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The Vsr Endonuclease of *Escherichia coli*: an Efficient DNA Repair Enzyme and a Potent Mutagen

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The Vsr endonuclease of *Escherichia coli* initiates the repair of T/G mismatches caused by deamination of 5-methylcytosine to thymine. In this paper, we examine the capacity of Vsr to prevent CG-to-TA mutations in cells with increased transcription of the cytosine methylase gene (*dcm*). We find that sufficient Vsr is produced by a single chromosomal copy of *vsr* to prevent mutagenesis. We also investigate the cause of the transition and frameshift mutations in cells overproducing Vsr. Neither the absence of the *dcm* methylase nor its overproduction affects Vsr-stimulated mutagenesis. However, addition of *mutS*, *mutL*, or *mutH* on multicopy plasmids has a significant effect: *mutL* or *mutH* decreases the number of mutations, while *mutS* stimulates mutagenesis. The *mut*-containing plasmids have the same effect in cells treated with 2-aminopurine and in cells made defective in DNA proofreading, two experimental situations known to cause transition and frameshift mutations by saturating mismatch repair.

The Vsr endonuclease in *Escherichia coli* cleaves DNA at T/G mismatches in C(T/G)WGG and related sequences (W = A or T), producing a single-strand nick 5′ of the T. This is the first step in very short patch (VSP) repair, the end result of which is the replacement of the mismatch with a normal CG base pair (reviewed in reference 17). Since the *dcm* cytosine methylase of *E. coli* methylates the second C of the sequence CCWGG, the main function of VSP repair is probably the prevention of CG-to-TA mutations caused by deamination of 5-methylcytosine to thymine. Cells which contain *dcm* but not *vsr* have high frequencies of C-to-T mutations at 5-methylcytosine (16, 20).

The chromosomal arrangement of *dcm* and *vsr* is unusual: the 5′ end of *vsr* overlaps the 3′ end of *dcm* in a +1 reading frame (25). The two genes are probably transcribed as a single mRNA, with translation of *vsr* depending on translation of *dcm* (7). The arrangement of the genes suggests that coordinated production of the two proteins is important. For example, it could ensure that Vsr is present whenever the methylase is present, thereby minimizing mutations caused by 5-methylcytosine deamination.

Recently, we uncoupled the expression of *vsr* from *dcm* by cloning *vsr* into a multicopy plasmid under the control of the strong, constitutive *trc* promoter (8). We had hypothesized that elevated levels of Vsr in the cell would increase competition between VSP repair and postreplication mismatch repair (MMR) for the correction of T/G mismatches formed by DNA polymerase errors and that this would result in increased numbers of CTAGG-to-CCAGG mutations. As predicted, these mutations were elevated. Surprisingly, however, overproduction of Vsr also increases transition mutations in general and dramatically stimulates frameshifts. Thus, the unusual genetic arrangement of *dcm* and *vsr* may also serve to minimize mutations caused by Vsr. For example, the overlap between the coding regions of the two genes may decrease the efficiency of *vsr* translation.

One purpose of the current study was to further investigate the biological reason for the cotranscription of *dcm* and *vsr*. Specifically, we wished to determine the extent of methylase- and endonuclease-stimulated mutagenesis resulting from overexpression of *dcm* and *vsr* either alone or as part of the operon. We found that a single chromosomal copy of *vsr* produces sufficient Vsr to protect cells from the mutagenic effects of increased transcription of *dcm* but that increased production of the methylase does not protect cells from the majority of mutations caused by large amounts of Vsr. The second purpose was to investigate the mechanism by which increased production of Vsr causes widespread mutagenesis. Since the pattern of mutation is very similar to that produced in cells defective in MMR (8), we examined the effects of *mutS*, *mutL*, and *mutH* on Vsr-stimulated mutagenesis. The results support the hypothesis that Vsr-stimulated mutagenesis is caused indirectly by disruption of MMR.

