



1
00:00:14,009 --> 00:00:07,320

[Music]

2
00:00:18,690 --> 00:00:16,590

okay so pictured here is a cartoon of

3
00:00:20,730 --> 00:00:18,700

the modern nitrogen cycle so nitrogen is

4
00:00:23,700 --> 00:00:20,740

an essential element for life we need it

5
00:00:25,350 --> 00:00:23,710

for DNA we need it for proteins but even

6
00:00:27,510 --> 00:00:25,360

though our atmosphere is almost entirely

7
00:00:29,490 --> 00:00:27,520

composed of nitrogen most organisms

8
00:00:33,119 --> 00:00:29,500

can't use it in this form so nitrogen

9
00:00:35,759 --> 00:00:33,129

needs to be reduced or fixed and this

10
00:00:38,810 --> 00:00:35,769

process is carried out by a small

11
00:00:41,910 --> 00:00:38,820

selection of microbes that basically

12
00:00:43,799 --> 00:00:41,920

provide all of the fixed nitrogen nearly

13
00:00:46,469 --> 00:00:43,809

all of the naturally fixed nitrogen to

14

00:00:49,610 --> 00:00:46,479

the biosphere so biological nitrogen

15

00:00:53,039 --> 00:00:49,620

fixation is also an exceedingly ancient

16

00:00:55,320 --> 00:00:53,049

metabolic process and it originated with

17

00:00:57,270 --> 00:00:55,330

the evolution of the nitrogenase enzyme

18

00:00:59,460 --> 00:00:57,280

so this is the only enzyme that's

19

00:01:01,410 --> 00:00:59,470

capable of fixing nitrogen and it

20

00:01:03,990 --> 00:01:01,420

evolved somewhere between two and three

21

00:01:05,399 --> 00:01:04,000

or more billion years ago and so really

22

00:01:07,500 --> 00:01:05,409

it's the activity of this nitrogen

23

00:01:09,810 --> 00:01:07,510

fixing enzyme nitrogenase that has

24

00:01:12,630 --> 00:01:09,820

helped to sustain the biosphere over

25

00:01:16,520 --> 00:01:12,640

potentially nearly half or also perhaps

26

00:01:18,899 --> 00:01:16,530

the majority of Earth's history

27

00:01:20,639 --> 00:01:18,909

alright so pictured here on the left is

28

00:01:22,830 --> 00:01:20,649

the nitrogenous protein complex

29

00:01:24,660 --> 00:01:22,840

nitrogenous is also interesting because

30

00:01:26,580 --> 00:01:24,670

it's a metallo enzyme and that means

31

00:01:28,080 --> 00:01:26,590

that it incorporates metal clusters in

32

00:01:30,539 --> 00:01:28,090

order to catalyze the reduction of

33

00:01:32,550 --> 00:01:30,549

nitrogen I'm so pictured here this is

34

00:01:34,980 --> 00:01:32,560

the cofactor in this little insert image

35

00:01:36,779 --> 00:01:34,990

and this particular cofactor is called

36

00:01:39,179 --> 00:01:36,789

the iron molybdenum cofactor and it's

37

00:01:41,940 --> 00:01:39,189

called that because it incorporates one

38

00:01:44,910 --> 00:01:41,950

unique atom of molybdenum that's the the

39

00:01:47,069 --> 00:01:44,920

kind of teal colored circle but there

40

00:01:49,109 --> 00:01:47,079

are other forms of nitrogenous and so

41

00:01:51,029 --> 00:01:49,119

the molybdenum four is by far the most

42

00:01:54,120 --> 00:01:51,039

common it's also the most efficient but

43

00:01:56,249 --> 00:01:54,130

there are also two other rarer forms so

44

00:01:58,440 --> 00:01:56,259

they instead incorporate an atom of

45

00:02:01,520 --> 00:01:58,450

vanadium or an additional atom of iron

46

00:02:03,629 --> 00:02:01,530

in place of molybdenum and so

47

00:02:05,520 --> 00:02:03,639

