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Endosymbiotic bacteria

GroEL buffers against deleterious mutations

GroEL, a heat-shock protein that acts as a molecular chaperone[1], is over-produced in endosymbiotic but not in free-living bacteria[2–4], presumably to assist in the folding of conformationally damaged proteins. Here we show that the overproduction of GroEL in *Escherichia coli* masks the effects of harmful mutations that have accumulated during a simulated process of vertical transmission. This molecular mechanism, which may be an adaptation to the bacterium's intracellular lifestyle, is able to rescue lineages from a progressive fitness decline resulting from the fixation of deleterious mutations under strong genetic drift[5,6].

Endosymbiotic bacteria have small population sizes, do not undergo recombination, and are maternally transmitted through tight population bottlenecks[7], causing the fixation of deleterious mutations due to genetic drift and hence an irreversible decline in fitness[8]. However, endosymbiosis is surprisingly stable and persists over long periods[9], which has led to the suggestion[5] that *groE* (the GroEL-encoding operon) could be buffering the mutational loss of functionally active proteins because, unlike other endosymbiont genes, it is subject to strong purifying selection[5].

To test this idea, we investigated the effects of overexpression of *groE* in a set of *E. coli* strains (a free-living bacterium close to several endosymbionts[9]) with mutations randomly accumulated throughout the genome. These spontaneous mutations were fixed by random genetic drift in a process that simulated the vertical transmission of a single endosymbiont between hosts.

We studied the accumulation of mutations in 12 replicate lines of two *E. coli* B genotypes, one of which had a 3.3-fold-increased mutation rate[10]. These genotypes had already adapted to a simple environment (DM25 medium) for 10,000 generations[11], suggesting that mutation accumulation might result in a decline in fitness. After 3,240 generations of mutation accumulation, we measured the fitness of the strains evolved on DM25 relative to their respective ancestors. As expected, the
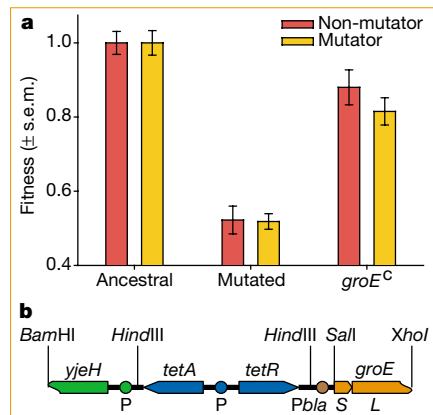

Figure 1 Effect of the overexpression of the *groE* operon on the fitness of randomly mutated strains of *Escherichia coli*. **a,** The fitness values for the ancestral strains of mutators and non-mutators are the same (left); 'mutated', fitness loss after 3,240 generations of mutation accumulation; '*groE*ᶜ', fitness recovery of strains in which *groE* is overexpressed. Culture conditions, fitness assays and phenotypic markers are described elsewhere[11], as is the mutation-accumulation protocol[12]. **b,** The regulated *E. coli groE* operon was changed to a constitutive form (*groE*ᶜ) by putting the structural genes under the control of the β-lactamase gene promoter (P*bla*). Recombinant genotypes were obtained by transduction using P1*vir* (ref. 13). The *groE*ᶜ construct contains part of the flanking *yjeH* gene, the inducible tetracycline-resistance operon (*tetA/R*) for transductant selection, the P*bla* promoter, and the *groE* coding regions (S and L). Restriction-enzyme cutting sites are indicated; P, promoter. Further details are available from the authors.

mutated strains lost fitness (Fig. 1a) as a result of the accumulation of deleterious mutations ($\overline{W} = 0.5168 \pm 0.0159$ for mutators; $\overline{W} = 0.5208 \pm 0.0087$ for non-mutators; paired *t*-tests, $P < 0.0001$).

We then replaced the regulated *groE* operon of the mutated strains with a constitutive allele (*groE*ᶜ; Fig. 1b). To estimate the fitness of the *groE*ᶜ strains, we ran competition experiments against the corresponding ancestors on DM25. Surprisingly, none of the *groE*ᶜ strains reached the expected cell density during growth overnight. A simple explanation could be that an overproduction of GroEL of about 86 ± 16-fold is deleterious because it diverts amino acids away from other cellular functions.

To test this, we grew each *groE*ᶜ strain and its ancestor in DM25 supplemented with increasing concentrations of tryptone (a mixture of peptides and amino acids). We found that *groE* overexpression was deleteri-

ous in the presence of small amounts of tryptone, but that there were no significant differences in cell density between *groE*ᶜ strains and their non-mutated ancestors at tryptone concentrations of 0.1% or higher. In endosymbionts, amino acids are not limiting because these are abundant in their intracellular environment[9].

To determine whether fitness estimates depend on the environment, we estimated fitness on DM25 and on DM25 + 0.1% tryptone. Both fitness estimates were correlated (partial correlation test, $P < 0.0001$).

Figure 1a shows that the average fitness of the *groE*ᶜ strains derived from non-mutator strains ($\overline{W} = 0.8801 \pm 0.0214$) was 75.9% greater than that of the mutated strains (paired *t*-test, $P < 0.0001$), but was 12% less than that of the ancestors ($P = 0.0002$); the average fitness of the *groE*ᶜ strains derived from evolved mutators was $\overline{W} = 0.8152 \pm 0.0167$, which is 61.6% greater than that of the mutated strains (paired *t*-test, $P < 0.0001$) but 18.48% less than that of the ancestral strains ($P < 0.0001$).

Is fitness recovery a result of the buffering of deleterious effects by GroEL, or is it simply a general benefit associated with increased concentrations of GroEL? In favour of the first possibility, the advantage of GroEL overproduction is evident only in an amino-acid-rich environment; also, if mutation compensation is occurring, we would expect a positive correlation between the extents of fitness loss and recovery, as evidenced by their partial correlation (1-tailed test, $P = 0.0089$). We conclude that GroEL overexpression is likely to be of help in maintaining these endosymbionts by protecting them against the harmful effects of accumulated mutations.

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