The MutHLS pathway recognizes most base-base mispairs, by homologous genetic recombination (reviewed in Refs. 1-4). MutH activation is sensitive to DNA topology. Circular heteroduplexes are better substrates than linear molecules, and activity of DNAs of the latter class depends on placement of the mismatch and d(GATC) site within the molecule. MutH activation is supported by a 6-kilobase linear heteroduplex in which the mismatch and d(GATC) site are centrally located and separated by 1 kilobase, but a related molecule, in which the two sites are located near opposite ends of the DNA, is essentially inactive as substrate. We conclude that MutH activation represents the initiation stage of methyl-directed repair and suggest that interaction of a mismatch and a d(GATC) site is provoked by MutS binding to a mispair, with subsequent ATP-dependent translocation of one or more Mut proteins along the helix leading to cleavage by a d(GATC) sequence on either side of the mismatch.

The Escherichia coli methyl-directed, MutHLS-dependent mismatch repair system controls genetic variability by correcting DNA biosynthetic errors and ensuring the fidelity of homologous genetic recombination (reviewed in Refs. 1-4). The MutHLS pathway recognizes most base-base mispairs, as well as insertion-deletion mismatches involving a small number of nucleotides, but the efficiency of repair varies with the nature of the mismatch and can be influenced by sequence context. The system processes mispairs in a strand-specific manner, with discrimination of the two DNA strands being based on the state of adenine methylation of d(GATC) sequences. Repair of a hemimethylated heteroduplex is restricted to the unmodified strand, symmetrically modified DNA is not processed, and a mismatch within an unmethylated helix is corrected with little strand bias. One exception to the rule of methyl direction has been demonstrated: the requirement for a hemimethylated/unmethylated d(GATC) site can be bypassed by a persistent strand break, in which case the associated DNA termini suffice to target correction to the incised strand (5, 6).

The methyl-directed reaction has been reconstituted in a purified system comprised of E. coli MutH, MutL, MutS, DNA helicase II, single strand DNA-binding protein, exonuclease I, DNA polymerase III holoenzyme (preparations used possessed associated 5' to 3' exonuclease activity (7, 8) DNA ligase, ATP, the four dNTPs, and the ligase cofactor NAD+ (6). Although the overall mechanism of the reaction remains to be established, analysis of MutH, MutL, and MutS has indicated that these three proteins are responsible for specific action with the two DNA sites involved in a methyl-directed repair event. Thus, MutS binds to mismatched base pairs in a reaction that is independent of the presence of a d(GATC) sequence within the DNA (9-11). Welsh et al. (12) have shown that MutH possesses a Mg2+-dependent endonuclease that incises 5' to d(GATC) sequences in a methyl-directed but mismatch-independent manner. Fully methylated sequences are resistant to attack, incision at hemimethylated sites is restricted to the unmodified strand, and unmethylated d(GATC) sequences are incised on either DNA strand. Despite the mismatch independence and the extremely weak nature (turnover number < 1 h-1) of the MutH-associated endonuclease, Welsh et al. (12) proposed that this activity is indicative of MutH function in mismatch repair, with the protein undergoing mismatch-dependent activation during assembly of a repair complex. The function of MutH as a d(GATC) endonuclease in mismatch correction was confirmed with the demonstration that a persistent strand break not only circumvents the requirement for the d(GATC) strand signal in mismatch repair, but bypasses the requirement for MutH as well (5, 6).

While attempts to assign specific enzymatic or DNA binding activities to MutL have yielded negative results, the protein has been shown to interact with the MutS-mismatch complex, provided that ATP or ATPyS is present (13, 14). In this paper we report a second biochemical effect of MutL and demonstrate mismatch-dependent activation of the MutH-associated endonuclease in a reaction requiring MutL, MutS, and ATP.
Initiation of Mismatch Repair

Proteins and DNA—E. coli MutH, MutL, and MutS were prepared as described previously (9, 12, 13). E. coli topoisomerase I was a gift from Dr. James Wang (Harvard University). E. coli DNA ligase, restriction endonucleases, and T4 polynucleotide kinase were from commercial sources.