phylogenetic analyses have established

48

00:02:07,679 --> 00:02:05,530

that each of these three nitrogenous

49

00:02:10,380 --> 00:02:07,689

forms are genetically distinct but they

50

00:02:13,050 --> 00:02:10,390

are evolutionarily related and so then

51
00:02:14,970 --> 00:02:13,060
that presents the question well what was

52
00:02:17,670 --> 00:02:14,980
the metal usage of ancestral

53
00:02:20,040 --> 00:02:17,680
nitrogenases and so the reason why this

54
00:02:22,080 --> 00:02:20,050
question is particularly interesting

55
00:02:24,840 --> 00:02:22,090
from an astrobiological perspective is

56
00:02:26,970 --> 00:02:24,850
it kind of requires that we probe this

57
00:02:28,080 --> 00:02:26,980
potential ancient and interaction

58
00:02:30,569 --> 00:02:28,090
between

59
00:02:32,429 --> 00:02:30,579
marine geochemistry through time as well

60
00:02:35,280 --> 00:02:32,439
as the evolution of nitrogen fixation

61
00:02:37,830 --> 00:02:35,290
and other metabolic processes so these

62
00:02:39,509 --> 00:02:37,840
three metals that are incorporated in

63
00:02:42,660 --> 00:02:39,519

the nitrogenous cofactor are themselves

64

00:02:44,879 --> 00:02:42,670

redox sensitive and so their abundances

65

00:02:47,610 --> 00:02:44,889

in in the Earth's oceans through time

66

00:02:49,559 --> 00:02:47,620

have changed largely as a function of

67

00:02:52,679 --> 00:02:49,569

Earth's surface oxygenation so for

68

00:02:55,920 --> 00:02:52,689

example molybdenum is one example its

69

00:02:58,170 --> 00:02:55,930

abundance in the Earth's oceans has was

70

00:03:00,149 --> 00:02:58,180

probably not very high until after the

71

00:03:02,369 --> 00:03:00,159

great oxidation event and so this

72

00:03:04,140 --> 00:03:02,379

observation then led to the hypothesis

73

00:03:06,149 --> 00:03:04,150

that perhaps ancestral forms of

74

00:03:08,220 --> 00:03:06,159

nitrogenase that originated prior to the

75

00:03:10,319 --> 00:03:08,230

great oxidation event maybe these were

76
00:03:11,580 --> 00:03:10,329
in fact molybdenum independent because

77
00:03:14,670 --> 00:03:11,590
there wasn't enough molybdenum to go

78
00:03:16,530 --> 00:03:14,680
around basically still more recent

79
00:03:18,839 --> 00:03:16,540
phylogenetic analyses have instead

80
00:03:21,089 --> 00:03:18,849
suggested that the molybdenum form

81
00:03:23,280 --> 00:03:21,099
preceded the evolution of the vanadium

82
00:03:24,869 --> 00:03:23,290
and iron forms and maybe this didn't

83
00:03:27,690 --> 00:03:24,879
happen until after the great oxidation

84
00:03:29,699 --> 00:03:27,700
event or somewhere close to it so we

85
00:03:31,830 --> 00:03:29,709
wanted to further explore this history

86
00:03:34,559 --> 00:03:31,840
of nitrogenous metal usage and we wanted

87
00:03:36,930 --> 00:03:34,569
to specifically be able to infer the

88
00:03:40,229 --> 00:03:36,940

metal dependence of reconstructed

89

00:03:42,089 --> 00:03:40,239

ancestral nitrogenous enzymes and so we

90

00:03:44,309 --> 00:03:42,099

use a method called ancestral sequence

91

00:03:46,710 --> 00:03:44,319

reconstructions so basically this

92

00:03:48,330 --> 00:03:46,720

entails collecting a modern data set of

93

00:03:50,879 --> 00:03:48,340

in this case nitrogenase protein

94

00:03:53,640 --> 00:03:50,889

sequences using that information to

