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Genetics

Repair of DNA heteroduplexes containing small heterologous sequences in *Escherichia coli*

(*mnt* gene/MutS/DNA mismatch repair/deletion mutations)

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ABSTRACT Plasmid heteroduplexes were constructed that contain 1, 2, 3, 4, or 5 unpaired bases within the *mnt* gene. These were used to assess the efficiency of repair of small heterologous sequences ("heterologies") in DNA by the *Escherichia coli* Dam-directed mismatch repair system. Heteroduplexes in defined states of methylation at d(GATC) sites were used to transform a repair-proficient indicator strain (which has a *mnt–lac* fusion coding for a nonfunctional *mnt* repressor) and its isogenic *mutH*, *-L*, and *-S* derivatives. Using this *in vivo* transformation system, we scored for repair on the basis of colony color: correction in favor of the strand bearing mnt^+ coding information gives rise to colonies that are white, whereas correction on the opposite strand (mnt^-) yields colonies that are red when grown on MacConkey agar. Failure to repair a heterology yields colonies that are both red and white ("mixed"). The correction efficiencies of two heteroduplexes, each containing a single G·T mismatch within *mnt*, were also monitored for purposes of comparison. Our results show that *mutHLS*-dependent, methyl-directed repair of heteroduplexes with 1-, 2-, and 3-base deletions is as highly efficient as the repair of G·T mismatches. Heteroduplexes with a 4-base deletion are marginally repaired and DNA with a 5-base deletion is not detectably repaired. In addition, we show that purified MutS protein from *Salmonella typhimurium*, which can substitute for *E. coli* MutS *in vivo*, binds to oligonucleotide duplexes containing 1, 2, 3, and 4 unpaired bases of a sequence identical with that used for the *in vivo* studies. Specific binding of MutS to homoduplex DNA and to DNA that had undergone a 5-base deletion was not observed.

The mismatch repair systems of *Escherichia coli* are involved in the correction of DNA containing mismatched bases (for reviews, see refs. 1–3). Mismatched bases can be generated through a number of processes: spontaneous deamination of 5-methylcytosine to thymine giving rise to G·T mispairs, allelic differences in recombination intermediates, and biosynthetic errors that arise during DNA replication. Dam-dependent (methyl-directed) mismatch repair corrects most base·base mismatches in a process characterized by long excision-repair tracts up to and in excess of one kilobase in length (4, 5). This system relies on the methylation of adenine in d(GATC) sequences to direct the strandedness of repair; this is accomplished by DNA adenine methyltransferase (Dam). In hemimethylated heteroduplexes, repair is directed to the unmethylated strand by the ability of the MutH protein to incise 5′ to the d(GATC) sequence on the unmethylated strand (4, 6). Among the other proteins involved in the initiation of mismatch repair are MutS, which recognizes mismatched bases in DNA (7, 8), and MutL, which may act to interface the activity of MutS with that of MutH (4, 9). Mismatch repair has been performed *in vitro* by a purified system (10).

Although the correction of base·base mismatches in DNA by Dam-directed mismatch repair is well documented (1–3), little is known about the specificity of repair of small sequences of heterologous DNA ("heterologies"). The present study examines the correction of insertion/deletions of 1 to 5 bases to determine the size constraints of the repair pathway both *in vivo* and *in vitro*.

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MATERIALS AND METHODS

Plasmid, Phage, and Bacterial Strains. Plasmid pPY97 and the numbering of bases in the *mnt* gene and *mnt* operator have been described (11). The fl phage R408 was a gift from M. Russel (12); λGM110 contains a mnt^-::*lacZ* operon fusion and will be described in detail elsewhere. *E. coli* strains GM4331 [F^- *thr-1* Δ(*lac–pro*)$_{XIII}$ *tsx-33 supE44 galK2 hisG4*(Oc) *rfb-1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1* (λGM110)], GM4348 [GM4331 but *mutL*::Km *Stu* (13)], GM4349 [GM4331 but *mutH471*::Tn*5*], GM4388 [GM4331 but *mutS201*::Tn*5* (14)], and GM1690 [F42/*dam-16*(Del; Km^R) *thr-1 ara-14 leuB6* Δ(*gpt–proA*)62 *lacY1 tsx-33 supE44 galK2 hisG4*(Oc) *rfb-1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*] were constructed in this laboratory. The genotype of JC9239 is described elsewhere (15).