Covalently closed, circular heteroduplex DNAs were prepared in supercoiled form as described previously. Phages fMR8 and fMR9 were used to prepare C-C and G-G heteroduplexes and phages fMR1 and fMR2 to prepare G-T and A-C heteroduplexes (10). The structure of these molecules is illustrated in Fig. 1. A control homoduplex containing a G-C base pair instead of a mismatch was prepared in the same way and used as control for mismatch repair reactions (12). The demonstration that a persistent strand break bypasses the requirements for MutH and for a G-T heteroduplex (methylated on the complement ary DNA strand). Reactions were quenched by the addition of 2 μl of 60 mM EDTA, and 2 μl was spotted onto polyethyleneimine-cellulose plates (EM Separations), which were developed in 0.5 M LiCl, 0.3 M acetic acid. Spots were visualized by autoradiography, and radioactivity associated with ATP and Pi was determined by liquid scintillation counting of appropriate portions of the polyethyleneimine-cellulose plate.

RESULTS

MutL, MutS, and ATP Activate the MutH-associated d(GATC) Endonuclease—As discussed above, purified MutH possesses an extremely weak, mismatch-independent, but nevertheless tightly associated d(GATC) endonuclease activity (12). The demonstration that a persistent strand break bypasses the requirements for MutH and for a d(GATC) strand signal in mismatch repair has confirmed a role for MutH as a d(GATC) endonuclease in this process (5, 6). MutH-specific endonuclease activity in the buffer used for mismatch repair assay corresponds to 0.0017 incision events/min/monomer equivalent. However, the specific activity of MutH with respect to mismatch repair in the reconstructed methyl-directed system is 20–70-fold higher (~0.04–0.12 repair events/min/monomer for a G-T mismatch, the most efficiently processed base-base mismatch (6)). We have therefore tested the proposal (12) that MutH endonuclease may undergo activation during assembly of a repair complex on a heteroduplex.

As shown in Table I, the combination of MutH, MutL, MutS, and ATP results in the appearance of an endonuclease activity that incises the unmethylated strand of a hemimethylated G-T heteroduplex in the vicinity of its single d(GATC) site. MutH, MutL, MutS, ATP, and a divalent cation are each required for this effect since activation was not detectable in reactions lacking one or more of the individual components. The intrinsic MutH activity is not detectable at the low concentration of the protein used in the experiments of Table

2 The abbreviations used are: RF, replicative form; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; bp, base pair(s); 2-AP, 2-aminopurine.
I (12), but it is evident that the degree of nuclease activation exceeds 30-fold when MutL, MutS, and ATP are also present. It is pertinent that the concentrations of the four components used in these experiments ("Materials and Methods") are identical to those used to study mismatch repair in a defined system (6). Therefore, the rate of the endonuclease reaction can be compared with that for in vitro mismatch correction. This point is considered below.

The intrinsic MutH activity incises hemimethylated or unmethylated duplexes immediately 5' to an unmethylated d(GATC) sequence (pNPgApTgP) to produce a strand break with 3'-OH and 5'-P termini (12). Table II demonstrates that the MutHLS-dependent, activated endonuclease responds to d(GATC) modification in a similar manner: each of the two hemimethylated configurations of a G-T heteroduplex was incised on the unmethylated strand, the symmetrically modified heteroduplex was resistant to cleavage, and the unmethylated substrate was subject to incision on either strand of the helix. In the case of the unmodified heteroduplex, d(GATC) cleavage during early stages of the reaction was largely limited to single strand incision, with prolonged incubation resulting in cleavage of the other strand to yield a double strand break (Table III). Double strand cleavage of unmethylated DNA by the activated MutH endonuclease is of interest in view of the finding that 2-aminopurine killing of dam mutants requires mutH, mutL, and mutS function (15). The possible relationship of these two observations will be considered below.