95

00:03:55,979 --> 00:03:53,650

build a phylogenetic tree and then using

96

00:03:57,780 --> 00:03:55,989

statistical methods to then infer an

97

00:04:01,050 --> 00:03:57,790

ancestral sequence that corresponds to

98

00:04:03,270 --> 00:04:01,060

some ancestral node of interest and then

99

00:04:04,649 --> 00:04:03,280

with that sequence you can analyze it

100

00:04:07,170 --> 00:04:04,659

for interesting features and you can

101

00:04:10,259 --> 00:04:07,180

also use it to model ancestral protein

102

00:04:11,969 --> 00:04:10,269

structures and so what we wanted to do

103

00:04:13,979 --> 00:04:11,979

is we wanted to use this method to

104

00:04:16,170 --> 00:04:13,989

specifically analyze the active site

105

00:04:18,330 --> 00:04:16,180

sequence and structural features that

106

00:04:20,490 --> 00:04:18,340

had previously been used to classify the

107

00:04:22,890 --> 00:04:20,500

metal dependence of modern nitrogenases

108

00:04:26,700 --> 00:04:22,900

and instead apply it to our inferred

109

00:04:28,560 --> 00:04:26,710

ancestors so pictured here on the left

110

00:04:30,240 --> 00:04:28,570

this is our nitrogenase phylogenetic

111

00:04:32,129 --> 00:04:30,250

tree so this was built from a

112

00:04:33,870 --> 00:04:32,139

concatenated data set of molybdenum

113

00:04:37,379 --> 00:04:33,880

vanadium and the iron nitrogenous

114

00:04:40,019 --> 00:04:37,389

protein sequences oops so most of the

115

00:04:41,609 --> 00:04:40,029

the tree is made up of molybdenum

116

00:04:44,159 --> 00:04:41,619

dependent forms I mentioned this these

117

00:04:46,619 --> 00:04:44,169

are the most common but up here on the

118

00:04:49,139 --> 00:04:46,629

top this lineage this is where the iron

119

00:04:51,719 --> 00:04:49,149

and vanadium nitrogenous forms cluster

120

00:04:56,069 --> 00:04:51,729

um and what's also interesting is they

121

00:04:58,769 --> 00:04:56,079

seem to branch after the divergence of

122

00:05:00,239 --> 00:04:58,779

this purple group here at M BMC so this

123

00:05:02,729 --> 00:05:00,249

is another smaller group of molybdenum

124

00:05:04,979 --> 00:05:02,739

dependent nitrogenases and so this

125

00:05:06,809 --> 00:05:04,989

topology seems to be consistent with the

126

00:05:08,969 --> 00:05:06,819

idea that iron and vanadium forms

127

00:05:11,699 --> 00:05:08,979

evolved after the evolution of

128

00:05:13,529 --> 00:05:11,709

molybdenum usage but at the base of this

129

00:05:15,359 --> 00:05:13,539

lineage or to other clues so there's

130

00:05:16,859 --> 00:05:15,369

one in yellow one in green these are

131

00:05:18,869 --> 00:05:16,869

quote-unquote uncharacterized

132

00:05:20,879 --> 00:05:18,879

nitrogenases and so they're called this

133

00:05:24,059 --> 00:05:20,889

because we don't have really any

134

00:05:27,389 --> 00:05:24,069

experimental evidence or any any

135

00:05:29,459 --> 00:05:27,399

assessment of what metals that these

136

00:05:32,129 --> 00:05:29,469

nitrogenases incorporate and so they're

137

00:05:33,449 --> 00:05:32,139

a little bit of a mystery and so along

138

00:05:36,449 --> 00:05:33,459

this phylogeny

139

00:05:39,239 --> 00:05:36,459

transect we might expect some kind of

140

00:05:41,189 --> 00:05:39,249

historical change in metal usage and so

