Mechanisms of ROS requirement in SIM

Damaged bases are replicated across by Trans-Lesion Polymerases (TLPs)

TLPs are error-prone

DinB TLP is required for SNV SIM

Mutations previously thought to form through action of DinB TLP on undamaged template

New Model:

TLPs compete for a place at the fork

-Perhaps DNA DSB needed to start replication in stressed cells

-DinB (and other TLPs) recruited when they encounter damaged base

We will test whether TLS activity of *dinB* is required for SIM

1
00:00:10,709 --> 00:00:08,870
so i really want to thank actually lucy

2
00:00:12,790 --> 00:00:10,719
and kristen for their introductions

3
00:00:13,990 --> 00:00:12,800
because they made my introduction a lot

4
00:00:14,789 --> 00:00:14,000
simpler

5
00:00:16,790 --> 00:00:14,799
so

6
00:00:19,269 --> 00:00:16,800
what we know about evolution is that it

7
00:00:21,510 --> 00:00:19,279
is at heart a genetic process

8
00:00:23,509 --> 00:00:21,520
and there are two parallel parallel

9
00:00:25,590 --> 00:00:23,519
pathways of evolution

10
00:00:28,310 --> 00:00:25,600
the first are single nucleotide

11
00:00:30,550 --> 00:00:28,320
variations now these are changes in one

12
00:00:32,630 --> 00:00:30,560
nucleotide at a time

13
00:00:35,430 --> 00:00:32,640

and these change dna and protein

14
00:00:38,229 --> 00:00:35,440
function bit by bit by accumulating

15
00:00:41,110 --> 00:00:38,239
until eventually you do get a modified

16
00:00:42,709 --> 00:00:41,120
or changed function or regulation

17
00:00:44,869 --> 00:00:42,719
now the second type

18
00:00:46,150 --> 00:00:44,879
is what we call a gross chromosomal

19
00:00:49,510 --> 00:00:46,160
rearrangement

20
00:00:51,830 --> 00:00:49,520
now these are changes of large segments

21
00:00:55,189 --> 00:00:51,840
of dna where they either invert

22
00:00:56,790 --> 00:00:55,199
amplify or completely disappear

23
00:00:58,549 --> 00:00:56,800
now these can

24
00:01:00,470 --> 00:00:58,559
affect evolution in multiple ways the

25
00:01:02,549 --> 00:01:00,480
first of which is you change copy number

26
00:01:03,910 --> 00:01:02,559
which gives you a rapid change in

27
00:01:06,630 --> 00:01:03,920
expression level

28
00:01:08,950 --> 00:01:06,640
or you can reassert reassort a gene and

29
00:01:10,789 --> 00:01:08,960
regulatory elements or and this is

30
00:01:12,710 --> 00:01:10,799
perhaps the most important ways by

31
00:01:14,550 --> 00:01:12,720
providing redundant sequence so if you

32
00:01:16,870 --> 00:01:14,560
have an essential gene

33
00:01:19,350 --> 00:01:16,880
that the organism needs to survive but

34
00:01:21,109 --> 00:01:19,360
by amplification you get a second copy

35
00:01:22,870 --> 00:01:21,119
then that organism is free to make

36
00:01:25,670 --> 00:01:22,880
changes in the second copy while

37
00:01:28,230 --> 00:01:25,680
retaining the essential function until

38
00:01:30,230 --> 00:01:28,240

eventually you have the change function

39
00:01:31,670 --> 00:01:30,240
taking over

40
00:01:33,590 --> 00:01:31,680
so what i'd like to introduce to you

41
00:01:36,310 --> 00:01:33,600
today is the phenomena of environmental

42
00:01:38,230 --> 00:01:36,320
stress induced mutation so like we hear

43
00:01:39,350 --> 00:01:38,240
about darwin we hear about survival of

44
00:01:40,870 --> 00:01:39,360
the fittest

45
00:01:43,350 --> 00:01:40,880
where you have random stochastic

46
00:01:45,109 --> 00:01:43,360
mutations in a genome that are selected

47
00:01:47,429 --> 00:01:45,119
for by the environment because they

48
00:01:49,350 --> 00:01:47,439
confer some advantage but what we've

49
00:01:51,830 --> 00:01:49,360
actually been elucidating over the last

50
00:01:54,149 --> 00:01:51,840
20 to 30 years is that

51
00:01:56,870 --> 00:01:54,159
the environment plays a very large part

52
00:01:58,870 --> 00:01:56,880
in how these mutations are formed so you

53
00:02:00,950 --> 00:01:58,880
have a microbe that is specifically

54
00:02:02,630 --> 00:02:00,960
adapted to its environment such as e

55
00:02:04,630 --> 00:02:02,640
coli to earth

56
00:02:06,469 --> 00:02:04,640
when you place this microbe in a

57
00:02:07,990 --> 00:02:06,479
different environment a different ph

58
00:02:08,949 --> 00:02:08,000
different temperature different carbon

59
00:02:11,510 --> 00:02:08,959
source

60
00:02:14,150 --> 00:02:11,520
this organism becomes stressed this

61
00:02:17,830 --> 00:02:14,160
stress induces stress responses stress

62
00:02:20,070 --> 00:02:17,840
responses are large collections of genes

63
00:02:22,229 --> 00:02:20,080

that together serve individual functions

64
00:02:23,190 --> 00:02:22,239
that help that organism adapt to that

65
00:02:26,949 --> 00:02:23,200
stress

66
00:02:29,830 --> 00:02:26,959
responses and microbes you find that you

67
