JOURNAL OF BACTERIOLOGY, Aug. 2004, p. 4862–4863 Vol. 186, No. 15
0021-9193/04/$08.00+0 DOI: 10.1128/JB.186.15.4862–4863.2004

Rebuttal: Growth under Selection Stimulates Lac$^+$ Reversion (Roth and Andersson)

Susan M. Rosenberg[1,2,3]* and P. J. Hastings[1]

Departments of Molecular and Human Genetics,[1] Biochemistry and Molecular Biology,[2] and Molecular Virology and Microbiology,[3] Baylor College of Medicine, Houston Texas 77030-3411

Roth's and Andersson's model (12) supports the conservative neo-Darwinist precept of constant and gradual evolutionary change. They exclude the possibility that mutation rates (per base pair replicated) may be affected by environmental stress (and are thus forced to argue the unimportance of those cells with demonstrably increased mutation rates). However, their model requires that we ignore much important data that contradict it; moreover, the data that they cite do not really support their conclusions. Here, space allows us to point out just a few such instances.

First, the quantification of their model requires that they diverge from commonly accepted values both for mutation rate and amplification copy number. To generate 100 Lac$^+$ point mutant colonies per 10^8 cells plated by day 5, they propose that 10^{-2} of the cells plated (10^6 cells) carry a preexisting gene duplication that becomes amplified and that one Lac$^+$ point mutation occurs when the "standard unselected mutation rate" of "10^{-8}/cell/division" acts on 10^8 *lac* copies, "for example. . .100 clones (colonies) of 10^4 cells, each with 100 copies of *lac*." This would mean that, on average, colonies of 10^6 cells with 100 *lac* copies would have one Lac$^+$ point mutant. The problem is that the reversion rate of this *lac* allele was $\sim 10^{-9}$/cell/generation in Cairns' and Foster's original paper (1) and between 10^{-9} and 10^{-10}/cell/generation in six subsequent papers from one of our laboratories (3–5, 8–10), that is, 10 to100 times lower than they suggest. (The reference that they cite for the abnormally high rate is by Foster, but from the same data, she derives a rate 10-times lower [2]). Similarly, they state a number of *lac* copies per amplified array (100) that is higher than the ~30 that is widely reported (their data, ours, and others). If the commonly accepted reversion rates and amplification copy number are used, then colonies of *lac*-amplified cells would have to reach 3×10^7 to 3×10^8 cells before they generated on average a single Lac$^+$ point mutant. This is incompatible with the data that most (>98%, their data) to all (Foster's and our data) cells in a visible Lac$^+$ colony (about 10^7 cells) are point mutants, not amplified.

Second, and also regarding quantification of their model, we and others found that Lac$^+$ point mutants carry high levels of unselected mutations, i.e., are hypermutated, relative to cells that starved on the same plates but did not become Lac$^+$. To achieve their model's key feature of no increase in mutation rate, Roth and Andersson advocate the hypothesis (not demonstrated; see references 2 and 11) that only 10% of Lac$^+$ point mutants descend from the transiently hypermutating cell subpopulation and 90% arise from cells with normal mutation rates (such that the hypermutable cell subpopulation can be imagined to be unimportant). However, they also suggest that in the (proposed) cells that are not hypermutating but produce Lac$^+$ point mutations "the same process operates but reversion occurs later in colony development and unstable (*lac*-amplified) Lac$^+$ cells predominate." These, they suggest, are the colonies that we call *lac* amplified. The problem is that these *lac*-amplified clones are not a 90% majority of Lac$^+$ colonies as their quantification would demand but rather are only 5 to 15% of the day 5 colony count (see Fig. 1 of reference 11). Both of their suggestions cannot be true: that *most* Lac$^+$ point mutants come from cells with a normal mutation rate and that these are the ones that we call *lac* amplified, which are a minority class. Conversely, if all those that we call "point mutant" are descended from the hypermutable subpopulation, as they state, then this would dictate that most Lac$^+$ colonies arose from that hypermutable subpopulation, because the point mutants are the majority (see Fig. 1 in reference 11). This would make their model like ours: a hypermutation (HM) model in which most Lac$^+$ point mutations come from cells with an increased mutation rate.

