Letter to the Editor

Adaptive Mutation of a *lacZ* Amber Allele

Patricia L. Foster* and John Cairns†

**Department of Environmental Health, Boston University School of Public Health, Boston, Massachusetts 02118 and †Clinical Trial Service Unit, Radcliffe Infirmary, Oxford OX2 6HE, United Kingdom*

IN a recent letter, McKenzie *et al.* (1998) reported that the late-arising "adaptive" Lac$^+$ revertants of our *Escherichia coli* strain, FC40, are not slow to grow on lactose medium and concluded that the mutants must therefore have arisen after plating on lactose. We had reached the same conclusion on different grounds, namely, that in FC40: (i) very few Lac$^+$ revertant colonies appear within 2 days of plating on lactose; (ii) despite the absence of any measurable increase in cell numbers, revertant colonies accumulate from day 2 onward, at a constant rate for at least the next 5 days; (iii) the distribution of these colonies is Poisson, not Luria-Delbrück; and, (iv) their cells produce the same amount of β-galactosidase as early arising revertants (Cairns and Foster 1991; Foster 1994; Foster and Trimarchi 1994). In addition, our finding that adaptive, but not growth-dependent, reversion of FC40 requires functions of RecA, *E. coli*'s recombinase, led us to conclude that in this strain adaptive mutations occur by a different mechanism from growth-dependent mutations (Cairns and Foster 1991).

The issue of slow growth has, however, been raised in connection with reversion of a *lacZ* amber strain (SM195), originally described in Cairns *et al.* (1988). Lac$^+$ mutants of SM195 that appear after plating on lactose medium include both intragenic revertants and extragenic suppressors (Cairns *et al.* 1988; Foster and Cairns 1992). Prival and Cebula (1996) reported that of the late-arising revertants of SM195 (*i.e.*, those that make their appearance 3–5 days after plating), 67% are slow-growing ochre suppressors that could have arisen during prior nonselective growth of the cells. Therefore, these revertants should not be classed as adaptive. On this basis, McKenzie *et al.* (1998) conclude that in the case of SM195 "the hypothesis of mutations occurring by a different mechanism after plating is not supported." That is not an accurate summary of the situation. Prival and Cebula (1996) confirmed our finding that fast-growing intragenic revertants of SM195 appear after plating on lactose, and they concluded that growth on the plate "may not be sufficient to account for all such revertants."

In fact, Prival and Cebula's experiments provide strong evidence that the late-arising intragenic revertants of SM195 are *not* due to growth on the plate. During days 3–5 of lactose selection, the rate of appearance of colonies of slow-growing ochre suppressors declined steadily, whereas the rate of appearance of fast-growing intragenic revertants steadily increased. From the median number of intragenic revertants appearing on day 2, the preselection mutation rate of intragenics in Prival and Cebula's experiments was 1.6 per 10^9 cell divisions [using Equation 36 of Lea and Coulson (1949)]. Prival and Cebula reported that a median of 11 or 12 intragenic revertants per 10^9 cells appeared from days 3 to 5 after plating. Thus, the Lac$^-$ population would have had to increase sevenfold to produce this number of late-arising intragenic revertants by growth-dependent mutation. This amount of growth would have been plainly visible. Furthermore, if growth were the reason for the late appearance of intragenics, it surely also would have produced ochre suppressors. This suggests that the process that generates mutations in stationary phase is better at producing intragenic revertants of the episomal *lacZ* allele than chromosomal ochre suppressors.

Finally, the late appearance of all classes of Lac$^+$ revertants of SM195 is affected by functions encoded in the *uvrB-bio* region of the chromosome (Cairns *et al.* 1988). Although the reason for this has not been determined, it suggests that for SM195, too, adaptive mutations may occur by a different mechanism from growth-dependent mutations.

Corresponding author: Patricia L. Foster, S107, Boston University School of Public Health, Boston University School of Medicine, 715 Albany St., Boston, MA 02118-2394. E-mail: pfoster@bu.edu

LITERATURE CITED

Cairns, J., and P. L. Foster, 1991 Adaptive reversion of a frameshift mutation in *Escherichia coli.* Genetics **128:** 695–701.

Cairns, J., J. Overbaugh and S. Miller, 1988 The origin of mutants. Nature **335:** 142–145.

Foster, P. L., 1994 Population dynamics of a Lac$^-$ strain of *Escherichia coli* during selection for lactose utilization. Genetics **138:** 253–261.

Foster, P. L., and J. Cairns, 1992 Mechanisms of directed mutation. Genetics **131:** 783–789.

Foster, P. L., and J. M. Trimarchi, 1994 Adaptive reversion of a

frameshift mutation in *Escherichia coli* by simple base deletions in homopolymeric runs. Science **265:** 407–409.

Lea, D. E., and C. A. Coulson, 1949 The distribution of the numbers of mutants in bacterial populations. J. Genet. **49:** 264–285.

McKenzie, G. J., M.-J. Lombardo and S. M. Rosenberg, 1998 Recombination-dependent mutation in *Escherichia coli* occurs in stationary phase. Genetics **149:** 1163–1165.

Prival, M. J., and T. Cebula, 1996 Adaptive mutation and slow-growing revertants of an *Escherichia coli lacZ* amber mutant. Genetics **144:** 1337–1341.

Communicating editor: R. Maurer