Preparation of Heteroduplexes and Transformation Procedure. pPY97 derivatives with deletions of 1, 2, 3, 4, or 5 base pairs within *mnt* were prepared by site-directed mutagenesis using T7 DNA polymerase (16). Methylated plasmid DNA was isolated from JC9239, while that devoid of methylation was isolated from GM1690 by using Qiagen maxi-columns as recommended by the manufacturer (Qiagen; Chatsworth, CA). Single-stranded plasmid DNA devoid of methylation at d(GATC) sites was extracted and purified from phage R408 virions by using plasmid-containing GM1690 as host (17).

The construction of covalently closed supercoiled heteroduplexes containing 1, 2, 3, 4, or 5 unpaired bases within *mnt* and the G·T mismatches at positions 40 and 41 in *mnt* were prepared by the method of Lu *et al.* (18). We used hemimethylated heteroduplexes involving two different constructs; one in which unmethylated circular single-strand wild-type pPY97 was annealed with its complementary methylated linear duplex DNA that had undergone deletions of 1–5 base pairs or G·C to A·T transitions, and the other in which unmethylated circular single strand with deletions of 1–5 bases was annealed with its complementary methylated linear pPY97 DNA. The hemimethylated heteroduplexes were tested for resistance to *Mbo* I and *Sau*3A digestion.

Fully unmethylated heteroduplexes were formed by annealing unmethylated duplex linear DNA that had undergone deletions with unmethylated circular single-strand pPY97 DNA. To prepare fully methylated heteroduplexes, hemimethylated heteroduplexes, constructed by annealing methylated mutant DNA with unmethylated single-strand pPY97, was methylated *in vitro* by using Dam methylase (New

England Biolabs) in the presence of *S*-adenosylmethionine. Digestion by *Sau*3A and *Dpn* I indicated that more than 95% of the d(GATC) sequences were fully methylated as determined by gel electrophoresis. Competent cells of strains GM4331, GM4348, GM4349, and GM4388 were prepared freshly when needed as described by Carraway *et al.* (19). Approximately 1 ng of heteroduplex was used for each transformation by the $CaCl_2$ procedure. The transformants were spread onto MacConkey agar plates containing 60 μg of ampicillin per ml and placed at 37°C for 18 hr. Colonies were scored for repair on the basis of their color.

MutS Band-Shift Assay. The [γ-^{32}P]ATP (Amersham) end labeling of the "bottom" strand (see below) by T4 polynucleotide kinase (New England Biolabs), annealing of the oligonucleotides (Operon Technologies, Alameda, CA), MutS binding reactions, and band-shift assays were carried out essentially as outlined by Jiricny *et al.* (20). Purified *Salmonella typhimurium* MutS protein (21) was a generous gift from Graham C. Walker (Massachusetts Institute of Technology). The 5-μl MutS binding reaction mixture consisted of 0.6 pmol of 36-base heteroduplex DNA and 2, 4, 6, 8, 10, or 12 pmol of MutS and was incubated at 0°C for 30 min. The 36-base oligonucleotides used to make the duplexes were as follows: 5′-GCATACGGAAGTTAAAGTGCGGATCATCTCTAGCCA-3′ was the bottom strand and represents the sequence complementary to the "top" strand, 5′-TGGCTAGAGATGATC**CGCACT**TTAACTTCCGTATGC-3′. The bold sequence in the top strand corresponds to that given in Fig. 1, which shows the base deletions used to create the 1-, 2-, 3-, 4-, and 5-base deletion duplexes. The G·T mismatch at position 40 was constructed by annealing 5′-TGGCTAGAGATGATCCGTACTTTAACTTCCGTATGC-3′ with the bottom strand.

