Assembly and Molecular Activities of the MutS Tetramer*

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Analytical equilibrium ultracentrifugation indicates that *Escherichia coli* MutS exists as an equilibrating mixture of dimers and tetramers. The association constant for the dimer-to-tetramer transition is $2.1 \times 10^7$ M$^{-1}$, indicating that the protein would consist of both dimers and tetramers at physiological concentrations. The carboxyl terminus of MutS is required for tetramer assembly because a previously described 53-amino acid carboxyl-terminal truncation (MutS800) forms a limiting species of a dimer (Ohmolova, G., Ban, C., Hsieh, P., and Yang, W. (2000) *Nature* 407, 703–710; Lamers, M. H., Perrakis, A., Enzlin, J. H., Winterwerp, H. H., de Wind, N., and Sixma, T. K. (2000) *Nature* 407, 711–717). MutS800 binds a 20-base pair heteroduplex an order of magnitude more weakly than full-length MutS, and at saturating protein concentrations, the heteroduplex-bound mass observed with MutS800 is only half that observed with the full length protein, indicating that the subunit copy number of heteroduplex-bound MutS is twice that of MutS800. Analytical equilibrium ultracentrifugation using a fluorescein-tagged 20-base pair heteroduplex demonstrated that native MutS forms a tetramer on this single site-sized heteroduplex DNA. Equilibrium fluorescence experiments indicated that dimer-to-tetramer assembly promotes mismatch binding by MutS and that the tetramer can bind only a single heteroduplex molecule, implying nonequivalence of the two dimers within the tetramer. Compared with native MutS, the ability of MutS800 to promote MutL-dependent activation of MutH is substantially reduced.

MutS homologs participate in multiple genetic stabilization pathways by virtue of their ability to recognize a spectrum of DNA lesions. Recognition of base pair mismatches by MutS homologs has been implicated in the rectification of DNA biosynthetic errors and in the dissolution of recombination events involving diverged sequences (1–5). Recognition of damaged base pairs by MutS homologs is also involved in the transcription-coupled repair of DNA damage (2, 4) and has been implicated in the activation of checkpoint and apoptotic responses to certain types of DNA damage in mammalian cells (6). Members of the MutS family can thus be viewed as molecular sentinels and then communicate presence of that lesion to downstream activities (1, 7–9).

Events downstream of MutS recognition have been delineated for the *Escherichia coli* pathway responsible for correction of DNA biosynthetic errors, and this reaction has been reconstituted in *vitro* using near homogeneous components. Although a number of mechanistic details remain to be established, basic roles of the individual activities have been defined. MutS recruits MutL to the heteroduplex in a reaction requiring ATP (10–12). Assembly of this ternary complex is sufficient to activate the latent endonuclease activity of MutH, which incises the unmethylated strand at a hemimethylated d(GATC) strand signal (13). In addition, this complex activates the unwinding activity of DNA helicase II, which loads at the MutH incision with an orientation bias so that helix unwinding proceeds toward the mismatch (14, 15). The unwound portion of the incised strand is hydrolyzed by a single-strand exonuclease. If unwinding proceeds from a strand break located 5′ to the mispair, the displaced single strand is degraded by the 5′-to-3′ hydrolytic activity of ExoVII or RecJ exonuclease (16, 17). When helicase unwinding initiates at a nick 3′ to the mismatch, the unwind strand is degraded by the 3′-to-5′ activity of ExoI, ExoVII, or ExoX (16–19). Excision terminates at a number of sites centered about 50 base pairs beyond the mismatch, the ensuing gap is repaired by DNA polymerase III holoenzyme in the presence of single-strand binding protein, and DNA ligase restores covalent integrity to the repaired strand (18, 20).

In addition to its mismatch recognition activity, bacterial MutS harbors a weak ATPase (21) that is stimulated by DNA (22). Because the rate-limiting step for ATP hydrolytic turnover is sensitive to the presence of a mismatch (22), the nucleotide and DNA binding sites of MutS are in close communication. Furthermore, the affinities of MutS for heteroduplex and homoduplex DNAs have been shown to be differentially modulated by binding of ADP or nonhydrolyzable analogues of ATP (23). *E. coli* MutS800 crystallizes as a dimer in which the two subunits are nonequivalent with respect to DNA interaction and nucleotide binding. Only one of the homodimeric MutS800 subunits is involved in recognition of the mismatched base, and the nucleotide-binding site of only this subunit is occupied by ADP-Mg (8, 24).