The specificity of phosphodiester bond hydrolysis by the MutHLS-dependent endonuclease is also identical to that of the intrinsic MutH activity. Strand breaks introduced by the activated species are efficiently closed by E. coli DNA ligase demonstrating that, as in the case of MutH-associated activity, hydrolysis produces 3'-OH and 5'-P termini (data not shown). Furthermore, as illustrated in Fig. 2, the MutHLS-dependent activity cleaves immediately 5' to the d(GATC) site when the d(GATC) site was quantitated by virtue of their fluorescence using a photometric grade, cooled CCD imager equipped with a 600-nm band pass filter (Photometrics, Inc.). A second portion of each reaction was subjected to indirect end labeling ("Materials and Methods") to determine the total yield of d(GATC) incision events in each DNA strand. The yield of single-cleaved molecules shown corresponds to the latter value after correction for double-strand events.

**FIG. 2. Specificity of cleavage by the activated MutHLS-dependent endonuclease.** Endonuclease reactions, which utilized the circular fMR1/fMR3 G-T heteroduplex bearing d(GATC) methylation on the complementary strand, were performed as described under "Materials and Methods." Products were hydrolyzed with HincII (Fig. 1) as indicated, and the DNA subjected to electrophoresis on a 6% polyacrylamide sequencing gel. Dideoxy sequencing markers (lanes A, C, G, and T) were obtained using fMR8 RF DNA, Sequenase Version 2.0 (United States Biochemical), [α-32P]dATP as the labeled nucleotide, and 5' phosphorylated oligomer II (nucleotides 1-17 of the viral DNA strand) primer. An additional marker was prepared by digesting unmethylated fMR8 RF DNA with HincII and MboI (the latter activity cleaves immediately 5' to the dG of the single d(GATC) site within the molecule). DNA was electroblotted from the sequencing gel onto a nylon membrane and probed with 5'-32P-oligomer I.
activities differ in their response to the presence of a mismatch. The rate of d(GATC) cleavage by the intrinsic MutH activity is unaffected by the presence of a mispair within the substrate (12), but the MutS requirement for endonuclease activation suggested that mismatch recognition might be involved in this process. Table IV shows that this is the case. The four different heteroduplexes tested were incised by the MutHLS-dependent activity at characteristic rates, with their relative rates of cleavage correlating with their differential sensitivity to methyl-directed repair (G-T > G-G > A-C > C-C) (6, 10).

Although the C-C heteroduplex used in these experiments is not subject to detectable methyl-directed repair (6, 10), it was subject to a low level of MutHLS-dependent cleavage at the hemimethylated d(GATC) site (Table IV, top), and a similar degree of cleavage was observed with a hemimethylated G-C construct that was prepared in the same manner ("Materials and Methods"). In contrast to the behavior of the G-T, G-G, and A-C heteroduplexes, the rate of cleavage of hemimethylated G-C and C-C DNAs decreased rapidly after \( \approx 10\% \) of the molecules had been incised, suggesting that only a subpopulation was sensitive to MutHLS cleavage. We have considered three potential explanations for the low level activity observed with the latter two DNAs: (i) sensitivity of the system to natural fluctuations in base pair conformation occurring within the helix; (ii) MutHLS recognizable damage incurred during heteroduplex preparation; (iii) presence of unknown base pair mismatches due to natural variation within the two DNA populations that are denatured and reannealed to produce the heteroduplex.

These possibilities were examined by testing activity of the system on unmethylated fMR3 RF DNA obtained from a dam host. In contrast to the efficient incision observed on the two strands of unmethylated G-T heteroduplex (Table II), no detectable d(GATC) cleavage was observed on either strand of unmethylated circular or linear RF DNA (Table IV, bottom). However, alkaline denaturation and reannealing of linear RF DNA resulted in low, but detectable cleavage on each strand at the d(GATC) site of the reannealed species. In view of these findings and the subpopulation observation mentioned above, we attribute low level MutHLS cleavage of the hemimethylated G-C and C-C substrates to damage associated with their preparation and/or natural variation within the DNAs used for their construction.

