
AbSciCon
2019

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

2
00:00:12,160 --> 00:00:09,240
[Applause]

3
00:00:14,110 --> 00:00:12,170
I'm Tom dear from the University of

4
00:00:16,660 --> 00:00:14,120
Arkansas and I'd like to thank you all

5
00:00:19,450 --> 00:00:16,670
for coming and thank the organizers for

6
00:00:22,150 --> 00:00:19,460
the opportunity to present the work

7
00:00:25,390 --> 00:00:22,160
going on in our lab so sulfur specific

8
00:00:29,589 --> 00:00:25,400
maturation of nitrogenous in a

9
00:00:31,210 --> 00:00:29,599
methanogens I've got some to be pretty

10
00:00:32,679 --> 00:00:31,220
exciting results to share with you but

11
00:00:36,400 --> 00:00:32,689
first I want to go through a little bit

12
00:00:39,010 --> 00:00:36,410
of background so Earth's geochemistry

13
00:00:43,180 --> 00:00:39,020

has shifted pretty radically over

14
00:00:47,230 --> 00:00:43,190
geologic time early on we think there

15
00:00:52,500 --> 00:00:47,240
were locally abundant sources of reduced

16
00:00:55,710 --> 00:00:52,510
iron and sulfide and that ancient cells

17
00:00:58,060 --> 00:00:55,720
broadly made use of these and

18
00:01:00,460 --> 00:00:58,070
incorporating clusters into iron sulfur

19
00:01:03,490 --> 00:01:00,470
proteins to carry out important

20
00:01:06,550 --> 00:01:03,500
reactions for life and with the rise of

21
00:01:09,310 --> 00:01:06,560
atmospheric oxygen these became much

22
00:01:12,370 --> 00:01:09,320
less attractive cofactors so where

23
00:01:15,940 --> 00:01:12,380
possible Arabs have minimised their use

24
00:01:18,370 --> 00:01:15,950
but modern anaerobes particularly strict

25
00:01:20,710 --> 00:01:18,380
Anna ropes like methanogens still make

26
00:01:23,110 --> 00:01:20,720
use of loads of higher and sulfur

27
00:01:26,020 --> 00:01:23,120
proteins and so they sort of represent a

28
00:01:31,680 --> 00:01:26,030
window into the past you know get some

29
00:01:38,680 --> 00:01:35,830
methanogens are also extremely important

30
00:01:41,230 --> 00:01:38,690
in studying the nitrogen cycle thanks to

31
00:01:44,649 --> 00:01:41,240
some really great work by eric boyd and

32
00:01:47,320 --> 00:01:44,659
john peters and many others there's good

33
00:01:49,600 --> 00:01:47,330
evidence that the nitrogenase enzyme

34
00:01:53,649 --> 00:01:49,610
that's responsible for the biological

35
00:01:57,969 --> 00:01:53,659
fixation of dinitrogen first arose in

36
00:02:01,990 --> 00:01:57,979
methanogens so a broad outline of the

37
00:02:05,560 --> 00:02:02,000
way that the the reaction progresses

38
00:02:07,919 --> 00:02:05,570

dinitrogen is reduced to ammonia at the

39
00:02:12,460 --> 00:02:07,929
expense of reducing equivalents and

40
00:02:15,460 --> 00:02:12,470
quite a bit of energy and there are iron

41
00:02:18,850 --> 00:02:15,470
sulfur clusters some somewhat bizarre

42
00:02:21,900 --> 00:02:18,860
ones compared to the broad range of iron

43
00:02:23,940 --> 00:02:21,910
sulfur proteins that are required

44
00:02:28,410 --> 00:02:23,950
to carry this out and then methanogens

45
00:02:31,590 --> 00:02:28,420
notably code for a lot of other iron

46
00:02:34,140 --> 00:02:31,600
sulfur proteins that are of interest to

47
00:02:37,130 --> 00:02:34,150
astrobiologists and people studying

48
00:02:39,170 --> 00:02:37,140
early Earth including Farah dachshunds

49
00:02:43,350 --> 00:02:39,180
hydrogenase and carbon monoxide

50
00:02:45,090 --> 00:02:43,360
dehydrogenase but given that all of

51
00:02:46,890 --> 00:02:45,100
these iron sulfur clusters are needed in

52
00:02:48,330 --> 00:02:46,900
methanogens we know little to nothing