Analysis of the cooperative interactions between the MutS nucleotide hydrolytic cycle and DNA interaction is complicated by the fact that, unlike MutS800, dimers of full-length MutS assemble into higher order structures (22, 24–27). *Thermus thermophilus* and *Thermus aquaticus* MutS proteins have been shown to assemble from dimers to tetramers in an equilibrium manner (26, 27). Velocity sedimentation and gel filtration have indicated that *E. coli* MutS dimers also assemble into a higher order structure and that assembly is promoted by the presence of ATP (22, 25). In this study, we show that the *E. coli* MutS
dimer assembles in an equilibrium fashion to form a tetrameric species, with the equilibrium constant for association indicating that the protein exists as a mixture of dimers and tetramers under physiological conditions. These studies also show that mismatch binding is facilitated by tetramer formation and that this species is capable of binding only one heteroduplex molecule, implying nonequivalence of the two dimers within the tetramer.

EXPERIMENTAL PROCEDURES

Proteins and DNA—E. coli MutS800 that has been crystallized (24) consists of the N-terminal 800 residues of the 853 amino acids of the natural protein. An expression plasmid for MutS800 was constructed by PCR (25) of the appropriate fragment of the MutS gene of plasmid pMS1 (25, 28) followed by subcloning into a pT7-3 expression vector using EcoRI and BamHI endonuclease restriction sites. E. coli MutS and MutS800 proteins were purified as described (23) and stored as concentrated stocks in 50 mM KPO4, pH 7.4, 150 mM KC1, 1 mM 2-mercaptoethanol, and 50% (v/v) glycerol at −20 °C before injection. The system was judged to be at equilibrium (30) by imaging for the appearance of the MutS800 aequorin chimera (24). Sedimentation equilibrium analysis performed in the presence of chip-bound DNA with increasing concentrations of MutS or MutS800 concentrations were determined from the absorbance at 280 nm using extinction coefficients of 69,420 m⁻¹ cm⁻¹ and 62,450 m⁻¹ cm⁻¹ calculated from primary amino acid sequences (29).

Oligodeoxynucleotides were purchased from Oligos Etc. (Wilsonville, OR). The 5’-terminus of the 20-mer sequence d(TAGCA-GCTTCTGAGTTCTGAGTC) was modified with fluorescein or biotin as indicated. 20-mer homoduplexes were prepared by annealing this sequence with its complement in 10 mM Tris-HCl, pH 8.0, 100 mM KC1, and 1 mM EDTA at 90 °C and slow cooling to room temperature. Concentrated stock solutions (100 μM) were stored at 4 °C. 20-mer G-T heteroduplexes were prepared in a similar manner, except that the 20-mer complement contained a centrally located G residue placed opposite the thymine indicated by bold italic in the sequence above. The annealed fluorescein-labeled duplexes were purified by high pressure liquid chromatography on a Waters Gen-Pak column eluted with a NaCl gradient (0.3–1 M) in 10 mM Tris-HCl, pH 8.0, and 1 mM EDTA. A 15-base pair G-T heteroduplex was derived from the internal sequence of this 20-base heteroduplex was determined at a MutS concentration of 5 μM. The association constant for the complex was calculated from the total MutS concentration, S, was calculated from the total MutS concentration, S, was calculated from the total MutS concentration, S, and well above the dissociation constant for the complex. The concentration of MutS800 dimers, S, was calculated from the total MutS concentration, S, and well above the dissociation constant for the complex.

**Equilibrium Fluorescence Spectroscopy Analysis**—Equilibrium fluorescence spectroscopy performed on a SLM 9100 spectrofluorometer was used to monitor the extent of MutS binding to a 15-base pair CyIII-derivatized G-T heteroduplex in 25 mM Tris-HCl, pH 7.6, 1 mM dithiothreitol, 100 mM KC1, 5 mM MgCl2, and 10% (v/v) glycerol. CyIII was excited at 549 nm and fluorescence emission intensity was monitored at 563 nm.

Binding of MutS to the CyIII-end labeled heteroduplex resulted in a 50% increase in fluorescence intensity and a shift in the wavelength maxima from 561 to 563 nm. A longer 20-base pair CyIII-labeled G-T heteroduplex did not give a signal change upon MutS binding (data not shown), suggesting that the distance from the fluorophore to the mismatch is critical for the observed fluorescence change. Equilibrium binding analysis was performed by titrating CyIII-labeled heteroduplex (50 μM) with increasing concentrations of MutS. The stoichiometry of binding of the 15-base pair CyIII-derivatized G-T heteroduplex was determined at a MutS concentration of 5 μM (monomer), conditions under which the protein is predominantly a tetramer and well above the dissociation constant for the complex. The concentration of MutS dimers, L, is defined by 20 base pairs.

