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Methyl directed DNA mismatch repair in *Vibrio cholerae*

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Abstract. Mismatches in DNA occur either due to replication error or during recombination between homologous but non-identical DNA sequences or due to chemical modification of bases. The mismatch in DNA, if not repaired, result in high spontaneous mutation frequency. The repair has to be in the newly synthesized strand of the DNA molecule, otherwise the error will be fixed permanently. Three distinct mechanisms have been proposed for the repair of mismatches in DNA in prokaryotic cells and gene functions involved in these repair processes have been identified. The methyl-directed DNA mismatch repair has been examined in *Vibrio cholerae,* a highly pathogenic gram negative bacterium and the causative agent of the diarrhoeal disease cholera. The DNA adenine methyltransferase encoding gene (*dam*) of this organism which is involved in strand discrimination during the repair process has been cloned and the complete nucleotide sequence has been determined. *Vibrio cholerae dam* gene codes for a 21·5 kDa protein and can substitute for the *Escherichia coli* enzyme. Overproduction of *Vibrio cholerae* Dam protein is neither hypermutable nor lethal both in *Escherichia coli* and *Vibrio cholerae.* While *Escherichia coli dam* mutants are sensitive to 2-aminopurine, *Vibrio cholerae* 2-aminopurine sensitive mutants have been isolated with intact GATC methylation activity. The mutator genes *mut*S and m*ut*L involved in the recognition of mismatch have been cloned, nucleotide sequence determined and their products characterized. Mutants of *mut*S and *mut*L of *Vibrio cholerae* have been isolated and show high rate of spontaneous mutation frequency. The *mut*U gene of *Vibrio cholerae,* the product of which is a DNA helicase II, codes for a 70 kDa protein. The deduced amino acid sequence of the *mut*U gene hs all the consensus helicase motifs. The DNA cytosine methyltransferase encoding gene *(dam)* of *Vibrio cholerae* has also been cloned. The *dcm* gene codes for a 53 kDa protein. This gene product might be involved in very short patch (VSP) repair of DNA mismatches. The *vsr* gene which is directly involved in VSP repair process codes for a 23 kDa protein. Using these information, the status of DNA mismatch repair in *Vibrio cholerae* will be discussed.

Keywords. Spontaneous mutation frequency; mutator genes; strand discrimination; methylation; nucleotide sequence; very short patch repair.

1. Introduction

Fidelity of DNA synthesis is pivotal in maintaining the genetic integrity of an organism. While the organism must replicate and repair its DNA with high degree

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of accuracy, a few errors must be permitted for species evolution. The overall accuracy of DNA synthesis is guided by the fidelity of DNA polymerase and its proof reading activity (Loeb and Kunkel 1982). The DNA polymerase makes an error at the rate of 10^{-3} per base pair added and the average rate by which a misincorporated base is overlooked by the proof reading activity of the DNA polymerase is again of the order of 10^{-3}. Hence, the biosynthetic error leads to a spontaneous mutation frequency of the order of 10^{-6}. For most markers, this rate is much higher than the actual values of spontaneous mutation frequency which ranges between 10^{-9}–10^{-11} per base pair replicated (Drake 1969; Cox 1976). Thus, mismatches in the DNA are repaired and the efficiency of the repair process dictates the spontaneous mutation frequency. The contribution of mismatch repair towards the reduction of spontaneous mutation frequency is of the order of 10^{-3}–10^{-4}.

Evidences for the repair of mismatched bases in prokaryotic cells came from studies on transformation in *Streptococcus pneumoniae* (Fox and Allen 1964) and from transfection of *Escherichia coli* cells by artificially constructed heteroduplexes of phage DNA in which the two strands could be distinguished genetically (Wildenberg and Meselson 1975; Wagner and Meselson 1976; Pukkila *et al* 1983; Dohet *et al* 1986). These experiments demonstrated the correction of heteroduplexes prior to the onset of replication. Several mutants of *E. coli* and *S. pneumoniae* were isolated which showed high rate of spontaneous mutation frequency. These mutants mapped in different loci in the *E. coli* genetic map and were collectively designated as mutator genes (Cox 1976). Evidences have accumulated to suggest that the processing of mismatches within recombination intermediates contribute to: (i) gene conversion, *i.e.* non-reciprocal transfer of genetic traits from one DNA molecule to another, (ii) co-conversion, (iii) map extension effects, (iv) localized negative interference, and (v) post-meiotic segregation (Meselson and Radding 1975; Holliday 1974; Finchman and Holliday 1970).