00:02:31,670 --> 00:02:29,840
transiently increase mutation rates by

68
00:02:34,390 --> 00:02:31,680
orders of magnitude

69
00:02:36,790 --> 00:02:34,400
until such a time that a mutation is

70
00:02:39,190 --> 00:02:36,800
made that allows that organism to adapt

71
00:02:41,110 --> 00:02:39,200
to that stressor mutation rates then

72
00:02:43,910 --> 00:02:41,120
fall back to normal

73
00:02:45,190 --> 00:02:43,920
leaving you with an adapted evolved life

74
00:02:47,110 --> 00:02:45,200
form

75
00:02:49,509 --> 00:02:47,120
so what we in our lab do is study the

76
00:02:51,910 --> 00:02:49,519
mechanisms of stress-induced mutation

77
00:02:54,550 --> 00:02:51,920
aiming to understand the inherent

78
00:02:56,470 --> 00:02:54,560
molecular mechanism behind the formation

79
00:02:58,710 --> 00:02:56,480
of these mutations and today i'm going

80
00:03:01,030 --> 00:02:58,720
to present to you some new discoveries

81
00:03:03,430 --> 00:03:01,040
where we found that reactive oxygen

82
00:03:05,990 --> 00:03:03,440
species is required for environmental

83
00:03:08,070 --> 00:03:06,000
stress-induced mutation and i'll discuss

84
00:03:09,990 --> 00:03:08,080
not only how they promote mutation but

85
00:03:12,550 --> 00:03:10,000
what this means for the evolution of

86
00:03:15,110 --> 00:03:12,560
microbes under stress

87
00:03:17,589 --> 00:03:15,120
so we work in a well-characterized

88
00:03:19,509 --> 00:03:17,599

system that selects for starvation

89
00:03:22,070 --> 00:03:19,519
stress induced mutants and e coli so you

90
00:03:24,309 --> 00:03:22,080
have an e coli cell that has a complete

91
00:03:27,190 --> 00:03:24,319
deletion of its lactose genes this means

92
00:03:29,110 --> 00:03:27,200
it cannot use lactose as a carbon source

93
00:03:32,470 --> 00:03:29,120
it can't eat it and then on its

94
00:03:34,630 --> 00:03:32,480
conjugated plasmid has a lac plus one

95
00:03:36,949 --> 00:03:34,640
mutation so this is the lac genes with

96
00:03:39,110 --> 00:03:36,959
an extra nucleotide

97
00:03:42,229 --> 00:03:39,120
so these cells are phenotypically lac

98
00:03:45,910 --> 00:03:42,239
minus they can't eat lactose so when we

99
00:03:47,509 --> 00:03:45,920
place them in a lactose only environment

100
00:03:48,949 --> 00:03:47,519
the only cells that are going to form

101
00:03:51,670 --> 00:03:48,959
colonies are those that have made

102
00:03:54,470 --> 00:03:51,680
compensatory mutations

103
00:03:55,670 --> 00:03:54,480
so what you see on right here

104
00:03:58,070 --> 00:03:55,680
laser pointer

105
00:04:00,470 --> 00:03:58,080
what you see right here is

106
00:04:03,350 --> 00:04:00,480
black plus colonies over the total cells

107
00:04:06,550 --> 00:04:03,360
plated over days of incubation so we

108
00:04:08,550 --> 00:04:06,560
grow up ourselves in a medium that they

109
00:04:10,070 --> 00:04:08,560
can use they can't eat until they reach

110
00:04:12,149 --> 00:04:10,080
a starvation

111
00:04:14,070 --> 00:04:12,159
very dense population

112
00:04:16,229 --> 00:04:14,080
at this point we put them in the

113
00:04:18,870 --> 00:04:16,239

presence of only lactose and count the

114
00:04:20,150 --> 00:04:18,880
number of colonies or mutants that arise

115
00:04:21,509 --> 00:04:20,160
on days two

116
00:04:23,590 --> 00:04:21,519
through seven

117
00:04:26,070 --> 00:04:23,600
now the great thing about this system is

118
00:04:27,510 --> 00:04:26,080
it can detect both cnvs

119
00:04:29,590 --> 00:04:27,520
and gcrs

120
00:04:32,629 --> 00:04:29,600
so cmvs those single nucleotide

121
00:04:34,790 --> 00:04:32,639
variations are going to be um negative

122
00:04:37,590 --> 00:04:34,800
one they just lose a base pair

123
00:04:41,030 --> 00:04:37,600
you reset you get all the lactose

124
00:04:42,870 --> 00:04:41,040
metabolism activity you need a gcr what

125
00:04:45,510 --> 00:04:42,880
the cell does is it actually takes this

126
00:04:47,909 --> 00:04:45,520
allele and amplifies it until you get 20

127
00:04:50,070 --> 00:04:47,919
or more copies you have just enough

128
00:04:50,950 --> 00:04:50,080
activity to survive and so what we can

129
00:04:52,950 --> 00:04:50,960
do

130
00:04:55,510 --> 00:04:52,960
is we can actually detect you take the

131
00:04:57,749 --> 00:04:55,520
total lac plus population

132
00:05:00,550 --> 00:04:57,759
you put them through a colorimetric

133
00:05:02,710 --> 00:05:00,560
assay and you can see that the cmvs

134
00:05:05,990 --> 00:05:02,720
which are very stable mutations give you

135
00:05:08,150 --> 00:05:06,000
solid blue colonies where the gcrs give

136
00:05:10,469 --> 00:05:08,160
you these fraction colonies as the array

137
00:05:12,870 --> 00:05:10,479
breaks down

138
00:05:14,950 --> 00:05:12,880

so i'm not going to get into the really

139