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S = \frac{1}{L} + \frac{1}{4K_D S^2} \quad (\text{Eq. 1})
\]

\[
\Delta F_{\text{obs}} - \Delta F_{\text{max}} = \frac{K_D S + K_D S^2 + 1 - \sqrt{K_D S^2 - K_D S - 1} - 4K_D S^2}{2K_D S} \quad (\text{Eq. 2})
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**RESULTS**

**Equilibrium Sedimentation of MutS Indicates Assembly of Dimers to Tetramers**—Sedimentation equilibrium profiles of MutS and a 53-residue carboxyl-terminal truncation (MutS800) are compared in Fig. 1 (initial concentrations of 20 μM as monomer equivalents). Although the MutS800 polypeptide is 94% of the mass of full-length MutS, the sedimentation equilibrium profiles of the two proteins differ dramatically. For comparison purposes, the data of Fig. 1 were fit to a model assuming a single ideal (non associating) species to yield a weight average Mr of 360,000 ± 20,000 (3.8 monomer equivalents) for full-length MutS and 220,000 ± 12,000 (2.4 monomer equivalents) for MutS800.
self-assembly reaction (22). At high concentrations (30 μM), the protein behaves as a uniform species of high molecular weight, but the oligomeric state of this species has not been established. The data of Fig. 1 suggest that the assembly end state is a tetramer of 95-kDa monomers, and as described below, functional analysis of the protein is consistent with this view. To further evaluate the nature of the MutS assembly reaction, sedimentation equilibrium analysis was performed at four different initial concentrations (2.5, 5.0, 10, and 20 μM monomer) at two different speeds (5000 and 7000 rpm). The eight data sets obtained in this manner were globally fit to a dimer-tetramer self-association model (WinNonlin, “Experimental Procedures”). As shown in Fig. 2, this model provides an excellent fit to the observed data and yields an association constant for the dimer to tetramer transition of 2.1 ± 0.1 × 10^7 M^-1 (parameter L1 in Equation 1). Although a small fraction of higher order oligomers cannot be ruled out by this analysis, it is clear that MutS is primarily tetrameric at the highest concentrations tested.

An assembly model that allows monomer and trimer intermediates also provides an adequate fit to the data of Fig. 2 (not shown). However, we regard such a model as unlikely because we have been unable to detect free monomer at the lowest MutS concentration and highest rotor speed tested (2.5 μM and 10,000 rpm, data not shown). Indeed, gel filtration and equilibrium sedimentation analysis of *T. aquaticus* MutS has led to the conclusion that this protein also exists in solution as an equilibrium mixture of dimers and tetramers (27).

**MutS Sediments As A Tetramer When Bound to a Single Site-sized Heteroduplex**—The nature of MutS oligomeric states active in heteroduplex binding was examined in sedimentation equilibrium experiments that monitored the apparent molecular mass of a fluorescein-derivatized, 20-base pair G-T heteroduplex as a function of MutS concentration (as monomer) and for initial concentrations of 10 μM (green) and 20 μM (tan). Black lines overlaid on the experimental data correspond to the global nonlinear least-squares fit to a dimer to tetramer assembly model (see “Experimental Procedures”). The fitting routine used takes into account the different extinction coefficients of the protein at the two scanning wavelengths used. The dimerization constant (L1) obtained from this analysis is 0.1 ± 0.01 × 10^7 M^-1. The square root of the variance for these eight data sets is 0.012 absorbance units, indicating a good fit to the model. Residues from this analysis are plotted above A as a function of radial cell position and are shown in B as a function of MutS concentration.