MATERIALS AND METHODS

Bacterial strains. CC101 to -106 (4), CC107 to -111 (5), and CC113 (8) are all derivatives of CSH142 [*ara* Δ(*gpt-lac*)*5 thi*], and contain an F′ *lacZYA proAB* episome. The strains differ only in the nature of the mutation in the *lacZ* gene. Isogenic mutant derivatives of these strains were constructed by using P1 transduction to introduce the mutant alleles into CSH142, then adding the appropriate F′ *lacZYA proAB* episomes by conjugation with the original CC strains. The *dnaQ49* allele was transduced from NR9695 (provided by R. M. Schaaper, National Institute of Environmental Health Sciences), and *mutS201*::Tn*5*, *mutH471*::Tn*5*, and *mutL211*::Tn*5* were transduced from GW3732, GW3733, and GW3734 (provided by G. C. Walker, Massachusetts Institute of Technology), respectively. Antibiotic-resistant transductants were tested for a mutator phenotype on Luria broth (LB) plates with rifampin. A *dcm-vsr* deletion [Δ(*supD-dcm-fla*) *zee3129*::Tn*10*] was transduced from CC221 (8); the deletion was verified by Southern hybridization with a *dcm-vsr* probe.

CC112 is *ara* Δ(*gpt-lac*)*5 thi gyrA argE*(Am) *rpoB thi* and contains a plasmid with a synthetic tRNA gene (21). The tRNA inserts a glutamic acid at TAG (amber) codons. As a result of this nonsense suppression, a CAG-to-TAG mutation at codon 461 of *lacZ* produces a Lac^+ phenotype. A Δ(*dcm-vsr*) version of CC112 was made by P1 transduction of the deletion from CC221 as described above. The SMB strains are *lacZ* derivatives of MC4100 (F^- *araD139 rpsL150 relA flbB5301 ptsF25 deoC1 thi-1 rbsR*). SMB2061 and SMB2062 contain the *lacZ* alleles from CC101 and CC102; SMB2065 to SMB2069 contain the *lacZ* alleles from CC105 to CC109. *lacZ* is carried by the F episome in the CC strains and on the chromosome in the SMB strains. SMB strains were constructed by S. M. Benson (University of Maryland, College Park).

Plasmids. Overexpression of *dcm* and/or *vsr* was accomplished by cloning the gene(s) into pKK233-2 (Clontech), where they are transcribed from the synthetic *trc* promoter. pKK-V (8), pKK-D, and pKK-DV contain *vsr*, *dcm*, and the *dcm-vsr* operon, respectively. The pKK plasmids could not be used in CC112, since the strain already contains a plasmid with a ColE1 origin of replication.

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TABLE 1. Effect of *dcm* and/or *vsr* overexpression on CCAGG-to-CTAGG mutations in wild-type and Δ(*dcm-vsr*) strains

Plasmid[a]	Cloned gene(s)	Promoter	No. of Lac$^+$ colonies/10^8 viable cells	
			CC112Δ[b]	CC112
pACYC184	None	NA[c]	1.8	2.3
pDV101	*dcm*	*dcm*	37.5	1
pDV104	*dcm*	*trc*	263	1.2
pDV102	*dcm-vsr*	*dcm*	3.6	0
pDCM28	*vsr*	*dcm*	0	1
pDV108	*vsr*	*trc*	0.3	1

[a] All plasmids contain the p15a origin of replication.
[b] CC112 containing a deletion of *dcm-vsr*.
[c] NA, not applicable.

Therefore, *Hin*dIII/*Bam*HI fragments containing the gene(s) and the *trc* promoter were subcloned from the pKK plasmids into pACYC184, a plasmid with a ColE1-compatible p15a origin (pDV series). pACYC184-based plasmids containing *dcm* (pDV101) and *dcm-vsr* (pDV102) expressed from the *dcm* promoter were constructed by subcloning from pDW and pDVW, respectively (20). pDCM28, a pACYC184-based plasmid containing *vsr* expressed from the *dcm* promoter, was obtained from A. S. Bhagwat (25).

Additional copies of *mutL*, *mutS*, and *mutH* were introduced into cells by transforming with pMQ339, pMQ341, and pMQ348, respectively (26). pMQ plasmids are derived from pACYC184, so they are compatible with pKK-V. Each pMQ plasmid was verified by its ability to complement the mutator phenotype of a strain with a Tn*5* in the corresponding chromosomal *mut* gene.

Mutagenesis assays. The frequency of occurrence of specific base substitution and frameshift mutations in the CC and SMB strains was measured by using a Lac reversion assay (4, 5, 8, 21). Each strain reverts from Lac$^-$ to Lac$^+$ via a unique point mutation in *lacZ* (see Table 2). For quantitative assays, 100-μl aliquots of saturated overnight cultures were spread on minimal lactose plates, and the number of colonies was counted after 36 h of incubation. For rapid screening, 5-μl aliquots were spotted onto minimal lactose plates and/or papillation plates (18, 19). The choice of plate was determined by the strain being assayed. Papillation plates are more sensitive, but they are difficult to use with strains, such as CC102 and CC110, which produce pale-blue colonies on plates containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). Viability was determined by spreading 100 μl or spotting 5 μl of a 10^{-6} dilution of the overnight culture on LB plates. All assays were done in triplicate from at least three independent transformants.

