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Rebuttal: Adaptive Mutation in *Escherichia coli* (Foster)

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If mutations arose in nongrowing cells, as proposed in Foster's paper (1), then stress-induced mutagenesis would seem inescapable. The plated population certainly grows very little. However, no evidence is provided that reversion occurs in this population and no effort is made to counter evidence to the contrary (see below)—that mutations actually arise in cells growing under selection within developing clones (rendering mutagenesis dispensable).

(i) Reversion requires that the *lac* allele be leaky and lactose be present in the selection medium, suggesting a growth requirement.

(ii) General mutagenesis is not induced by simple starvation (3) but is seen in the Cairns system when lactose is provided, suggesting dependence on growth.

(iii) Clonally related unstable and stable lac^+ cells are found within all revertant colonies, consistent with both types arising sequentially within a growing colony.

(iv) Revertant number is strongly reduced if one inhibits growth of cells carrying a *lac* amplification.

(v) Revertant number increases if a *lac* duplication is provided in the parent cells.

(vi) Results of the respreading experiment are not inconsistent with reversion within growing clones because single duplication-bearing cells have a low (10^{-4}) probability of forming a visible revertant colony.

The genomic position of *lac* affects behavior of this system. Cited results suggest that general mutagenesis under selection is independent of *lac* position and therefore must reflect a genome-wide stress response. However, we have only seen general mutagenesis when the *lac* locus under selection is on the F'_{128} plasmid located *cis* to $dinB^+$ (allowing coamplification). It has also been argued that the *lac* reversion rate is 100-fold higher on F than in the chromosome, and the stress-induced increase is only apparent when applied to the higher basal *lac* rate on the plasmid (J. Cairns and P. L. Foster, Letter, Genetics **165:**2317-2318, 2003). In contrast, we find the same *lac* reversion rate on F'_{128} as at any of 30 chromosomal sites (J. R. Roth, E. Kofoid, F. P. Roth, O. G. Berg, J. Seger, and D. I. Andersson, Letter, Genetics **165:**2319-2321, 2003). Both conflicts may reflect problems in the strain with a chromosomal *lac* allele (2, 5). The structure of F'_{128} makes it unexpectedly difficult to move the triply marked *lac* allele from F'_{128} to the chromosome.

The higher reversion rate of a *tetA* frameshift observed on F during selection may reflect coamplification of *tetA* with *lac* rather than mutation directed to F. Greater mutagenesis of F seems a minor point, since mutagenesis makes such a small contribution to reversion (see our rebuttal of Rosenberg and Hastings [6]).

Our experience with *Salmonella* suggests that the effect of an *rpoS* mutation on revertant number (seen in *Escherichia coli*) is likely to be indirect rather than an alteration of a programmed response to stress. It has previously been shown that this mutation reduces growth under selective conditions (4); alternatively, it could reduce the amplification rate, expression of the *tra* operon, or the extent of SOS induction.

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