141

00:05:44,249 --> 00:05:41,199

it's here where we specifically

142

00:05:46,079 --> 00:05:44,259

reconstructed and analyzed ancestral

143

00:05:48,359 --> 00:05:46,089

nitrogenase sequences in order to

144

00:05:51,329 --> 00:05:48,369

basically track this history of metal

145

00:05:53,249 --> 00:05:51,339

usage ok so the first thing we wanted to

146

00:05:54,749 --> 00:05:53,259

look at was the active site sequence

147

00:05:57,179 --> 00:05:54,759

composition and see if we could find

148

00:06:00,359 --> 00:05:57,189

anything that appeared to correlate with

149

00:06:02,159 --> 00:06:00,369

metal binding so on the bottom these are

150

00:06:05,040 --> 00:06:02,169

pairwise loops these are pairwise

151
00:06:07,709 --> 00:06:05,050
comparisons of extant nitrogenous forms

152
00:06:09,600 --> 00:06:07,719
against each other it's a heat map so

153
00:06:12,689 --> 00:06:09,610
the lighter colors indicate that the

154
00:06:14,759 --> 00:06:12,699
active site sequences lower identity the

155
00:06:16,649 --> 00:06:14,769
darker colors indicate higher sequence

156
00:06:19,049 --> 00:06:16,659
identity and so you can see that these

157
00:06:20,999 --> 00:06:19,059
comparisons do a decent job at

158
00:06:24,359 --> 00:06:21,009
differentiating the molybdenum forms

159
00:06:26,489 --> 00:06:24,369
from the vanadium or iron forms and so

160
00:06:28,139 --> 00:06:26,499
we then bring that up to the top portion

161
00:06:30,839 --> 00:06:28,149
of this plot where we're comparing the

162
00:06:33,209 --> 00:06:30,849
ancestors that's a through e with the

163
00:06:35,459 --> 00:06:33,219

modern forms and we find that most of

164

00:06:37,739 --> 00:06:35,469

the ancestors B through e seem to look a

165

00:06:40,829 --> 00:06:37,749

lot more like the molybdenum forms than

166

00:06:43,229 --> 00:06:40,839

the iron and vanadium forms perhaps with

167

00:06:45,749 --> 00:06:43,239

the exception of ancestor a at the top

168

00:06:47,759 --> 00:06:45,759

which happens to be the direct ancestor

169

00:06:50,669 --> 00:06:47,769

of the iron vanadium forms and so it's

170

00:06:52,930 --> 00:06:50,679

resemblance to those modern nitrogenase

171

00:06:55,790 --> 00:06:52,940

is quite reasonable

172

00:06:58,070 --> 00:06:55,800

all right so we also use these sequences

173

00:07:00,230 --> 00:06:58,080

to generate models of these ancestor

174

00:07:02,600 --> 00:07:00,240

ancestral proteins so here they are

175

00:07:04,880 --> 00:07:02,610

aligned and superimposed on one another

176
00:07:07,040 --> 00:07:04,890
but we were specifically interested in

177
00:07:09,440 --> 00:07:07,050
calculating the volume of the active

178
00:07:11,420 --> 00:07:09,450
site pocket and the reason we were

179
00:07:12,740 --> 00:07:11,430
interested so that's what's pictured in

180
00:07:14,480 --> 00:07:12,750
pink right there the reason we were

181
00:07:16,700 --> 00:07:14,490
interested again was that this parameter

182
00:07:19,190 --> 00:07:16,710
was previously used to classify metal

183
00:07:21,940 --> 00:07:19,200
dependence of modern nitrogenases again

184
00:07:24,440 --> 00:07:21,950
we wanted to apply this to the ancestors

185
00:07:26,630 --> 00:07:24,450
okay so in this rather complicated plot

186
00:07:29,930 --> 00:07:26,640
these are pocket volumes both for

187
