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d(GATC) sequences influence *Escherichia coli* mismatch repair in a distance-dependent manner from positions both upstream and downstream of the mismatch

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ABSTRACT

The role of d(GATC) sites in determining the efficiency of methyl-directed mismatch repair in Escherichia coli was investigated. Transfection of host bacteria, both proficient and deficient in mismatch repair, with a series of artificially constructed M13 heteroduplexes showed that a decrease in the total number of d(GATC) sequences within these vectors lowered the efficiency of repair in vivo. Single hemimethylated d(GATC) sequences were still able to direct the correction event to the unmethylated strand, providing that the mismatch to d(GATC) site distance was shorter than approximately 1 kb. In excess of this distance, the effect of hemimethylated d(GATC) sites on mismatch correction was almost unnoticeable. The directionality of the repair event could be dictated by d(GATC) sequences situated both upstream and downstream of the mispair, suggesting that this important antimutagenic pathway can proceed bidirectionally.

INTRODUCTION

The efficient correction of base-base mismatches, appearing in newly-replicated DNA from polymerase errors, requires that the repair machinery be able to discriminate between the parent and the daughter strands. Wagner and Meselson (1) proposed that in E. coli this strand discrimination may be mediated by DNA methylation. As the newly-synthesized DNA strand is transiently undermethylated, a methylation-sensitive mismatch correction system, acting on a hemimethylated DNA substrate, could direct the repair event to this strand. Support for this hypothesis came from the work of Marinus and Morris (2,3), who showed that E. coli strains deficient in the production of Dam methylase, which modifies the exocyclic amino group of adenine residues within dam-sites, d(GATC), possessed mutator phenotypes. Moreover, strains that were Dam overproducers were also found to be spontaneous mutators (4), suggesting that both unmethylated and fully-methylated DNA

substrates are either erroneously or inefficiently repaired. Second-site mutants of the Dam⁻ phenotype, isolated as 2-aminopurine-resistant clones, were shown to possess mutations in the mutH, mutL and mutS genes, which indicated a close connection between dam methylation and these mutator loci (5,6). Further evidence came from the experiments of Radman et al. (7), Pukkila et al. (8), Wagner et al. (9) and Kramer et al. (10), who demonstrated that mismatch correction in hemimethylated substrates was heavily biased in favour of the methylated strand. Transfection of artificially-constructed mismatch-containing M13 substrates into mutator E. coli strains (10) furthermore demonstrated that the mutS and mutL species were fully mismatch correction-deficient, whereas the mutator phenotype of the mutH strain was shown to be, at least in part, the result of its inability to differentiate between the methylated and the unmethylated strands during the correction of mispairs. Consistent with this latter hypothesis is the fact that efficient mismatch correction in E. coli requires the presence in the DNA of at least one hemimethylated dam-site (11-14). DNA substrates that were either fully-methylated or devoid of dam-sites were found to be refractory to mismatch correction (12,14). What are the possible mechanisms of methylation-directed mismatch correction and what is the precise role of the hemimethylated d(GATC) sequences in this pathway? Based on the findings that the purified E. coli MutS protein binds to duplex DNA containing mismatched nucleotides (15), and that a homogeneous preparation of the MutH protein is able to nick the unmethylated DNA strand at hemimethylated d(GATC) sites (16), Modrich (17) proposed three plausible mechanisms for this repair event. In all of these, the binding of the MutS protein at the mismatch site, and its subsequent interaction with the MutH protein is presumed to result in an endonucleolytic cleavage of the unmethylated strand immediately 5′ from the d(GATC) sequence, and thus in the initiation of a strand displacement or degradation reaction (see also 12), which would remove the errant nucleotide from the mismatch. Although it is at the moment unclear how this protein-protein interaction takes place, available experimental data suggest that the methylation status of the DNA can exert its influence over distances in excess of 1

kb, at least in the absence of replication in vitro (14, 18).

The situation in vivo is a little less clear. The correction of the mismatch in the transfected DNA substrate has to compete with DNA replication, and it is therefore conceivable that the efficiency of mismatch correction in this system falls off with an increase in the mismatch to dam-site distance in a more dramatic fashion than in the cell-free system. Moreover, it remains to be established whether hemimethylated sites can exert their influence on the strandedness of a mismatch repair event from positions both upstream (5') or downstream (3') from the mispair (on the unmethylated strand). Our efforts to address these latter questions are described below.