Activated MutH Turns Over in the d(GATC) Endonuclease Reaction—Turnover of the intrinsic endonuclease activity of purified MutH has not been demonstrated (12). In contrast, turnover of the activated species on heteroduplex DNA was observed during extended incubation at low MutH concentrations (Table V), with an apparent turnover number of 0.1 min\(^{-1}\) mol\(^{-1}\). Since we have not attempted to saturate the system with heteroduplex DNA, this determination may well be an underestimate. This value can be compared with \( k_{cat} \) values ranging from 0.1 to 100 min\(^{-1}\) mol\(^{-1}\) for Type II restriction endonucleases (16), activities that catalyze much simpler reactions.

**MutH Activation Is Dependent on ATP Hydrolysis**—ATP is required for methyl-directed repair (6, 17) and serves as cofactor for DNA helicase II (18) and DNA polymerase III holoenzyme (19), both of which are required for the reaction (6). As shown in Table I and Fig. 3, ATP is also required for activation of MutH endonuclease, with half-maximal activity achieved at 0.3 mM. dATP at a concentration of 2 mM was as effective as 2 mM ATP with respect to activating the system, but GTP, CTP, UTP, dGTP, dCTP, and dTTP were inactive at this concentration (<0.5 fmol/20 min under standard assay conditions). ATPγS also failed to support activation and was found to be a potent inhibitor of the reaction promoted by ATP (Table I). As shown in Fig. 4 (upper panel), ATPγS

### Table IV

**Mismatch dependence of d(GATC) endonuclease activation**

| Heteroduplex | Methylated strand | d(GATC) cleavage |
|--------------|------------------|-----------------|
| G-T          | C                | 1.3             |
| G-G          | C                | 1.1             |
| A-C          | C                | 0.41            |
| C-C          | C                | 0.15            |
| G-C          | C                | 0.14            |

| Substrate    | Methylated strand | d(GATC) cleavage |
|--------------|------------------|-----------------|
| G-C (supercoiled) | Neither | <0.025 (C), <0.025 (V) |
| G-C (linear control) | Neither | <0.025 (C), <0.025 (V) |
| G-C (denatured and reannealed) | Neither | \( \approx 0.03 \) (C), \( \approx 0.03 \) (V) |

### Table V

**Turnover of activated MutH**

| MutH Incubation | d(GATC) cleavage/MutH |
|-----------------|-----------------------|
| 10.4            | 20                    | 1.6 |
| 2.1             | 60                    | 6.5 |

![Fig. 3. Dependence of endonuclease activation on ATP concentration.](image)
inhibited endonuclease activation as well as ATP hydrolysis that occurs in such reactions, with the coordinate inhibition of the two activities strongly suggesting that MutH activation requires hydrolysis of ATP.

Associated ATPase activity has not been identified in near homogeneous preparations of MutH or MutL (13), but MutS displays weak ATPase in the presence or absence of DNA (20). While genetic evidence suggests that MutS ATPase has a functional role in mismatch repair (20), it is premature to conclude that nucleotide hydrolysis associated with MutH activation is due solely to the mutS gene product. Nevertheless, it is interesting to note that the MutS activity responds to ATPγS inhibition in a manner similar to that of the MutHLS-dependent endonuclease (Fig. 4, lower panel).

