DNA Chain Length Dependence of Formation and Dynamics of hMutSα·hMutLα·Heteroduplex Complexes*

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Formation of a ternary complex between human MutSα, MutLα, and heteroduplex DNA has been demonstrated by surface plasmon resonance spectroscopy and electrophoretic gel shift methods. Formation of the hMutLα·hMutSα·heteroduplex complex requires a mismatch and ATP hydrolysis, and depends on DNA chain length. Ternary complex formation was supported by a 200-base pair G-T heteroduplex, a 100-base pair sub-strand was somewhat less effective, and a 41-base pair length. Ternary complex formation was supported by a 200-base pair G-T heteroduplex, a 100-base pair sub-

The MutS and MutL homologs, which are required for the initiation of mismatch repair, have been implicated in the correction of DNA biosynthetic errors, the transcription-coupled repair of DNA damage, and the fidelity of genetic recombina-

The MutSα and MutLα expressions of a human cDNA in SF9 cells. The two subunits of hMutSα were expressed from baculovirus constructs expressing the appropriate human cDNAs in SF9 cells. The two subunits of hMutSα were expressed from a single virus constructed using the Dual Bsh system (Life Technologies, Inc.). Briefly, hMSH2 cDNA (provided by Bert Vogelstein, Johns Hopkins Oncology Center, Baltimore, MD) was inserted into the Neor site just downstream of the p10 promoter, while hMSH6 cDNA (a gift from Rick Fishel, Thomas Jefferson University, Philadelphia, PA) was provided by Mike Liskay, Oregon Health Sciences University, Portland, OR. hMLH1 and PMS2 cDNAs for human MLH1 and PMS2 (generously provided by Nick Liskay, Oregon Health Sciences University, Portland, OR) were expressed from individual viral constructs prepared using the pFastBac I system (Life Technologies, Inc.). hMLH1 was expressed from the polyhedrin promoter by insertion between the BamHI and SalI sites. cDNAs for human MLH1 and PMS2 (generously provided by Mike Liskay, Oregon Health Sciences University, Portland, OR) were expressed from individual viral constructs prepared using the pFastBac I system (Life Technologies, Inc.). hMLH1 was expressed from the polyhedrin promoter by insertion between the BamHI and SalI sites. cDNAs for human MLH1 and PMS2 (generously provided by Mike Liskay, Oregon Health Sciences University, Portland, OR) were expressed from individual viral constructs prepared using the pFastBac I system (Life Technologies, Inc.). hMLH1 was expressed from the polyhedrin promoter by insertion between the BamHI and SalI sites. cDNAs for human MLH1 and PMS2 (generously provided by Mike Liskay, Oregon Health Sciences University, Portland, OR) were expressed from individual viral constructs prepared using the pFastBac I system (Life Technologies, Inc.). hMLH1 was expressed from the polyhedrin promoter by insertion between the BamHI and SalI sites. cDNAs for human MLH1 and PMS2 (generously provided by Mike Liskay, Oregon Health Sciences University, Portland, OR) were expressed from individual viral constructs prepared using the pFastBac I system (Life Technologies, Inc.). hMLH1 was expressed from the polyhedrin promoter by insertion between the BamHI and SalI sites. cDNAs for human MLH1 and PMS2 (generously provided by Mike Liskay, Oregon Health Sciences University, Portland, OR) were expressed from individual viral constructs prepared using the pFastBac I system (Life Technologies, Inc.). hMLH1 was expressed from the polyhedrin promoter by insertion between the BamHI and SalI sites. cDNAs for human MLH1 and PMS2 (generously provided by Mike Liskay, Oregon Health Sciences University, Portland, OR) were expressed from individual viral constructs prepared using the pFastBac I system (Life Technologies, Inc.). hMLH1 was expressed from the polyhedrin promoter by insertion between the

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sites. hPFM2 was also expressed from the polyhedrin promoter after insertion between BacH1 and X01 sites.