MATERIALS AND METHODS

All restriction endonucleases used in this study were purchased from Boehringer Mannheim or New England Biolabs, and used according to manufacturer's instructions.

E. coli and phage strains: JM101 (Δlac-pro thi supE (F' traD36 proAB, lacI^{q}Z M15) (19), BMH71-18 wt, mutS, mutH and mutL (source: H.-J. Fritz, see (10)), GM1674 (Δlac-pro tsx78 supE44 galK2 galT22 thi dam3 dcm6 (F' lacI^{q}Z M15 pro) source: M. Marinus), bacteriophage M13mp9 (19).

Oligonucleotide-directed mutagenesis: The oligonucleotides were synthesized on an Applied Biosystems model 380A DNA synthesizer. They were purified and phosphorylated with polynucleotide kinase as described previously (13). Site-directed mutagenesis was carried out as described by Zoller and Smith (21, 22), except that the mismatch repair-deficient strain BMH71-18/mutS was used in the transfection experiments (20). Thus starting from the wt (6-dam) M13mp9, the oligonucleotides (all written in 5'→3'direction) CCGGGGTACCGTCGACCTGCA, GAAGGGCGGTCGGTGC, CAGGAAGGTCGCACTC, TGAGAGGTCTACAAAG, TTGCGGGGTCGTCACC and CGTAACGGTCTAAAGT were used to generate the variant M13 phage 5-dam, 4-dam, 3-dam, 2-dam, 1-dam and 0-dam (figure 2a), respectively. The second series of variants, 1-dam$_1$ → 1-dam$_6$, each containing a single d(GATC) site (figure 2b), were constructed from M13mp9/0-dam, using the oligos AATTCCCGGGGATCCGTCGACCTGCAGCCA, GAAGGGCGATCGGTGC, CAGGAAGATCGCACTC, TGAGAGATCGACAAAG, TTGCGGGATCGTCACC and CGTAACGATCTAAAGT,

respectively. Conversion of the arg$_7$ CGA codon to an opal (TGA) stop codon was achieved with the oligos GATCCGTCAACCTGCA on wt M13mp9 and CCGGGGTACCGTCAACCTGCA on all the other phage variants.

Construction of M13mp9/1-dam$_7$: M13mp9/0-dam and M13mp9/op7/0-dam (each 1 μg, 2 pmole) were digested with HindIII (2 units) and recovered by ethanol precipitation. The HindIII/BamHI adapter AGCTCGGATCCG was kinased (see above), 10 pmole were supended in annealing buffer (10 mM Tris, pH 8; 5 mM $MgCl_2$; 50 mM NaCl; 10 μl total volume), heated to 80°C (5 min.) and allowed to cool to room temperature (20 min.). 0.2 pmole of the duplex solution were added to a mixture containing 0.1 pmole of the HindIII-digested M13 DNAs in the same buffer, as well as 1 mM ATP and 0.1 mM DTT (9 μl total volume). T4-DNA ligase was added (1 μl, 4 units), and the reaction was allowed to proceed at 11°C for 16 hrs. 5 μl of these mixtures were then subjected to a HindIII digest, in order to eliminate those DNA molecules, which were religated without the adapter. An aliquot of this mixture was used to transfect competent JM101 E. coli as previously described (13). Blue plaques of M13mp9/1-dam$_7$ and colourless plaques of M13mp9/op7/1-dam$_7$ were picked and the isolated phage DNA was sequenced by the dideoxy method (23) using the universal M13 sequencing primer GTAAAACGACGGCCAGT. Interestingly, both the 1-dam$_7$ variants suffered a deletion of the 4th lacZ (Thr) codon. The sequence of the modified polylinker of these phages is therefore shown in full in figure 1.