00:05:16,550 --> 00:05:14,960
hard core genetic mechanism behind these

140
00:05:18,070 --> 00:05:16,560
mutation formations but you need to know

141
00:05:19,270 --> 00:05:18,080
a couple things

142
00:05:21,670 --> 00:05:19,280
first of all

143
00:05:24,310 --> 00:05:21,680
these mutations require three stress

144
00:05:26,230 --> 00:05:24,320
responses in e coli now these sense the

145
00:05:28,390 --> 00:05:26,240
stress they see that e coli is in an

146
00:05:29,990 --> 00:05:28,400
environment cannot survive in these

147
00:05:31,189 --> 00:05:30,000
stress responses

148
00:05:33,909 --> 00:05:31,199
turn on

149
00:05:35,670 --> 00:05:33,919
error-prone dna translation polymerases

150
00:05:37,350 --> 00:05:35,680
so rather than the regular polymerase

151
00:05:39,830 --> 00:05:37,360
that replicates the genome in a very

152
00:05:41,830 --> 00:05:39,840
faithful manner these are prone to

153
00:05:44,629 --> 00:05:41,840
inserting mutations even when they're on

154
00:05:46,870 --> 00:05:44,639
an undamaged template

155
00:05:48,710 --> 00:05:46,880
and lastly you need dna transactions you

156
00:05:51,110 --> 00:05:48,720
need a dna double stranded break which

157
00:05:53,590 --> 00:05:51,120
is where both backbones of the double

158
00:05:55,830 --> 00:05:53,600
helix break and then you need the dna

159
00:05:57,029 --> 00:05:55,840
damage repair event to repair that break

160
00:05:59,510 --> 00:05:57,039
so

161
00:06:00,310 --> 00:05:59,520
in a healthy growing easily replicating

162
00:06:02,550 --> 00:06:00,320
cell

163
00:06:05,270 --> 00:06:02,560

when you get a dna double stranded break

164
00:06:07,350 --> 00:06:05,280
it repairs it in a very faithful manner

165
00:06:09,029 --> 00:06:07,360
but in a starving cell that has all

166
00:06:10,390 --> 00:06:09,039
these error prone polymerases up

167
00:06:13,749 --> 00:06:10,400
regulated

168
00:06:15,510 --> 00:06:13,759
you get error prone repair of that break

169
00:06:17,029 --> 00:06:15,520
you get mutations

170
00:06:18,550 --> 00:06:17,039
and then that's how you get your lac

171
00:06:20,230 --> 00:06:18,560
plus mutants

172
00:06:21,510 --> 00:06:20,240
so the big questions our lab tries to

173
00:06:24,710 --> 00:06:21,520
answer is one

174
00:06:27,189 --> 00:06:24,720
how to stress regulate evolution and two

175
00:06:29,350 --> 00:06:27,199
what dictates the choice between a gross

176
00:06:32,070 --> 00:06:29,360
chromosomal rearrangement and a single

177
00:06:34,070 --> 00:06:32,080
nucleotide variation

178
00:06:36,950 --> 00:06:34,080
so we have a new component of this

179
00:06:38,870 --> 00:06:36,960
mechanism emerging where a damaged base

180
00:06:40,950 --> 00:06:38,880
in the dna is required for

181
00:06:43,270 --> 00:06:40,960
stress-induced mutation and this is an

182
00:06:46,309 --> 00:06:43,280
unexpected constraint on both the

183
00:06:48,550 --> 00:06:46,319
mechanism of single nucleotide variation

184
00:06:50,070 --> 00:06:48,560
in gcrs

185
00:06:52,790 --> 00:06:50,080
so while we were investigating the

186
00:06:55,590 --> 00:06:52,800
nuclear associated proteins

187
00:06:57,909 --> 00:06:55,600
we discovered that deletion of dps

188
00:07:00,150 --> 00:06:57,919

inhibits stress-induced uh

189
00:07:01,350 --> 00:07:00,160
nucleotide variations and gcr so what

190
00:07:02,710 --> 00:07:01,360
you're going to see and this is the kind

191
00:07:03,909 --> 00:07:02,720
of data you're going to see the rest of

192
00:07:07,029 --> 00:07:03,919
the talk

193
00:07:08,309 --> 00:07:07,039
is where you have a rates of mutation

194
00:07:10,469 --> 00:07:08,319
formation

195
00:07:12,629 --> 00:07:10,479
normalized to wild type so what you can

196
00:07:14,790 --> 00:07:12,639
see is that when we delete dps on the

197
00:07:17,110 --> 00:07:14,800
right we get more mutation so whatever

198
00:07:19,430 --> 00:07:17,120
dps is doing normally it's inhibiting

199
00:07:21,510 --> 00:07:19,440
mutation and we find that this holds

200
00:07:23,589 --> 00:07:21,520
true for both c and v's

201
00:07:25,189 --> 00:07:23,599
and gcrs so i'm not going to split up

202
00:07:27,029 --> 00:07:25,199
the data for the rest of the talk but i

203
00:07:29,749 --> 00:07:27,039
want you to keep in mind that everything

204
00:07:32,150 --> 00:07:29,759
i tell you is true for both types

205
00:07:34,230 --> 00:07:32,160
of mutations

206
00:07:35,350 --> 00:07:34,240
so then we wanted to make sure that we

207
00:07:37,589 --> 00:07:35,360
weren't

208
00:07:40,550 --> 00:07:37,599
allowing an alternative mutagenesis

209
00:07:43,430 --> 00:07:40,560
pathway to happen so we in so we added

210
00:07:45,830 --> 00:07:43,440
deletions of known required genes such

211
00:07:47,430 --> 00:07:45,840
as that rpos that i talked about

212
00:07:49,189 --> 00:07:47,440