In *E. coli* DNA mismatches are recognized by a multienzyme complex and the repair system does not repair all mismatches with equal efficiency (Brown and Jiricny 1988). The recognition of different base mismatches by the same enzyme complex poses an interesting stereochemical problem. Transition mismatches are apparently better repaired than transversion mismatches and for a given mismatch, repair efficiency increases with increasing GC content in the neighbouring nucleotide sequences (Radman and Wagner 1986). Three distinct mechanisms have been proposed for the repair of mismatches in the DNA in prokaryotic cells (Claverys and Lacks 1986; Radman and Wagner 1986; Modrich 1987, 1989). These are: (i) methyl directed repair, (ii) short patch repair, and (iii) recombinational repair *via* double strand breaks in the DNA. All the three mechanisms require the functions of two mutator genes, *mut*L and *mut*S. These gene functions are involved in steps common to all the mechanisms, *i.e.* the recognition of the mismatch.

2. Methyl-adenine directed mismatch repair

To correct replication errors, the mismatch repair process must distinguish newly synthesized strands from parental strands and act only on the new strand, for otherwise the error will be permanently fixed. Strand discrimination is done in different organisms in different ways. Methylation, a post-replicational phenomenon

plays the key role in strand discrimination in *E. coli.* The newly synthesized strand is transiently undermethylated. Studies using artificially constructed hemimethylated heteroduplexes showed strong bias on repair of the unmethylated strand both *in vivo* (Pukkila *et al* 1983; Kramer *et al* 1984) and *in vitro* (Lu *et al* 1983). The fully methylated parental strand is not the substrate for the mismatch repair enzyme complex (Wagner and Meselson 1976). The methylase activity (MTase) involved in this process is an adenine methylase, coded by the DNA adenine methyltransferase encoding gene, *dam,* which methylates the adenine residue in the sequence 5'-GATC-3' at the N-6 position. Mutation in the *dam* gene increases the spontaneous mutation frequency in *E. coli* (Cox 1970). The role of Dam MTase in strand discrimination in *E. coli* was confirmed from the observation that overproduction of this protein exhibits hypermutability (Herman and Modrich 1981; Marinus 1984). Besides the Dam MTase, the methyl directed repair mechanism requires the function of *mut*S, *mut*L, *mut*H, *mut*U/*uvr*D, single stranded DNA binding protein (SSB), DNA polymerase III holoenzyme, exonuclease I, exonuclease VII or RecJ exonuclease (Copper *et al* 1993) and DNA ligase. Mutations in the *mut* genes have identical mutational spectrum. Mutation in the *dam* gene exhibits 10-fold less mutation than *mut* mutants. *In vitro* studies using purified proteins and NTPs showed that this set of proteins can repair mismatches in the DNA in a strand specific reaction that is directed by the state of methylation of a single-GATC-sequence located even 1 kb away from the mismatched base pair (Lahue *et al* 1989). The methyl directed mismatch repair is initiated by MutHLS dependent cleavage of the unmodified strand at a hemimethylated d(GATC) sequence and the reaction is independent of the polarity of the unmodified strand (Grilley *et al* 1993). If the d(GATC) sequence is located 5'- to the mismatch, mismatch correction requires 5'-3' hydrolytic activity of exonuclease VII or RecJ exonuclease. On the other hand, if the d(GATC) sequence is located 3'- to the mismatch 3'-5' exonuclease I activity is required. Exonuclease I, VII and RecJ are each highly specific for single stranded DNA (Chase and Richardson 1974; Lovett and Kolodner 1989). The excision reaction also requires DNA helicase II encoded by *mut*U *(uvr*D) gene.

3. DNA mismatch repair in Vibrio cholerae

The methyl-adenine directed DNA mismatch repair has recently been examined in *V. cholerae,* a highly pathogenic gram negative bacterium and the causative agent of the diarrhoeal disease cholera, and genes involved in this repair process have been identified. The present report reviews the status of DNA mismatch repair in this organism.