Cells containing the temperature-sensitive *dnaQ49* allele were grown at 37°C to disable proofreading; controls were grown at 30°C. Treatment of cells with 2-aminopurine (2-AP) has been described previously (4).

RESULTS

Overexpression of *dcm*, *vsr*, and the *dcm-vsr* operon. It is known that the presence of a plasmid containing *dcm* (expressed from its own promoter) in a cell lacking chromosomal copies of *dcm* and *vsr* increases CCAGG-to-CTAGG mutations (16, 20). Presumably, the absence of VSP repair leaves the cells unable to correct T/G mismatches caused by deamination of 5-methylcytosine to thymine. We wished to determine whether transcription of *dcm* from a stronger promoter (*trc*) would increase mutation and whether normal levels of *vsr* transcription were sufficient to counteract mutagenesis.

The results in column 4 of Table 1 show that the mutation frequency, measured as the number of Lac$^-$ cells per 10^8 which revert to Lac$^+$ via a CCAGG-to-CTAGG mutation in *lacZ*, does increase when *dcm* is transcribed from the *trc* instead of the *dcm* promoter in a *vsr* strain (Table 1, compare pDV101 with pDV104). However, a single copy of *vsr* on the chromosome is sufficient to counteract the mutagenesis caused by both pDV101 and pDV104 (Table 1, column 5). Cotranscription of *dcm* and *vsr* with the *dcm* promoter (in pDV102) produces background levels of mutation regardless of the presence or absence of the genes on the chromosome. Plasmids containing *vsr* alone, either with the *dcm* promoter (in pDCM28) or the *trc* promoter (in pDV108), do not stimulate CCAGG-to-CTAGG mutations in either *dcm*$^+$ or *dcm* mutant cells.

TABLE 2. Effect of overexpression of *dcm* and/or *vsr* on mutagenesis

F′	Mutation	No. of Lac$^+$ colonies/10^8 viable cells			
		pKK233-2[a]	pKK-D	pKK-V	pKK-DV
CC101	AT→CG	1	0	0.1	0.5
CC102	GC→AT	0.5	0.4	27	21
CC103	GC→CG	0	0	0	0
CC104	GC→TA	1.1	2.8	2	6
CC105	AT→TA	0.4	0.3	0.3	0.4
CC106	AT→GC	0	0.4	12	6
CC107	+G	20	91	10,942	10,717
CC108	−G	6	11	1,124	1,435
CC109	−(CG)	73	89	1,556	1,375
CC110	+A	1	1.8	68	134
CC111	−A	11	11	907	779
CC113	CTAGG→CCAGG	2	3.6	223	26

[a] All plasmids contain the ColE1 origin of replication.

We have shown previously that overexpressing *vsr* by cloning it in a multicopy plasmid (pKK233-2) under the control of the *trc* promoter (in pKK-V) stimulates both transition and frameshift mutations (8). Since *vsr* is normally cotranscribed with *dcm* from a promoter 5′ of *dcm*, we explored the possibility that an imbalance between the methylase and the endonuclease contributes to mutagenesis. We therefore cloned *dcm* alone (pKK-D) and the *dcm-vsr* pair (pKK-DV) into pKK233-2 and measured mutagenesis by using a Lac reversion assay (4, 5, 8). As shown in Table 2, cells transformed with pKK-DV show elevated frequencies of transition and frameshift mutations very similar to those seen in cells containing pKK-V. Only the CTAGG-to-CCAGG mutation (CC113) is stimulated more by the *vsr* plasmid than by the *dcm-vsr* plasmid. Overproduction of the methylase does not contribute to mutagenesis; cells containing pKK-D have a low level of mutation similar to that of control cells containing just the cloning vector (pKK233-2). The presence or absence of the *dcm-vsr* operon on the chromosome has no effect on the relative frequency of the various types of mutation with any of the plasmids (data not shown).

