Letter to the Editor

Adaptive Mutation Requires No Mutagenesis—Only Growth Under Selection: A Response

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In a bacterial system devised by Cairns and Foster (1991), a *lac* mutant population, starved in the presence of lactose, gives rise to ~100 revertant colonies over 6 days. In this time, the plated population is not growing and is not experiencing general mutagenesis. Three models have been proposed to explain this. The directed mutation model (DMM) suggests that stress induces mutagenesis that is focused on the relevant target (*lac*, or the F′ plasmid that carries it) to the exclusion of the chromosome at large (Cairns *et al.* 1988; Cairns and Foster 1991; Foster and Cairns 1992). The hypermutable state model (HSM) suggests that stress induces genome-wide mutagenesis in a subset of the population (10^5 cells); this generates Lac^+ revertants but kills the rest of the mutagenized population (Hall 1990; Torkelson *et al.* 1997). According to the HSM, mutation appears directed because only Lac^+ revertants survive mutagenesis. We have proposed (see below) the amplification mutagenesis model (AMM), in which growth under selection increases revertant number with no required change in the rate or target specificity of mutation (Andersson *et al.* 1998; Hendrickson *et al.* 2002; Slechta *et al.* 2003).

The article under discussion (Roth *et al.* 2003) examines some quantitative predictions of the HSM and finds that this model requires an implausibly high intensity of genome-wide mutagenesis—vastly higher than that estimated experimentally (Rosche and Foster 1999; Slechta *et al.* 2002b). If realized, this mutation rate would add so many lethal mutations that one could not recover the number of *lac* mutations that are observed. We argued that the behavior of the Cairns-Foster system must be explainable by some means other than the HSM.

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The letter from Cairns and Foster (2003, this issue) suggests that our calculations are in error because we extrapolated from rates observed or inferred for genes on an F′ plasmid to predict the number of mutations that would be caused in the chromosome. Their argument is based on three assumptions: (1) that mutation rates are ~100-fold lower in the chromosome than on the F′, (2) that stress induces mutagenesis regardless of where *lac* is located, and (3) that reversion is not detectably enhanced when *lac* is in the chromosome, simply because the basal reversion rate of a *lac* allele at that position is too low. We do not accept these assumptions. We have moved the *lac* allele used in the Cairns experiment to 34 different sites in the chromosome and compared unselected reversion rates to that of the same allele on the F′ plasmid. At all chromosomal sites the unselected reversion rates clustered around the 10^{-8} value found for *lac* on F'_{128} (Slechta *et al.* 2002a; S. Slechta, unpublished data). The chromosomal reversion rates were not affected by the presence of F'_{128} (Slechta *et al.* 2003). The 100-fold lower mutation rate suggested for chromosomal *lac* in the Cairns-Foster letter would be reasonable for a typical chromosomal frameshift mutation (Bull *et al.* 2001), but the *lac* allele used in this system is not typical. That allele has a constellation of three mutations—an I^Q promoter, a +1 frameshift in *lacI*, and a deletion that fuses the *lacI* and *lacZ* genes (Cairns and Foster 1991). This combination provides a leaky Lac^- phenotype that can be corrected by any −1 mutation (or other mutation having the same effect on reading frame) in ~100 bp (about one-tenth of the *lacI* gene sequence). This allele would therefore be expected to revert at ~100 times the rate of a typical +1 frameshift, and our measurements suggest that it does. There seems to be no effect of gene position on reversion in the absence of selection.

When selection is imposed, a position effect is seen. When *lac* is on the F′ plasmid, 100 revertants accumulate

over 6 days and these revertants show an average 20- to 50-fold increase in associated mutations distributed genome-wide (Torkelson *et al.* 1997; Rosche and Foster 1999; Slechta *et al.* 2002a). In contrast, when selection is applied to strains with *lac* in the chromosome, very few revertants appear and we find that these few revertants show no increase in associated mutations (Slechta *et al.* 2002a). We conclude that selection enhances reversion (and causes associated mutations) only when *lac* is on a conjugative plasmid. The HSM model does not address this position effect, but assumes that general mutagenesis is induced in a subset of the stressed population and is responsible for the *lac* revertants. We ignored this shortfall of HSM and simply tested the quantitative implications of that model as it has been enunciated (Rosenberg 2001).

The assumptions underlying the above critique appear to be based on reversion tests of *lac* at a single chromosomal site (Rosche and Foster 2000). There the reversion rate ($\sim 10^{-10}$) was lower than that of *lac* on F'_{128} (10^{-8}) and the very few recovered *lac* revertants did show evidence of general mutagenesis. We have not seen unselected mutations associated with reversion of a chromosomal *lac* allele either in an *Escherichia coli* strain like that used (Rosche and Foster 2000) or in Salmonella strains with *lac* at chromosomal sites. We do not know why the particular strain tested by Rosche and Foster showed a lower reversion rate or associated mutations, but we suggest that problems may have arisen in the course of genetically transferring the triply mutant *lac* allele from the F′ plasmid to the chromosome; this procedure sometimes generates a duplication of the chromosomal *lac-din* region, because a duplication join point is inherent in the structure of F'_{128} (Kofoid *et al.* 2003). If this occurred, the behavior of their strain may be explainable by the amplification model (see below).