53
00:02:54,420 --> 00:02:48,340
about how they're actually being

54
00:02:56,910 --> 00:02:54,430
assembled so what we do know about iron

55
00:03:01,710 --> 00:02:56,920
sulfur cluster biogenesis we mostly know

56
00:03:04,920 --> 00:03:01,720
from bacteria and eukaryotes so there

57
00:03:07,170 --> 00:03:04,930
are three described systems with some

58
00:03:09,240 --> 00:03:07,180
commonalities running through them

59
00:03:11,610 --> 00:03:09,250
the first system described was actually

60
00:03:14,790 --> 00:03:11,620
the NIF system this is specific for

61
00:03:19,230 --> 00:03:14,800
nitrogenase described in his Oda dr. van

62
00:03:22,890 --> 00:03:19,240
LAN di next described was the is C or

63
00:03:26,150 --> 00:03:22,900

ystem and so this is quite similar to

64
00:03:28,890 --> 00:03:26,160
NIF but more widely distributed among

65
00:03:31,170 --> 00:03:28,900
bacteria and the mitochondria of

66
00:03:33,360 --> 00:03:31,180
eukaryotes this is more of a

67
00:03:35,580 --> 00:03:33,370
housekeeping system for general iron

68
00:03:38,580 --> 00:03:35,590
sulfur cluster biogenesis and then last

69
00:03:41,670 --> 00:03:38,590
described was the sough system and this

70
00:03:44,520 --> 00:03:41,680
is especially important today in strict

71
00:03:47,730 --> 00:03:44,530
anaerobes strict anaerobic bacteria and

72
00:03:51,300 --> 00:03:47,740
in the chloroplasts of plants common to

73
00:03:54,840 --> 00:03:51,310
all three systems is the overall

74
00:03:57,060 --> 00:03:54,850
reaction scheme which is a cysteine d

75
00:04:01,140 --> 00:03:57,070
sulfurous to take sulfur from cysteine

76
00:04:03,120 --> 00:04:01,150
and then to help assemble with some iron

77
00:04:05,030 --> 00:04:03,130
coming from somewhere it varies

78
00:04:07,830 --> 00:04:05,040
depending on the organism in the system

79
00:04:12,240 --> 00:04:07,840
to assemble a cluster on a scaffold

80
00:04:15,420 --> 00:04:12,250
protein so for NIF and ISC the cysteine

81
00:04:18,510 --> 00:04:15,430
t sulfur aces are the S proteins the u

82
00:04:19,860 --> 00:04:18,520
proteins of the scaffolds and then from

83
00:04:22,260 --> 00:04:19,870
the scaffold the cluster can be handed

84
00:04:25,530 --> 00:04:22,270
off to a target able protein the

85
00:04:27,900 --> 00:04:25,540
subsystem works the same way in it's

86
00:04:31,680 --> 00:04:27,910
been described in bacteria but the

87
00:04:35,610 --> 00:04:31,690
scaffold is a multi mer of B C or B C

88
00:04:38,879 --> 00:04:35,620

and D soft proteins but all of

89
00:04:40,950 --> 00:04:38,889
have in common the facts that free iron

90
00:04:43,830 --> 00:04:40,960
and sulfide are broadly toxic to most

91
00:04:47,129 --> 00:04:43,840
cells so controlled biogenesis is

92
00:04:49,350 --> 00:04:47,139
required and for all three of these

93
00:04:52,260 --> 00:04:49,360
systems as described everything kind of

94
00:04:57,530 --> 00:04:52,270
runs through cysteine cysteine is the

95
00:05:00,110 --> 00:04:57,540
sulfur donor now methanogens

96
00:05:03,360 --> 00:05:00,120
categorically seem to lack an if type

97
00:05:05,670 --> 00:05:03,370
dedicated biogenesis system for

98
00:05:08,219 --> 00:05:05,680
nitrogenase which is sort of surprising

99
00:05:10,350 --> 00:05:08,229
because you can find the the core

100
00:05:13,830 --> 00:05:10,360
nitrogenous genes in members of all

101
00:05:17,040 --> 00:05:13,840
seven orders of extant methanogens the

102
00:05:19,590 --> 00:05:17,050
two deeply rooted orders are somewhat

103
00:05:22,379 --> 00:05:19,600
metabolically restricted to co2

104
00:05:24,390 --> 00:05:22,389