Fig. 3 shows sedimentation equilibrium profiles of 5 μM fluorescein-labeled heteroduplex as a function of MutS concentration, with data fit to a model that assumes the presence of a single ideal species. For initial MutS concentrations of 20 and 40 μM (monomer), this analysis yielded weight average values for the protein-DNA complexes of Mr 335,000 ± 12,000 and 465,000 ± 30,000. These values are much larger than that expected for a MutS dimer bound to a single heteroduplex (204, 000), but are similar to those expected for a MutS tetramer bound to one or two molecules of heteroduplex DNA (394 or 408 kDa). It is important to note that a limiting species indicative of MutS tetramer binding was also observed with a fluorescein-
labeled, 15-base pair heteroduplex ($M_r = 362,000 \pm 20,000$, data not shown). The similar $M_r$ values obtained with 15- and 20-base pair heteroduplexes argues against a model in which a tetramer-heteroduplex stoichiometry is simply a result of the contiguous binding of two MutS dimers on the same short piece of DNA. Furthermore, although it is clear that the tetramer is the predominant form of MutS bound to heteroduplex under these conditions, it is possible that a small fraction of the complexes observed at the highest MutS concentration tested (40 $\mu$M) may involve higher order assemblies. Experiments described below, which provide an independent evaluation of MutS-heteroduplex stoichiometry at more physiological protein concentrations (32), confirm activity of the MutS tetramer in heteroduplex recognition.

**The Heteroduplex-bound Molecular Mass of MutS is Twice That of MutS800**—Because surface plasmon resonance spectroscopy monitors mass change upon ligand binding, we have used this method to compare the relative mass equivalents of MutS and MutS800, which are bound by a 20-base pair G-T heteroduplex at protein concentrations much lower than those used in the sedimentation equilibrium experiments. As shown in Fig. 4, both proteins bind with higher affinities to the small

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**Fig. 3. Co-sedimentation of MutS with a 20-base pair heteroduplex.** Equilibrium sedimentation profiles of a 20-base pair fluorescein-end-labeled G-T heteroduplex (see "Experimental Procedures") were scanned at 494 nm. Initial heteroduplex concentration was 5 $\mu$M, and sedimentation was performed in the presence of 20 and 40 $\mu$M MutS (5000 rpm, $A$ and $B$, respectively). The apparent molecular mass of the DNA oligonucleotide in the presence of MutS was determined for each sample and the fit to a single ideal species ("Experimental Procedures") is overlaid on each data set. The apparent values for the MutS-DNA complex were $M_r$, 335,000 $\pm$ 12,000 at 20 $\mu$M and $M_r$, 465,000 $\pm$ 30,000 at 40 $\mu$M MutS.
heteroduplex than to an otherwise identical A-T homoduplex, and at saturating protein concentrations, the heteroduplex-bound mass observed with native MutS is twice (2.0 ± 0.5) that observed with MutS800. Because MutS800 is known to recognize a mismatch as a dimer (24, 33), this mass ratio implies that native MutS is binding to the heteroduplex as the tetramer, consistent with the differences in molecular mass observed for the two proteins during equilibrium centrifugation (Fig. 1). It is also noteworthy that although MutS800 retains mismatch specificity, this dimeric protein binds 18-fold more weakly than native MutS (Fig. 4, $K_d$ of 18 ± 1 nM for MutS and 331 ± 8 nM for MutS800).

The MutS Tetramer Binds a Single Heteroduplex Molecule—To further clarify the role of MutS assembly in DNA binding and to assess the stoichiometry of this interaction, we have developed an equilibrium fluorescence assay that monitors binding of the protein to a 15-base pair G-T heteroduplex, which contains a CyIII fluorophore on the 5'-terminus of one strand (see “Experimental Procedures”). As shown in Fig. 5, MutS binds to this small heteroduplex, resulting in a 30% enhancement of CyIII fluorescence intensity. For purposes of comparison, Fig. 5 also shows the MutS binding profile for the 15-base pair heteroduplex, and the corresponding A-T homoduplex, as determined by surface plasmon resonance spectroscopy.

The affinity of MutS for this small DNA is significantly less than that for the 20 base pair G-T heteroduplex from which the 15mer was derived as an internal sequence, although specificity for the mismatch is retained. Fits of this data to a simple hyperbolic mechanism (Fig. 5, dashed lines) yielded apparent $K_d$ values for the 15-mer of 100 ± 15 and 270 ± 57 nM for surface plasmon resonance and fluorescence assays, respectively; these values can be compared with the $K_d$ for the 20-base pair substrate of 18 nM (Fig. 4). However, the data sets of Fig. 5 deviate appreciably from a simple hyperbolic model, with binding profiles for this small heteroduplex displaying apparent positive cooperativity. We attribute this cooperative behavior to facilitation of binding by MutS assembly (i.e. heteroduplex binding drives tetramer formation (see “Discussion”)). Indeed, fits of the data sets of Fig. 5 to a cooperative dimer-to-tetramer assembly model (solid lines) are in excellent agreement with experimental observations. As is evident in Fig. 5, binding scored by equilibrium fluorescence seems to be more tightly coupled to MutS assembly than that observed by surface plasmon resonance spectroscopy. This presumably reflects differences in the molecular events scored by the two methods. For example, because surface plasmon resonance scores heteroduplex-bound MutS mass, binding of either the dimer or the tetramer will yield a signal in this assay. This may not be the case for the fluorescence assay: production of a fluorescent signal upon tetramer, but not dimer, binding would result in an apparent enhancement of cooperativity like that observed. It is also possible that biotin- or CyIII-derivatization of the 15-base pair DNA for the two types of assay may differentially affect MutS binding to the small heteroduplex.

