
AbSciCon
2017
MESA, ARIZONA

1
00:00:12,250 --> 00:00:06,150
you

2
00:00:12,260 --> 00:00:15,630
[Music]

3
00:00:20,290 --> 00:00:18,130
situation is that there's only a single

4
00:00:23,320 --> 00:00:20,300
planet that we know of that actually

5
00:00:26,230 --> 00:00:23,330
harbors life so we're constrained in our

6
00:00:28,480 --> 00:00:26,240
thinking about what constitutes life by

7
00:00:32,710 --> 00:00:28,490
what we know on earth and surprisingly

8
00:00:35,259 --> 00:00:32,720
enough we know actually quite little we

9
00:00:40,259 --> 00:00:35,269
know about the three domains of life on

10
00:00:44,979 --> 00:00:40,269
Earth and that life probably originated

11
00:00:48,549 --> 00:00:44,989
at once as and a there's a single

12
00:00:52,709 --> 00:00:48,559
genetic code and all of the diversity of

13
00:00:58,090 --> 00:00:52,719

life that we see are based on this

14
00:01:01,600 --> 00:00:58,100
single genetic code we don't really know

15
00:01:04,359 --> 00:01:01,610
very much about evolution in the early

16
00:01:06,520 --> 00:01:04,369
stages and by the early stages I would

17
00:01:09,700 --> 00:01:06,530
say the first half of the history of our

18
00:01:12,550 --> 00:01:09,710
planet and particularly confounding has

19
00:01:14,139 --> 00:01:12,560
been the fact that there's been a

20
00:01:16,990 --> 00:01:14,149
tremendous amount of lateral gene

21
00:01:19,890 --> 00:01:17,000
transfer and that has confounded our

22
00:01:21,910 --> 00:01:19,900
understanding of evolutionary processes

23
00:01:24,340 --> 00:01:21,920
there's been a tremendous amount of

24
00:01:26,679 --> 00:01:24,350
evolution during this time and most of

25
00:01:28,870 --> 00:01:26,689
the metabolic processes have evolved

26
00:01:32,200 --> 00:01:28,880
during this time including oxygenic

27
00:01:35,770 --> 00:01:32,210
photosynthesis methanogenesis all sorts

28
00:01:38,499 --> 00:01:35,780
of respiration photo trophy and probably

29
00:01:42,399 --> 00:01:38,509
the most fundamental biological process

30
00:01:45,249 --> 00:01:42,409
which is chemiosmotic coupling all of

31
00:01:47,819 --> 00:01:45,259
these processes require pigments and

32
00:01:50,260 --> 00:01:47,829
there are a number of different pigments

33
00:01:52,840 --> 00:01:50,270
and the ones that I'm going to talk

34
00:01:55,420 --> 00:01:52,850
about primarily are the carotenoids

35
00:01:59,550 --> 00:01:55,430
and in particular retinol the visual

36
00:02:03,219 --> 00:01:59,560
pigment also I'll compare them to

37
00:02:06,039 --> 00:02:03,229
Flavin's and porphyrins one of the main

38
00:02:07,929 --> 00:02:06,049

questions that we have in our mind is in

39
00:02:11,020 --> 00:02:07,939
an evolutionary sense when did these

40
00:02:12,580 --> 00:02:11,030
different pigments arise because if some

41
00:02:14,410 --> 00:02:12,590
of the pigments arose earlier than

42
00:02:17,410 --> 00:02:14,420
others then we should be aware of that

43
00:02:21,940 --> 00:02:17,420
as we search for biosignatures

44
00:02:23,979 --> 00:02:21,950
elsewhere so the other hypothesis that I

45
00:02:25,660 --> 00:02:23,989
keep in mind is that generally speaking

46
00:02:30,240 --> 00:02:25,670
life has evolved

47
00:02:33,220 --> 00:02:30,250
from simplicity to complexity and the

48
00:02:37,449 --> 00:02:33,230
beta carotene and particular its

49
00:02:41,680 --> 00:02:37,459
oxidative product retinol are two of the

50
00:02:43,540 --> 00:02:41,690
