Overexpression of vsr in *Escherichia coli* Is Mutagenic

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Received 16 October 1995/Accepted 8 May 1996

Overexpression of vsr in *Escherichia coli* stimulates transition and frameshift mutations. The pattern of mutations suggests that mutagenesis is due to saturation or inactivation of dam-directed mismatch repair.

*Escherichia coli* has a DNA repair system dedicated to converting T/G mismatches into C · G base pairs in (T/G)WGG and related sequences (8, 12). Very short patch (VSP) repair is mediated by a sequence- and mismatch-specific endonuclease (10), the product of the *vsr* gene (20). The second C in CCWGG sequences in methylated in *E. coli* by the Dcm methylase. Thus, the main function of VSP repair is probably to correct CTWGG (1, 14).

C(T/G)WGG heteroduplexes can also arise from errors in DNA replication. Such errors are normally corrected by the dam-directed repair system in favor of the base on the template strand (16). Thus, a T/G mismatch arising from misreplication of CTWGG should be repaired to T · A. However, there is good evidence that VSP repair outcompetes dam-directed repair for C(T/G)WGG mutations (11).

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The purpose of our research was to determine whether overexpression of vsr would stimulate CTAGG-to-CCAGG mutations (Fig. 1). Overexpression of vsr was accomplished by subcloning it from pDVW (17) into pKK233-2 (Pharmacia), under the control of the strong, constitutive trc promoter (pKK-V). vsr-lacZ fusion plasmids were used to estimate levels of vsr expression from the synthetic trc promoter (pKK-VZ) and from the wild-type promoter 5' of the overlapping dcm gene (7) (pVZ). All four plasmids are shown in Fig. 2. CC221 cells containing pKK-VZ produce about 6,000 U of β-galactosidase, while cells containing pVZ produce about 300 U, indicating that pKK-V overproduces Vsr.

CC221 [ara Δ(gpt-lac)5 thi Δ(supD-dcm-fla) zee3129::Tn10], a derivative of CSH142 (15), contains a large chromosomal deletion which removes *dcm, vsr*, and at least 20 kb of surrounding sequence. The deletion was introduced by P1 transduction from RP4182 (courtesy of A. S. Bhagwat) using a tetacycline resistance marker from CAG12099 (19). The deletion was verified by Southern hybridization using a *dcm-vsr* probe. Mutagenesis assays were done by introducing pKK-V and pKK233-2 into a set of CC221 derivatives containing F′ *lacZ lacY* A′ proA B′ episomes. Each episome has a point mutation in lacZ which makes the cell Lac⁺; reversion to Lac⁻ requires a specific base substitution or frameshift mutation. F′ CC101 to CC111 have been described previously (4, 6). F′ CC113 was constructed for this study.

F′ CC113 contains an amber mutation at codon 999 in *lacZ*, introduced by site-directed mutagenesis as described previously (5). The substitution of an amber codon (TAG) for the wild-type tryptophan codon (TGG) eliminates β-galactosidase activity. It also converts the *dcm* methylase recognition sequence (CCTGG) overlapping codons 998 (TCC) and 999 (TGG) of *lacZ* to CCTAG. Trp-999 is part of the active site of β-galactosidase (9). We have found that it is essential for enzyme activity; cells must contain a tryptophan codon at position 999 in *lacZ* to be Lac⁺ (7a). Thus, cells containing F′ CC113 revert from Lac⁻ to Lac⁺ only via an A · T-to-G · C transition mutation which restores the wild-type CCTGG sequence. This is equivalent to a CTAGG-CCAGG mutation on the complementary strand. We have verified the assay by amplifying a fragment of *lacZ* surrounding codon 999 from randomly chosen Lac⁺ revertants and either digesting it with

![FIG. 1. Competition between dam-directed repair and VSP repair for the correction of (T/G)AGG mismatches. Misinsertion of G opposite T during replication of the top strand of structure I produces structure II, a substrate for both dam-directed and VSP repair. Correction of the T/G mismatch by dam-directed repair restores structure I. Processing of the mismatch by VSP repair yields structure III and results in a TA-to-CG mutation. An increase in VSP repair caused by overexpression of vsr or a decrease in dam-directed repair should increase the yield of structure III.](http://jb.asm.org/)

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EcoRII or sequencing it. In all cases, the expected CCTAGG-to-CCTGG mutation had occurred.

Table 1 shows that CC221 containing F' CC113 mutates from Lac⁻ to Lac⁺ at a 20-fold-higher frequency when transformed with pKK-V than it does when transformed with the parental plasmid, pKK233-2. This result is consistent with our original hypothesis that overexpression of vsr would stimulate CTAGG-to-CCAGG mutations (Fig. 1). However, to our surprise, Vsr-stimulated mutagenesis is not confined to a specific base substitution mutation in a restricted sequence context. pKK-V stimulates transition mutations in general (F' CC102 and CC106) and causes a dramatic increase in numbers of frameshift mutations (F' CC107 to CC111).

Given that VSP repair is specific for T/G mismatches in a fairly narrow range of sequence contexts (8, 12), it seems unlikely that excess Vsr causes mutagenesis directly by blocking access of dam-directed repair to most mismatched or unpaired bases. Instead, we suggest that overexpression of vsr causes mutagenesis indirectly by saturating or disabling dam-directed repair. This hypothesis is consistent with the fact that the spectrum and levels of mutations shown in Table 1 are very similar to those produced by a mutH version of CSH142 assayed with F' CC101 to CC111 (4). Conditions that increase the numbers of base pair mismatches are known to increase numbers of transition and frameshift mutations by saturating dam-directed repair (4, 13, 18). However, it is difficult to envision a mechanism by which excess Vsr would stimulate mismatch formation. One attractive possibility is that excess Vsr endonuclease inactivates dam-directed repair by sequestering MutS and/or MutL, two proteins which are required for both dam-directed repair and VSP repair (12). We are currently testing this hypothesis.

Vsr is apparently present in the cell in very small amounts (7). This may be one of the reasons why 5-methylcytosines are hotspots for C-to-T mutation even in cells with a functional VSP repair system (3). Our finding that excess Vsr is highly mutagenic suggests that suboptimal VSP repair is the price the cell pays to avoid Vsr-stimulated mutagenesis.

We thank Georgina Macintyre for useful discussions. This work was supported by grants to C.G.C. from the National Cancer Institute of Canada (with funds from the Canadian Cancer Society) and the Natural Sciences and Engineering Research Council of Canada.

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