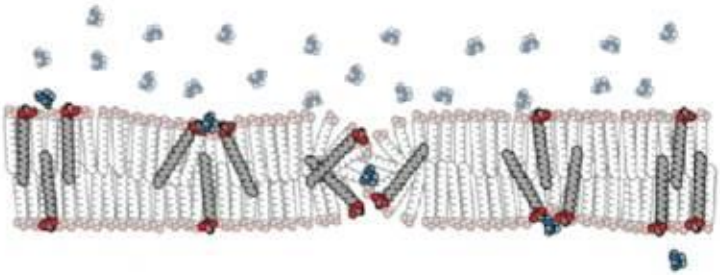
Spontaneous Membrane Permeation:



- short acyl chain
- cone-shaped
- unsaturated or branched

NASA Astrobiology Institute

Director's Seminar Series
11/3/2008 – Jack Szostak

1
00:00:08,230 --> 00:00:04,390
good morning or afternoon everyone

2
00:00:10,790 --> 00:00:08,240
welcome to the nai director seminar

3
00:00:12,950 --> 00:00:10,800
i am really really pleased to be able to

4
00:00:15,030 --> 00:00:12,960
introduce jack shaw stack today i think

5
00:00:16,550 --> 00:00:15,040
jack shaw stack is probably well known

6
00:00:18,310 --> 00:00:16,560
to everybody and doesn't need much of an

7
00:00:19,429 --> 00:00:18,320
introduction

8
00:00:22,470 --> 00:00:19,439
but

9
00:00:24,390 --> 00:00:22,480
i will say that jack is associated not

10
00:00:26,710 --> 00:00:24,400
only with the harvard medical school

11
00:00:28,630 --> 00:00:26,720
where he's in the department of genetics

12
00:00:29,990 --> 00:00:28,640
but also the massachusetts general

13
00:00:32,709 --> 00:00:30,000

hospital where he's in the department of

14
00:00:35,350 --> 00:00:32,719
molecular biology and he's also a howard

15
00:00:37,670 --> 00:00:35,360
hughes medical institute investigator

16
00:00:41,190 --> 00:00:37,680
jack is very well known for his work on

17
00:00:43,030 --> 00:00:41,200
rna evolution and most recently he has

18
00:00:45,270 --> 00:00:43,040
been doing some very exciting work on

19
00:00:47,270 --> 00:00:45,280
designing an artificial cell

20
00:00:48,950 --> 00:00:47,280
and what jack is going to tell us today

21
00:00:50,470 --> 00:00:48,960
is what we can learn about the origin of

22
00:00:52,790 --> 00:00:50,480
life from efforts to design an

23
00:00:55,510 --> 00:00:52,800
artificial cell and jack i will turn it

24
00:00:56,830 --> 00:00:55,520
directly over to you

25
00:00:59,830 --> 00:00:56,840
okay thank

26
00:01:00,790 --> 00:00:59,840
you and uh thanks for the opportunity to

27
00:01:02,869 --> 00:01:00,800
uh

28
00:01:06,789 --> 00:01:02,879
talk to everybody today

29
00:01:08,870 --> 00:01:06,799
uh so what i'd like to

30
00:01:12,230 --> 00:01:08,880
tell you about is some of the recent

31
00:01:14,710 --> 00:01:12,240
work for my lab that's aimed at

32
00:01:15,990 --> 00:01:14,720
the synthesis of a very

33
00:01:19,670 --> 00:01:16,000
simple

34
00:01:21,429 --> 00:01:19,680
minimal artificial cell

35
00:01:22,230 --> 00:01:21,439
as made out of

36
00:01:24,710 --> 00:01:22,240
just

37
00:01:26,950 --> 00:01:24,720
chemical simple chemicals

38
00:01:28,550 --> 00:01:26,960

so in general what we're

39
00:01:31,590 --> 00:01:28,560
trying to do

40
00:01:32,390 --> 00:01:31,600
is get a better idea of what's required

41
00:01:34,550 --> 00:01:32,400
to

42
00:01:37,990 --> 00:01:34,560
make that move from complicated

43
00:01:40,469 --> 00:01:38,000
chemistry to really simple biology

44
00:01:42,789 --> 00:01:40,479
and so the main thing that that means to

45
00:01:45,109 --> 00:01:42,799
me is that we want to see the

46
00:01:47,990 --> 00:01:45,119
spontaneous emergence of darwinian

47
00:01:50,469 --> 00:01:48,000
evolutionary processes

48
00:01:52,830 --> 00:01:50,479
from mixtures of the right kinds of

49
00:01:55,670 --> 00:01:52,840
chemical building blocks

50
00:01:57,270 --> 00:01:55,680
so more specifically what we're trying

51
00:01:58,469 --> 00:01:57,280
to do is test

52
00:02:01,749 --> 00:01:58,479
uh

53
00:02:03,910 --> 00:02:01,759
particular aspects of a model that we

54
00:02:05,429 --> 00:02:03,920
have in mind for what a very

55
00:02:07,510 --> 00:02:05,439
early primitive

56
00:02:09,270 --> 00:02:07,520
protocol might have looked like

57
00:02:11,830 --> 00:02:09,280
and so

58
00:02:13,750 --> 00:02:11,840
on this uh next slide

59
00:02:17,270 --> 00:02:13,760
we have a schematic

60
00:02:19,030 --> 00:02:17,280
diagram of the kind of

61
00:02:20,470 --> 00:02:19,040
construct that we're trying to put

62
00:02:24,070 --> 00:02:20,480
together

63
00:02:26,390 --> 00:02:24,080

so this is a a diagram of what we think

64
00:02:28,070 --> 00:02:26,400
are the minimal requirements

65
00:02:30,229 --> 00:02:28,080
of a very early cell and it has

66
00:02:32,790 --> 00:02:30,239
basically two

67
00:02:34,150 --> 00:02:32,800
components a bilayer

68
00:02:38,710 --> 00:02:34,160
membrane

69
00:02:40,949 --> 00:02:38,720
and inside it uh some kind of uh

70
00:02:44,150 --> 00:02:40,959
nucleic acid or related molecule that

71
00:02:47,589 --> 00:02:44,160
can carry information in the sequence

72
00:02:51,509 --> 00:02:47,599
of its monomer building blocks

73
00:02:53,430 --> 00:02:51,519
okay so um this conception of an early

74
00:02:56,470 --> 00:02:53,440
cell builds uh

75
00:03:00,150 --> 00:02:56,480
heavily on the ideas of

76
00:03:02,309 --> 00:03:00,160
self-assembly both chemical and physical

77
00:03:04,390 --> 00:03:02,319
so we know that bilayer membranes can

78
00:03:06,309 --> 00:03:04,400
self-assemble

79
00:03:07,670 --> 00:03:06,319
spontaneously from

80
00:03:09,270 --> 00:03:07,680
ancient

81
00:03:11,190 --> 00:03:09,280
small molecules

82
00:03:13,589 --> 00:03:11,200
and we know that

83
00:03:18,710 --> 00:03:13,599
given activated nucleotides and the

84
00:03:23,910 --> 00:03:20,309
we can

85
00:03:25,270 --> 00:03:23,920
spontaneously form uh long rna

86
00:03:25,990 --> 00:03:25,280
polymers

87
00:03:27,990 --> 00:03:26,000
so

88
00:03:29,670 --> 00:03:28,000

in addition to these self-assembly

89
00:03:31,110 --> 00:03:29,680
processes though

90
00:03:33,110 --> 00:03:31,120
what we need

91
00:03:35,670 --> 00:03:33,120
for cellular behavior and for

92
00:03:37,509 --> 00:03:35,680
evolutionary behavior is the ability of

93
00:03:40,630 --> 00:03:37,519
the whole system

94
00:03:42,550 --> 00:03:40,640
to grow and divide to replicate

95
00:03:44,070 --> 00:03:42,560
and so if we think first about the

96
00:03:45,670 --> 00:03:44,080
bilayer

97
00:03:47,830 --> 00:03:45,680
cell membrane

98
00:03:48,949 --> 00:03:47,840
there have to be physical processes that

99
00:03:50,949 --> 00:03:48,959
allow

100
00:03:52,949 --> 00:03:50,959
for additional monomers to become

101
00:03:54,710 --> 00:03:52,959
incorporated into the bilayer so that it

102
00:03:57,350 --> 00:03:54,720
can grow larger

103
00:04:00,229 --> 00:03:57,360
and then also some kind of

104
00:04:02,390 --> 00:04:00,239
process that will mediate division into

105
00:04:04,710 --> 00:04:02,400
smaller daughter cells

106
00:04:07,190 --> 00:04:04,720
and of course the tricky part is that

107
00:04:09,670 --> 00:04:07,200
all of this has to happen without any uh

108
00:04:11,589 --> 00:04:09,680
pre-existing complicated biological

109
00:04:13,429 --> 00:04:11,599
machinery and we know that all modern

110
00:04:16,229 --> 00:04:13,439
cells of course devote

111
00:04:18,150 --> 00:04:16,239
a lot of infrastructure to the growth

112
00:04:19,110 --> 00:04:18,160
and division of their of their cell

113
00:04:21,830 --> 00:04:19,120

membrane

114
00:04:23,030 --> 00:04:21,840
in addition same kinds of considerations

115
00:04:25,189 --> 00:04:23,040
apply

116
00:04:27,030 --> 00:04:25,199
to the genetic material

117
00:04:29,430 --> 00:04:27,040
so in modern biology of course we have a

118
00:04:31,270 --> 00:04:29,440
lot of complicated enzymology uh

119
00:04:34,629 --> 00:04:31,280
dedicated to the

120
00:04:36,150 --> 00:04:34,639
uh replication of our genome

121
00:04:37,830 --> 00:04:36,160
but in this case

122
00:04:39,830 --> 00:04:37,840
we're trying to think of how something

123
00:04:42,150 --> 00:04:39,840
could emerge from a chemical system and

124
00:04:44,390 --> 00:04:42,160
so what we've been focusing on are

125
00:04:47,030 --> 00:04:44,400
purely chemical systems which would

126
00:04:50,870 --> 00:04:47,040
allow a template strand to be copied

127
00:04:54,950 --> 00:04:50,880
into a duplex and the strands come apart

128
00:04:56,629 --> 00:04:54,960
and copy those single strands again so

129
00:05:00,390 --> 00:04:56,639
that in the end you have

130
00:05:02,150 --> 00:05:00,400
um java duplexes that can be distributed

131
00:05:04,469 --> 00:05:02,160
to the daughter cell

132
00:05:06,790 --> 00:05:04,479
and so this cycle can go around and

133
00:05:11,749 --> 00:05:06,800
around indefinitely

134
00:05:15,749 --> 00:05:14,070
sequences will diverge and eventually

135
00:05:17,990 --> 00:05:15,759
some kind of

136
00:05:21,270 --> 00:05:18,000
sequence will arise it does something

137
00:05:23,430 --> 00:05:21,280
helpful to the cell in some way

138
00:05:25,990 --> 00:05:23,440

improve the efficiency of replication

139
00:05:28,230 --> 00:05:26,000
either of the genetic material or the

140
00:05:30,629 --> 00:05:28,240
cell itself or the whole structure

141
00:05:31,830 --> 00:05:30,639
and such molecules should have an

142
00:05:33,510 --> 00:05:31,840
advantage

143
00:05:35,909 --> 00:05:33,520
and gradually come to take over the

144
00:05:38,070 --> 00:05:35,919
population and that kind of

145
00:05:41,430 --> 00:05:38,080
genetic change in population structure

146
00:05:42,950 --> 00:05:41,440
is the essence of darwinian evolution

147
00:05:45,189 --> 00:05:42,960
okay so this is the kind of thing that

148
00:05:47,110 --> 00:05:45,199
we'd like to put together

149
00:05:48,070 --> 00:05:47,120
in a laboratory

150
00:05:49,830 --> 00:05:48,080
and

151
00:05:51,909 --> 00:05:49,840
so that means we have to start thinking

152
00:05:54,150 --> 00:05:51,919
about the kinds of molecules that are

153
00:05:55,430 --> 00:05:54,160
going to go into these structures the

154
00:05:57,670 --> 00:05:55,440
cell membrane

155
00:05:59,909 --> 00:05:57,680
and the genetic material

156
00:06:02,790 --> 00:05:59,919
and also we have to think about

157
00:06:03,909 --> 00:06:02,800
energy sources

158
00:06:07,270 --> 00:06:03,919
and

159
00:06:10,710 --> 00:06:07,280
so just to touch on that of course this

160
00:06:12,550 --> 00:06:10,720
kind of system is inherently a

161
00:06:14,870 --> 00:06:12,560
far from equilibrium system there are a

162
00:06:18,070 --> 00:06:14,880
lot of ways that energy can

163
00:06:19,590 --> 00:06:18,080

go into the system so we'll be supplying

164
00:06:22,790 --> 00:06:19,600
from the environment activated

165
00:06:25,029 --> 00:06:22,800
nucleotides with chemical energy

166
00:06:27,350 --> 00:06:25,039
in the activated nucleotides we have

167
00:06:28,550 --> 00:06:27,360
possibility for mechanical energy to

168
00:06:30,469 --> 00:06:28,560
mediate

169
00:06:32,230 --> 00:06:30,479
cell division

170
00:06:35,110 --> 00:06:32,240
energy is released and the phase

171
00:06:37,110 --> 00:06:35,120
transfer is new molecules integrate into

172
00:06:40,790 --> 00:06:37,120
the bilayer

173
00:06:42,950 --> 00:06:40,800
membrane structure and and so on

174
00:06:44,710 --> 00:06:42,960
so so both

175
00:06:46,870 --> 00:06:44,720
matter in the form of new building

176
00:06:49,350 --> 00:06:46,880
blocks and energy

177
00:06:51,430 --> 00:06:49,360
will be flowing through this system and

178
00:06:54,950 --> 00:06:51,440
mediating the overall growth and

179
00:06:59,189 --> 00:06:57,670
okay so one other aspect of this is this

180
00:07:02,469 --> 00:06:59,199
is such a simple

181
00:07:04,309 --> 00:07:02,479
uh minimal cellular structure

182
00:07:06,070 --> 00:07:04,319
that is

183
00:07:09,589 --> 00:07:06,080
growth and division relies on a

184
00:07:10,870 --> 00:07:09,599
correspondingly complicated environment

185
00:07:14,550 --> 00:07:10,880
and

186
00:07:17,029 --> 00:07:14,560
so in this conception we're thinking of

187
00:07:18,390 --> 00:07:17,039
environmental chemistry is supplying the

188
00:07:21,110 --> 00:07:18,400

various building blocks that are

189
00:07:23,430 --> 00:07:21,120
required for these processes

190
00:07:25,909 --> 00:07:23,440
as well as the various uh

191
00:07:28,150 --> 00:07:25,919
sources of energy