The Incised d(GATC) Sequence May Lie Either 3' or 5' to the Mismatch on the Unmethylated DNA Strand—An unmodified d(GATC) sequence can reside either 3' or 5' to the mismatch as viewed along the unmethylated strand. MutH activation might therefore depend on a particular orientation of the two sites. Since the experiments described above used circular substrates and do not address this issue, we have tested linear heteroduplexes for their ability to support this reaction. The G-T heteroduplex was hydrolyzed with Eco47III, which cleaves the circular molecule within the longer path separating the two DNA sites (Fig. 1), to yield a blunt-ended, 6111-bp linear molecule in which the mispair and the d(GATC) site are separated by 1024 bp. As shown in Fig. 5, both hemimethylated configurations of the linear heteroduplex were efficiently incised by the MutHLS-dependent activity. Since the d(GATC) sequence on the viral DNA strand of the molecule lies 3' to the mismatch while that on the complementary strand is located 5' to the mispair, these results indicate that activation lacks obligate polarity. An alternate interpretation is that polarity associated with interaction of the two DNA sites can be bypassed by transient circle formation via interaction of the blunt ends of the linear molecule, but this possibility is excluded by experiments described immediately below.

Dependence of MutH Activation on Topology of the Heteroduplex—During the course of these experiments, we noticed that the MutHLS system appeared to be less active on linear heteroduplex DNAs than on circular molecules. The activity of the system on several different substrate configurations is summarized in Table VI. Heteroduplexes that are routinely used to study in vitro mismatch correction are prepared in a negatively supercoiled form (10, 17). Cleavage of a hemimethylated circular G-T heteroduplex with Eco47III yields a linear in which the mismatch and d(GATC) site are centrally located with a separation distance of 1 kilobase (Fig. 1). The activity of this linear DNA was consistently lower than that of the supercoiled species. Moreover, cleavage of the supercoil

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**Fig. 4. Inhibition of endonuclease activation and ATPase by ATPγS.** Upper panel, ATP hydrolysis occurring in reactions containing MutH, MutL, MutS, and heteroduplex DNA was determined as described under "Materials and Methods" except that ATPγS was present as indicated. Values shown are corrected for tripolyphosphate hydrolysis by a contaminating ATPase present in the MutL preparation (13). Cleavage at the d(GATC) site of the G-T heteroduplex (methylated on the complementary DNA strand) was determined under identical conditions except that unlabeled ATP was used. Measurements were corrected for a small amount of d(GATC) cleavage that was mediated by MutH alone and evident at the higher concentration of the protein used in these experiments. ●, ATPase; ○, d(GATC) endonuclease. Lower panel, ATP hydrolysis by MutS was determined as described under “Materials and Methods” except that MutH and MutL were omitted from the reaction. Hemi-
methylated G-T heteroduplex (modified on complementary strand) and ATPγS were present as indicated. ●, no DNA; ○, with hetero-
duplex.

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**Fig. 5. Incision of linear heteroduplexes by the MutHLS-dependent endonuclease.** Linear G-T heteroduplexes in the two hemimethylated configurations were prepared by cleavage of circular DNAs with Eco47III. Endonuclease reactions were incubated for 20 min and terminated by addition of 5 μl of 0.2 N NaOH, 0.04 M EDTA, 10% Ficoll 400, 0.1% brom cresol green, without subsequent restriction endonuclease digestion. Marker DNA in the left lane was prepared by digestion of unmethylated pMR3 RF with Eco47III and MboI. Upper panel, the membrane was probed for cleavage on the complementary strand using 5'-32P-oligomer II. Lower panel, the same membrane was probed with 5'-32P-oligomer I to detect cleavage on the viral DNA strand.

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\(^{5}\) K. G. Au and P. Modrich, unpublished observations.
Initiation of Mismatch Repair


d(GATC) sites (17, 22-24), and extract experiments have shown that repair is associated with mismatch- and MutHLS-dependent incision of an unmethylated strand in the vicinity of a d(GATC) sequence (22). Since a persistent strand break bypasses both the d(GATC) and the MutH requirements of the reaction, d(GATC) endonuclease activity has been attributed to the mutH gene product (5, 6). This conclusion is consistent with the finding that MutH possesses a weak d(GATC) endonuclease, but only if this latent activity undergoes mismatch-dependent activation (12). We have shown that this is the case.

