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

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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 10⁸ cells plated by day 5, they propose that 10⁻² of the cells plated (10⁹ 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 10⁸ lac copies, "for example...100 clones (of colonies) of 10⁸ cells, each with 100 copies of lac." This would mean that, on average, colonies of 10⁶ cells with 100 lac copies would have one Lac⁺ point mutant. The problem is that the reversion rate of this lac allele was ~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 to 100 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 × 10⁸ to 3 × 10⁹ 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 10⁷ 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 contraindicates 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 up-regulates 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-

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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 produc-
ing 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 colo-

There 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.

Fourth, we disagree with Roth and Anderson 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.

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