simplest pigments Flavin's which are

51
00:02:46,690 --> 00:02:43,550
involved in respiration processes and

52
00:02:50,110 --> 00:02:46,700
many organisms is also relatively simple

53
00:02:52,270 --> 00:02:50,120
compared to what are the porphyrins

54
00:02:56,670 --> 00:02:52,280
which are involved in a wide variety of

55
00:03:04,780 --> 00:02:56,680
processes and are some of the most

56
00:03:08,140 --> 00:03:04,790
interesting pigments out there so if you

57
00:03:09,580 --> 00:03:08,150
look at the spectra of these different

58
00:03:11,740 --> 00:03:09,590
pigments especially the chlorophylls

59
00:03:14,110 --> 00:03:11,750
versus the retinols

60
00:03:15,759 --> 00:03:14,120
the chlorophyll pigments are there which

61
00:03:17,770 --> 00:03:15,769
there are a variety which are tuned to

62
00:03:20,880 --> 00:03:17,780
specific environments as we'll hear

63
00:03:24,490 --> 00:03:20,890

about in the next talk have to

64
00:03:26,949 --> 00:03:24,500
absorptions maxima one in the blue and

65
00:03:29,920 --> 00:03:26,959
one in the red and therefore the color

66
00:03:31,690 --> 00:03:29,930
green is observed in the case of the

67
00:03:34,030 --> 00:03:31,700
retinal pigment there's a single peak

68
00:03:39,180 --> 00:03:34,040
which is in the green region and

69
00:03:41,920 --> 00:03:39,190
therefore those pigments are purple the

70
00:03:47,470 --> 00:03:41,930
retinal pigments the prototype vector

71
00:03:51,009 --> 00:03:47,480
Dobson is the simplest biological system

72
00:03:52,660 --> 00:03:51,019
for energy transduction so again we

73
00:03:55,390 --> 00:03:52,670
think that these may have evolved very

74
00:03:58,150 --> 00:03:55,400
early it's a single protein with a

75
00:04:01,539 --> 00:03:58,160
single chromophore it is a light driven

76
00:04:03,640 --> 00:04:01,549
proton pump and it generates a proton

77
00:04:08,680 --> 00:04:03,650
motive gradient and that proton motive

78
00:04:11,050 --> 00:04:08,690
gradient can be used for akp synthesis

79
00:04:15,340 --> 00:04:11,060
and a wide variety of other by energetic

80
00:04:17,550 --> 00:04:15,350
processes so the organisms the extreme

81
00:04:21,819 --> 00:04:17,560
halophiles are yellow philic archaea are

82
00:04:24,360 --> 00:04:21,829
a very nice system in terms of by

83
00:04:28,360 --> 00:04:24,370
signatures to be thinking about retinal

84
00:04:31,360 --> 00:04:28,370
pigments and carotenoids so I think many

85
00:04:34,270 --> 00:04:31,370
of you have seen these ponds in South

86
00:04:38,420 --> 00:04:34,280
San Francisco Bay which are highly

87
00:04:41,210 --> 00:04:38,430
saline the airplane when

88
00:04:43,040 --> 00:04:41,220

lands in San Francisco generally flies

89
00:04:45,740 --> 00:04:43,050
over at least when you're coming from

90
00:04:47,360 --> 00:04:45,750
the East Coast over these ponds and

91
00:04:50,510 --> 00:04:47,370
these ponds have been the subject of

92
00:04:53,210 --> 00:04:50,520
much interest over the years the ponds

93
00:04:54,680 --> 00:04:53,220
are of different colors depending on the

94
00:04:58,760 --> 00:04:54,690
types of organisms and their salinity

95
00:05:05,770 --> 00:04:58,770
and they are some of them are red orange

96
00:05:10,220 --> 00:05:05,780
or purple the JPL a virus collection has

97
00:05:15,290 --> 00:05:10,230
actually kept ten years of data on those

98
00:05:18,830 --> 00:05:15,300
ponds and the the ponds are differing by

99
00:05:22,250 --> 00:05:18,840
the concentration of salt from about 20