Effect of *mutH*, *mutL*, and *mutS* on Vsr-stimulated mutagenesis. The high numbers of transition and frameshift mutations in cells overexpressing Vsr (Table 2) are very similar to those seen in cells defective in MMR (5). Therefore, it is possible that excess Vsr causes mutation by interfering with MMR. If so, addition of pKK-V to cells with a defect in one of the mismatch repair genes should not increase mutagenesis above that caused by the loss of MMR. The data in Table 3 confirm this prediction: transition and frameshift mutations in *mutS*, *mutL*, or *mutH* strains are not increased when the cells are transformed with pKK-V.

If MMR is affected by overproduction of Vsr, then addition of plasmids containing the *mutS*, *mutL*, or *mutH* gene may influence mutation. We therefore transformed cells containing pKK-V with plasmids containing one of the three *mut* genes (pMQ341, pMQ339, and pMQ348) and measured the number of Lac$^+$ revertants per 10^8 viable cells due to a GC-to-AT transition mutation, a +1 frameshift mutation in a run of six A's, or a CTAGG-to-CCAGG mutation. The data were normalized to the numbers of mutants produced by cells containing the control plasmid, pACYC184. Figure 1 shows that the *mutS*, *mutH*, and *mutL* plasmids have no statistically significant effect on the CTAGG-to-CCAGG mutation (Fig. 1, group IV).

TABLE 3. Overexpression of *vsr* does not increase mutation in cells defective in MMR

Strain	Plasmid	No. of Lac$^+$ colonies/10^8 viable cells		
		F′ CC102	F′ CC107	F′ CC110
mutH::Tn*5*	pKK233-2	220	21,827	833
	pKK-V	215	15,525	785
mutL::Tn*5*	pKK233-2	231	46,512	885
	pKK-V	173	41,402	750
mutS::Tn*5*	pKK233-2	204	36,487	687
	pKK-V	255	43,459	769

However, the numbers of GC-to-AT transition (Fig. 1, group II) and +A frameshift (Fig. 1, group III) mutations are decreased substantially by the addition of the *mutL* or the *mutH* plasmid. In contrast, addition of the *mutS* plasmid increases the number of mutations. The *mutS* plasmid does not stimulate mutation in cells without pKK-V (data not shown).

The extremely high mutation rate characteristic of cells defective in DNA proofreading (*mutD5*) is due in large measure to saturation of MMR (22). Addition of a cloned *mutH* or *mutL* from *Salmonella typhimurium* was found to decrease mutation levels (as measured by rifampin or nalidixic acid resistance), while addition of the *S. typhimurium mutS* was either neutral or slightly mutagenic (23), results remarkably similar to those seen in Fig. 1 for cells overproducing Vsr. In order to make a direct comparison, we used the Lac reversion assay to assess mutagenesis in a *dnaQ* strain of *E. coli* containing a plasmid with the *E. coli mutS*, *mutL*, or *mutH* gene. As shown in Fig. 2, the *mutH* or *mutL* plasmid reduces the AT-to-GC transition (CC106) and the +A frameshift (CC110) in the proofreading mutant while the *mutS* plasmid increases both mutations. (The AT-to-GC transition is illustrated rather than GC to AT due to the difficulty in photographing dark-blue papillae on the pale-blue colony produced by CC102.)

It has been suggested that the large number of frameshift mutations caused by treatment of *E. coli* with base analogs is

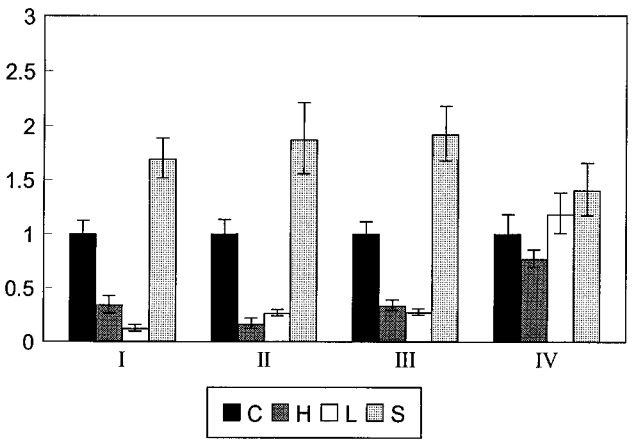

FIG. 1. Effect of cloned *mut* genes on 2-AP- and Vsr-stimulated mutations. Groups: I, CC110 grown in the presence of 700 μg of 2-AP/ml; II to IV, CC102, CC110, and CC113 transformed with pKK-V. Cells were transformed (I) or cotransformed (II to IV) with one of the following plasmids: pACYC184 (C), pMQ348 (H), pMQ339 (L), or pMQ341 (S). Data are normalized to the values obtained for cells containing the control plasmid (pACYC184): 480 (I), 19 (II), 928 (III), and 53 (IV) Lac$^+$ colonies per 10^8 viable cells.