reduction these do not use cysteine as a

105
00:05:26,790 --> 00:05:24,400
soul sulfur source when they're growing

106
00:05:29,279 --> 00:05:26,800
and there actually is some evidence

107
00:05:31,320 --> 00:05:29,289
showing that inorganic sulfide is the

108
00:05:33,420 --> 00:05:31,330
direct donor for iron sulfur clusters so

109
00:05:34,830 --> 00:05:33,430
that flies in the face of what I just

110
00:05:40,379 --> 00:05:34,840
showed you for everything we know from

111
00:05:42,000 --> 00:05:40,389
bacteria and eukaryotes so they are not

112
00:05:46,260 --> 00:05:42,010
predicted to code for a cysteine sulfur

113
00:05:47,550 --> 00:05:46,270

race just the course of proteins and

114
00:05:48,779 --> 00:05:47,560
I'll go ahead and tell you now those

115
00:05:50,909 --> 00:05:48,789
soft proteins seem like they're

116
00:05:54,270 --> 00:05:50,919
universally conserved in methanogens and

117
00:05:57,779 --> 00:05:54,280
even more broadly in archaea so the five

118
00:06:01,589 --> 00:05:57,789
later evolving orders of methanogens so

119
00:06:03,810 --> 00:06:01,599
also have those soft core proteins many

120
00:06:07,830 --> 00:06:03,820
of them also appear to have the core

121
00:06:10,379 --> 00:06:07,840
misc system as well these are more

122
00:06:14,300 --> 00:06:10,389
flexible they some of them are capable

123
00:06:17,490 --> 00:06:14,310
of multiple methanogenesis pathways and

124
00:06:19,230 --> 00:06:17,500
have briefly been reported several of

125
00:06:22,200 --> 00:06:19,240
them to be able to grow on cysteine or

126
00:06:24,689 --> 00:06:22,210
sulfide as a sole sulfur source and for

127
00:06:27,210 --> 00:06:24,699
some of them even more compounds than

128
00:06:30,450 --> 00:06:27,220
that in our lab we mainly work with

129
00:06:32,760 --> 00:06:30,460
members of the genus Mathias Arsena

130
00:06:33,659 --> 00:06:32,770
order matheno sarsen Allie's and I'm

131
00:06:37,589 --> 00:06:33,669
gonna tell you a little bit more about

132
00:06:39,960 --> 00:06:37,599
the one that we love the most next so

133
00:06:41,400 --> 00:06:39,970
matheno SAR saina heceta for ends or

134
00:06:44,850 --> 00:06:41,410
asset difference depending on who you're

135
00:06:47,580 --> 00:06:44,860
talking to is a metal metabolically

136
00:06:48,969 --> 00:06:47,590
flexible model meth antigen it's a great

137
00:06:51,279 --> 00:06:48,979
model system

138
00:06:53,800 --> 00:06:51,289

for one thing there's a strong genetic

139
00:06:59,409 --> 00:06:53,810
system that's been developed so that we

140
00:07:01,749 --> 00:06:59,419
can manipulate the genome and also it

141
00:07:03,909 --> 00:07:01,759
just has lots of copies of everything so

142
00:07:06,730 --> 00:07:03,919
there are as many as maybe three copies

143
00:07:11,290 --> 00:07:06,740
of the ISC system two copies of the

144
00:07:14,200 --> 00:07:11,300
subsystem and this is sort of unusual if

145
00:07:15,790 --> 00:07:14,210
you look in bacteria to have many copies

146
00:07:18,339 --> 00:07:15,800
of things but it looks like it's not the

147
00:07:22,179 --> 00:07:18,349
only method of SARS on a species that

148
00:07:24,760 --> 00:07:22,189
has this and we can confirm that it can

149
00:07:26,709 --> 00:07:24,770
grow on cysteine or sulfide or a

150
00:07:29,529 --> 00:07:26,719
combination of the two sulfur sources

151
00:07:33,070 --> 00:07:29,539
and does just fine

152
00:07:35,529 --> 00:07:33,080
it also appears to have all three known

153
00:07:38,760 --> 00:07:35,539
versions of nitrogenous molybdenum

154
00:07:41,559 --> 00:07:38,770
vanadium and the iron only nitrogenase

155
00:07:44,860 --> 00:07:41,569