100
00:05:25,430 --> 00:05:22,260
PPT to over 350 PPT very close to

101
00:05:31,190 --> 00:05:25,440
saturation so if you look at the

102
00:05:33,560 --> 00:05:31,200
reflectance spectra in these ponds you

103
00:05:39,560 --> 00:05:33,570
can see absorption for the carotenoids

104
00:05:43,190 --> 00:05:39,570
and BR you can also see absorption for

105
00:05:46,340 --> 00:05:43,200
chlorophylls however the carotenoids in

106
00:05:51,070 --> 00:05:46,350
BR are are much more prevalent in the

107
00:05:53,450 --> 00:05:51,080
highest salt concentrations and the the

108
00:05:55,520 --> 00:05:53,460
concentration of cells in these ponds is

109
00:05:58,340 --> 00:05:55,530
extremely high it's maybe ten to the

110
00:06:02,060 --> 00:05:58,350
eleventh cells per mil so we think that

111
00:06:07,700 --> 00:06:02,070
this is a pretty good system to think

112
00:06:10,070 --> 00:06:07,710
about developing a way to assay for

113
00:06:15,290 --> 00:06:10,080

biosignatures in this route in this

114
00:06:19,100 --> 00:06:15,300
range so these organisms are growing in

115
00:06:22,660 --> 00:06:19,110
extremely se line brine and I think you

116
00:06:27,290 --> 00:06:22,670
could see the the cells between the the

117
00:06:29,510 --> 00:06:27,300
salt crystals the cells can actually

118
00:06:35,000 --> 00:06:29,520
survive desiccated in the salt crystals

119
00:06:37,340 --> 00:06:35,010
and when you plate them you see highly

120
00:06:39,710 --> 00:06:37,350
pigmented colonies and one can also

121
00:06:42,890 --> 00:06:39,720
observe many mutants and that's been our

122
00:06:45,680 --> 00:06:42,900
approach okay so our approach to

123
00:06:47,210 --> 00:06:45,690
studying these halo bacterial pigments

124
00:06:48,439 --> 00:06:47,220
has been the isolation of pigment

125
00:06:50,420 --> 00:06:48,449
mutants

126
00:06:53,719 --> 00:06:50,430
then we did characterization

127
00:06:55,670 --> 00:06:53,729
biochemically and genetically and in the

128
00:06:57,969 --> 00:06:55,680
recent years we have just been able to

129
00:06:59,809 --> 00:06:57,979
sequence a whole genome to analyze them

130
00:07:02,540 --> 00:06:59,819
and then we've also done some

131
00:07:05,140 --> 00:07:02,550
spectroscopic analysis both absorption

132
00:07:07,969 --> 00:07:05,150
and reflection and characterized

133
00:07:09,650 --> 00:07:07,979
different genes and pathways so I want

134
00:07:15,400 --> 00:07:09,660
to share with you some of the data that

135
00:07:18,170 --> 00:07:15,410
we've accumulated on these so the

136
00:07:21,290 --> 00:07:18,180
carotenoid and retinol by synthetic

137
00:07:24,050 --> 00:07:21,300
pathway starts with the isoprenoid

138
00:07:26,360 --> 00:07:24,060

pathway and then ultimately gets to

139
00:07:30,890 --> 00:07:26,370
lycopene lycopene is actually the color

140
00:07:34,129 --> 00:07:30,900
of tomato then it goes on to beta

141
00:07:37,159 --> 00:07:34,139
carotene and then ultimately to retinol

142
00:07:39,800 --> 00:07:37,169
and the retinol is allows the cells to

143
00:07:42,770 --> 00:07:39,810
grow phototrophic lee the other branch

144
00:07:46,249 --> 00:07:42,780
from lycopene goes to the effector

145
00:07:51,050 --> 00:07:46,259
Ruben's and these are c50 pigments which

146
00:07:53,480 --> 00:07:51,060
have function in repair of DNA damage so

147
00:07:55,760 --> 00:07:53,490
we were able to isolate mutants that are

148
00:07:59,540 --> 00:07:55,770