192
00:07:30,390 --> 00:07:28,160
okay so i know there's a very particular

193
00:07:32,790 --> 00:07:30,400
model there are a lot of different ideas

194
00:07:33,990 --> 00:07:32,800
not everyone accepts this

195
00:07:35,430 --> 00:07:34,000
you know there's a lot of people that

196
00:07:36,710 --> 00:07:35,440
think that all the building blocks would

197
00:07:38,950 --> 00:07:36,720
have to arise

198
00:07:40,629 --> 00:07:38,960
internally from localized chemical

199
00:07:42,390 --> 00:07:40,639
reactions

200
00:07:44,790 --> 00:07:42,400
but we're

201
00:07:46,309 --> 00:07:44,800
in our experience not really directly

202
00:07:48,390 --> 00:07:46,319
concerns with the origins of the

203
00:07:50,550 --> 00:07:48,400
building blocks a particular assumption

204
00:07:52,390 --> 00:07:50,560
is they come from external environmental

205
00:07:53,909 --> 00:07:52,400
chemistry but we're really only trying

206
00:07:54,869 --> 00:07:53,919
to test ideas

207
00:07:56,950 --> 00:07:54,879
about

208
00:07:59,589 --> 00:07:56,960
how these molecules interact and

209
00:08:01,430 --> 00:07:59,599
generate larger structures

210
00:08:03,670 --> 00:08:01,440
that

211
00:08:05,110 --> 00:08:03,680
might have the properties of the living

212
00:08:05,990 --> 00:08:05,120
cell

213
00:08:08,309 --> 00:08:06,000

okay

214
00:08:11,270 --> 00:08:08,319
so uh what i'd like to do then is just

215
00:08:14,150 --> 00:08:11,280
briefly go through

216
00:08:16,790 --> 00:08:14,160
the the stages involved in building up

217
00:08:18,469 --> 00:08:16,800
this kind of uh structure and the

218
00:08:19,430 --> 00:08:18,479
associated processes of growth and

219
00:08:21,589 --> 00:08:19,440
division

220
00:08:24,230 --> 00:08:21,599
um so we have to begin with the two

221
00:08:26,869 --> 00:08:24,240
basic components and think about

222
00:08:28,869 --> 00:08:26,879
how the vesicle uh membrane itself could

223
00:08:31,350 --> 00:08:28,879
grow and divide

224
00:08:35,670 --> 00:08:33,750
separately we can think about

225
00:08:38,149 --> 00:08:35,680
the genetic materials

226
00:08:39,829 --> 00:08:38,159
and how they could replicate

227
00:08:41,269 --> 00:08:39,839
and then things start to actually get

228
00:08:43,589 --> 00:08:41,279
more interesting

229
00:08:45,509 --> 00:08:43,599
when you can think about what's required

230
00:08:48,630 --> 00:08:45,519
to put these things together

231
00:08:50,470 --> 00:08:48,640
and have them work in a compatible way

232
00:08:52,829 --> 00:08:50,480
and so in fact today i'll be talking

233
00:08:54,790 --> 00:08:52,839
quite a bit about compatibility

234
00:08:57,190 --> 00:08:54,800
issues such as

235
00:08:58,870 --> 00:08:57,200
how the nucleotide building blocks for

236
00:09:00,389 --> 00:08:58,880
the genetic material might get across

237
00:09:02,310 --> 00:09:00,399
the membrane

238
00:09:04,870 --> 00:09:02,320

to allow internal

239
00:09:06,630 --> 00:09:04,880
replication of the genetic material

240
00:09:08,949 --> 00:09:06,640
to talk about that

241
00:09:12,310 --> 00:09:08,959
simply and chemistry

242
00:09:14,310 --> 00:09:12,320
and its compatibility with the

243
00:09:16,150 --> 00:09:14,320
chemistry of the bilayer

244
00:09:18,150 --> 00:09:16,160
and

245
00:09:20,949 --> 00:09:18,160
finally the issue of how to get the

246
00:09:23,350 --> 00:09:20,959
strands apart so that after template

247
00:09:24,470 --> 00:09:23,360
copying you can go to uh subsequent

248
00:09:26,230 --> 00:09:24,480
rounds

249
00:09:28,150 --> 00:09:26,240
and uh

250
00:09:30,310 --> 00:09:28,160
i probably won't have time today to talk

251
00:09:32,790 --> 00:09:30,320
about the highest level of

252
00:09:35,110 --> 00:09:32,800
interactions which are the

253
00:09:37,350 --> 00:09:35,120
more cooperative or positive

254
00:09:38,710 --> 00:09:37,360
interactions

255
00:09:40,310 --> 00:09:38,720
these are things we're just starting to

256
00:09:47,670 --> 00:09:40,320
work on

257
00:09:52,070 --> 00:09:49,990
okay so i'm going to start off by giving

258
00:09:53,670 --> 00:09:52,080
some background about

259
00:09:55,350 --> 00:09:53,680
the kinds of vesicles and the kinds of

260
00:09:56,470 --> 00:09:55,360
molecules

261
00:09:59,350 --> 00:09:56,480
that we're

262
00:10:02,310 --> 00:09:59,360
using to study

263
00:10:03,750 --> 00:10:02,320

vesicles that can grow and divide

264
00:10:05,350 --> 00:10:03,760
okay and so the kinds of molecules we

265
00:10:08,470 --> 00:10:05,360
use they're basically very

266
00:10:11,430 --> 00:10:08,480
uh simple uh fatty acids

267
00:10:13,670 --> 00:10:11,440
and the reason that we're not using

268
00:10:16,550 --> 00:10:13,680
uh molecules you might be more familiar

269
00:10:18,470 --> 00:10:16,560
with like phospholipids and cholesterol

270
00:10:21,910 --> 00:10:18,480
single and throw on the components of

271
00:10:24,470 --> 00:10:21,920
modern biological membranes is that

272
00:10:26,470 --> 00:10:24,480
modern cell membranes are designed

273
00:10:28,550 --> 00:10:26,480
to be very good barriers

274
00:10:29,670 --> 00:10:28,560
so that the protein machinery can

275
00:10:31,430 --> 00:10:29,680
control

276
00:10:33,030 --> 00:10:31,440
everything that gets in and out of

277
00:10:35,590 --> 00:10:33,040
modern cell

278
00:10:37,430 --> 00:10:35,600
but of course in the first cell

279
00:10:39,110 --> 00:10:37,440
there was no machinery to mediate the

280
00:10:41,670 --> 00:10:39,120
transport of molecules across the

281
00:10:43,110 --> 00:10:41,680
membrane and so we need to make

282
00:10:46,550 --> 00:10:43,120
membranes that have more dynamic

283
00:10:48,150 --> 00:10:46,560
structures and which can allow building

284
00:10:49,910 --> 00:10:48,160
blocks to get across

285
00:10:52,470 --> 00:10:49,920
and waste products to

286
00:10:53,990 --> 00:10:52,480
get into the cell spontaneously

287
00:10:56,150 --> 00:10:54,000
and these are the kinds of molecules

288
00:10:58,829 --> 00:10:56,160

that turns out

289
00:11:01,509 --> 00:10:58,839
that have the right properties so if we

290
00:11:03,750 --> 00:11:01,519
use uh oleic acid or the shorter chain

291
00:11:05,509 --> 00:11:03,760
meristeleic acid

292
00:11:07,110 --> 00:11:05,519
for a lot of our

293
00:11:09,430 --> 00:11:07,120
model studies

294
00:11:11,670 --> 00:11:09,440
and down here at the bottom you see uh

295
00:11:12,630 --> 00:11:11,680
caprication saturated

296
00:11:14,790 --> 00:11:12,640
uh

297
00:11:16,150 --> 00:11:14,800
shorter chain fatty acid

298
00:11:18,790 --> 00:11:16,160
which is something that's a little bit

299
00:11:21,590 --> 00:11:18,800
more prebiotically reasonable than those

300
00:11:23,030 --> 00:11:21,600
longer chain unsaturated fatty acids but

301
00:11:24,470 --> 00:11:23,040
all of these molecules will

302
00:11:28,790 --> 00:11:24,480
self-assemble

303
00:11:31,910 --> 00:11:28,800
into a bilayer membranes which can clog

304
00:11:36,949 --> 00:11:31,920
up and make vesicles uh

305
00:11:42,470 --> 00:11:39,269
okay so these uh fatty acid vesicles

306
00:11:44,949 --> 00:11:42,480
have a lot of uh interesting properties

307
00:11:47,269 --> 00:11:44,959
and uh among them

308
00:11:50,230 --> 00:11:47,279
the most important is the fact

309
00:11:52,550 --> 00:11:50,240
that the fatty acids will self-assemble

310
00:11:53,670 --> 00:11:52,560
uh into these larger much larger

311
00:11:55,430 --> 00:11:53,680
structures

312
00:11:57,829 --> 00:11:55,440
and so that's just

313
00:11:59,829 --> 00:11:57,839

it here

314
00:12:02,550 --> 00:11:59,839
typically when they're

315
00:12:03,670 --> 00:12:02,560
at high ph fatty acids will form

316
00:12:04,710 --> 00:12:03,680
small

317
00:12:06,629 --> 00:12:04,720
aggregates

318
00:12:09,430 --> 00:12:06,639
called micelle

319
00:12:10,790 --> 00:12:09,440
as you lower the ph and

320
00:12:12,629 --> 00:12:10,800
start to protonate more of the

321
00:12:14,870 --> 00:12:12,639
carboxylates

322
00:12:16,629 --> 00:12:14,880
these micelles can start to interact

323
00:12:19,269 --> 00:12:16,639
with each other

324
00:12:20,870 --> 00:12:19,279
assemble into small sheets

325
00:12:22,550 --> 00:12:20,880
and eventually when the sheets grow

326
00:12:24,949 --> 00:12:22,560
large enough

327
00:12:27,110 --> 00:12:24,959
the thermal fluctuations will ensure and

328
00:12:29,269 --> 00:12:27,120
know gradually eventually they'll round

329
00:12:32,550 --> 00:12:29,279
up and close in on themselves to make

330
00:12:38,470 --> 00:12:34,870
so this process is actually quite

331
00:12:40,069 --> 00:12:38,480
interesting and a little bit complicated

332
00:12:43,269 --> 00:12:40,079
there's a lag phase

333
00:12:45,750 --> 00:12:43,279
due to this nucleation step

334
00:12:48,470 --> 00:12:45,760
but interestingly the

335
00:12:50,949 --> 00:12:48,480
reaction then becomes autocatalytic

336
00:12:52,790 --> 00:12:50,959
in that these intermediate structures on

337
00:12:54,230 --> 00:12:52,800
the final vesicles will actually

338
00:12:56,069 --> 00:12:54,240

catalyze

339
00:12:57,269 --> 00:12:56,079
accelerate the rate of formation of new

340
00:12:59,590 --> 00:12:57,279
vesicles

341
00:13:01,509 --> 00:12:59,600
and that's something we'll come back to

342
00:13:03,829 --> 00:13:01,519
uh shortly

343
00:13:05,750 --> 00:13:03,839
okay so here is

344
00:13:07,350 --> 00:13:05,760
an image that shows you

345
00:13:09,509 --> 00:13:07,360
some of these

346
00:13:11,670 --> 00:13:09,519
vesicles in the background

347
00:13:13,430 --> 00:13:11,680
and illustrates again this important

348
00:13:15,590 --> 00:13:13,440
phase transition

349
00:13:16,870 --> 00:13:15,600
the fact that you go from myself to high

350
00:13:18,949 --> 00:13:16,880
ph

351
00:13:19,750 --> 00:13:18,959
and then these interact with each other

352
00:13:21,910 --> 00:13:19,760
and

353
00:13:23,750 --> 00:13:21,920
undergo this phase transition

354
00:13:24,870 --> 00:13:23,760
to a bilateral state

355
00:13:25,829 --> 00:13:24,880
at lower

356
00:13:27,509 --> 00:13:25,839
ph

357
00:13:29,350 --> 00:13:27,519
and this is very important because this

358
00:13:30,629 --> 00:13:29,360
allows us to

359
00:13:32,829 --> 00:13:30,639
feed

360
00:13:35,829 --> 00:13:32,839
pre-existing

361
00:13:37,910 --> 00:13:35,839
vesicles uh with new material in the

362
00:13:41,829 --> 00:13:37,920
form of fatty acids and that is

363
00:13:44,949 --> 00:13:42,949

okay

364
00:13:45,910 --> 00:13:44,959
one of the things that i find

365
00:13:47,910 --> 00:13:45,920
kind of

366
00:13:48,870 --> 00:13:47,920
fascinating about these structures is

367
00:13:51,269 --> 00:13:48,880
that

368
00:13:53,509 --> 00:13:51,279
they're very long live individual

369
00:13:57,430 --> 00:13:53,519
vesicles last

370
00:13:59,189 --> 00:13:57,440
indefinitely at least weeks or months

371
00:14:00,069 --> 00:13:59,199
but we know from other experiments that

372
00:14:04,550 --> 00:14:00,079
the

373
00:14:08,389 --> 00:14:04,560
exchange

374
00:14:13,509 --> 00:14:10,790
so in this image you see two populations

375
00:14:15,509 --> 00:14:13,519
of vesicles labeled with different dyes

376
00:14:17,670 --> 00:14:15,519
they've been mixed for a day

377
00:14:20,069 --> 00:14:17,680
and you can see that the red and green

378
00:14:22,310 --> 00:14:20,079
vesicles have remained separate

379
00:14:24,230 --> 00:14:22,320
so they're not constantly fusing and

380
00:14:26,310 --> 00:14:24,240
separating they maintain their

381
00:14:28,310 --> 00:14:26,320
individual identity

382
00:14:31,910 --> 00:14:28,320
even though the fatty acid molecules go

383
00:14:31,920 --> 00:14:36,150
very rapidly

384
00:14:37,910 --> 00:14:37,030
okay

385
00:14:40,310 --> 00:14:37,920
so

386
00:14:42,389 --> 00:14:40,320
i mentioned before that the

387
00:14:43,670 --> 00:14:42,399
self-assembled self-assembly of these

388
00:14:45,590 --> 00:14:43,680

vesicles

389
00:14:49,430 --> 00:14:45,600
is auto catalytic