Third, the argument that most Lac$^+$ point mutants have arisen from cells with "normal" mutation rates disregards the evidence that 85% of Lac$^+$ point mutation requires a special error-prone DNA polymerase, DinB/Pol IV, that is not required for spontaneous mutation in growing cells. This contradicts cryptic-growth (CG) models such as theirs and supports HM models.

Fourth, the idea that neither amplification nor point mutation is a stress response and that both occur in normal growing cells (as in CG models such as Roth's and Andersson's) is incompatible with the demonstration that both require the general stress-response and stationary-phase transcription factor RpoS (8). RpoS is required specifically for amplification and for mutation in stationary phase but not in growing cells, and independently of many types of possible indirect effects. These data support HM models in which increased mutation is part of a stress response. Moreover, the fact that RpoS upregulates error-prone DinB/Pol IV strongly supports the idea of stress/stationary-phase-induced mutagenesis (7).

Also, we disagree with the interpretation of some of the evidence cited as specific support for the Roth-Andersson model; again, just a few examples are given:

First, experiments interpreted as indicating that the *lac* region must be amplified for Lac$^+$ point mutation to occur—that selection against multiple copies of a *tetA* gene placed near *lac* inhibited Lac$^+$ mutation—are also compatible with our error-

* Corresponding author. Mailing address: Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, BCM-S809A, Mail Stop BCM225, Houston, TX 77030-3411. Phone: (713) 798-6924. Fax: (713) 798-8967. E-mail: smr@bcm.tmc.edu.

prone double-strand-break repair (DSBR) model for Lac$^+$ point mutation. DSBR requires that the cells have more than one copy of the *lac* region for repair, and selection of cells with few or one copy would select against those capable of producing point mutants in our model.

Second, experiments that were interpreted as showing that young colonies carry a high proportion of *lac*-amplified cells whereas older colonies carry fewer, in apparent support of the idea that point mutants overgrow *lac*-amplified cells in colonies, are subject to a different interpretation: The young colonies are a smaller fraction of all cells on the constant volume of agar taken from the selection plate and analyzed, such that contaminating, unrelated neighbor colonies of *lac*-amplified cells will be a greater fraction of all the cells present than in older (bigger) colonies. We suggest that the *lac*-amplified CFU were from unrelated microcolonies (a point not tested in these experiments).

Third, the argument that most Lac$^+$ adaptive mutation, but not most hypermutation of unselected genes, occurs in the absence of DinB error-prone DNA polymerase represents, in our opinion, a misinterpretation of the data that show that in the absence of DinB (or SOS, which upregulates DinB), Lac$^+$ colonies have lower frequencies of unselected mutations. The problem is that DinB is required for most point mutation, but not for adaptive *lac* amplification, and genome-wide hypermutation is not found in *lac*-amplified clones (6). Thus, in DinB$^-$ or SOS$^-$ cells, point-mutant colonies are reduced drastically leaving *lac*-amplified colonies to predominate, and these, as demonstrated previously, are not hypermutated. This is also why we disagree with Roth and Andersson on how much of Lac$^+$ adaptive mutation is removed by blocking DinB: we measure point mutants and *lac*-amplified clones separately and find that 85% of point mutants disappear in DinB$^-$ cells (while none of the *lac*-amplified clones does), whereas they lump the two together and so interpret the decrease in the number of (total) Lac$^+$ colonies as smaller.

Fourth, we disagree with the interpretation of experiments presented as evidence that *dinB*$^+$ and *lac* must be located next to each other for hypermutation to occur. The strains compared with *dinB* and *lac* in *cis* or in *trans* were not sufficiently isogenic. Importantly, those with *dinB* in *trans* (which showed little mutation) also carried a large *Salmonella* plasmid shown previously by the Roth lab to inhibit RecA-dependent Lac reversion. The differences in mutation are likely to have been caused by the plasmid.