and that then b air

213
00:07:51,830 --> 00:07:49,199

error prone polymerase i talked about

214
00:07:53,670 --> 00:07:51,840
and when you put those in a dps deletion

215
00:07:56,390 --> 00:07:53,680
background you lose

216
00:07:59,350 --> 00:07:56,400
all enhanced mutagenesis so all of that

217
00:08:00,869 --> 00:07:59,360
extra mutations were our characterized

218
00:08:03,589 --> 00:08:00,879
error-prone

219
00:08:06,230 --> 00:08:03,599
double-stranded brake repair

220
00:08:08,469 --> 00:08:06,240
so what is dps

221
00:08:10,390 --> 00:08:08,479
dps is a nuclear associated protein

222
00:08:12,469 --> 00:08:10,400
these are the closest e coli has to

223
00:08:14,550 --> 00:08:12,479
compaction proteins this is the closest

224
00:08:16,790 --> 00:08:14,560
they're ever going to get to a histone

225
00:08:19,110 --> 00:08:16,800
it's induced by rpos and stationary

226
00:08:21,670 --> 00:08:19,120
phase and it does two things it

227
00:08:24,150 --> 00:08:21,680
physically co-crystallizes with dna in a

228
00:08:26,230 --> 00:08:24,160
very unique hyper-condensed way

229
00:08:28,469 --> 00:08:26,240
and two it protects against reactive

230
00:08:30,950 --> 00:08:28,479
oxygen species by creating protein

231
00:08:32,630 --> 00:08:30,960
shells around ferrous iron to sequester

232
00:08:33,670 --> 00:08:32,640
them from the cytoplasm

233
00:08:36,230 --> 00:08:33,680
and then

234
00:08:39,029 --> 00:08:36,240
if fentanyl reactions do occur they

235
00:08:41,430 --> 00:08:39,039
occur within that protein shell

236
00:08:43,509 --> 00:08:41,440
and dps actually forms a tryptophan

237
00:08:47,430 --> 00:08:43,519
radical instead of releasing it into the

238
00:08:49,269 --> 00:08:47,440

cytoplasm to damage other macromolecules

239
00:08:52,150 --> 00:08:49,279
so we wanted to see how does dps

240
00:08:54,550 --> 00:08:52,160
suppress mutagenesis so there really are

241
00:08:55,750 --> 00:08:54,560
just two options it's a physical

242
00:09:00,150 --> 00:08:55,760
protection

243
00:09:02,630 --> 00:09:00,160
for the chemical because frankly the

244
00:09:04,870 --> 00:09:02,640
chemical was easier to test

245
00:09:07,269 --> 00:09:04,880
so we looked and see whether or not the

246
00:09:09,110 --> 00:09:07,279
role of dps is protection

247
00:09:10,070 --> 00:09:09,120
against reactive oxygen species and

248
00:09:12,550 --> 00:09:10,080
which

249
00:09:15,110 --> 00:09:12,560
means that reactive oxygen species are

250
00:09:16,949 --> 00:09:15,120
in fact a required chemical species for

251
00:09:19,190 --> 00:09:16,959
mutagenesis to occur

252
00:09:21,750 --> 00:09:19,200
so the first easy thing we did

253
00:09:22,470 --> 00:09:21,760
was we added known reducing agents to

254
00:09:25,430 --> 00:09:22,480
our

255
00:09:27,829 --> 00:09:25,440
assay we added two two by pyridine which

256
00:09:30,070 --> 00:09:27,839
chelates ferrous iron specifically and

257
00:09:32,630 --> 00:09:30,080
thiourea which is an ros scavenger

258
00:09:35,430 --> 00:09:32,640
specifically for hydroxyl radicals and

259
00:09:37,670 --> 00:09:35,440
what you see is that we get a reduction

260
00:09:40,470 --> 00:09:37,680
of mutagenesis in a dosage-dependent

261
00:09:42,470 --> 00:09:40,480
manner for both chemical treatments and

262
00:09:44,870 --> 00:09:42,480
more than that if you look at the orange

263
00:09:47,190 --> 00:09:44,880

set of bars that's that dps deletion

264
00:09:50,150 --> 00:09:47,200
mutant i talked about so when we add

265
00:09:53,030 --> 00:09:50,160
thiourea we are completely cancelling

266
00:09:55,509 --> 00:09:53,040
the effect of delta dps our first line

267
00:09:58,550 --> 00:09:55,519
of evidence that yes dps is inhibiting

268
00:10:00,389 --> 00:09:58,560
via ros protection

269
00:10:02,710 --> 00:10:00,399
so the second thing

270
00:10:05,110 --> 00:10:02,720
is we used uh two mutants that are

271
00:10:07,030 --> 00:10:05,120
constitutively active for the ros

272
00:10:09,430 --> 00:10:07,040
responses now what constitutively active

273
00:10:12,230 --> 00:10:09,440
means is e coli has two

274
00:10:15,110 --> 00:10:12,240
ways of detoxifying ros radicals it has

275
00:10:17,670 --> 00:10:15,120
what we call the sox rs response

276
00:10:20,389 --> 00:10:17,680
which is activated by sox r that

277
00:10:22,230 --> 00:10:20,399
activates superoxide disc mutases which

278
00:10:24,790 --> 00:10:22,240
take care of superoxide and then you

279
00:10:27,269 --> 00:10:24,800
have the oxy-r response which takes care

280
00:10:28,470 --> 00:10:27,279
of hydrogen peroxide and hydroxyl

281
00:10:31,110 --> 00:10:28,480
radicals

282
00:10:34,310 --> 00:10:31,120
well constitutive active alleles mean

283
00:10:36,630 --> 00:10:34,320
that even in the absence of inducing ros

284