RESULTS

The *in Vivo* Repair Assay Can Determine the Repair Efficiency of Small DNA Heterologies. The plasmid pPY97 is a pBR322 derivative with an M13 origin of replication so that single-strand DNA can be prepared (11). It confers ampicillin resistance and contains an *mnt–tet* operon fusion that places *tet* expression under the control of Mnt. Site-directed mutagenesis was used to create 1-, 2-, 3-, 4-, and 5-base deletions in and around the His-6 codon within the *mnt* structural gene (Fig. 1); this histidine residue is critical for proper binding to O_{mnt} (22). The *mnt* gene was sequenced for each mutant to confirm both that the correct mutant *mnt* sequence had been isolated and that no other mutations were present.

A system has been developed to detect accurately the correction of DNA containing insertion/deletion heterologies as well as base·base mismatches (Fig. 1). Correction of plasmid DNA heteroduplexes is scored on the basis of color changes in colonies after an indicator strain has been transformed with heteroduplex and incubated on MacConkey/ampicillin agar. The indicator strain GM4331, and its isogenic *mutH*, *-L*, and *-S* derivatives, is a λ lysogen containing *lacZYA* and the *mnt* gene and its operator from bacteriophage P22. The promoter for *lacZYA* is negatively controlled by Mnt repressor; however, in GM4331 the *mnt* gene codes for a nonfunctional repressor, allowing *lacZYA* to be constitutively expressed. GM4331 forms red colonies on MacConkey agar as it ferments the lactose in the medium. Transformation of the indicator strain with a plasmid coding for a nonfunctional Mnt repressor also results in *lacZYA* expression, thus forming red colonies, and in addition, it confers plasmid-encoded tetracycline resistance (Fig. 1). Conversely, a plasmid containing a functional Mnt repressor, which binds to the *mnt* operator (O_{mnt}) on both the chromosome and the plasmid, effectively blocks expression of *lacZYA* and tetracycline resistance, and thus colonies are white (Fig. 1).

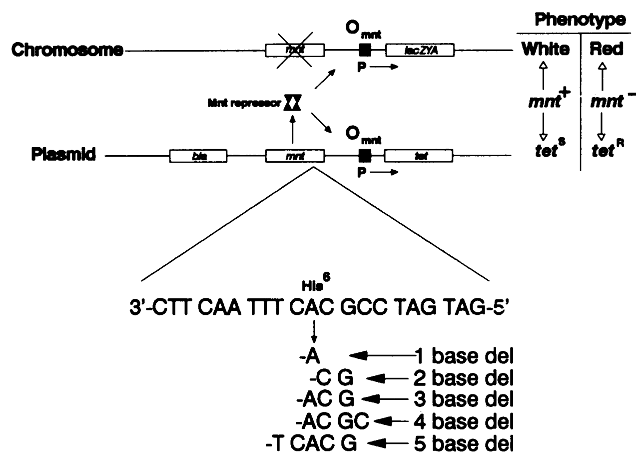

FIG. 1. The indicator system used to evaluate efficiency of repair is based on two major components, plasmid-encoded Mnt and chromosomal *mnt–lacZYA* fusion operon as described in the text. The plasmid-encoded *bla* gene confers resistance to ampicillin. The DNA sequence of *mnt* shows the base(s) that were deleted in the strand complementary to single-strand pPY97 as well as the surrounding sequence. These mutations were constructed so that the His-6 residue within *mnt* would be eliminated, thus producing a nonfunctional Mnt repressor. The sequence is oriented 3′ to 5′ to indicate the direction of transcription of *mnt*. *P* represents the promoter region and O_{mnt} the operator, and the arrow indicates the direction of transcription for *tet* and *lacZYA* genes.

When a heteroduplex of a defined methylation state consisting of a strand containing the wild-type *mnt* sequence and its complementary strand containing an *mnt* sequence with a deletion mutation is introduced into an indicator strain, we observe three possible outcomes: transformants giving rise to red, white, or red plus white ("mixed") colonies. Correction of a small DNA heterology is indicated by the presence of either white or red colonies after transformation of the indicator strain with hemimethylated heteroduplexes in which the methylated strand d(GATC) is wild type or mutant, respectively. No repair is indicated by the presence of mixed colonies, since replication of the heteroduplex that is refractory to repair should yield colonies that harbor both wild-type and mutant forms of pPY97.