Infected SF9 cells for hMutSa isolation were grown by KemBio Technologies, Inc. (Frederick, MD). Frozen cell pellets were thawed and suspended (10 mg of cells) in 25 ml HEPE5-KOH, pH 7.5, 0.1 mM EDTA, and 100 mM NaCl. The extract was centrifuged at 22,000 

-32P-labeled complementary strand sequence derived by PCR from f1MR1 or f1MR3 (9). Forward and reverse primers for PCR were (5'-GTCCTTCCCTCCTTTCCGCTC-3') and (5'-AACTTTTCTGCGGTGC-3'). The -32P-labeled, 150-mer residue viral strand sequence from f1MR1 was combined with the complementary sequence (0.45 mg each in 100 

duplex or G-T heteroduplex DNAs described above, in which one strand was labeled with [32P]ATP (3000 Ci/mmol, PerkinElmer Life Sciences) to a specific activity of 1 x 10^7 cpm/pmole. Poly d(T)/d(C) was purchased from Amersham Pharmacia Biotech.

A 41-mer G-T heteroduplex used for gel shift analysis was prepared by mixing, in a 100-

were lysed with 20 strokes using a Dounce B pestle, the suspension was adjusted to 100 

mRNA level, 80 nM top strand 5-32P-d(AGCCGAATTTC-

G-T homo-
Fig. 1. Ternary complex formation requires a mismatch and hMutSα. SPRS analysis was performed as described under “Experimental Procedures” using a SA sensor chip derivatized with 143 RU of a 200-bp G-T heteroduplex (blue lines) and 146 RU of an otherwise identical 200-bp A-T homoduplex (red lines). Protein solutions contained 20 mM Tris, pH 7.6, 100 mM KCl, 1 mM dithiothreitol, 5 mM MgCl₂, 0.005% surfactant P20, 100 μM ATP, 200 nM hMutSα alone (red and blue solid lines), 200 nM hMutLo alone (hyphenated blue line), or 200 nM of hMutSα and 200 nM hMutLo (dashed red and blue lines). Chip-bound protein is expressed as mass equivalents of hMutSα (see “Experimental Procedures”), where one mass equivalent corresponds to 258 kDa. hMutSα and hMutLo: Heteroduplex Complexes

Experimental Procedures.

Heteroduplex-bound protein, as compared with that observed with hMutSα alone. This mass enhancement is mismatch-dependent since it was not observed with the A-T homoduplex control, and experiments described below demonstrate that it is not a simple consequence of increased hMutS binding due to presence of hMutLo. This effect requires the simultaneous presence of hMutSα and hMutLo since no enhancement of heteroduplex-bound protein was observed in experiments in which the two proteins were passed over the chip in a sequential manner (data not shown).

The hMutLo-dependent mass enhancement was only observed in the presence of ATP, where it displayed a strong dependence on DNA chain length (Fig. 2). In the absence of ATP (lower panel), ∼1 hMutSα heterodimer was bound/41-bp G-T heteroduplex, and this value increased to approximately two equivalents with the 200-bp heteroduplex. Under these conditions in the absence of ATP, the protein mass bound by 41-, 100-, and 200-bp heteroduplexes was unaffected by inclusion of hMutLo along with hMutSα, as compared with that observed with hMutSα alone.

As observed previously (18, 20, 26), the presence of ATP resulted in a dramatic reduction in hMutSα binding to the 41-bp heteroduplex, and the presence of hMutLo was without effect (Fig. 2, compare upper and lower panels). However, the extent of hMutSα binding to 100- and 200-bp heteroduplexes in the presence of ATP was similar to that observed in the absence of nucleotide, and the presence of hMutLo resulted in a substantial enhancement of protein mass bound by these two heteroduplex DNAs. Based on experiments described below, and previous observations with bacterial and yeast mismatch repair proteins (11–13, 16, 17), we have concluded that this enhancement of heteroduplex-bound protein mass reflects mismatch-, ATP-, and hMutSα-dependent presence of hMutLo in a nucleoprotein complex with heteroduplex DNA. The stoichiometry of formation of this hMutLo-hMutSα-heteroduplex ternary complex will be considered below.

Ternary complex formation was demonstrable in the presence of ATP-Mg²⁺, but we have been unable to detect a hMutLo-dependent increase in the mass of heteroduplex-bound protein in the presence of AMPPNP-Mg²⁺ (data not shown). This finding, which is similar to previous observations with bacterial MutS and MutL (13), strongly suggests that ternary complex formation is dependent upon ATP hydrolysis by one or both proteins.