so the molybdenum nitrogenase is the one

156
00:07:47,170 --> 00:07:44,870
you find in all desert ropes the

157
00:07:49,300 --> 00:07:47,180
vanadium and iron only nitrogenases are

158
00:07:51,179 --> 00:07:49,310
the alternative nitrogenases and i'm not

159
00:07:53,679 --> 00:07:51,189
going to talk any more about them today

160
00:07:58,079 --> 00:07:53,689
so from here forward I'll be talking

161
00:08:03,339 --> 00:07:58,089
about the molybdenum nitrogenase and I

162
00:08:05,740 --> 00:08:03,349
started first looking at the is c2 gene

163
00:08:09,070 --> 00:08:05,750

cluster these are the proteins that have

164
00:08:11,619 --> 00:08:09,080
been detected at the highest levels most

165
00:08:13,559 --> 00:08:11,629
consistently in proteomic studies of a

166
00:08:17,889 --> 00:08:13,569
messy divorce

167
00:08:19,929 --> 00:08:17,899
so I briefly I cloned the two core

168
00:08:23,619 --> 00:08:19,939
components the cysteine d sulphurous and

169
00:08:26,230 --> 00:08:23,629
the scaffold proteins s2 and u 2 into e

170
00:08:29,800 --> 00:08:26,240
coli overexpressed purified to

171
00:08:33,639 --> 00:08:29,810
homogeneity and i did the same for a

172
00:08:35,949 --> 00:08:33,649
massive runs as a Cana tastes just to

173
00:08:39,699 --> 00:08:35,959
have a physiologically relevant target

174
00:08:42,579 --> 00:08:39,709
protein to load cluster into so we can

175
00:08:46,420 --> 00:08:42,589
build cluster on the scaffold you

176
00:08:48,400 --> 00:08:46,430
protein using the S protein and then

177
00:08:52,509 --> 00:08:48,410
incubated with April a Cana tastes and

178
00:08:55,180 --> 00:08:52,519
monitor for activity and what we see is

179
00:08:56,490 --> 00:08:55,190
we only get robust activation of April

180
00:09:00,250 --> 00:08:56,500
that kind of taste when it's being

181
00:09:04,000 --> 00:09:00,260
incubated with cluster loaded scaffold

182
00:09:06,090 --> 00:09:04,010
so on its own there's basically no act

183
00:09:09,070 --> 00:09:06,100
and if we incubate it with equivalent

184
00:09:11,410 --> 00:09:09,080
amounts of iron and sulfide in a

185
00:09:14,530 --> 00:09:11,420
reducing environment to what would be

186
00:09:16,990 --> 00:09:14,540
found on the scaffold we basically have

187
00:09:18,550 --> 00:09:17,000
no activity again so in vitro this looks

188
00:09:22,630 --> 00:09:18,560

like a bonafide iron sulfur cluster

189
00:09:25,350 --> 00:09:22,640
biogenesis system so we can knock out

190
00:09:27,880 --> 00:09:25,360
these genes and we did that using

191
00:09:30,900 --> 00:09:27,890
essentially standard techniques and what

192
00:09:34,060 --> 00:09:30,910
we see is moderately impaired growth

193
00:09:35,860 --> 00:09:34,070
when this mutant has grown with cysteine

194
00:09:37,240 --> 00:09:35,870
as the sole sulfur source there's a

195
00:09:39,430 --> 00:09:37,250
little bit of variability from

196
00:09:42,750 --> 00:09:39,440
experiment to experiment but

197
00:09:47,050 --> 00:09:42,760
consistently the mutant prefers sulfide

198
00:09:48,880 --> 00:09:47,060
we can actually a say for cysteine T

199
00:09:51,430 --> 00:09:48,890
sulfurous activity in sulfur eli sites

200
00:09:53,610 --> 00:09:51,440
and when we do that we see significantly

201
00:09:56,260 --> 00:09:53,620
reduced cysteine d sulphureus activity

202
00:09:58,540 --> 00:09:56,270
and the mutant compared to the parent

203
00:10:04,870 --> 00:09:58,550
regardless of what Sulphurs horse the

204
00:10:06,850 --> 00:10:04,880
cells groove on so we see what we think

205
00:10:09,570 --> 00:10:06,860
is some altered sulfur metabolism

206
00:10:12,670 --> 00:10:09,580
