JOURNAL OF BACTERIOLOGY, Apr. 1998, p. 1944–1946 Vol. 180, No. 7
0021-9193/98/$04.00+0
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# Levels of the Vsr Endonuclease Do Not Regulate Stationary-Phase Reversion of a Lac$^-$ Frameshift Allele in *Escherichia coli*

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Received 30 October 1997/Accepted 29 January 1998

**Vsr endonuclease, which initiates very short patch repair, has been hypothesized to regulate mutation in stationary-phase cells. Overexpression of Vsr does dramatically increase the stationary-phase reversion of a Lac$^-$ frameshift allele, but the absence of Vsr has no effect. Thus, at least in this case, Vsr has no regulatory role in stationary-phase mutation, and the effects of Vsr overproduction are likely to be artifactual.**

When *Escherichia coli* and other microorganisms are subjected to nonlethal selective pressure, mutations can arise even though the cells are not dividing (2). This phenomenon has been called directed, adaptive, or stationary-phase mutation (reviewed in reference 6). Recently, it has been shown that in one case of stationary-phase mutation, reversion of an episomal frameshift mutation in *E. coli*, the mutations are not truly adaptive but may arise in a subpopulation of cells experiencing a transient state of enhanced mutation (7, 20). Methyl-directed mismatch repair (MMR), which is an important contributor to replication fidelity in dividing cells (17), is equally important in reducing stationary-phase mutations; thus, MMR activity cannot be globally limiting in nutritionally deprived or stationary-phase cells (reviewed in reference 8). Nonetheless, it has been suggested that MMR could become limiting in a subpopulation of cells and that these cells could then give rise to stationary-phase mutations (10).

Very short patch repair (VSR) is a DNA repair pathway that corrects T · G mismatches to C · G at CC(A/T)GG and similar sequences (reviewed in reference 13). The 5 position of the internal C's of CC(A/T)GG sites in *E. coli* is methylated by the Dcm methylase, which is encoded by the *dcm* gene (reviewed in reference 16). Because deamination of 5-methylcytosine produces thymine, Dcm recognition sites are hot spots for mutation (3, 5). Although it is not known why *E. coli* methylates CC(A/T)GG sites, it is clear that VSR prevents G · C-to-A · T mutations that can result from this methylation (12, 18).

VSR is initiated by the Vsr endonuclease, which is encoded by the *vsr* gene. When Vsr is overproduced, a wide variety of mutations result independently of Dcm methylation (4, 15). Efficient VSR requires MutS and MutL, two proteins that participate in MMR (11); excess Vsr appears to deplete MMR proteins, particularly MutL, resulting in a general mutational state (15). VSR is active in stationary-phase cells (14), and it is possible that stationary-phase mutations are due to reduction of MMR caused by upregulation of VSR in a subpopulation of cells (10, 15). The experiments reported here refute this hypothesis by showing that in the complete absence of Vsr, cells experience a normal level of stationary-phase mutation.

The mutational target for stationary-phase mutation in our strain, FC40, is a +1 frameshift affecting the *lacZ* gene carried on an F′ episome (1). When plated on lactose minimal medium, FC40 does not divide but does produce Lac$^+$ mutations at a constant rate for several days. Unlike growth-dependent (preplating) mutations, stationary-phase (postplating) Lac$^+$ mutations are dependent on recombination functions and consist mainly of −1-bp frameshifts in runs of iterated bases (reviewed in reference 8).

When FC40 was transformed with a plasmid overproducing Vsr, both the preplating and the postplating mutation rates increased about $10^2$-fold (Table 1, experiment 1). This increase is equivalent to that produced by loss of MMR (1). As shown in Table 1, experiment 2, Vsr overproduction had no effect on stationary-phase mutation in a *mutL* mutant strain, supporting the hypothesis that Vsr overproduction depletes MMR activity. Interestingly, unlike normal postplating mutation in this strain, the Vsr-induced Lac$^+$ mutations were not totally dependent on *recA* (Table 1, experiment 3).

