed to happen you

163
00:07:16,340 --> 00:07:15,030

should see like fans up here so our base

164
00:07:17,780 --> 00:07:16,350
yield what we started out with is

165
00:07:20,090 --> 00:07:17,790
twenty-five percent but with each

166
00:07:22,070 --> 00:07:20,100
additional cycle so basically each

167
00:07:27,110 --> 00:07:22,080
additional day we should get more and

168
00:07:28,940 --> 00:07:27,120
more like ated product so we thought

169
00:07:31,670 --> 00:07:28,950
this was a you know great start but

170
00:07:33,320 --> 00:07:31,680
before going further into cycling

171
00:07:35,860 --> 00:07:33,330
experiments we were to try increasing

172
00:07:39,170 --> 00:07:35,870
that base yield that twenty five percent

173
00:07:41,060 --> 00:07:39,180
so it already briefly went over

174
00:07:42,680 --> 00:07:41,070
different temperatures you can get

175
00:07:44,840 --> 00:07:42,690
between twenty-five to thirty percent

176
00:07:48,530 --> 00:07:44,850
depending on if you're between negative

177
00:07:51,320 --> 00:07:48,540
20 or negative 10 we tried looking at

178
00:07:53,690 --> 00:07:51,330
sugars of millimolar amounts of glucose

179
00:07:57,050 --> 00:07:53,700
sucrose lactose your halos well it

180
00:07:58,520 --> 00:07:57,060
didn't help it didn't hurt we tried

181
00:08:00,920 --> 00:07:58,530
looking at different buffers pipis

182
00:08:02,450 --> 00:08:00,930
sodium phosphate Tris and as long as

183
00:08:05,960 --> 00:08:02,460
they were the same pH they were

184
00:08:08,390 --> 00:08:05,970
comparable pH pH is important so our

185
00:08:12,050 --> 00:08:08,400
standard is ph 8 and if you go a little

186
00:08:14,000 --> 00:08:12,060
higher it didn't change the a yield but

187
00:08:19,040 --> 00:08:14,010
if you go lower it'll start decreasing

188
00:08:20,360 --> 00:08:19,050

that yield and if you go about 5.5 it

189
00:08:23,150 --> 00:08:20,370
just killed the reaction there's just no

190
00:08:24,980 --> 00:08:23,160
ligation going on now amino acids now

191
00:08:28,940 --> 00:08:24,990
why would I be talking about amino acids

192
00:08:31,700 --> 00:08:28,950
if if this is in the context of the RNA

193
00:08:35,350 --> 00:08:31,710
world well let's face it I doubt early

194
00:08:39,709 --> 00:08:35,360
Earth was as clean as our test tubes so

195
00:08:41,870 --> 00:08:39,719
we decided to look at what would happen

196
00:08:45,020 --> 00:08:41,880
if we added or contaminated our samples

197
00:08:48,260 --> 00:08:45,030
with amino acids so we tried looking at

198
00:08:51,110 --> 00:08:48,270
the 20 standard amino acids and I didn't

199
00:08:53,800 --> 00:08:51,120
list all of them but 18 out of 20 fell

200
00:08:55,660 --> 00:08:53,810
in this category here none of them

201
00:08:57,310 --> 00:08:55,670
increased the ligation yield but none of

202
00:09:00,730 --> 00:08:57,320
them dropped it below twenty percent

203
00:09:02,500 --> 00:09:00,740
only two of them drastically reduced our

204
00:09:05,560 --> 00:09:02,510
yield which is tryptophan and isoleucine

205
00:09:08,260 --> 00:09:05,570
and overall I think this is good news

206
00:09:10,530 --> 00:09:08,270
because the the earliest amino acids at

207
00:09:12,910 --> 00:09:10,540
least the ones that are proposed

208
00:09:17,380 --> 00:09:12,920
earliest amino acid they're on this side

209
00:09:19,390 --> 00:09:17,390
of the list and the last thing we tried

210
00:09:21,460 --> 00:09:19,400
was anions this might sound a little

211
00:09:24,640 --> 00:09:21,470
strange because with RNA studies usually

212
00:09:26,380 --> 00:09:24,650
focus more on cation cation effects and

213
00:09:28,240 --> 00:09:26,390

that's because RNA is negatively charged

214
00:09:32,829 --> 00:09:28,250
cations are positive so you expect

215
00:09:35,829 --> 00:09:32,839
interactions we didn't think that anions

216
00:09:40,210 --> 00:09:35,839
would contribute very much but well we

217
00:09:43,360 --> 00:09:40,220
were wrong so depending on which sodium

218
00:09:46,360 --> 00:09:43,370
salt you used you get very different

219
00:09:48,190 --> 00:09:46,370
results our best ones were from acetate

220
00:09:49,360 --> 00:09:48,200
for weed and citrate and we think it has

221
00:09:52,240 --> 00:09:49,370
something to do with their carboxyl

222
00:09:56,200 --> 00:09:52,250
groups fluoride chloride bromide and

223
00:09:57,760 --> 00:09:56,210
iodide they seem to follow some general

224
00:09:59,380 --> 00:09:57,770
patterns like fluorine and chlorine are

225
00:10:01,780 --> 00:09:59,390
supposed to be like more electronegative

226
00:10:04,030 --> 00:10:01,790
than bromine and iodine they're also