Restriction enzyme analysis of DNAs: All the variant DNAs were grown in both dam$^+$ (JM101) and dam$^-$ (GM1674) E. coli hosts, isolated by the method of Messing (19), and the replicative-form (RF) DNAs were subjected to restriction enzyme analysis. The DNAs of the variants 5-dam → 0-dam, as well as of the wt M13mp9 were digested with Sau3AI and the fragments were separated by electrophoresis on 0.8% agarose gels (figure 2a). Cleavage of the 1-dam$_{1-7}$ variant DNAs with BalI and Sau3AI gave rise in each case to two fragments of various lengths, indicative of the position of the d(GATC) with respect to the BalI site (figure 2b). The results of similar experiments with MboI in place of Sau3AI, where the former enzyme was shown to cleave solely the unmethylated DNAs at d(GATC) sites, confirmed the different state of

methylation of the dam sites in the DNAs isolated from the two hosts (not shown).

Preparation of hemimethylated nicked duplexes: This procedure is outlined in figure 3. The β-gal$^+$ (blue) M13mp9 variant DNAs were grown in GM1674 (dam$^-$ host), and the (RF) DNAs were digested with BalI. These linearized molecules were mixed with five mole-equivalents of the appropriate β-gal$^-$ (colourless) methylated ss DNA, isolated from a dam$^+$ (JM101) host, in a buffer containing 25 mM Tris.HCl pH 8, 375 mM KCl. The mixture was heated in a boiling water bath for 3 min. and then allowed to anneal at 65°C for 10 min. (10). The excess ss DNA was removed from the mixture by filtration through nitrocellulose filters (24), the nicked duplex DNA was recovered by ethanol precipitation and resuspended in TE buffer (10 mM Tris.HCl pH 8, 1 mM EDTA).

Transfection/reinfection experiments: The procedure described by Wood et al. (13) was used without modification.

RESULTS AND DISCUSSION.

Our recent work showed that a G/T mismatch, present at nucleotide position 6619 of the filamentous bacteriophage M13mp9 (i.e. within the coding sequence of the lacZ insert, figure 1) was very efficiently repaired from a hemimethylated heteroduplex construct (13). M13mp9 contains six dam-sites, situated 5, 135, 231, 664, 2361 and 2693 bp downstream from the mispair on the viral (+) strand. In order to define the contributions of the individual dam-sites on the efficiency of correction of this mismatch, we sequentially removed these sites by oligonucleotide-directed mutagenesis. A series of M13mp9 phages were thus obtained that possessed five, four, three, two, one and no d(GATC) sequences (dam-sites), designated 5-dam, 4-dam, 3-dam, 2-dam, 1-dam and 0-dam, respectively. Double-stranded replicative form DNAs were subjected to hydrolysis with the restriction endonuclease Sau3AI, which cleaves immediately 5′ from the d(GATC) sequences. This analysis confirmed that the above phage were indeed depleted of dam-sites (figure 2a).

G/T-containing hemimethylated duplexes were constructed such that phage DNA (M13 (+) strand), grown in a dam$^+$ host (JM101), was annealed to the appropriate (-) strand obtained by denaturing

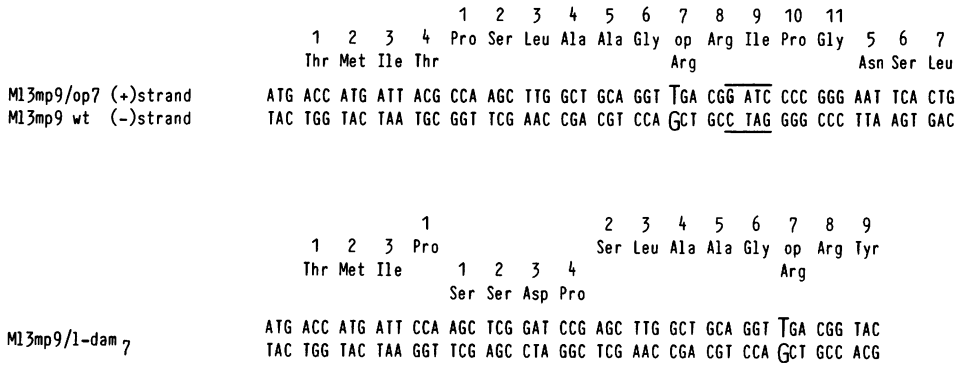

Fig. 1: The sequence of the polylinker region of the lacZ insert of M13mp9 and its opal variant M13mp9/op7. The single G/T mispair is shown in large type. The sequence of the corresponding region of M13mp9/1-dam7, containing the HindIII/BamHI adapter, is also shown. Note that the BamHI site of the wt M13mp9 (underlined) has in all variants been converted to a GGTACC KpnI site.

linearized RF (ds) DNA grown in a dam⁻ host (GM1674) (see Materials and methods). In all these constructs the T-containing (+) strand coded for the β-gal⁻ phenotype (colourless), while the G-containing (-) strand encoded the β-gal⁺ phenotype. In addition, the (-) strand in all the constructs was nicked at the BalI site at position 5081, i.e. 1.5kb to the 3'-side of the guanine residue of the G/T mismatch (see figure 3).