00:07:32,060 --> 00:07:29,940
ancestral nitrogenases and for extant

188
00:07:34,370 --> 00:07:32,070

nitrogenases and i just want to draw

189

00:07:36,410 --> 00:07:34,380

your attention to the right side for the

190

00:07:38,570 --> 00:07:36,420

the modern ones and what was kind of

191

00:07:40,910 --> 00:07:38,580

surprising was that we see that the

192

00:07:43,460 --> 00:07:40,920

vanadium nitrogenases as well as one

193

00:07:45,770 --> 00:07:43,470

major group of the molybdenum dependent

194

00:07:48,770 --> 00:07:45,780

ones seem to occupy a very similar

195

00:07:50,450 --> 00:07:48,780

volume range in fact they're so similar

196

00:07:51,650 --> 00:07:50,460

and nearly indistinguishable that

197

00:07:54,320 --> 00:07:51,660

they're actually more similar to each

198

00:07:57,350 --> 00:07:54,330

other than are the two different groups

199

00:07:59,150 --> 00:07:57,360

of molybdenum nitrogenases also

200

00:08:01,430 --> 00:07:59,160

problematically as it seems like a lot

201
00:08:03,650 --> 00:08:01,440
of our ancestors also occupy the same

202
00:08:07,070 --> 00:08:03,660
range and so kind of in contrast to a

203
00:08:09,920 --> 00:08:07,080
previous study we don't seem to see any

204
00:08:12,950 --> 00:08:09,930
way to use this information to infer

205
00:08:16,340 --> 00:08:12,960
metal usage particularly with regard to

206
00:08:17,960 --> 00:08:16,350
our ancestors alright so the last thing

207
00:08:20,420 --> 00:08:17,970
we did was we were interested in the

208
00:08:23,000 --> 00:08:20,430
distribution of proteins that today are

209
00:08:25,400 --> 00:08:23,010
involved with the synthesis of the

210
00:08:28,520 --> 00:08:25,410
molybdenum cofactor in nitrogenase and

211
00:08:29,900 --> 00:08:28,530
so these two proteins to two of the

212
00:08:32,600 --> 00:08:29,910
various proteins that are involved in

213
00:08:34,760 --> 00:08:32,610

this pathway NIF II and if n so we

214

00:08:36,740 --> 00:08:34,770

mapped their distribution on taxa that

215

00:08:39,170 --> 00:08:36,750

are represented represented in our

216

00:08:41,630 --> 00:08:39,180

phylogeny and what we found is that

217

00:08:43,280 --> 00:08:41,640

these two kind of strange clays the

218

00:08:45,590 --> 00:08:43,290

yellow and green on characterized

219

00:08:48,890 --> 00:08:45,600

nitrogenases they seem to have either a

220

00:08:51,830 --> 00:08:48,900

non-existent or incomplete set of NIF E

221

00:08:53,540 --> 00:08:51,840

and if N and so by that information you

222

00:08:57,710 --> 00:08:53,550

might infer that they are unable to use

223

00:09:00,140 --> 00:08:57,720

molybdenum perhaps parsimoniously we

224

00:09:03,230 --> 00:09:00,150

might also infer that the oldest

225

00:09:05,000 --> 00:09:03,240

ancestors may similarly not have nifion

226

00:09:08,080 --> 00:09:05,010

if n and so may

227

00:09:10,970 --> 00:09:08,090

maybe also molybdenum in the pendant

228

00:09:13,340 --> 00:09:10,980

okay so as a quick recap it seems like

229

00:09:15,170 --> 00:09:13,350

our oldest ancestor active site sequence

230

00:09:17,240 --> 00:09:15,180

compositions resemble the molybdenum

231

00:09:19,520 --> 00:09:17,250

ones more than the vanadium and iron

232

00:09:22,520 --> 00:09:19,530

ones but we have this seemingly

233

00:09:25,610 --> 00:09:22,530