An advantage of equilibrium fluorescence over surface plasmon resonance spectroscopy is that the concentrations of either binding partner can be varied independently. We have exploited this fact to establish the stoichiometry of binding of the 15-mer heteroduplex to the MutS tetramer. Fig. 6 shows a titration of 5 μM MutS (monomer) with the 15-base pair CyIII-labeled G-T heteroduplex. At this concentration, MutS is predominantly a tetramer (90%, Equation 1; see “Experimental Procedures”), and because this concentration is well above the binding constant for the 15-mer (Fig. 5), heteroduplex-MutS interaction under these conditions will be nearly stoichiometric. Fit of this titration data to Equation 2 ("Experimental Procedures") yielded an interaction stoichiometry of 1.13 ± 0.09 μM DNA bound per 5 μM MutS monomer equivalents; i.e. one molecule of 15-base pair heteroduplex bound for every 4.4 ± 0.4 MutS monomers. Thus, the process of dimer-to-tetramer assembly renders the two dimers non-equivalent with respect to the ability to bind heteroduplex. Tetramer formation enhances the heteroduplex affinity of one dimer but reduces the heteroduplex affinity of the other.
were fit to a Langmuir binding isotherm (Equation 2, Experimental Procedures), which yielded a binding stoichiometry of 1.13 ± 0.09 μM DNA bound per 5 μM MutS monomer equivalents.

Dimer-forming MutS800 Is Compromised in the MutH Activation Assay—Expression of MutS800 has been reported to suppress the mutator phenotype of mutS mutant cells. However, these results were obtained when the MutS800 gene was present on high copy number plasmid (24). Because we have been unable to detect MutS800 assembly beyond a dimer, we have compared the biochemical activity of this protein with that of native MutS in the initial step of methyl-directed mismatch repair: the mismatch-, MutS-, MutL-, and ATP-dependent activation of the MutH d(GATC) endonuclease (13). As expected from the biological observations, MutS800 does support activation of MutH endonuclease (Fig. 7), but its activity is compromised in this respect. The concentration of MutS800 necessary to achieve half maximal MutH activation is about six times that observed for full-length MutS. Although this phenomenon may be caused in part by the reduced affinity of MutS800 for a heteroduplex (Fig. 4), it is also evident that maximal rate of MutH activation supported by saturating concentrations of MutS800 is reduced relative to that of the wild-type protein.

DISCUSSION

Previous studies have shown that T. aquaticus and T. thermophilus activities undergo dimer-to-tetramer assembly, although the functional consequences of oligomerization have not been addressed (26, 27). A growing body of evidence now exists for oligomeric states of E. coli MutS beyond that of the dimer. Early velocity sedimentation experiments showed that MutS exists as 7 S and 10 S forms, and subsequent gel filtration analysis demonstrated a concentration-dependent increase in the molecular size of E. coli MutS, with the plateau value consistent with a tetramer or hexamer (22, 25). Furthermore, MutS assembly has been shown to be promoted by adenine nucleotides (22, 34). Response of the MutS ATPase to heteroduplex activation has also suggested that the functional form of the protein is a higher order oligomer than a dimer. ATPase activation saturates at a ratio of one heteroduplex per four to six MutS monomer equivalents, suggesting that the cooperative unit for ATP hydrolysis by the native protein is at least a tetramer (22).

The equilibrium sedimentation experiments described here show that E. coli MutS also assembles to a form a tetramer.