RESULTS

Mismatch-, ATP-, and Chain Length-dependent Formation of a Heteroduplex Complex—Ternary complexes of yeast MutSα and MutLo with synthetic heteroduplexes of ~50 bp in size have been demonstrated by electrophoretic gel shift (16, 17). However, we have been unable to reproducibly detect ternary complexes involving bacterial MutS and MutL, or human MutSα and MutLo, utilizing synthetic heteroduplexes 41 bp in length. Since there is abundant evidence for ternary complex formation between the bacterial mismatch repair proteins and heteroduplexes of 143 bp or longer (11–13), we reasoned that this problem might be due to the small size of synthetic heteroduplexes. Using surface plasmon resonance spectroscopy, specific binding of hMutSα to a 200-bp G-T heteroduplex (see “Experimental Procedures”) was evident in the presence of ATP-Mg²⁺, with the heteroduplex signal approximately 3 times that observed with an otherwise identical A-T homoduplex (Fig. 1). Although hMutLo did not bind detectably to the 200-bp heteroduplex under these conditions, passage of a mixture of hMutSα and hMutLo over the chip resulted in a substantial enhancement of the mass of heteroduplex-bound protein, as compared with that observed with hMutSα alone. This mass enhancement is mismatch-dependent since it was not observed with the A-T homoduplex control, and experiments described below demonstrate that it is not a simple consequence of increased hMutS binding due to presence of hMutLo. This effect requires the simultaneous presence of hMutSα and hMutLo since no enhancement of heteroduplex-bound protein was observed in experiments in which the two proteins were passed over the chip in a sequential manner (data not shown).

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Apparent hMutSα and hMutLα Affinities and Stoichiometry of Ternary Complex Formation—Ternary complex formation requires ATP and is dependent on DNA chain length. Ternary complex formation between hMutSα and hMutLα and 41-, 110-, and 200-bp G-T heteroduplex DNA substrates (183, 165, and 166 RU, respectively) was monitored by SPRS as described under “Experimental Procedures” and in the legend to Fig. 1. hMutSα (200 nM), hMutLα (200 nM), and ATP (1 mM) were present as indicated. Progress curves are shown for 41-bp (blue), 110-bp (green), and 200-bp (red) G-T heteroduplexes. Solutions contained hMutSα only (solid lines) or both hMutSα and hMutLα (dashed lines). The solid and dashed lines are essentially coincident with the 41-bp heteroduplex (blue) in the presence or absence of ATP.

Fig. 2. Ternary complex formation between hMutSα, hMutLα, and a heteroduplex requires ATP and is dependent on DNA chain length. Ternary complex formation between hMutSα and hMutLα and 41-, 110-, and 200-bp G-T heteroduplex DNA substrates (183, 165, and 166 RU, respectively) was monitored by SPRS as described under “Experimental Procedures” and in the legend to Fig. 1. hMutSα (200 nM), hMutLα (200 nM), and ATP (1 mM) were present as indicated. Progress curves are shown for 41-bp (blue), 110-bp (green), and 200-bp (red) G-T heteroduplexes. Solutions contained hMutSα only (solid lines) or both hMutSα and hMutLα (dashed lines). The solid and dashed lines are essentially coincident with the 41-bp heteroduplex (blue) in the presence or absence of ATP.

Like hMutSα binding to the 200 bp G-T heteroduplex in the presence of ATP, ternary complex formation was a hyperbolic function of hMutLα concentration (Fig. 3, lower panel), characterized by an apparent $K_d$ of 70 nM in the presence of a hMutSα concentration of 200 nM. At this MutSα concentration, which is approximately equal to the $K_d$ for binding to heteroduplex DNA (above), the SPRS results shown in Figs. 1–3 indicate a relative ternary complex stoichiometry of 0.6 and 0.8 mol of hMutLα/mol of hMutSα for the 100- and 200-bp heteroduplexes, respectively. This calculation is based on the extent of hMutSα binding to heteroduplex DNA after correction for nonspecific complexes formed with homoduplex controls (Figs. 1 and 3). It is noteworthy that a similar hMutLα-dependent enhancement of heteroduplex-bound mass was observed at 800 nM hMutSα (4 times the $K_d$), providing additional evidence that the observed mass increase is due to hMutLα binding.
hMutSα/hMutLα: Heteroduplex Complexes