To determine the reliability of this method, we transformed the indicator strains with heteroduplex DNA and spread the transformants onto MacConkey/ampicillin agar. The purity of the transformants was evaluated by isolating plasmid DNA from red, white, and mixed colonies and retransforming the indicator strain GM4331. The color of the retransformed colonies reflects the plasmid purity. Plasmid DNA isolated from 20 red colonies gave rise to only red colonies after retransformation, indicating that these transformants contained mutant pPY97. Plasmid DNA isolated from mixed colonies gave rise to either red or white colonies after retransformation, indicating that replication of a heteroduplex proceeded in the absence of repair. All mixed colonies grew well on plates containing tetracycline at 10 μg/ml. Plasmid DNA isolated from most white colonies also gave rise to white colonies after retransformation; however, DNA from some white colonies gave rise to both white and red colonies, indicative of a mixed colony. Therefore, since true white colonies are sensitive to tetracycline and mixed colonies are resistant, we determined the concentration of tetracycline required to separate true white colonies from "false white" or mixed colonies. True white colonies will not grow on plates that contain tetracycline at 3 μg/ml, and retransformation using plasmid DNA isolated from these colonies gives rise to white colonies only. Conversely, false white colonies are resistant to tetracycline and since they give both

Table 1. Genetic analysis of hemimethylated heteroduplex transformations

Heteroduplex		Wild type			*mutL*::Km *Stu*			*mutH471*::Tn*5*			*mutS201*::Tn*5*		
Mutant/WT methylation	Type	% R	% W	% M	% R	% W	% M	% R	% W	% M	% R	% W	% M
+/−	G·T at 40	98	1	1	4	4	92	25	22	53	4	5	91
+/−	T·G at 41	98	1	1	6	9	85	26	28	46	6	8	86
+/−	1 Δ	99	0	1	4	4	92	25	21	54	4	4	92
+/−	2 Δ	98	1	1	2	8	90	21	19	60	3	3	94
+/−	3 Δ	97	1	2	6	5	89	15	14	71	7	5	88
+/−	4 Δ	39	4	57	2	4	94	5	6	89	6	5	89
+/−	5 Δ	7	3	90	5	3	92	4	7	89	5	5	90
−/+	1 Δ	3	95	2	5	3	92	37	25	38	4	4	92
−/+	2 Δ	5	93	2	4	6	90	33	19	48	6	7	87
−/+	3 Δ	6	90	4	3	4	93	21	11	68	6	3	91
−/+	4 Δ	3	18	79	6	6	88	7	5	88	6	7	87
−/+	5 Δ	5	7	88	8	3	89	5	4	91	5	4	91

The percentages of red (% R), white (% W), and mixed (% M) colonies in each of the four recipient indicator strains were calculated from the total population of transformants, which ranged from 400 to greater than 1300 total colonies per transformation. The state of adenine methylation is indicated in the first column: +/−, methylated strand with a deletion annealed with unmethylated circular wild-type (WT) single strand of pPY97; −/+, unmethylated circular single strand with a deletion annealed with methylated wild-type pPY97 strand. Positions of mismatches or number of bases deleted (1 Δ, etc.) are indicated in the second column.

red and white colonies on retransformation, they are scored as mixed colonies.

Transformations of GM4331 (wild type) and the *mnt*⁻ strains with homoduplex (pPY97), the various heteroduplexes (and states of methylation), and a 1:10 mixture of homoduplex (pPY97) and heteroduplex revealed no significant loss of heteroduplex viability that might occur as a result of lethal processing of deletion mutations.