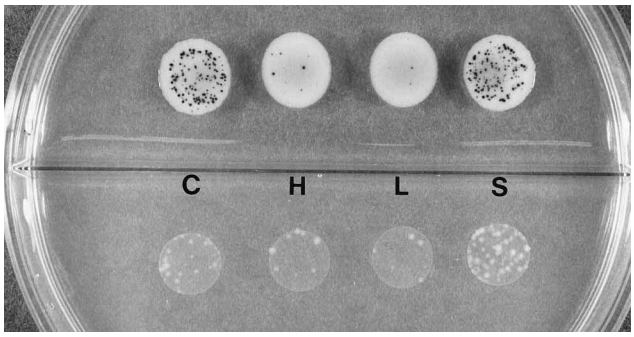

FIG. 2. Effect of *mut* genes on base substitution and frameshift mutations in a *dnaQ* strain. The top half of the plate shows CC106 on papillation medium. The bottom half of the plate shows CC110 on minimal lactose medium. Cells are transformed with the following plasmids: pACYC184 (C), pMQ348 (H), pMQ339 (L), and pMQ341 (S).

due to saturation of MMR (5), although this hypothesis has never been explicitly tested. For example, in 2-AP-treated cells, DNA polymerase replication errors are probably left unrepaired due to the presence of excess 2-AP–C base pairs (5), a lesion known to be subject to MMR (10). The results in Fig. 1 show that the +A frameshift mutation caused by treatment of cells with 2-AP is reduced by the addition of plasmid-borne *mutL* or *mutH* but increased by addition of cloned *mutS* (Fig. 1, group I). These results are consistent with those seen in cells defective in proofreading (Fig. 2) and in cells overproducing Vsr (Figure 1, group III).

Vsr-stimulated mutagenesis is not confined to *lacZ* alleles on an F′ episome. Some criticism has been levelled at studies which measure mutations occurring in target genes located on an F episome (9). Therefore, we determined the effect of overproduction of Vsr on the occurrence of mutations in *lacZ* on the chromosome; the *lacZ* alleles used to measure specific transition and frameshift mutations are identical to those used in the F′-based assay. Comparison of the data in Fig. 3A and B with the data in Table 2 shows that overexpression of Vsr stimulates the same types of mutation, to the same relative extent, in the chromosomal gene as in the episomal gene and that mutation is decreased by the addition of plasmids containing either *mutL* or *mutH* and increased by the *mutS* plasmid. pKK-V also stimulates mutations in *rpoB*, the gene which codes for the β subunit of RNA polymerase, as shown by increased numbers of rifampin-resistant colonies (Fig. 3C). Mutation to Rif^r is influenced by the *mut*-containing plasmids in the same way as reversion to Lac$^+$.

DISCUSSION

The *dcm* cytosine methylase and the *vsr* endonuclease of *E. coli* both have the potential to cause mutation. It has been known for many years that methylation of cytosine in *E. coli* increases the frequency of CG-to-TA mutations (3) and that the mutation frequency is elevated in cells which lack *vsr* (16, 20). Strangely, although Vsr is required in order to avoid mutations at 5-methylcytosine, overexpression of Vsr causes transition and frameshift mutations throughout the genome (8). How does the cell exploit the DNA repair capacity of Vsr without suffering from its mutagenic effects? The solution may lie in the unusual genetic arrangement of *dcm* and *vsr*, in which the 5′ end of *vsr* overlaps the 3′ end of *dcm* (25). We therefore compared the frequency of occurrence of methylase-specific and Vsr-specific mutations in cells transformed with a multicopy plasmid containing the intact *dcm-vsr* operon and cells