227
00:10:06,100 --> 00:10:04,040
smaller in size then bromine and iodine

228
00:10:08,230 --> 00:10:06,110
and in the context of chemical abundance

229
00:10:11,350 --> 00:10:08,240
there's a lot more fluorine and chlorine

230
00:10:13,420 --> 00:10:11,360
than bromine and iodine and we also took

231
00:10:16,090 --> 00:10:13,430
this as another piece of good news

232
00:10:19,540 --> 00:10:16,100
because based on this we don't want a

233
00:10:21,970 --> 00:10:19,550
lot of bromine and iodine around we're

234
00:10:26,110 --> 00:10:21,980
still not sure exactly how the anions

235
00:10:29,470 --> 00:10:26,120
are affecting the the ligation yield but

236
00:10:31,810 --> 00:10:29,480
we but we are exploring some ideas so

237
00:10:33,820 --> 00:10:31,820
that's where we are experimentally I'd

238
00:10:37,840 --> 00:10:33,830

like to end this talk by summarizing

239
00:10:40,180 --> 00:10:37,850
that ice preserves RNA and it also

240
00:10:43,150 --> 00:10:40,190
preserved an enzymatic functions of your

241
00:10:45,880 --> 00:10:43,160
RNA particularly ligation what we're

242
00:10:49,150 --> 00:10:45,890
looking at and you don't need to die

243
00:10:51,240 --> 00:10:49,160
valent cations to do it and it this

244
00:10:54,579 --> 00:10:51,250
ligation reaction also tolerates the

245
00:10:57,070 --> 00:10:54,589
majority of amino acids the osmolytes

246
00:10:58,900 --> 00:10:57,080
that we looked at and you can try using

247
00:11:01,480 --> 00:10:58,910
different buffers in different salts and

248
00:11:04,500 --> 00:11:01,490
you can still get ligation you can

249
00:11:07,180 --> 00:11:04,510
increase the base ligation yield by

250
00:11:07,630 --> 00:11:07,190
freeze-thaw cycles and really this last

251
00:11:09,760 --> 00:11:07,640
point

252
00:11:12,550 --> 00:11:09,770
our next step it's to combine thermal

253
00:11:14,530 --> 00:11:12,560
cycling with our best ligation yield and

254
00:11:16,360 --> 00:11:14,540
that was that forty percent from acetate

255
00:11:19,270 --> 00:11:16,370
so we want to get above that twenty

256
00:11:33,700 --> 00:11:19,280
percent which is our best so far so

257
00:11:35,890 --> 00:11:33,710
that's it that was really cool have you

258
00:11:38,320 --> 00:11:35,900
given any insight into how this might

259
00:11:40,510 --> 00:11:38,330
vary with pressure because both the

260
00:11:43,270 --> 00:11:40,520
pennsbury I hypothesis and the idea of

261
00:11:46,570 --> 00:11:43,280
delivery of volatile to the earth could

262
00:11:48,640 --> 00:11:46,580
mean that if RNA is kind of having fun

263
00:11:51,610 --> 00:11:48,650

ligating itself on comets for a long

264
00:11:54,970 --> 00:11:51,620
time it could be safe there um I don't

265
00:11:56,860 --> 00:11:54,980
know about low pressure but I did look a

266
00:12:00,270 --> 00:11:56,870
little bit into very high pressure and

267
00:12:04,300 --> 00:12:00,280
that could that could actually affect

268
00:12:07,030 --> 00:12:04,310
the way that ice is formed so this this

269
00:12:11,710 --> 00:12:07,040
type of ice it's normal pressure and

270
00:12:13,390 --> 00:12:11,720
it's what phase 1 H ice but with high

271
00:12:16,360 --> 00:12:13,400
pressure you get different types of

272
00:12:18,250 --> 00:12:16,370
phases of ice I and I don't know I've

273
00:12:20,800 --> 00:12:18,260
never I haven't tried different

274
00:12:30,990 --> 00:12:20,810
pressures it's just what was in our lab

275
00:12:36,100 --> 00:12:33,790
so going off of the pressure in the ice

276
00:12:38,770 --> 00:12:36,110
bit you were mentioning that this

277
00:12:40,090 --> 00:12:38,780
reaction only occurs under these frozen

278
00:12:41,650 --> 00:12:40,100
conditions where you have the solid

279
00:12:43,510 --> 00:12:41,660
phase and you're attributing that to an

280
00:12:44,740 --> 00:12:43,520
increase in the concentration so we

281
00:12:50,290 --> 00:12:44,750
reason to think it might be an effective

282
00:12:53,350 --> 00:12:50,300
surface chemistry with the ice um we

283
00:12:55,750 --> 00:12:53,360
don't know if like for instance we don't

284
00:12:58,930 --> 00:12:55,760
know if the RNA is somehow like

285
00:13:01,720 --> 00:12:58,940
concentrating near the surface the the

286
00:13:05,470 --> 00:13:01,730
the walls of the of those tunnels we

287
00:13:07,390 --> 00:13:05,480
don't know so far all we know is that it

288
00:13:10,380 --> 00:13:07,400

is there is a concentrating effect and

289
00:13:14,430 --> 00:13:10,390
you can visibly see that if you stain it

290
00:13:17,350 --> 00:13:14,440
if you stay in the RNA and you detect

291
00:13:22,960 --> 00:13:17,360
fluorescence but