FIG. 3. Apparent $K_d$ values for assembly of hMutSα/hMutLα heteroduplex and hMutLα/hMutSα heteroduplex complexes. Upper panel, binding of hMutSα to a 200-bp G-T heteroduplex (175 RU) and an A-T homoduplex (174 RU) was determined in the presence of 1 mM ATP. Plateau values of SPRS sensograms obtained as a function of hMutSα concentration are shown as a function of the concentration of the protein. Data were fit to a square hyperbola using a nonlinear least squares routine. Apparent $K_d$ values for the G-T heteroduplex (○) and the A-T homoduplex (●) are 205 and 420 nM, respectively. The data are also plotted after subtraction of homoduplex values from those obtained with heteroduplex in order to correct heteroduplex binding for nonspecific effects (●). Hyperbolic fit of this corrected data yielded an apparent $K_d$ of 140 nM and an asymptotic value of 3 equivalents of hMutSα/200-bp heteroduplex. Lower panel, the SA chip was derivatized with streptavidin complexes. The presence of end blocks at both termini stabilized binary hMutSα/hMutLα ternary complexes. This implies that the levels of these species observed by SPRS prior to termination of protein flow correspond to a dynamic steady state, i.e., the complexes are turning over rapidly via dissociation and reassociation. It is important to emphasize that the multiphasic dissociation kinetics observed with both heteroduplex and homoduplex DNAs imply the existence of several distinct types of specific and nonspecific complexes.

hMutLα:hMutSα:Heteroduplex Ternary Complexes by Gel Shift Analysis—The chain length dependence of formation and the nature of hMutLα:hMutSα:heteroduplex ternary complexes was also examined by electrophoretic gel shift assays. As observed by SPRS, electrophoretic assay indicated that hMutLα did not bind detectably to 41-, 100-, or 200-bp G-T heteroduplexes (Fig. 5). In the presence of hMutSα, specific complexes were evident with each of these heteroduplexes, and the presence of both proteins led to production of one or more supershifted species. A hMutLα-dependent supershifted complex was produced with the 41-bp heteroduplex, as well as its homoduplex control. Although production of this species required presence of hMutSα, the lack of a mismatch requirement indicates that it is nonspecific in nature. Three supershifted species were observed with 100- and 200-bp DNAs in the presence of hMutSα and hMutLα (these were in addition to the hMutSα/hMutLα:heteroduplex binary complex, which was evident at a low level with the 200-bp substrate). Two of these (Fig. 5, asterisks) were produced with both homoduplex and heteroduplex, and as observed with 41-bp DNAs, production of these nonspecific complexes was dependent on the presence of both hMutSα and hMutLα. However, with both 100- and 200-bp DNAs, a heteroduplex-specific, supershifted complex was also produced (Fig. 5, arrows). Combined Southern and Western blot analysis confirmed the presence of both hMutSα and hMutLα in specific and nonspecific ternary complexes produced with the 100-bp G-T heteroduplex (Fig. 6).

These observations confirm the chain length dependence of specific ternary complex formation observed in SPRS experiments. By contrast, nonspecific complexes of the sort observed by gel shift assay were not detected as a mass enhancement in SPRS experiments with homoduplex DNA (Fig. 1). The reason for this is not clear, but as noted above, the multiphasic dissociation kinetics observed by SPRS with homoduplex DNA could be indicative of several classes of nonspecific complex.

The stability of ternary complexes was evaluated by challenge of preformed complexes with poly(dI)·poly(dC). As shown in Fig. 7, poly(dI)·poly(dC) challenge resulted in a dramatic reduction in ternary complex formation with the 200-bp G-T (lanes 4 and 5). The yield of binary hMutSα:heteroduplex complexes was also extremely low under these conditions when ATP was present in the reaction. However, distinct results were obtained when the two ends of the duplex were blocked with biotin-streptavidin complexes. The presence of end blocks at both heteroduplex termini stabilized binary hMutSα:heteroduplex DNA in the presence of hMutSα and hMutLα displayed similar multiphasic dissociation kinetics (Fig. 4 lower curve). The major species (~60%) dissociated rapidly with a $t_{1/2}$ of ~1 s, the second component (~16%) dissociated more slowly ($t_{1/2}$ ~ 19 s), with the residual (~24%) dissociating too slowly to permit an estimate of lifetime.