Several observations lead us to infer that the mismatch- and MutHLS-dependent partial reaction described here represents the initiation step in methyl-directed mismatch correction (3). The excision-repair mode of the reaction (25) implies a requirement for at least one strand break (or DNA end). In fact, analysis of the fate of closed circular heteroduplex DNA in the purified system has failed to reveal significant covalent alteration of the substrate if MutS, MutL, or MutH is omitted from the reaction (6). Each of these proteins is therefore necessary for initiation of the initial strand break that occurs during the course of repair. As shown in this paper, MutH, MutL, and MutS are also sufficient to mediate a cleavage event that has been previously implicated in methyl-directed repair, with the substrate specificity of the MutHLS endonuclease being similar to that of the overall reaction.

In order to conclude that MutHLS-dependent cleavage represents the initial step in methyl-directed mismatch correction, it is also necessary to show that d(GATC)-incised heteroduplex is produced rapidly enough to be a kinetically significant intermediate in the overall reaction. To permit this sort of comparison, most of our experiments utilized protein and DNA concentrations identical to those used previously to characterize the reconstituted methyl-directed reaction (6). The rate of MutHLS cleavage under these conditions compares favorably with the rate of mismatch repair. For example, cleavage of the two hemimethylated configurations of the G-T heteroduplex used in the experiments of Tables II and IV occurred at rates of 1.3 and 1.7 fmol min⁻¹ (viral and complementary strands, respectively) as compared to 1.2 and 0.4 fmol min⁻¹ for mismatch repair of these molecules (6). A similar case holds for the other mismatches in Table IV, and we conclude that incision by the activated MutH endonuclease is sufficiently fast for the product to be an intermediate in the overall repair reaction. It is pertinent to note that although MutHLS cleavage is somewhat more efficient on the complementary DNA strand in the case of both hemimethylated and unmethylated heteroduplexes (Table II), an opposite preference has been observed for repair occurring on these DNAs (6, 10). However, since repair directed by a strand break also occurs more efficiently on the viral strand (6), the strand preference evident in the overall reaction presumably reflects events subsequent to incision.

Double strand cleavage by the intrinsic MutH endonuclease has not been observed (12), but the activated form of the protein can cleave both DNA strands at a d(GATC) site in an unmethylated heteroduplex (Table III). This finding is of interest in view of the controversy concerning the mechanism of killing of dam⁺ mutants by 2-aminopurine (2-AP). Since mutH⁺, mutL⁻, and mutS⁻ mutations that block mismatch repair also alleviate 2-AP lethality in methylase mutants, Glickman and Radman (26) have attributed killing to undirected repair of mismatches involving the base analogue. This proposal presumes that such mispairs occur with a sufficiently high frequency so that a lethal double strand break may ensue

| Table VI: Dependence of MutH activation on DNA topology |
|-----------------------------------------------|
| Heteroduplex topology | d(GATC) cleavage (fmol/min) |
|----------------------|-----------------------------|
| Experiment 1         |                             |
| Supercoiled          | 1.3                         |
| Relaxed              | 2.5                         |
| Eco47III linear      | 0.64                        |
| HincII linear        | <0.10                       |
| Experiment 2         |                             |
| Relaxed              | 18                          |
| Eco47III linear      | 6.5                         |
| HincII linear        | 16                          |
| Relaxed +            | 8.8                         |
| Eco47III linear      | 2.3                         |
| HincII linear        | 8.5                         |

with HincII, which places the two sites near opposite ends of a blunt-ended linear at a separation distance of 5.4 kilobases (Fig. 1), resulted in virtual loss of substrate activity. The reduced activity of the two linear molecules is not due to the absence of superhelical stress as the corresponding relaxed circular heteroduplex was an excellent substrate. The presence of a diffusable inhibitor in the linear DNA preparations was also excluded since circles were preferentially incised by the activated endonuclease in reactions containing equimolar concentrations of circular and linear DNAs (Table VI). The MutHLS system thus responds to placement of the mismatch and the d(GATC) site within the heteroduplex. The mechanistic basis of this effect has not been defined, but it is seemingly dependent on the proximity of one or both sites to a DNA end and/or the distance by which the two sites are separated along the helix contour.