***In Vivo* Correction of Small DNA Heterologies by Mismatch Repair Is Dam-Directed.** The mismatch repair-proficient indicator strain, GM4331, and its isogenic *mutH*, *-L*, and *-S* strains were transformed with hemimethylated heteroduplexes, in which the mutant strand contains methylated d(GATC) sequences, containing 1-, 2-, 3-, 4-, or 5-base heterologies. A G·T mismatch at position 40 and a T·G mismatch at position 41 of *mnt* were used as positive controls. We chose to compare the efficiency of correction of DNA containing small heterologies with that of the G·T mismatches since the Dam-directed repair system has been shown to correct them with high efficiency. In addition, the G·T mismatches do not lie within sequences that favor very short patch (VSP) repair (23). Table 1 shows that 1-, 2-, and 3-base deletion heteroduplexes are repaired by wild-type mismatch repair-proficient cells in a *mutHLS*-dependent, methyl-directed process with an efficiency similar to that of the G·T mismatches, since 97–99% of the wild-type transformants give rise to red colonies. The 4-base heterology is marginally corrected, since 39% of the transformants are red colonies and only 4% are white colonies, whereas 5-base heterologies are refractory to repair, since 90% of the transformants give rise to mixed colonies. Interestingly, our system detected a low level of methyl-independent correction after transformation of the *mutH* (GM4349) indicator strain. This will be addressed in more detail below.

To demonstrate the dependence of this system on methylated d(GATC) sequences to direct the strandedness of repair, we switched the methylation state so that the wild-type strand (white) is methylated rather than the mutant strand (red). In this case, 90–95% of the wild-type transformations with hemimethylated heteroduplexes containing 1-, 2-, and 3-base heterologies gave rise to white colonies, indicative of repair using the methylated strand as template (Table 1). The results also show that the 4-base deletion is poorly corrected, while the 5-base deletion is not corrected.

We isolated plasmid DNA from 20 red colonies from the wild-type transformations in Table 1 and sequenced the *mnt* gene to confirm that the red colonies were the result of directed repair of the deletion mutation present in the heteroduplex. This was repeated for 16 red colonies taken from *mutH* transformations. In every case, plasmids isolated from red colonies that arose as a result of transformation with heteroduplexes containing 1- to 5-base insertion/deletion heterologies contained the appropriate deletion mutation.

***In Vivo* Correction of Unmethylated Heteroduplexes Proceeds Without Strand Bias.** The Dam-dependent repair pathway, while maintaining the ability to correct DNA, loses its ability to discriminate the wild-type strand from the deletion strand when DNA is devoid of adenine methylation in d(GATC) sequences (6). Table 2 shows the results of transformations with unmethylated heteroduplexes. The G·T mismatches and 1-, 2-, and 3-base deletion heteroduplexes show repair consistent with the loss of strand bias, since transformation of the mismatch-proficient strain gave rise to nearly equivalent numbers of either red or white colonies and a very

Table 2. Genetic analysis of unmethylated heteroduplex transformations

Heteroduplex		Wild type			*mutL*::Km *Stu*			*mutH471*::Tn*5*			*mutS201*::Tn*5*		
Mutant/WT methylation	Type	% R	% W	% M	% R	% W	% M	% R	% W	% M	% R	% W	% M
−/−	G·T at 40	42	53	5	7	6	87	23	21	56	7	7	86
−/−	T·G at 41	39	47	14	8	7	85	27	21	52	6	9	85
−/−	1 Δ	47	49	4	5	7	88	26	27	47	5	4	91
−/−	2 Δ	49	47	4	3	6	91	23	19	58	7	6	87
−/−	3 Δ	47	42	11	5	8	87	12	13	76	4	5	91
−/−	4 Δ	18	31	51	7	9	84	9	11	80	9	10	81
−/−	5 Δ	6	10	84	7	8	85	6	7	87	5	8	87

Conventions are as in Table 1. The total population of transformants ranged from 450 to greater than 1000 total colonies per transformation. −/− indicates that both the deletion strand and the complementary wild-type strand are devoid of adenine methylation.

