

Growth Phase-Dependent Regulation of Vsr Endonuclease May Contribute to 5-Methylcytosine Mutational Hot Spots in *Escherichia coli*

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Using rabbit polyclonal antibodies, we have shown that the Dcm cytosine methylase of *Escherichia coli* is maintained at a constant level during cell growth, while Vsr endonuclease levels are growth phase dependent. Decreased production of Vsr relative to Dcm during the log phase may contribute substantially to the mutability of 5-methylcytosine.

Many organisms, although by no means all, methylate selected cytosines in their genomes. Such methylation is important, particularly in mammalian cells, where it is required for normal development (5). However, the tendency of 5-methylcytosine (5-MeC) to deaminate to thymine adds a significant mutational burden to the cell. Despite the fact that organisms which methylate their genomes have DNA repair systems dedicated to correcting deamination-induced T/G mismatches (6, 14), C-to-T transitions at methylated cytosines are among the most prevalent spontaneous mutations (1, 11). *Escherichia coli* K-12 is a case in point. This bacterium has a single DNA (cytosine-5) methyltransferase (Dcm), a product of the *dcm* gene, which methylates the second C of the sequence CCWGG (W = A or T). T/G mismatches which result from deamination of the methylated C are corrected to CG base pairs by a process known as very-short-patch (VSP) repair (reviewed in reference 7). The process is initiated by a single-stranded endonuclease (Vsr) which cleaves 5' of the mismatched T (4). Removal of several bases 3' of the nick and their resynthesis by DNA polymerase I complete the repair process. However, despite VSP repair, 5-MeCs remain hot spots for mutation in *E. coli* (1).

It has been shown that inefficient VSP repair in replicating cells is a major contributor to mutation at 5-MeC in *E. coli* (8). Recently, we proposed that the inefficiency is a deliberate strategy for mutation avoidance (9). This counterintuitive hypothesis is based on our finding that artificially increased production of Vsr results in very high levels of mutation at non-CCWGG sequences (3). The types of mutation which occur (transitions and frameshifts), the frequency with which they occur, and the fact that their frequency is reduced by addition of plasmids containing *mutH* or *mutL* (9) all suggest that Vsr-stimulated mutation is caused by interference with mismatch repair. If this is indeed the case, then Vsr production may have to be maintained at low levels in dividing cells to ensure optimal correction of DNA replication errors. Unfortunately, this strategy could also result in suboptimal VSP repair and a concomitant increase in C-to-T mutations at 5-MeC.

We have now made a rabbit polyclonal antibody to Vsr and used it to measure production of the protein during the growth of a culture of *E. coli*. CSH142 cells [*ara* (*gpt-lac*)5 *thi*] were

grown in Luria-Bertani (LB) medium (10), and samples were taken at various times throughout the log phase and stationary phase, as shown in Fig. 1A. Cell extracts containing equal amounts of protein were electrophoresed on sodium dodecyl sulfate-polyacrylamide gels, and the separated proteins were transferred to nylon membranes for Western analysis (12). Antigen-antibody complexes were detected with a chemiluminescent substrate, following the procedure outlined by the supplier (NEN Life Science Products). Figure 1B shows that Vsr is undetectable during the log phase, appearing only as the cells enter the stationary phase.

The 5' end of *vsr* overlaps the 3' end of *dcm* in a +1 reading frame (13), and the genes are apparently cotranscribed from a promoter 5' of *dcm* (2). If the stationary-phase-dependent regulation of Vsr production were transcriptional, one would expect Dcm production to follow the same pattern as Vsr.

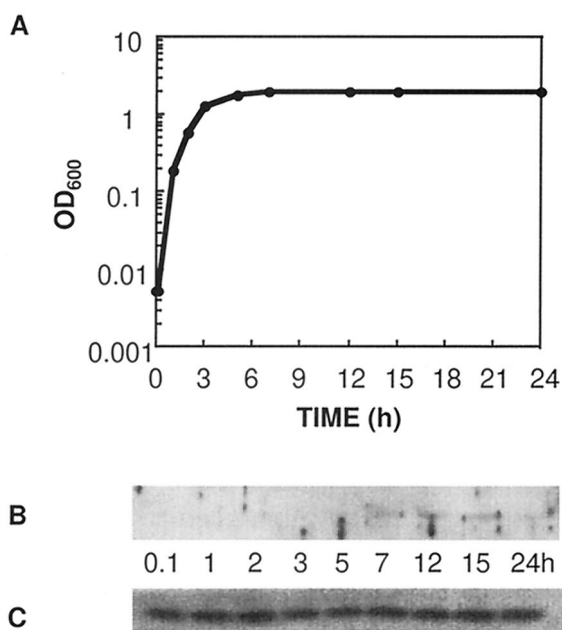


FIG. 1. Production of Vsr and Dcm in growing and stationary-phase *E. coli* cells. Cultures were initiated from a 1:100 dilution of an overnight, saturated culture. Culture growth was measured by optical density at 600 nm (OD₆₀₀) (A). Samples were taken from the culture at the times shown, and protein extracts were subjected to Western analysis with antibodies to Vsr (B) or to Dcm (C).