The nicked duplex DNAs were transfected into mismatch repair -proficient (BMH71-18) and -deficient (BMH71-18/mutS) E. coli hosts. As described earlier (13), correction of the G/T mismatch to a G/C (i.e. in favour of the unmethylated (-) strand) gives rise to plaques with a blue (β-galactosidase⁺) phenotype, while repair favouring the methylated (+) strand generates an opal stop codon in the lacZ reading frame to give rise to colourless (β-galactosidase⁻) plaques (figure 1). Unrepaired mismatches give rise to mottled plaques containing both types of phage, which increase the yield of the dominant (blue) marker in the transfection assay. The contribution of mottled plaques to the observed marker yield of the transfection experiments can be estimated from a subsequent reinfection assay (10, see also Materials and methods), which uses progeny phage for host infection, giving rise to pure phage bursts only. Thus poorly repaired mispairs

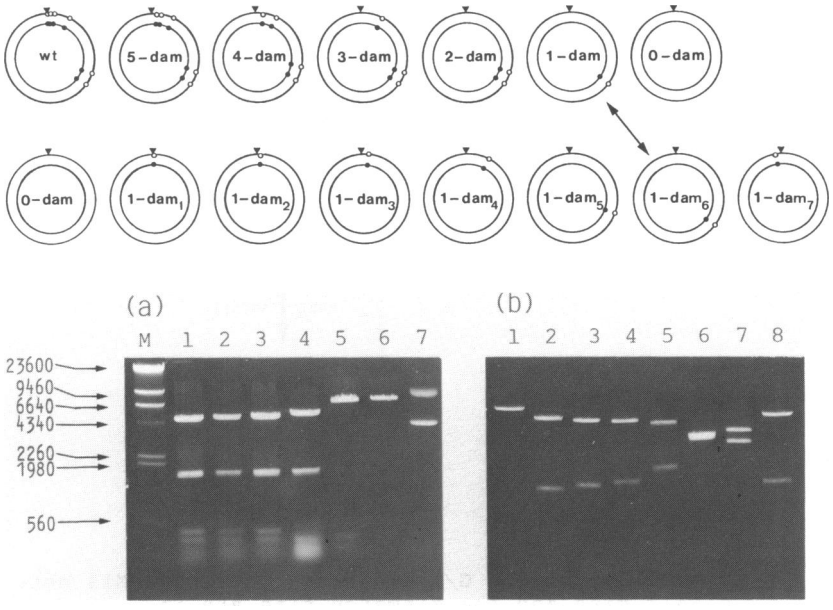

Fig. 2: The structures and the restriction enzyme analysis of the M13mp9 variants. The mismatch position (▲), as well as the positions of the methylated (●) and unmethylated (○) dam-sites are indicated.
(a) Hydrolysis of the wt and dam-variant M13mp9 DNAs with Sau3AI yielded the following fragments (lengths in base-pairs): 4911, 1696, 434, 332, 132 and 94 (wt mp9, lane 1); 5043, 1696, 434, 332 and 94 (5-dam, lane 2); 5137, 1696, 434 and 332 (4-dam, lane 3); 5571, 1696 and 332 (3-dam, lane 4); 7267 and 332 (2-dam, lane 5); 7599 (1-dam$_6$, lane 6). The variant 0-dam was not cleaved by this enzyme (lane 7). The lengths of the marker fragments (lane M, lambda HindIII digest) are shown on the left.
(b) The combined BalI/Sau3AI hydrolysis of the M13mp9 variants containing single dam-sites produced the following fragments (in base-pairs): 7599 (0-dam, lane 1); 6056, 1543 (1-dam$_1$, lane 2); 5926, 1673 (1-dam$_2$, lane 3); 5830, 1769 (1-dam$_3$, lane 4); 5396, 2203 (1-dam$_4$, lane 5); 3899, 3700 (1-dam$_5$, lane 6); 4231, 3368 (1-dam$_6$, lane 7) and 6101, 1507 (1-dam$_7$, lane 8).

show large differences between the yields of the (-) strand marker in the two assays, while efficient repair produces similar amounts of (-) strand marker phenotype in both assays.