contradictory observation that the basal

234

00:09:27,410 --> 00:09:25,620

and characterize nitrogenases lack the

235

00:09:30,020 --> 00:09:27,420

associated proteins for molybdenum

236

00:09:32,330 --> 00:09:30,030

dependence so let's you solve this

237

00:09:34,490 --> 00:09:32,340

discrepancy is maybe two main options

238

00:09:37,070 --> 00:09:34,500

that we can go with perhaps these oldest

239

00:09:39,500 --> 00:09:37,080

ancestors did in fact use molybdenum and

240

00:09:41,780 --> 00:09:39,510

these uncharacterized clades may have

241

00:09:43,190 --> 00:09:41,790

secondarily lost these cofactor proteins

242

00:09:45,170 --> 00:09:43,200

or maybe they use a different pathway

243

00:09:48,080 --> 00:09:45,180

for molybdenum usage that we currently

244

00:09:49,670 --> 00:09:48,090

don't know anything about the second

245

00:09:52,640 --> 00:09:49,680

option is that perhaps the oldest

246

00:09:54,470 --> 00:09:52,650

ancestors did not use molybdenum because

247

00:09:55,970 --> 00:09:54,480

they were not able to synthesize the

248

00:09:57,890 --> 00:09:55,980

molybdenum cofactor they didn't have

249

00:10:00,230 --> 00:09:57,900

enough a they didn't have enough n and

250

00:10:02,360 --> 00:10:00,240

the sequence resemblances that we

251
00:10:04,550 --> 00:10:02,370
observe may not be entirely definitive

252
00:10:06,770 --> 00:10:04,560
and that molybdenum usage really did

253
00:10:08,690 --> 00:10:06,780
evolve with Nephi and if n once

254
00:10:10,910 --> 00:10:08,700
molybdenum became available and not

255
00:10:14,360 --> 00:10:10,920
until after the divergence of these

256
00:10:16,760 --> 00:10:14,370
uncharacterized clades so for a few

257
00:10:18,680 --> 00:10:16,770
reasons we prefer the second scenario of

258
00:10:21,110 --> 00:10:18,690
the first thing is that we actually do

259
00:10:23,660 --> 00:10:21,120
have isotopic signatures of nitrogenous

260
00:10:25,400 --> 00:10:23,670
activity in the geologic record so these

261
00:10:28,550 --> 00:10:25,410
were recently reported and they date

262
00:10:30,500 --> 00:10:28,560
prior to our estimates for marine

263
00:10:33,350 --> 00:10:30,510

molybdenum availability and they're also

264

00:10:36,710 --> 00:10:33,360

dated older than the molecular molecular

265

00:10:38,090 --> 00:10:36,720

clock ages and if EMF in a second it's

266

00:10:40,370 --> 00:10:38,100

been previously proposed the

267

00:10:42,650 --> 00:10:40,380

nitrogenases prior to the evolution of

268

00:10:44,840 --> 00:10:42,660

Na Fianna fen may still have been

269

00:10:47,540 --> 00:10:44,850

functional so able to fix nitrogen and

270

00:10:50,030 --> 00:10:47,550

this model kind of still leaves some

271

00:10:52,940 --> 00:10:50,040

room that could be possible that early

272

00:10:54,980 --> 00:10:52,950

molybdenum usage may have been may have

273

00:10:57,440 --> 00:10:54,990

been doable by some different currently

274

00:11:00,250 --> 00:10:57,450

unknown pathway so we can illustrate

275

00:11:03,230 --> 00:11:00,260

this model on our phylogeny

276

00:11:05,540 --> 00:11:03,240

so first potentially we started with a

277

00:11:08,300 --> 00:11:05,550

nitrogenous that had no associated NIF

278

00:11:10,340 --> 00:11:08,310

lan offend proteins therefore was not

279

00:11:12,680 --> 00:11:10,350