although there's no gross cluster

207
00:10:16,320 --> 00:10:12,680
assembly defect so if we just look at

208
00:10:19,180 --> 00:10:16,330
acid labile sulfide to see how much

209
00:10:20,980 --> 00:10:19,190
cluster content there is and the mutant

210
00:10:22,830 --> 00:10:20,990
versus the parent there's no significant

211
00:10:26,170 --> 00:10:22,840
difference regardless of sulfur source

212
00:10:27,700 --> 00:10:26,180
when we look at sulfate sulfur so this

213
00:10:29,620 --> 00:10:27,710

is mechanistically important for the

214
00:10:31,900 --> 00:10:29,630
cysteine ki sulfurous it's sort of how

215
00:10:33,700 --> 00:10:31,910
it works and this is also has

216
00:10:36,490 --> 00:10:33,710
implications for the sulfur relay system

217
00:10:39,100 --> 00:10:36,500
in sulfur metabolism when we look at

218
00:10:40,290 --> 00:10:39,110
sulfate and sulfur we see a significant

219
00:10:43,210 --> 00:10:40,300
reduction

220
00:10:46,870 --> 00:10:43,220
anytime cysteine is present as a sulfur

221
00:10:49,260 --> 00:10:46,880
source so that's sort of an interesting

222
00:10:51,490 --> 00:10:49,270
thing to see in combination with

223
00:10:55,450 --> 00:10:51,500
basically no difference in cluster

224
00:10:57,820 --> 00:10:55,460
content so switching back to the

225
00:11:00,340 --> 00:10:57,830
wild-type real fast a massive difference

226
00:11:02,230 --> 00:11:00,350
can fix nitrogen regardless of what it's

227
00:11:05,490 --> 00:11:02,240
using as a sulfur source cysteine versus

228
00:11:08,800 --> 00:11:05,500
sulfide maybe somewhat surprisingly

229
00:11:11,470 --> 00:11:08,810
neither cysteine nor alanine which is

230
00:11:14,950 --> 00:11:11,480
the sort of side product of 16t sulfur

231
00:11:16,750 --> 00:11:14,960
ace neither one of these amino acids

232
00:11:21,310 --> 00:11:16,760
by having an amino group can serve as a

233
00:11:24,460 --> 00:11:21,320
nitrogen source for in assitive wrens so

234
00:11:27,760 --> 00:11:24,470
when we grow under nitrogen replete

235
00:11:29,140 --> 00:11:27,770
conditions or under nitrogen fixing

236
00:11:31,150 --> 00:11:29,150
conditions where there's no ammonium

237
00:11:33,670 --> 00:11:31,160
present there doesn't seem to be a huge

238
00:11:37,420 --> 00:11:33,680

difference our preference for sulfur

239
00:11:40,000 --> 00:11:37,430
source and when we do a Western blot and

240
00:11:42,490 --> 00:11:40,010
go looking for the catalytic subunit one

241
00:11:45,760 --> 00:11:42,500
of catalytic subunits of the molybdenum

242
00:11:49,450 --> 00:11:45,770
nitrogenase we see it show up only when

243
00:11:51,790 --> 00:11:49,460
ammonium is absent and it's being

244
00:11:55,240 --> 00:11:51,800
produced abundantly whether cysteine or

245
00:11:59,610 --> 00:11:55,250
sulfide is the sulfur source so the

246
00:12:01,930 --> 00:11:59,620
mutant strain deleted of ISC 2 when we

247
00:12:04,660 --> 00:12:01,940
grow it under nitrogen fixing conditions

248
00:12:08,500 --> 00:12:04,670
with sulfide as a sulfur source there is

249
00:12:11,250 --> 00:12:08,510
no phenotype whatsoever however if we

250
00:12:15,070 --> 00:12:11,260
try growing it with cysteine there is a

251
00:12:17,050 --> 00:12:15,080
pronounced growth defect again that

252
00:12:18,610 --> 00:12:17,060
varies somewhat from experiment to

253
00:12:22,090 --> 00:12:18,620
experiment this is probably the most

254
00:12:25,210 --> 00:12:22,100
severe iteration of it that we've seen

255
00:12:28,300 --> 00:12:25,220
but there is a strong strong preference

256
00:12:31,650 --> 00:12:28,310
for growth with sulfide as the sulfur

257