The results of the transfections of the mismatch repair-proficient strain BMH71-18 and its isogenic mismatch repair-deficient mutant BMH71-18/mutS are represented in figure 4. The influence of the d(GATC) sequences on mismatch correction in the

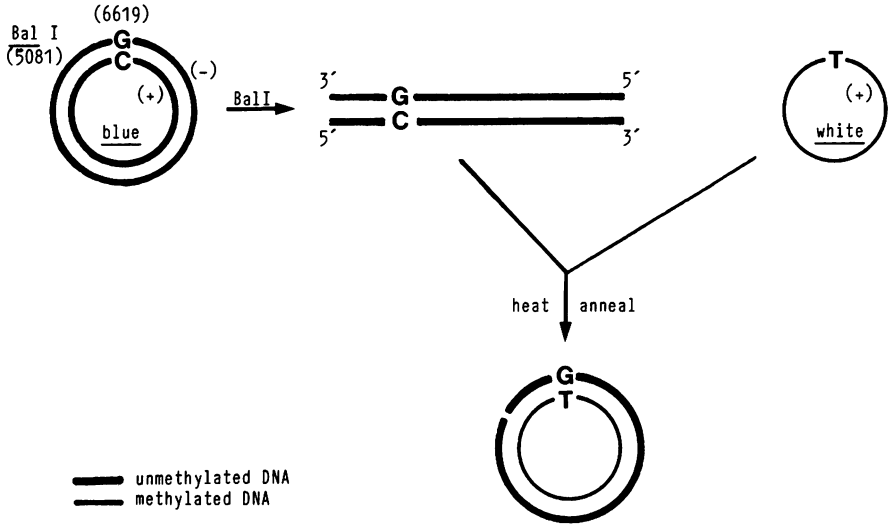

Fig. 3: Construction of the G/T mismatch containing M13 heteroduplex. The BalI site and the mismatch site are indicated, the numbers corresponding to their respective nucleotide positions. The signs (+) and (-) designate the viral and the complementary strands, respectively. The words blue and white refer to the β-galactosidase$^+$ and β-galactosidase$^-$ phenotypes, respectively.

repair-proficient host can be clearly seen to fall off with the increase in the mismatch to dam-site distances and with the accompanying decrease in the total number of dam-sites . The results of the transfection (figure 4, filled bars) and reinfection experiments (not shown) were similar, indicating that all the M13mp9 constructs were efficiently repaired, and that the observed increase in the yield of the minus strand marker was thus the result of decreasing strand-directionality of the correction process. By contrast, the (-) strand marker yields obtained from the transfection experiments with the mutS strain, which is fully deficient in mismatch correction, remained around 90% for all the variants (figure 4, plain bars). Furthermore, the reinfection experiments (data not shown) gave rise to (-) strand marker yields of 65-70%, suggesting that a large proportion of plaques arose from unrepaired mismatched duplexes. Due to the non-symmetrical mode of M13 replication, which slightly favours the (-) strand (25), the latter marker yields correspond