white these occur some of them occur

149
00:08:03,800 --> 00:07:59,550
spontaneously and the white mutants when

150
00:08:06,350 --> 00:08:03,810
analyzed genetically have been shown to

151
00:08:09,290 --> 00:08:06,360
have a defect in life being elongate so

152
00:08:15,520 --> 00:08:09,300
that's the first step in the conversion

153
00:08:17,779 --> 00:08:15,530
of lycopene into vector Rubens another

154
00:08:20,060 --> 00:08:17,789
type of mutant that we've been able to

155
00:08:23,899 --> 00:08:20,070
isolate our retinal mutants in this case

156
00:08:25,700 --> 00:08:23,909
the phenotypes are much more subtle

157
00:08:29,209 --> 00:08:25,710
the cells on the left are the wild-type

158
00:08:34,250 --> 00:08:29,219
cells on the right are unable to make

159
00:08:36,319 --> 00:08:34,260
retinol and they have a defect in the

160
00:08:42,529 --> 00:08:36,329
enzyme for beta-carotene mono oxygenase

161
00:08:45,230 --> 00:08:42,539
and we initially knocked out the gene

162
00:08:47,780 --> 00:08:45,240
that we believed to be responsible for

163
00:08:52,579 --> 00:08:47,790

this because the VRP gene and you can

164
00:08:56,000 --> 00:08:52,589
see in number two that the retinol is

165
00:08:58,020 --> 00:08:56,010
reduced but not completely gone beta

166
00:09:00,300 --> 00:08:58,030
carotene is actually induced

167
00:09:02,550 --> 00:09:00,310
compared to the wild type there's a

168
00:09:04,530 --> 00:09:02,560
second gene it turns out that also

169
00:09:06,780 --> 00:09:04,540
encodes a beta-carotene mono oxygenase

170
00:09:08,460 --> 00:09:06,790
and when you do a double mutant then you

171
00:09:14,040 --> 00:09:08,470
can see that it has knocked out retinal

172
00:09:17,010 --> 00:09:14,050
entirely so we've gone on and isolated

173
00:09:22,860 --> 00:09:17,020
other mutants the halo vector and vector

174
00:09:25,110 --> 00:09:22,870
tops and mutants and we've isolated a br

175
00:09:27,690 --> 00:09:25,120
over producers so this is a sucrose

176
00:09:31,980 --> 00:09:27,700
gradient showing the relative proportion

177
00:09:33,630 --> 00:09:31,990
of carotenoids to vector Group B R if

178
00:09:36,600 --> 00:09:33,640
you compare that to the next two which

179
00:09:38,130 --> 00:09:36,610
has the wild-type you can see that

180
00:09:42,240 --> 00:09:38,140
there's a considerably lower amount of

181
00:09:44,250 --> 00:09:42,250
BR compared to the carotenoids and then

182
00:09:46,740 --> 00:09:44,260
we also isolated an orange mutant and

183
00:09:49,800 --> 00:09:46,750
colorless mutants and when we analyze

184
00:09:55,890 --> 00:09:49,810
these we see that the orange mutants

185
00:09:59,310 --> 00:09:55,900
have an insertions into the protein gene

186
00:10:01,980 --> 00:09:59,320
for vector Dobson and the colorless

187
00:10:06,360 --> 00:10:01,990
mutants have insertions in the bat gene

188
00:10:08,160 --> 00:10:06,370

for which is a regulatory gene for not

189
00:10:10,230 --> 00:10:08,170
just the protein but also for the

190
00:10:11,850 --> 00:10:10,240
pigment so we've done a fair amount of

191
00:10:13,970 --> 00:10:11,860
analysis of this type and we're putting

192
00:10:18,690 --> 00:10:13,980
together the pathway for these

193
00:10:20,280 --> 00:10:18,700
production of the carotenoids and this

194
00:10:22,650 --> 00:10:20,290
is the portion that I've mentioned

195
00:10:25,620 --> 00:10:22,660
before the lycopene is converted to

196
00:10:29,970 --> 00:10:25,630
retinol which binds to the vector opsin