So far, our results confirm for stationary-phase mutations the results obtained previously for growth-dependent mutations (15). However, the hypothesis that Vsr normally determines the rate of stationary-phase mutation predicts that stationary-phase mutations should decline or disappear in a *vsr* mutant. To test this prediction, we moved into our strain by P1 transduction a deletion that includes the overlapping *vsr* and *dcm* genes. The donor was a strain (CC221/F′) in which this deletion is linked to a *d*Tn*10* element (4), and the recipient was the F$^-$ parent of FC40, FC36 (1). After selection for tetracycline resistance (Tet$^r$, encoded by the *d*Tn*10* element), Tet$^r$ isolates were screened for loss of motility (Mot$^-$), which is also conferred by the deletion. The Lac$^-$ frameshift-carrying episome was then mated into Mot$^-$ and Mot$^+$ isolates, creating FC1106 and FC1107 (the latter control demonstrated that the transposon itself had no effect on mutation; see Table 2, experiment 4). To confirm that the *dcm* gene was, indeed, lost in Mot$^-$ transductants, the strains were transformed with a plasmid and plasmid DNA was isolated and digested with endonuclease *Eco*RII, which is blocked by Dcm methylation (16). Only when isolated from FC1106 was plasmid DNA sensitive to cleavage by *Eco*RII (data not shown). Although only one pair of strains was used for the experiments presented here, several other paired isolates, produced as described above or by backcrossing from FC1106, were shown to have the same phenotypes.

As shown in Table 2, experiment 4, loss of *vsr* and *dcm* had

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TABLE 1. Effect of overproduction of $Vsr^+$ on stationary-phase reversion of a $Lac^-$ frameshift allele[a]

| Expt and strain | Relevant allele(s) | Plasmid | $n$[b] | Mean no. of $Lac^+$ revertants/$10^8$ cells plated ± SEM | |
|---|---|---|---|---|---|
| | | | | Day 2[c] | Days 3–5[d] |
| 1 | | | | | |
| FC899 | Wild type | Control | 5 | 13 ± 2 | 51 ± 4 |
| FC900 | Wild type | $Vsr^+$ | 5 | 819 ± 368 | 3,218 ± 324 |
| 2 | | | | | |
| FC1150 | *mutL* | Control | 4[e] | 124 ± 79 | 1,956 ± 251 |
| FC1151 | *mutL* | $Vsr^+$ | 5 | 280 ± 63 | 1,874 ± 195 |
| 3 | | | | | |
| FC1154 | *recA* | Control | 5 | 0.3 ± 0.2 | 0.5 ± 0.2 |
| FC1155 | *recA* | $Vsr^+$ | 5 | 200 ± 20 | 56 ± 12 |
| FC1159 | *recA mutL* | | 5 | 86 ± 30 | 15 ± 4 |

[a] Cells ($10^8$ or $10^6$) of the revertible strains were mixed with cells ($10^9$) of a nonrevertible scavenger strain and plated in 2.5 ml of top agar on M9 lactose plates as previously described (1). The $Vsr^+$ plasmid was pKKV, and the control plasmid was pKK223-2 (4; obtained from C. G. Cupples, Concordia University). The scavenger strain carried the control plasmid where appropriate. *mutL211*::Tn*5* was transduced from NR9360 (obtained from R. Schaaper, National Institute of Environmental Health Sciences); the *recA* allele was *recA938*::Tn*9* (obtained from the *E. coli* Genetic Stock Center). Except for the relevant alleles, the strains are isogenic, with the following two exceptions, neither of which influences mutation rates: (i) all but the *mutL* strains are rifampin resistant; and (ii) the episomes in FC1154 and FC1155 carry a $Tet^s$ *d*Tn*10* element (7).

[b] Number of independent cultures in the experiment.

[c] Mean number of $Lac^+$ mutants appearing 2 days after plating; these are due to growth-dependent mutations.

[d] Mean number of $Lac^+$ mutants appearing each day from day 3 to day 5; these are due to stationary-phase mutations.

[e] One culture with a jackpot of $Lac^+$ mutants was eliminated.