00:10:38,630 --> 00:10:36,640
we still get high levels of expression

285
00:10:40,870 --> 00:10:38,640
of all those detoxifying genes so we're

286
00:10:43,670 --> 00:10:40,880
decreasing the basal level

287
00:10:46,310 --> 00:10:43,680
of ros in these cells and we find that

288
00:10:48,069 --> 00:10:46,320

in both cases we dramatically reduce

289
00:10:50,150 --> 00:10:48,079
mutation formation

290
00:10:53,030 --> 00:10:50,160
and more than that when we add the

291
00:10:54,470 --> 00:10:53,040
oxyare constitutive allele with the dps

292
00:10:56,470 --> 00:10:54,480
deletion again

293
00:10:58,870 --> 00:10:56,480
we completely nullify

294
00:11:01,350 --> 00:10:58,880
that increase in mutation so we know

295
00:11:03,910 --> 00:11:01,360
that ros is required for stress induced

296
00:11:06,470 --> 00:11:03,920
mutagenesis to occur and that dps is

297
00:11:07,910 --> 00:11:06,480
acting through ros

298
00:11:09,670 --> 00:11:07,920
so the first question is whether or not

299
00:11:11,750 --> 00:11:09,680
the role of ros was to induce the

300
00:11:13,990 --> 00:11:11,760
general stress response maybe it was a

301
00:11:15,990 --> 00:11:14,000
signaling molecule that just told the

302
00:11:17,990 --> 00:11:16,000
cell you're really stressed out you're

303
00:11:18,870 --> 00:11:18,000
in a really bad shape

304
00:11:20,470 --> 00:11:18,880
so

305
00:11:22,069 --> 00:11:20,480
rssb

306
00:11:25,190 --> 00:11:22,079
is

307
00:11:27,430 --> 00:11:25,200
a negative regulator of rpos rpos that

308
00:11:29,990 --> 00:11:27,440
general stress response that we need

309
00:11:34,230 --> 00:11:30,000
rpos tags it and leads it to degradation

310
00:11:36,230 --> 00:11:34,240
so when we delete rssb we give more rpos

311
00:11:37,590 --> 00:11:36,240
more of those stress responses and more

312
00:11:41,110 --> 00:11:37,600
mutation

313
00:11:43,110 --> 00:11:41,120

but as you can see even when we put

314
00:11:45,670 --> 00:11:43,120
that delta rssb in the presence of

315
00:11:48,630 --> 00:11:45,680
increasing thiourea dosages we can't

316
00:11:50,150 --> 00:11:48,640
counter it so whatever ros is doing it's

317
00:11:52,710 --> 00:11:50,160
not for the induction of the general

318
00:11:55,269 --> 00:11:52,720
stress response

319
00:11:57,110 --> 00:11:55,279
so that leads us to how does reactive

320
00:11:59,269 --> 00:11:57,120
oxygen species cause mutation formation

321
00:12:01,750 --> 00:11:59,279
well they are incredibly damaging

322
00:12:04,230 --> 00:12:01,760
biochemical species they damage every

323
00:12:05,910 --> 00:12:04,240
macro every every macromolecule within

324
00:12:07,670 --> 00:12:05,920
the cell but i'm going to talk to you

325
00:12:09,190 --> 00:12:07,680
about two main

326
00:12:10,629 --> 00:12:09,200
hypotheses one

327
00:12:13,030 --> 00:12:10,639
is they are required for protein

328
00:12:15,670 --> 00:12:13,040
oxidation and two they're required for

329
00:12:18,790 --> 00:12:15,680
dna oxidation and then we break it down

330
00:12:20,949 --> 00:12:18,800
into how that dna oxidation could impact

331
00:12:23,910 --> 00:12:20,959
and i'll get to that later

332
00:12:27,750 --> 00:12:23,920
so the first one the easiest one was do

333
00:12:30,310 --> 00:12:27,760
our ros promoting sim um through protein

334
00:12:32,389 --> 00:12:30,320
damage so when you oxidize proteins you

335
00:12:34,870 --> 00:12:32,399
do alter the function of those proteins

336
00:12:37,030 --> 00:12:34,880
and it was a possibility that perhaps

337
00:12:38,949 --> 00:12:37,040
that altered function was

338
00:12:41,910 --> 00:12:38,959

required for mutation formation so we

339
00:12:43,910 --> 00:12:41,920
used a hyper accurate ribosomal allele

340
00:12:45,750 --> 00:12:43,920
that decreases the amount

341
00:12:48,150 --> 00:12:45,760
of oxidized proteins in cell and found

342
00:12:50,629 --> 00:12:48,160
that it had no effect so ross are not

343
00:12:53,750 --> 00:12:50,639
required for protein oxidation

344
00:12:56,389 --> 00:12:53,760
so the second one does ros promote uh

345
00:12:57,110 --> 00:12:56,399
mutagenesis through dna damage

346
00:12:59,430 --> 00:12:57,120
so

347
00:13:01,990 --> 00:12:59,440
the first one is can it saturate

348
00:13:04,949 --> 00:13:02,000
mismatch repair so so we all know that

349
00:13:07,990 --> 00:13:04,959
guanine pairs of cytosine normally

350
00:13:10,150 --> 00:13:08,000
well oxidized dna damage normally takes

351
00:13:12,550 --> 00:13:10,160
a form of eight oxo g formation which is

352
00:13:14,949 --> 00:13:12,560
oxidized guanine when you get oxidized

353
00:13:17,590 --> 00:13:14,959
guanine you have a slight propensity to

354
00:13:18,949 --> 00:13:17,600
mispair with adenine so we thought that

355
00:13:21,829 --> 00:13:18,959
maybe

356
00:13:23,670 --> 00:13:21,839