197
00:10:32,610 --> 00:10:29,980
and produces BR the lye and another gene

198
00:10:36,300 --> 00:10:32,620
rub converse lycopene into becsher

199
00:10:38,720 --> 00:10:36,310
rubriz and then if you go backwards for

200
00:10:42,230 --> 00:10:38,730
the carotenoid pathway there are other

201
00:10:44,670 --> 00:10:42,240
genes that are involved in conversion of

202
00:10:49,260 --> 00:10:44,680
Filene and general general power

203
00:10:52,250 --> 00:10:49,270
phosphate into lycopene and then before

204
00:10:54,690 --> 00:10:52,260
that there's a complicated pathway for

205
00:10:57,890 --> 00:10:54,700
isoprenoid goes all the way from

206
00:11:02,970 --> 00:10:57,900
basically acetate up to general journal

207
00:11:05,829 --> 00:11:02,980
so just to put that

208
00:11:08,530 --> 00:11:05,839
pathway into context we think that this

209
00:11:11,050 --> 00:11:08,540
is actually a relatively simple pathway

210
00:11:14,460 --> 00:11:11,060
for production of pigments and may have

211
00:11:17,319 --> 00:11:14,470
originated fairly early in evolution and

212
00:11:20,829 --> 00:11:17,329
the carotenoid pathway is producing both

213
00:11:22,780 --> 00:11:20,839

the vector Rubens and retinols before

214
00:11:28,389 --> 00:11:22,790
the carotenoid pathways the isoprenoid

215
00:11:32,050 --> 00:11:28,399
pathway which is taking STL Co a to the

216
00:11:33,490 --> 00:11:32,060
general general power phosphate and we

217
00:11:36,460 --> 00:11:33,500
think that this is maybe one of the

218
00:11:39,220 --> 00:11:36,470
original or early by synthetic pathways

219
00:11:43,000 --> 00:11:39,230
on earth for pigments and remember these

220
00:11:48,060 --> 00:11:43,010
pigments are involved both in generating

221
00:11:51,810 --> 00:11:48,070
energy as well as protection of DNA the

222
00:11:55,389 --> 00:11:51,820
okay I'm sure this is the last slide so

223
00:11:57,490 --> 00:11:55,399
the another branch of that pathway leads

224
00:12:00,490 --> 00:11:57,500
to membrane lipids so it makes sense

225
00:12:02,350 --> 00:12:00,500
that under these circumstances you would

226
00:12:05,170 --> 00:12:02,360
be able to generate something that looks

227
00:12:07,540 --> 00:12:05,180
like a protocell and produce a protein

228
00:12:11,350 --> 00:12:07,550
with a chromophore that would be able to

229
00:12:13,689 --> 00:12:11,360
generate energy for life and this entire

230
00:12:16,240 --> 00:12:13,699
pathway of course is just direct

231
00:12:22,540 --> 00:12:16,250
offshoot from central metabolism from

232
00:12:24,400 --> 00:12:22,550
glycolysis and TCA so we think that

233
00:12:27,189 --> 00:12:24,410
there may be a temporal appearance of

234
00:12:30,009 --> 00:12:27,199
these pigments on earth the carotenoids

235
00:12:32,889 --> 00:12:30,019
might have appeared first retinal

236
00:12:34,960 --> 00:12:32,899
pigments appeared subsequently and then

237
00:12:37,360 --> 00:12:34,970
ultimately the chlorophyll and you can

238
00:12:39,340 --> 00:12:37,370

see that there is a complementarity

239
00:12:43,329 --> 00:12:39,350
between the retinal pigments and the

240
00:12:47,380 --> 00:12:43,339
chlorophyll pigments so I think I'll

241
00:12:50,560 --> 00:12:47,390
stop there and acknowledge my co-authors

242
00:12:53,410 --> 00:12:50,570
Priya Victoria and also our

243
00:12:57,009 --> 00:12:53,420
collaborators at Kenyon College and also

244
00:12:59,170 --> 00:12:57,019
thank NASA exobiology and the mirrors