molybdenum dependent at least not in the

280

00:11:14,210 --> 00:11:12,690

typical way and that this ancestral

281

00:11:16,790 --> 00:11:14,220

state is still exhibited by

282

00:11:17,470 --> 00:11:16,800

uncharacterized clades that still exists

283

00:11:19,780 --> 00:11:17,480

in the

284

00:11:21,699 --> 00:11:19,790

hello then we have this stepwise

285

00:11:24,189 --> 00:11:21,709

addition of these co factor proteins

286

00:11:26,170 --> 00:11:24,199

perhaps starting with NIF e and this is

287

00:11:28,629 --> 00:11:26,180

still reflected by the uncharacterized

288

00:11:30,879 --> 00:11:28,639

clades in green and it wasn't until we

289

00:11:33,610 --> 00:11:30,889

have both Nephi and NIF and that today

290

00:11:36,040 --> 00:11:33,620

our harbored by the molybdenum dependent

291

00:11:38,590 --> 00:11:36,050

forms in purple and also in blue that we

292

00:11:41,079 --> 00:11:38,600

had actual true molybdenum usage and

293

00:11:42,850 --> 00:11:41,089

then finally it wasn't after until after

294

00:11:44,680 --> 00:11:42,860

the evolution of molybdenum usage that

295

00:11:50,290 --> 00:11:44,690

we get the diversification of the iron

296

00:11:53,079 --> 00:11:50,300

and in vanadium forms alright so in

297

00:11:55,120 --> 00:11:53,089

conclusion it seems like our ancestors

298

00:11:57,310 --> 00:11:55,130

at least just looking at the sequences

299

00:12:00,100 --> 00:11:57,320

seem to resemble molybdenum nitrogenases

300

00:12:01,960 --> 00:12:00,110

but our phylogenetic analyses suggest

301
00:12:04,930 --> 00:12:01,970
that canonic canonical of molybdenum

302
00:12:07,060 --> 00:12:04,940
usage evolved with a cofactor synthesis

303
00:12:10,329 --> 00:12:07,070
mechanism Nephi and a fen and probably

304
00:12:13,000 --> 00:12:10,339
not until after the divergence of these

305
00:12:14,710 --> 00:12:13,010
uncharacterized clades and finally I

306
00:12:16,569 --> 00:12:14,720
just want to highlight that even though

307
00:12:18,879 --> 00:12:16,579
the work I'm presented here today is

308
00:12:20,740 --> 00:12:18,889
entirely computational this method of

309
00:12:23,620 --> 00:12:20,750
ancestral sequence reconstruction also

310
00:12:26,139 --> 00:12:23,630
permits us to synthesize their encoding

311
00:12:28,329 --> 00:12:26,149
genes and quote-unquote resurrect these

312
00:12:30,309 --> 00:12:28,339
enzymes in the laboratory and so we can

313
00:12:32,230 --> 00:12:30,319

continue to experimentally test these

314

00:12:37,870 --> 00:12:32,240

hypotheses so please stay tuned for

315

00:12:39,730 --> 00:12:37,880

future work ok so to just and here I'd

316

00:12:40,740 --> 00:12:39,740

like to acknowledge our research group

317

00:12:44,889 --> 00:12:40,750

at the University of Arizona

318

00:12:46,540 --> 00:12:44,899

particularly my PI Batool Kjar please

319

00:12:47,889 --> 00:12:46,550

visit our website if you're interested

320

00:12:49,600 --> 00:12:47,899

in lots of other fun projects we're

321

00:12:52,300 --> 00:12:49,610

working on and I'd like to acknowledge

322

00:12:55,059 --> 00:12:52,310

our funding sources in particular the

323

00:12:57,300 --> 00:12:55,069

NASA Astrobiology postdoc program so

324

00:13:00,050 --> 00:12:57,310

thank you very much

325

00:13:02,890 --> 00:13:00,060

[Music]