390
00:14:51,670 --> 00:14:49,440
and just go back to that diagram

391
00:14:53,990 --> 00:14:51,680
several years ago when

392
00:14:55,110 --> 00:14:54,000
marty hanchik and shelly fujicallo were

393
00:14:57,110 --> 00:14:55,120
in my lab

394
00:14:59,030 --> 00:14:57,120
they were thinking about the auto

395
00:15:00,870 --> 00:14:59,040
catalytic aspect of this process i

396
00:15:02,389 --> 00:15:00,880
should mention that was first

397
00:15:06,310 --> 00:15:02,399
uh

398
00:15:08,470 --> 00:15:06,320
worked out in the lab of purely lucy

399
00:15:10,310 --> 00:15:08,480
and in fact the fact that fatty acids

400
00:15:12,629 --> 00:15:10,320
could make vesicles like this was worked

401
00:15:14,949 --> 00:15:12,639
out a long time ago uh in dave steemer's

402
00:15:17,030 --> 00:15:14,959
life so all of our work

403
00:15:19,990 --> 00:15:17,040
really builds on the pioneering efforts

404
00:15:21,750 --> 00:15:20,000
of the deemer and luisi labs

405
00:15:22,629 --> 00:15:21,760
okay so marty and shelly were thinking

406
00:15:24,870 --> 00:15:22,639
about

407
00:15:27,189 --> 00:15:24,880
the auto catalytic nature of bicycle

408
00:15:28,310 --> 00:15:27,199
self-assembly and they thought well

409
00:15:30,069 --> 00:15:28,320
maybe

410
00:15:31,829 --> 00:15:30,079
it's something to do

411
00:15:34,069 --> 00:15:31,839
with these surfaces there's some

412
00:15:36,550 --> 00:15:34,079
property of these negatively charged

413
00:15:39,030 --> 00:15:36,560

surfaces that

414
00:15:40,550 --> 00:15:39,040
in some way catalyzes the assembly of

415
00:15:42,949 --> 00:15:40,560
new membranes

416
00:15:45,350 --> 00:15:42,959
and so that made them think about what

417
00:15:49,189 --> 00:15:45,360
other kinds of surfaces might catalyze

418
00:15:52,829 --> 00:15:51,910
and so they start to think about various

419
00:15:55,670 --> 00:15:52,839
mineral

420
00:15:56,949 --> 00:15:55,680
surfaces and probably the most famous

421
00:15:59,269 --> 00:15:56,959
mineral

422
00:16:02,870 --> 00:15:59,279
at least in the prebiotic chemistry

423
00:16:04,310 --> 00:16:02,880
community is a clay mineral

424
00:16:05,990 --> 00:16:04,320
montmorillonite

425
00:16:08,230 --> 00:16:06,000
which was shown

426
00:16:10,150 --> 00:16:08,240
many years ago by jim ferriss when he

427
00:16:11,829 --> 00:16:10,160
was doing the sabbatical let's lovely

428
00:16:14,550 --> 00:16:11,839
orga

429
00:16:18,150 --> 00:16:14,560
to catalyze the assembly of activated

430
00:16:21,030 --> 00:16:18,160
nucleotides into long strands of rna

431
00:16:23,189 --> 00:16:21,040
so we happen to have sort of a sample of

432
00:16:25,509 --> 00:16:23,199
this clay from jim

433
00:16:28,470 --> 00:16:25,519
and uh so marty and we just decided to

434
00:16:31,269 --> 00:16:28,480
see if that clay would catalyze this

435
00:16:34,150 --> 00:16:31,279
classical assembly uh reaction

436
00:16:35,749 --> 00:16:34,160
and uh sure enough it did

437
00:16:37,350 --> 00:16:35,759
under some conditions that can

438
00:16:40,470 --> 00:16:37,360

accelerate

439
00:16:41,990 --> 00:16:40,480
vesicle assembly by over a hundred fold

440
00:16:44,629 --> 00:16:42,000
and when they went to look at the

441
00:16:46,550 --> 00:16:44,639
resulting vesicles in the microscope

442
00:16:48,150 --> 00:16:46,560
one of the really cool things they could

443
00:16:49,269 --> 00:16:48,160
see is that there are little particles

444
00:16:50,710 --> 00:16:49,279
of clay

445
00:16:53,030 --> 00:16:50,720
that end up

446
00:16:53,829 --> 00:16:53,040
trapped inside the vesicles that they

447
00:17:01,829 --> 00:16:53,839
have

448
00:17:04,549 --> 00:17:03,189
so

449
00:17:06,309 --> 00:17:04,559
that made them think

450
00:17:07,829 --> 00:17:06,319
about trying another experiment which

451
00:17:10,069 --> 00:17:07,839
was to

452
00:17:11,590 --> 00:17:10,079
absorb some rna molecules onto the

453
00:17:13,669 --> 00:17:11,600
surface of the clay

454
00:17:16,390 --> 00:17:13,679
so mimicking a situation in which the

455
00:17:18,470 --> 00:17:16,400
clay had actually catalyzed rna assembly

456
00:17:22,309 --> 00:17:18,480
and then see if that rna coded client

457
00:17:24,150 --> 00:17:22,319
could also catalyze vesicle assembly

458
00:17:25,189 --> 00:17:24,160
and uh

459
00:17:26,150 --> 00:17:25,199
it did

460
00:17:29,830 --> 00:17:26,160
and

461
00:17:32,150 --> 00:17:29,840
that those experiments resulted in

462
00:17:34,470 --> 00:17:32,160
some of these beautiful pictures where

463
00:17:35,750 --> 00:17:34,480

you can see here

464
00:17:38,950 --> 00:17:35,760
in orange

465
00:17:40,870 --> 00:17:38,960
are clay particles that are

466
00:17:43,190 --> 00:17:40,880
bearing

467
00:17:44,470 --> 00:17:43,200
fluorescently tagged rna molecules on

468
00:17:46,950 --> 00:17:44,480
their surface

469
00:17:48,789 --> 00:17:46,960
and you can see that this uh clay

470
00:17:51,510 --> 00:17:48,799
particle is trapped

471
00:17:52,549 --> 00:17:51,520
uh within this large

472
00:17:54,230 --> 00:17:52,559
vesicle

473
00:17:56,950 --> 00:17:54,240
which in turn is filled with hundreds of

474
00:17:58,789 --> 00:17:56,960
smaller vesicles all of which have been

475
00:18:02,630 --> 00:17:58,799
assembled under the influence of the

476
00:18:04,150 --> 00:18:02,640
surface of this mineral particle

477
00:18:05,430 --> 00:18:04,160
and the next slide just shows another

478
00:18:06,710 --> 00:18:05,440
example

479
00:18:09,029 --> 00:18:06,720
of that

480
00:18:11,909 --> 00:18:09,039
so again a clay particle

481
00:18:15,750 --> 00:18:11,919
with rna molecules on its surface

482
00:18:18,390 --> 00:18:15,760
inside a large vesicle in this case many

483
00:18:19,669 --> 00:18:18,400
closely spaced phyla membranes

484
00:18:21,750 --> 00:18:19,679
so

485
00:18:24,630 --> 00:18:21,760
these experiments

486
00:18:27,669 --> 00:18:24,640
in essence are showing that there's a

487
00:18:29,830 --> 00:18:27,679
simple very common abundant mineral that

488
00:18:31,029 --> 00:18:29,840

on the one hand can catalyze the

489
00:18:33,830 --> 00:18:31,039
assembly

490
00:18:35,029 --> 00:18:33,840
of a genetic material rna as shown by

491
00:18:37,110 --> 00:18:35,039
jim ferris

492
00:18:40,310 --> 00:18:37,120
on the other hand can catalyze the

493
00:18:43,590 --> 00:18:40,320
assembly of bilayer membrane

494
00:18:44,390 --> 00:18:43,600
as shown by uh marty and shelly

495
00:18:46,390 --> 00:18:44,400
and

496
00:18:48,310 --> 00:18:46,400
amazingly can actually bring them all

497
00:18:51,350 --> 00:18:48,320
together so it's bringing together the

498
00:18:53,909 --> 00:18:51,360
two main components of what we think of

499
00:18:54,830 --> 00:18:53,919
as a simple protocol

500
00:18:58,789 --> 00:18:54,840
so

501
00:19:00,310 --> 00:18:58,799
it's hard not to um

502
00:19:01,830 --> 00:19:00,320
to take the lesson from that the

503
00:19:06,230 --> 00:19:01,840
minerals may have played an important

504
00:19:10,390 --> 00:19:07,990
okay so

505
00:19:12,230 --> 00:19:10,400
that brings us that gives us the basic

506
00:19:13,350 --> 00:19:12,240
structure but it doesn't really tell us

507
00:19:15,190 --> 00:19:13,360
very much

508
00:19:17,430 --> 00:19:15,200
about

509
00:19:19,430 --> 00:19:17,440
how the membrane could grow and divide

510
00:19:21,590 --> 00:19:19,440
we have a continuous cycle of growth and

511
00:19:23,510 --> 00:19:21,600
division

512
00:19:25,110 --> 00:19:23,520
it also doesn't tell us anything about

513
00:19:27,430 --> 00:19:25,120

how we're going to get that genetic

514
00:19:30,470 --> 00:19:27,440
material to replicate

515
00:19:32,310 --> 00:19:30,480
okay so uh first i want to go over

516
00:19:33,590 --> 00:19:32,320
some additional experiments that were

517
00:19:36,950 --> 00:19:33,600
done by

518
00:19:40,150 --> 00:19:36,960
marty hanchik and shelly fujikawa

519
00:19:41,430 --> 00:19:40,160
on uh vertical growth and division

520
00:19:42,230 --> 00:19:41,440
all right

521
00:19:43,430 --> 00:19:42,240
very

522
00:19:46,150 --> 00:19:43,440
quickly

523
00:19:47,270 --> 00:19:46,160
uh the basic idea as i alluded to

524
00:19:49,590 --> 00:19:47,280
earlier

525
00:19:51,750 --> 00:19:49,600
is that you can take prove this thing

526
00:19:52,630 --> 00:19:51,760
that's a cult

527
00:19:54,710 --> 00:19:52,640
which

528
00:19:56,630 --> 00:19:54,720
basically just sits there if they're

529
00:19:58,630 --> 00:19:56,640
left alone

530
00:20:00,710 --> 00:19:58,640
and you can add

531
00:20:01,990 --> 00:20:00,720
additional fatty acids in the form of

532
00:20:03,830 --> 00:20:02,000
micelles

533
00:20:06,710 --> 00:20:03,840
and when they're mixed

534
00:20:08,870 --> 00:20:06,720
molecules from the micelles

535
00:20:11,430 --> 00:20:08,880
tend to integrate into the bilayer

536
00:20:12,870 --> 00:20:11,440
membrane so that it grows

537
00:20:15,190 --> 00:20:12,880
and

538
00:20:17,909 --> 00:20:15,200

that was first seen in cryo-em

539
00:20:19,190 --> 00:20:17,919
experiments by the luigi lab

540
00:20:21,669 --> 00:20:19,200
and

541
00:20:23,750 --> 00:20:21,679
we tend to

542
00:20:25,350 --> 00:20:23,760
use a fluorescence-based assay to follow

543
00:20:27,110 --> 00:20:25,360
the membrane growth

544
00:20:28,630 --> 00:20:27,120
and here the idea is just that

545
00:20:32,149 --> 00:20:28,640
intercalated

546
00:20:35,190 --> 00:20:32,159
donor and acceptor fluorescence sides

547
00:20:37,430 --> 00:20:35,200
get spread apart as they get diluted

548
00:20:40,230 --> 00:20:37,440
when additional exophiles enter into

549
00:20:42,789 --> 00:20:40,240
this membrane so the change in fret

550
00:20:44,549 --> 00:20:42,799
efficiency gives you a real-time assay

551
00:20:45,750 --> 00:20:44,559
that you can use to follow vesicle

552
00:20:48,149 --> 00:20:45,760
growth

553
00:20:49,430 --> 00:20:48,159
and in doing lots of experience of that

554
00:20:51,750 --> 00:20:49,440
sort

555
00:20:52,470 --> 00:20:51,760
marty and shelly were able to show that

556
00:20:57,029 --> 00:20:52,480
the

557
00:20:59,909 --> 00:20:57,039
sense that as much as 90

558
00:21:03,110 --> 00:20:59,919
of the newly added fatty acids

559
00:21:04,870 --> 00:21:03,120
could be incorporated into a pre-formed

560
00:21:08,390 --> 00:21:04,880
vesicle

561
00:21:11,990 --> 00:21:08,400
okay so once they're grown

562
00:21:14,310 --> 00:21:12,000
it turns out that there's a very easy

563
00:21:16,870 --> 00:21:14,320

and deficient and somewhat artificial

564
00:21:18,310 --> 00:21:16,880
way of making them divide and that is to

565
00:21:19,750 --> 00:21:18,320
simply take

566
00:21:22,390 --> 00:21:19,760
large vesicles

567
00:21:23,430 --> 00:21:22,400
and force them to small pores

568
00:21:25,750 --> 00:21:23,440
and

569
00:21:26,789 --> 00:21:25,760
somehow on the other side become small

570
00:21:29,110 --> 00:21:26,799
ethical

571
00:21:33,669 --> 00:21:31,750
exactly what happens it's still not

572
00:21:36,549 --> 00:21:33,679
completely clear

573
00:21:39,750 --> 00:21:36,559
but we do know that most of the contents

574
00:21:41,590 --> 00:21:39,760
remain inside okay the surface to volume

575
00:21:43,029 --> 00:21:41,600
ratio changes so of course you have to

576
00:21:45,270 --> 00:21:43,039
lose some

577
00:21:47,190 --> 00:21:45,280
of the contents but basically

578
00:21:48,950 --> 00:21:47,200
the the uh

579
00:21:51,830 --> 00:21:48,960
the aqueous contents whatever they are

580
00:21:53,750 --> 00:21:51,840
retain remain at the same concentration

581
00:21:57,190 --> 00:21:53,760
so that tells us that the vesicle is

582
00:21:58,950 --> 00:21:57,200
nothing ripped apart and then reforming

583
00:22:00,070 --> 00:21:58,960
once it exits the

584
00:22:01,430 --> 00:22:00,080
pore

585
00:22:03,430 --> 00:22:01,440
but rather

586
00:22:07,029 --> 00:22:03,440
a spherical vesicle is probably being

587
00:22:08,870 --> 00:22:07,039
elongated and then pinched apart

