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Rebuttal: Adaptive Point Mutation (Rosenberg and Hastings)

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The Rosenberg-Hastings paper (4) defends a model much like that of Foster (1) and argues that the observed mutagenesis contradicts predictions of the amplification model. On the contrary, amplification indirectly causes weak mutagenesis and allows that mutagenesis to have a detectable effect on *lac* reversion. However, this mutagenesis is a minor side effect that is neither sufficient nor necessary to explain reversion. Mutagenesis has distracted attention from the main message of this system—a target number increase during growth under selection.

SELECTION INCREASES REVERTANT YIELD 10^4-FOLD

Seen in the light of the amplification mutagenesis (AM) model, 100 *lac*$^+$ revertant colonies arise from 10^6 plated cells that carry a *lac* duplication and can therefore grow under selection. This revertant frequency (10^{-4}) is 10^4-fold higher than that seen without selection (10^{-8}). Selection increases both target number and (indirectly) mutation rate. The whole effect of selection is the product of these two factors, because the mutation rate affects all *lac* copies. Target number increase (amplification and growth) provides a factor of more that 10^3; mutagenesis provides a factor of 4 or 5.

FIVEFOLD EFFECT OF MUTAGENESIS

Elimination of *dinB* (and general mutagenesis) reduces revertant yield less than fivefold. Without mutagenesis, selection still causes a RecA-dependent 25-fold increase in revertant yield (based on revertant colony number). This importance of RecA in the absence of mutagenesis suggests that recombination (i.e., amplification) is central (7).

MUTAGENESIS CAUSES 80% OF REVERTANTS

To be responsible for 80% of point mutations, DinB must increase the basal mutation rate only fivefold (6). This small increase would produce five *lac* revertants if applied to 10^8 nongrowing cells as suggested by the HM model. However, it produces 100 revertants if applied to 10^7 growing cells (within colonies), each cell with 200 *lac* copies (2).

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INDUCTION OF DinB

We suggest that the mutation rate increases fivefold when one *dinB* gene is induced (SOS) by single-stranded DNA produced by the F′ replication origin aided by DNA fragments released by amplification segregation (6). This small increase explains revertant number, if applied to a pool of target *lac* copies enlarged by growth and amplification, but is too low to be detectable (by the methods used) as an increase in frequency of associated mutations.

ORIGINS OF ASSOCIATED MUTATIONS

A detectable level of associated unselected mutations forms in about 10% of *lac* revertants (3)—the subset that coamplifies *dinB* with *lac*. In these clones, SOS induces many *dinB*$^+$ copies and thereby increases the mutation rate several hundred fold.

GROWTH—WITH OR WITHOUT SELECTION

This system showcases effects of selection on mutation in growing cells. With selection, cells reach the goal (Lac$^+$) by a succession of genetic events, each allowing a clonal expansion. Each event is made more frequent by increases in target number provided by the previous expansion. Without selection (or without growth), the same goal can be reached only by rare single-step events. A diagram of this process is in reference 5.

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