TABLE 2. Loss of *vsr* and *dcm* does not affect stationary-phase reversion of a $Lac^-$ frameshift allele[a]

| Expt and strain | Relevant allele | Plasmid | $n$[b] | Mean no. of $Lac^+$ revertants/$10^8$ cells plated ± SEM | |
|---|---|---|---|---|---|
| | | | | Day 2[c] | Days 3–5[d] |
| 4 | | | | | |
| FC40 | Wild type | | 10 | 1 ± 0.3 | 20 ± 2 |
| FC1107 | Wild type | | 10 | 2 ± 0.3 | 25 ± 2 |
| FC1106 | Δ(*vsr dcm*) | | 10 | 2 ± 0.6 | 22 ± 1 |
| 5 | | | | | |
| FC1141 | Wild type | Control | 5 | 4 ± 2 | 21 ± 2 |
| FC1142 | Wild type | $Vsr^+$ | 5 | 316 ± 76 | 2,111 ± 268 |
| FC1139 | Δ(*vsr dcm*) | Control | 4[e] | 3 ± 1 | 28 ± 2 |
| FC1140 | Δ(*vsr dcm*) | $Vsr^+$ | 5 | 407 ± 57 | 3,008 ± 319 |
| 6 | | | | | |
| FC1147 | Wild type | Control | 5 | 6 ± 1 | 43 ± 2 |
| FC1148 | Wild type | $Dcm^+$ | 5 | 10 ± 1 | 48 ± 3 |
| FC1143 | Δ(*vsr dcm*) | Control | 4[e] | 11 ± 2 | 34 ± 2 |
| FC1144 | Δ(*vsr dcm*) | $Dcm^+$ | 5 | 6 ± 1 | 21 ± 1 |

[a] Experimental protocols and the $Vsr^+$ plasmid and its control were as described in Table 1, footnote *a*. In experiment 4, two scavenger strains were used, one wild type and one Δ(*vsr dcm*); this made no difference, so the results were combined. In experiment 6, the control plasmid was a $Tet^s$ derivative of pBR322 (pSTL174; obtained from S. T. Lovett, Brandeis University) and the $Dcm^+$ plasmid was pDcm21 (19; obtained from A. S. Bhagwat, Wayne State University). FC1107, FC1141, and FC1142 carry the same chromosomal *d*Tn*10* element as the Δ(*vsr dcm*) strains.

[b] Number of independent cultures in the experiment.

[c] Mean number of $Lac^+$ mutants appearing 2 days after plating; these are due to growth-dependent mutations.

[d] Mean number of $Lac^+$ mutants appearing each day from day 3 to day 5; these are due to stationary-phase mutations.

[e] One culture with a jackpot of $Lac^+$ mutants was eliminated.

no effect on stationary-phase reversion of the $Lac^-$ frameshift allele. The stationary-phase $Lac^+$ mutations in the Δ(*vsr dcm*) strain were normal in that they were totally dependent on *recA*. [In a small-scale experiment (9), the numbers of postplating $Lac^+$ mutants per sector were 0.2 ± 0.2 for a Δ(*vsr dcm*) *recA* strain and 0.9 ± 0.3 for a $vsr^+$ $dcm^+$ *recA* control.] Because the mutagenic effect of Vsr overproduction is independent of Dcm methylation (15; see below), these results disprove, at least for the $Lac^-$ frameshift allele, the hypothesis that the level of Vsr determines stationary-phase mutation.

We additionally tested whether Vsr or Dcm overproduction would affect mutation in the Δ(*vsr dcm*) background (Table 2). Overproduction of Vsr was equally mutagenic in the deletion strain and in the wild-type strain (Table 2, experiment 5), confirming that Vsr-induced mutation does not depend on *dcm* methylation (4). Overproduction of Dcm had a small inhibitory effect on $Lac^+$ mutation in the Δ(*vsr dcm*) strain (Table 2, experiment 6) (although it was within the range of variation of our experiments, we have confirmed this result in several additional experiments). This might be due to loss of viability, but, at least during the course of this experiment, we detected no difference in viability between the Δ(*vsr dcm*) and wild-type strains.

In conclusion, overproduction of Vsr is as powerful a mutator in stationary-phase cells as it is in growing cells. In addition, our data suggest that Vsr overproduction may induce some postplating $Lac^+$ mutations by a *recA*-independent pathway. In contrast, overproduction of Dcm in the absence of Vsr has a small inhibitory effect on stationary-phase mutation. However, these effects appear to be artifacts of overproduction because loss of *vsr* and *dcm* does not prevent the normal level of $Lac^+$ reversion in stationary-phase cells. Thus, at least in this case, Vsr has no regulatory role in stationary-phase mutation.