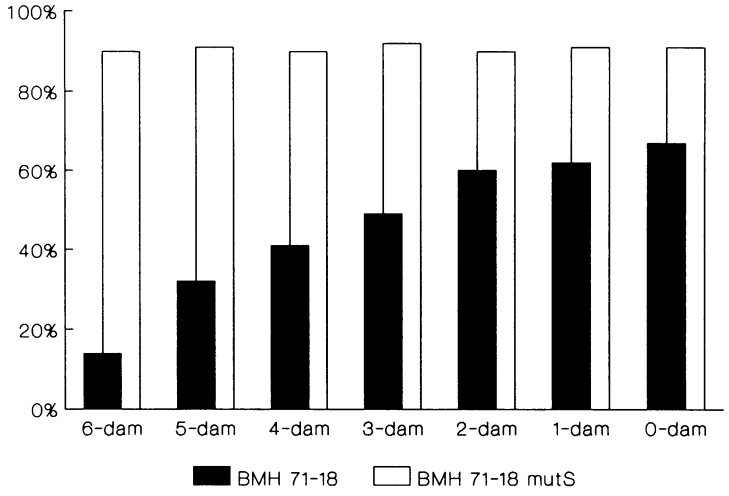

Fig. 4: (-) Strand marker yields of transfections of BMH 71-18 and BMH 71-18 mutS with dam-site depleted M13mp9 heteroduplexes. The values represent the average yields obtained from three separate experiments. In each experiment, more than 500 plaques were counted.

to the expected yield of progeny phage arising from the replication of an unrepaired mismatch-containing M13 DNA heteroduplex (see also ref. 10 for discussion).

The above data demonstrate quite unambiguously that dam-sites play an important role in determining the efficiency and the strandedness of the mismatch correction event in vivo. Nonetheless, the observed effects could have been caused either by the increase in the mismatch to dam-site distance, by the concomitant decrease in the number of these sites present in the substrate molecule, or by a combination of both these phenomena. The results of similar experiments carried out in vitro (14) showed that the kinetics of the repair process were quite clearly dependent on the number of d(GATC) sequences. We attempted to determine the relative contribution of the distance factor to the overall efficiency of mismatch correction in vivo by constructing a second series of M13mp9 variants, where we reintroduced one of the original d(GATC) sequences into the dam-site-depleted variant 0-dam by oligonucleotide-directed mutagenesis. Restriction enzyme analysis of these variants with Sau3AI and BalI confirmed that

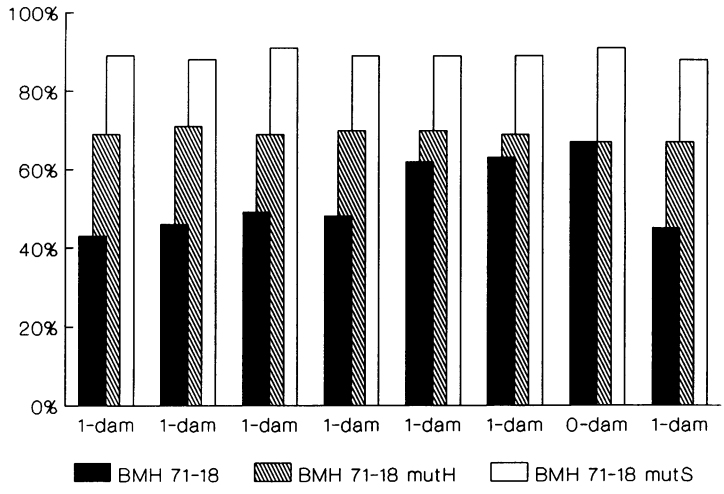

Fig. 5: (-) Strand marker yields of transfections of BMH 71-18, BMH 71-18 mutH and BMH 71-18 mutS with M13mp9 heteroduplexes containing single d(GATC) sites. The average values of three separate experiments are shown, each deriving from more than 500 plaques.

these molecules contained single dam-sites (figure 2b).

We could thus assemble a set of six mismatch-containing heteroduplexes, 1-dam$_{1-6}$, each of which carried a single hemimethylated d(GATC) sequence at a distance of 5, 135, 231, 664, 2361 and 2693 bp downstream (3') from the mismatch. In addition, we inserted a BamHI/HindIII adapter $^{5'}$AGCTCGGATCCG$^{3'}$ into the HindIII site of M13mp9/0-dam so as to generate a new variant, 1-dam$_7$, with a single dam-site 21bp upstream of the G/T mispair (see Materials and methods).