00:12:36,610 --> 00:12:31,660
source under nitrogen fixing conditions

258
00:12:40,690 --> 00:12:36,620
especially with this mutant so that has

259
00:12:43,420 --> 00:12:40,700
led us to a proposed model of cluster

260
00:12:46,150 --> 00:12:43,430
biogenesis in the methanogens M assitive

261
00:12:50,950 --> 00:12:46,160
runs in which cysteine is routed through

262
00:12:54,970 --> 00:12:50,960
the ISC system and then can be used to

263
00:12:58,420 --> 00:12:54,980

load cluster into proteins as needed and

264
00:13:02,680 --> 00:12:58,430
that inorganic sulfide may be going

265
00:13:05,950 --> 00:13:02,690
through perhaps a sort of primordial

266
00:13:07,330 --> 00:13:05,960
subsystem because these subsystems I

267
00:13:10,660 --> 00:13:07,340
told you they're pretty universal in

268
00:13:12,970 --> 00:13:10,670
archaea but they're never really paired

269
00:13:16,330 --> 00:13:12,980
with a suffice cysteine to sulphurous so

270
00:13:20,920 --> 00:13:16,340
it seems like the subsystem is maybe

271
00:13:23,950 --> 00:13:20,930
primordial in archaea and and not really

272
00:13:25,360 --> 00:13:23,960
geared towards using cysteine so the

273
00:13:27,130 --> 00:13:25,370
results that i've showed are consistent

274
00:13:27,530 --> 00:13:27,140
we are still being able to load cluster

275
00:13:35,230 --> 00:13:27,540
in

276
00:13:39,800 --> 00:13:35,240
but there is a serious problem once we

277
00:13:42,170 --> 00:13:39,810
cut off the ISE system and that specific

278
00:13:45,499 --> 00:13:42,180
is c2 is C 1 and is C 3 are still

279
00:13:50,990 --> 00:13:45,509
present but apparently they are not able

280
00:13:53,059 --> 00:13:51,000
to provide for normal maturation of the

281
00:13:57,290 --> 00:13:53,069
molybdenum molybdenum nitrogenase when

282
00:14:01,249 --> 00:13:57,300
cysteine is the sulfur source and so I'd

283
00:14:03,680 --> 00:14:01,259
like to thank my advisor P I dam Lesnar

284
00:14:05,449 --> 00:14:03,690
other members of the lesner lab our

285
00:14:07,720 --> 00:14:05,459
collaborator ever doin who's done some

286
00:14:09,499 --> 00:14:07,730
great biophysical characterization of

287
00:14:11,499 --> 00:14:09,509
proteins for us that I didn't really

288
00:14:14,870 --> 00:14:11,509

have time to share with you today and

289
00:14:16,819 --> 00:14:14,880
then also thank the funding agencies for

290
00:14:22,370 --> 00:14:16,829
their generosity and with that I'll take

291
00:14:24,949 --> 00:14:22,380
any questions thanks Thomas you have

292
00:14:27,889 --> 00:14:24,959
time for a few questions yeah that was

293
00:14:31,490 --> 00:14:27,899
really nice he mentioned that your

294
00:14:35,600 --> 00:14:31,500
bacterium has all three different types

295
00:14:38,629 --> 00:14:35,610
of nitrogenases i didn't can't perhaps

296
00:14:41,269 --> 00:14:38,639
already said this but are you looking at

297
00:14:43,280 --> 00:14:41,279
a deleted version that only has one at a

298
00:14:46,280 --> 00:14:43,290
time or have you tried building such a

299
00:14:48,350 --> 00:14:46,290
thing - I see whether some of them might

300
00:14:49,689 --> 00:14:48,360
be more affected by the by the various

301
00:14:52,780 --> 00:14:49,699
conditions that you've been describing

302
00:14:56,509 --> 00:14:52,790
we are looking into that we don't have

303
00:14:59,059 --> 00:14:56,519
anything where we have actually deleted

304
00:15:01,639 --> 00:14:59,069
the nitrogenase but it's possible to

305
00:15:04,250 --> 00:15:01,649
grow under conditions where you don't

306
00:15:07,030 --> 00:15:04,260
give them any molybdenum and then they

307
00:15:11,939 --> 00:15:07,040