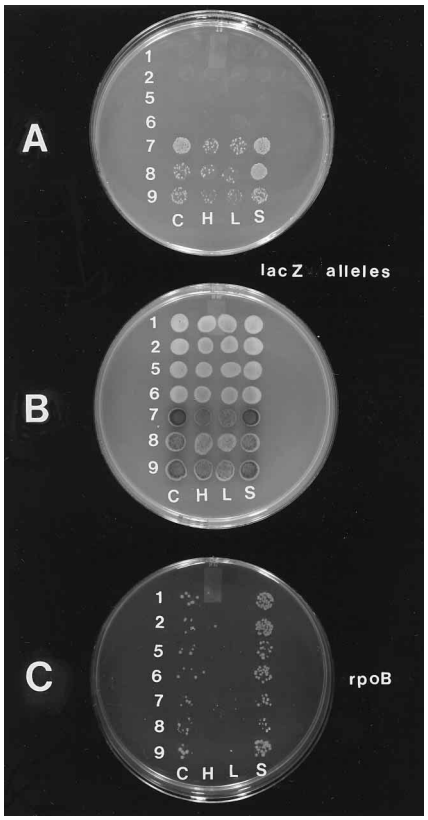

FIG. 3. Effect of *mut* plasmids on Vsr-stimulated mutations in chromosomal *lacZ* alleles (A and B) and *rpoB* (C). The strains, each transformed with pKK-V, are arranged in rows: SMB2061 (1), SMB2062 (2), and SMB2065 to SMB2069 (5 to 9). Strains in the same column are cotransformed with the following plasmids: pACYC184 (C), pMQ348 (H), pMQ339 (L), and pMQ341 (S). The strains were grown on lactose minimal medium (A), papillation medium (B), and LB with rifampin (C).

transformed with plasmids containing *dcm* or *vsr* alone. The cloned operon and the cloned individual genes are transcribed from the *trc* promoter. The combination of a high-copy-number plasmid and a strong promoter considerably augments production of the proteins (8, 14). Transformed cells were tested, with or without the *dcm-vsr* operon on the chromosome, by using an assay in which cells revert from Lac^- to Lac^+ via specific base substitution or frameshift mutations in *lacZ*.

Methylase-stimulated mutagenesis. Increased transcription of *dcm* stimulates only 5-methylcytosine-to-thymine mutations (Table 1); it has no effect on other types of base substitution and frameshift mutations (Table 2). The CCAGG-to-CTAGG mutagenesis depends on whether or not *vsr* is present. In cells with neither a chromosomal nor a plasmid-borne *vsr* gene, the presence of *dcm* stimulates the mutations. The frequency of mutation increases approximately sixfold when the gene is expressed from the *trc* promoter (Table 1). We have estimated from *dcm*::*lacZ* fusions that pDV104 produces about sixfold more methylase than pDV101 (14). This correspondence between levels of methylase and levels of mutagenesis suggests either that the target cytosine is normally poorly methylated or that the methylase actively contributes to the occurrence of mutations, as suggested by in vitro data (24).

Table 1 shows that a plasmid with both *dcm* and *vsr* (pDV102) does not stimulate mutation in cells lacking a chromosomal copy of *vsr* (CC112Δ). However, it should be noted that this result is at odds with data published by Bandaru and coworkers. They find that a *dcm-vsr* plasmid increases mutagenesis 26-fold in a strain which is Vsr^- due to a nonsense mutation in *dcm* (the *dcm-6* allele) (1). The discrepancy is not due to the different alleles used in the two studies: a *dcm-6* version of CC112 transformed with pDV102 gives the same results (not shown) as CC112Δ (Table 1). The mutagenesis that the other group saw may have been due to mutations other than CCAGG to CTAGG resulting from increased production of Vsr. We have ruled out this possibility in our assay (pDV108).

A plasmid containing *dcm* alone does not stimulate mutation, even when overproduced, so long as there is a copy of *vsr* on the chromosome (e.g., Table 1, pDV101 and pDV104). This result, too, is at odds with data published by Bandaru and coworkers. They find that a *dcm*-containing plasmid, with the *dcm* promoter, produces a 47-fold increase in mutations in a vsr^+ strain (2). There are several possible explanations for this discrepancy. The CCAGG target site in CC112 may be inefficiently methylated or it may be unusually resistant to deamination. Alternatively, the requirement for nonsense suppression in order to generate a Lac^+ phenotype may reduce the sensitivity of our assay.

5-Methylcytosines are hotspots for mutation in *E. coli* (3). Several hypotheses have been put forward to explain these hotspots, including the possibility that repair of T/G mismatches caused by deamination of 5-methylcytosine to thymine is inefficient. Our results suggest that VSP repair is actually remarkably efficient.

