Letter to the Editor

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

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Manuscript received October 19, 2003
Accepted for publication November 5, 2003

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 (10^6 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.

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^{-3} value found for lac on F′ (Slechta et al. 2002a; Slechta, unpublished data). The chromosomal reversion rates were not affected by the presence of F′ (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 R^2 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 conjugal 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 (≈10⁻¹⁸) was lower than that of lac on F⁺ (10⁻⁶) 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 insertion in this gene (within Tn10) on F⁺ lac (Foster 1997). However, the Tn10 element used in this F⁺ experiment is actually inserted very close to lac in the mhpC gene (Kofoid et al. 2003). A Tn10 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⁺ do not appear to co-amplify (Hendrickson et al. 2002). Thus the high reversion rate seen for lac on F⁺ 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⁺ 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 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 10⁴-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 reversion yield decreases only slightly when general mutagenesis is eliminated by a dinB or lexA⁺ 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 reversion 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'⁺) 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 Tn10) on F' lac (Foster 1997). However, the Tn10 element used in this F' experiment is actually inserted very close to lac in the mhpC gene (Kofoid et al. 2003). A Tn10 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' do not appear to co-amplify (Hendrickson et al. 2002). Thus the high reversion rate seen for tetA on F' 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.
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 (Kofoi 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).

LITERATURE CITED


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