Table 3. Genetic analysis of fully methylated heteroduplex transformations

Heteroduplex		Wild type			*mutL*::Km *Stu*			*mutH471*::Tn*5*			*mutS201*::Tn*5*		
Mutant/WT methylation	Type	% R	% W	% M	% R	% W	% M	% R	% W	% M	% R	% W	% M
+/+	G·T at 40	35	27	38	9	8	83	30	31	39	9	5	86
+/+	T·G at 41	31	25	44	6	5	89	24	30	46	7	13	80
+/+	1 Δ	31	30	39	6	10	84	26	29	45	8	6	86
+/+	2 Δ	29	23	48	5	3	92	25	19	56	7	5	88
+/+	3 Δ	28	19	53	6	5	89	19	25	56	6	7	87
+/+	4 Δ	8	12	80	8	6	86	8	9	83	7	7	86
+/+	5 Δ	6	8	86	4	5	88	7	6	87	7	10	83

Conventions are as in Table 1. The total population of transformants ranged from 300 to 800 total colonies per transformation. +/+ indicates that both the deletion strand and the complementary wild-type strand are fully methylated at more than 95% of the d(GATC) sequences.

low percentage of mixed colonies. The data obtained for the 4-base deletion heteroduplex suggest that it is a weak substrate for repair, while the 5-base deletion heteroduplex is not repaired.

***In Vivo* Correction of Symmetrically Methylated Heteroduplexes Proceeds Independently of Dam Methylation and MutH.** Table 3 shows the data from transformations of the indicator strains with heteroduplexes containing symmetrically methylated d(GATC) sequences. Since these methylated sequences are resistant to nicking by MutH endonuclease, correction of mismatched bases and insertion/deletion heterologies should be greatly reduced (6, 24). The results in Table 3 do not support this prediction. Transformation of the mismatch-proficient indicator strain with the symmetrically methylated heteroduplexes revealed a pattern of methylation-independent repair similar to that observed with *mutH* transformations in Tables 1 and 2. This repair process requires the gene products of *mutL* and *-S*, since more than 80% of the transformants of these strains give rise to mixed colonies. Lack of correction of 4- and 5-base insertion/deletion heteroduplexes in wild-type and *mutH* strains is also consistent with the data observed in Tables 1 and 2. Therefore, Dam-independent repair follows the same specificity as the Dam-dependent system, albeit at reduced efficiencies.

MutS Binds to Small Insertion/Deletion Heterologies *in Vitro*. Since MutS binds to mismatched bases and is required for correction of small insertion/deletion heterologies *in vivo* (Tables 1–3), we investigated the possibility that MutS might also play a part in the recognition of insertion/deletion heterologies. Fig. 2 displays the results of band-shift assays involving 36-base heteroduplexes containing 1 (unpaired T), 2 (unpaired GC), 3 (unpaired TGC), 4 (unpaired TGCG), or 5 (unpaired AGTGC) -base insertion/deletion heterologies or the G·T mismatch and *S. typhimurium* MutS protein. *S. typhimurium* MutS has been shown to complement *E. coli* MutS *in vivo* (ref. 21; unpublished data). The band-shift assays of Fig. 2 show that MutS recognizes and binds to 1-, 2-, 3-, and 4-base heterologies in addition to the G·T mismatch. Specific binding to the 5-base deletion and homoduplex (data not shown) was not observed. Densitometric scans of the autoradiographs reveal that the hierarchy for MutS binding to the above mutations followed a surprising pattern: 1-base deletion > 2-base deletion > 3-base deletion ≈ G·T mispair > 4-base deletion. The binding of MutS to 36-base heteroduplexes is a reversible process, as MutS–heteroduplex complexes can be dissociated by SDS (data not shown). Furthermore, MutS does not bind to 36-base single-strand DNA within the parameters of our experimental system.

DISCUSSION

Genetic studies provided initial evidence supporting the correction of small [but not large (25)] heterologies by *E. coli* mismatch repair (26–29). However, there was no direct evidence until Dohet *et al.* (30) demonstrated that 1-base insertions and deletions are efficiently repaired *in vivo*. Learn and Grafstrom (31), using an *in vitro* system, have demonstrated that 1-, 2-, and 3-base deletions can be repaired as efficiently as G·T mismatches in a *mutHLS*-dependent, methyl-directed process. Mismatch repair systems in other cell types have also been implicated in the repair of small insertion/deletion mutations. The Hex system of *Streptococcus pneumoniae* efficiently corrects 1- and 2-base heterologies and marginally corrects 3-base heterologies (32), while mismatch repair in *Saccharomyces cerevisiae* corrects single-base frameshift mutations (33, 34).