we were getting so many ros miss pairs

357
00:13:26,470 --> 00:13:23,680
that we were titrating out mismatch

358
00:13:28,790 --> 00:13:26,480
repair pathways that that were letting

359
00:13:30,710 --> 00:13:28,800
those translation polymers polymerases

360
00:13:32,310 --> 00:13:30,720
make those mutations and they weren't

361
00:13:33,829 --> 00:13:32,320
getting repaired

362
00:13:35,829 --> 00:13:33,839
so when we added

363
00:13:37,269 --> 00:13:35,839

over expression of mismatch repair which

364
00:13:39,350 --> 00:13:37,279
is the p-mutel

365
00:13:41,910 --> 00:13:39,360
with thio urea treatment

366
00:13:43,910 --> 00:13:41,920
we actually got an additive effect which

367
00:13:45,910 --> 00:13:43,920
means that whatever ros is doing

368
00:13:47,350 --> 00:13:45,920
whatever mismatch repair is doing

369
00:13:49,910 --> 00:13:47,360
they're in completely different pathways

370
00:13:51,110 --> 00:13:49,920
they're doing different things

371
00:13:53,190 --> 00:13:51,120
so two

372
00:13:54,550 --> 00:13:53,200
do ros underlie

373
00:13:56,310 --> 00:13:54,560
the formation of that dna

374
00:13:59,590 --> 00:13:56,320
double-stranded break that we mentioned

375
00:14:01,509 --> 00:13:59,600
was required for mutation formation

376
00:14:03,509 --> 00:14:01,519
so this is an analogous assay to that

377
00:14:06,790 --> 00:14:03,519
lac plus one only this is a tech plus

378
00:14:09,350 --> 00:14:06,800
one so we're selecting for tetracycline

379
00:14:11,590 --> 00:14:09,360
resistant mutants which is an antibiotic

380
00:14:14,230 --> 00:14:11,600
so what you see and so what we did is we

381
00:14:16,389 --> 00:14:14,240
took a plasmid containing cat g which is

382
00:14:17,350 --> 00:14:16,399
one of those catalases that detoxifies

383
00:14:19,430 --> 00:14:17,360
ros

384
00:14:21,509 --> 00:14:19,440
and we over expressed it in cells so

385
00:14:24,470 --> 00:14:21,519
we're getting lots and lots of catalase

386
00:14:26,230 --> 00:14:24,480
and hopefully less and less ros

387
00:14:28,150 --> 00:14:26,240
and even when we put this in the

388
00:14:30,069 --> 00:14:28,160

presence of a dna double-stranded break

389
00:14:31,030 --> 00:14:30,079
so we're saying okay we'll give you a

390
00:14:33,110 --> 00:14:31,040
break

391
00:14:34,230 --> 00:14:33,120
do you still need ros

392
00:14:35,910 --> 00:14:34,240
well they did

393
00:14:38,310 --> 00:14:35,920
so as you can see in that last bar

394
00:14:40,150 --> 00:14:38,320
that's the over expression of cat g in

395
00:14:40,949 --> 00:14:40,160
the presence of a dna double straight up

396
00:14:44,790 --> 00:14:40,959
break

397
00:14:46,949 --> 00:14:44,800
so again whatever ros is doing it's not

398
00:14:49,430 --> 00:14:46,959
substituting uh for it's not through

399
00:14:52,150 --> 00:14:49,440
double strand break formation

400
00:14:55,590 --> 00:14:52,160
so lastly we said okay

401
00:14:58,710 --> 00:14:55,600
maybe it's to create a damaged base and

402
00:15:01,670 --> 00:14:58,720
that that damage base the presence of it

403
00:15:03,590 --> 00:15:01,680
is required for mutations to form

404
00:15:05,670 --> 00:15:03,600
so we did the same kind of experiment

405
00:15:08,310 --> 00:15:05,680
only instead of overexpressing catalase

406
00:15:11,509 --> 00:15:08,320
we overexpressed mute m

407
00:15:14,389 --> 00:15:11,519
mute m encodes for dna glycosalase

408
00:15:17,910 --> 00:15:14,399
specifically removes eight oxygen from

409
00:15:19,590 --> 00:15:17,920
dna it's very specific fredoxo g

410
00:15:21,829 --> 00:15:19,600
so what we found is that when we

411
00:15:23,430 --> 00:15:21,839
overexpress mudem

412
00:15:25,590 --> 00:15:23,440
and we

413
00:15:26,550 --> 00:15:25,600

increase the amount of excision of eight

414
00:15:29,430 --> 00:15:26,560
oxygen

415
00:15:31,670 --> 00:15:29,440
we lose almost all of our mutation

416
00:15:35,430 --> 00:15:31,680
so from this we know that ros is

417
00:15:37,189 --> 00:15:35,440
promoting sim through damaging dna after

418
00:15:38,870 --> 00:15:37,199
double strand break formation and that

419
00:15:41,910 --> 00:15:38,880
to be effective

420
00:15:44,470 --> 00:15:41,920
those ros damage bases have to remain

421
00:15:46,710 --> 00:15:44,480
within the dna

422
00:15:48,470 --> 00:15:46,720
so from this i hopefully have convinced

423
00:15:50,790 --> 00:15:48,480
you that not only are reactive oxygen

424
00:15:53,269 --> 00:15:50,800
species required it's specifically

425
00:15:55,110 --> 00:15:53,279
required to create eight oxygen lesions

426
00:15:57,670 --> 00:15:55,120
and for those lesions to remain

427
00:15:58,389 --> 00:15:57,680
unrepaired in the dna

428
00:15:59,990 --> 00:15:58,399
so

429
00:16:02,150 --> 00:16:00,000
a possible mechanism we know that

430