The results of the transfection experiments are represented in figure 5. A comparison of the data in figures 4 and 5 enabled us to determine the extent of the contribution of dam-site number to the overall repair efficiency in vivo. For example, the (-) strand marker yields obtained from the transfections of BMH71-18 with the heteroduplexes wt and 1-dam$_1$ differ by 29% (figure 4, column 1 cf figure 5, column 1). As the nearest dam-site in both these constructs lies merely 5bp downstream from the mismatch, the difference in the (-) strand marker yield represents the contribution of the five additional dam-sites in the former sub-

strate to the overall efficiency of repair. This contribution can be seen to progressively diminish, until it becomes hardly noticeable in the case of the 1-dam$_5$ and 1-dam$_6$ constructs (figure 5, columns 5 & 6, cf figure 4, columns 5 & 6). In the latter M13mp9 variant series, the effect of the increasing mismatch to dam-site distance could be subdivided into three groups: (i) all the sites situated within 1kb of the mismatch were able to exert a similar influence on the efficiency of the repair event; (ii) the sites lying in excess of 2 kb downstream from the G/T mismpair were found to be less effective in directing the strandedness of mismatch correction, while (iii) the removal of the last remaining dam-site gave rise to (-) strand marker yields consistent with the repair taking place in the absence of strand discrimination. This latter finding agrees with the results of transfections of the BMH71-18 mutH strain, which is unable to differentiate between dam-methylated and unmethylated DNA. These experiments gave rise to (-) strand marker yields that remained nearly constant for all the heteroduplex constructs (figure 5, hatched columns). As the reinfection experiments produced similar results (not shown), indicating a low incidence of mottled plaques, we were able to confirm the findings of Kramer et al. (10), which showed this mutH mutant to be relatively proficient in mismatch repair, its mutator phenotype being most likely the result of its inability to discriminate between the methylated and the unmethylated strands during mismatch correction. It should be said, however, that cell-free extracts of the mutH mutant KMBL3773 exhibited no mismatch repair activity in an in vitro assay (14), suggesting that our findings may be limited to only a subset of mutH mutants. By contrast, transfections of the isogenic mutS strain with the heteroduplex constructs resulted in all cases in ~90% (-) strand marker yields (figure 5, plain columns), a large proportion of the plaques being mixed bursts as shown by the results of the reinfection experiments (not shown). Such an effect is consistent with the lack of mismatch correction in the mutS strain.

The two sets of results described above demonstrate that the efficiency of mismatch correction in E. coli is dependent not only on the number of dam-sites in the substrate, but also on the

distance between the mismatch and the nearest dam-site. Thus, although the repair tracts in vivo (as well as in vitro) were found to be up to 3 kb long (1), our results demonstrate that the influence of strand methylation decreases significantly as the mismatch to dam-site distance exceeds 1 kb.

The question that still remained to be answered concerned the directionality of the repair event. In all the constructs in our first series (6-dam to 1-dam_6) the dam-sites were situated within 3 kb downstream of the G/T mispair, i.e. in excess of 5 kb in the opposite direction on the 7.6 kb circular substrates. These constructs do not enable us to establish whether the repair machinery can also take account of d(GATC) sequences lying upstream of the mismatch. We attempted to address this question by constructing the variant 1-dam_7, which contains a new dam-site 21bp upstream from the G/T mispair (figure 1). As shown in figure 5 (column 7), the presence of this site in the heteroduplex construct gave rise to yields of the (-) strand marker at levels similar to those observed for the 1-dam_{1-4} variants. Furthermore, control experiments showed that this repair was also dependent on functional mutH,L,S genes (data not shown), indicating that the repair proceeded via the same pathway as in the case of the constructs containing dam-sites downstream of the mismatch. Our results thus demonstrate that the directionality of a mismatch correction event, facilitated by the E. coli methylation-directed mismatch repair system, can be influenced by d(GATC) sequences lying both upstream and downstream from the mispair.

A number of possible mechanisms of E. coli methylation-directed mismatch repair have been proposed (12, 17). The initial event, common to all these hypotheses, involves the recognition and the binding of the mismatch by a protein complex. In one suggested pathway, this complex is presumed to consist of the 97 kd product of the mutS gene, required for mismatch recognition, as well as of the other constituents of the repair machinery, the proteins coded by the mutL, mutH and uvrD genes. The next stage would involve the "reeling-in" of the DNA from one or both sides of the bound complex, until a hemimethylated d(GATC) sequence was reached. The unmethylated strand would be nicked at this site by the mutH protein, and the DNA would then be "reeled-out". Had the