4. Dam methylation

Dam methylation has so far been reported only in enterobacteriaceae such as *E. coli, S. typhimurium* and *S. pneumoniae.* The presence of *dam* in *V. cholerae,* an organism belonging to the family *Vibrionaceae* has recently been reported (Bandyopadhyay *et al* 1989) and mutants sensitive to DNA base analogues such as 2-aminopurine (2-AP) with intact DAM MTase activity and normal spontaneous mutation frequency were isolated. These mutants, are sensitive to UV light and alkylating agents. All the mutant phenotypes can be suppressed by introducing the

E. coli dam gene into the mutant cells. The *dam* gene of *V. cholerae* has been cloned and the complete nucleotide sequence has been determined. *V. cholerae dam* encodes a 21·5 kDa protein and is directly involved in methyl directed DNA mismatch repair (Bandyopadhyay and Das 1994).

The Dam MTase of *V. cholerae* is different from that of *E. coli* in several aspects. The *V. cholerae* protein (21·5kDa) is smaller in size than the *E. coli* protein (31 kDa). In *E. coli,* the Dam MTase activity and sensitivity to base analogues like 2AP are coupled and in general 2AP sensitive cells are *dam* mutants (Claverys and Lacks 1986; Radman and Wagner 1986; Modrich 1989). In contrast, all the 2AP sensitive *V. cholerae* mutants isolated so far have intact Dam MTase activity (Bandyopadhyay *et al* 1989). All attempts to isolate *dam* mutants in *V. cholerae* have so far been unsuccessful. In view of the fact that *dam rec* double mutants in *E. coli* are nonviable, it is possible that wild type *V. cholerae* cells might have a defect in one of the *rec* genes. The *recA* gene of *V. cholerae* has been cloned and characterized (Paul *et al* 1986; Ghosh *et al* 1992). The status of RecBC nuclease in this organism is not clear. No detectable UV induced DNA degradation occurs in these cells and the recombinant processes are in general not efficient. If the failure to isolate *V. cholerae dam* mutants is due to a defect in a *rec* gene, it would be possible to isolate *dam* mutants using a *mut* mutant since *rec mut dam* triple mutants are viable (Schlagman and Hattman 1983). A *mut*S mutant of *V. cholerae* has been isolated (unpublished observation) and will be used to isolate *dam* mutants.

Overproduction of *E. coli* Dam MTase results in hypermutable phenotype (Marinus 1984). V. *cholerae dam* gene expression is high in both *V cholerae* and in *E. coli* cells and the overproduction is neither lethal nor displays hypermutability (Bandyopadhyay and Das 1994). This property of V. *cholerae* Dam MTase is similar to that of phage T4, the overproduction of which does not result in hypermutability. However, the T4 *dam* gene can not suppress the hypermutable phenotype of *E. coli dam* mutants, but does suppress all the other phenotypic traits associated with such mutants (Schlagman *et al* 1986). It is possible that the rate of methylation in *V. cholerae* is slower than that in *E. coli* and hence even in the presence of a large number of Dam MTase molecules, the repair enzyme complex gets sufficient time to repair mismatches in the newly synthesized strand. The *E. coli* dam gene is expressed as a distal gene of an operon (Jonczyk *et al* 1989). The *V. cholerae dam* gene, on the other hand, has the consensus Shine-Dalgarno sequence and two -5''GATC-3'-sequences in tandem at the -35 position (Bandyopadhyay and Das 1994). This might be responsible for the overproduction of the protein.

A unique feature of the *V. cholerae dam* gene is its vector dependent expression. If *V. cholerae dam* gene is cloned in a high copy number vector, the Dam MTase synthesized can not confer 2AP resistance to *E. coli dam* mutants while the Dam MTase activity and the spontaneous mutation frequency are restored.