This work was initiated by a suggestion from C. G. Cupples, for which we thank her. We also thank M. Berlyn, A. S. Bhagwat, C. G. Cupples, S. T. Lovett, J. H. Miller, and R. M. Schaaper for strains and plasmids.

This work was supported by grant MCB-9214137 from the U.S. National Science Foundation.

## REFERENCES

1. **Cairns, J., and P. L. Foster.** 1991. Adaptive reversion of a frameshift mutation in *Escherichia coli*. Genetics **128:**695–701.
2. **Cairns, J., J. Overbaugh, and S. Miller.** 1988. The origin of mutants. Nature (London) **335:**142–145.
3. **Coulondre, C., J. H. Miller, P. J. Farabough, and W. Gilbert.** 1978. Molecular basis of base substitution hotspots in *Escherichia coli*. Nature (London) **274:**775–780.
4. **Doiron, K. M., S. Viau, M. Koutroumanis, and C. G. Cupples.** 1996. Overexpression of *vsr* in *Escherichia coli* is mutagenic. J. Bacteriol. **178:**4294–4296.
5. **Duncan, B. K., and J. H. Miller.** 1980. Mutagenic deamination of cytosine residues in DNA. Nature (London) **287:**560–561.
6. **Foster, P. L.** 1993. Adaptive mutation: the uses of adversity. Annu. Rev. Microbiol. **47:**467–504.
7. **Foster, P. L.** 1997. Nonadaptive mutations occur on the F′ episome during adaptive mutation conditions in *Escherichia coli*. J. Bacteriol. **179:**1550–1554.
8. **Foster, P. L.** Adaptive mutation: has the unicorn landed? Genetics, in press.
9. **Foster, P. L., J. M. Trimarchi, and R. A. Maurer.** 1996. Two enzymes, both of which process recombination intermediates, have opposite effects on adaptive mutation in *Escherichia coli*. Genetics **142:**25–37.
10. **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.
11. **Lieb, M.** 1987. Bacterial genes *mutL*, *mutS*, and *dcm* participate in repair of

mismatches at 5-methylcytosine sites. J. Bacteriol. **169:**5241–5246.
12. **Lieb, M.** 1991. Spontaneous mutation at a 5-methylcytosine hotspot is prevented by very short patch (VSP) mismatch repair. Genetics **128:**23–27.
13. **Lieb, M., and A. S. Bhagwat.** 1996. Very short patch repair: reducing the cost of cytosine methylation. Mol. Microbiol. **20:**467–473.
14. **Lieb, M., and S. Rehmat.** 1997. 5-Methylcytosine is not a mutation hot spot in nondividing *Escherichia coli*. Proc. Natl. Acad. Sci. USA **94:**940–945.
15. **Macintyre, G., K. M. Doiron, and C. G. Cupples.** 1997. The Vsr endonuclease of *Escherichia coli*: an efficient DNA repair enzyme and a potent mutagen. J. Bacteriol. **179:**6048–6052.
16. **Marinus, M. G.** 1996. Methylation of DNA, p. 782–791. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, D.C.
17. **Modrich, P., and R. Lahue.** 1996. Mismatch repair in replication fidelity, genetic recombination, and cancer biology. Annu. Rev. Biochem. **65:**101–133.
18. **Petropoulos, L., J. J. Vidmar, E. Passi, and C. G. Cupples.** 1994. A simple assay for monitoring the mutagenic effects of 5-methylcytosine deamination in *Escherichia coli*. Mutat. Res. **304:**181–185.
19. **Sohail, A., M. Lieb, M. Dar, and A. S. Bhagwat.** 1990. A gene required for very short patch repair in *Escherichia coli* is adjacent to the DNA cytosine methylase gene. J. Bacteriol. **172:**4214–4221.
20. **Torkelson, J., R. S. Harris, M.-J. Lombardo, J. Nagendran, C. Thulin, and S. M. Rosenberg.** 1997. Genome-wide hypermutation in a subpopulation of stationary-phase cells underlies recombination-dependent adaptive mutation. EMBO J. **16:**3303–3311.