have to use alternative nitrogenases so

308
00:15:16,679 --> 00:15:14,009
I think we have time for other questions

309
00:15:19,499 --> 00:15:16,689
if there are any thanks for the great

310
00:15:21,509 --> 00:15:19,509
talk and the data were coming pretty

311
00:15:23,549 --> 00:15:21,519
quickly so I'm sorry if I oh sure miss

312
00:15:27,090 --> 00:15:23,559
something but it seems like in the is

313
00:15:30,389 --> 00:15:27,100

situ mutant eat undergrowth with

314
00:15:32,340 --> 00:15:30,399
sulphide you had and maybe even an

315
00:15:35,369 --> 00:15:32,350
increased amount of sulphate sulphur and

316
00:15:38,819 --> 00:15:35,379
you also saw some sort of ISC to like

317
00:15:41,059 --> 00:15:38,829
activity does that suggest there's

318
00:15:44,329 --> 00:15:41,069
another group of proteins that are

319
00:15:47,309 --> 00:15:44,339
accomplishing this function yes

320
00:15:51,479 --> 00:15:47,319
certainly for the second part of your

321
00:15:54,199 --> 00:15:51,489
question the most parsimonious

322
00:15:57,809 --> 00:15:54,209
explanation would be those other is see

323
00:16:03,619 --> 00:15:57,819
clusters have bonafide cysteine t sulfur

324
00:16:06,239 --> 00:16:03,629
races a more complicated answer would be

325
00:16:08,309 --> 00:16:06,249
in the methanogens that we don't really

326
00:16:10,409 --> 00:16:08,319
work with and that are predicted not to

327
00:16:11,819 --> 00:16:10,419
have cysteine to sulfur ace if you crack

328
00:16:15,359 --> 00:16:11,829
them open and look for assisting to self

329
00:16:16,470 --> 00:16:15,369
race activity you'll find it so we

330
00:16:19,169 --> 00:16:16,480
probably don't know everything that's

331
00:16:20,999 --> 00:16:19,179
going on I think it would be really

332
00:16:23,669 --> 00:16:21,009
great to look at that latter at class of

333
00:16:29,069 --> 00:16:23,679
methanogens with these assays mm-hmm

334
00:16:32,220 --> 00:16:29,079
it's really exciting Thank You Thomas

335
00:16:36,479 --> 00:16:32,230
all right thanks so our next speaker

336
00:16:38,249 --> 00:16:36,489
Miriam accessibility is she is around so

337
00:16:39,780 --> 00:16:38,259
it doesn't look like she might have made

338
00:16:43,679 --> 00:16:39,790

it to the conference so I apologize

339
00:16:45,030 --> 00:16:43,689
after that talk isn't gonna be coming up

340
00:16:48,090 --> 00:16:45,040
so we have a few minutes if there's

341
00:16:50,329 --> 00:16:48,100
other questions or waiting for the next

342
00:16:52,470 --> 00:16:50,339
talk for the speakers that have already

343
00:16:59,000 --> 00:16:52,480
presented happy to field a few questions

344
00:17:08,130 --> 00:17:04,020
Chirk Shawn I have a question for you

345
00:17:10,320 --> 00:17:08,140
yeah go home before the oxygenation of

346
00:17:13,920 --> 00:17:10,330
the atmosphere what would be the source

347
00:17:17,340 --> 00:17:13,930
of sulfite I I kind of thought that it

348
00:17:20,040 --> 00:17:17,350
went to either sulfate or sulfide but is

349
00:17:22,590 --> 00:17:20,050
there a sulfite component and what we

350
00:17:25,590 --> 00:17:22,600
expect to be coming out from volcanoes

351
00:17:28,380 --> 00:17:25,600
and then going into the ocean yeah so I

352
00:17:30,360 --> 00:17:28,390
won't bring it up but you know sulfur

353
00:17:31,800 --> 00:17:30,370
dioxide so I mentioned the disproportion

354
00:17:34,410 --> 00:17:31,810
ation reaction where it goes to sulfate

355
00:17:35,640 --> 00:17:34,420
as well as hydrogen sulfide but that's

356
00:17:37,080 --> 00:17:35,650
at higher temperatures at lower

357
00:17:40,470 --> 00:17:37,090