The relative extents of binding to heteroduplex and homoduplex DNAs (Figs. 1, 3, and 4) indicate that 60–70% of the protein mass bound to the heteroduplex under these conditions is dependent on the presence of a single mismatch. We therefore think it likely that the more rapidly dissociating heteroduplex species ($t_{1/2}$ values of 1 and 10 s, 80% by mass) correspond to several classes of mismatch-dependent ternary complexes. This implies that the levels of these species observed by SPRS prior to termination of protein flow correspond to a dynamic steady state, i.e., the complexes are turning over rapidly via dissociation and reassociation. It is important to emphasize that the multiphasic dissociation kinetics observed with both heteroduplex and homoduplex DNAs imply the existence of several distinct types of specific and nonspecific complexes.
complexes in the presence of ATP (compare lanes 2 and 3 with lanes 8 and 9), confirming previous observations in this respect (20, 27). The terminal end block also stabilized the MutL\(\alpha\)/MutS\(\alpha\) heteroduplex ternary complexes, but a single end block did not; that fraction of the heteroduplex that contained only one end block was recovered as free DNA after poly(dI)-poly(dC) challenge, whereas heteroduplexes with streptavidin-biotin blocks at both duplex termini were not (compare lanes 10 and 11). These observations are consistent with the conclusion discussed above that ternary complexes are dynamic in nature.

Due to the size of the heteroduplex and the presence of streptavidin end blocks, the supershifted complex observed in the presence of MutS\(\alpha\) and MutL\(\alpha\) was not resolved into specific and nonspecific components (lanes 10 and 11, compare with lane 9). However, a surprising effect of streptavidin-biotin end blocks became evident upon examination of nonspecific interactions with the 200-bp homoduplex. Two classes of nonspecific complex are produced with homoduplex DNA in the presence of MutS\(\alpha\), MutL\(\alpha\), and ATP (Fig. 5, lane 6; Fig. 7, lane 8). Unexpectedly, the corresponding nonspecific complexes were not detectable under conditions where the homoduplex molecules were blocked at both ends with streptavidin-biotin complexes (lane 12). These observations suggest that presence of free duplex DNA termini have an important role in the production of nonspecific complexes that are observed by gel shift assay.

**DISCUSSION**

Although MutS homologs bind readily to small synthetic heteroduplexes (10, 18, 23, 24, 29–31), the experiments described here indicate that formation of the MutL\(\alpha\)/MutS\(\alpha\)/MutS\(\alpha\) heteroduplex requires ATP (and probably its hydrolysis) and depends on DNA chain length. The 200-bp heteroduplex used in this report supports efficient ternary complex formation, 100- and 110-bp heteroduplexes appear to be somewhat less effective in this regard, and we have been unable to detect mismatch-dependent ternary complex formation with a 41-bp heteroduplex substrate. We have also obtained similar results with *E. coli* MutS and MutL.\(^3\)

The SPRS and gel shift analyses described here show that

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\(^3\) L. J. Blackwell and P. Modrich, unpublished experiments.
the hMutLo-hMutSo-heteroduplex ternary complex is dynamic, undergoing rapid dissociation and reassociation in the presence of ATP-Mg$^{2+}$ at near physiological ionic strength. In fact, the kinetics of dissociation of ternary complexes are similar to those observed with the binary hMutSo-heteroduplex in the presence of ATP. As observed for ternary complexes (Fig. 4), dissociation of binary complexes as monitored by SPRS was multiphasic (data not shown); the major amplitude dissolved rapidly (~60% of complexes, $t_{1/2} = 3$ s), a second species dissolved more slowly (~26%, $t_{1/2} = 50$ s), and a third component (~14%) dissolved too slowly to determine an accurate rate. These observations are similar to those obtained previously by Galio et al. (13) in SPRS studies of bacterial MutS and MutL. As described here for hMutSo and hMutLo, these earlier studies led to the conclusion that ternary complexes of MutS, and MutL, with a 149-bp heteroduplex turn over rapidly as compared with the lifetime of MutS-heteroduplex complexes. In the case of the human system, we have also shown that hMutLo-hMutSo-heteroduplex complexes can be kinetically stabilized by placement of a physical block at each end of a linear DNA. The simplest interpretation of this finding is that turnover of the ternary complex depends on movement of one or both mismatch repair proteins along the helix with dissociation occurring at free ends. By contrast, the recent work of Hsieh and colleagues (32) has led to the conclusion that bacterial MutL stabilizes mismatch-bound MutS in the presence of ATP, resulting in a much longer lifetime for the MutL-MutS-heteroduplex ternary complex as compared with that of the MutS-heteroduplex (32). The basis of these differing conclusions is uncertain, although different methods were used to monitor dissociation kinetics. Our conclusions and those of Galio et al. (13) are based on real time analysis using SPRS, whereas Schofield et al. (32) monitored dissociation kinetics by gel shift assay after addition of a heteroduplex trap.