326

00:13:06,980 --> 00:13:02,900

we have time for a couple of questions

327

00:13:08,840 --> 00:13:06,990

yeah a gene families are sometimes put

328

00:13:10,910 --> 00:13:08,850

it just super gene families if you look

329

00:13:12,650 --> 00:13:10,920

deeper and you've lower your homology

330

00:13:17,060 --> 00:13:12,660

threshold and I'm wondering have you

331

00:13:18,590 --> 00:13:17,070

looked at a possibly related gene family

332

00:13:20,270 --> 00:13:18,600

to the nitrogenases that you looked at

333

00:13:23,720 --> 00:13:20,280

and if so that would give you an

334

00:13:25,670 --> 00:13:23,730

out-group for your tree yes so so our

335

00:13:27,920 --> 00:13:25,680

tree is rooted with an out-group in this

336

00:13:29,420 --> 00:13:27,930

case we used proteins that are they're

337

00:13:30,950 --> 00:13:29,430

called light independent proto

338

00:13:32,600 --> 00:13:30,960

chlorophyll l'd reductases they're

339

00:13:34,700 --> 00:13:32,610

involved with the synthesis of bacteria

340

00:13:36,410 --> 00:13:34,710

chlorophyll basically and so they do

341

00:13:39,110 --> 00:13:36,420

share distant homology there's also

342

00:13:41,780 --> 00:13:39,120

other enzymes that seem to be fairly

343

00:13:44,420 --> 00:13:41,790

related those are called nitrogenous

344

00:13:46,010 --> 00:13:44,430

like enzymes and as of yet I don't think

345

00:13:48,410 --> 00:13:46,020

we know exactly what they do but they

346

00:13:49,670 --> 00:13:48,420

seem to be in methanogens do they have

347

00:13:53,270 --> 00:13:49,680

any metal cofactor

348

00:13:55,910 --> 00:13:53,280

yes they do have metal cofactors they do

349

00:13:59,540 --> 00:13:55,920

not look very much like the nitrogenous

350

00:14:01,010 --> 00:13:59,550

cofactor I can't describe it to you off

351

00:14:07,520 --> 00:14:01,020

the top of my head but yes they are

352

00:14:10,300 --> 00:14:07,530

metallo enzymes yeah is what another

353

00:14:12,910 --> 00:14:10,310

possibility be that the ancestral

354

00:14:15,020 --> 00:14:12,920

nitrogenase sort of could use multiple

355

00:14:16,490 --> 00:14:15,030

cofactors multiple enzymes and that

356

00:14:20,360 --> 00:14:16,500

instead in that what you're seeing is

357

00:14:22,490 --> 00:14:20,370

sort of specializing and canalization to

358

00:14:25,760 --> 00:14:22,500

being assembled in particular ways and

359

00:14:27,440 --> 00:14:25,770

maybe getting better as a result yeah so

360

00:14:29,300 --> 00:14:27,450

we have thought about this kind of

361

00:14:32,000 --> 00:14:29,310

interesting idea of like an ancestral

362

00:14:34,940 --> 00:14:32,010

generalist enzyme and it's kind of cool

363

00:14:38,360 --> 00:14:34,950

to think about it's it's a little bit

364

00:14:40,490 --> 00:14:38,370

difficult to reconcile with with the

365

00:14:43,190 --> 00:14:40,500

modern processes of building that

366

00:14:45,460 --> 00:14:43,200

cofactor which you know rely on a kind

367

00:14:49,070 --> 00:14:45,470

of very hierarchical structure of

368

00:14:52,400 --> 00:14:49,080

associated proteins that do the job

369

00:14:55,340 --> 00:14:52,410

basically and so one potential option is

370

00:14:57,710 --> 00:14:55,350

that there was only a small subset of

371

00:15:01,460 --> 00:14:57,720

those proteins that directed perhaps

372

00:15:03,079 --> 00:15:01,470

just the core of the cofactor deficient

373

00:15:07,820 --> 00:15:03,089

of molybdenum maybe deficient of

374

00:15:09,350 --> 00:15:07,830

vanadium but it's I'm not sure of any

375

00:15:11,550 --> 00:15:09,360

way that you could sort of get other

376

00:15:13,560 --> 00:15:11,560

metals transiently associating

377

00:15:15,510 --> 00:15:13,570

the cofactor but maybe by looking at

378

00:15:18,360 --> 00:15:15,520

these other uncharacterized nitrogenases

379

00:15:20,280 --> 00:15:18,370

we can sort of expand our idea of the

380

00:15:23,820 --> 00:15:20,290

possibilities of how this nitrogenase

381

00:15:25,110 --> 00:15:23,830

could have functioned all right all

382

00:15:27,740 --> 00:15:25,120

right if there are no other questions

383

00:15:28,470 --> 00:15:27,750

let's thank again our speakers