00:16:04,230 --> 00:16:02,160
damaged bases are replicated across by

431
00:16:06,310 --> 00:16:04,240
translation polymerases that these

432
00:16:08,710 --> 00:16:06,320
polymerases are error pro and that we

433
00:16:10,470 --> 00:16:08,720
require them for mutation formation

434
00:16:12,949 --> 00:16:10,480
previously we thought they were creating

435
00:16:13,990 --> 00:16:12,959
mutations by acting on an undamaged

436
00:16:16,310 --> 00:16:14,000
template it was just kind of a

437
00:16:18,230 --> 00:16:16,320
stochastic mutation formation

438
00:16:20,069 --> 00:16:18,240

but now what we're thinking

439
00:16:22,790 --> 00:16:20,079
is that maybe since these are starving

440
00:16:24,870 --> 00:16:22,800
cells don't have replication going we

441
00:16:27,350 --> 00:16:24,880
need the dna double stranded break to

442
00:16:29,590 --> 00:16:27,360
start replication again but that these

443
00:16:32,550 --> 00:16:29,600
translation polymerases aren't called

444
00:16:35,110 --> 00:16:32,560
into action until they come to an

445
00:16:36,870 --> 00:16:35,120
oxidatively damaged base so we're going

446
00:16:38,710 --> 00:16:36,880
to actually test that by seeing whether

447
00:16:41,509 --> 00:16:38,720
or not the translation synthesis

448
00:16:43,430 --> 00:16:41,519
activity of dimby is required for

449
00:16:45,829 --> 00:16:43,440
mutation formation now what does this

450
00:16:46,949 --> 00:16:45,839
mean to the astrobiology conference that

451
00:16:49,590 --> 00:16:46,959
i'm at

452
00:16:52,470 --> 00:16:49,600
so microbes are the first forms of life

453
00:16:53,990 --> 00:16:52,480
on a first on a new planet right

454
00:16:56,230 --> 00:16:54,000
the main thing that's going to drive

455
00:16:58,069 --> 00:16:56,240
their evolution is the environment that

456
00:16:59,590 --> 00:16:58,079
they're in and the stress that that puts

457
00:17:01,509 --> 00:16:59,600
them under now we think it's pretty

458
00:17:03,670 --> 00:17:01,519
unlikely that reactive oxygen species

459
00:17:06,150 --> 00:17:03,680
are universally required because there

460
00:17:07,750 --> 00:17:06,160
are plenty of environments where oxygen

461
00:17:10,309 --> 00:17:07,760
is not present

462
00:17:11,909 --> 00:17:10,319
but it is possible that an unrepaired

463
00:17:14,150 --> 00:17:11,919

damaged base

464
00:17:15,909 --> 00:17:14,160
is universally required for environment

465
00:17:18,710 --> 00:17:15,919
stress-induced evolution so we're going

466
00:17:21,429 --> 00:17:18,720
to test this by taking away oxidation

467
00:17:23,669 --> 00:17:21,439
and damaging our dna in another way

468
00:17:25,990 --> 00:17:23,679
like alkylating agents and see if we can

469
00:17:28,069 --> 00:17:26,000
suppress this requirement but all

470
00:17:32,230 --> 00:17:28,079
together this shows that a requirement

471
00:17:33,990 --> 00:17:32,240
for unrepaired damaged bases if general

472
00:17:36,630 --> 00:17:34,000
would appear to present a constraint on

473
00:17:38,390 --> 00:17:36,640
the mechanism of evolution across

474
00:17:41,029 --> 00:17:38,400
all organisms in multiple types of

475
00:17:43,029 --> 00:17:41,039
environments that dna damage be present

476
00:17:44,630 --> 00:17:43,039
and persist

477
00:17:47,350 --> 00:17:44,640
so with that i'd like to thank both of

478
00:17:56,710 --> 00:17:47,360
my labs and of course nasa for all their

479
00:18:02,870 --> 00:17:57,990
do we have some questions from the

480
00:18:08,310 --> 00:18:05,669
um i'm a little confused as to how the

481
00:18:10,070 --> 00:18:08,320
gcr pathway is helpful in particular

482
00:18:12,070 --> 00:18:10,080
because you've got like a single

483
00:18:13,669 --> 00:18:12,080
insertion mutant right so if you just

484
00:18:15,909 --> 00:18:13,679
make more copies of that gene you're

485
00:18:18,310 --> 00:18:15,919
just going to have more genes that are

486
00:18:20,470 --> 00:18:18,320
frame shifted and going to make nonsense

487
00:18:22,390 --> 00:18:20,480
proteins how is that good how does the

488
00:18:24,789 --> 00:18:22,400

how do the cells survive via that

489
00:18:26,630 --> 00:18:24,799
mechanism okay so

490
00:18:28,630 --> 00:18:26,640
you get that one to two percent it's a

491
00:18:31,029 --> 00:18:28,640
frame shift so every so often you get a

492
00:18:32,390 --> 00:18:31,039
slippage you get a read through you get

493
00:18:34,070 --> 00:18:32,400
a functional protein so it's not like

494
00:18:36,390 --> 00:18:34,080
we're getting a lot of nonsense proteins

495
00:18:37,190 --> 00:18:36,400
that are they just don't make anything

496
00:18:39,830 --> 00:18:37,200
so

497
00:18:41,590 --> 00:18:39,840
if you've got 20 copies 30 copies 60

498
00:18:43,029 --> 00:18:41,600
copies of this thing and each one of

499
00:18:44,310 --> 00:18:43,039