The in vivo concentration of MutS in exponential E. coli cells has been estimated to be 0.5 μM in monomer equivalents (32). The dimerization constant of 2.1 ± 1 × 10^{-12} M^{-1} obtained from sedimentation equilibrium indicates that ~50% of the MutS would be present as tetramer at this concentration (Equation 1). Insofar as this fraction will be dependent on actual in vivo conditions, we note that this value is almost certainly an underestimate for two reasons. As discussed above, the dimer-to-tetramer transition is promoted by the presence of ATP (22, 34). In addition, the results shown in Fig. 5 indicate that heteroduplex binding also promotes tetramer formation. This finding accounts for the observation in Fig. 4 that full-length MutS binds to the high-affinity, 20-base pair heteroduplex as a tetramer with saturation occurring well below the estimated in vivo concentration of 0.13 μM. If MutS assembly were uncoupled from DNA binding, then the protein would initially bind to DNA as a dimer. However, no discernible MutS dimer-DNA intermediate complex was formed. Although the data in Fig. 4 are not demonstrably cooperative at the lowest MutS concentrations tested, we attribute this to the much higher intrinsic affinity of MutS for the 20-base pair DNA used in the experiments of Fig. 4 compared with the shorter, 15-base pair molecule. The reduced affinity of MutS for the smaller DNA permits visualization of cooperativity at low protein concentrations (Fig. 5).

Although the MutS dimer can clearly recognize a mismatch, the findings that ATP and heteroduplex binding promote tetramer formation strongly suggest a functional role for this oligomeric state in mismatch repair. Functionality is also suggested by the observation that the two dimers within the tetramer are not equivalent; i.e., like the MutS dimer, the tetramer is capable of binding only one molecule of heteroduplex (Fig. 6). Indeed, if MutS tetramers were inactive in mismatch repair, then in vivo overexpression of the protein would be expected to result in a mutator phenotype, but this is not the case (35). Substrate-induced protein assembly has been invoked as a mechanism that renders the cell more responsive to small changes in protein concentration (36). Our calculations suggest
that dimer and tetramer concentrations may be finely tuned at physiological concentrations of MutS, such that a decrease in concentration of the protein will lead to a greater-than-first-order decrease in the tetramer concentration. In view of the higher mismatch affinity of the tetramer, this would lead to a reduction in the efficiency of mismatch recognition. In this regard, it is noteworthy that the concentration of MutS (but not that of MutL or MutH) is down-regulated during metabolic stress (32).

Previous electron microscopic analysis has demonstrated the MutS-, ATP-, and mismatch-dependent formation of DNA loops with 6.4-kilobase heteroduplex (37). A bi-lobed E. coli MutS complex stabilized DNA loops at the base, and loop size was observed to increase with time in an ATP-dependent manner. Because the mismatch was often present within the DNA loop, these observations led to the suggestion that in the presence of ATP, MutS translocates from the mismatch in a bidirectional fashion. Based on the projected shadow size in electron micrographs, the MutS complex observed at the loop base was estimated to be a dimer (37). However, in view of the clamp-like structure of the DNA-bound MutS dimer (24, 33) and the results presented here, it seems likely that the bi-lobed complex observed in the electron microscope was actually the tetramer. The MutS tetramer is readily accommodated into a bidirectional translocation, because such a mechanism would be dependent on at least two potential DNA binding sites. In this regard, it is interesting to note that MutS800, which is incapable of assembling into tetramers, displays only half the maximal stimulated rate of MutH activation compared with the native protein (Fig. 7).

Alternate functions for the MutS tetramer can also be envisioned. For example, it has been suggested that one dimer within the MutS tetramer may bind to the mispair, whereas the other participates in a search for the strand discrimination signal (8). Indeed, there is ample evidence that MutS functions during several discrete steps during mismatch repair. It is required for the mismatch- and MutL-dependent activation of the MutH endonuclease, which incises the unmethylated strand at the d(GATC) strand signal (13) and for the mismatch- and MutL-dependent loading of DNA helicase II at the ensuing strand break (15, 38). Recent experiments have demonstrated that MutS can also interact with the β-clamp of DNA polymerase III holoenzyme (39) and therefore may be involved in the polymerase specifity of the reaction. It is thus clear that MutS plays essential rules in multiple steps of the repair reaction that involve several DNA sites and other proteins. It is important to note, however, that there is no mechanistic evidence

indicating that the same MutS molecule that is responsible for initial mismatch recognition event is the same molecule responsible for subsequent MutS functions during the repair reaction. Inasmuch as cooperative interactions are evident across the tetramer dimer-mispair interface, coordination of repair events could in principle be mediated through the transient synapses of different MutS dimers.

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