588
00:22:11,029 --> 00:22:08,880

more like a biological

589
00:22:13,750 --> 00:22:11,039
division process

590
00:22:15,590 --> 00:22:13,760
so that's also very efficient and that

591
00:22:17,350 --> 00:22:15,600
allows you to

592
00:22:19,029 --> 00:22:17,360
combine the

593
00:22:21,669 --> 00:22:19,039
process of growth

594
00:22:24,390 --> 00:22:21,679
and division to make a cycle

595
00:22:26,149 --> 00:22:24,400
that can be repeated indefinitely

596
00:22:28,789 --> 00:22:26,159
so for example in this experiment we

597
00:22:30,310 --> 00:22:28,799
start off with small vesicles

598
00:22:31,669 --> 00:22:30,320
that grow

599
00:22:32,630 --> 00:22:31,679
divided

600
00:22:37,350 --> 00:22:32,640
grow

601
00:22:38,830 --> 00:22:37,360
cetera

602
00:22:41,830 --> 00:22:38,840
and in every

603
00:22:44,310 --> 00:22:41,840
generation the contents are distributed

604
00:22:46,549 --> 00:22:44,320
to daughter classical and the membrane

605
00:22:48,070 --> 00:22:46,559
material is also distributed to the

606
00:22:49,350 --> 00:22:48,080
daughter vesicle

607
00:22:51,750 --> 00:22:49,360
so

608
00:22:53,350 --> 00:22:51,760
in that sense it's like a primitive form

609
00:22:55,990 --> 00:22:53,360
of cell division

610
00:22:58,149 --> 00:22:56,000
okay but only focusing on the membrane

611
00:22:59,430 --> 00:22:58,159
part of the cell

612
00:23:02,310 --> 00:22:59,440
okay

613
00:23:04,310 --> 00:23:02,320

all right so uh so what about

614
00:23:06,390 --> 00:23:04,320
the genetic contents we need to have

615
00:23:07,590 --> 00:23:06,400
some kind of molecule that can encode

616
00:23:10,710 --> 00:23:07,600
information

617
00:23:13,510 --> 00:23:10,720
and have the potential to do something

618
00:23:17,590 --> 00:23:13,520
useful for the cell in its cycle of

619
00:23:23,750 --> 00:23:21,110
and so here everything that we've done

620
00:23:26,149 --> 00:23:23,760
completely built on the pioneering work

621
00:23:27,750 --> 00:23:26,159
of the late leslie orgo

622
00:23:29,510 --> 00:23:27,760
who for decades

623
00:23:31,190 --> 00:23:29,520
studied a lot with his students and

624
00:23:35,669 --> 00:23:31,200
colleagues

625
00:23:38,789 --> 00:23:35,679
the chemistry of non-enzymatic

626
00:23:41,510 --> 00:23:38,799
copying of templates primarily rna but

627
00:23:43,350 --> 00:23:41,520
also dna and occasionally

628
00:23:45,510 --> 00:23:43,360
you ventured a little bit further away

629
00:23:48,630 --> 00:23:45,520
into some related polymers which is

630
00:23:50,630 --> 00:23:48,640
where we've taken up the excel

631
00:23:51,669 --> 00:23:50,640
what i'm showing you here is a classical

632
00:23:54,630 --> 00:23:51,679
type of

633
00:23:59,350 --> 00:23:57,750
chemical uh copying of an rna template

634
00:24:01,430 --> 00:23:59,360
strand this is an experiment done by

635
00:24:02,470 --> 00:24:01,440
david horning several years ago in the

636
00:24:05,750 --> 00:24:02,480
lab

637
00:24:07,190 --> 00:24:05,760
and it shows you that uh this kind of

638
00:24:08,470 --> 00:24:07,200

copying is

639
00:24:13,190 --> 00:24:08,480
uh

640
00:24:17,510 --> 00:24:13,200
reasonably efficient so here we're

641
00:24:19,190 --> 00:24:17,520
adding g's to copy a short stretch of

642
00:24:21,669 --> 00:24:19,200
sea template

643
00:24:23,430 --> 00:24:21,679
and you can see over a couple of days

644
00:24:25,190 --> 00:24:23,440
the primer grows by a couple of

645
00:24:26,950 --> 00:24:25,200
nucleotides

646
00:24:28,630 --> 00:24:26,960
it works pretty well and pretty

647
00:24:30,710 --> 00:24:28,640
accurately

648
00:24:34,950 --> 00:24:30,720
when with c and g's

649
00:24:37,590 --> 00:24:34,960
a lot more slowly with ace and used

650
00:24:39,590 --> 00:24:37,600
and when you have the possibility for gu

651
00:24:42,630 --> 00:24:39,600
level space fairing

652
00:24:44,310 --> 00:24:42,640
the accuracy also tends to go down

653
00:24:46,470 --> 00:24:44,320
so the basic problem

654
00:24:48,870 --> 00:24:46,480
that we've been trying to overcome

655
00:24:51,669 --> 00:24:48,880
are to find a chemical

656
00:24:54,630 --> 00:24:51,679
replication system that is fast

657
00:24:56,870 --> 00:24:54,640
efficient and accurate

658
00:24:59,190 --> 00:24:56,880
in order to do that we're stepping away

659
00:25:01,269 --> 00:24:59,200
from the requirements that everything

660
00:25:03,590 --> 00:25:01,279
absolutely has to be prebiotically

661
00:25:06,310 --> 00:25:03,600
reasonable we just want to find

662
00:25:07,990 --> 00:25:06,320
some chemical system that works

663
00:25:10,310 --> 00:25:08,000

and the hope is that eventually that

664
00:25:12,789 --> 00:25:10,320
will give us ideas that will let us go

665
00:25:17,510 --> 00:25:12,799
back to more plausible

666
00:25:22,310 --> 00:25:21,269
okay so uh in order to do that

667
00:25:23,590 --> 00:25:22,320
what we're

668
00:25:25,590 --> 00:25:23,600
looking at

669
00:25:28,149 --> 00:25:25,600
are monomers

670
00:25:31,750 --> 00:25:30,390
different in several ways from

671
00:25:33,909 --> 00:25:31,760
the modern

672
00:25:36,390 --> 00:25:33,919
nucleophile triphosphates that are used

673
00:25:37,990 --> 00:25:36,400
to make rna and dna

674
00:25:39,830 --> 00:25:38,000
so over

675
00:25:41,430 --> 00:25:39,840
on this side we have the modern

676
00:25:43,669 --> 00:25:41,440
biological monomers with their

677
00:25:46,149 --> 00:25:43,679
triphosphates

678
00:25:48,310 --> 00:25:46,159
and these require very sophisticated

679
00:25:50,230 --> 00:25:48,320
enzymes to get the large reed

680
00:25:52,149 --> 00:25:50,240
enhancements that are required to make

681
00:25:55,110 --> 00:25:52,159
rna and dna

682
00:25:58,549 --> 00:25:56,870
they're also very highly charged which

683
00:26:00,549 --> 00:25:58,559
is good because that way they don't leak

684
00:26:03,990 --> 00:26:00,559
out itself

685
00:26:06,230 --> 00:26:04,000
what we need is something that is

686
00:26:08,470 --> 00:26:06,240
less charged so it can get across the

687
00:26:10,630 --> 00:26:08,480
membrane into the cell

688
00:26:13,430 --> 00:26:10,640

we want it to be more reactive

689
00:26:15,830 --> 00:26:13,440
so we use a harder leaving group things

690
00:26:18,710 --> 00:26:15,840
like imidazole

691
00:26:21,190 --> 00:26:18,720
we use a harder nucleophile so typically

692
00:26:22,789 --> 00:26:21,200
an amine instead of a hydroxyl it's a

693
00:26:25,110 --> 00:26:22,799
nucleophile

694
00:26:27,590 --> 00:26:25,120
we also can play around with the nuclear

695
00:26:30,470 --> 00:26:27,600
base to try to

696
00:26:34,710 --> 00:26:30,480
modulate the strength of base pairing

697
00:26:37,830 --> 00:26:35,590
we

698
00:26:38,789 --> 00:26:37,840
can also play around with the sugar part

699
00:26:40,789 --> 00:26:38,799
of the

700
00:26:43,190 --> 00:26:40,799
monomer so that we make polymers with

701
00:26:46,630 --> 00:26:43,200
different sugar phosphate backbone

702
00:26:47,430 --> 00:26:46,640
and that way we can play around with

703
00:26:49,110 --> 00:26:47,440
the

704
00:26:50,710 --> 00:26:49,120
effects of

705
00:26:52,470 --> 00:26:50,720
conformational constraints or

706
00:26:54,230 --> 00:26:52,480
flexibility

707
00:26:56,950 --> 00:26:54,240
and so on

708
00:26:58,390 --> 00:26:56,960
so when we use these modified building

709
00:27:00,470 --> 00:26:58,400
blocks

710
00:27:03,830 --> 00:27:00,480
these are the kinds of

711
00:27:08,630 --> 00:27:03,840
nucleic acids that we end up making so

712
00:27:13,269 --> 00:27:10,630
phosphate diester types

713
00:27:16,310 --> 00:27:13,279

of polymers

714
00:27:18,549 --> 00:27:16,320
at the bottom you see the corresponding

715
00:27:24,070 --> 00:27:18,559
nitrogen substituted versions which are

716
00:27:30,549 --> 00:27:24,950
so

717
00:27:33,110 --> 00:27:30,559
the false remedy analog of dna

718
00:27:35,190 --> 00:27:33,120
next to it we have the same thing except

719
00:27:37,510 --> 00:27:35,200
with the two prime five prime

720
00:27:38,870 --> 00:27:37,520
linkage instead of the standard three

721
00:27:40,789 --> 00:27:38,880
prime linkage

722
00:27:42,149 --> 00:27:40,799
and this is interesting because in a lot

723
00:27:44,470 --> 00:27:42,159
of

724
00:27:45,990 --> 00:27:44,480
organelles experiments they found

725
00:27:47,430 --> 00:27:46,000
that

726
00:27:48,630 --> 00:27:47,440
with rna

727
00:27:50,549 --> 00:27:48,640
monomers

728
00:27:52,630 --> 00:27:50,559
you made a mixture of two prime and

729
00:27:57,110 --> 00:27:52,640
three prime linkages which seems that

730
00:28:00,470 --> 00:27:58,389
over here

731
00:28:02,630 --> 00:28:00,480
we have a slightly different sugar of

732
00:28:04,789 --> 00:28:02,640
four carbon sugar three oh

733
00:28:08,630 --> 00:28:04,799
so this makes q a

734
00:28:10,789 --> 00:28:08,640
um a nucleic acid uh first made in the

735
00:28:12,950 --> 00:28:10,799
essential group

736
00:28:15,510 --> 00:28:12,960
which remarkably has a shorter

737
00:28:25,750 --> 00:28:15,520
backbone repeat unit and yet it's still

738
00:28:32,789 --> 00:28:28,789

on the far side we have glycerol nucleic

739
00:28:36,710 --> 00:28:32,799
acid with an acyclic

740
00:28:39,750 --> 00:28:36,720
backbone repeat unit again

741
00:28:41,750 --> 00:28:39,760
very different in structure from dna

742
00:28:44,549 --> 00:28:41,760
but also a perfectly good base faring

743
00:28:46,870 --> 00:28:44,559
system

744
00:28:49,750 --> 00:28:46,880
so by looking at these different

745
00:28:52,549 --> 00:28:49,760
polymers we can assess

746
00:28:53,590 --> 00:28:52,559
different factors that might contribute

747
00:28:57,430 --> 00:28:53,600
to

748
00:28:59,430 --> 00:28:57,440
replication efficiency and accuracy

749
00:29:00,710 --> 00:28:59,440
such as how well constrained or how

750
00:29:03,510 --> 00:29:00,720
flexible

751
00:29:05,669 --> 00:29:03,520
the template is how pre-organized

752
00:29:08,310 --> 00:29:05,679
templates are

753
00:29:10,710 --> 00:29:08,320
how the helical geometry influences the

754
00:29:12,389 --> 00:29:10,720
reaction rate and so on

755
00:29:14,710 --> 00:29:12,399
so i'm not going to go through all of

756
00:29:17,990 --> 00:29:14,720
the chemistry involved

757
00:29:19,269 --> 00:29:18,000
to make these polymers and the activated

758
00:29:20,950 --> 00:29:19,279
monomers

759
00:29:24,070 --> 00:29:20,960
i'm just going to show you a couple of

760
00:29:26,070 --> 00:29:24,080
examples from our more most successful

761
00:29:28,230 --> 00:29:26,080
experiments

762
00:29:30,149 --> 00:29:28,240
okay so

763
00:29:31,269 --> 00:29:30,159

so here's one example

764
00:29:32,549 --> 00:29:31,279
where we're

765
00:29:36,549 --> 00:29:32,559
copying

766
00:29:37,510 --> 00:29:36,559
a dna template which is a stretch of c

767
00:29:40,789 --> 00:29:37,520
using

768
00:29:41,909 --> 00:29:40,799
this monomer which is basically standard

769
00:29:43,590 --> 00:29:41,919
g

770
00:29:46,470 --> 00:29:43,600
uh hooked up

771
00:29:48,549 --> 00:29:46,480
to deoxyribose with an immunogroup in

772
00:29:50,950 --> 00:29:48,559
the two prime position

773
00:29:53,190 --> 00:29:50,960
and over here on the phosphate we have

774
00:29:55,669 --> 00:29:53,200
imidazole as a leaving group

775
00:29:58,389 --> 00:29:55,679
so it's a very reactive monomer

776
00:30:01,830 --> 00:29:58,399
when you add it to this primer template

777
00:30:04,470 --> 00:30:01,840
you can watch the primer get extended

778
00:30:07,110 --> 00:30:04,480
with a chain of g's

779
00:30:09,430 --> 00:30:07,120
as it copies the

780
00:30:12,149 --> 00:30:09,440
see a section of the template and then

781
00:30:13,830 --> 00:30:12,159
the reaction stops when it hits the a

782
00:30:15,590 --> 00:30:13,840
you can see that over here here's the

783
00:30:17,830 --> 00:30:15,600
starting primer

784
00:30:19,669 --> 00:30:17,840
over the course of a few hours you see

785
00:30:21,110 --> 00:30:19,679
all the intermediates