them kind of reads through one to two

500
00:18:47,669 --> 00:18:44,320
percent of the time

501
00:18:49,990 --> 00:18:47,679
it works and more than that

502
00:18:51,110 --> 00:18:50,000
gcr is a lot of what we see in things

503
00:18:53,430 --> 00:18:51,120
like

504
00:18:54,870 --> 00:18:53,440
cancer formation things that lead to

505
00:18:57,029 --> 00:18:54,880
these increased mutation rates are

506
00:18:58,950 --> 00:18:57,039
actually amplification of required

507
00:19:01,909 --> 00:18:58,960
of like caretaker genes and gatekeeper

508
00:19:05,669 --> 00:19:01,919
genes smv is kind of not the predominant

509
00:19:10,950 --> 00:19:08,630
we have a a question from sagan net

510
00:19:14,310 --> 00:19:10,960
yes we do uh let me make sure you guys

511
00:19:18,789 --> 00:19:16,630
can you hear me okay um this comes from

512
00:19:20,549 --> 00:19:18,799
donald burke he asks what mutational

513
00:19:23,270 --> 00:19:20,559

densities can you achieve through these

514
00:19:27,110 --> 00:19:24,710
i'm sorry can you say that again i will

515
00:19:29,590 --> 00:19:27,120
say that again what mutational densities

516
00:19:32,230 --> 00:19:29,600
can you achieve through these stresses

517
00:19:33,669 --> 00:19:32,240
through these stresses um

518
00:19:35,750 --> 00:19:33,679
not really sure what he means by

519
00:19:41,909 --> 00:19:35,760
mutational densities okay i'll ask him

520
00:19:46,630 --> 00:19:44,390
okay can the mutational density get high

521
00:19:48,710 --> 00:19:46,640
enough to create a library of mutated

522
00:19:53,909 --> 00:19:48,720
proteins for applied evolution of the

523
00:19:59,029 --> 00:19:56,310
man um

524
00:20:00,870 --> 00:19:59,039
the best i can tell you is that in our

525
00:20:02,549 --> 00:20:00,880
assay

526
00:20:04,549 --> 00:20:02,559
we have looked

527
00:20:07,909 --> 00:20:04,559
to see whether or not some of these

528
00:20:08,789 --> 00:20:07,919
stress and successfully mutated genomes

529
00:20:11,029 --> 00:20:08,799
have

530
00:20:13,029 --> 00:20:11,039
other types of mutations in other things

531
00:20:14,150 --> 00:20:13,039
that you know we look for oxytropes and

532
00:20:16,630 --> 00:20:14,160
things like that i hope i don't get in

533
00:20:18,310 --> 00:20:16,640
trouble for mentioning this

534
00:20:19,029 --> 00:20:18,320
the quick answer is we don't often find

535
00:20:21,510 --> 00:20:19,039
them

536
00:20:23,830 --> 00:20:21,520
usually we find the mutation that fixed

537
00:20:24,789 --> 00:20:23,840
it and everything goes back to normal

538
00:20:26,630 --> 00:20:24,799

so

539
00:20:29,909 --> 00:20:26,640
thanks i hope that helps

540
00:20:31,750 --> 00:20:29,919
hi um fantastic talk by the way i

541
00:20:33,830 --> 00:20:31,760
was just curious

542
00:20:35,590 --> 00:20:33,840
you mostly talked about the conditions

543
00:20:37,830 --> 00:20:35,600
of the general stress response i was

544
00:20:40,070 --> 00:20:37,840
just curious if

545
00:20:41,830 --> 00:20:40,080
whether or not the sort of phenomena the

546
00:20:43,750 --> 00:20:41,840
reactive oxygen species and the higher

547
00:20:45,350 --> 00:20:43,760
rate of mutation may also would also

548
00:20:46,870 --> 00:20:45,360
necessarily occur in say a more specific

549
00:20:50,630 --> 00:20:46,880
stress response like the syringe

550
00:20:52,390 --> 00:20:50,640
response under amino acid scarcity

551
00:20:54,870 --> 00:20:52,400
oh that's so awesome that's a good

552
00:20:56,789 --> 00:20:54,880
question um so in terms of strange

553
00:20:58,710 --> 00:20:56,799
response i don't think i'm at liberty to

554
00:21:00,070 --> 00:20:58,720
talk about that but keep watching

555
00:21:03,430 --> 00:21:00,080
publications and maybe you'll see

556
00:21:04,870 --> 00:21:03,440
something um the great thing about it is

557
00:21:09,350 --> 00:21:04,880
that

558
00:21:12,549 --> 00:21:09,360
today rpos rec all this stuff we don't

559
00:21:15,029 --> 00:21:12,559
just see it in e coli in this assay

560
00:21:17,029 --> 00:21:15,039
we see it in bioresistance in salmonella

561
00:21:19,909 --> 00:21:17,039
we see the multi-class antibiotic drug

562
00:21:22,710 --> 00:21:19,919
resistance we see it in hypoxia induced

563
00:21:25,430 --> 00:21:22,720

cancer tumor initiation and progression

564
00:21:27,590 --> 00:21:25,440
so the things that i'm talking about

565
00:21:30,149 --> 00:21:27,600
multiple types of stresses multiple

566
00:21:32,470 --> 00:21:30,159
microbes multiple environments

567
00:21:34,710 --> 00:21:32,480
this looks to be like a fairly conserved

568
00:21:37,990 --> 00:21:34,720
pathway for a lot of different things

569
00:21:40,230 --> 00:21:38,000
which makes it even cooler right so

570
00:21:41,750 --> 00:21:40,240
thank you uh and with that i would like