temperatures that can just hydrate

358
00:17:43,050 --> 00:17:40,480
it's a sulfuric acid as well with water

359
00:17:47,190 --> 00:17:43,060
and so that can specie 8 depending on

360
00:17:49,980 --> 00:17:47,200
you know the pH it could be at lower pH

361
00:17:51,780 --> 00:17:49,990
sulfur dioxide or sulfuric acid at high

362
00:17:54,480 --> 00:17:51,790
rpm I so fight already

363
00:17:55,650 --> 00:17:54,490

still higher pH sulfite so there's a

364
00:17:58,560 --> 00:17:55,660
potential that you could have sulfite

365
00:18:01,130 --> 00:17:58,570
there and without oxygen or ferric iron

366
00:18:03,630 --> 00:18:01,140
present it could potentially be stable

367
00:18:08,520 --> 00:18:03,640
and I had a diagram on an extra slide

368
00:18:18,510 --> 00:18:10,470
sure I think there's a question in the

369
00:18:25,650 --> 00:18:21,660
I was so we know that for example in eco

370
00:18:28,830 --> 00:18:25,660
light the soft system is assigned for

371
00:18:33,360 --> 00:18:28,840
the de novo cluster synthesis compared

372
00:18:36,660 --> 00:18:33,370
to IOC which is basically for repair

373
00:18:40,320 --> 00:18:36,670
when one iron falls off you just use ISC

374
00:18:43,620 --> 00:18:40,330
to put that one on and back and like in

375
00:18:48,680 --> 00:18:43,630
the lab for example we we put our enzyme

376
00:18:51,890 --> 00:18:48,690
is C D G G for reduction and iron and it

377
00:18:55,350 --> 00:18:51,900
basically repairs the cluster without

378
00:18:58,110 --> 00:18:55,360
the need for anything else so is there

379
00:19:02,340 --> 00:18:58,120
anything similar like these two jobs for

380
00:19:04,920 --> 00:19:02,350
like are the two in your box doing the

381
00:19:10,110 --> 00:19:04,930
same thing or they are all like doing

382
00:19:12,480 --> 00:19:10,120
just then although synthesis not

383
00:19:15,840 --> 00:19:12,490
entirely short so the the very first

384
00:19:18,840 --> 00:19:15,850
part of what you were saying that the

385
00:19:20,580 --> 00:19:18,850
different jobs different roles for ISC

386
00:19:24,750 --> 00:19:20,590
and stuff and E coli yep

387
00:19:29,520 --> 00:19:24,760
so my understanding is that is C is that

388
00:19:32,700 --> 00:19:29,530

the general system and stuff is more

389
00:19:36,480 --> 00:19:32,710
expressed if there is oxidative stress

390
00:19:40,830 --> 00:19:36,490
or a lack of iron is that the other way

391
00:19:43,350 --> 00:19:40,840
round but yeah okay yeah I was just

392
00:19:45,240 --> 00:19:43,360
wondering if that's the case here or

393
00:19:47,340 --> 00:19:45,250
they are just like two systems doing

394
00:19:51,750 --> 00:19:47,350
exactly the same job probably not doing

395
00:19:54,360 --> 00:19:51,760
exactly the same job so if you were

396
00:19:55,140 --> 00:19:54,370
talking about a little bit of oxidative

397
00:19:57,540 --> 00:19:55,150
stress

398
00:20:01,580 --> 00:19:57,550
we're like one iron has fallen out of

399
00:20:05,100 --> 00:20:01,590
the cluster then yeah I would guess that

400
00:20:08,520 --> 00:20:05,110
probably either one could could handle

401
00:20:09,780 --> 00:20:08,530
that kind of repair but well and for

402
00:20:12,660 --> 00:20:09,790
instance with the connotates if you

403
00:20:14,400 --> 00:20:12,670
don't really make it a Poe and fully

404
00:20:16,890 --> 00:20:14,410
strip it out usually what's happening is

405
00:20:19,010 --> 00:20:16,900
one irons being lost and you can

406
00:20:22,399 --> 00:20:19,020
reactivate it like pretty easily just by

407
00:20:24,230 --> 00:20:22,409
incubation with DDT

408
00:20:27,440 --> 00:20:24,240
so yeah that probably doesn't answer