Very Short Patch Mismatch Repair Activity Associated with Gene dcm Is Not Conferred by a Plasmid Coding for EcoRII Methylase

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The only cytosine methylase in Escherichia coli K-12 methylates the second cytosine in the sequence CC (A/T)GG and is encoded by gene dcm. Methylation and very short patch mismatch repair activities lacking in a dcm mutant of E. coli were restored by a plasmid containing the cloned dcm gene. In contrast, plasmids with the gene for EcoRII methylase, which is a homolog of dcm, restored only mismatch activity and not mismatch repair.

A very short patch (VSP) repair system in Escherichia coli recognizes T·G mismatches when they occur in certain DNA sequences (7, 9). Such T·G mismatches are corrected to C·G in a unidirectional manner (5, 7). For example, T·G mismatches in 5'CGGCC or 5'CCGCCC are corrected by VSP repair to yield 5'CC(A/T)GG. The product of the dcm (deoxycytosine methylase) gene adds methyl groups to the internal cytosines of this sequence. Spontaneous deamination of 5-methylcytosine produces a repair-prone T·G mismatch. It has been reported that a dcm mutant (the dcm-6 mutant) is unable to perform VSP repair (5, 8, 11), supporting the suggestion that dcm may play a role in preventing mutations that occur when 5-methylcytosine is spontaneously deaminated to produce thymine (5, 7).

Another cytosine methylase, whose methylation sites are identical to those of dcm methylase, is part of the EcoRII restriction-modification system. The genes for the EcoRII enzymes lie on an R plasmid, N3 (4). Recently, the EcoRII methylase gene and dcm were cloned on multicopy plasmids. Southern blot analysis with a labeled EcoRII methylase probe suggested that gene dcm is significantly similar to the EcoRIII sequence. Here we show that a plasmid containing dcm, but not plasmids carrying the EcoRII methylase gene, restores VSP repair activity to dcm-bacteria.

VSP repair activity is monitored most conveniently by examining the progeny of four-factor crosses between two lambda phages having closely linked mutations in the repressor (cl) gene (6). Bacteria were infected with approximately five Nam and five Oam phages; the N- and O- parental phages contained different mutations in gene cl, which lies between N and O. Progeny phage were plated on a dcm mutant strain that lacks an amber suppressor and thus allows only N+ O- recombinant phage to form plaques. N+ O- recombinants that are cl+ due to recombination between the cl markers form turbid plaques, while cl- phage form clear plaques. When one of the cl mutations is susceptible to VSP repair, the frequency of cl+ recombinants is much higher than expected on the basis of the physical distance between the markers (4). The excess recombinants are produced by the correction of mismatches in DNA heteroduplexes that arise during phage recombination.

pDCM1 is a pBR322 derivative carrying the dcm+ gene (1), and pR215 is a pACYC184 derivative carrying the functional EcoRII methylase gene (A. Bhagwat, unpublished data). Both plasmids were constructed from multicopy vectors (2, 3). The methylase activities in extracts of bacterial strains containing either plasmid were greater than the activity in extracts of a dcm+ strain (GM30); cells carrying pCDM1 contained 30-fold-higher activity, and those carrying pR215 contained 6-fold-higher activity. When phage lambda was grown on the isogenic dcm-6 strain GM31 (10) containing either plasmid, all CC(A/T)GG sequences in phage DNA were methylated, as shown by the resistance of the DNA to digestion by EcoRII endonuclease (data not shown).

Recombination between mutation am6 (a C-to-T transition at the internal C in a 5' CCAGG sequence) and mutation 330 (5 bases from am6) was very infrequent when the cross was made in the dcm mutant strain (Fig. 1, cross A). The frequency of cl+ recombinants was significantly higher in a dcm+ host and increased more than 20-fold when the cross was made in dcm mutant bacteria containing the dcm+ plasmid. Thus, a single copy of dcm does not confer maximal VSP repair activity. Similar results were obtained in a cross of am6 with another nearby marker (Fig. 1, cross B). The frequency of recombination between mutations CP7 and am302, neither of which is susceptible to VSP repair, was identical in all three bacterial strains (Fig. 1, cross C).

In contrast, a plasmid carrying the gene for EcoRII methylase (pR215) did not increase the frequency of cl+ recombinants in crosses of am6 with CP7 (compare Fig. 2, crosses 1 and 2, with Fig. 1, cross B). If the role of the dcm product in VSP repair involves binding to the CC(A/T)GG or related DNA sequences, EcoRII methylase may inhibit this by direct competition. If the binding affinities of the two methylases to the target sequence are comparable, the presence of pR215 could decrease the amount of VSP repair in a dcm+ strain. Crosses 4 and 5 (Fig. 2) showed that this is not the case. Although we consider it unlikely, we cannot at this time rule out the possibility that the affinity of EcoRII methylase for its substrate is much lower than that of dcm methylase for the same substrate.

Plasmid pDCM1 contains about 11 kilobases of bacterial DNA which is likely to include genes in addition to dcm. The products of these genes may participate in VSP repair. We considered the possibility that R factor N3, which codes for the EcoRII restriction-modification system, might encode such genes, which were lost during the construction of pR215. To test this, we transformed N3 into GM31 and used the transformant as a host for crosses of am6 with mutation.

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Bacteria for EcoRII were plated to were at No VSP CP7. Known sequences of ampicillin, and tetracycline of aGM25034 from the National Institutes of Health Service Foundation are involved in the National Institutes of Health Service Foundation as described in the legend. Relevant Genotype Plasmid

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<th>O^*parent</th>
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**FIG. 1.** Effect of pDCMI on mismatch repair in dcm^- and dcm-6 bacteria. Crosses were made as described previously (3). Progeny phage were plated on strain GM2142, a derivative of GM31 that lacks an amber suppressor. A total of 2,000 to 10,000 plaques were screened in each experiment to determine the frequency of cI^+ (turbid) plaques. Standard deviations are shown as lines to the right of the bars. All crosses were performed at least three times.

**FIG. 2.** Mismatch repair in strains containing plasmids coding for EcoRII methylase. Plasmid N3 is about 60 kilobase pairs in size. Known genes include those for resistance to sulfanilamide, streptomycin, and tetracycline and factors required for conjugative transfer. Bacteria were infected with a 1:1 mixture of λ Nam53 clam6 and λ c1CP7 Oam29 (total multiplicity of infection, 10). Progeny phage were screened as described in the legend to Fig. 1.

CP7. No VSP repair activity was associated with the presence of the R factor (Fig. 2, cross 3).

We conclude that the ability to recognize and methylate CC(A/T)GG sequences is not sufficient for the participation of a protein in VSP repair. Further, these data suggest that gene dcm contains sequences crucial for its role in VSP repair that are missing from the EcoRII methylase or that E. coli contains one or more additional genes linked to dcm that are involved in VSP repair. In the latter case, dcm-6 would be a pleotropic mutation affecting genes in addition to dcm. Sequence analysis of dcm^- and dcm-6 can help to settle this question.

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**LITERATURE CITED**