Finally, we wish to note a nonobvious concordance between their view and ours. Although Roth and Anderson state that their model does not direct mutation to the F′ plasmid and imply that Foster's (and perhaps our) model does, we note that all three contributors to this Dialog invoke the same role of the F′ plasmid: increasing mutation (by error-prone DSBR in Foster's and our papers or by increasing amplification in Roth's and Andersson's) by virtue of frequent double-strand-end (DSE) formation at the transfer origin. All authors suggest that this merely enhances a process that happens by a similar mechanism, but less frequently, in the chromosome (where DSEs also occur, but less frequently). Thus the implied disagreement on this point does not exist. Understanding this can help with a related point of genuine disagreement. We disagree about whether there was ever any appearance of *lac*-directed mutation in this Lac system: we think not, because unselected chromosomal genes are mutated via a similar recombination protein- and DinB/Pol IV-dependent mechanism (we showed); and they think so because the frequency of unselected chromosomal mutations is lower than on F′. According to all of our views, this is exactly what would be expected for a general, genome-wide mutation mechanism that happens to occur more frequently on F′ because of excess double-stranded ends on the episome—thus, mutations have no appearance of being directed to the *lac* gene.

We think that the closer look presented here at the data behind the models makes HM models inescapable in this system and that, ultimately, the data on these and other mutation mechanisms will influence our views of evolution.

REFERENCES

1. **Cairns, J., and P. L. Foster.** 1991. Adaptive reversion of a frameshift mutation in *Escherichia coli.* Genetics **128:**695–701.
2. **Foster, P. L.** 2004. Adaptive mutation in *Escherichia coli.* J. Bacteriol. **186:** 4846–4852.
3. **Harris, R. S., G. Feng, K. J. Ross, R. Sidhu, C. Thulin, S. Longerich, S. K. Szigety, M. E. Winkler, and S. M. Rosenberg.** 1997. Mismatch repair protein MutL becomes limiting during stationary-phase mutation. Genes Dev. **11:** 2426–2437.
4. **Harris, R. S., S. Longerich, and S. M. Rosenberg.** 1994. Recombination in adaptive mutation. Science **264:**258–260.
5. **Harris, R. S., K. J. Ross, and S. M. Rosenberg.** 1996. Opposing roles of the Holliday junction processing systems of *Escherichia coli* in recombination-dependent adaptive mutation. Genetics **142:**681–691.
6. **Hastings, P. J., H. J. Bull, J. Klump, and S. M. Rosenberg.** 2000. Adaptive amplification: an inducible chromosomal instability mechanism. Cell **103:** 723–731.
7. **Layton, J. C., and P. L. Foster.** 2003. Error-prone DNA polymerase IV is controlled by the stress-response sigma factor, RpoS, in *Escherichia coli.* Mol. Microbiol. **50:**549–561.
8. **Lombardo, M.-J., I. Aponyi, and S. M. Rosenberg.** 2004. General stress response regulator RpoS in adaptive mutation and amplification in *Escherichia coli.* Genetics **166:**669–680.
9. **McKenzie, G. J., P. L. Lee, M.-J. Lombardo, P. J. Hastings, and S. M. Rosenberg.** 2001. SOS mutator DNA polymerase IV functions in adaptive mutation and not adaptive amplification. Mol. Cell **7:**571–579, 1119.
10. **McKenzie, G. J., D. B. Magner, P. L. Lee, and S. M. Rosenberg.** 2003. The *dinB* operon and spontaneous mutation in *Escherichia coli.* J. Bacteriol. **185:**3972–3977.
11. **Rosenberg, S. M., and P. J. Hastings.** 2004. Adaptive point mutation and adaptive amplification pathways in the *Escherichia coli* Lac system: stress responses producing genetic change. J. Bacteriol. **186:**4838–4843.
12. **Roth, J. R., and D. I. Andersson.** 2004. Adaptive mutation: how growth under selection stimulates Lac$^+$ reversion by increasing target copy number. J. Bacteriol. **186:**4855–4860