786
00:30:23,669 --> 00:30:21,120
and then by six hours you're

787
00:30:25,430 --> 00:30:23,679
accumulating full length products

788
00:30:27,510 --> 00:30:25,440

and at the end of the day

789
00:30:33,190 --> 00:30:27,520
the reaction's pretty much stopped

790
00:30:38,310 --> 00:30:35,269
this is a reaction that is done

791
00:30:40,389 --> 00:30:38,320
completely without any enzyme

792
00:30:46,070 --> 00:30:40,399
it's just a chemical

793
00:30:51,750 --> 00:30:48,149
what we would like is to have something

794
00:30:54,549 --> 00:30:51,760
like this that it's completely general

795
00:30:57,430 --> 00:30:54,559
that works with all bases it works with

796
00:31:00,230 --> 00:30:57,440
mixed sequence templates

797
00:31:02,470 --> 00:31:00,240
and it works very high accuracy

798
00:31:04,070 --> 00:31:02,480
and so we're not there yet but that's

799
00:31:07,990 --> 00:31:04,080
what we're going to be working on a lot

800
00:31:11,909 --> 00:31:09,110
the next

801
00:31:13,750 --> 00:31:11,919
slide here shows you a similar kind of

802
00:31:15,669 --> 00:31:13,760
example

803
00:31:16,870 --> 00:31:15,679
where the monomer

804
00:31:20,070 --> 00:31:16,880
has

805
00:31:21,909 --> 00:31:20,080
the reactive nucleophile on the normal

806
00:31:23,669 --> 00:31:21,919
three prime position

807
00:31:25,750 --> 00:31:23,679
and in this case

808
00:31:27,590 --> 00:31:25,760
the monomer has is an a

809
00:31:29,750 --> 00:31:27,600
residue and this

810
00:31:30,950 --> 00:31:29,760
activated monomer can copy a stretch of

811
00:31:33,350 --> 00:31:30,960
keys

812
00:31:35,350 --> 00:31:33,360
and so again over here

813
00:31:38,549 --> 00:31:35,360

you can see over

814
00:31:41,110 --> 00:31:38,559
the course of a day or two

815
00:31:43,350 --> 00:31:41,120
the accumulation of full length

816
00:31:46,310 --> 00:31:43,360
copied template

817
00:31:48,149 --> 00:31:46,320
the thing that's a little bit ironic is

818
00:31:51,350 --> 00:31:48,159
that

819
00:31:53,269 --> 00:31:51,360
this three prime x the two prime amino

820
00:31:55,830 --> 00:31:53,279
nucleotides

821
00:31:58,950 --> 00:31:55,840
system works well in copying a's and

822
00:32:00,710 --> 00:31:58,960
keys but pooling for g's and c's

823
00:32:02,950 --> 00:32:00,720
the one i showed you before works well

824
00:32:04,630 --> 00:32:02,960
with j's and c so poorly

825
00:32:06,789 --> 00:32:04,640
for age of keys

826
00:32:09,110 --> 00:32:06,799
we don't really understand yet

827
00:32:11,350 --> 00:32:09,120
completely why that's the case

828
00:32:14,549 --> 00:32:11,360
um but we have some ideas we're trying

829
00:32:16,630 --> 00:32:14,559
to sort this out and hopefully in the

830
00:32:20,070 --> 00:32:16,640
end we'll converge on a system that can

831
00:32:23,029 --> 00:32:20,080
copy any mix sequence

832
00:32:25,909 --> 00:32:23,039
okay but for now we can use this kind of

833
00:32:29,590 --> 00:32:25,919
chemical template copier

834
00:32:31,509 --> 00:32:29,600
as a model to look at some of the

835
00:32:34,710 --> 00:32:31,519
more interesting questions about the

836
00:32:37,590 --> 00:32:34,720
interaction of nucleic acid replication

837
00:32:39,590 --> 00:32:37,600
and vesicle replication

838
00:32:44,549 --> 00:32:39,600

okay so that's what i want to head into

839
00:32:48,870 --> 00:32:47,190
so one of the main issues

840
00:32:51,110 --> 00:32:48,880
in thinking about this

841
00:32:52,149 --> 00:32:51,120
is how to get those activated building

842
00:32:54,549 --> 00:32:52,159
blocks

843
00:32:56,950 --> 00:32:54,559
across the membrane bilayer to the

844
00:32:59,029 --> 00:32:56,960
inside of the vesicle where we want

845
00:33:01,669 --> 00:32:59,039
nucleic acid replication to be taking

846
00:33:05,669 --> 00:33:03,669
and we kind of avoided getting into

847
00:33:07,269 --> 00:33:05,679
these experiments for a long time

848
00:33:09,350 --> 00:33:07,279
because we were just used to thinking

849
00:33:11,990 --> 00:33:09,360
about modern biological membranes and

850
00:33:14,470 --> 00:33:12,000
the idea that something as large

851
00:33:17,509 --> 00:33:14,480
as poor and charged as a nucleotide

852
00:33:19,669 --> 00:33:17,519
would get across a bilayer membrane

853
00:33:20,870 --> 00:33:19,679
that any kind of help from a channel or

854
00:33:23,029 --> 00:33:20,880
a pump

855
00:33:25,509 --> 00:33:23,039
seemed kind of crazy

856
00:33:27,029 --> 00:33:25,519
but in fact uh when you make the

857
00:33:29,990 --> 00:33:27,039
membranes out of the right building

858
00:33:30,950 --> 00:33:30,000
blocks it turns out that it can work

859
00:33:31,990 --> 00:33:30,960
okay

860
00:33:34,630 --> 00:33:32,000
so

861
00:33:36,549 --> 00:33:34,640
we started looking at permeability

862
00:33:38,630 --> 00:33:36,559
several years ago

863
00:33:39,830 --> 00:33:38,640

with somewhat smaller molecules just the

864
00:33:41,430 --> 00:33:39,840
sugars

865
00:33:43,590 --> 00:33:41,440
and these are experiments that were done

866
00:33:44,630 --> 00:33:43,600
uh by michael sacrado when he was in my

867
00:33:46,230 --> 00:33:44,640
lab

868
00:33:47,669 --> 00:33:46,240
and michael used

869
00:33:49,750 --> 00:33:47,679
a very simple

870
00:33:53,110 --> 00:33:49,760
intuitive asset

871
00:33:55,909 --> 00:33:53,120
that is based on making vesicles with an

872
00:33:59,269 --> 00:33:55,919
encapsulated fluorescent dye

873
00:34:02,389 --> 00:33:59,279
when you add a solute such as the sugar

874
00:34:04,070 --> 00:34:02,399
two vesicles with this dye calcium

875
00:34:06,549 --> 00:34:04,080
the first thing that happens is that

876
00:34:09,430 --> 00:34:06,559
water rushes out of the vesicles

877
00:34:11,750 --> 00:34:09,440
to equalize the osmotic pressure

878
00:34:14,950 --> 00:34:11,760
the result is that the dye on the inside

879
00:34:15,909 --> 00:34:14,960
gets more complicated more concentrated

880
00:34:17,669 --> 00:34:15,919
and

881
00:34:19,829 --> 00:34:17,679
the fluorescence in this case is

882
00:34:21,669 --> 00:34:19,839
self-clenching so this fluorescence

883
00:34:23,750 --> 00:34:21,679
intensity goes down

884
00:34:26,069 --> 00:34:23,760
and then more slowly

885
00:34:28,629 --> 00:34:26,079
as solute and water

886
00:34:31,990 --> 00:34:28,639
gradually diffuse into the vesicle

887
00:34:34,310 --> 00:34:32,000
it relaxes back to its initial

888
00:34:35,510 --> 00:34:34,320

spherical state and the dye becomes

889
00:34:37,270 --> 00:34:35,520
diluted

890
00:34:39,510 --> 00:34:37,280
the crunching is reduced and the

891
00:34:42,069 --> 00:34:39,520
fluorescence intensity returns to the

892
00:34:44,310 --> 00:34:42,079
original value

893
00:34:46,069 --> 00:34:44,320
okay so i'm just going to show you

894
00:34:47,190 --> 00:34:46,079
one of michael's experiments in which he

895
00:34:49,270 --> 00:34:47,200
compared

896
00:34:50,550 --> 00:34:49,280
a set of four sugars

897
00:34:51,829 --> 00:34:50,560
and these are

898
00:34:54,470 --> 00:34:51,839
the four

899
00:34:57,910 --> 00:34:54,480
ribose and it's three diastereomers

900
00:34:59,910 --> 00:34:57,920
elixirs ravenous and xylose so they're

901
00:35:02,870 --> 00:34:59,920
very sugars very

902
00:35:05,510 --> 00:35:02,880
similar sugars they differ only in

903
00:35:08,150 --> 00:35:05,520
whether the two and three prime hydroxyl

904
00:35:10,630 --> 00:35:08,160
are facing up or down relative to the

905
00:35:14,829 --> 00:35:10,640
plane of the of the ring

906
00:35:16,630 --> 00:35:14,839
so the results were pretty surprising

907
00:35:17,670 --> 00:35:16,640
and

908
00:35:21,109 --> 00:35:17,680
the main

909
00:35:23,750 --> 00:35:21,119
thing we saw was that ribose

910
00:35:25,430 --> 00:35:23,760
entered these vesicles three to ten

911
00:35:27,829 --> 00:35:25,440
times faster

912
00:35:32,069 --> 00:35:27,839
than its close relative

913
00:35:36,470 --> 00:35:34,550

so we still don't exactly know why that

914
00:35:38,550 --> 00:35:36,480
is we have some models there are

915
00:35:39,589 --> 00:35:38,560
performance that could be done to test

916
00:35:40,870 --> 00:35:39,599
us

917
00:35:44,390 --> 00:35:40,880
but the

918
00:35:46,950 --> 00:35:44,400
strikingly faster permeability of rivas

919
00:35:49,829 --> 00:35:46,960
i think is very suggestive

920
00:35:51,750 --> 00:35:49,839
and maybe the idea is that maybe this

921
00:35:53,829 --> 00:35:51,760
kind of unexpected

922
00:35:55,349 --> 00:35:53,839
physical property

923
00:35:57,510 --> 00:35:55,359
was just one of

924
00:36:00,630 --> 00:35:57,520
perhaps many contributing factors that

925
00:36:02,550 --> 00:36:00,640
led to the emergence of ribose as the

926
00:36:04,150 --> 00:36:02,560
dominant sugar in

927
00:36:05,750 --> 00:36:04,160
genetic polymers

928
00:36:07,430 --> 00:36:05,760
so the idea is if you had a very

929
00:36:08,950 --> 00:36:07,440
primitive cell with some internal

930
00:36:12,550 --> 00:36:08,960
metabolism

931
00:36:15,190 --> 00:36:12,560
that made use of sugars from the outside

932
00:36:18,630 --> 00:36:15,200
any cell that relied on ribose

933
00:36:21,190 --> 00:36:18,640
would have much easier faster access to

934
00:36:23,910 --> 00:36:21,200
that substrate than a competing cell

935
00:36:25,109 --> 00:36:23,920
that required for example xylo or

936
00:36:27,109 --> 00:36:25,119
abnormal

937
00:36:29,109 --> 00:36:27,119
okay so just a physical

938
00:36:31,190 --> 00:36:29,119

difference in the permeability of argos

939
00:36:32,630 --> 00:36:31,200
would confer an advantage

940
00:36:34,230 --> 00:36:32,640
maybe that

941
00:36:36,069 --> 00:36:34,240
is something

942
00:36:38,630 --> 00:36:36,079
relevant to the emergence of the rna

943
00:36:42,310 --> 00:36:40,470
okay so

944
00:36:45,510 --> 00:36:42,320
having seen that we could get molecules

945
00:36:48,150 --> 00:36:45,520
of polar and sugars across these uh

946
00:36:50,710 --> 00:36:48,160
across fatty acid membranes

947
00:36:53,990 --> 00:36:50,720
uh reasonably rapidly

948
00:36:55,990 --> 00:36:54,000
it encouraged us to look at larger and

949
00:36:57,270 --> 00:36:56,000
more polar molecules namely the

950
00:36:59,829 --> 00:36:57,280
nucleotides

951
00:37:02,470 --> 00:36:59,839
that we need for uh

952
00:37:04,870 --> 00:37:02,480
for internal replication

953
00:37:07,270 --> 00:37:04,880
and so this is work that was done by

954
00:37:10,550 --> 00:37:07,280
sharif nancy when he was a postdoc in

955
00:37:11,510 --> 00:37:10,560
the lab up until about a year ago

956
00:37:14,390 --> 00:37:11,520
and

957
00:37:16,550 --> 00:37:14,400
what sharif did to look at

958
00:37:18,390 --> 00:37:16,560
the movement of nucleotides across a

959
00:37:21,270 --> 00:37:18,400
bilayer membrane was just to prepare

960
00:37:24,790 --> 00:37:21,280
vesicles that were loaded up

961
00:37:27,109 --> 00:37:24,800
with a particular nucleotide

962
00:37:29,910 --> 00:37:27,119
and then he would just measure the rate

963
00:37:31,109 --> 00:37:29,920

at which material leaked out using size

964
00:37:33,190 --> 00:37:31,119
exclusion

965
00:37:35,190 --> 00:37:33,200
chromatography

966
00:37:37,190 --> 00:37:35,200
and what he found was that

967
00:37:38,710 --> 00:37:37,200
you can see from these these top lines

968
00:37:39,589 --> 00:37:38,720
represent data

969
00:37:40,470 --> 00:37:39,599
um

970
00:37:42,950 --> 00:37:40,480
from

971
00:37:44,630 --> 00:37:42,960
normal nucleotide monophosphates so

972
00:37:46,630 --> 00:37:44,640
these molecules have two negative

973
00:37:49,589 --> 00:37:46,640
charges on the phosphate

974
00:37:51,750 --> 00:37:49,599
and they leak out very slowly only a few

975
00:37:54,790 --> 00:37:51,760
percent over a day

976
00:37:57,109 --> 00:37:54,800
but when you look at the

977
00:37:58,630 --> 00:37:57,119
activated nucleotides

978
00:38:00,950 --> 00:37:58,640
the ones that have something like

979
00:38:03,030 --> 00:38:00,960
imidazole on the phosphate