Our finding that heterologies larger than 4 bases are not recognized and processed by Dam-dependent mismatch repair contradicts an earlier report showing Dam-dependent correction of 10-base heterologies (35). In support of our interpretation, we have found that MutS fails to bind specifically to 7- and 9-base heterologies *in vitro* and, in addition, that *in vivo* correction of heteroduplexes containing 7-, 9-,

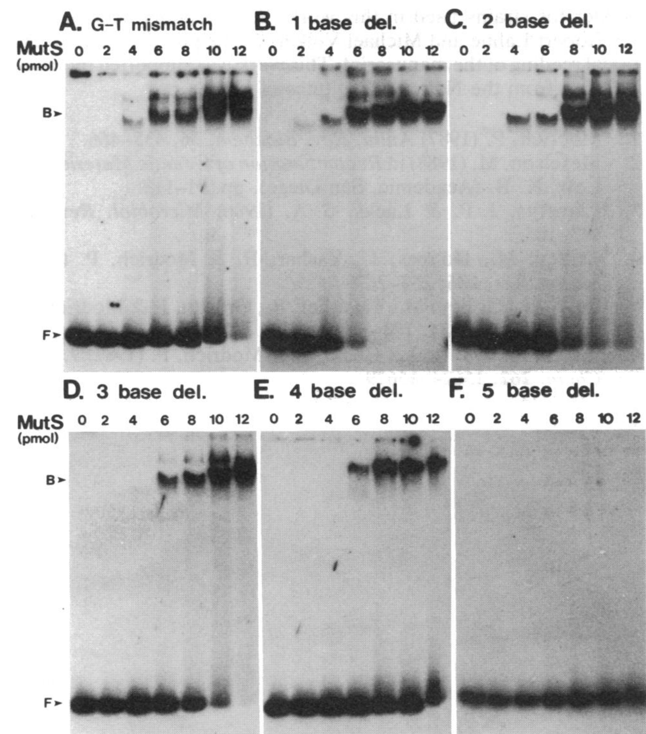

FIG. 2. MutS band-shift assays. Increasing concentrations of *S. typhimurium* MutS were added to 0.6 pmol of ^{32}P-labeled 36-base duplexes containing 1-, 2-, 3-, 4-, or 5-base heterologies or a G·T mismatch at position 40. The binding reaction was analyzed on a 6% nondenaturing polyacrylamide gel followed by autoradiography. B represents MutS protein bound to heteroduplex. F represents unbound (free) 36-base duplex.

and 11-base heterologies is not observed (B.O.P., M. Carraway, and M.G.M., unpublished observations). Therefore, the most likely explanation is that the 10-base heterology (an *Xho* I linker) induced a secondary structure recognized by MutS due not to the number of bases involved but to some other feature.

The methyl-independent, *mutL/mutS*-dependent repair observed (Tables 1–3) is probably best explained by the presence of strand breaks or nicks that might have been introduced into the closed circular heteroduplex before, during, or after the transformation process. Single-strand breaks are sufficient to direct the strandedness of repair for mismatch correction in the absence of either MutH or d(GATC) sequences (10, 36). An alternative, albeit less likely, explanation might be that components of other pathways involved in localized repair are assisting MutL and MutS in correcting small heterologies. Methyl-independent repair was not affected by mutations in *recF, recA, dcm-6*, or the triple mutant *xthA nfo nth* (data not shown).

Densitometric scans of the autoradiographs in Fig. 2 reveal that MutS binds to 1- and 2-base deletions with higher affinity than to the position 40 G·T mismatch or 3-base deletion. Competition studies between 1-, 2-, and 3-base deletions and a position 40 G·T mismatch agree with these results (data not shown). However, it should be recalled from Table 1 that close to 99% of the 1-, 2-, and 3-base heterologies and G·T mispairs were corrected *in vivo*. This raises the possibility that the repair system may already have corrected as many mispairs as possible prior to plasmid replication, thus making it difficult to clearly define the relationship between MutS binding and correction efficiency. Examining the kinetics of this repair process should help clarify this issue.

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