We agree with Cairns and Foster that our cost estimate ignores any contribution to *lac* reversion by the nonhypermutable majority of plated cells. We ignored it because it is ignored by the HSM (which we were testing). However, we did consider this population *as described by HSM* in showing (Roth *et al.* 2003, Table 1, line 11) that 10^8 cells with the normal mutation rate are expected to produce <1 Lac^+ revertant under the HSM model. We also considered the predicted result if that population experienced the fourfold increase estimated by Bull *et al.* (2001); then the HSM predicts four revertants. Further increasing general mutagenesis of the whole population (Roth *et al.* 2003, Table 1, lines 9 and 10), can ultimately predict the observed 100 mutants, but eliminates the apparent directedness of mutation that is a hallmark of this system (*i.e.*, the population at large would show as many associated mutations as do the Lac^+ revertants).

We considered the demonstration by Rosche and Foster (1999) that the majority of *lac* revertants (90%) experience little or no general mutagenesis while 10% experience a 200-fold increase (an average 20-fold increase). We agreed with their conclusion and pointed out that the low average rate they estimated is in stark contrast to the 10^5-fold increase required by HSM, leading to our conclusion that HSM is unlikely to explain the observed *lac* revertants. Further support for this conclusion is the observation that revertant yield decreases only slightly when general mutagenesis is eliminated by a *dinB* or $lexA^{Ind}$ mutation (McKenzie *et al.* 2000; Slechta *et al.* 2002b, 2003; Tompkins *et al.* 2003). We imagine that Cairns and Foster would agree with us—general mutagenesis is neither necessary nor sufficient to explain selection-enhanced revertant frequency in their system.

If general mutagenesis as posited by HSM is set aside as the cause of reversion, how then can one explain the lac^+ revertants that arise under selection? The DMM (Foster 1993) posits that selection induces mutagenesis focused on *lac* (or on the F′ plasmid that carries it). Such directed mutagenesis could certainly explain reversion without costly associated mutations. This model was initially supported by experiments in which starvation of the *lac* mutant (on F'_{128}) had very little effect on reversion of chromosomal *tetA* frameshifts in the nonrevertant parent population (Bull *et al.* 2001), but caused a striking increase in unselected revertants of a *tetA* frameshift inserted (within Tn*10*) on F′ *lac* (Foster 1997). However, the Tn*10* element used in this F′ experiment is actually inserted very close to *lac* in the *mhpC* gene (Kofoid *et al.* 2003). A Tn*10* insertion in this gene has been shown to co-amplify with *lac* during selection for improved growth on lactose (Godoy and Fox 2000); insertions far from *lac* on F'_{128} do not appear to co-amplify (Hendrickson *et al.* 2002). Thus the high reversion rate seen for *tetA* on F'_{128} during lactose selection (like that of the *lac* mutation) is likely to reflect selected amplification of the target site (*tetA*) with *lac* (see below) rather than mutagenesis directed to the plasmid.

We suggest that the Cairns-Foster phenomenology requires no induced mutagenesis, directed or general. The AMM proposes that rare preexisting cells with a *lac* duplication grow slowly when placed under selection and improve their growth by further *lac* amplification within each developing colony. Ultimately there are so many copies of *lac* (within colonies) that reversion can occur without any increase in the underlying (per base pair) mutation rate (Andersson *et al.* 1998; Hendrickson *et al.* 2002). Selection appears to direct mutations to *lac* because this gene is amplified during growth under selection and because only the lac^+ revertant allele is maintained during subsequent selected loss of the many (now deleterious) copies of the mutant *lac* allele. This process is enhanced for *lac* on F'_{128} because the transfer origin of this plasmid makes DNA ends that stimulate duplication, amplification, and segregation (Galitski and Roth 1995; Slechta *et al.* 2002a). We have recently

presented evidence that the genome-wide general mutagenesis experienced by 10% of *lac* revertant clones (Rosche and Foster 1999) occurs because these few clones include within their amplified *lac* region the nearby *dinB* gene for an error-prone polymerase (Kofoid *et al.* 2003; Slechta *et al.* 2003); the majority (90%) of revertants arise without mutagenesis by amplifying *lac* alone. Thus the interesting predictions of Ninio do not seem to be required here (Ninio 1991). We submit that selection increases the number of *lac* revertants primarily by increasing the number of mutational targets (AMM), not by increasing the general mutation rate (as proposed by HSM) and not by directing mutation (as proposed by DMM).

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