980
00:38:05,829 --> 00:38:03,040
they have one less charge

981
00:38:06,870 --> 00:38:05,839
and now you see that they leak out a lot

982
00:38:09,190 --> 00:38:06,880
faster

983
00:38:11,030 --> 00:38:09,200
in fact they equilibrate over with a

984
00:38:14,069 --> 00:38:11,040
half time of about

985
00:38:18,950 --> 00:38:15,109
so

986
00:38:20,630 --> 00:38:18,960
system which are these

987
00:38:22,150 --> 00:38:20,640
14 carbon

988
00:38:23,670 --> 00:38:22,160

fatty acids

989
00:38:25,829 --> 00:38:23,680
and

990
00:38:27,589 --> 00:38:25,839
mixed in with the glycerol ester

991
00:38:30,829 --> 00:38:27,599
we can look at the same thing using a

992
00:38:33,349 --> 00:38:30,839
more prebiotically plausible mixture

993
00:38:36,790 --> 00:38:33,359
of anti-files

994
00:38:38,069 --> 00:38:36,800
and that data is shown here so this is a

995
00:38:39,750 --> 00:38:38,079
set of

996
00:38:42,310 --> 00:38:39,760
anti-files that are

997
00:38:44,230 --> 00:38:42,320
10 carbon saturated chains

998
00:38:47,349 --> 00:38:44,240
and we see very similar

999
00:38:50,069 --> 00:38:47,359
data almost superimposable

1000
00:38:52,710 --> 00:38:50,079
so activated nucleotides again

1001
00:38:55,349 --> 00:38:52,720
can can get across these these membranes

1002
00:38:57,670 --> 00:38:55,359
in a reasonable time scale

1003
00:38:59,990 --> 00:38:57,680
okay so now we have

1004
00:39:01,430 --> 00:39:00,000
in hand the chemistry of template

1005
00:39:03,910 --> 00:39:01,440
copying

1006
00:39:07,030 --> 00:39:03,920
we have this data which shows that the

1007
00:39:09,030 --> 00:39:07,040
monomers can get the theory into the

1008
00:39:11,270 --> 00:39:09,040
inside of the vesicle

1009
00:39:13,750 --> 00:39:11,280
and so we thought maybe we could just

1010
00:39:15,910 --> 00:39:13,760
put it all together

1011
00:39:17,349 --> 00:39:15,920
sorry before i get to that let me just

1012
00:39:19,910 --> 00:39:17,359
tell you how we think things are

1013
00:39:23,270 --> 00:39:19,920

actually getting across the membrane

1014
00:39:25,349 --> 00:39:23,280
so these are uh two earlier

1015
00:39:26,550 --> 00:39:25,359
models it's a classical defaultation

1016
00:39:29,030 --> 00:39:26,560
model where

1017
00:39:31,190 --> 00:39:29,040
to get a small molecule or an ion across

1018
00:39:32,470 --> 00:39:31,200
the membrane you strip off the water of

1019
00:39:33,670 --> 00:39:32,480
solvation

1020
00:39:35,589 --> 00:39:33,680
essentially dissolve it in the

1021
00:39:37,670 --> 00:39:35,599
hydrophobic interior

1022
00:39:39,670 --> 00:39:37,680
and then it exits the other side

1023
00:39:41,270 --> 00:39:39,680
that's just way too energetically

1024
00:39:42,870 --> 00:39:41,280
expensive

1025
00:39:44,870 --> 00:39:42,880
to be plausible

1026
00:39:47,190 --> 00:39:44,880
the rate of this kind of process would

1027
00:39:49,670 --> 00:39:47,200
be vanishingly small

1028
00:39:52,390 --> 00:39:49,680
the other possibility is that

1029
00:39:54,470 --> 00:39:52,400
these membranes make transient force

1030
00:39:56,069 --> 00:39:54,480
that sort of open up and let stuff of

1031
00:39:58,069 --> 00:39:56,079
course

1032
00:39:58,950 --> 00:39:58,079
we know that's not really the case

1033
00:40:02,150 --> 00:39:58,960
because

1034
00:40:04,230 --> 00:40:02,160
if the pores had any significant size at

1035
00:40:07,510 --> 00:40:04,240
least they would let anything across

1036
00:40:09,030 --> 00:40:07,520
equally we wouldn't see specificity just

1037
00:40:11,670 --> 00:40:09,040
such as we see between the different

1038
00:40:14,870 --> 00:40:11,680

sugars or the different nucleotides

1039
00:40:17,349 --> 00:40:14,880
so the model that we favor

1040
00:40:19,510 --> 00:40:17,359
is a kind of hybrid

1041
00:40:21,430 --> 00:40:19,520
transient poor

1042
00:40:23,349 --> 00:40:21,440
model in which

1043
00:40:25,829 --> 00:40:23,359
the solutes

1044
00:40:28,069 --> 00:40:25,839
approach the surface of the membrane

1045
00:40:29,829 --> 00:40:28,079
uh form polar interactions with the head

1046
00:40:32,310 --> 00:40:29,839
groups of the lipids and non-polar

1047
00:40:34,870 --> 00:40:32,320
interactions with the acell chain

1048
00:40:35,990 --> 00:40:34,880
and then there's a converted concerted

1049
00:40:38,630 --> 00:40:36,000
inversion

1050
00:40:39,510 --> 00:40:38,640
of the transient complex to the other

1051
00:40:40,230 --> 00:40:39,520
side

1052
00:40:41,270 --> 00:40:40,240
so

1053
00:40:43,190 --> 00:40:41,280
this

1054
00:40:45,670 --> 00:40:43,200
model involves highly curved

1055
00:40:48,710 --> 00:40:45,680
intermediate uh states

1056
00:40:52,150 --> 00:40:48,720
and and that's supported by

1057
00:40:56,069 --> 00:40:52,160
the fact that more cone-shaped exophiles

1058
00:40:58,550 --> 00:40:56,079
greatly increase permeability

1059
00:41:02,150 --> 00:40:58,560
okay so let's go back imagine this issue

1060
00:41:04,470 --> 00:41:02,160
of the compatibility of template copying

1061
00:41:07,430 --> 00:41:06,470
vesicle structure

1062
00:41:10,150 --> 00:41:07,440
okay

1063
00:41:11,990 --> 00:41:10,160

so here's the experiment that i was

1064
00:41:14,870 --> 00:41:12,000
building up to before

1065
00:41:15,750 --> 00:41:14,880
in this case we prepare vesicles that

1066
00:41:18,790 --> 00:41:15,760
have

1067
00:41:21,190 --> 00:41:18,800
the primer template on the inside

1068
00:41:23,589 --> 00:41:21,200
and we're adding the activated monomer

1069
00:41:25,270 --> 00:41:23,599
to the outside

1070
00:41:27,190 --> 00:41:25,280
okay so here's the chemistry of the

1071
00:41:29,190 --> 00:41:27,200
reaction exactly the same as what you

1072
00:41:30,550 --> 00:41:29,200
saw before

1073
00:41:31,510 --> 00:41:30,560
over

1074
00:41:33,910 --> 00:41:31,520
here

1075
00:41:35,030 --> 00:41:33,920
is the solution control so we see the

1076
00:41:37,750 --> 00:41:35,040
primer

1077
00:41:39,670 --> 00:41:37,760
converting to the plus 15 product over

1078
00:41:42,870 --> 00:41:39,680
the course of about a day

1079
00:41:44,309 --> 00:41:42,880
and then on this side we see exactly the

1080
00:41:46,950 --> 00:41:44,319
same reaction

1081
00:41:48,550 --> 00:41:46,960
going on inside vesicles

1082
00:41:50,550 --> 00:41:48,560
and you can see that it's a little bit

1083
00:41:53,430 --> 00:41:50,560
slower

1084
00:41:55,109 --> 00:41:53,440
it takes a bit longer to accumulate full

1085
00:41:57,750 --> 00:41:55,119
length material but at the end of the

1086
00:42:00,390 --> 00:41:57,760
day we still have mostly

1087
00:42:01,510 --> 00:42:00,400
fully copied templates on the inside

1088
00:42:03,109 --> 00:42:01,520

so the

1089
00:42:05,829 --> 00:42:03,119
slight delay

1090
00:42:08,069 --> 00:42:05,839
reflects the extra time it takes for the

1091
00:42:10,390 --> 00:42:08,079
activated nucleotides to go from outside

1092
00:42:12,710 --> 00:42:10,400
the vesicle across the membrane to the

1093
00:42:15,430 --> 00:42:12,720
inside where they can take part in the

1094
00:42:22,230 --> 00:42:15,440
template copying chemistry

1095
00:42:26,790 --> 00:42:25,589
so this is a big step forward to showing

1096
00:42:30,390 --> 00:42:26,800
that

1097
00:42:32,309 --> 00:42:30,400
at the beginning

1098
00:42:33,910 --> 00:42:32,319
is at least reasonable in the sense that

1099
00:42:35,990 --> 00:42:33,920
we can add

1100
00:42:38,790 --> 00:42:36,000
activated building blocks to the outside

1101
00:42:41,270 --> 00:42:38,800
and see copying of genetic materials on

1102
00:42:44,710 --> 00:42:41,280
the inside

1103
00:42:46,550 --> 00:42:44,720
in this particular exam the membranes

1104
00:42:48,550 --> 00:42:46,560
are

1105
00:42:50,790 --> 00:42:48,560
one of our favorite models which is a

1106
00:42:52,870 --> 00:42:50,800
c14 unsaturated

1107
00:42:54,950 --> 00:42:52,880
fatty acid

1108
00:42:56,950 --> 00:42:54,960
in this experiment

1109
00:42:58,390 --> 00:42:56,960
we see a more

1110
00:42:59,990 --> 00:42:58,400
prebiotic

1111
00:43:01,270 --> 00:43:00,000
photographer files again they're

1112
00:43:03,510 --> 00:43:01,280
saturated

1113
00:43:05,750 --> 00:43:03,520

10 carbon chains

1114
00:43:07,990 --> 00:43:05,760
very similar

1115
00:43:10,550 --> 00:43:08,000
time course and at the end of the day we

1116
00:43:13,109 --> 00:43:10,560
have mostly full length copied templates

1117
00:43:16,230 --> 00:43:13,119
inside these vesicles

1118
00:43:19,589 --> 00:43:16,240
and then finally here is

1119
00:43:23,270 --> 00:43:19,599
the same kind of experiment except using

1120
00:43:25,109 --> 00:43:23,280
vesicles made of modern photolipids

1121
00:43:28,710 --> 00:43:25,119
and in this case the nucleotides can't

1122
00:43:31,030 --> 00:43:28,720
get across and you see absolutely no

1123
00:43:33,109 --> 00:43:31,040
copying of the

1124
00:43:35,190 --> 00:43:33,119
internal template so the primer does not

1125
00:43:36,710 --> 00:43:35,200
get elongated at all

1126
00:43:38,870 --> 00:43:36,720
so

1127
00:43:42,150 --> 00:43:38,880
in order for these systems to be

1128
00:43:44,390 --> 00:43:42,160
compatible you you really do have to use

1129
00:43:48,630 --> 00:43:44,400
the right building blocks to make the

1130
00:43:51,349 --> 00:43:50,069
okay

1131
00:43:55,589 --> 00:43:51,359
all right so

1132
00:43:57,589 --> 00:43:55,599
so we can do a template copier

1133
00:43:59,190 --> 00:43:57,599
if you just had efficient copying

1134
00:44:02,870 --> 00:43:59,200
chemistry and

1135
00:44:04,710 --> 00:44:02,880
and ended up with a full length duplex

1136
00:44:07,109 --> 00:44:04,720
that would be a dead end unless there

1137
00:44:09,109 --> 00:44:07,119
was a way to get the strands apart

1138
00:44:11,270 --> 00:44:09,119

so that the separated strands could be

1139
00:44:14,150 --> 00:44:11,280
copied again

1140
00:44:16,630 --> 00:44:14,160
the obvious way to do that is by thermal

1141
00:44:18,470 --> 00:44:16,640
cycling just like we do for pcr

1142
00:44:20,550 --> 00:44:18,480
reactions

1143
00:44:22,550 --> 00:44:20,560
again for years we were kind of afraid

1144
00:44:24,550 --> 00:44:22,560
to do that experiment

1145
00:44:27,190 --> 00:44:24,560
because we thought that if we

1146
00:44:29,430 --> 00:44:27,200
heated our delicate fatty acid vesicles

1147
00:44:31,910 --> 00:44:29,440
up to 95 degrees they would just fall

1148
00:44:34,630 --> 00:44:31,920
apart and everything would leak out and

1149
00:44:35,349 --> 00:44:34,640
the experiment would be over

1150
00:44:38,309 --> 00:44:35,359
so

1151
00:44:41,430 --> 00:44:38,319
uh it wasn't until sharifambi actually

1152
00:44:44,390 --> 00:44:41,440
did the experiments uh that we realized

1153
00:44:45,430 --> 00:44:44,400
uh things weren't so bad after all so

1154
00:44:47,670 --> 00:44:45,440
here

1155
00:44:48,710 --> 00:44:47,680
in this experiment what sharif did

1156
00:44:51,670 --> 00:44:48,720
was to

1157
00:44:53,430 --> 00:44:51,680
make vesicles with an encapsulated

1158
00:44:55,910 --> 00:44:53,440
fragment of dna

1159
00:44:58,390 --> 00:44:55,920
and just monitor the rate at which it

1160
00:45:01,270 --> 00:44:58,400
looks out

1161
00:45:04,150 --> 00:45:01,280
over time so in this case he heated the

1162
00:45:05,750 --> 00:45:04,160
vesicles for an hour at the indicated

1163
00:45:08,230 --> 00:45:05,760

temperature

1164
00:45:10,550 --> 00:45:08,240
and so you see the plane fatty acid

1165
00:45:11,910 --> 00:45:10,560
vesicles start to leak at around 60 or

1166
00:45:13,670 --> 00:45:11,920
70 degrees

1167
00:45:15,910 --> 00:45:13,680
and kind of fall apart

1168
00:45:17,829 --> 00:45:15,920
between 80 and 90.

1169
00:45:20,309 --> 00:45:17,839
if you mix in a little of the

1170
00:45:22,630 --> 00:45:20,319
corresponding fatty alcohol

1171
00:45:24,710 --> 00:45:22,640
the vesicles get more stable

1172
00:45:27,270 --> 00:45:24,720
they don't really start to fall apart

1173
00:45:29,750 --> 00:45:27,280
until between 90 and 100.