The homology between the nucleotide and the deduced amino acid sequences of *E. coli* and *V. cholerae dam* genes was only 30–35%. In spite of the lack of nucleotide and amino acid sequence homology *V. cholerae* Dam can substitute for the *E. coli* enzyme and can suppress the phenotypic traits associated with *E. coli dam* mutants. The possible secondary structures of *V. cholerae* and *E. coli* Dam proteins were predicted from the deduced amino acid sequences by calculating the propensities for each amino acid to be in α-helix, β-sheet or ß-(reverse) turn. A

comparison of Novotany plots (Novotany and Auffray 1984) based on Chou and Fasman (1978) algorithm of *V. cholerae* and *E. coli* Dam MTase showed that the propensities of amino acid residues 58–94 and 98–152 of *V. cholerae* protein are almost identical with those of 100-136 and 196-249 residues of *E. coli* protein respectively (figure 1). The probabilities of the conformational state (Gamier *et al* 1978) of the amino acid residues of these two regions of *V. cholerae* and *E. coli* Dam MTase are similar (figure 2) and the primary amino acid sequences of these two domains of the two proteins also have 70 % homology. It is possible that these domains of the Dam MTase might be responsible for the functional complementation. However, structural predictions from primary sequences are highly speculative and the reliability of such predictions is questionable. Determination of the three-dimensional structure of *V. cholerae* Dam MTase is now in progress.

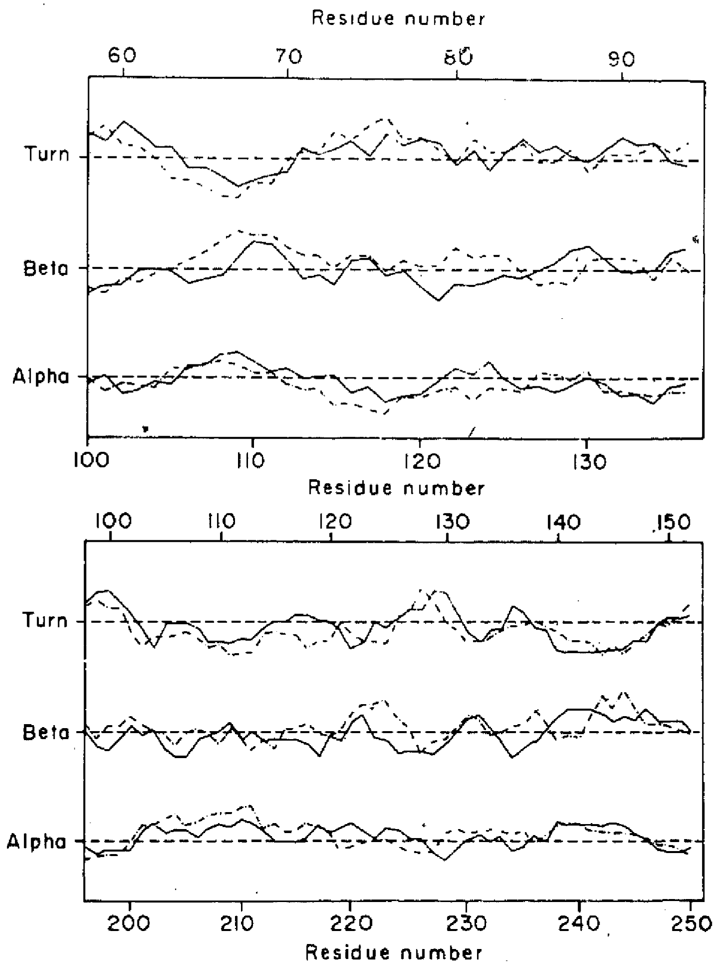

Figure 1. The comparison of NOVOTANY plots of the amino acid residues in two domains of the *dam* gene products of *V cholerae* (solid line) and *E. coli* (dotted line). Probabilities of amino acids to be in α-helix, β-sheet or β-turn were calculated in 4-residue window and plotted against residue numbers. In both panels the upper numbers represent the amino acid residues of *V. cholerae* protein and the lower numbers represent that of *E. coli*.