DNA been nicked 5′ from the mismatch, the "reeling-out" would be accompanied by nick-translation, which would proceed through and past the mismatch to generate a long repair tract. The nicking of an unmethylated d(GATC) sequence 3′ from the bound complex could initiate an exonucleolytic reaction, which would hydrolyze (or simply displace) the unmethylated strand in a 3′→5′ direction with the help of DNA helicase, the product of the uvrD gene, and a second protein, perhaps a DNA polymerase, possessing a 3′→5′ exonuclease activity. The single-stranded region thus generated would be stabilized by the single-strand binding protein Ssb until the gap was filled-in by the appropriate polymerase. The efficiency of this pathway would to a great extent be determined by the distance between the mismatch and the nearest hemimethylated d(GATC) sequence. However, our results (figures 4 & 5) and those of others (14, 26) indicate that the efficiency of repair rises with an increase in the number of d(GATC) sites within the DNA substrates.

An alternative pathway, which might account for this effect, could involve two distinct DNA binding events. The binding of a protein complex, consisting of the MutS and (probably) MutL proteins, at the mismatch site would be accompanied by the binding of a second complex at any hemimethylated d(GATC) sequence. This latter complex could consist of the MutH and UvrD proteins. The interaction of the two complexes, either following the above-described "reeling-in" of the DNA separating them, or by looping of the DNA, would initiate a cascade of events similar to those described above. DNA looping, recently shown to take place during the interaction of trans-acting transcription activation factors (27, 28) is energetically undemanding and could facilitate the rapid interaction of the two protein complexes over large distances. Moreover, this model does not require that all the dam-sites be occupied. The presence of multiple dam-sites in the DNA substrate would increase the probability that at least one of these sequences will be associated with the MutH protein so as to mediate its interaction with the mismatch-bound complex and thus initiate the repair event. As to which of these two possible mechanisms represents the true mismatch correction pathway will only become clear from

the results of *in vitro* mismatch repair experiments using a system reconstituted from the purified constituent protiens of the mismatch repair machinery of *E. coli*.

Our data, using M13 heteroduplexes containing single *dam*-sites 5 to 2693 bp 3' from the G/T mismatch (figure 5), suggest that their influence on the directionality of mismatch repair remains constant for the constructs where the distance between the mispair and the hemimethylated d(GATC) sequence is less than 1 kb. Indeed, it is still observable, albeit weakly, when the distance increases to more than 2.6 kb.

As the correction of the mismatch in the transfected heteroduplex is in direct competition with DNA replication, we considered the possibility that the apparent inefficiency of repair of the substrates 1-dam_5 and 1-dam_6 was the result of marker separation in the cases where replication preceeded repair. This would be expected to give rise to a large amount of mixed plaques in the transfection experiments. As this was not the case, we concluded that an increase in the distance between the mispair and the *dam*-site led mainly to a loss of directionality of the process. Support for this hypothesis came from the transfection/reinfection experiments of BMH 71-18 *mutH*. In this strain, the transfected DNA can presumably not be nicked at the hemimethylated d(GATC) sequence, yet the single G/T mismatch within it appeared to be efficiently corrected. These findings would suggest that the repair machinery used other, specific or non-specific, strand breaks in the heteroduplex DNA constructs as initiation points for the correction event, a conclusion reached by other groups (12, 29). It should be said, however, that the nick-directed repair pathway may be distinct from the methylation-dependent *mutHLS* correction mechanism (14). Our model of the repair event allows for initiation of the methylation-directed correction process taking place both 5' and 3' from the mismatch (on the unmethylated strand). This proposal was substantiated by the results of transfection assays involving the heteroduplexes 1-dam_1 and 1-dam_7. Although the single d(GATC) sequence in these heteroduplex constructs was situated 5bp downstream from the G/T mismatch in the former and 21bp upstream in the latter, the (-) strand marker yields following transfection of the mismatch

repair-proficient host were similar (figure 5, columns 1 and 7, respectively). This finding agrees with the recently reported results of *in vitro* mismatch correction experiments (26) and suggests that the methylation-directed mismatch correction pathway of *E. coli* has no preferred polarity.

Transfection experiments, using artificially-constructed mismatch-containing hemimethylated heteroduplexes, have provided us with a number of invaluable insights into mismatch correction. However, the elucidation of its precise mechanism must await the isolation and characterization of intermediates arising during the course of this important antimutagenic pathway.

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