1174
00:45:31,589 --> 00:45:29,760
if you mix in some of the glycerol after

1175
00:45:33,510 --> 00:45:31,599
of the fatty acid

1176
00:45:34,630 --> 00:45:33,520
it's amazing i mean you can take these

1177
00:45:37,190 --> 00:45:34,640
vesicles

1178
00:45:40,550 --> 00:45:37,200
and boil them for an hour and none of

1179
00:45:42,870 --> 00:45:40,560
the dna leaks out at all

1180
00:45:45,270 --> 00:45:42,880
even these less stable vesicles you can

1181
00:45:47,430 --> 00:45:45,280
do normal pcr type service cycling where

1182
00:45:50,550 --> 00:45:47,440
you just heat up for a minute or so and

1183
00:45:52,550 --> 00:45:50,560
nothing leaks out under those conditions

1184
00:45:54,390 --> 00:45:52,560
pretty much the same thing is true with

1185
00:45:55,990 --> 00:45:54,400
the shorter chain

1186
00:45:58,470 --> 00:45:56,000
saturated

1187
00:46:00,710 --> 00:45:58,480
fatty acid mixtures

1188
00:46:02,390 --> 00:46:00,720

fatty investigates by themselves are

1189
00:46:04,230 --> 00:46:02,400
unstable

1190
00:46:05,349 --> 00:46:04,240
they become more stable when you add the

1191
00:46:06,950 --> 00:46:05,359
alcohol

1192
00:46:08,790 --> 00:46:06,960
and much more stable when you add the

1193
00:46:10,710 --> 00:46:08,800
glycerol out there so

1194
00:46:11,829 --> 00:46:10,720
interestingly these more complicated

1195
00:46:14,950 --> 00:46:11,839
mixtures

1196
00:46:17,190 --> 00:46:14,960
uh always seem to work better

1197
00:46:19,349 --> 00:46:17,200
they're not as stable

1198
00:46:21,430 --> 00:46:19,359
as the previous said

1199
00:46:23,910 --> 00:46:21,440
but and this isn't heating at an hour

1200
00:46:25,829 --> 00:46:23,920
and monitoring leakage of a dna fragment

1201
00:46:27,670 --> 00:46:25,839
but they're perfectly fine when heated

1202
00:46:30,870 --> 00:46:27,680
up for a minute or two

1203
00:46:31,670 --> 00:46:30,880
to 95 degrees nothing leaks out

1204
00:46:33,670 --> 00:46:31,680
so

1205
00:46:35,109 --> 00:46:33,680
those experiments

1206
00:46:37,430 --> 00:46:35,119
imply

1207
00:46:40,309 --> 00:46:37,440
that an encapsulated

1208
00:46:42,710 --> 00:46:40,319
nucleic acid duplex could be heated up

1209
00:46:44,069 --> 00:46:42,720
it could have the strands come apart

1210
00:46:46,150 --> 00:46:44,079
cool down

1211
00:46:48,309 --> 00:46:46,160
and then have template copying chemistry

1212
00:46:50,230 --> 00:46:48,319
go on at the lower temperature

1213
00:46:52,309 --> 00:46:50,240

so we wanted to be sure

1214
00:46:54,309 --> 00:46:52,319
that that that this strand separation

1215
00:46:56,710 --> 00:46:54,319
would actually take place

1216
00:46:58,230 --> 00:46:56,720
and so the way that sharif tested that

1217
00:47:01,670 --> 00:46:58,240
is shown here

1218
00:47:05,589 --> 00:47:01,680
he encapsulated a short dna duplex

1219
00:47:08,790 --> 00:47:05,599
in which a small fraction of the

1220
00:47:13,030 --> 00:47:08,800
dna duplexes were labeled on each strand

1221
00:47:14,309 --> 00:47:13,040
with a donor and a quencher fluorophore

1222
00:47:17,430 --> 00:47:14,319
so

1223
00:47:19,990 --> 00:47:17,440
quenched at the beginning of the

1224
00:47:22,390 --> 00:47:20,000
experiment because every donor has a

1225
00:47:23,670 --> 00:47:22,400
nearby pressure most of the molecules

1226
00:47:25,270 --> 00:47:23,680
are not labeled

1227
00:47:26,950 --> 00:47:25,280
when you heat them up

1228
00:47:29,510 --> 00:47:26,960
the strands come apart

1229
00:47:31,750 --> 00:47:29,520
everything floats around separately

1230
00:47:34,630 --> 00:47:31,760
when they're cooled down the strands

1231
00:47:37,430 --> 00:47:34,640
gradually re-anneal but now the donor

1232
00:47:39,430 --> 00:47:37,440
and cruncher are usually separated that

1233
00:47:40,790 --> 00:47:39,440
results in an increase in fluorescence

1234
00:47:43,670 --> 00:47:40,800
intensity

1235
00:47:46,150 --> 00:47:43,680
and that's exactly what he saw

1236
00:47:48,390 --> 00:47:46,160
its data correspond to

1237
00:47:49,990 --> 00:47:48,400
complete separation and randomization of

1238
00:47:53,190 --> 00:47:50,000

the strands

1239
00:47:55,670 --> 00:47:53,200
okay so the thermocycling inside these

1240
00:47:58,069 --> 00:47:55,680
uh very simple primitive vesicles looks

1241
00:48:01,030 --> 00:47:58,079
quite uh plausible

1242
00:48:02,790 --> 00:48:01,040
as an added bonus it turns out

1243
00:48:04,069 --> 00:48:02,800
that the membranes become much more

1244
00:48:06,309 --> 00:48:04,079
permeable

1245
00:48:08,069 --> 00:48:06,319
to polar molecules like nucleotides at

1246
00:48:09,750 --> 00:48:08,079
these high temperatures

1247
00:48:12,069 --> 00:48:09,760
and so

1248
00:48:14,550 --> 00:48:12,079
what at room temperature

1249
00:48:18,309 --> 00:48:14,560
took hours to a day

1250
00:48:20,390 --> 00:48:18,319
uh occurs in a few minutes at 90 degrees

1251
00:48:21,589 --> 00:48:20,400
so if we have a

1252
00:48:24,309 --> 00:48:21,599
short

1253
00:48:26,710 --> 00:48:24,319
temperature exclusion uh go up to say 90

1254
00:48:29,670 --> 00:48:26,720
degrees the strands come apart uh

1255
00:48:32,710 --> 00:48:29,680
nucleotides from outside can rapidly

1256
00:48:35,589 --> 00:48:32,720
enter uh in the course of a few minutes

1257
00:48:37,670 --> 00:48:35,599
and then as the temperature goes down

1258
00:48:40,950 --> 00:48:37,680
the membrane seals up and template

1259
00:48:43,190 --> 00:48:40,960
copying chemistry could happen so we can

1260
00:48:45,829 --> 00:48:43,200
start to imagine a very

1261
00:48:49,589 --> 00:48:45,839
simple environmentally driven

1262
00:48:53,109 --> 00:48:49,599
cell cycle if you will that is driven by

1263
00:48:55,510 --> 00:48:53,119

uh by fluctuations in the supply of

1264
00:49:00,390 --> 00:48:55,520
cooling blocks for the for the membrane

1265
00:49:06,870 --> 00:49:02,950
okay so just to uh

1266
00:49:09,030 --> 00:49:06,880
summarize go over the main uh points

1267
00:49:11,030 --> 00:49:09,040
uh what we what we found in the course

1268
00:49:13,349 --> 00:49:11,040
of doing these experiments

1269
00:49:14,630 --> 00:49:13,359
is that we actually have uh multiple

1270
00:49:16,870 --> 00:49:14,640
pathways

1271
00:49:19,349 --> 00:49:16,880
for vesicle growth and division

1272
00:49:21,270 --> 00:49:19,359
i've only described one pathway for each

1273
00:49:23,670 --> 00:49:21,280
today but

1274
00:49:25,589 --> 00:49:23,680
turns out they're actually very robust

1275
00:49:27,190 --> 00:49:25,599
in the sense that there are

1276
00:49:29,510 --> 00:49:27,200
there are multiple ways in which close

1277
00:49:31,990 --> 00:49:29,520
growth and division can happen

1278
00:49:33,670 --> 00:49:32,000
we know that nucleotides can get into

1279
00:49:38,390 --> 00:49:33,680
vesicles

1280
00:49:40,549 --> 00:49:38,400
enough for strand separation

1281
00:49:41,990 --> 00:49:40,559
the chemistry of template copying is

1282
00:49:43,829 --> 00:49:42,000
compatible with

1283
00:49:45,270 --> 00:49:43,839
the existence and integrity of the

1284
00:49:46,069 --> 00:49:45,280
vesicles

1285
00:49:46,950 --> 00:49:46,079
and

1286
00:49:49,750 --> 00:49:46,960
uh

1287
00:49:52,069 --> 00:49:49,760
it's early days but we think that the

1288
00:49:54,230 --> 00:49:52,079

chemical approach to

1289
00:49:55,430 --> 00:49:54,240
copying nucleic acid

1290
00:49:56,829 --> 00:49:55,440
sequences

1291
00:49:58,790 --> 00:49:56,839
looks quite

1292
00:50:01,510 --> 00:49:58,800
promising and

1293
00:50:04,390 --> 00:50:01,520
therefore this bypasses the need to get

1294
00:50:07,589 --> 00:50:04,400
started with a complicated uh dry design

1295
00:50:11,829 --> 00:50:09,910
okay so um

1296
00:50:14,309 --> 00:50:11,839
what if all this is really relevant to

1297
00:50:15,990 --> 00:50:14,319
the origin of life and to me i think the

1298
00:50:18,470 --> 00:50:16,000
most

1299
00:50:20,470 --> 00:50:18,480
general and important lesson is that as

1300
00:50:23,670 --> 00:50:20,480
we do we and others do these kinds of

1301
00:50:27,030 --> 00:50:23,680
experiments we're continuously

1302
00:50:29,829 --> 00:50:27,040
coming across very surprising unexpected

1303
00:50:32,309 --> 00:50:29,839
physical chemical phenomena that might

1304
00:50:35,829 --> 00:50:32,319
have played an important role

1305
00:50:36,870 --> 00:50:35,839
and um so the ones i mentioned are shown

1306
00:50:39,190 --> 00:50:36,880
here this

1307
00:50:41,349 --> 00:50:39,200
selective membrane permeability that

1308
00:50:44,790 --> 00:50:41,359
favors ribose

1309
00:50:47,670 --> 00:50:44,800
the unexpected thermal stability of

1310
00:50:50,230 --> 00:50:47,680
fatty acid membranes

1311
00:50:52,309 --> 00:50:50,240
the unexpected permeability for example

1312
00:50:55,910 --> 00:50:52,319
a few other things i didn't have time to

1313
00:50:57,750 --> 00:50:55,920

talk about or earlier work by uh

1314
00:50:59,990 --> 00:50:57,760
well i didn't mention jim ferriss's work

1315
00:51:01,270 --> 00:51:00,000
but also worked by uh

1316
00:51:03,109 --> 00:51:01,280
pierre elaine

1317
00:51:03,990 --> 00:51:03,119
on showing that

1318
00:51:07,910 --> 00:51:04,000
you can

1319
00:51:10,950 --> 00:51:07,920
get rna polymerization catalyzed by

1320
00:51:13,349 --> 00:51:10,960
by freezing monomer solutions

1321
00:51:14,790 --> 00:51:13,359
uh there are new ways of concentrating

1322
00:51:18,950 --> 00:51:14,800
dilute

1323
00:51:20,470 --> 00:51:18,960
chemicals that are quite interesting

1324
00:51:24,950 --> 00:51:20,480
and so on there's just

1325
00:51:27,750 --> 00:51:24,960
many many uh unexpected but very simple

1326
00:51:29,670 --> 00:51:27,760
chemical and physical phenomena

1327
00:51:31,670 --> 00:51:29,680
that i think could be very relevant to

1328
00:51:33,589 --> 00:51:31,680
our understanding of the origin of life

1329
00:51:36,390 --> 00:51:33,599
and we're not going to find these

1330
00:51:39,190 --> 00:51:36,400
unless people who do these kinds of

1331
00:51:41,829 --> 00:51:39,200
synthetic experiments

1332
00:51:42,630 --> 00:51:41,839
and so finally i just sort of mentioned

1333
00:51:44,950 --> 00:51:42,640
uh

1334
00:51:47,349 --> 00:51:44,960
again the people that did all this work

1335
00:51:49,990 --> 00:51:47,359
all of this was done by a lot of really

1336
00:51:51,750 --> 00:51:50,000
brilliant graduate students and postdocs

1337
00:51:52,950 --> 00:51:51,760
i've tried to mention many of them as i

1338
00:51:55,990 --> 00:51:52,960

went along

1339
00:51:58,790 --> 00:51:56,000
um a lot of the more recent work on vets

1340
00:52:00,710 --> 00:51:58,800
goals has been done by ting tsu

1341
00:52:01,589 --> 00:52:00,720
and the nucleic acid work by jason

1342
00:52:05,670 --> 00:52:01,599
schrum

1343
00:52:07,670 --> 00:52:05,680
jesse chen alonso ricardo

1344
00:52:09,750 --> 00:52:07,680
cherise manzi did a lot of the

1345
00:52:11,270 --> 00:52:09,760
permeability in term of stability

1346
00:52:12,470 --> 00:52:11,280
experiments

1347
00:52:17,990 --> 00:52:12,480
and

1348
00:52:25,430 --> 00:52:19,670
thank you very much let's all thank our

1349
00:52:28,630 --> 00:52:27,190
thank you very much for a great talk i

1350
00:52:31,510 --> 00:52:28,640
would encourage everybody to go to

1351
00:52:34,150 --> 00:52:31,520
jack's website because jack has posted a

1352
00:52:36,630 --> 00:52:34,160
number of movies that illustrate

1353
00:52:38,790 --> 00:52:36,640
some of those processes and particularly

1354
00:52:41,109 --> 00:52:38,800
the processes by which my cells can

1355
00:52:44,470 --> 00:52:41,119
merge and and form vesicles and also the

1356
00:52:47,030 --> 00:52:44,480
transport across the uh the membrane and

1357
00:52:49,270 --> 00:52:47,040
i think you'd find that very interesting

1358
00:52:51,990 --> 00:52:49,280
i'd like to ask the uh

1359
00:52:54,150 --> 00:52:52,000
the first question and i would like to

1360
00:52:56,549 --> 00:52:54,160
ask everybody else to raise your hand on

1361
00:52:58,790 --> 00:52:56,559
webex and then we'll call on you

1362
00:53:00,870 --> 00:52:58,800
to ask jack questions but just before i

1363
00:53:03,430 --> 00:53:00,880

do i'd just like to announce that the

1364
00:53:05,990 --> 00:53:03,440
next nai director seminar is going to be

1365
00:53:08,870 --> 00:53:06,000
in just three weeks from now on november

1366
00:53:11,670 --> 00:53:08,880
24th and it'll be roger summons talking

1367
00:53:15,030 --> 00:53:11,680
about the mother of all extinctions the

1368
00:53:17,510 --> 00:53:15,040
permian triassic extinction and

1369
00:53:18,470 --> 00:53:17,520
his work on the mechanisms that caused

1370
00:53:20,549 --> 00:53:18,480
that

1371
00:53:22,870 --> 00:53:20,559
so jack the question i'd like to ask you

1372
00:53:25,349 --> 00:53:22,880
is what are your future directions and

1373
00:53:28,150 --> 00:53:25,359
where do you think

1374
00:53:32,950 --> 00:53:28,160
you will be on this line of work let us

1375
00:53:38,150 --> 00:53:36,309
uh well a lot of our work now is focused

1376
00:53:40,390 --> 00:53:38,160
on the chemistry of nucleic acid

1377
00:53:43,270 --> 00:53:40,400
replication so

1378
00:53:45,349 --> 00:53:43,280
um that's the big push

1379
00:53:46,630 --> 00:53:45,359
i'm reasonably optimistic that we'll

1380
00:53:48,549 --> 00:53:46,640
find

1381
00:53:50,950 --> 00:53:48,559
um

1382
00:53:52,150 --> 00:53:50,960
a system that lets us

1383
00:53:54,390 --> 00:53:52,160
do

1384
00:53:56,790 --> 00:53:54,400
replication well enough that we can

1385
00:53:59,510 --> 00:53:56,800
combine it

1386
00:54:01,349 --> 00:53:59,520
with a replicating vesicle system and

1387
00:54:04,549 --> 00:54:01,359
what we really want to be seeing is the

1388
00:54:06,150 --> 00:54:04,559

emergence of the spontaneous emergence

1389
00:54:08,549 --> 00:54:06,160
of sequences that

1390
00:54:10,950 --> 00:54:08,559
contribute that are selected

1391
00:54:12,710 --> 00:54:10,960
whether we'll be there in five years or

1392