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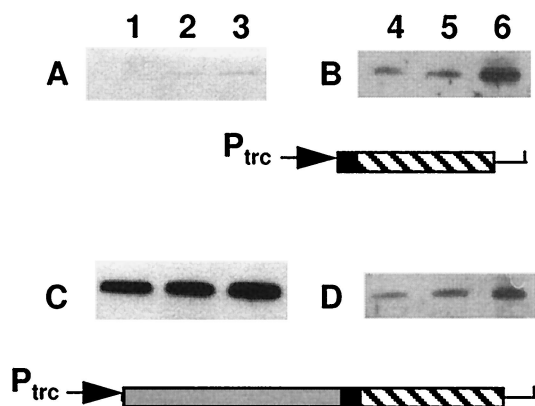


FIG. 2. Growth phase-dependent production of Vsr is independent of the *dcm* promoter and of operon structure. Cells were transformed with pKK-V (A and B) or pKK-DV (C and D); maps of the corresponding plasmid inserts are shown (shaded bar, *dcm*; black bar, overlap between *dcm* and *vsr*; hatched bar, *vsr*). Cultures were grown in LB medium plus ampicillin from a 1:100 dilution of a saturated overnight culture. Samples were taken in the early log (lanes 1 and 4), late log (lanes 2 and 5), and stationary (lanes 3 and 6) phases, and protein extracts were subjected to Western analysis. The top half of each blot was treated with antibodies to Dcm (A and C), and the bottom half was treated with antibodies to Vsr (B and D). The small amount of Dcm visible in Fig. 2A is the product of the chromosomal *dcm* gene.

Figure 1C shows the results of Western analysis with a polyclonal antibody that we made specific for Dcm. Unlike Vsr, the cellular levels of Dcm are independent of growth phase, strongly suggesting that regulation of Vsr production is post-transcriptional. To confirm this, we used a plasmid, pKK-DV (9), in which *dcm* and *vsr* are expressed from a plasmid-borne promoter (*trc*). Transcription of the operon is constitutive since the strain is *lacI*. pKK-DV transformants, grown in LB medium plus ampicillin (100 μ g/ml), were collected in the early log, late log, and stationary phases and subjected to Western analysis. Production of Dcm from the plasmid remained independent of growth phase (Fig. 2C); production of Vsr remained dependent on growth phase (Fig. 2D).

Vsr disappears very rapidly when stationary-phase cells are diluted into fresh medium (Fig. 1B), suggesting that the protein is actively degraded as cells prepare to reenter log phase. The subsequent slow buildup of the protein could be due to inefficient translation, occasioned by the fact that the *vsr* ribosome binding site (RBS) is within the 3' end of the *dcm* coding region (13). To determine whether this is the case, we measured Vsr production in cells transformed with pKK-V (3). In this plasmid, *vsr* is transcribed directly from the *trc* promoter and translated from a plasmid-borne RBS. Although pKK-V transformants produce higher levels of Vsr than pKK-DV transformants in the stationary phase (compare lanes 6 in Fig. 2B and D), the pattern of reduced Vsr expression during the log phase is maintained (Fig. 2B).

We do not yet know what mechanism controls growth phase-dependent production of Vsr. However, the fact that the *dcm* promoter is not required and that the operon arrangement of *dcm* and *vsr* does not contribute significantly suggests that a large part of the regulation is posttranslational. The rapidity with which the protein disappears when cells leave the station-

ary phase suggests active degradation. The targeted proteolysis of the *E. coli* sigma factor, σ^S , during the log phase and its stabilization during the stationary phase provide an attractive model for this form of regulation (15).

Based on our previous observation that high levels of Vsr are mutagenic (3), we had hypothesized that levels of the Vsr endonuclease are tightly controlled in growing cells (9). In particular, we suggested that mutagenesis could be avoided by keeping levels of Vsr low during periods of DNA replication in order to avoid interference with mismatch repair. The results presented in this paper show that, as predicted, the amount of Vsr is very low in growing cells and only increases as cells enter the stationary phase.

It has always been assumed that the operon arrangement of *dcm* and *vsr* serves to minimize mutation by coordinating production of the methylase and the repair endonuclease (2, 13). Clearly, this is not the case. Instead, limited amounts of Vsr are available in the log phase to repair deamination damage. This could be a major contributor to 5-MeC mutability in *E. coli*.

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