Vsr-stimulated mutagenesis. Of the mutations we measured, only the CCAGG-to-CTAGG mutation was unaffected by overproduction of Vsr (Table 1). This lack of mutation is not due to a defect in the plasmid or to low copy number: pDV108 gives the same level of mutation as pKK-V when introduced into CC110 (data not shown). However, the reverse mutation, CTAGG to CCAGG, is stimulated, as is a broad spectrum of other base substitution and frameshift mutations in both episomal genes (Table 2) and chromosomal genes (Fig. 3).

Our data indicate that the mechanism by which overproduction of Vsr stimulates T-to-C mutations at CTAGG sites is distinct from that which stimulates other transition mutations and frameshifts. First, only the CTAGG-to-CCAGG mutation (CC113) is decreased by the presence of *dcm* with *vsr* on the plasmid (pKK-DV versus pKK-V); other types of mutation are unaffected by *dcm* on either the plasmid or the chromosome (Table 2). This protective effect of *dcm* may be due to binding of the methylase to C(T/G)AGG and exclusion of Vsr: it is known that some bacterial cytosine methylases bind tightly to recognition sites that contain mismatches (13, 27). Second, the CTAGG-to-CCAGG mutation is unaffected by the addition of plasmids containing *mutH*, *mutL*, or *mutS* (Fig. 1, group IV) while the other mutations are significantly affected (Fig. 1, groups II and III). We have suggested that the CTAGG-to-CCAGG mutations are caused by direct competition between VSP repair and MMR for T/G mismatches caused by misreplication of CTAGG (8). Although one might expect that adding extra Mut proteins would reduce mutation by stimulating MMR, it is possible that they stimulate VSP repair as much or more.

In our previous paper on Vsr-mediated mutagenesis (8), we noted that strains overproducing Vsr and strains defective in MMR show a remarkably similar mutagenic spectrum in the Lac reversion assay. This led us to suggest that the majority of Vsr-stimulated mutations are caused indirectly by disruption of MMR. The data in Table 3, which show that introduction of pKK-V into cells defective in *mutS*, *mutL*, or *mutH* does not increase base substitution or frameshift mutations beyond that

caused by inactivation of MMR, support this hypothesis. We also suggested that the disruption was due to a decrease in the availability of MMR proteins in cells overproducing Vsr (8). This hypothesis was based on genetic evidence that MutS and MutL contribute to VSP repair (12, 15, 28). Results presented in this paper strengthen this hypothesis.

It is known that inactivation of proofreading stimulates mutation by saturating MMR (23). There is good evidence that the saturation is due to exhaustion of one or more of the MMR proteins (6). We used a single assay (Lac reversion), and a set of isogenic strains, to directly compare mutagenesis in cells overproducing Vsr (Fig. 1, groups II and III) and cells defective in proofreading due to a mutation in *dnaQ* (Fig. 2) following the addition of plasmids containing the *E. coli mutH*, *mutL*, and *mutS* genes. Since saturation of MMR has also been proposed as the mechanism for frameshift mutations produced by treatment of cells with base analogs (5), we performed the same experiment on cells treated with 2-AP (Fig. 1, group I). The striking similarities among the results of the three experiments suggest that MMR is inactivated through a common mechanism.

Our complementation experiments (this paper), and those of Schaaper and Radman with proofreading mutants (23), show that MutS is not limiting. The more likely cause of mutation is thus depletion of MutL and/or MutH. The involvement of MutL, but not MutH, in VSP repair tends to point the finger of suspicion at MutL. It is also interesting that increased mutation in stationary phase is associated with a decrease in the level of functional MutL protein (11). Stimulation of mutation by *mutS*-containing plasmids may indicate that MutL is inactivated by interaction with MutS. MutH may decrease mutation by increasing the number of productive MutS-MutL interactions (i.e., those that result in MMR). However, the true nature of the interactions among the Mut proteins, and of their interaction with Vsr, will require further genetic and biochemical data.

The Dcm methylase poses less of a threat to the integrity of the *E. coli* genome than does the Vsr endonuclease, ostensibly a DNA repair enzyme. Our results indicate that increased transcription of the *dcm-vsr* operon would not increase Dcm-mediated mutations at 5-methylcytosines but would result in a Vsr-mediated, genome-wide increase in other transition and frameshift mutations. We suspect that Vsr production is probably tightly regulated in the normal cell to avoid mutagenesis. However, it is worth considering that Vsr could provide the cell with a mechanism for transiently increasing its mutation rate under adverse circumstances, including stationary phase.

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