00:54:13,910 --> 00:54:12,720
not is a little bit hard to say of

1393
00:54:15,990 --> 00:54:13,920
course

1394
00:54:18,230 --> 00:54:16,000
uh there is one new direction that we've

1395
00:54:21,430 --> 00:54:18,240
been having a lot of fun thinking about

1396
00:54:25,349 --> 00:54:23,510
just knowing what we know from these

1397
00:54:28,470 --> 00:54:25,359
experiments can we actually start to

1398
00:54:34,230 --> 00:54:31,109
primitive living systems that would be

1399
00:54:37,510 --> 00:54:34,240
chemically very different

1400
00:54:43,270 --> 00:54:40,390
things that would uh would live in a

1401
00:54:45,030 --> 00:54:43,280
solvent other than water

1402
00:54:47,270 --> 00:54:45,040
and

1403
00:54:51,030 --> 00:54:47,280
it's a really interesting exercise it

1404
00:54:56,230 --> 00:54:53,910
the qualities of dna and and and the

1405
00:55:00,069 --> 00:54:56,240
forces that go into

1406
00:55:02,950 --> 00:55:00,079
uh membrane behavior and and uh and

1407
00:55:06,549 --> 00:55:02,960
nucleic acid duplex behavior so it's an

1408
00:55:08,390 --> 00:55:06,559
interesting exercise anyway

1409
00:55:12,950 --> 00:55:08,400
thank you jack marco do we have hands

1410
00:55:16,790 --> 00:55:14,790
i wonder why you thought the clay

1411
00:55:18,790 --> 00:55:16,800
catalyzed the formation of the micelles

1412
00:55:22,230 --> 00:55:18,800
was it the ph of the clay itself that

1413
00:55:28,309 --> 00:55:26,069

we think it is that the clay

1414
00:55:31,109 --> 00:55:28,319
works because of its surface charge so

1415
00:55:33,510 --> 00:55:31,119
it's not something that's very specific

1416
00:55:35,910 --> 00:55:33,520
to that clay in fact

1417
00:55:38,710 --> 00:55:35,920
almost any mineral surface that has a

1418
00:55:40,950 --> 00:55:38,720
negative surface charge will work

1419
00:55:43,510 --> 00:55:40,960
and so we think that because of the

1420
00:55:44,470 --> 00:55:43,520
electrical double layer at the surface

1421
00:55:46,309 --> 00:55:44,480
there's

1422
00:55:48,470 --> 00:55:46,319
such an electrostatic effect that

1423
00:55:50,789 --> 00:55:48,480
concentrates myself

1424
00:55:52,230 --> 00:55:50,799
uh close to the surface making it easier

1425
00:55:54,150 --> 00:55:52,240
for them to

1426
00:55:56,470 --> 00:55:54,160
start to interact with each other

1427
00:55:58,309 --> 00:55:56,480
it's really just a

1428
00:55:59,750 --> 00:55:58,319
kind of hand waving model at this point

1429
00:56:00,470 --> 00:55:59,760
so that's that's what we think are going

1430
00:56:03,430 --> 00:56:00,480
on

1431
00:56:11,190 --> 00:56:03,440
okay thanks

1432
00:56:14,630 --> 00:56:12,710
hi can you hear me this is neither

1433
00:56:18,390 --> 00:56:14,640
sahara at wisconsin

1434
00:56:20,069 --> 00:56:18,400
yes um hi um i have a question about

1435
00:56:21,589 --> 00:56:20,079
what you might think be the role of

1436
00:56:23,109 --> 00:56:21,599
magnesium

1437
00:56:25,990 --> 00:56:23,119
uh in your

1438
00:56:28,069 --> 00:56:26,000

nucleic acid polymerization reactions as

1439
00:56:29,750 --> 00:56:28,079
well as for the overall permeability of

1440
00:56:31,510 --> 00:56:29,760
your

1441
00:56:33,109 --> 00:56:31,520
membrane being that they're negatively

1442
00:56:35,270 --> 00:56:33,119
charged is also an electric double layer

1443
00:56:37,589 --> 00:56:35,280
associated with the outside of the

1444
00:56:39,750 --> 00:56:37,599
membrane and that might have been

1445
00:56:42,230 --> 00:56:39,760
probably higher magnesium in seawater as

1446
00:56:44,950 --> 00:56:42,240
well as plenty of sodium

1447
00:56:46,950 --> 00:56:44,960
than one has no

1448
00:56:49,829 --> 00:56:46,960
you see what i'm getting at with this

1449
00:56:53,349 --> 00:56:49,839
question yeah yeah yeah so uh so a word

1450
00:56:54,870 --> 00:56:53,359
from dave diemer's lab uh

1451
00:56:58,309 --> 00:56:54,880
showed that

1452
00:57:00,950 --> 00:56:58,319
these fatty acid-base membranes really

1453
00:57:03,109 --> 00:57:00,960
don't tolerate very high concentrations

1454
00:57:05,430 --> 00:57:03,119
of magnesium

1455
00:57:06,470 --> 00:57:05,440
so i i think these kinds of membranes

1456
00:57:08,630 --> 00:57:06,480
would not

1457
00:57:11,670 --> 00:57:08,640
work in seawater

1458
00:57:15,430 --> 00:57:11,680
i think there has to be a more deadly is

1459
00:57:16,630 --> 00:57:15,440
more of a freshwater type of system

1460
00:57:18,789 --> 00:57:16,640
with the right mixtures of

1461
00:57:21,670 --> 00:57:18,799
anthropophiles you can tolerate

1462
00:57:24,309 --> 00:57:21,680
up to say four millimolar magnesium

1463
00:57:26,230 --> 00:57:24,319

uh so that for a long time seemed like a

1464
00:57:28,150 --> 00:57:26,240
big problem when we were stuck thinking

1465
00:57:30,309 --> 00:57:28,160
about getting started

1466
00:57:31,430 --> 00:57:30,319
with ribosomes because when we try to

1467
00:57:33,510 --> 00:57:31,440
select

1468
00:57:35,910 --> 00:57:33,520
new ribosomes that do for example

1469
00:57:38,630 --> 00:57:35,920
polymerization

1470
00:57:39,430 --> 00:57:38,640
chemistry they tend to require very very

1471
00:57:41,670 --> 00:57:39,440
high

1472
00:57:43,109 --> 00:57:41,680
concentrations of divalent cations like

1473
00:57:45,190 --> 00:57:43,119
magnesium

1474
00:57:46,870 --> 00:57:45,200
which would not be compatible with the

1475
00:57:49,510 --> 00:57:46,880
structure of the membrane

1476
00:57:51,990 --> 00:57:49,520
when we go to these uh phosphoramine

1477
00:57:53,190 --> 00:57:52,000
polymers and the amino sugars

1478
00:57:55,829 --> 00:57:53,200
it turns out

1479
00:57:58,789 --> 00:57:55,839
that chemistry is completely independent

1480
00:57:59,990 --> 00:57:58,799
of diagnostics so we can actually do

1481
00:58:03,430 --> 00:58:00,000
everything

1482
00:58:05,190 --> 00:58:03,440
uh in an environment that

1483
00:58:06,789 --> 00:58:05,200
contains a certain amount of salt and

1484
00:58:16,870 --> 00:58:06,799
some buffers but

1485
00:58:16,880 --> 00:58:20,870
we have a question at uw

1486
00:58:25,109 --> 00:58:23,190
yeah uh my question is have you looked

1487
00:58:26,630 --> 00:58:25,119
at any chemical controls on the size of

1488
00:58:28,630 --> 00:58:26,640

these vesicles and whether or not it

1489
00:58:30,950 --> 00:58:28,640
would be possible to have a chemical

1490
00:58:32,470 --> 00:58:30,960
mechanism for division as opposed to a

1491
00:58:33,589 --> 00:58:32,480
physical one or is it something where

1492
00:58:37,990 --> 00:58:33,599
you actually need the physical

1493
00:58:45,109 --> 00:58:41,589
we've been looking at different athletes

1494
00:58:50,950 --> 00:58:47,990
mostly i guess looking at

1495
00:58:53,190 --> 00:58:50,960
simple environmental ways uh in which

1496
00:58:55,510 --> 00:58:53,200
you know environmental punctuation

1497
00:58:57,589 --> 00:58:55,520
i think they're much more plausible ways

1498
00:58:59,030 --> 00:58:57,599
of doing it than this extrusion through

1499
00:59:00,789 --> 00:58:59,040
small force that's really just a

1500
00:59:02,549 --> 00:59:00,799
laboratory model

1501
00:59:04,390 --> 00:59:02,559
but in more recent experiments that

1502
00:59:06,390 --> 00:59:04,400
aren't published yet we can

1503
00:59:09,109 --> 00:59:06,400
we have systems where larger vesicles

1504
00:59:11,430 --> 00:59:09,119
divide very easily

1505
00:59:12,950 --> 00:59:11,440
just with gentle shear forces

1506
00:59:15,670 --> 00:59:12,960
now

1507
00:59:18,230 --> 00:59:15,680
there's an interesting possibility for a

1508
00:59:19,349 --> 00:59:18,240
more chemically mediated

1509
00:59:21,990 --> 00:59:19,359
division

1510
00:59:24,470 --> 00:59:22,000
based on the phase separation of lipids

1511
00:59:26,549 --> 00:59:24,480
in the bilayer so if you make domains if

1512
00:59:28,870 --> 00:59:26,559
you say separate so there are domains

1513
00:59:30,710 --> 00:59:28,880

with different lipid compositions then

1514
00:59:33,190 --> 00:59:30,720
there's a high energy

1515
00:59:35,270 --> 00:59:33,200
boundary between those domains and

1516
00:59:36,470 --> 00:59:35,280
minimization of that line tension can

1517
00:59:37,990 --> 00:59:36,480
actually

1518
00:59:41,750 --> 00:59:38,000
drive division

1519
00:59:44,069 --> 00:59:41,760
and that's been seen in in phospholipids

1520
00:59:47,510 --> 00:59:44,079
single lipid cholesterol mixtures which

1521
00:59:49,589 --> 00:59:47,520
haven't been able uh to

1522
00:59:51,430 --> 00:59:49,599
make a system that works that way with

1523
00:59:53,589 --> 00:59:51,440
single-chain anti-files

1524
00:59:55,670 --> 00:59:53,599
yes but i think that would be a really

1525
00:59:57,589 --> 00:59:55,680
cool way of doing it i think it's still

1526
01:00:00,069 --> 00:59:57,599
worth looking into and there may of

1527
01:00:06,549 --> 01:00:00,079
course be many other other ways of

1528
01:00:06,559 --> 01:00:10,309
okay is there another question that aims

1529
01:00:14,950 --> 01:00:13,109
yeah hi jack this is david um i was just

1530
01:00:16,470 --> 01:00:14,960
thinking about ph when you were talking

1531
01:00:18,150 --> 01:00:16,480
about your experiments and of course at

1532
01:00:20,150 --> 01:00:18,160
some point the ph difference may have

1533
01:00:20,870 --> 01:00:20,160
something to do with energy harvesting

1534
01:00:23,190 --> 01:00:20,880
but

1535
01:00:24,549 --> 01:00:23,200
are there any considerations about pa ph

1536
01:00:35,270 --> 01:00:24,559
in your experiments

1537
01:00:40,549 --> 01:00:38,789
we supply a new material new fatty acids

1538
01:00:42,789 --> 01:00:40,559

as an alkaline

1539
01:00:46,390 --> 01:00:42,799
solution that goes into a buffered

1540
01:00:48,549 --> 01:00:46,400
suspension of ethical and then

1541
01:00:50,789 --> 01:00:48,559
the my cells that we're the stable phase

1542
01:00:52,470 --> 01:00:50,799
of high ph now are a thermodynamically

1543
01:00:54,630 --> 01:00:52,480
unstable phase so

1544
01:00:57,270 --> 01:00:54,640
so it's energetically downhill for those

1545
01:00:58,470 --> 01:00:57,280
molecules to now transfer into the

1546
01:00:59,990 --> 01:00:58,480
bilayer

1547
01:01:02,150 --> 01:01:00,000
phase

1548
01:01:02,950 --> 01:01:02,160
so so that's one aspect of it the other

1549
01:01:04,789 --> 01:01:02,960
is

1550
01:01:07,190 --> 01:01:04,799
i didn't have time to go into it but

1551
01:01:08,950 --> 01:01:07,200
under certain conditions

1552
01:01:11,589 --> 01:01:08,960
during growth

1553
01:01:13,349 --> 01:01:11,599
you actually drive the formation of ph

1554
01:01:15,349 --> 01:01:13,359
and ion gradient

1555
01:01:17,349 --> 01:01:15,359
and that's because

1556
01:01:19,670 --> 01:01:17,359
uh new modules

1557
01:01:21,030 --> 01:01:19,680
come in first to the outer leaflet of

1558
01:01:22,950 --> 01:01:21,040
the bilayer

1559
01:01:26,390 --> 01:01:22,960
but then they have to flip-flop to the

1560
01:01:28,950 --> 01:01:26,400
inside and it's generally the

1561
01:01:30,470 --> 01:01:28,960
protonated neutral uh form of the fatty

1562
01:01:33,270 --> 01:01:30,480
acid will do that many orders of

1563
01:01:34,950 --> 01:01:33,280

magnitude faster than the ionized form

1564
01:01:37,030 --> 01:01:34,960
and then it'll re-equilibrate on the

1565
01:01:39,030 --> 01:01:37,040
inside so as you grow

1566
01:01:40,710 --> 01:01:39,040
you're essentially pumping protons into

1567
01:01:41,829 --> 01:01:40,720
the interior

1568
01:01:43,990 --> 01:01:41,839
now

1569
01:01:47,270 --> 01:01:44,000
so that results in a ph gradient which

1570
01:01:49,030 --> 01:01:47,280
could be used uh to perhaps

1571
01:01:51,030 --> 01:01:49,040
that some of that energy

1572
01:01:55,670 --> 01:01:51,040
could be tapped to do something useful

1573
01:01:57,750 --> 01:01:55,680
for example take up an amine substrate

1574
01:02:00,549 --> 01:01:57,760
it's a little tricky because ph

1575
01:02:02,710 --> 01:02:00,559
gradients decay very rapidly if there

1576
01:02:04,150 --> 01:02:02,720
are free fatty acids around

1577
01:02:05,910 --> 01:02:04,160
but maybe some

1578
01:02:07,910 --> 01:02:05,920
slightly more advanced membrane

1579
01:02:10,069 --> 01:02:07,920
composition with uh for example

1580
01:02:11,990 --> 01:02:10,079
phosphorylated

1581
01:02:14,789 --> 01:02:12,000
monomers

1582
01:02:16,630 --> 01:02:14,799
those retain a ph gradient for longer

1583
01:02:17,670 --> 01:02:16,640
and might allow that

1584
01:02:24,390 --> 01:02:17,680
that

1585
01:02:24,400 --> 01:02:34,309
okay are there any other questions

1586
01:02:37,910 --> 01:02:36,069
okay if there are no other questions

1587
01:02:45,109 --> 01:02:37,920
let's thank our speaker again jack that

1588
01:02:50,549 --> 01:02:47,910

uh jack's seminar will be up on our

1589
01:02:52,309 --> 01:02:50,559
website the podcast usually is up within

1590
01:02:54,230 --> 01:02:52,319
about three or four days so by the end

1591
01:02:56,230 --> 01:02:54,240
of this week or early next week if you'd

1592
01:02:58,870 --> 01:02:56,240
like to hear his seminar again you can

1593
01:03:00,789 --> 01:02:58,880
do so and if you'd like to recommend a

1594
01:03:02,950 --> 01:03:00,799
seminar to anybody who missed it this

1595
01:03:04,950 --> 01:03:02,960
time they'll be able to see it in its

1596
01:03:07,109 --> 01:03:04,960
entirety and i encourage you to also go

1597
01:03:09,750 --> 01:03:07,119
back and take a look at the archives of

1598
01:03:12,150 --> 01:03:09,760
all the past seminars we've had a great

1599
01:03:15,029 --> 01:03:12,160
run of speakers earlier this year and

1600
01:03:17,349 --> 01:03:15,039
last year norm sleep was started us off

1601
01:03:18,950 --> 01:03:17,359
this year so just go to the website take

1602
01:03:21,029 --> 01:03:18,960
a look and you'll see a lot of great

1603
01:03:23,510 --> 01:03:21,039
seminars archived and i hope to see you

1604
01:03:25,190 --> 01:03:23,520
all in three weeks from now for roger

1605
01:03:26,470 --> 01:03:25,200
summon seminar and jack thanks once