**Discussion**

Provocation of a methyl-directed repair event requires a mismatch and one or more hemimethylated or unmethylated d(GATC) sites (17, 22-24), and extract experiments have shown that repair is associated with mismatch- and MutHLS-dependent incision of an unmethylated strand in the vicinity of a d(GATC) sequence (22). Since a persistent strand break bypasses both the d(GATC) and the MutH requirements of the reaction, d(GATC) endonuclease activity has been attributed to the mutH gene product (5, 6). This conclusion is consistent with the finding that MutH possesses a weak d(GATC) endonuclease, but only if this latent activity undergoes mismatch-dependent activation (12). We have shown that this is the case.

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*Although competition experiments have not been done, the relaxed circular G-T heteroduplex has consistently proven to be a better substrate than a negatively supercoiled DNA (Table VI). Since the MutHLS system probably recognizes base pair mismatches in their intrahelical conformations (21), this effect may be due to destabilization of the intrahelical G-T conformation in underwound molecules.
when nearby mismatches provoke excision on opposite DNA strands. Grafstrom et al. (27) have questioned this view with the argument that the 2-AP-C mismatch, a likely candidate for provocation of the MutHLS system, occurs too infrequently to result in overlap of excision tracts. Furthermore, Grafstrom and colleagues (27) have identified mutH mutations that, while capable of suppressing the lethal effect of 2-AP in dam−1 strains, are not associated with a major defect in methyl-directed repair. The nature of these mutations thus suggests that killing by the base analogue does not result from the normal course of methyl-directed correction. These divergent observations can be rationalized by our finding that activated MutH endonuclease can cleave both strands of an unmethylated d(GATC) site, assuming that double strand cleavage in this manner is associated with a significant lethal cross section. Such a mechanism would account for the mutH, mutL, and mutS dependence observed by Glickman and Radman (28), with a single mismatch being sufficient to promote a lethal event. The phenotype of the mutH mutations mentioned above can also be accommodated within this scheme if their effect is to simply reduce the efficiency of second strand cleavage by activated MutH nuclease.

The finding that d(GATC) incision by the MutHLS activity can occur either 3' or 5' to the mismatch on the unmethylated DNA strand implies that the mechanism of activation of MutH endonuclease lacks obligate directionality. This suggests that the methyl-directed system may possess a bidirectional capability, with a d(GATC) site located on either side of the mismatch being sufficient to target repair to the unmodified strand. This possibility is consistent with several in vivo observations (28, 29) and, in fact, has recently been confirmed by the finding that both hemimethylated configurations of a linear G-T heteroduplex with a single d(GATC) site (molecules similar to those used in Fig. 5) are subject to methyl-directed repair in E. coli extracts and in the purified reconstituted system.

Since a strand break is sufficient to determine strand specificity of mismatch repair (5, 6), the simplest mechanism invokes initiation of excision at the site of MutH cleavage (3, 4). Such a mechanism requires that the repair system assess the absolute orientation of the heteroduplex so that excision from the incised d(GATC) site proceeds with appropriate directionality. This requirement can be met only if interaction between the mismatch and the d(GATC) site is mediated along the helix contour. Two possibilities have been proposed in this respect (21): directional protein-DNA translocation or polymerization of an asymmetric protein along the helix. Several results reported here are consistent with potential involvement of translocation in the MutHLS-dependent cleavage reaction. Directed translocation requires energy input, and as described above, ATP hydrolysis is apparently necessary for MutH activation. The unusual response of the MutHLS endonuclease to substrate topology can also be interpreted in this manner. Our finding that linear substrates are consistently less active than circular molecules is reminiscent of similar observations with Type I restriction endonucleases (30), activities in which DNA translocation is a key element of mechanism (31, 32). Possible involvement of translocation in the MutHLS reaction is currently being examined using more direct methods.

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