The molecular basis of the chain length dependence for ternary complex formation is not clear, but there are a number of potential explanations for this effect. One possibility is that the presence of flanking homoduplex is necessary to accommodate both hMutSo and hMutLo. The formation of nucleoprotein complexes containing hMutLo that we have detected require hMutSo; however, the ATPase of bacterial MutL is known to be activated in the absence of MutS by single strands and to a

**Fig. 6.** Specific and nonspecific ternary complexes contain hMutSo and hMutLo. Gel shift reactions (see “Experimental Procedures”) contained 200 nM hMutSo, 200 nM hMutLo, 32P-labeled 100-bp G-T heteroduplex (lanes 1 and 3 of each experiment) or A-T homoduplex (lane 2 of each experiment). ATP (0.5 mM) was present in reactions shown in lanes 2 and 3, but omitted from samples shown in lane 1. After electrophoresis, protein and DNA were electrotransferred to nitrocellulose and DEAE membranes (see “Experimental Procedures”). DNA bound to the DEAE membrane was visualized by autoradiography and nitrocellulose-bound mismatch repair polypeptides identified by Western blot. In the experiment shown at the top, the nitrocellulose membrane was probed as indicated with anti-MLH1, and then with anti-MSH6 after stripping. The experiments shown in the center and at the bottom were performed in a similar manner except that stripping was not used; parallel gels were examined individually for DNA and MSH2, or for DNA and PMS2. Specific complexes are indicated by arrows and nonspecific complexes by asterisks. In the experiment shown at the top, the nonspecific complex that runs more slowly than the specific component is barely visible in the DNA and MSH6 panels, but is evident in the MLH1 panel. The faster migrating species in lane 1 of each DNA panel corresponds to the hMutSo-DNA complex. That portion of the gel where free DNA runs is not shown.

**Fig. 7.** The hMutLo/hMutSo-heteroduplex ternary complex is dynamic. Gel shift analysis was performed as described under “Experimental Procedures” in the presence or absence of 0.5 mM ATP using 32P-labeled 200-bp G-T heteroduplex (lanes 1–5 and 7–11) or a control 200-bp A-T homoduplex (lanes 6 and 12). Both DNAs contained a 5′-terminal biotin on each DNA strand. Reactions in the right panel contained 0.5 mg/ml streptavidin and were preincubated 10 min prior to addition of mismatch repair proteins to allow conjugation of biotin. Reactions were initiated by addition of hMutSo and hMutLo as indicated, to a final concentration of 200 nM. After 10 min at room temperature, a poly(dI-dC) competitor was added to a final concentration of 25 μg/ml. Reactions were terminated after an additional 5-min incubation subjected to polyacrylamide gel electrophoresis (see “Experimental Procedures”). The location of the specific ternary complex formed in the absence of streptavidin is shown by an arrow. Mobilities of free DNA with 0, 1, or 2 bound streptavidin molecules are also indicated.
lesser extent by duplex DNA (33, 34), implying presence of a DNA binding center. MutL and hMutLo are large asymmetric proteins (Stokes radii of 61 and 74 Å, respectively (Refs. 11 and 19)) and are potentially capable of occluding a substantial segment of helix. It is also possible that ternary complex formation involves oligomerization (or polymerization along the helix) of hMutSc or hMutLo. Indeed, we have concluded that ternary complexes with 200-bp heteroduplex DNA contain several copies of each heterodimer, but as discussed above, the presence of multiple protein copies can also be explained by a mechanism that invokes movement of repair protein complexes along the helix contour. A third interesting possibility is that the chain length requirement is indicative of a major DNA conformational transition associated with ternary complex formation, e.g. the opening of a significant length of helix or the introduction of a substantial bend, perhaps due to a partial wrapping of DNA about one of the repair activities.

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Addendum—While this manuscript was in preparation, we learned of a paper in press by Hsieh and colleagues (52) demonstrating chain length-dependent effects with respect to bacterial MutL modulation of MutS-heteroduplex interaction. The DNA chain length effects observed in the two systems are not surprising since early work with the bacterial proteins demonstrated that the MutS footprint in the presence of DNase I expands dramatically from 20 bp to ~100 bp in the presence of MutL and ATP (11).

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