a

```
V. cholerae :  (58) SADGQNQ-RREWMVQCEN-LSYRP--RSELNASCTQWWPPF (94)
                    --  *=- **--=-   * =-*-   * =*-=-  --  **
E. coli     : (100) KS--QDPFRRAVLFLYLNRYGYNGLCRYNLRGEF-NV--PF (135)

V. cholerae :  (98) W-SQSSDVT-EA-GREIHQ-A-A-TS-HACDEVRLKSAK-SS-S-TLL-RPAPLRLM-VRLALTTLS (152)
                    = =-*  -* *  = -= = * =     *     * * *-  =  = - - * *-* == ** -=== -
E. coli     : (196) YHTNS--FTLEQQA-HLAEIAEGLVERHIP--V-LISNHDTMLTREWYQR-AKLHVVKVRRSISSNG (255)
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b

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V. cholerae :  (58) CTTCCCCTTTEEEEE-TTTTCCTTETTTTTTTTTEETT (94)
                     ****     ***** ****     *    **   ****
E. coli     : (100) TTTCCHEEEEEEEEEETTTTTEEEEEEEEETTEEEEETT (137)

V. cholerae :  (98) TCCCCCCCHHHHHHHHHHHHHHHHHHHHHHHT-CEEEEEEECHHEEEEEHHHHHEEEEEEEEE (159)
                    ******  ******************        ******   **     ************
E. coli     : (196) TCCCCC--HHHHHHHHHHHHHHHHHHHHHHEEEEEEECCCEEE---HHHHHH-HHHHHEEEEEEEEEC (252)
```

Figure 2. **(a)** Comparison of the amino acid sequences of *V, cholerae* and *E. coli* Dam proteins in the domains exhibiting similarities in secondary structures. Identical (*) or similar (=) residues and conservative substitutions (-) are shown. Numbers indicate the positions of the residues in the original sequence. **(b)** A comparison of the maximum probabilities of amino acids to be in different conformational states between the *E. coli* and *V. cholerae* proteins in the specified domains. **C,** random coil; **T,** β-turn; **E,** β-sheet; H, α-helix. The maximum probability was evaluated following the method of Garnier *et al* (1978). Residues with the probabilities of having identical conformations are shown (*). Numbers indicate the positions of the residues in the original sequence.

5. Mutator genes

Using interspecific complementation of *E. coli mut*L and *mut*S mutants with plasmids containing the gene bank of *V. cholerae,* the *mut*L and *mut*S genes of this organism have been identified (Bera *et al* 1989). The *mut*L and *mut*S genes of *V. cholerae* encode 62 and 92 kDa proteins respectively. The complete nucleotide sequence of the *mut*L gene has been determined. Mutations in either *mut*L or *mut*S increased the spontaneous mutation frequency in *V. cholerae.* Recently, the *mut*U gene of *V. cholerae* coding for a 70 kDa DNA helicase II has been cloned and its nucleotide sequence has been determined (unpublished observation). The deduced amino acid sequence of the *mut*U gene exhibits all the consensus helicase motifs.

6. Very short patch repair

In *E. coli* the product of cytosine methyl transferase gene *(dcm)* methylates the inner cytosine residue in the sequence 5'CC(A/T)GG. 5-methyl cytosine is unstable and its deamination results in T/G mismatches which if not repaired correctly prior to replication produce C- > T transition (Coulondre *et al* 1978). An efficient repair system which can counteract the mutagenic effect of 5-methyl cytosine deamination in fully GATC methylated DNA has been demonstrated in *E. coli* (Lieb 1983, 1985; Jones *et al* 1987). This process known as very short patch (VSP) repair depends on the functions of *vsr, dcm, pol*A, *mut*L and *mut*S. VSP repair is fully operative in *mut*K and *mut*U mutants (Lieb 1987; Zell and Fritz 1987). The involvement of the *dcm* gene product in this repair process is not clear (Bhagwat *et al* 1986; Lieb 1987). VSP mismatch repair is greatly reduced in *dcm* mutants

(Bhagwat *et al* 1986). It is possible that *dcm* might have a repair function in addition to methyl transferase activity. The *vsr* gene product has been shown to be an endonuclease which nicks double stranded DNA within the sequence (CT(A/T)GN or NT(A/T)GG next to the underlined thymidine residue which is mismatched to 2-deoxyguanosine (Sohail *et al* 1990; Hennecke *et al* 1991). The incision is strand specific and Vsr induced incision marks the initiation step of VSP repair.

The existence of cytosine methyl transferase in *V. cholerae* has already been reported (Bandyopadhyay *et al* 1989). Recently, the *dcm* and *vsr* genes have been cloned (unpublished observation). Similar to *dcm* gene product of *E. coli,* the *dcm* gene of V. *cholerae* encodes a 53 kDa protein. The Vsr of *V. cholerae* is a 23 kDa protein compared to 18 kDa of *E. coli*.

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